Highlights:

- Upland methanogenesis increased with greater O_2 consumption and/or lower O_2 diffusion.
- We developed a novel, automated CH_4 stable isotope pool dilution method for lab studies.
- We found the driest intact mineral soils shown to host methanogenesis, <30% water-filled porespace.
- Methanogenesis was a better predictor of NO_3- and NH_{4+} than moisture, N_2O, or CO_2 flux.
- Upland anaerobicity appears to be widespread and to affect N-cycling

Data is archived at:

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Impacts of Moisture, Soil Respiration, and Agricultural Practices on Methanogenesis in Upland Soils as Measured with Stable Isotope Pool Dilution

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Abstract

Anoxic microsites can alter the habitat of upland soils and host diverse anaerobic processes that affect greenhouse gas production, nitrogen dynamics, and biodiversity. Microsites that are methanogenic indicate deeply reducing conditions that may have especially strong impacts on soil function. However, there have not been controlled studies to determine the regulators of methanogenic microsite formation or persistence and most studies have been limited to tropical or high organic matter soils. We hypothesized that upland methanogenesis, as an indicator of anaerobic activity, is primarily affected by soil moisture and organic matter. To test this hypothesis, we examined relationships between soil properties, rates of methanogenesis, and biogeochemical responses in an incubation experiment that manipulated soil source (semi-arid and mesic ecosystems), agricultural practice (conventional, no-till, and organic), and moisture (10% to 95% water-filled porespace) of intact soil cores. Methanogenesis was correlated with factors related to both increased O_2 demand (e.g., soil respiration) and decreased O_2 diffusion (e.g., water-filled porespace), and the relative importance of these different mechanisms changed over four months. While the highest rates of methanogenesis occurred above 75% water-filled porespace, we observed methanogenesis over the full range of soil moistures. These are the driest soils shown to host methanogenesis, outside of biological soil crusts. Cores from plots with organic amendments had the highest rates of methanogenesis. Comparisons of methanogenesis and N-cycling revealed new relationships in upland soils: stronger methanogenesis was associated with more soil NH_4^+ and higher N_2O emissions but less NO_3^-, likely due to reduced conditions causing increased denitrification and/or decreased nitrification. Our findings show that upland methanogenesis can arise from either increased O_2 demand or decreased O_2 diffusion, similar to wetland ecosystems, and that the presence of anoxic microsites appears to alter N-cycling. The current paradigm is that upland anaerobicity is generally a minor or moisture-related event, but we demonstrate here that it can be persistent, occur across the

full range of soil moisture, and may result in significant impacts on nutrient availability. These and other anaerobic impacts on soil function and biodiversity may occur over the entire landscape of temperate ecosystems.

1. Introduction

Anoxic microsites occur frequently in upland, unsaturated soils and can host a range of anaerobic processes including denitrification, iron reduction, and fermentation (Sexstone et al., 1985, von Fischer et al., 2007, Fimmen et al., 2008, Keiluweit et al., 2016, Keiluweit et al., 2018)., however it is not well understood how these reduced regions are able to persist despite the presence of high O_2 concentrations in the surrounding pores. Nonetheless, researchers have collected consistent indirect evidence of anaerobic activity in upland soils - even methanogenesis, which only occurs under strongly reducing conditions, has been observed in the relatively well-drained soils of forests, grasslands, deserts, and croplands across an array of climates (von Fischer and Hedin, 2007, Kammann et al., 2009, Angel et al., 2011, Angel et al., 2012, Yang and Silver, 2016). Perhaps because of the very fine scales of heterogeneity associated with this phenomenon (Sey et al., 2008, Liptzin and Silver, 2015, Keiluweit, et al. 2018), we lack a practical understanding of the factors that drive anoxic microsite formation and persistence. There is also little mechanistic evidence describing how the presence anoxic microsites translates to impacts on redox-sensitive soil processes.

Theoretically, we understand that anoxic regions can form when the diffusion of O_2 into a volume of soil is less than the O_2 demand within that volume (Jorgenson, 1977, Sexstone et al., 1985). Thus, relative to nearby oxic soil volumes, anoxia develops in microsites (e.g., in soil aggregates, pores, or organic material) due to locally elevated O_2 consumption, decreased O_2 diffusion, or a combination of both (Keiluweit, et al. 2018). Rates of O_2 consumption increase with more live, respiring biomass (microbial and faunal populations) and energy substrates (electron donors such as labile organic matter). In contrast, rates of O_2 diffusion vary with soil architecture, which defines the pore network available for gas diffusion, and soil water content, which affects the volume of pores open to gaseous diffusion at any given time (Moyano et al., 2013). Previous studies support the idea that soil water content and organic

matter affect formation and prevalence of anoxic microsites (Linn and Doran, 1984, Sexstone et al., 1985, van der Lee et al., 1999, Chacon et al., 2006, von Fischer and Hedin, 2007, Loecke and Robertson, 2009, Wagner, 2017, Keiluweit, et al. 2018), but none have quantified the relationships of these drivers over an array of replicated experimental factors. Even less is known about how anoxic microsites persist over time. Since rates of decomposition and biological activity in soils are temporally dynamic their impacts on soil anoxia should change in kind.

Anoxic microsites alter the biogeochemistry of redox-sensitive compounds because they have the potential to host every anaerobic process. They increase emissions of anaerobically produced greenhouse gases CH_4 and N_2O (Sexstone et al., 1985, von Fischer et al., 2007), and decrease rates of organic matter decomposition and preferentially preserve lipids (Keiluweit et al., 2016). However, the greatest impacts of anoxic microsites on vegetation are likely to be changes to N-cycling. Since Jansson and Clarke (1952) first theorized that upland denitrification occurred in "a micro-mosaic of anaerobic and aerobic spots", both field (Ball et al., 1999, Hall et al., 2012) and lab (Parkin, 1987, van der Lee et al., 1999, Jäger et al., 2011) studies have shown that anoxia and its likely controls (e.g., moisture, organic matter) increase upland denitrification. Anoxic microsites may also slow nitrification, as occurs in fully anoxic habitats (Tallec et al., 2008, Ge et al., 2014). Anoxic conditions that result in increased denitrification and decreased nitrification frequently lead to lower concentrations of NO_3^- and higher concentrations of NH_4^+ in wetlands and aquatic sediments (Seitzinger, 2006, Reddy and DeLaune, 2008, Maltais-Landry et al., 2009). In addition, anoxic microsites in an oxic soil should create more interfaces between nitrifying and denitrifying regions and thus may enhance N_2O emissions from denitrification. The ability of anoxic microsites to alter upland N-cycling could be important for nutrient availability, yet this has not been investigated, to our knowledge.

Although methanogenesis is typically studied because of its impact on greenhouse gas cycling, this obligate anaerobic process can also be used as a strong indicator of anoxia. And since it occurs in the most reduced of environments its presence in oxic soil indicates that all other anaerobic processes can also be present (Megonigal et al., 2003). Viewing methanogenesis as an anchor indicator of upland anoxia is a relatively underutilized way to characterize anoxic microsites that has only been used a handful of times (von Fischer and Hedin, 2007, Kammann et al., 2009, Wagner, 2017). In the past, characterization of anoxic microsites was primarily accomplished through measurements with oxygen and redox micro-electrodes which have several drawbacks including high cost, limited sample sizes and repeatability, and constrained functionality in field settings. Methanogenesis has been difficult to measure in upland soils because CH_4 consumption masks, and usually surpasses, the observable impact of methanogenesis on a soil's net, surface CH_4 flux. Stable isotope pool dilution provides a direct approach to measure methanogenesis by allowing separate quantification of methanogenesis and methanotrophy. Quantification of methanogenesis is a promising approach to estimate the impact of anoxia on soil function because it integrates across the volume of a study soil, measures a function instead of only chemistry, is non-destructive, and can be automated in the lab and extended to the field.

Agricultural soils are excellent systems to investigate how soil properties and biota affect anoxic microsite prevalence and activity because common agronomic practices alter soil properties that affect O_2 concentration. Any impact that microsites have on cropland plant nutrition could affect yield and food supply. Tillage destroys soil aggregates and decreases organic matter content, which should increase O_2 diffusion and decrease its consumption simultaneously. However tillage can also increase bulk density of soil, decreasing O_2 diffusion from the atmosphere. Organic and no-till practices typically increase organic matter content of soils, which provides more substrate for decomposition and increases the capacity for soils to retain moisture (Bayer et al., 2012). These dual changes should increase O_2 consumption while

118 slowing its diffusion. Soil macrofauna (e.g., larvae, earthworms) contribute to upland soil anoxia because
119 they can host anaerobic processes in their gut (Šustr and Šimek, 2009, Kamman et al., 2017). Since no-till
120 soils host greater soil faunal populations (House and Parmelee, 1985), faunal abundance may mediate
121 agronomic effects on anoxic microsite abundance.

122 The goals of this study were to, a) measure effects of agricultural practice, soil source, and soil
123 moisture on rates of methanogenesis, b) determine how soil properties and treatments affect the
124 formation and persistence of methanogenic microsites, and, c) evaluate relationships between
125 methanogenesis and other redox-sensitive processes including inorganic N concentrations, N_2O emission,
126 and microbial biomass. To achieve these goals, we collected soil cores from fields managed for
127 conventional, no-till, and organic crop production at two long-term agricultural field sites with contrasting
128 climates that are common for U.S. croplands: a mesic site in central Michigan and a more xeric site in
129 eastern Colorado. Soil cores were incubated under a range of soil WFPS (water-filled porespace) for five
130 months. During that period we measured, gross CH_4 fluxes and net CO_2 and N_2O fluxes at week 6 and
131 week 21. We characterize the extent of anoxic microsite activity by measuring methanogenic activity, i.e.,
132 gross CH_4 production. To quantify gross CH_4 production and consumption we developed and
133 implemented a method of high-throughput, automated isotope pool dilution measurements with
134 dynamic volume headspaces, building on a manual method with static headspace volumes (von Fischer
135 and Hedin, 2002). We characterized soil faunal activity throughout the incubation period and measured
136 soil redox potential, inorganic nitrogen, pH, and microbial biomass at the end of the incubation.

137 Following from established relationships of environmental properties and wetland
138 methanogenesis, we hypothesize that the presence of methanogenic microsites and the magnitude of
139 gross CH_4 production fluxes will be positively correlated with factors related to O_2 consumption (soil
140 respiration, organic matter, microbial biomass) and negatively correlated with O_2 diffusion (WFPS, tillage

history). We chose to manipulate soil moisture over a range of WFPS rather than gravimetric moisture because WFPS is a volumetric measure of moisture that directly reflects amount of porespace closed to air diffusion and it also allowed us to compare moisture treatments across agronomic treatments that had different bulk densities. Changes in O_2 consumption and WFPS are not mutually exclusive and it is likely that both affect soil anoxia, but it is not clear if one is the dominant control of upland methanogenesis. We also hypothesize that cores with greater faunal activity will have higher rates of methanogenesis.

Beyond presence and rates of methanogenesis, we investigated temporal patterns in methanogenesis because biological demand for O_2 changes over time (Keiluweit, et al. 2018). We held the physical factors of soil moisture and structure constant over time while expecting biological activity (measured as soil respiration) to peak in the first two weeks and gradually decrease in the following 15-20 weeks (Cotrufo et al., 1994, Alster et al., 2016). Previous work has shown that even when ample labile C and high WFPS are present methanogenesis may not begin for 30-80 days (Peters and Conrad, 1995, Yao et al., 1999), so we chose to measure methanogenesis and its covariates at 6 and 21 weeks into the incubation. These two time points allowed for a contrast of early methanogenesis, coinciding with high rates of respiration, with late methanogenesis, occurring well after the peak respiration.

To explore implications of anoxic microsites we examined relationships between their presence and impacts on redox sensitive soil properties. Most importantly, we tested the hypothesis that greater methanogenesis will coincide with higher NH_4^+ concentrations and lower NO_3^- concentrations that are expected when anaerobic activities occur. We also analyzed the impact of methanogenesis on N_2O emission and microbial biomass.

2. Methods

2.1. Field sites

Soils were collected from the USDA-ARS Central Great Plains Research Station, Akron, Colorado, USA (40.15°N, 103.15°W, 1384 m elevation), hereafter "Akron", and the Kellogg Biological Station LTER, Hickory Corners, Michigan, USA (42.4° N, 85.4°W, 288 m), hereafter "KBS". Mean annual precipitation is 418mm and 900mm and mean annual temperature is 9.0^{o}C and 9.7^{o}C for Akron and KBS, respectively (Mikha et al., 2013, Robertson and Hamilton, 2015). Akron soils are Weld silt loam (fine, smectitic, mesic Aridic Paleustolls; or Calcic Kastanozems in the Food and Agriculture Organization World Reference Base, WRB) with textures ranging from 27-32% sand, 43-50% silt, and 22-28% clay (Mikha et al., 2013) and KBS soils are Kalamazoo loam (fine-loamy, mixed, mesic Typic Hapludalfs; or Luvisols, WRB) with texture of 43% sand, 38% silt, and 19% clay (Robertson and Hamilton, 2015).

