Quantification of potential methane emissions associated with organic matter amendments following oxic soil inundation

Brian Scott[1], Andrew H. Baldwin[1], Stephanie A. Yarwood[1]

[1]Environmental Science and Technology Department, University of Maryland, College Park, 20742, USA

Correspondence to: Brian Scott (bscott33@umd.edu)

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, composted yard waste, and wood mulch were evaluated at three different levels. Using 1-liter glass microcosms, we measured the production of biogenic gases over 60 days in two soils designated by texture: 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, and CH_4 production was more pronounced in the SL. We observed 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 higher total gas and nearly equal amounts of CH_4 and CO_2. If this is generally true in soils, it may be appropriate to report CH_4 emissions in the context of inundation duration. The CH_4 from the SCL soil ranged from $0.003 - 0.8$ cm^3 Kg^{-1} day in Phase 1 to $0.75 - 28$ cm^3 Kg^{-1} day in Phase 2, and from the SL range from $0.03 - 16$ cm^3 Kg^{-1} day in Phase 1 to $1.8 - 64$ cm^3 Kg^{-1} day in Phase 2. Adding fresh organic matter

24   (e.g., hay) increased ferrous iron (Fe^{2+}) concentrations whereas in some cases composted

25   organic matter decreased both Fe^{2+} concentrations and CH_4 production. Methanogenesis

26   normally increases following the depletion of reduceable Fe; however, we observed

27   instances where this was not the case, suggesting other biogeochemical mechanisms

28   contributed to the shift in gas production.

29   **Keywords** Methane emissions, mitigation wetlands, organic amendments
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31   **1 Introduction**
32
33   The ecological benefits of wetlands are well documented, including their role as

34   carbon (C) sinks to stabilize global climate (Mitsch et al., 2015). Driven in part by this

35   ecological contribution, from 1970 to 2015 human-made wetlands have increased 233%

36   (Darrah et al., 2019). Between 2004 and 2009 the United States saw a net gain of 16,670

37   hectares of freshwater wetlands: 360,820 hectares of new wetlands to offset 344,140

38   hectares of existing (presumably C-sink) wetlands that were destroyed (Dahl, 2011).

39   Although created or restored wetlands may effectively sequester C, it may take hundreds

40   of years to offset their radiative forcing due to methane (CH_4) emissions (Neubauer,

41   2014). With such a large number of human-made wetlands, and their potential to increase

42   global warming, it is vital to consider factors that may contribute to CH_4 emissions.

43   Organic amendments such as straw, wood mulch, manure, and biosolids, mixed

44   into the soil, are thought to accelerate C storage by enhancing the conversion of plant-

45   derived compounds to microbial residues (Richardson et al., 2016). Microbial residues,

46   largely aliphatic-C from cell membrane lipids, can accumulate in soil and are not directly

47   accessible by methanogens (Chen et al., 2018). Plants contribute both above and

48   belowground organic matter (OM). Belowground plant materials are preferentially

49   converted to soil organic carbon (SOC) (Mazzilli et al., 2015). In saturated soils root

50   residues of wetland plants contain suberin and cutin (Watanabe et al., 2013), which

51   persist, reducing biogenic gas production (Mikutta et al., 2006). Before contributing to

52   SOC, standing litter in natural wetlands is partially decomposed by fungi (Kuehn et al.,

53   2011), and further decomposed by aerobic bacteria (Yarwood, 2018). Allochthonous

54   organic amendments are derived from above-ground material, but they have not been

55   subjected to wetland biogeochemical processes. Studies suggest these materials are less

56   amenable to soil C stabilization compared to natural plant inputs and may increase CH_4

57   production (Scott et al., 2020). In addition to increasing CH_4 production directly, organic

58   amendments may cause SOC priming that produces additional CH_4 (Nottingham et al.,

59   2009), and can lead to an increase in iron (Fe) reduction and toxicity (Saaltink et al.,

60   2017).

61   Iron oxides play multiple roles in anoxic soils, being both an electron acceptor for

62   organic C metabolism (Straub et al., 2001), and a stabilizing agent for SOC on mineral

63   surfaces (Lehmann and Kleber, 2015). As a metabolite, Fe reduction competes with CH_4

64   production (Huang et al., 2009) and can facilitate sulfur recycling (which also competes

65   with CH_4 production) in freshwater sediments (Hansel et al., 2015). However, recent

66   literature suggests the relationship of Fe reduction and methanogenesis is more complex.

67   Some methanogens appear capable of switching between methanogenesis and Fe

68   reduction (Sivan et al., 2016). In cultures with *Methanosarcina acetivorans*, adding Fe

69   oxides increased methane production (Ferry, 2020), presumably by the utilization of a

70   metabolic pathway where electron flow is bifurcated with some electrons going toward

71   Fe reduction to increase energy yield (Zhuang et al., 2015; Prakash et al., 2019). In

72   systems that are near pH neutral, Fe reduction does not necessarily have an energetic

73   competitive advantage over CH_4 production (Bethke et al., 2011). In addition to

74   influencing metabolic pathways, metal-oxide surfaces can stabilize organic matter,

75   making it less bioavailable, which can affect both Fe reduction (Poggenburg et al., 2018),

76   C mineralization (Amendola et al., 2018; Lalonde et al., 2012) and production of CH_4.

77   We carried out a lab experiment using organic amendments commonly used in

78   wetland restoration (biosolids (Bloom®) - B, manure - M, composted yard waste

79   (LeafGro®) - L, wood chips - W, and hay - H) and measured how they affected CH_4

80   production and Fe reduction. One-liter (1-L) glass jar microcosms were incubated with

81   two different soils collected from sites where freshwater wetlands were recently created.

82   The microcosms were kept under anaerobic conditions to compare the ability of these

83   substrates to support anaerobic metabolism. We hypothesized that organic amendments

84   would stimulate dissimilatory Fe-reduction in soils (measured as soluble ferrous iron,

85   Fe^{2+}). Further, we hypothesized that amendments promoting Fe reduction would limit

86   methanogenesis. We also tested differences between cured (i.e., aged/composted) and

87   uncured (fresh) organic amendments and hypothesized that uncured amendments would

88   increase Fe reduction due to the presence of more labile, soluble, compounds. In the

89   United States organic amendments are often required in mitigation wetlands, that is,

90   wetlands created or restored to offset wetland losses; however, there has not been a

4

91 systematic evaluation of whether or not amendments promote hydric soil conditions (Fe

92 reduction), lead to Fe toxicity (from Fe reduction), or increase CH_4 production.

93 **2 Materials and methods**

94 **2.1 Microcosm setup**

95 Saturated incubations were established using soil from two recent mitigation

96 wetlands located in Maryland, USA. The first site (76°50'40.35"W, 38°47'5.41"N) was

97 most recently a horse pasture and will be referred to as SCL denoting the texture (sandy

98 clay loam). The second site (75°47'40.20"W, 39°1'52.42"N) was most recently a corn/soy

99 farm with tile drains and was likely a wetland prior to conversion to farmland. The

100 second site will be referred to as SL (sandy loam). Both sites had been recently graded to

101 establish wetland topography, so the upper portion of the soils, where soil samples were

102 collected, were mixed endo- and umbr-aquic horizons but with no ped structure. Soil was

103 collected from these recently constructed surface horizons to a depth of 15 cm, a typical

104 depth for mixing-in organic amendments, sieved (2mm) and homogenized prior to use.