At Akron, soils were collected from long-term tillage study plots in 2-year wheat/fallow rotation that were under conventional tillage (mold-board plowing) with chemical fertilizers (*Akron Conventional*) and no-till with chemical fertilizers (*Akron No-till*), see Mikha et al. (2013) and Halvorson et al. (1996) for management details. Soils were also collected from recently established (2010) organically managed wheat-fallow plots (*Akron Organic*), see Calderón et al. (2017) for management details. In October 2012, prior to wheat planting, (20 months prior to soil sampling) Akron Conventional and No-till plots received 13.6 kg ha^{-1} of urea (46-0-0) and Akron Organic received 109,000 kg ha^{-1} of composted beef feedlot manure. Total carbon of Akron Conventional and No-till soils is 6.55 g C kgdw^{-1} (kg dry weight) and 7.55 C g kgdw^{-1}, respectively (0-20cm; Mikha et al. 2013) while Organic contains 19.4 g C kgdw^{-1} (0-30cm; Calderón et al., 2017). Mikha et al. (2013) also found that Akron No-till soils (0-20cm) have roughly twice the mass of macroaggregates (>250 μm) and less microaggregates and silt and clay fraction compared to Akron Conventional.

185 At KBS, soils were collected from the Main Cropping System Experiment plots, treatments one
186 (conventional), two (no-till), and four (organic). The conventional plots are tilled (chisel plow) and receive
187 chemical fertilizers (*KBS Conventional*), no-till plots receive chemical fertilizers (*KBS No-till*), and the
188 organic plots receive chisel tillage with only winter leguminous cover crops as fertilizer (*KBS Organic*), see
189 Syswerda et al. (2011) and Robertson and Hamilton (2015) for management details. The 3-year rotation
190 for KBS Conventional and KBS No-till is corn/soybean/winter wheat while the rotation for KBS Organic is
191 corn/ryegrass/soybean/winter wheat/red clover, with ryegrass and red clover as winter cover crops. KBS
192 Conventional and KBS No-till plots received 47 kg K_2O ha^{-1} April 18th, 2014 and 32.8 kg N ha^{-1} and 22.3 kg
193 P_2O_5 ha^{-1} on May 12th, 2014. Soil total carbon of these soils (top 25cm) in 2001 was 10.4 g C $kgdw^{-1}$, 11.5
194 g C $kgdw^{-1}$, and 12.2 g C $kgdw^{-1}$ for KBS Conventional, No-till, and Organic plots, respectively (Syswerda et
195 al., 2011). Aggregation varies between KBS treatments with Organic and No-till having more large
196 macroaggregates (2000-8000 µm) than Conventional, No-till having more macroaggregates (250-2000
197 µm) than the other two treatments, and Conventional having the greatest amounts of the two smallest
198 particle sizes (53-250 µm, <53 µm; Grandy and Robertson, 2007).

199 2.2. Intact soil core removal and processing

200 We assayed intact soil cores in this experiment to maintain the soil structures which might affect
201 methanogenesis and anoxia and to ensure our study units were similar to soils under field conditions. At
202 each site, three field (i.e., plot) replicates of each agricultural treatment were visited and six intact soil
203 cores in PVC pipe sleeves were removed from each plot (n=18 plots; n=108 cores extracted). Cores were
204 extracted at Akron on May 21st 2014, which was the year of fallow rotation and followed the wheat
205 rotation, and from KBS on June 2nd-3rd 2014, which was the year of corn rotation and followed the wheat
206 rotation. The cores were collected using 18cm lengths of PVC pipe (7.62 cm i.d.) that had beveled ends
207 to facilitate insertion into soil. During core removal, the PVC pipes were slowly driven into the soil by

hand in order to minimize compaction and fracturing of core soils. PVC pipe soil cores were inserted 15±1cm deep and were then excavated out to minimize disturbance to the soil structure, placed in plastic zip-closure bags, and stored on ice in coolers until returned to the lab (<72 hours). The intact cores were then stored at 4°C for less than 14 days until initial soil processing or the start of the pre-incubation. Only areas of bare soil without plants were chosen for sampling, at KBS these were inter-row areas.

A subset of cores from each field plot were processed to obtain measurements of the initial conditions of the soils (n=36). Within 1 week of the field extraction of cores, two of the six cores from each plot were sacrificed to characterize initial conditions; they were sieved (4.25mm), measured for bulk density and subsampled for pH, inorganic nitrogen, and gravimetric moisture. Measurements of bulk density and gravimetric moisture of initial conditions cores were used to determine the percent WFPS at field moisture for each plot and to determine the target water masses for incubation moisture treatment cores. The four remaining cores from each plot were assigned moisture targets across the range of 15-65% water holding capacity. Because each core differed slightly in porosity and because the true porosity of each core was not known until final sacrifice, a relatively uniform distribution of experimental WFPS from 10-95% was imposed.

2.3. Incubation

Cores for the incubation (n=72) were permanently sealed on the bottom (one layer of 127µm HDPE film and two layers of 254µm PVC pipe tape) and placed in Percival I-35LVL model incubators set to 23^{o}C for a 3-7 day pre-incubation (see table S1 for schedule of incubation). Cores were adjusted to target moistures by air drying or regular moisture additions. Most cores had reached the targets by week 6 of the incubation, although 13 of the 72 cores required more than 15% WFPS change between weeks 6 and 21 to reach targets. Once cores reached target moisture levels the pipe tops were covered with perforated HDPE film (removed for gas flux measurements) to allow gas exchange while avoiding

increased evaporation caused by incubator fans; tests confirmed film did not create a barrier to diffusion. To maintain cores at the target WFPS levels soil moisture was adjusted every 2-5 weeks. Cores were stored in the incubators at 23^{o}C for the duration of the incubation period. The soils did not contain living plants, if any germinated during the incubation they were removed.

At the end of core incubations, we first made gravimetric and redox measurements of the intact cores and then sieved and subsampled the soils. During measurements of soil redox potential, the bottom seal of the PVC pipe was removed and the soil core was placed on a wet, porous mat connected to the reference electrode of the redox array (see below). We measured redox potential at three depths (surface, 3cm, 8cm) with 5 probes (n=15 measurements per core). Fifteen of the soil cores were too dry and hard to pierce with probes so these were not measured. Occasionally probes were not able to pierce deeper soils or had electrical malfunctions and that data was excluded. The majority of soil volumes were oxic in each core so we used only the minimum (i.e., most reducing) redox value observed of each core in our analyses since that value should indicate the likelihood of methanogenesis and other anaerobic processes. After final redox potentials were recorded soils were sieved (4.25mm), homogenized, and subsampled. Subsamples were stored at -20^{o}C for subsequent pH measurement, maintained at 4^{o}C for inorganic N, and microbial biomass measures.

2.4. Soil properties

We measured pH in a 1:1 w/w solution of dry soil and deionized H_2O. Inorganic N was extracted over 24 hours in a solution of 10 gdw (g dry weight) soil and 45ml 1M KCl incubated in a 60ml *Whirlpak* bag (Nasco Inc., Fort Atkinson, WI, USA). It was then stored at -20^{o}C until NH_4^+ and NO_3^- were quantified colorimetrically (Alpkem Flow Solution IV, O.I. Analytical, College Station, TX, USA). Microbial biomass C and N were extracted using a chloroform slurry technique adapted from Fierer and Schimel (2003). Our method differs in that jars were shaken for 4 hours at 200 RPM and that cell lysis extracts were sparged

with N_2 gas to volatilize any residual chloroform. Microbial biomass extracts were stored at -20^{o}C until analysis on a Shimadzu TOC-L (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Redox measurements were made with an array modified from Rabenhorst (2009): platinum electrodes were connected to a calomel reference electrode with operational amplifiers (MAX406BCSA+), powered by a 9V battery. The reference electrode was connected to the base of the moistened soil cores via a KCl salt bridge. Probes had 5mm long tips made of 0.5mm diameter Pt wire and potentials were measured with a high precision voltmeter (ThermoScientific Orion 3 Star Benchtop pH meter).

We documented soil faunal activity throughout the experiment, which primarily amounted to the presence of worm castings on the soil surface or the rare emergence of a coleopteran. Prior to sacrifice, any bioturbation of the soil surface was recorded, and during the sacrifices the presence of earthworms, small worms (i.e., Enchytraeids or nematodes), coleopterae, larvae, and ants was recorded; when possible, these creatures were removed from the sieved soil so their biomass would not impact post-sacrifice chemical measurements. For subsequent statistical analysis, we categorized the impact of soil fauna: category 1, large (>1mm girth) macrofauna found during incubation (i.e., coleopteran emergence) or sacrifice, or worm castings on soil surface; category 2, evidence of small soil fauna/mesofauna or their activity (i.e., sub-millimeter bioturbation observed on soil surface or macroscopic worms, <1mm girth, observed at sacrifice); category 3, no evidence of soil fauna during incubation or sacrifice.

2.5. Net CO_2 and N_2O fluxes

Measurements of net gas fluxes were made one or seven days prior to gross CH_4 measurements and were performed by sealing the cores in plastic containers, taking two headspace gas samples over 4-5 hours, quantifying the change in headspace gas concentrations, and calculating the flux rates. The high-density polyethylene containers were 24.5 cm tall with a diameter of 12.4 cm and a volume of 3.05 liters (U.S. Plastics), lids were fitted with black butyl septa for syringe sampling and sealed with high vacuum

277 grease. 30ml of room air was added to the headspace to pressure balance the gas removed at the first
278 sampling. Headspace and soil air were allowed to equilibrate for 45 minutes, then the first headspace gas
279 sample was collected, another 4-5 hours passed, then the second gas sample was collected. Following this
280 sampling the cores were removed from the jars and returned to the incubator. At each sampling, 30ml of
281 headspace gas was removed and injected into 20ml glass vials sealed with gray butyl septa.

282 Headspace gas concentrations (CO_2 and N_2O) were measured on a custom hybrid gas
283 chromatograph/laser absorption spectroscopy system; N_2O was measured on a Shimadzu 14B gas
284 chromatograph (GC) with electron capture detector, while CO_2 was measured on a Los Gatos Research
285 Greenhouse Gas Analyzer (LGR GGA). In a novel configuration that we developed, gas was drawn from a
286 single gas sample vial twice: first with injection into the GC, then with injection into a stream of zero air
287 (i.e., 80% N_2, 20% O_2, no trace gases) via an injection port on the inlet line to the LGR GGA. The GC was
288 internally plumbed in a standard configuration with on-column injection of sample via injection port into
289 an N_2 carrier gas stream. The LGR GGA injection gas was drawn from the inlet line through an open split
290 by the instrument's internal pump. Sample CO_2 moved through the LGR GGA as a pulse over time that
291 were transmitted by the analog-out ports on the LGR GGA and integrated by Shimadzu EZstart software.
292 During sample injection, a CTC Analytics AOC-5000 Combi-PAL autosampler injected 5ml of sample gas
293 into the separate injection ports of the GC and LGR GGA. All samples were calibrated against certified lab
294 standard gases (National Institute of Standards and Technology, USA).

295 2.6. Isotope pool dilution for measurement of gross CH_4 production

296 2.6.1. Overview

297 To make measurements of gross CH_4 production (i.e. methanogenesis) in week 6 and 21 we
298 developed and utilized a novel dynamic headspace volume CH_4 isotope pool dilution method. This new
299 method builds on the approach of von Fischer and Hedin (2002), the main difference is that where that

older method enclosed soil samples in rigid jars of a static volume, our new method enclosed soil samples in flexible foil-layered polyester bags whose volume decreases with each headspace gas sampling. This dynamic volume approach allows for automated sampling with a laser based spectrometer (MCIA; Methane Carbon Isotope Analyzer, ABB - Los Gatos Research Inc., San Jose, CA, USA) that requires large (i.e. >700ml) gas samples to accurately quantify carbon CH_4 stable isotope ratios. The flexible bag shrinks in volume during gas sampling periods, preventing pressure changes.

2.6.2. Numerical solution for a dynamic headspace with discrete losses of volume

Because volume was removed in discrete events (e.g., 100ml removed every 5 hours during a 2 minute sampling) the number of moles was re-calculated at the end of each gas removal event (i.e., sampling event). But in the periods between sampling events the headspace can be treated as static. To begin, we adapt an equation for a static headspace from von Fischer and Hedin (2002) eq. 4, using a slightly different nomentclature which we define below. The static headspace used by von Fischer and Hedin (2002) allowed them to derive a simplified equation where concentration and mass (i.e., moles) of CH_4 were interchangeable. However, since volume is not static in our method, this equivalency is no longer valid. To calculate expected fluxes we derived the formulae with the volume of CH_4 defining the net flux, and production and consumption constants. We modify eq. 4 from von Fischer and Hedin (2002),

$$\frac{dm}{dt} = P - k \cdot m \quad (1)$$

from a mass to volumetric formulation,

$$\frac{dM_v(t)}{dt} = P_v - k_v \cdot C(t) \quad (2)$$

$$= P_v - k_v \cdot \frac{M_v(t)}{V_S} \quad (3)$$

Where,

322 $\frac{dM_v(t)}{dt}$ is the change in the volume of methane per time,

323 P_v is the production rate of CH_4 in volume per time,

324 k_v is the first order rate constant for consumption, i.e., the rate of consumption of CH_4, in volume per

325 time per concentration CH_4,

326 V_S is the total bag headspace volume of all gases (constant during an individual time period),

327 $C(t) = \Phi(t) = \frac{M_v(t)}{V_S}$ is the concentration of CH_4 at time t.

328 To scale constants correctly all concentrations are in vol/vol, not parts per million.

329 Since the original static headspace solution, eq. 5 from von Fischer and Hedin (2002), was,

330 $$M(t) = \frac{P}{k} - \left(\frac{P}{k} - M_0\right) \cdot e^{-k \cdot t} \quad (4)$$

331 and the only change needed to modify the original flux equation (eq. 1) for dynamic gas volumes was,

332 $k = \frac{k_v}{V_S}$, , we can substitute that into eq. 4 to produce a solution for $M_v(t)$,

333 $$M_v(t) = \frac{V_S \cdot P_v}{k_v} - \left(\frac{V_S \cdot P_v}{k_v} - M_0\right) \cdot e^{-\frac{k_v \cdot t}{V_S}} \quad (5)$$

334 Eq. 5 should be used to calculate the change in $M_v(t)$ during a single time period (i.e., between two

335 measurements). Then $M_v(t)$and V_S are adjusted for the next time period by subtracting the methane

336 volume and total bag volume lost during gas sampling.

337 Eq. 5 can be altered slightly to use the initial concentration instead of moles:

338 $$M_v(t) = \frac{V_S \cdot P_v}{k_v} - \left(V_S \cdot \left(\frac{P_v}{k_v} - \Phi_0\right)\right) \cdot e^{-\frac{k_v \cdot t}{V_S}} \quad (6)$$

339 Where,

340 $\Phi_0 = \frac{M_0}{V_S}$, i.e. the CH_4 concentration at t_0 in vol/vol.

In order to implement this solution (eq. 6) we calculated the expected P_{12} and k_{12} for CH_4 with the above model and then calculated the ^{13}C production and consumption (i.e., the isotopic signature of methanogenesis and the fractionation due to methanotrophy) as a proportion of P_{12} and k_{12}, ,

$$P_{13} = 0.01055627 \cdot P_{12} \quad (7)$$

$$k_{13} = 0.98 \cdot k_{12} \quad (8)$$

We followed von Fischer and Hedin (2002) and chose to use C fractionation factors of -60 (eq. 7) and -20‰ (eq. 8) for production and consumption of CH_4, respectively. These commonly observed values fall in the middle of the ranges measured in cultures and environmental samples (Whiticar, 1993, Snover and Quay, 2000, Templeton et al., 2006, Kinnaman et al., 2007). We performed a sensitivity analysis across the range of published *P* and *k* fractionation factors and found little impact on our estimates of *P* and *k*, see supplementary material for details.

To estimate *P* and *k*, an iterative optimization was performed where model estimates were compared to the data, a goodness of fit term was calculated (weighted sum of the squared error of the isotopic ratio and total CH_4), and that term was minimized through iterative variation of P_{12} and k_{12}.We report here values for P_{12} converted to mass as rates of methanogenesis. See data archive for code of the model implementation and optimization in R.

2.6.3. Measurement array

The measurement array consists of multiple sample and standard polyester bags connect via high density polyethylene lines to interconnected gas manifolds, with their final output going to the MCIA. The MCIA is set to run constantly and record all data. Gas manifold management was performed by an Arduino MEGA microcontroller (*arduino.cc*, Arduino S.R.L., Italy). This independent microcontroller provided more flexibility of sampling timing as compared to the MCIA internal manifold management software. The system is illustrated in fig. S1. The 13 liter polyester foil layered soil sample bags

(www.sorbentsystems.com, IMPAK Corp., Los Angeles, California, USA) were fitted with 1/4 inch bulkheads (Swagelok, Solon, Ohio, USA) to enable gastight connection with manifolds.

2.6.4. Array operation

First, the MCIA was powered on and allowed to reach a stable temperature. Then soil cores were placed in polyester sample bags that were closed by heat-sealing; two or three no-soil control bags were added to each run. Additions of a $^{12}CH_4$ + $^{13}CH_4$ mixture brought the bag headspace to ~10ppm $^{12}CH_4$ and ~1ppm $^{13}CH_4$. SF_6 was added at the same time to quantify initial headspace volume. Gas ports were sealed and bags allowed to stand for 4 hours to allow headspace and soil air gases to equilibrate, then a 30ml gas vial sample was taken for later SF_6 quantification via the gas chromatograph. Sample, check standard, and calibration bags were then connected to gas manifolds. Sampling cycles commenced, with gas sampling of the calibration bags, then each soil sample bag; the check standard bag was sampled in between pairs of sample bags to provide information for drift corrections. Sampling cycles were separated by ~11 hours and a total of 6-8 cycles were run. Gas sampling lasted 400 sec for each bag, resulting the removal of 726 ml of gas volume from the bags. Each full cycle with 18 sample bags was ~5 hours in duration. After the final sampling cycle, soil cores were removed from the bags and returned to storage incubators. Since a group of cores (i.e., Akron or KBS) had 36 sample cores each, the group was split in half with the first 18 sample cores quantified via IPD for ~3 days followed by the second 18 cores.

2.6.5. Isotope pool dilution data processing and model fit

Data was exported from the MCIA in raw form and a run log of sampling times was calculated according to the sampling schedule. MCIA data and run logs were imported to a database for efficient data handling. Every gas sampling was averaged over the final 150 sec of sampling (the first 250 sec data are discarded) to average over instrument noise and obtain a value of concentration of $^{12}CH_4$ and $^{13}CH_4$ for each sampling time. These initial concentrations were calibrated against the gas calibration standards

used at the beginning of the cycle, different calibrations were used for each cycle to correct for inter-cycle instrument drift. Finally, calibrated sample concentrations were corrected for intra-cycle drift, if present, with check standard bag data. The sample bag concentration data and sampling times were exported to model fitting software (e.g., Microsoft Excel or R)

The data were fit to the above derived dynamic headspace volume isotope pool dilution model by iterative optimization (see fig. S2 for example fit). The model goodness of fit was assessed via fit statistics (e.g. r^2) and visually. Poor fits from the same cycle were analyzed for similar trends, when drift correction did not improve consistency of check standards data from that cycle was excluded from model fits. Gross production (methanogenesis) and consumption (methanotrophy) fluxes were calculated from the model P and K fits. Detection limits were defined as the mean estimated flux plus one standard deviation of control (i.e., no-soil bags) bag fluxes; fluxes below that limit were assigned a value of half the limit, a common approach used with chemical data to avoid censoring and losing the related information (Croghan and Egeghy, 2003). Gross CH_4 consumption rates were not included in this analysis because the focus is on methanogenesis as an indicator of anoxia.

2.7. Statistics

When the error structures were log-normal, as was the case for NH_4^+, NO_3^-, microbial biomass, soil organic carbon, CO_2 emission, N_2O emission, and methanogenesis, we log transformed data prior to statistical analyses. We regressed soil properties and gas emissions over experimental factors (site, agronomic practice, WFPS) separately to evaluate how they were distributed and compared to methanogenesis. We analyzed the rates and presence of methanogenesis in two ways, 1), a second-degree generalized linear mixed model (GLMM) over all experimental factors, 2) single factor linear regression over factors connected to potential mechanisms (i.e., WFPS, soil respiration, soil faunal activity). The GLMM allowed us to test how the experimental factors related to agriculture affected

methanogenesis and was designed with agronomic practice, site, and WFPS treatment at fixed variables, and plot number as a random variable. The GLMMs had gamma error distribution with log link function for rate models and a binomial error structure with logit link for presence/absence models. With the linear regressions we were able to explore the relationships between methanogenesis and the soil properties and gas fluxes related to mechanisms of methanogenic microsite formation and maintenance, log methanogenic rates were used for these regressions.

We categorized cores into one of four groups based on the temporal patterns of methanogenesis from week 6 to week 21. This allowed us to test how factors are related to presence or dynamics of microsites and how the drivers may change over time. The categories, illustrated in fig. 1, were: *Consistent* (methanogenesis rates above detection limit at week 6 and 21; n=17 of 72 cores), *Quick* (methanogenesis detected only week 6; n=18), *Slow* (methanogenesis detected only week 21; n=8), and *None* (methanogenesis never detected; n=29). We constructed a multinomial model of these categories over the experimental treatments and performed likelihood ratio tests. This led to a multiple comparisons model of the proportion of cores within each category from each agronomic practice.

To test for relationships between methanogenesis and a set of soil properties and biogeochemical processes (N-cycling, redox potential, microbial biomass) we regressed these variables over the log of the 6 and 21 week mean of methanogenic rates. Methanogenic rates from week 21 were used for the microbial biomass regression, the mean rate regression had a lower r^2, to show that the relationship did not have strong explanatory power at any point in the incubation. We tested more complex regressions (i.e., multiple regression including combinations of methanogenesis, site, agricultural practice, WFPS), but these did not yield different inferences or greater understanding than the single-factor regressions provide. Statistical analyses were performed in R statistical software, version 3.4.2 (R core team, 2017).

3. Results

3.1. Soil properties

Soil physical and chemical properties primarily varied over agronomic practice and WFPS, and some varied by site. Soils from Organic plots had higher NO_3^-, NH_4^+, and microbial biomass than No-till and Conventional soils, indicating that organic amendments had significantly altered the soil environment (Table 1). No-till soils had lower pH than Conventional and Organic. Akron soils had higher pH, NO_3^-, and microbial biomass than KBS soils, the latter two differences driven by much higher NO_3^- and microbial biomass in Akron's Organic cores. Significant site-based differences in porosity across treatments were driven by porosities around 60% for Akron's Conventional and Organic plots; all other plots had 40-45% mean porosity. Both No-till and Organic cores had 2 to 3-fold more soil faunal activity than Conventional cores.

3.2. Net CO_2 and N_2O fluxes

Emissions of CO_2 and N_2O all increased with WFPS and were generally greater in Organic plots than in Conventional and No-till (fig. S3). Between weeks 6 and 21, soil respiration decreased 69% from 636 to 199 nmol CO_2 gdw^{-1} d^{-1} while N_2O fluxes decreased 57% from 0.40 to 0.17 nmol N_2O gdw^{-1} d^{-1}. Neither gas emission differed significantly between sites.

3.3. Methanogenesis

Of the 72 cores that we analyzed, 43 exhibited rates of methanogenesis that were quantifiable (i.e., above the method's detection limit of 5.84 nmol CH_4 day^{-1} $kgdw^{-1}$). The mean rate of methanogenesis during the experiment was 26.0 nmol CH_4 day^{-1} $kgdw^{-1}$ (including cores below the detection limit) and did not differ between weeks 6 and 21, but rates of individual cores did show temporal patterns. Methanogenesis occurred across the range of WFPS and in all agricultural practices (fig. 1.c.). Our

measures of methanogenesis are not representative of the net CH_4 flux to or from the atmosphere as methane oxidation occurred simultaneously in nearly all cores (data not shown).

3.3.1. Week 6 methanogenesis rates and presence

The nature of this data, with both continuous variation in rates of methanogenesis and categorical behavior (i.e., presence/absence of methanogenesis) enables continuous and categorical analyses. At week 6, both magnitude and presence of methanogenesis were affected by agronomic practice and WFPS, while soil respiration was also a significant factor (table 2). Organic cores averaged twice the methanogenic rate of Conventional cores ($p=0.006$), while No-till rates did not differ from the other agronomic practices (fig. 2a) However, presence of methanogenesis was equally high in both Organic and No-till cores (n=15 and 14 of 24 cores, respectively) and less than half as frequent in Conventional cores (n=6 of 24 cores; $p=0.062$). Rates of methanogenesis increased exponentially with greater WFPS (Table 2; fig. 2c) with the greatest rates occurring at WFPS > 75%. Since previous studies found that anaerobic activities increase dramatically in upland soils with WFPS > 60% (Linn and Doran, 1984, Pennock et al, 2010, von Fischer and Hedin, 2007), we tested this as a threshold value for methanogenesis. Cores falling above this threshold in week 6 of our experiment were 65% more likely to be methanogenic than cores with WFPS < 60% ($p=0.03$; one-sided Fisher's exact test). Moisture also increased the likelihood methanogenesis was present in cores ($p=0.049$).