105 Additional soil information is shown on Supplemental Table S1.

106 Microcosm experiments were conducted in 1-L glass straight-sided wide-mouth

107 food canning jars. Each microcosm had a total of 600cc of solid material and was filled

108 with water for a total volume of 660cc. The volumes needed to be precise in order to

109 facilitate headspace and liquid sampling and to allow space for soil expansion. When

110 amendments were added, an equal volume of soil needed to be removed so the total

111 volume of solid material was a constant 600cc. At the start of the experiment, the

112 headspace was purged with nitrogen gas. The incubation temperature was 20°C. Jar lids

113   had precision drilled holes fitted with grey butyl rubber stoppers, making it possible to

114   non-destructively remove the overlying liquid (for Fe and pH analyses) using a 7.5 cm

115   needle. Since the head-space pressure increased due to biogenic gas production,

116   atmospheric pressure was re-established during gas sampling events by piercing the septa

117   with a 24-gauge needle connected to a 50mL gas-tight syringe. This procedure allowed us

118   to record the total volume of gas produced and collect gas samples (0.01 - 1000 μL)

119   under atmospheric pressure (Supplemental Figure S1). A small coating of silicone

120   applied to stoppers after piercing prevented leaks. All microcosm trials were run with

121   three replicates except where noted.

122   **2.2 Microcosm Experiments**

123   **2.2.1 Experiment 1**

124   We measured CH_4 and Fe^{2+} production with various organic amendments,

125   including composted yard waste (L), composted wood chips (W), class 1 biosolids - (B),

126   manure (M), and hay (H) at three treatment levels: 8.8% (v/v), 26%, and 53%, in two

127   soils, a SL and a SCL. We used horse M for the SCL incubations and cow M for the SL

128   incubations. This matched the wetland mitigation conditions at each field location. The

129   treatment levels reflect the Maryland Department of Environment (MDE)

130   recommendation for wetland restoration (60 cubic yards per acre assuming a 6" mixing

131   depth) = 1x, 3x, and 6x the MDE recommended level. All amendments were sieved to

132   5mm. Hay was chopped with a Wiley mill, blended, or cut with scissors until it could

133   easily pass a 5mm sieve.

134 **2.2.2 Experiment 2**

135   We measured CH_4 and Fe^{2+} production using cured (aged) and uncured (fresh)

136 organic materials. We used two amendments, B and M. The two cured materials were

137 from the same two sources as the fresh material but had been cured for a minimum of 3

138 months. We added the same amount of amendment to each microcosm based on OM

139 content. Each amendment was evaluated for OM by loss-on-ignition (LOI) (550°C for

140 2h). Based on the percent OM we adjusted the amount of amendment so the final loading

141 rate was 20g OM/ 600 cm^3 soil. The microcosm setup was the same as Experiment 1

142 except we used the same volume of soil (600 cm^3) in all microcosms. These microcosms

143 were incubated for 13 days and sampled periodically for Fe^{2+} and biogenic gases.

144 **2.2.3 Experiment 3**

145   We measured a) CH_4 and b) Fe^{2+} production as a function of pH. We used H

146 leachate as a substrate (McMahon et al., 2005). We leached 5.63 g H with 125 cm^3 cold

147 de-ionized water, shaking horizontally at 5°C for 24 hours. The leachate was filtered to

148 20 μm and immediately placed into jars with 600 cm^3 SL soil and incubated for 22 days.

149 The pH was adjusted to target levels of 5.6, 6.1, and 6.6 using a non-substrate buffer: 2-

150 (N-morpholino) ethanesulfonic acid (MES). To determine the necessary concentration of

151 MES, we titrated SL (pH 5.8) to our maximum desired pH (6.6). We determined that the

152 buffering capacity of the soils corresponded to ~ 2 mN in the 125 cm^3 of liquid (leachate

153 volume), so we prepared microcosms using 125 cm^3 of 20 mN MES buffer.

154 **2.2.4 Experiment 4**

155   We measured Fe^{2+} production using leached H as a substrate (as in Experiment 3)

156 but compared these finding to those with unleached H, and the H residuals.

2.3 Soil, Liquid, and Gas Analyses

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158   Prior to the start of the experiments, we analyzed the SL and SCL for soil texture,

159   percent soil C, and extractable Fe (Supplemental Table S1). Soil texture was determined

160   by adding 50 g soil to a 1000 ml cylinder with 0.5% hexametaphosphate. Sand settled

161   after 1 minute and silt after 24 hours. Soil moisture content was determined as weight

162   loss of approximately 5 g of soil dried at 105°C for 48 hours. We determined percent soil

163   C using thermal combustion at 950°C on a LECO CHN-2000 analyzer (LECO Corp., St.

164   Joseph, MI). Iron extractions were performed sequentially with 1 M hydroxylamine

165   hydrochloride (HHCL) in 25% v/v acetic acid; 50 g / 1 sodium dithionite in solution 0.35

166   M ace-tic acid / 0.2 M sodium citrate buffered to pH 4.8; 0.2 M ammonium oxalate / 0.17

167   M oxalic acid (pH 3.2) (Poulton and Canfield, 2005). The HHCL extraction targets

168   bioavailable iron, primarily ferrihydrite and lepidocrocite. Dithionite also includes more

169   crystalline iron oxide forms, hematite and goethite. Oxalate includes the bioavailable iron

170   oxides and magnetite.

171   Throughout the experiments we measured Fe^{2+}, pH, and biogenic gases in the

172   headspace. In some cases, Fe^{2+} and pH were measured only at the end of the incubation.

173   Using a 3" needle, we extracted 0.3 - 1 cm^3 (for Fe^{2+}) and 1 cm^3 (for pH) of the

174   supernatant liquid to avoid disturbing soil in the jars. Samples of liquid supernatant were

175   removed during gas sampling, when atmospheric pressure was maintained, to avoid loss

176   of biogenic gases and atmospheric contamination. For the final sample point the jar

177   contents were thoroughly mixed prior to sampling to include pore water and gases.

178   Ferrous iron in supernatant liquid was measured with a HACH DR4000

179 spectrophotometer. The spectrophotometer was also used to measure Fe in the Fe-oxide

180 extractions. Prior to analysis, extracted Fe-oxides were reduced by adding thioglycolic

181 acid. To confirm the spectrophotometer accuracy, a subset of samples was also analyzed

182 on a PerkinElmer PinAAcle 900T atomic absorption spectrometer. An Orion 9142BN

183 electrode was used to determine pH.

184 Gas samples were collected in 12 cm^3 N-purged exetainer vials and analyzed by

185 injecting 5 cm^3 into a Varian Model 450-GC gas chromatograph. Since sample volume

186 was typically 1 cm^3 or less, 5 cm^3 nitrogen gas was added to the vials immediately prior

187 to analysis for CO_2 and CH_4, and measured concentrations were corrected for dilution

188 and prior headspace gas concentrations. We also performed fluorescent spectral scans on

189 dissolved organic matter that was extracted from organic materials with 1:10 solid

190 (weight) / deionized water (volume) for 24 hours and filtered to 0.45 μm (Fischer et al.

191 2020). After diluting samples, emission spectra were recorded using an Aqualog

192 fluorometer (Horiba Scientific; Edison, NJ).

193 **2.4 Data analysis**

194 Unless otherwise noted, statistical determinations were done using ANOVA in R

195 or SAS. The Fe^{2+} concentrations were evaluated using contrasts for each of the

196 amendments compared to the control using the R multcomp package. The gas curves

197 were modelled as piecewise, bimodal linear functions using the R "Segmented" package

198 (Muggeo, 2008). Breakpoints were determined using the total gas curves but, in some

199 cases, Segmented could not identify a breakpoint in the total gas curve, so CH_4 curves

200 were used as noted in Supplemental Figures S2 & S3. Gas curves from H amendments

201 did not fit a piecewise model and were modelled as sigmoidal functions using the

202 SSgompertz function in R. However, SSgompertz is sensitive to data scatter, particularly

203 at the beginning and end of the curve, so the gas curves for H6x in the SL were fit with a

204 power function in Excel.