In regressions of week 6 methanogenic rates over mechanistic factors, WFPS ($p=0.001$; $r^2=0.14$) and soil respiration ($p=0.005$; $r^2= 0.11$) had similar levels of explanatory power (table 2). Logistic regression indicated that only WFPS ($p=0.018$) predicted presence/absence of methanogenesis.

Cores with faunal category 1 (i.e., evidence of large soil fauna) had higher rates of methanogenesis than category 3 (i.e., no evidence of any fauna) in week 6 ($p=0.054$; table 2). Three of the four cores that had rates of methanogenesis in the range expected from macrofauna (i.e., 80 to 1700 nmol CH_4 $kgdw^{-1}$

478 day^{-1}; Kammann et al., 2009) fell into category 1, the other core with this rate fell into category 2.
479 However, cores in faunal categories 1 and 2 were mostly from Organic and No-till treatments while those
480 from category 3 were mostly Conventional treatments (p=0.004), so there is co-variation of organic matter
481 and soil respiration with these soil faunal categories.

482 ### 3.3.2. Week 21 methanogenesis rates and presence

483 At week 21, WFPS and WFPS^2 were the primary significant predictors in the experimental GLMM
484 (p=0.049 and 0.001, respectively) and also among mechanistic regressions (p=0.0018). As was the case at
485 week 6, the greatest rates occurred above 75% WFPS (fig. 3c) and during week 21 cores with WFPS > 60%
486 were also 65% more likely to be methanogenic than drier cores. Agronomic practice affected
487 methanogenesis in interaction with WFPS as rates of methanogenesis increased much more at high WFPS
488 in Organic cores than No-till or Conventional cores (p=0.024, fig. 3d). The only impact field site had on
489 methanogenesis was in week 21 where KBS Organic cores averaged more than 20 times the rate of
490 methanogenesis of any other group (p=0.063). The presence/absence of methanogenesis at week 21 was
491 not predicted well by the experimental GLMM or mechanistic regressions (Table 2), though cores above
492 60% WFPS were 70% more likely to be methanogenic than drier cores (p=0.08; one-sided Fisher's exact
493 test).

494 ### 3.3.3. Methanogenesis dynamics categories

495 We categorized each core's methanogenic dynamics based on presence/absence of
496 methanogenesis at 6 and 21 weeks (fig. 1). We compare the characteristics of these categories to learn
497 about the causes of methanogenic microsite formation and persistence.

498 The distribution of agricultural practices across methanogenic dynamics categories is striking (fig.
499 3; table S2). Half of the Conventional cores did not produce CH_4 (i.e., were in the None group) and another
500 quarter of them only started producing CH_4 during the latter half of the incubation period (i.e., the Slow

group). Nearly all No-till and Organic cores that hosted methanogenesis during the study were methanogenic during week 6.

Compared to all other methanogenesis groups, the Consistent group was distinguished by redox characteristics: it had by far the highest proportion (41%) of sub-oxic redox measurements (i.e. <300mV; Megonigal et al., 1993, Megonigal and Rabenhorst, 2013) and contained all the cores with visible gleying (n=7). However, there are also some differences between Consistent and Quick cores that may help explain why the latter lost methanogenesis over the course of the incubation. There were very few (n=2 of 18) Quick cores from carbon poor Conventional plots, significantly less than the number of No-till cores ($p=0.049$, fig. 3). Quick were more likely to be in a lower WFPS treatment than Consistent cores: while Quick cores lost an average 15% WFPS from pre-incubation field moisture to their target incubation moisture, Consistent cores only lost 5% ($p=0.3$). And although this comparison is not significant, seven of the 12 Quick cores that started at field moistures above the 60% WFPS threshold dropped below 60% WFPS to their target moisture, while only three of the 13 Consistent cores starting above 60% WFPS had target moistures below 60% WFPS. The average 6 to 21 week decline in soil respiration of the Quick cores' was not significantly different than the decline in respiration of Consistent cores (not shown).

Almost all Slow cores were from Conventional plots (n=6 of 8), significantly more than from the Organic plots (n=0 of 8, $p=0.043$, fig. 3). The Slow set did not include any cores with sub-oxic redox potential and did not have higher respiration than any other groups. The week 21 average WFPS of Slow cores was 41%, 8% lower than any other methanogenesis category. Cores of the None group had the lowest mean WFPS (57%) prior to the incubation (i.e., field moisture content) of all other groups, which were between 64% to 68%.

3.4. Methanogenesis, N-cycling, and other soil properties

We performed single factor linear regressions between methanogenesis and soil properties to determine if higher rates of methanogenesis correlated with other soil properties that reflect increases in anaerobic activity and conditions. A principal components analysis (fig. S5) provides an overview of how all measured properties are related.

We found clear correlations between methanogenesis and inorganic N across the studied cores (fig. 6). Akron Organic plots had very high rate of manure application which resulted in initial core NO_3^- content eight times higher than any other site + agronomic practice treatment, which made this treatment an outlier in NO_3^- content with high influence on regressions. The other site+agronomic practice treatments did not significantly differ in inorganic N (fig. S8). Since the overwhelming control on NO_3^- content in Akron Organic cores was the agronomic treatment and our goal was to evaluate relationships between methanogenesis and inorganic N, we regressed the data both with and without Akron Organic data (table 3).

The concentration of NH_4^+ and ratio of NH_4^+ / NO_3^- were positively correlated with mean methanogenesis while NO_3^- was negatively correlated (fig. 4). These relationships were stronger when Akron Organic core data was excluded but significant regardless of their presence (table 3). The explanatory power of these fits of inorganic N over methanogenesis was much greater than those resulting from regressing inorganic N over mean soil respiration or N_2O fluxes (r^2 =0 to 0.13, not shown). Over the range of methanogenic rates observed, regressions predict an average ~9 fold decrease in NO_3^- content and a ~6 fold increase in NH_4^+ content. When averaged over weeks 6 and 21, rates of methanogenesis and N_2O emission had a significant but noisy correlation. Mean respiration (r^2=0.46) and WFPS (r^2=0.31 and 0.43, for weeks 6 and 21, respectively) were more strongly correlated with N_2O than methanogenesis is, so in this experiment methanogenesis may be an indicator, but not a superior predictor, of N_2O production.

We made multiple redox measurements per core and every core had multiple measurements >300mV. We restricted statistical analyses to the cores' minimum redox potentials because methanogenesis generally occurs in highly reduced soils. Low redox potentials were correlated with elevated rates of methanogenesis at both week 6 (r^2=0.35) and week 21 (r^2=0.23; fig. S6). Although redox potential does not directly measure any processes it reflects the mean redox state of terminal electron acceptors present, thus in cores with sub-oxic potentials it indicates very different microbial habitats present. Neither microbial biomass nor pH were significantly correlated with rates of methanogenesis.

4. Discussion

This study addresses fundamental questions regarding the causes of upland methanogenesis and its persistence, as well as applied questions of agricultural impacts on upland anaerobic processes and their relationship to nutrient availability. To characterize mechanisms of anoxia in unsaturated upland soils, we measured methanogenesis in soil cores from a range of agricultural practices, sites, and soil moistures. The presence of oxic redox potentials within each core confirmed that methanogenesis was occurring in a subset of the soil volumes, i.e., microsites. We found that both soil moisture and elevated soil respiration appeared to play roles in causing methanogenesis, and that the comparative importance of these drivers shifted over time. Soils under no-till and organic managements had a propensity for methanogenesis, especially when wet, and long-term methanogenesis was primarily driven by soil WFPS. However, methanogenesis also occurred at very low soil moistures, showing that it can persist in soils that are typically considered thoroughly oxic. Methanogenesis was associated with lower NO_3^- and higher NH_4^+, a novel finding for upland soils that suggests it may be an indicator of altered nutrient cycling.

4.1. Drivers of methanogenesis

We hypothesized that factors closely related to O_2 consumption within and diffusion into microsites (i.e., soil respiration and WFPS, respectively) would correlate with methanogenic rates and presence. Early in our incubation both soil respiration and WFPS were correlated with methanogenic rates while only WFPS predicted rates and persistence at later times. The coincidence of high soil respiration and high methanogenesis during week 6 is consistent with heavy microbial respiration causing strong O_2 consumption, CO_2 production, and ultimately anoxic microsites. Oxygen content in small pores can plunge within a two weeks of soil wetting (Keiluweit, et al. 2018) and microbial O_2 consumption has also been cited as the cause of correlation between CO_2 emission and methanogenesis (r^2=0.17) in a cornfield over

576 the course of a growing season (Yang and Silver, 2016). In the cornfield the relationship was attributed
577 to labile C from roots feeding O_2 consumption and methanogenesis. Notably, the positive relationship
578 between methanogenesis and soil respiration observed here is in contrast to the typical relationship found
579 in wetlands.

580 The positive relationship between rates of methanogenesis with WFPS in both weeks 6 and 21
581 suggests that soil water may have decreased O_2 diffusion by closing off pores. Soil moisture also typically
582 increases microbial activity and this dual effect of moisture was likely a factor in week 6 but moisture's
583 effect on O_2 diffusion appears to have been the primary control of methanogenesis later in the incubation
584 when soil respiration had declined. Our finding that rates of methanogenesis are highest above 75% WFPS
585 and that methanogenesis is 65% more likely to be present above 60% WFPS corresponds with previous
586 findings that anaerobic activity increases sharply above 60% WFPS in agricultural and forest soils (Linn and
587 Doran, 1984, von Fischer and Hedin, 2007) and that anoxic microsites can be widespread at 60% WFPS
588 (Keiluweit, et al. 2018). Soil moisture can also be an important control on methanogenesis in wetlands,
589 with methanogenic microsites protected above -100 kPa (Wagner, 2017). As with our study, most
590 observations of upland soils have been made of soil cores with sealed bottoms and sides acting as
591 boundaries to gas diffusion. Since these boundaries decrease oxygen diffusion slightly our measured
592 threshold of WFPS for anaerobic activity may be lower than in field settings. In the above-mentioned
593 cornfield study (Yang and Silver, 2016) and a study of soil cores from a temperate grassland (Kammann et
594 al., 2009), soil moisture was not correlated with methanogenesis. However, both studies compared
595 methanogenesis to gravimetric soil moisture while we used WFPS, a volumetric calculation of soil
596 moisture that reflects gas diffusivity better and is known to correlate well with anaerobic activity (Linn
597 and Doran, 1984, Castellano et al., 2010, Wagner, 2017, Keiluweit, et al. 2018). In fact, when our

598 methanogenesis data were regressed over gravimetric moisture we found no significant relationships (fig.
599 S7, see supplemental section 1.2 for a comparison of WFPS vs gravimetric moisture).

600 Despite the importance of WFPS in controlling higher rates of methanogenesis, it was also
601 apparent that high soil moisture was not a requirement for methanogenic activity. Methanogenesis was
602 present across the range of experimental WFPS (10%-95%) which suggests that instances of
603 methanogenesis observed in deserts and other arid soils (e.g., Angel et al., 2011) are not exceptions to a
604 rule, but rather are revealing a continuum of methanogenesis across soil moistures. The drivers of
605 methanogenesis at low moisture, however, are largely unknown.

606 Both a manipulative experiment (Kammann et al., 2017) and a field study (Kammann et al., 2009)
607 have found that anaerobic guts of *Amphamallin, Cetonia* and other arthropod larvae can contribute the
608 majority of methanogenesis in grassland soils. In our study, cores with soil faunal activity trended towards
609 being more likely to have high early methanogenesis and three-quarters of cores with very high
610 methanogenesis had macrofaunal activity, however, the effect of fauna was difficult to disentangle from
611 effects related to soil respiration and WFPS. Specifically, faunal activity was better maintained in moist
612 soils and was very uncommon in Conventional soils, which also had low respiration and contain less
613 organic matter. Earthworms were responsible for most faunal activity in our study, but most previous
614 work has not found them to host methanogenesis (Šustr and Šimek, 2009, but see Depkat-Jakob et al.
615 2012), so it appears unlikely that soil faunal guts hosted the majority of methanogenesis in these soils.
616 Experiments that control and manipulate soil fauna presence (*sensu* Kammann et al., 2017) over a variety
617 of organic matter concentrations would be able to better elucidate the role that larvae and earthworms
618 play in upland methanogenesis.

619 A recent study found that clay content was the primary determinant of anoxic microsite
620 prevalence across a range of 5.6%-13.3% clay (Keiluweit, et al. 2018), however we did not detect a

variation in methanogenesis due to the sites our soils came from, which have clay content ranging approximately 16%-25%. This may be due to mineral interactions that differ between soils or the array of soil properties that co-varied between our two sites.