205 **3 Results**

206 **3.1 Experiment 1a: Effect of organic amendments and soil type on CH_4 gas production**

207   Gas production occurred in two distinct steady-state gas production periods,

208 which we identified as Phase 1, and then after a breakpoint, Phase 2 (Figure 1) with

209 individual gas curves are shown in Supplemental Figures S2 (SCL) and S3 (SL). Some

210 CH_4 was produced almost immediately upon inundation (Table 1a), but after the

211 breakpoint (40 days in both the SL and SCL soils), there is a large increase in CH_4 as

212 well as an average $4.7x \pm 1.9$ increase in total gas production (Table 1b). One of our

213 amendments, H, did not fit the linear bimodal pattern, so we reported rates separately on

214 Table 1c.

215   Gas production varied by soil texture. In general, the SL soil produced 2.6 times

216 as much total gas (Figure 2a) and 2.4 times as much CH_4 as the SCL (Figure 2b). In the

217 SCL soil, CH_4 production in Phase 1 was 0.003 cm^3 CH_4^{-1} Kg soil^{-1} day and with

218 amendments increased to as much as 0.8 cm^3 CH_4^{-1} Kg soil^{-1} day (Table 1a). In Phase 2

219 1.9 cm^3 CH_4^{-1} Kg soil^{-1} day was produced in control soils and with amendments

220 increased to as much as 28 cm^3 CH_4^{-1} Kg soil^{-1} day (Table 1b). In the SL soil,

221 amendments increased the rate from 0.04 to 16 cm^3 CH_4^{-1} Kg soil^{-1} day Phase 1 and from

222 1.8 to 64 cm^3 CH_4^{-1} Kg soil^{-1} day in Phase 2.

223   Gas production rates generally increased with amendment loading rate (Table 1a

224   & b), as expected. With the exception of L in the SL, all amendments reduced the time

225   required to transition from Phase 1 to Phase 2 (i.e. the breakpoint). Biosolids caused the

226   largest shift, decreasing the breakpoint to as little as 5 days. While amendments generally

227   increased CH_4 production there were exceptions. Low loading rates of cured amendments

228   (L and W) had lower CH_4 production rates than unamended soil: L1 in Phase 1 in both

229   soils; L3 in the SL; L3 in the SCL (Phase 2 only); and W1 in the SCL (Phase 2).

230   Biosolids (B1) also lowered CH_4 production rates in the SL soil (Phase 1) (Table 1a). We

231   examined the normalized CH_4 production rates (per g C in soil), but in most cases results

232   were not statistically different at $p < 0.05$ (Supplemental Figure S4). The general trends

233   indicate uncured amendments (e.g. B and M) produce more methane per unit carbon than

234   cured amendments (L).

235   Using fresh H, biogenic gas production followed a sinusoidal pattern and we

236   reported maximum CH_4 production rate at the inflection point (Table 1c). Hay was prone

237   to floating at higher loading rates and was present in the water column above the surface

238   (not in contact with soil). In the instances where this occurred (H3 and H6 in the SCL),

239   there was a decrease in overall gas production rate and very low CH_4 – much lower than

240   unamended soils (Table 1c and Supplemental Figure S2z). Floating also occurred in one

241   replicated for H6 in SL – the pattern is shown on Supplemental Figures S2&3z, but not

242   used in the average reported value (Table 1c).

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

The type and loading rate of organic amendments affected total soluble Fe^{2+} production, compared to the unamended control, in a limited number of cases (Figure 3, Supplemental Table S2). In the SL soil, L caused a decrease ($p < 0.05$) in supernatant Fe^{2+} concentrations whereas H increased supernatant Fe^{2+} in both soils ($p < 0.05$). In a separate set of experiments, we documented the relationship between supernatant Fe and pore water Fe (Supplemental Figure S5). 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 the amount of hydrochloramine hydrochloride extractable Fe (FeHHCl) compared to the SL and had 7.6x more dithionite extractable Fe (Supplemental Table S1). Of the FeHHCl 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 FeHHCl in the SCL and 236 % in the SL was reduced to Fe^{2+} (Supplemental Table S2). 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 other amendments, Fe^{2+} production continued or even increased during periods of high CH_4 production. Figure 4 shows two examples that highlight this pattern and the complete set of curves is in Supplemental Figure S6.

3.3 Experiment 2a: Effect of cured versus fresh organic amendments on CH_4 gas 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 production rates of 18.2 and 27.8 cm^3 CH_4^{-1} Kg $soil^{-1}$ day in the SCL and SL soils, respectively

265 (Table 1c). The H3 and H6 loading rates would likely have been higher had some portion

266 of the H not floated. The M6 trials produced the most CH_4 at 27.7 and 64.0 cm^3 CH_4^{-1}

267 Kg soil^{-1} day in the SCL and SL soils, respectively. Of the amendments used, M was

268 cured the least (after fresh H, which was uncured). LeafGro, a commercial composted

269 yard waste, was cured the most and produced very little CH_4, in some cases less than the

270 controls. Since we could not specify precisely how long the organic material had been

271 cured, we conducted a separate experiment with organic materials of known curing

272 periods (at least 90 days), using B and M. Rather than use the same volumetric quantities,

273 we used the same loading rate based on OM content. The results confirmed that curing

274 has a strong influence on CH_4 production. Methane production was higher using fresh

275 material in both cases and cured material sometimes decreased CH_4 production (Table 2).

276 **3.4 Experiment 2b: Effect of cured versus fresh organic amendments on Fe^{2+} production**

277   In Experiment 1b, we observed that curing also had an effect on the amount of

278 Fe^{2+} produced. Hay was the only amendment that produced significantly more Fe^{2+} and L

279 produced a significant reduction in Fe^{2+} (Figure 3). In Experiment 2 we used biosolids

280 (B) and manure (M) that had been cured at least 3 months. Whether the material had been

281 cured had a strong influence on Fe^{2+} production and Fe^{2+} was higher using fresh material

282 in both cases (Figure 5).

283 **3.4.1 Spectral Analysis: Effect of organic amendments and soil type on CH_4 gas production**

284   We observed differences in CH_4 and Fe reduction rates when using organic

285 material that had been cured versus uncured. The fluorescent spectral signatures of the

286 cured materials (B and M) were similar as were the signatures of fresh material

13

287   (Supplemental Figure S7), so curing differentiated the materials more than the source.

288   The difference in signatures was indicative of higher concentrations of organic (humic)

289   acids and lower nominal oxidation state in the cured materials. We considered other

290   organic matter characterization methods such as the material's carbon to nitrogen ratio,

291   but we did not find another reliable predictor of CH_4 and Fe^{2+} production other than

292   curing.

293   **3.5 Experiment 3: Effect of pH on a) CH_4 and b) Fe^{2+} production**

294   The soil pH affected both CH_4 and Fe^{2+} production. In Experiment 1, we observed

295   that Fe^{2+} varied with pH in the SL soil ($p<0.001$; Supplemental Figure S8a), but there

296   was little variation in the SCL ($p=0.45$; Supplemental Figure S8b). In order to isolate the

297   effect of pH, we performed experiment 3 using a single substrate (H leachate) in the SL

298   soil. Higher pH increased the CH_4 production rate in both Phase 1 and 2 (Table 3) and

299   reduced the production of Fe^{2+} (Figure 6).

300   **3.6 Experiment 4: Leached versus unleached H and pH considerations**

301   In Experiment 4 we measured Fe^{2+} produced from H, H leachate, and H residuals

302   (Figure 7). We expected the soluble fraction to be more labile and produce more Fe^{2+};

303   however, the H residuals (solid fraction) appeared to produce more Fe^{2+} than the

304   leachate. As noted on the figure, separate leached fractions changed the system pH. Using

305   the results from Experiment 2, we predict that at comparable pH there would have been

306   no difference in Fe^{2+} production between H, H residuals, and leachate (Supplemental

307   Figure S9). Given the potentially strong influence of pH, we re-evaluated the results from

308   Experiment 2b, correcting for pH and confirmed that the organic material age accounts

309   for differences in Fe^{2+} production (Supplemental Figure S10). Similarly, we considered

310   whether pH may have affected the out-come of Experiment 1. A MANOVA analysis of

311   the Experiment 1 data (Supplemental Table S3) indicated that pH and soil type had a

312   small effect (p=0.30 and 0.81, respectively) compared to organic matter type and loading

313   rate (p<0.0001).

314   **4 Discussion**

315   Net CH_4 emissions are a primary factor that determines whether a wetland is a C

316   sink or contributes to long term global warming (Neubauer and Verhoeven, 2019). Soil

317   management practices, such as wetland restoration methods, can have a large impact on

318   CH_4 production and total greenhouse gas emissions (Paustian et al., 2016). Our data

319   indicate that organic amendments used in created or restored wetlands may have a large

320   influence on CH_4 production. Organic amendments that had been cured (L and W) only

321   slightly increased CH_4 emissions, but fresh material (M and H) resulted in large increases

322   (Tables 1a&b). This is consistent with field studies where comparable cured amendments

323   (composted wood and yard waste), did not result in increased CH_4 emissions (Winton and

324   Richardson, 2015), but straw (Ballantine et al., 2015) and peat bales (Green, 2014)

325   increased CH_4 emissions. Organic material is commonly cured, or composted, to remove

326   plant pathogens (Noble and Roberts, 2004) and to reduce the amount of cellulosic

327   material (Hubbe et al., 2010), which competes for oxygen, contributing to phytotoxicity

328   (Saidpullicino et al., 2007; Hu et al., 2011). Curing produces humic acids and increases

329   the nominal oxidation state (NOSC) of C (Guo et al., 2019). When cured material is then

330   subjected to anaerobic conditions, less CH_4 is produced (Yao and Conrad, 1999), which

331   would make composted material more suitable in a wetland restoration context.

332   Following soil inundation, we observed two distinct gas production phases (Phase

333   1 and 2). This pattern is difficult to distinguish in unamended soils but has been reported

334   previously (Yao and Conrad, 1999; Drake et al., 2009). Our breakpoint (5 – 45 days

335   Table 1b)) was similar to Yao and Conrad (1999) (5 – 36 days). The Phase 2 rates in

336   unamended soils were also similar: $0.96 – 3.98 \; cm^3 \; CH_4^{-1} \; Kg \; soil^{-1}$ day in Yao and

337   Conrad (1999) and $1.82 – 1.94 \; cm^3 \; CH_4^{-1} \; Kg \; soil^{-1}$ day in our study (Table 1b).

338   There are several explanations that could account for the observed gas production

339   pattern. One is the lag period required to re-establish populations of methanogenic

340   archaea, which are likely dormant under oxic conditions and regrowth can be on the order

341   of days (Jabłoński et al., 2015). In our study, B had the earliest shift to Phase 2 CH_4

342   production (Table 1b), possibly due to elevated levels of dormant methanogens present

343   from anaerobic digestion. The two-phase gas production could also be due to depletion

344   of bioavailable Fe-oxides, thus relieving the competition between Fe reducers and

345   methanogens (Megonigal et al., 2004). Our data were mixed, with some treatments

346   showing evidence of competition by Fe reducers, but in other cases we did not see

347   competition. In treatment M1, for example, ferrous Fe in the supernatant plateaued at

348   about the same time as the breakpoint (Figure 4b), after which CH_4 production increased.

349   In contrast, in W3 soluble Fe continued to be produced well after the breakpoint, and the

350   amount of bioavailable Fe used during the course of the incubation was less than $28 \pm 4\%$

351   (Figure 4b, Supplemental Table S2). In addition to quantifying Fe oxide concentrations,

352 the CO_2:CH_4 ratios can be indicative of interactions between methanogens and other

353 reducers (Bridgham et al. 2013). If Fe reduction or other reduction stops during Phase 2,

354 we would expect the CO_2:CH_4 ratio to be near 1:1 (Bridgham et al. 2013). However, we

355 observed notable exceptions. The SCL L1 treatment had a ratio of 73:1 in Phase 2 (Table

356 1b), yet still had the characteristic shift to higher overall gas production (4.67x). Other

357 treatments also had higher CO_2:CH_4 ratios: L3, L6, W1, B1, C, and W1-3 in the SL soil

358 (Table 1b). Our mixed observations may have been due to microsite formation. In high

359 producing microcosms, microsite development may have been disrupted by gas

360 ebullition, which was substantial enough in H amended trials to cause effervescence.

361 Amendments with low gas production and limited gas ebullition (e.g. L, W and C)

362 continued to produce Fe^{2+} after the breakpoint, possibly because methanogens were

363 active in undisturbed microsites, as described in Yang et al. (2017).

364 The increased gas production from organic amendments was more pronounced in

365 SL compared to SCL, where there was 2.4x higher CH_4 and 2.6x higher gas production

366 (Figures 2a & b). We observed a more pronounced effect than a recent rice field study

367 where there was more CH_4 from SL soils versus SCL, although in that study results were

368 not statistically significant (Kim et al., 2018). Yagi and Minami (1990) observed that

369 compost (approximate loading rate the same as our 1x treatment) increased respiration

370 rates by 1.8x in a SCL versus a loam soil. Maietta, Hondula, et al. (2020) observed that

371 respiration rates were higher in a sandy loam soil compared to a silty clay, with and

372 without 3.3% & 23% wetland hay amendments. Thus, we might conclude that in general

373 coarser grained (sandy) soil textures emit more CH_4; however, there are a number of

374 investigations where this was not the case (Yagi and Minami, 1990; Glissmann and

375 Conrad, 2002). Other factors may have contributed. In our experiment the SCL had 7.6x

376 dithionite extractable Fe, and 4.6x as much %C (Supplemental Table S1), so additional

377 studies would be needed to isolate texture as the controlling factor.

378 We considered the gas production from H microcosms separately because they

379 followed a different pattern than the other amendments, but the pattern was similar to

380 other studies using hay (Glissmann and Conrad, 2002) and wetland hay (Maietta et al.,

381 2020b). Our study adds to these findings by observing that H produced very low CH_4 in

382 the water column (after floating) compared to being mixed with soil (Table 1c). This may

383 merit further study because if this is generally true, applying fresh organic matter as a

384 mulch, rather than mixed into the soil, could greatly reduce the adverse consequence of

385 increased CH_4 emissions.

386 Reduction of Fe-oxides occurs in saturated soils in the presence of an organic

387 substrate and is a key biogeochemical process in wetland soils. With sufficient time,

388 hydric soils may develop redoximorphic features from Fe reduction; however, studies

389 have not shown lasting redoximorphic development due to organic amendments (Gray,

390 2010; Ott et al., 2020). Organizations responsible for constructing mitigation wetlands

391 have an interest in documenting Fe reduction prior to redoximorphic feature development

392 as evidence soils that are hydric. Some mitigation wetland practitioners experience

393 challenges meeting hydric soil testing standards. Although reports in the scientific

394 literature are rare, there are examples of sites meeting vegetation and hydrology wetland

395 indicators, but not hydric soils (Berkowitz et al., 2014). Both the soils we tested produced

396   sufficient Fe^{2+} and would have passed hydric soils tests, so a soil amendment would not

397   be needed.

398   We observed that fresh organic matter resulted in increased Fe^{2+} compared to

399   cured organic matter (Figure 3), likely due to the presence of labile carbon, allowing

400   access to more crystalline Fe-oxides (Lentini et al., 2012). In some soils, Fe-reducing

401   bacteria using fresh organic matter amendments could access crystalline Fe making it

402   more bioavailable. However, without an anoxic/oxic cycle, increased Fe^{2+} production

403   could lead to Fe^{2+} toxicity and ferrolysis (Kirk, 2004), similar to the way fresh organic

404   matter leads to SOC priming (Blagodatsky et al., 2010). Ferrolysis occurs when

405   bioavailable Fe-oxides are reduced to Fe^{2+} and are subject to hydraulic transport. We

406   observed that cured amendments, like L, lowered Fe^{2+} concentrations (Figure 3), possibly

407   due to the presence of humic acids that are generated during curing (Guo et al., 2019).

408   Humic acids often contain insufficient biogeochemical energy to drive dissimilatory Fe

409   reduction (Keiluweit et al., 2017), chelate Fe^{2+}, removing it from the liquid phase

410   (Catrouillet et al., 2014), and create insoluble precipitates (Shimizu et al., 2013).