4.2. Methanogenesis dynamics

Consistently methanogenic cores appear to have been hospitable to methanogens and other anaerobes across large portions of the cores because many had areas with low redox potentials and visible gleying. Faunal effects also cannot be ruled out as contributing methanogenesis to these cores. The Quick cores hosted methanogenesis in the first half of the incubation but, unlike Consistent cores, could not maintain this activity through week 21. This decline was likely driven by pre-incubation loss of soil moisture gradually increasing air-filled pore volume and O_2-diffusion, it did not appear to be caused due to a loss of O_2-consumption since soil respiration continued at levels similar to the other categories. In particular, seven Quick cores were above the 60% WFPS threshold at the initial soil sampling but were brought down to a target moisture below the threshold, while only three Consistent cores similarly crossed the 60% WFPS threshold.

The Slow group of cores were not significantly different from other groups in any of our measured soil properties, but their week 21 mean WFPS was the lowest of all groups. This makes their methanogenesis unexpected: neither soil respiration nor WFPS were high, there were not many cores with faunal activity, and very few were from No-till or Organic plots that supplied most other methanogenic cores. Some of the drier cores appears to shrink during the incubation and only changes in height were measured for WFPS calculations, so it is possible that decreased volume caused dry cores to have greater WFPS than we calculated, decreasing O_2 diffusion.

The Slow group may have required longer to reach measurable methanogenesis than other cores because of a lack of biological potential, i.e., there were few methanogens present when the incubation

started so it took longer for populations to grow large enough to reach measurable rates. Most Slow cores were from conventionally managed plots, and in a field study of the same Akron plots the Conventional soils hosted much lower methanogenesis than the No-till plots (P.E. Brewer, unpublished results). Compared to no-till soils, tilled soils have lower microbial biomass and altered community (Acosta-Martinez et al., 2010, Helgason et al., 2010, Muruganandam et al., 2010) so it is plausible that low biological potential may have delayed the onset of methanogenesis, explaining why methanogenesis was never observed in half of the Conventional cores. The absence of methanogenesis in the None cores may also have been the result of low biological potential since those cores had lower WFPS at soil sampling than the other groups and thus may have been inhospitable to methanogens for a long period of time prior to field sampling.

4.3. Agricultural and site effects on methanogenesis

Upland methanogenesis was affected by agricultural practices and site in a variety of ways, some of which appear to be related to respiration and WFPS. The soils from Organic plots were distinguished by high early rates of methanogenesis and high soil respiration, both likely due to the higher organic matter concentration of these soils (Syswerda et al., 2011, Calderón et al., 2017). However, the near identical frequency of methanogenic activity in Organic and No-till soil cores, indicates that robust soil respiration was not a requirement for methanogenesis to be present. No-till cores had much lower respiration than Organic at week 6, low enough to be similar to Conventional cores, so the early methanogenesis in No-till cores may have been more dependent on low O_2 diffusion than early methanogenesis in Organic cores was. Soils from the No-till plots contain more large aggregates than the Conventional and Organic plots (Grandy and Roberston, 2007, Mikha et al., 2013), so methanogenesis in No-till may have frequently been hosted in the centers of aggregates where O_2 is slow to diffuse from intra-aggregate pores (Sexstone et al.,1985, Sey et al., 2008). Elevated methanogenesis in aggregates has

667 been observed in both models and artificial aggregates (Ebrahimi and Or, 2016, Ebrahimi and Or, 2017).
668 If aggregates protected anoxic microsites at their centers, this would have made high organic matter and
669 respiration less important for No-till methanogenesis and WFPS more important.

670 The field site where the soil was from also appears to have affected methanogenesis at week 21
671 when site interacted with agricultural practice. Specifically, cores from KBS Organic plots averaged 20
672 times the rates of all other site-practice groups, including Organic cores from Akron. This divergence in
673 the response of Organic cores could be due to NO_3^- suppressing methanogenesis. The heavy field compost
674 amendments in Akron Organic plots provided substrate for decomposition and the production of much
675 more CO_2 and microbial biomass than KBS. A byproduct of high decomposition in Akron Organic cores was
676 high soil NO_3^-, which was nearly seven fold greater than in KBS Organic (not shown). These high NO_3^-
677 concentrations could have suppressed methanogenesis by allowing denitrifiers and other anaerobes to
678 outcompete methanogens for electron donors or create toxic intermediaries like NO_2^- (Klüber and Conrad,
679 1998, Yao et al., 1999, Nazaries et al., 2013).

680 Agricultural practices can also affect soil porosity and water-holding capacity which, in turn,
681 affects WFPS dynamics. While our study intentionally maintained the WFPS of all agricultural practices at
682 similar levels and distributions, soil WFPS under field conditions would likely vary among different
683 practices due to differences in bulk density, rate of evapotranspiration, or other factors. For example,
684 porosity of Akron No-till soil cores was 44% while it was ~59% for Akron's Conventional and Organic cores,
685 so we kept gravimetric moisture content at different levels in order to maintain the same WFPS targets –
686 this would not occur if a precipitation event deposited the same amount of water on all study soils. Since
687 effects agricultural practices have on soil porosity can alter relative rates of N_2O emission (Linn and Doran,
688 1984, Castellano et al., 2010) it would likely affect methanogenesis as well, future studies examining
689 anoxic microsites should measure WFPS or matric potential in addition to other soil moisture metrics.

4.4. Changes in N-cycling and soil properties related to methanogenesis

Methanogenesis is an indicator of anoxic conditions that provide ideal habitat for other anaerobic metabolisms that alter redox-sensitive soil properties. We performed a novel analysis and evaluated relationships between upland methanogenesis and soil N availability, gaseous loss via N_2O, and microbial biomass.

Methanogenesis had a significant positive correlation with NH_4^+ and negative correlation with NO_3^- concentrations. More importantly, methanogenesis was a better predictor of these inorganic N concentrations than WFPS, soil respiration or N_2O fluxes. This suggests that processes controlling N-cycling were not only closely related to methanogenesis, but that properties that control or correlate with most soil microbial functions (i.e., moisture and soil respiration) were more distantly related to N-cycling than methanogenesis was. Pools of NH_4^+ may have enlarged in anoxic regions because anaerobic metabolisms have lower carbon use efficiency than aerobic metabolisms (Šantrůčková et al., 2004, Mazoni et al., 2012), so greater N mineralization may follow. Anoxia inhibits nitrification, an aerobic process, so NH_4^+ may have also accumulated in methanogenic soils because it was not being converted to NO_3^-; this mechanism would explain both the NH_4^+ and NO_3^- relationships with methanogenesis. Alternatively, the negative relationship between NO_3^- and methanogenesis may have arisen from denitrifiers out-competing methanogens for carbon in NO_3^- rich environments or direct inhibition of methanogenesis, as mentioned earlier. Finally, dissimilatory nitrate reduction to ammonium (DNRA) may have converted NO_3^- to NH_4^+ in soils with greater anoxic volumes (Paul, 2015). All of the above mechanisms describe how presence of anoxic microsites can explain the data by having coincident impacts on inorganic N transformations and methanogenesis and they all predict high NH_4^+ and low NO_3^- with high methanogenesis. The ratio of NH_4^+/NO_3^- provides a simple metric to test this relationship and, indeed, there was a strong positive correlation between this ratio and methanogenesis, indicating

713 methanogenesis coincided with anaerobic microbial activities that altered N-availability in this
714 experiment.

715 Agronomic treatment can affect a soil's inorganic N content and soils with high or low
716 concentrations of inorganic N could have high influence that biases regressions, leading to incorrect
717 inference about relationships between methanogenesis and N-cycling. Since three of the four cores with
718 the highest rates of methanogenesis were from a single agronomic practice (Organic) we inspected the
719 regressions in three ways to verify the correlation between inorganic N and methanogenesis was not due
720 to an agronomic effect on N that was unrelated to anoxia. First, we determined that inorganic N in field
721 and incubation soils was not different between treatments, except for Akron Organic cores where
722 concentrations averaged eight times higher due to high rates of manure application. When Akron Organic
723 samples were excluded from regressions it did not alter the conclusions and, in fact, increases the r^2 of
724 the fits (table 3). Second, we excluded data from cores with the four highest rates of methanogenesis, the
725 regressions were still significant with slopes in the same direction. Lastly, we tested whether the inorganic
726 N patterns matched a mechanism other than an anoxic control: if cores with high methanogenesis also
727 had high inorganic N concentrations due to agronomic treatment that were unaffected by anoxia, those
728 soils would most likely be high in NO_3^- and low in NH_4^+ since nitrification is ubiquitous in these upland
729 soils. This situation would predict low values of NH_4^+/NO_3^- to occur with high rates of methanogenesis,
730 but the opposite was observed. These three tests of the relationships between methanogenesis and
731 inorganic N indicate that agronomic treatment effects on inorganic N did not create a bias, further support
732 that a shared control of anoxia drove the patterns of methanogenesis and inorganic N.

733 Correlations between N_2O fluxes and methanogenesis could have resulted from co-occurring
734 denitrification and methanogenesis since both require anoxic conditions. It is unlikely the two processes
735 would coincide at micro-scales (i.e. <50 μm) because denitrifiers would out-compete methanogens and

fermenters for organic carbon (Nazaries et al., 2013), but aggregates only 250 µm in size can simultaneously host methanogenesis and denitrification (Sey et al., 2008), ostensibly because these particles have an outer layer where denitrification occurs while the core is deeply reduced, enabling methanogenesis. Co-occurrence of N_2O and CH_4 production has also been observed in a study of upland gross methanogenesis (Kammann et al., 2009). The strength of the correlations from our dataset may be weak (r^2= 0.09) because denitrification can also occur when soils are not reduced enough to host methanogenesis or because N_2O reduction can suppress methanogenesis (Klüber and Conrad, 1998). Alternatively, if nitrification was the dominant source of N_2O instead of denitrification, it may have occurred in oxic microsites while methanogenesis occurred in anoxic microsites of soils with high organic matter and rates of respiration (Sey et al., 2008).

Low microbial biomass in flooded, anoxic soils has been observed (Unger et al., 2009), however we did not find a significant relationship between microbial biomass and rates of methanogenesis.

4.5. Implications for agricultural and natural ecosystems

Because anaerobic processes are fundamentally different than aerobic, they can bring unexpected changes to nutrient availability, decomposition, and greenhouse gas fluxes in upland agricultural and natural soils that are generally assumed to be oxic. Specifically, our findings show that upland methanogenesis may indicate the presence of other processes that decrease NO_3^- concentrations and increase both NH_4^+ concentrations and N_2O emissions. Therefore, greater frequency of anoxic microsites and anaerobic activity in upland soils may slow nutrient release or increase gaseous N loss. Anoxic microsites and their impacts occur more often in wet and high organic matter soils, such as those under organic agricultural production, but are also common in dry soils. Thus, biogeochemical impacts of anaerobicity can be expected in all ecosystems and may play unexamined roles in regulating nutrient availability.

Acknowledgements and Data Statement

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Tables

	Agronomic Practices				Site			WFPS		
	C	N	O		Akron	KBS				
				p			p	Sign	p	r^2
pH	6.73[a]	5.98[b]	6.71[a]	<0.001	6.76	6.19	<0.001		0.66	0
NH_4^+ (µg gdw^{-1})	1.1[b]	1.2[b]	2.3[a]	0.002	1.5	1.4	0.77	+	0.091	0.04
NO_3^- (µg gdw^{-1})	28.2[b]	30.8[b]	69.3[a]	0.01	56.1	27.3	0.007	-	0.001	0.14
%N as NO_3^-	91.5	91.9	87.8	0.715	90.8	90.0	0.85	-	<.001	0.20
Redox pot. (mV)	339	305	289	0.586	330	288	0.31	-	0.012	0.11
MBC (mg gdw^{-1})	0.32[b]	0.27[b]	0.81[a]	<0.001	0.55	0.30	0.002	-	<0.001	0.16
Porosity	49.3%[a]	41.9%[b]	49.7%[a]	0.002	53.9%	40.0%	<0.001			

Faunal Cat.	**Count**	**Count**	**Count**	**p**	**Count**	**Count**	**p**
1	3	9	9	0.004	8	13	0.26
2	2	8	7		11	6	
3	19	7	8		17	17	

Table 1: Soil property analyses are ANOVAs over agronomic practices, t-tests over site, linear regressions over WFPS, multinomial models over faunal category. NH_4^+, NO_3^-, MBC were all log transformed prior to analyses, but means are back-transformed to original units. Superscript letters are results of multiple comparisons analyses, levels without shared letters indicate significant differences. The soil faunal categories are: 1 = large macrofaunal activity, 2 = evidence of mesofaunal activity, 3 = no soil faunal activity observed. For all n=72 except redox potential n=57.