411   Regulating Fe^{2+} production, through the selection of the appropriate OM

412   amendment, could influence the growth of wetland plants. For example, rice growth may

413   be stimulated under low Fe^{2+} doses of 1 mg/L (Müller et al., 2015), but higher doses can

414   produce detrimental Fe plaque (Pereira et al., 2014). Some native wetland species are

415   adapted to high Fe^{2+} concentrations. *Juncus effusus* growth is stimulated at 25 mg/L Fe^{2+}

416   (Deng et al., 2009). North American native reed *Phragmites australis* ssp. *americanus*

417   was stimulated at 11 mg/L Fe^{2+} from ferrous sulfate (Willson et al., 2017), but the

418   invasive Eurasian lineage of *Phragmites australis* seedling growth was inhibited by Fe^{2+}

419   as low as 1 mg/L (Batty, 2003). Soils high in free Fe^{2+} adversely affected *P. australis*

420   growth by creating an Fe-oxide plaque on roots (Saaltink et al., 2017).
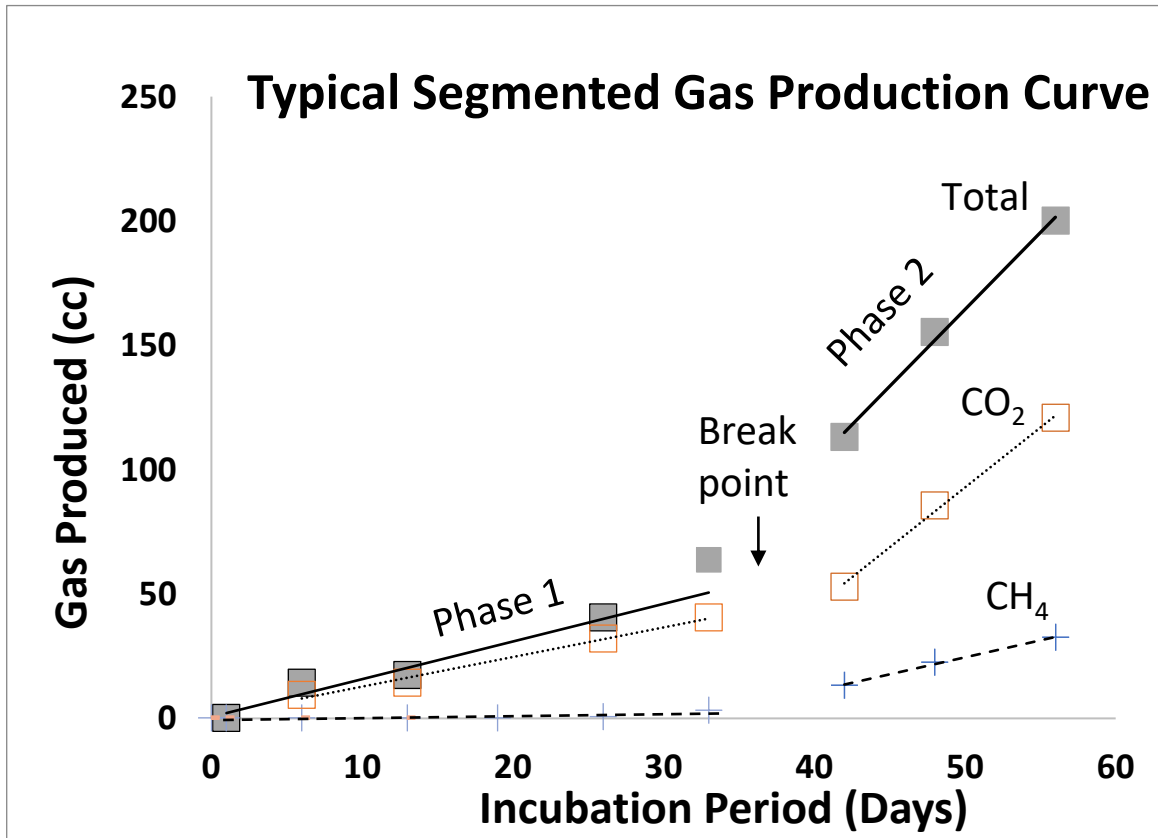
421   Our results show that pH has a significant effect on both the production of Fe^{2+}

422   (Figure 3) and CH_4 (Table 3). Between pH 5.6 and 6.6, the lower pH produced more Fe^{2+}

423   and less CH_4, consistent with thermodynamic predictions (Ye et al., 2012).

424   Hydrogenotrophic methanogens can maximize CH_4 production at pH 5 (Bräuer et al.,

425   2004). In rice paddy soils, CH_4 emissions had a clear peak at pH 7, but almost none

426   below pH 5.5 (Wang et al., 1993). The strong effect of pH underscores the need to take

427   this parameter into account when interpreting data from experiments evaluating Fe-

428   reduction and methanogenesis. Attempting to control the pH of soils could potentially

429   introduce confounding effects. We used an MES buffer with 10x the quantity we

430   estimated from a soil titration and still saw shifts in the pH after incubation. With a high

431   residual soil acidity, the amount of buffer needed to control soil pH may increase the

432   ionic strength to a level that could influence cellular sorption to mineral and Fe-oxide

433   surfaces (Mills et al., 1994) as well as enzyme activity (Leprince and Quiquampoix,

434   1996).

435   **5 Implications**

436   In our experiment, we observed that organic amendments can increase CH_4

437   production, particularly after extended anaerobic periods. We quantified CH_4 production

438   potential from several organic amendments, and in a separate field experiment

439   (unpublished) show that these results are useful in predicting field CH_4 production. There

440   is mounting concern that CH_4 from restored and created wetlands may result in net global

441   warming for decades to centuries (Neubauer, 2014). Our results suggest that not only do

442   organic amendments increase CH_4 gas production overall, but uncured amendments can

443   also decrease the time it takes before there is a large increase in both total gas production

444   and CH_4. Methane production is not constant and dramatically increases after several

445   weeks. Because of this, it may be beneficial to report wetland CH_4 data along with

446   inundation duration, which can strongly affect CH_4 (Hondula et al., 2021). It may be

447   possible to limit CH_4 in many wetland settings, particularly mitigation wetlands where

448   hydrology is part of the design: shorter flooding or inundation durations with alternating

449   drier conditions. This strategy has been proposed for rice paddy fields (Souza, 2021). Our

450   lab study demonstrates the potential for significant CH_4 emissions, but in a real system,

451   methanotrophic activity could attenuate some of the emissions (Chowdhury and Dick,

452   2013); however, this would not decrease the overall C loss from soils, it only changes the

453   pathway. If organic amendments are to be used, cured amendments may be preferrable

454   because they are not as prone to high CH_4 generation and may attenuate Fe^{2+} toxicity.

455   Amendments that lower the soil pH increase Fe reduction and limit methanogenesis

456   (Marquart et al., 2019). When deciding whether or not to use organic amendments for

457   wetland mitigation consideration should be given to whether or not the material has been

458   cured, the pH, the soil texture, and expected hydroperiod.

Figures

Typical Segmented Gas Production Curve

Gas Produced (cc) / Incubation Period (Days)

Total

Phase 2

Break point

CO_2

CH_4

Phase 1

462   Fig. 1 – Typical gas production in saturated soils amended with organic matter (All

463   Experiments).

464   Gases were best modeled using a segmented linear function. After a breakpoint the

465   average total gas production increased by a factor of ~5 whereas there is a sharp (>> 5x)

466   increase in methane production. Data presented is from the manure (1x) amended trials in

467   sandy clay loam soil. Note that hay amended trials exhibited a typical sinusoidal pattern

468   shown in Supplemental Figures S2&3h,i,j).

469

Total Gas Production (cc/Kg/day)
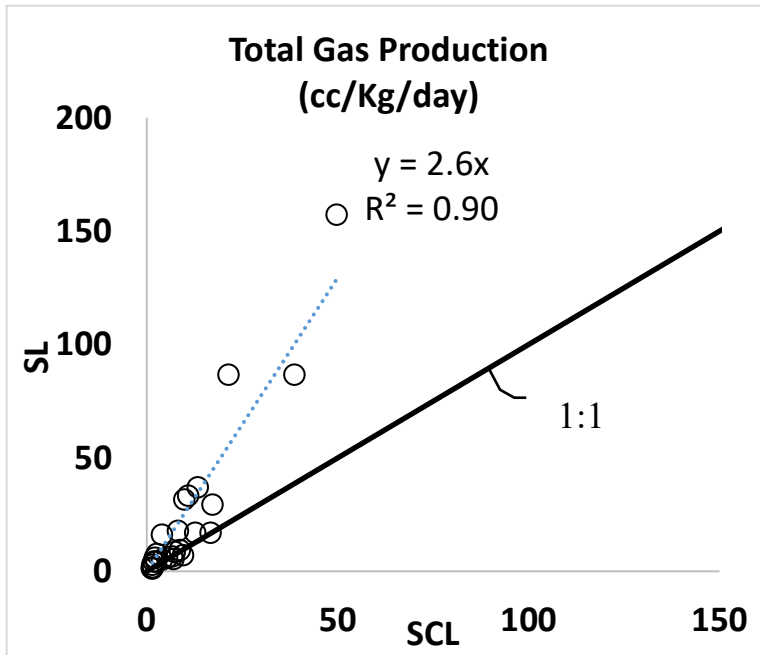
$y = 2.6x$
$R^2 = 0.90$

1:1

Fig. 2a. Experiment 1. Total biogenic gas production rate in the SL soil versus the SCL mesocosms. The SL mesocosms had, on average, 2.6 times higher gas production than the SCL.
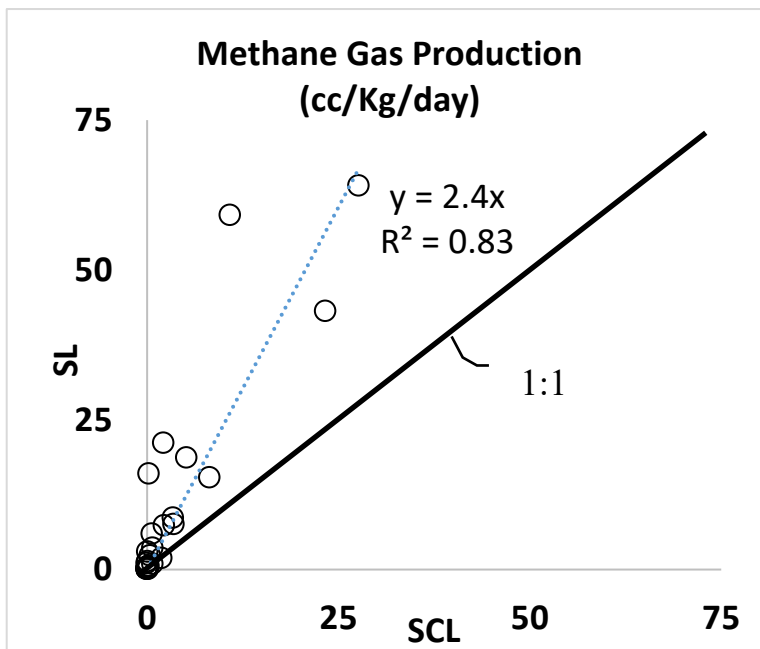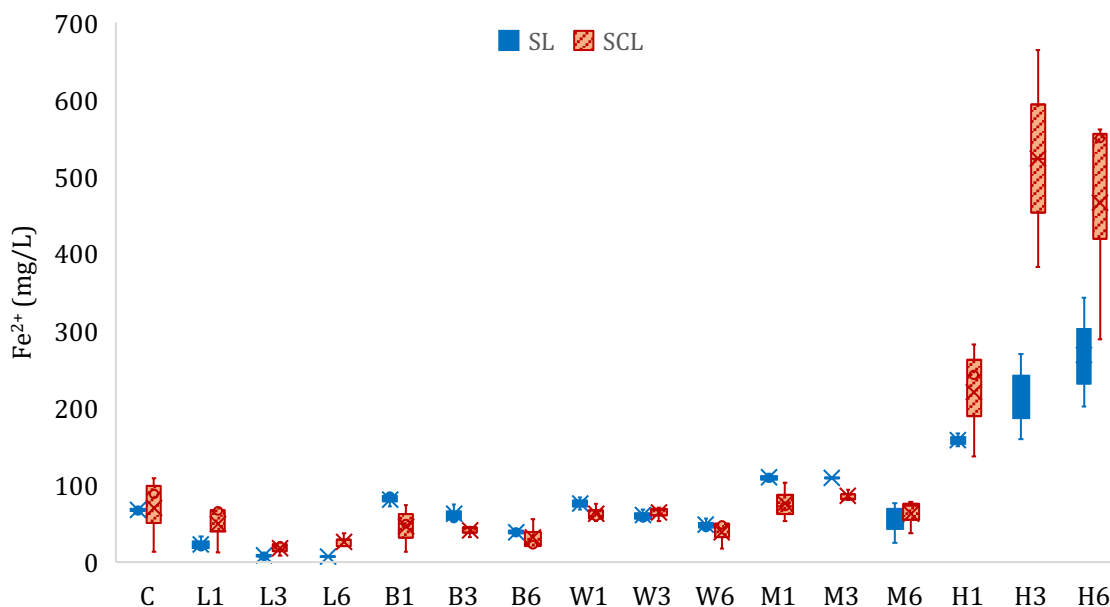

Methane Gas Production (cc/Kg/day)

$y = 2.4x$
$R^2 = 0.83$

1:1

Fig. 2b. Experiment 1. Biogenic methane gas production rate in the SL soil versus the SCL mesocosms. The SL mesocosms had, on average, 2.4 times higher methane gas production than the SCL.
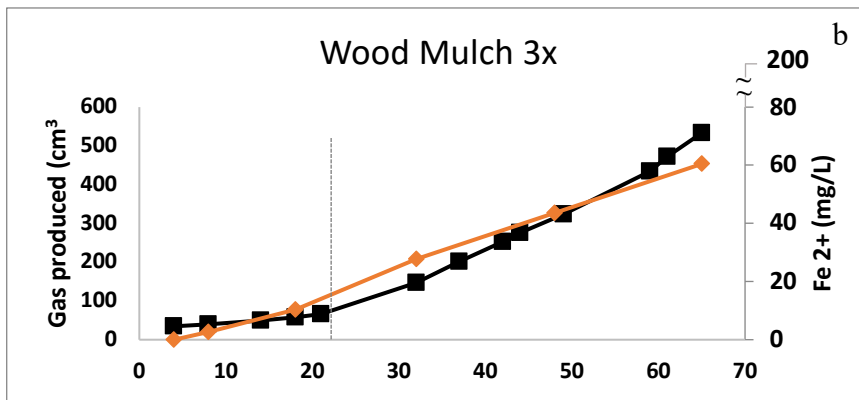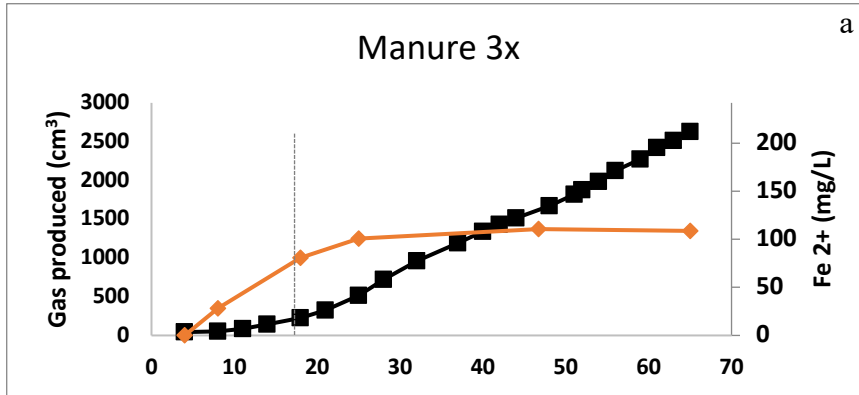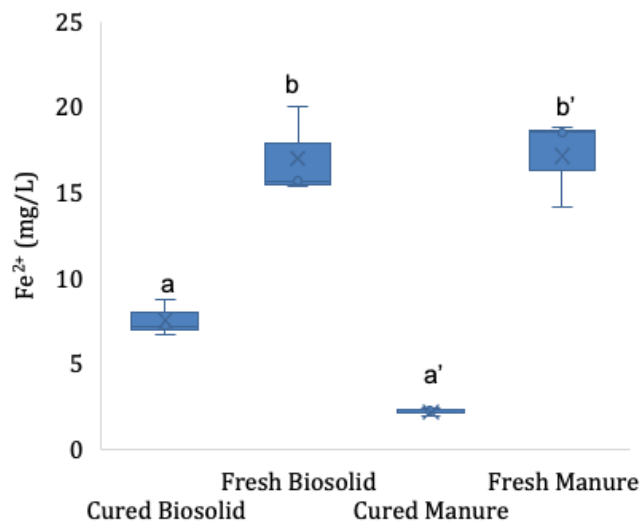
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484   Fig. 3 – (Experiment 1b) Ferrous iron (Fe^{2+}) concentration in the liquid phase at the end