Experimental GLMMs	**Response:**	**Rate week 6**	**Rate week 21**	**Presence week 6**	**Presence week 21**
	Df	Factor p			
Intercept	1	0.000	0.000	0.374	0.041
Site	1	0.190	0.194	0.160	0.114
Ag. practice	1	**0.006**	0.417	0.062	0.764
WFPS	2	**0.001**	**0.049**	**0.031**	0.482
WFPS x WFPS	1	**0.001**	**0.001**	0.169	0.362
Site x Ag. practice	1	0.801	0.063	0.462	0.780
Site x WFPS	2	0.253	0.960	0.755	0.695
Ag. practice x WFPS	1	0.455	**0.024**	0.106	0.797
Mechanism Regressions		Model p; r^2 where significant			
WFPS	2	**0.001; 0.14**	**0.018; 0.08**	**0.022; 0.05**	0.236
Soil respiration	2	**0.005; 0.11**	0.243	0.051	0.4
Soil faunal activity	3	0.054	0.485	0.212	0.525

Table 2: Top; model and predictor statistics for experimental factor GLMMs of methanogenesis metrics: rates and presence for week 6 to 21. Models were balanced and complete over Site and Agronomic practice (model df=12, error df =59, n=72). Bottom; single factor regressions of methanogenesis metrics over properties related to mechanisms, r^2 included where fit is significant.

	All data				Without Akron Organic			
	p	**r^2**	**Intercept**	**Slope**	***p***	**r^2**	**Intercept**	**Slope**
log NH_4^+	<0.0001	0.33	-0.174	0.387	<0.0001	0.47	-0.248	0.428
log NO_3^-	<0.0001	0.19	1.96	-0.417	<0.0001	0.39	1.84	-0.43
ratio NH_4^+/NO_3^-	<0.0001	0.36	-2.13	0.803	<0.0001	0.53	-2.089	0.859
log N_2O	0.0174	0.09	-1.86	0.524	0.0264	0.09	-1.96	0.521

Table 3: Results of regressions of N compounds over log of the mean rate of methanogenesis. Results on the left are from regressions with all data (lines plotted in fig. 4), results on the right are from regressions with all data except Akron Organic cores.

Figures

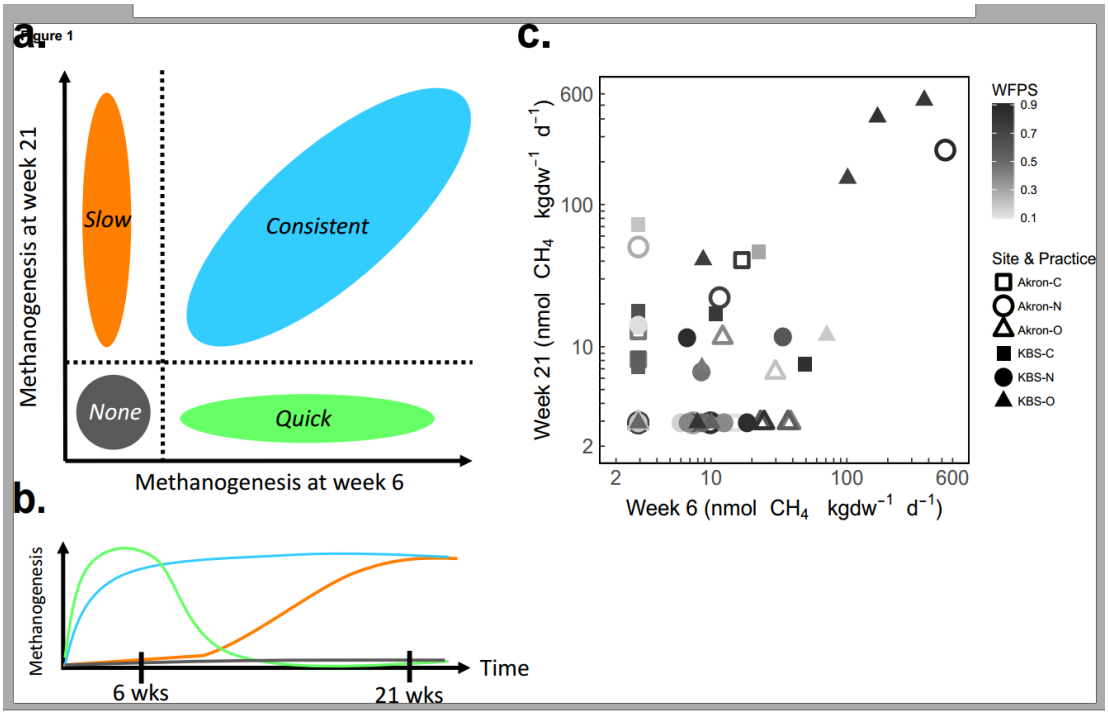


Figure 1: Conceptual diagram of groups based on methanogenic temporal dynamics (a&b). *Consistent* cores have detectable rates of methanogenesis at both 6 and 21 weeks, *Quick* cores have them only at week 6, *Slow* cores have them only at week 21, *None* cores do not have rates of methanogenesis above the detection limit (dotted line) at either 6 or 21 weeks. Rates of methanogenesis at week 21 over methanogenesis at week 6 (c). Cores falling on the x-axis are categorized as *Quick*, those falling on the y-axis are categorized as *Slow*, those falling on the origin (i.e., x,y=2.92) are categorized as *None*, all others are categorized as *Consistent*. Filled shapes are KBS, hollow shapes are Akron; squares are Conventional, circles are No-till, triangles are Organic; shapes are shaded by WFPS.

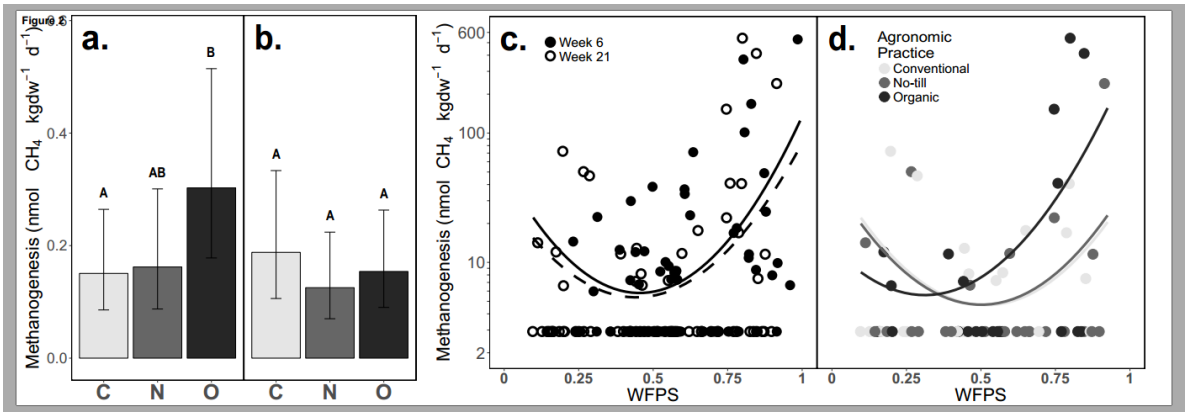
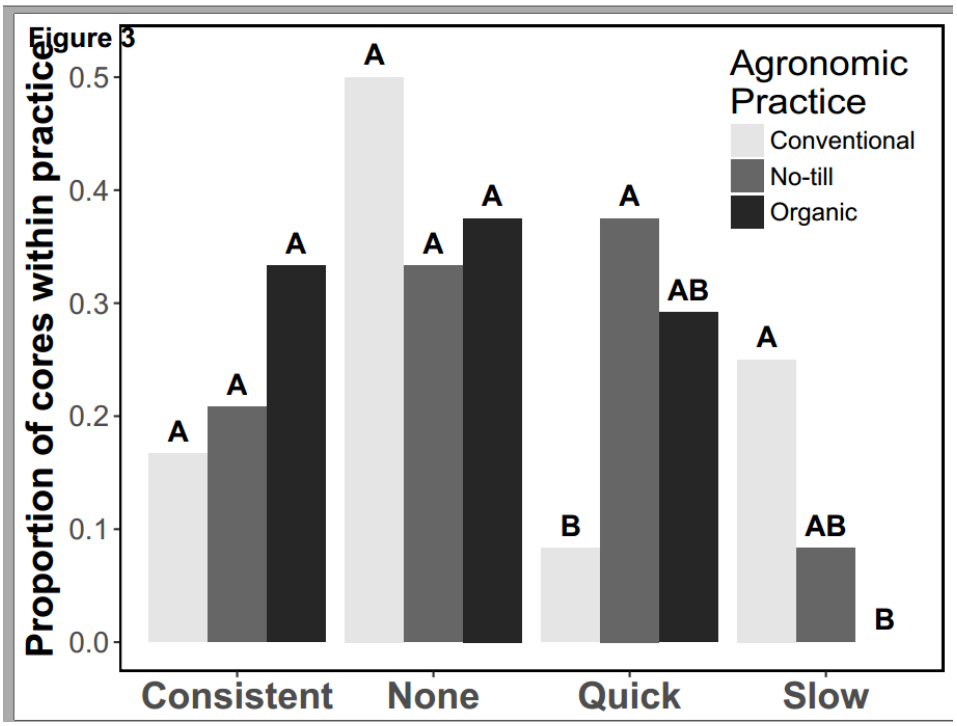


Figure 2: Results of the experimental GLMMs: methanogenic flux over agronomic practice in (a) week 6 and (b) week 21, and over (c) WFPS. C = conventional, N = no-till, O = organic practices. Tukey post-hoc levels that share the same letter are not significantly different. In (c), week 6 data are solid circles and week 6 model fit is the solid line, week 21 data are open circles and model fit is a dashed line. In (d), methanogenesis in week 21 is plotted over WFPS based on the GLMM fit. Each agronomic practice is fit separately to show the significant agronomic practice X WFPS interaction.



824

825 Figure 3: Frequency of agronomic practice within each methanogenic dynamics category.

826 Letters indicate post-hoc comparisons within each methanogenesis category, levels sharing letters are

827 not significantly different than others within that category.

828

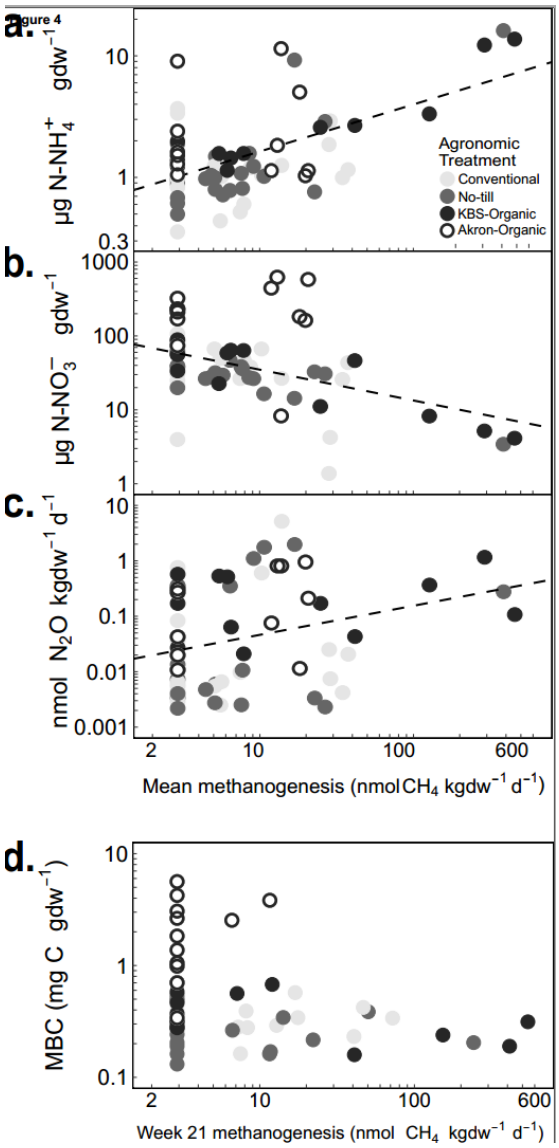


Figure 4: Regressions of (a) NH_4^+, (b) NO_3^-, and (c) mean N_2O emission over the mean rate of methanogenesis; and (d) microbial biomass regressed over week 21 methanogenesis. Plotted linear regressions are made with all data, Akron Organic is highlighted with circular markers. Rate of methanogenesis, NH_4^+, NO_3^-, and N_2O emission are log transformed. Five negative N_2O fluxes are excluded from the regression.

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Supporting Information for

Impacts of Moisture, Soil Respiration, and Agricultural Practices on Methanogenesis in Upland Soils as Measured with Stable Isotope Pool Dilution

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1.1 Sensitivity of IPD model fit to variation in fractionation factors

In the situation where the fractionation factors of the actual microbial CH_4 cycling are different from the values we chose above it is possible that the estimates of P and k would change significantly. To determine the potential impact of such a difference, we performed a sensitivity analysis by fitting the model with fractionation factor values that represent the upper and lower limits of isotopic fractionations that have been measured for CH_4 production and consumption. The range of production fractionations tested was -40 to -90‰ (Whiticar, 1993) and the range of consumption fractionations was -3 to -50‰ (Snover and Quay, 2000, Templeton et al., 2006, Kimmaman et al., 2007). We made estimates of P and k with each combination of these fractionation factors, then log transformed the P and k estimates (since all the analyses in this manuscript are based on log-transformed rates of methanogenesis), and compared the results to those from fits using the fractionation factors for our main analyses.