485   of the incubation period.

486   Microcosms receiving different organic amendment types and levels in Sandy Clay Loam

487   (SCL) and Sandy Loam (SL) soils. C = no amendment control, L = LeafGro (yard waste),

488   B = biosolids, W = wood chips, M = manure, H = hay. Numbers signify treatment level

489   (1, 3, or 6 times amount of organic matter equivalent to 60 yd^3 / acre to a depth of 6

490   inches). Different lower-case letters signify differences ($p < 0.05$) based on contrasts

491   compared to C and brackets signify all results in the bracketed group were not

492   statistically different. H increased total Fe^{2+} production compared to the C in both soils,

493   and L decreased total Fe^{2+} production compared to C (SL only).

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498   Fig. 4 – (Experiment 1b) Ferrous iron (Fe^{2+}) and methane (CH_4) in selected microcosms.

499   Depletion of Fe coincided with the breakpoint (dashed line) with manure, but not with

500   wood mulch. Other examples of this pattern are shown in Supplemental Figure S6. The

501   maximum value on the secondary x-axis is the maximum expected Fe^{2+} concentration
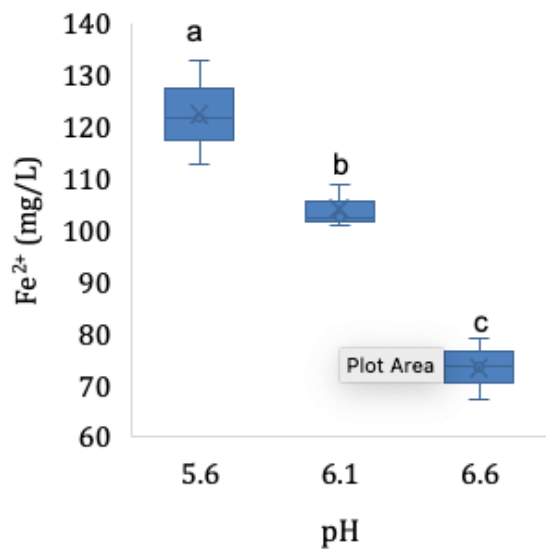
502   based on the HHCL extraction.

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509 Fig. 5 – (Experiment 2b) Ferrous iron (Fe^{2+}) concentration in the liquid phase at the end

510 of the incubation period (13 days).

511 Incubation was carried out in sandy loam soil. Different letters indicate a difference at

512 $p < 0.001$.
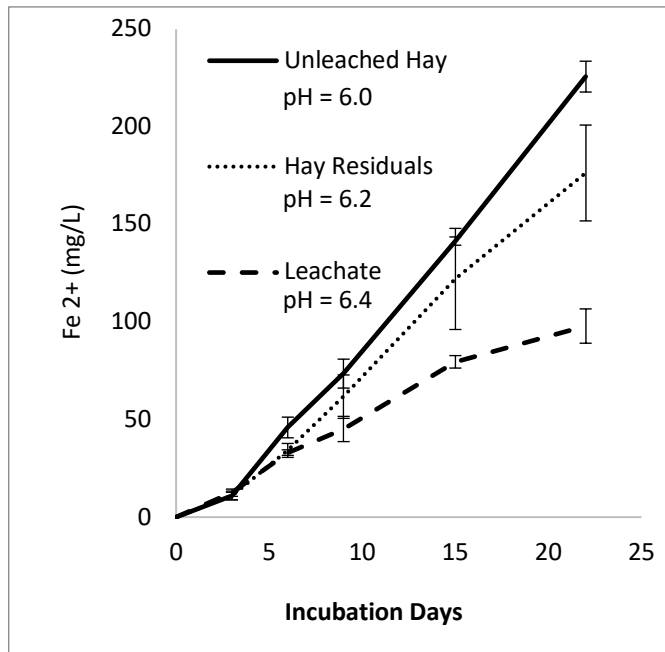
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516   Fig. 6 – (Experiment 3) Ferrous iron (Fe^{2+}) concentration in the liquid phase with varied

517   pH in microcosms receiving hay in Sandy Loam soils.

518   Different letters indicate a difference at $p < 0.05$.

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525 Fig. 7 – (Experiment 4) Ferrous iron (Fe^{2+}) concentration in the liquid phase with hay as

526 substrate.

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Tables

531   Table 1a – (Experiment 1a – Phase 1). Carbon dioxide (CO_2), methane (CH_4) and total

532   gas production. Organic amendment types: B (biosolids), M (manure), L (composted yard

533   waste), W (composted wood chips) and levels (1 = 60 yd^3 / acre equivalent: 3 = 180; 6 =

534   360) in silty clay loam (SCL) and sandy loam (SL) soils. Instances where organic

535   amendments did not increase CH_4 production are bolded. Note: $CO_2 : CH_4$ ratios are

536   based on calculated gas production rates, not total gas produced.

Soil	Treatment	Soil (g)	CO_2		CH_4		Total Gas		$CO_2:CH_4$
			cm^3/day	cm^3/Kg/day	cm^3/day	cm^3/Kg/day	cm^3/day	cm^3/Kg/day	
SCL	Control	621.63	0.97	1.56	0.002	0.003	0.99	1.59	520.0
SCL	B1	425.24	1.53	3.61	0.08	0.18	4.13	9.70	20.1
SCL	B3	544.53	1.50	2.76	0.44	0.80	3.85	7.06	3.5
SCL	B6	468.02	2.09	4.46	0.06	0.13	3.53	7.55	34.3
SCL	M1	583.40	0.74	1.27	0.02	0.04	1.33	2.27	31.8
SCL	M3	495.56	1.79	3.61	0.32	0.64	2.05	4.13	5.6
SCL	M6	394.39	1.49	3.77	0.12	0.30	4.35	11.03	12.6
SCL	L1	586.46	0.83	1.42	0.001	**0.001**	0.85	1.45	1420.0
SCL	L3	516.34	0.89	1.72	0.01	0.01	0.91	1.77	172.0
SCL	L6	410.17	0.67	1.63	0.04	0.09	0.80	1.95	18.1
SCL	W1	593.36	1.00	1.68	0.01	0.01	0.92	1.56	168.0
SCL	W3	539.61	0.98	1.81	0.10	0.19	1.39	2.58	9.5
SCL	W6	457.42	1.03	2.25	0.11	0.24	1.29	2.81	9.4
SL	Control	634.60	0.50	0.79	0.03	0.04	0.56	0.88	19.8
SL	B1	606.80	1.25	2.06	0.02	**0.04**	4.13	6.80	51.5
SL	B3	551.50	1.57	2.84	0.44	0.79	2.92	5.29	3.6
SL	B6	467.87	2.08	4.44	0.59	1.27	3.81	8.15	3.5
SL	M1	619.92	2.62	4.22	0.58	0.93	3.49	5.63	4.5
SL	M3	588.37	4.48	7.61	3.44	5.85	9.42	16.02	1.3
SL	M6	540.93	8.63	15.95	8.59	15.87	17.92	33.13	1.0
SL	L1	600.10	0.35	0.58	0.02	**0.03**	0.73	1.22	19.3
SL	L3	530.30	0.61	1.15	0.02	**0.03**	0.78	1.46	38.3
SL	L6	425.87	0.62	1.47	0.11	0.26	1.66	3.89	5.7
SL	W1	603.27	0.98	1.62	0.06	0.10	1.55	2.56	16.2
SL	W3	538.77	1.42	2.64	0.20	0.36	2.14	3.98	7.3
SL	W6	442.57	3.05	6.88	0.24	0.54	3.23	7.31	12.7