When our measurements were fit to the upper and lower limits of production and consumption fractionation factors, only 8 of 164 methanogenesis estimates had greater than 5% deviation from the methanogenic rates estimated in our main analysis. None of these were methanogenic rates with high leverage (i.e., all fell between the 25 and 75% quartiles) and the largest of these deviations was an increase of 10%. Eighty-eight percent of methanogenic rate estimates deviated less than 2.5%, including all of the top 10% of rates.

These deviations represent the largest changes in P and k possible if fractionation due to microbial activities in our cores fell to an extreme in the range of observed values. Even at these extremes the impacts on P and k are fairly small and confined to a small number of cores. Thus it

appears unlikely that inaccurate choices for fractionation factors would have a significant impact on results.

(Deviations in rates of CH_4 consumption were all less than 3%, however, CH_4 consumption was not analyzed in this study.)

1.2 Relationships between gravimetric moisture, WFPS (water-filled porespace), and rates of methanogenesis

While WFPS was a significant predictor of the rates of methanogenesis (p=0.0241, fig S7b), gravimetric moisture was not (p=.71, fig. S7c). This is a somewhat surprising outcome considering the high correlation of WFPS and gravimetric moisture (fig. S7a), but when considering the expected controls of anoxic microsites this relationship is less surprising. WFPS is a volumetric measure of moisture and is as much a metric of available space in soils as it is a measure of relative moisture, indeed, air-filled porespace is 1-WFPS. Since it reflects air-filled space it relates to soil gas diffusivity and, thus, the rate of diffusion of O_2 from the atmosphere. Thus calculations of WFPS are much more likely to indicate the likelihood of a soil containing anoxic regions than gravimetric moisture would.

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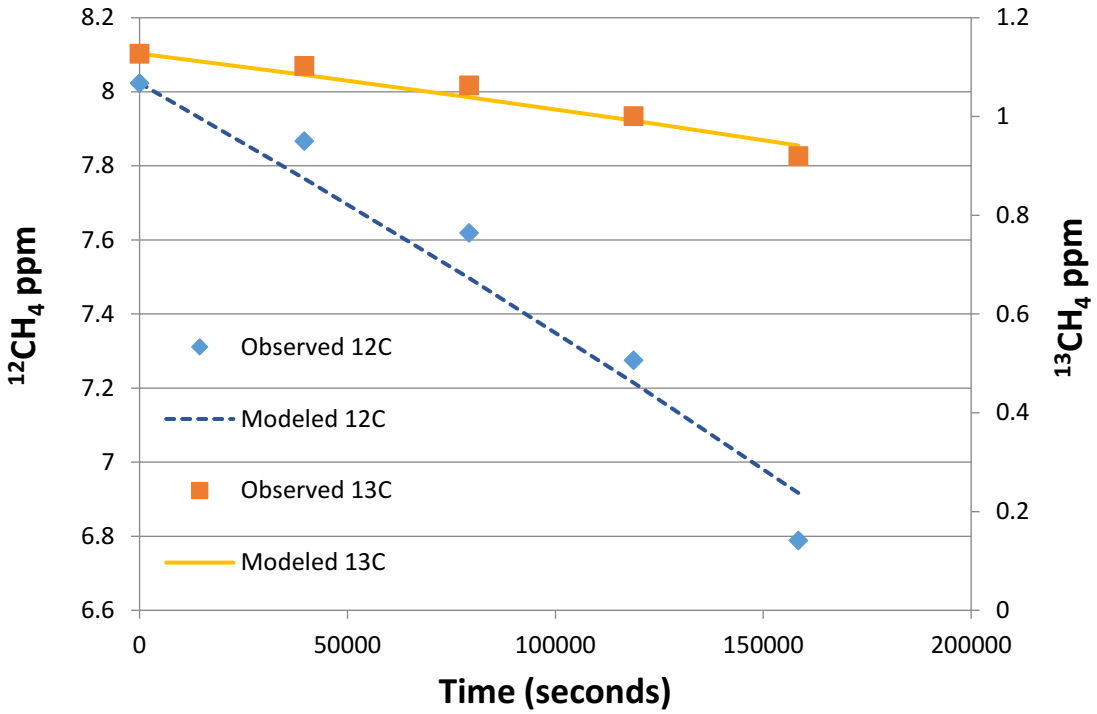
1095 Figure S1: Instrumentation used in CH_4 isotope pool dilution method with dynamic headspace. Polyester
1096 foil bags with soil cores inside (left), MCIA (center, top of cart), manufacturer supplied gas manifolds
1097 (center, bottom of cart), high capacity check standard gas bag (center, behind MCIA), Arduino MEGA
1098 and lab-made gas manifold (right, middle of cart), Arduino control computer (right, top of cart), gas
1099 calibration standard bags (right, bottom of cart).

1100



1101 Figure S2: Raw CH_4 isotope pool dilution data (observed) and model predicted $^{12}CH_4$ and $^{13}CH_4$
1102 concentrations. Data from the week 21 IPD analysis of core AK-N-2-F.

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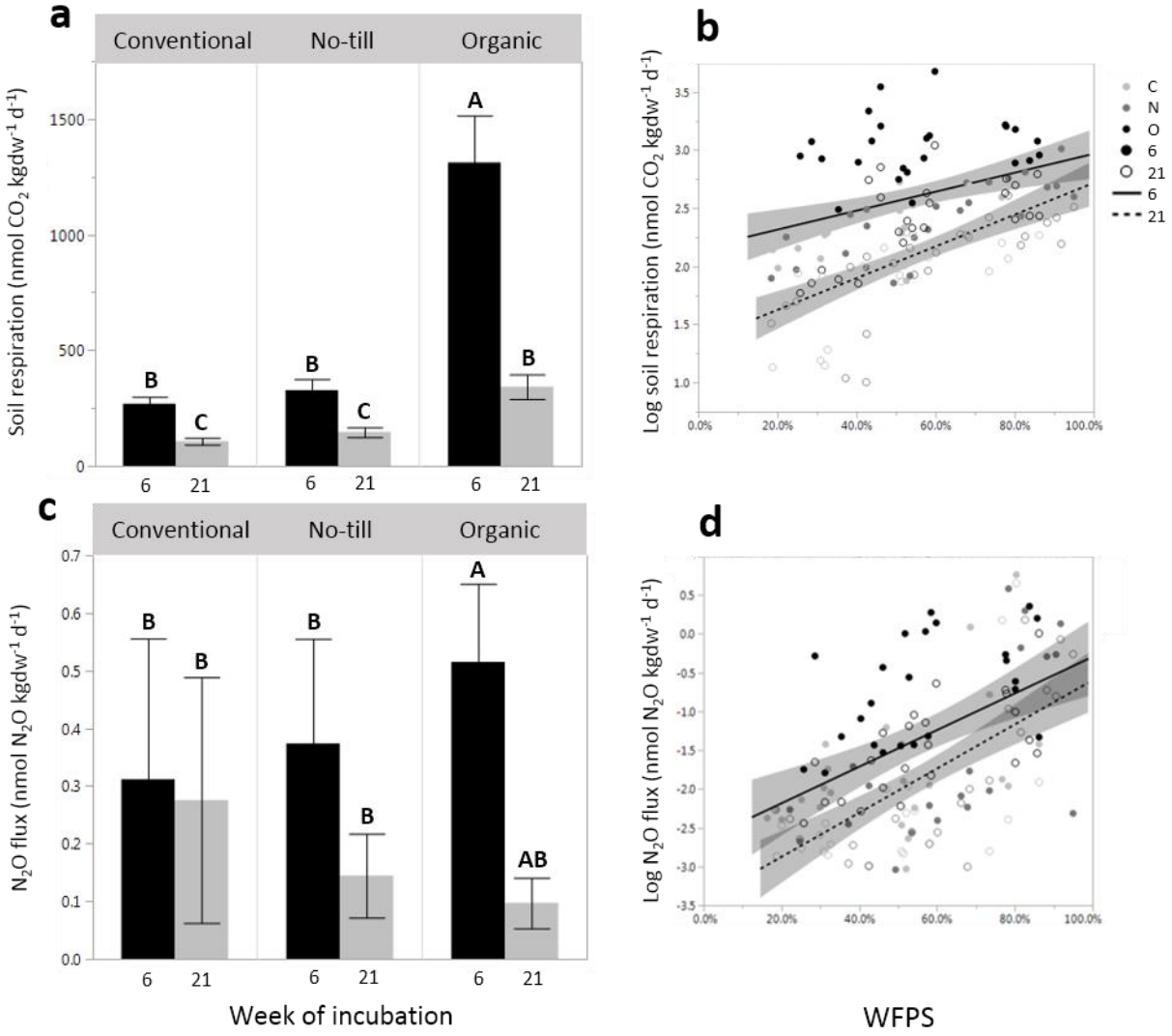


Figure S3: Soil respiration (CO_2 flux) and N_2O flux over agricultural practices and WFPS by sampling time. Panels (a) and (c) show untransformed fluxes with Tukey post-hoc connecting letters from ANOVAs performed on log-transformed fluxes (values that do not share the same letter are significantly different). Regressions in panel (b) r^2 are 0.14 and 0.49 for weeks 6 and 21 respectively; for panel (d) r^2 are 0.19 and 0.42 for weeks 6 and 21 respectively. Abbreviations are: C for Conventional, N for No-till, O for Organic, 6 for week 6, 21 for week 21.

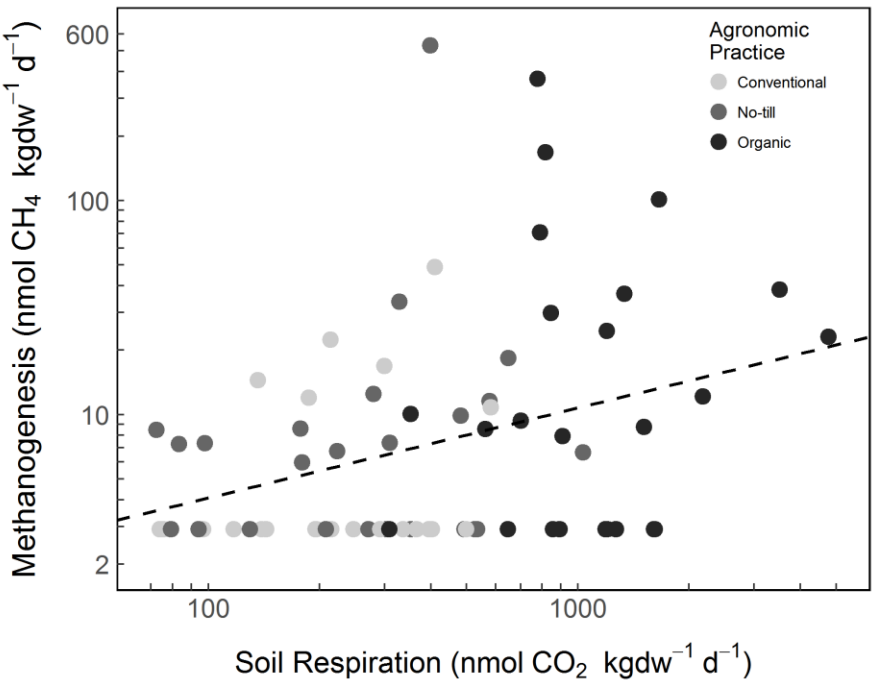


Figure S4: rates of methanogenesis from week 6 over CO_2 flux. All gas fluxes are log_{10} transformed.

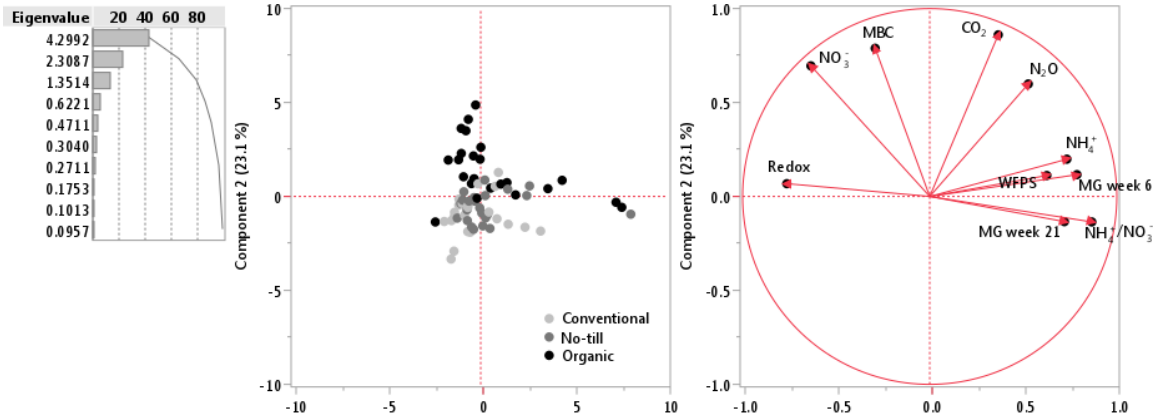


Figure S5: Principal components analysis of soil properties and gas fluxes. "MG" = rate of methanogenesis; "MBC" = microbial biomass carbon. Prior to analysis MBC, NH_4^+, NO_3^-, and all gas fluxes were log-transformed.