Table 1b – (Experiment 1a – Phase 2). Carbon dioxide (CO_2), methane (CH_4) and total gas production, and the Phase 1 : Phase 2 breakpoint. Organic amendment types: B (biosolids), M (manure), L (composted yard waste), W (composted wood chips) and levels (1 = 60 yd^3 / acre equivalent: 3 = 180; 6 = 360) in silty clay loam (SCL) and sandy loam (SL) soils. Instances where organic amendments did not increase CH_4 production are bolded. Note: r^2 values represent the combined best fit curve, using triplicate samples, for Phase 1 (Table 1a) and Phase 2.

Soil	Treatment	CO_2		CH_4		Total Gas		$CO_2:CH_4$	Break Point	r^2	Ph 2: Ph1
		cm³/day	cm³/Kg/day	cm³/day	cm³/Kg/day	cm³/day	cm³/Kg/day				
SCL	Control	2.06	3.31	1.20	1.94	2.54	4.09	1.7	40.0 ± 4.5	0.959	2.57
SCL	B1	5.58	13.13	1.47	3.45	5.49	12.91	3.8	29.3 ± 1.9	0.987	1.33
SCL	B3	3.74	6.86	4.45	8.17	9.48	17.40	0.8	20.1 ± 3.4	0.974	2.46
SCL	B6	7.42	15.85	10.90	23.29	18.20	38.89	0.7	10.3 ± 2.4	0.994	5.15
SCL	M1	2.26	3.88	1.29	2.22	5.82	9.97	1.7	40.2 ± 2.1	0.997	4.39
SCL	M3	4.64	9.37	5.39	10.89	10.69	21.58	0.9	20.8 ± 0.8	0.997	5.23
SCL	M6	5.85	14.83	10.91	27.67	19.69	49.93	0.5	22.1 ± 3.2	0.956	4.53
SCL	L1	3.85	6.57	0.05	**0.090**	3.96	6.76	73.0	32.2 ± 1.6	0.966	4.67
SCL	L3	4.21	8.16	0.39	**0.75**	4.54	8.79	10.9	32.0 ± 2.2	0.983	4.97
SCL	L6	5.90	14.39	0.92	2.24	6.95	16.95	6.4	32.0 ± 3.7	0.923	8.68
SCL	W1	1.56	2.63	0.27	**0.460**	3.22	5.42	5.7	34.0 ± 3.7	0.986	3.48
SCL	W3	1.93	3.58	1.90	3.52	4.51	8.35	1.0	24.2 ± 3.1	0.989	3.23
SCL	W6	2.19	4.79	2.36	5.15	6.22	13.60	0.9	13.0 ± 2.4	0.981	4.84
SL	Control	1.00	1.58	1.16	1.82	3.11	4.91	0.9	40.0 ± 3.2	0.957	5.55
SL	B1	4.44	7.31	5.16	8.50	10.19	16.79	0.9	8.6 ± 3.0	0.880	2.47
SL	B3	8.76	15.89	8.42	15.28	16.12	29.23	1.0	4.7 ± 1.8	0.989	5.53
SL	B6	12.61	26.96	20.15	43.07	40.39	86.33	0.6	9.1 ± 1.2	0.992	10.59
SL	M1	8.64	13.93	13.03	21.02	19.41	31.30	0.7	16.7 ± 0.7	0.998	5.56
SL	M3	15.23	25.88	34.77	59.10	50.79	86.33	0.4	17.2 ± 1.5	0.992	5.39
SL	M6	29.50	54.53	34.62	64.00	84.92	156.98	0.9	29.4 ± 1.4	0.974	4.74
SL	L1	1.35	2.24	1.71	2.85	3.76	6.26	0.8	38.3 ± 1.2	0.992	5.12
SL	L3	2.27	4.27	1.86	3.50	4.82	9.09	1.2	40.5 ± 2.0	0.977	6.22
SL	L6	4.25	9.99	3.07	7.21	7.15	16.78	1.4	44.8 ± 1.3	0.988	4.31
SL	W1	2.10	3.48	1.32	2.19	3.47	5.76	1.6	25.6 ± 7.6	0.762	2.25
SL	W3	6.58	12.22	4.05	7.51	9.46	17.56	1.6	23.2 ± 2.3	0.974	4.41
SL	W6	10.10	22.83	8.23	18.60	16.22	36.65	1.2	23.2 ± 1.1	0.991	5.02
									AVERAGE		4.7
									STDEV		1.9

Table 1c – Experiment 1a. Carbon dioxide (CO_2), methane (CH_4) and total gas production with hay (H) amendment. H amended trials fit a sigmoidal, not segmented, pattern, and therefore there was no breakpoint and we present p values for the sigmoidal fit, except H6 SL rates where we used a power function in Excel and report the r^2 value. Gas production rates (cm 3 gas Kg soil -1 day -1) represent maximum at the inflection point. The amendment floated to the surface in the SCL H3 and H6 trials, which resulted in unusually low CH_4 production rates.

Sigmoidal curve values			CO_2			CH_4			Total Gas			
Soil	Treatment	Soil (g)	cm^3/day	cm^3/Kg/day	p	cm^3/day	cm^3/Kg/day	p	cm^3/day	cm^3/Kg/day	p	CO_2:CH_4
SCL	H1	573.03	9.70	16.93	2.0E-16	10.40	18.15	0.164	37.1	64.75	1.3E-12	0.93
SCL	H3	477.85	7.50	15.70	3.0E-14	0.02	0.04	0.933	9.90	20.72	7.8E-6	393
SCL	H6	334.20	6.60	19.75	0.019	0.09	0.27	0.921	6.70	20.05	9.6E-13	73
SL	H1	582.57	8.90	15.28	5.5E-14	16.20	27.81	0.283	18.40	31.58	2.9E-4	0.55
SL	H3	478.00	20.80	43.51	1.8E-13	12.20	25.52	0.636	36.80	76.99	0.0093	1.7
SL	H6	321.13	50.71	158.0	0.93(r^2)	77.7	242.1	0.69(r^2)	79.79	248.47	0.74(r^2)	0.65

Table 2 – (Experiment 2a). Methane gas data for incubations with fresh and cured organic matter in sandy loam soil.

Control data (*) from Experiment 1a (Table 1a) included for reference. Different letters indicate a difference at $p<0.001$.

Treatment	Phase 1 Methane $(cm^3/Kg/day)$	Phase 2 Methane $(cm^3/Kg/day)$
Control*	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

– (Experiment 3). Methane gas data versus pH.

Microcosms receiving hay in Sandy Loam soils (Experiment 3). Different letters indicate a difference at $p<0.001$.

pH	Phase 1 CH_4 (cm³/Kg/day)	Phase 2 CH_4 (cm³/Kg/day)
5.6[a]	0.44	10.6
6.1[b]	1.0	13.0
6.6[c]	1.8	13.8

Declarations

Funding

Conflicts of interest/Competing interest

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.

Code availability

None

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