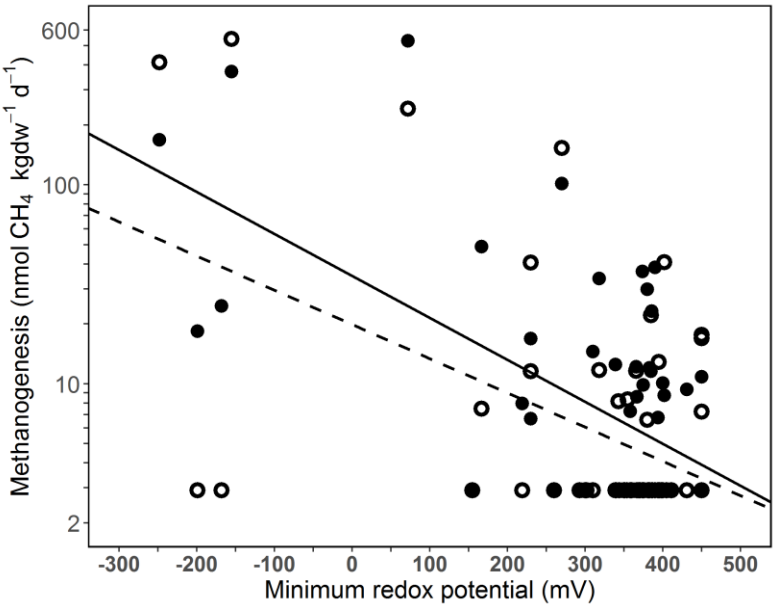
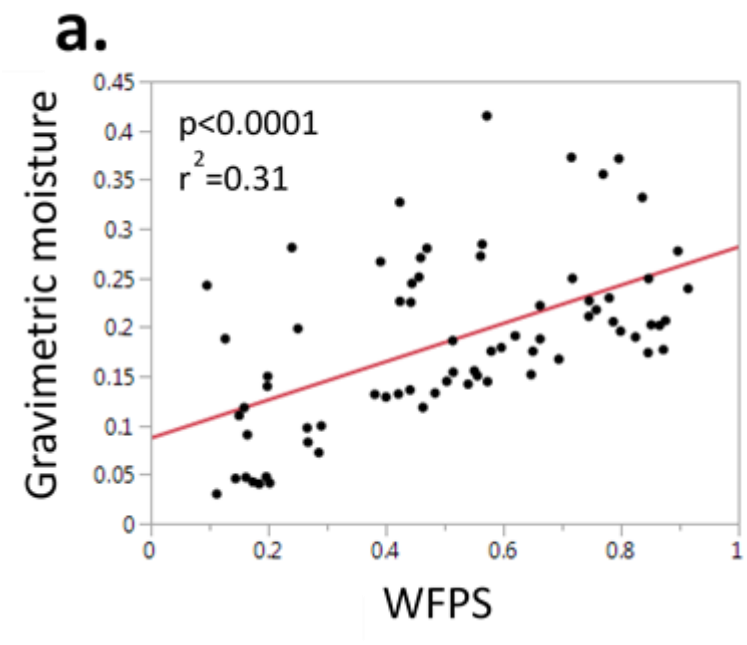
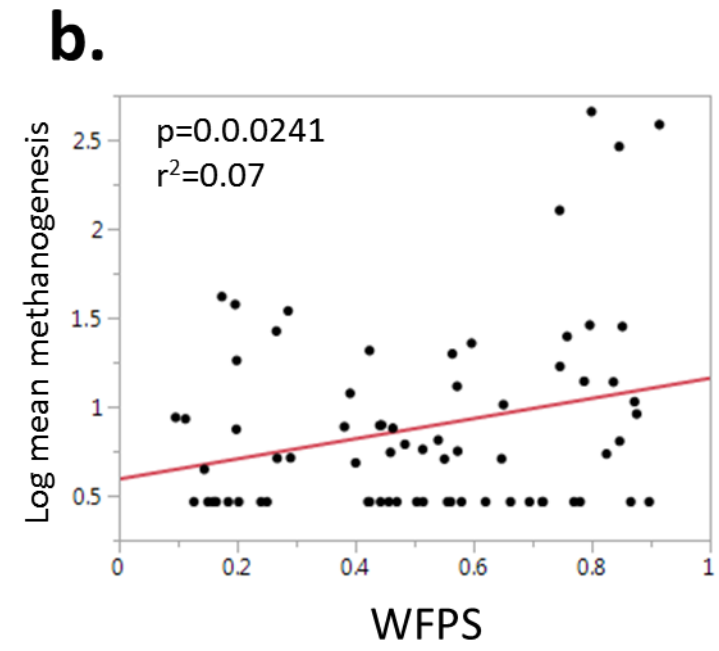


Figure S6: Week 6 and 21 methanogenic fluxes over the minimum redox potential of each core. Week 6 data are solid black and prediction is solid line, week 21 data are circles and prediction is a dotted line.


a.
Gravimetric moisture
p<0.0001
r 2=0.31
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.2
0.4
0.6
0.8
1
WFPS

1137


b.
Log mean methanogenesis
p=0.0.0241
r2=0.07
2.5
2
1.5
1
0.5
0
0.2
0.4
0.6
0.8
1
WFPS

1138

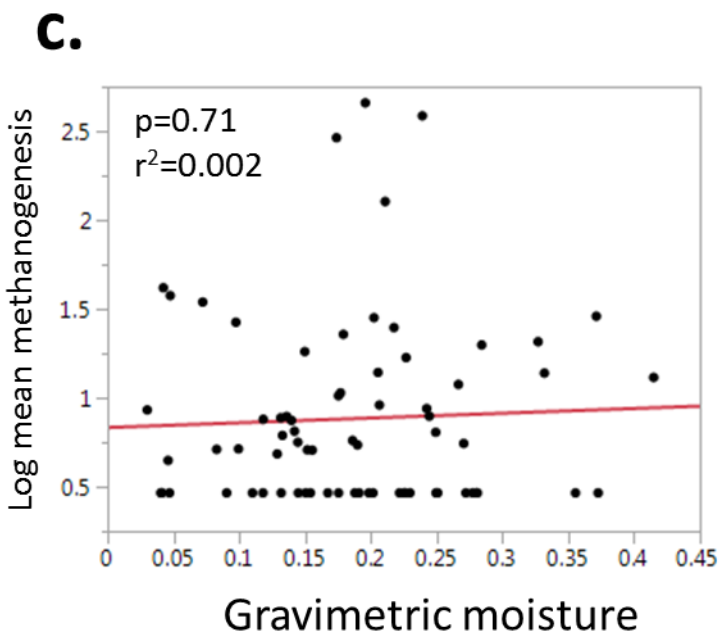
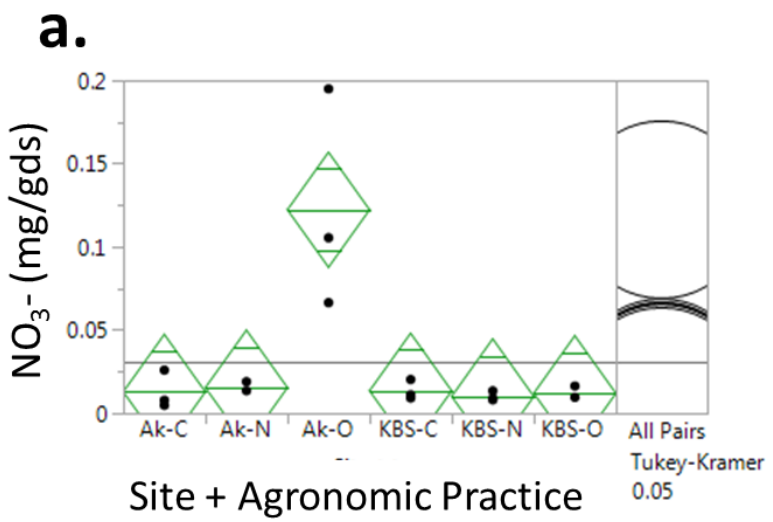


Figure S7: a, gravimetric moisture over WFPS; b, log mean methanogenesis over WFPS; and, c., log mean methanogenesis over percent gravimetric moisture. Quadratic fit of gravimetric moisture and log mean methanogenesis did not provide a significant regression (not shown).



Summary of Fit

Rsquare	0.767972
Adj Rsquare	0.671294
Root Mean Square Error	0.027501
Mean of Response	0.031036
Observations (or Sum Wgts)	18

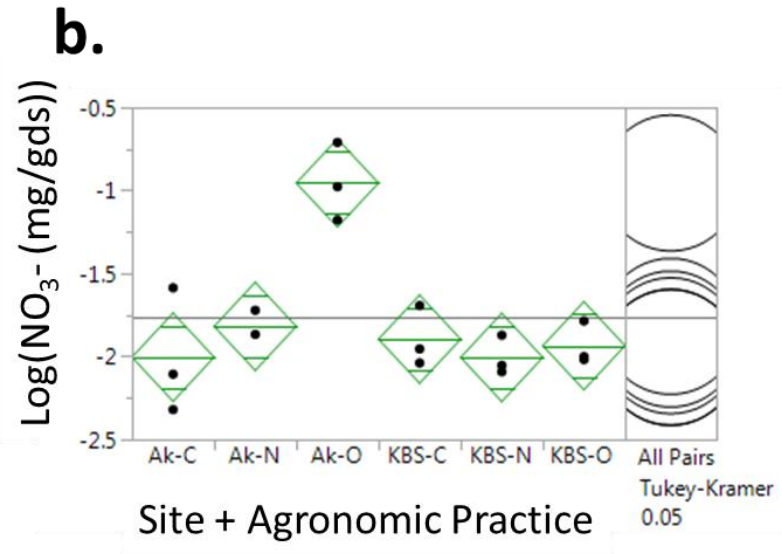
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site_trt	5	0.03003863	0.006008	7.9436	0.0016*
Error	12	0.00907559	0.000756		
C. Total	17	0.03911422			

Connecting Letters Report

Level		Mean
Ak-O	A	0.12231367
Ak-N	B	0.01540067
KBS-C	B	0.01352267
Ak-C	B	0.01285567
KBS-O	B	0.01198967
KBS-N	B	0.01013633

Levels not connected by same letter are significantly different.



Summary of Fit

Rsquare	0.821354
Adj Rsquare	0.746919
Root Mean Square Error	0.211124
Mean of Response	-1.76857
Observations (or Sum Wgts)	18

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site_trt	5	2.4592152	0.491843	11.0344	0.0004*
Error	12	0.5348823	0.044574		
C. Total	17	2.9940974			

Connecting Letters Report

Level		Mean
Ak-O	A	-0.954698
Ak-N	B	-1.818053
KBS-C	B	-1.894990
KBS-O	B	-1.934668
Ak-C	B	-2.003888
KBS-N	B	-2.005122

Levels not connected by same letter are significantly different.

Figure S8: ANOVA and multiple comparison analyses of the site+agronomic practice factors for NO_3^- and $\log(NO_3^-)$. These are from measurements of inorganic N in the initial cores measured prior to the incubation and shortly after extraction from the field plots. They show that Akron Organic plots had 8x the NO_3^- of any other plots.

Week from incubation start	Activities or measurements occurring
-2	Soil cores extracted from field plots
-1	Subset of soil cores processed to measure initial conditions of soils. Pre-incubation of remaining cores.
0	Start of incubation.
5 or 6	First measurement of CO_2 and N_2O net fluxes
6	First measurement of methanogenesis with IPD
20 or 21	Second measurement of CO_2 and N_2O net fluxes
21	Second measurement of methanogenesis with IPD
22	Destructive sampling of cores and measurements of redox potential, gravimetric moisture, pH, inorganic N, microbial biomass,

1155

1156 Table S1: Schedule of activities and measurements made during soil core incubations.

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Methanogenesis categories multinomial model

Predictor	Chi-square	df	p
Site	6.61	3	0.086
Agronomic practice	14.32	6	**0.026**
WFPS	9.80	3	**0.020**

Table S2: ANOVA table of a multinomial model of methanogenesis dynamics categories predicted by the three experimental factors, no interactions were included in the model.