Environmental Science Processes & Impacts

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Journal:	*Environmental Science: Processes & Impacts*
Manuscript ID	EM-ART-03-2020-000124.R2
Article Type:	Paper

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Environmental Significance Statement

Permafrost thaw will release abundant labile soil organic carbon substrates for anaerobic respiration and methanogenesis, presenting a potentially large positive feedback for warming. Our results reveal that divergent soil biogeochemical conditions will determine fate of these substrates and influence the ratio of carbon dioxide (CO_2) to methane (CH_4) production. In soils with circumneutral pH and few available electron acceptors, nearly all labile carbon could be mineralized via methanogenesis, producing a greenhouse gas mixture with a higher radiative forcing compared to respiration. In contrast, soils contain more abundant terminal electron acceptors convert more of the labile carbon to CO_2. A better understanding of these competing anaerobic processes could thus improve our ability to simulate feedbacks on Arctic warming using fine-scale modeling.

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Anaerobic respiration pathways and response to increased substrate availability of Arctic wetland soils

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Abstract

The availability of labile carbon (C) compounds in Arctic wetland soils is expected to increase due to thawing permafrost and increased fermentation as a result of decomposition of organic matter with warming. How microbial communities respond to this change will affect the balance of CO_2 and CH_4 emitted during anaerobic organic matter decomposition, and ultimately the net radiative forcing of greenhouse gas emissions from these soils. While soil water content limits aerobic respiration, the factors controlling methanogenesis and anaerobic respiration are poorly defined in suboxic Arctic soils. We conducted incubation experiments on two tundra soils from field sites on the Seward Peninsula, Alaska, with contrasting pH and geochemistry to determine the pathways of anaerobic microbial respiration and changes with increasing substrate availability upon warming. In incubation of soils from the circumneutral Teller site, the ratio of CO_2 to CH_4 dropped from 10 to <2 after 60 days, indicating rapid depletion of alternative terminal electron acceptors (TEAs). Addition of acetate stimulated production of CO_2 and CH_4 in a nearly 1:1 ratio, consistent with methanogenesis, and the composition of the microbial community shifted to favor clades capable of utilizing the added acetate such as the Fe(III)-reducing *Geobacter* and the methanogenic archaea *Methanosarcina*. In contrast, both CO_2 and CH_4 production declined with acetate addition during incubation of soils from the more acidic Council site, and fermentative microorganisms increased in abundance despite the high availability of fermentation products. These results demonstrate that the degree to which increasing substrate availability stimulates greenhouse gas production in tundra wetlands will vary widely depending on soil pH and geochemistry.

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1. Introduction

Wetland soils are among the largest sources of methane (CH_4) emissions, with an estimated flux of 180 Tg per year globally.[1] Arctic wetlands currently emit an estimated 15% of this global total, but are changing the most rapidly due to the magnification of warming at high latitudes and the thawing of permafrost.[2, 3] As permafrost thaws, an increasingly large carbon (C) pool will be exposed to decomposition, presenting an uncertain but potentially large positive feedback for warming.[4-6] The magnitude of this feedback will determine if terrestrial ecosystems remain a C sink or become a C source to the atmosphere in the next century.[7]

The net change in radiative forcing from decomposition of soil organic carbon (SOC) in tundra soils will depend in large part on the specific aerobic and anaerobic respiration pathways, because the warming potential of CH_4 is about 30 times larger than carbon dioxide (CO_2).[8, 9] However, the biogeochemical and microbial factors affecting anaerobic respiration are poorly understood and not explicitly represented in most models of SOM decomposition.[10, 11] For example, the anaerobic respiration of organic matter to CO_2 coupled to the reduction of terminal electron acceptors (TEAs) such as NO_3^-, SO_4^{2-}, and Fe(III) is not simulated in models. Depletion of TEAs due to increased respiration could cause an increasing fraction of C to be mineralized to CH_4, rather than CO_2, increasing the net radiative forcing from the soils. Currently, the median ratio of CO_2 to CH_4 produced during anaerobic incubation of tundra soils is 7,[12] much higher than the theoretical ratio of 1 obtained from the complete fermentation of carbohydrates to CO_2 and CH_4. This demonstrates that anaerobic respiration via TEAs makes a large contribution to SOM decomposition and mineralization in tundra soils, reducing CH_4 emissions. Predicting future greenhouse gas emissions from tundra soils will therefore require an understanding of TEA availability among different ecosystems and soil types.

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In addition to TEA availability, changes in CH_4 emissions will be mediated by increases or decrease in substrate availability. Methanogens utilize acetate through acetoclastic methanogenesis, single-carbon compounds through methylotrophic methanogenesis, or H_2 to reduce CO_2 via hydrogenotrophic methanogenesis. Alternatively, some microorganisms can use direct interspecies electron transfer (DIET) to oxidize soluble substrates and transfer electrons to methanogens by physical connections in the absence of preferred TEAs.[13] Acetate and H_2 are produced through fermentation of carbohydrates. Experiments [14] and models [11] indicate that the production of these substrates through hydrolysis and fermentation is generally the rate-limiting step in CH_4 production. Substrate availability is expected to increase through increased fermentation with warming and permafrost thaw, and permafrost itself can contain high concentrations of labile substrates such as acetate.[15-17]

The composition of the microbial community will also influence how tundra soil CO_2 and CH_4 production will respond to increased substrate availability. The relative abundance of acetoclastic and hydrogenotrophic methanogens depends strongly on environmental variables such as temperature,[18] nutrients,[19] and soil pH, so it is possible that abundant substrates will not be utilized due to a mismatch of substrates and the methanogens capable of utilizing them. For example, acetoclastic methanogenesis is unfavorable at low pH. In acidic soils, acetic acid often accumulates[20, 21] and can be toxic to methanogens.[22-24] Therefore, acidic tundra soils may not experience increases in CH_4 emissions in response to the elevated substrate availability under warming.

In this study, we conducted incubations to evaluate how increasing substrate availability will affect the CO_2 and CH_4 production potential of two contrasting organic-rich tundra wetland soils, an acidic pH 5.5 bog soil (Council) and a circumneutral pH 6.8 soil from the toe of a

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hillslope (Teller), both near Nome, Alaska, United States. We hypothesized that these soils differ in TEA availability, such as SO_4^{2-}, and geochemical characteristics because the Teller fen site is influenced by runoff from mineral soils along the hillslope, while the low pH of the Council site suggests its hydrology is primarily influenced by precipitation.[25, 26] Our objectives were therefore to determine (1) the TEA availability in each of these soils by quantifying the C mineralized using each TEA (NO_3^-, Fe(III), and SO_4^{2-}) via methanogenesis during incubation; and (2) how the predicted increase in substrate availability will affect CO_2 and CH_4 production in these soils.

2. Methods

2.1 Study site and soil collection

Active-layer soil cores were collected in August 2018 from the Teller Road Mile 27 site near Intensive Site 9 (64.729193°N, 165.944072°W), and from a thermokarst depression at the Council Road Mile 71 site (64.859618°N, 163.703477°W), using a gouge auger (AMS Inc.). At both sites, the water table was at or near the soil surface at the time of collection. The cores were separated into 10-cm segments in the field and shipped to Oak Ridge National Laboratory (Oak Ridge, TN) on ice packs. They were stored frozen at –22 °C until analysis and the start of the incubation experiment.

The frozen cores were processed in an anaerobic glove bag with ~98% N_2 and 2% H_2. Two 10-cm segments of each core were utilized, representing the 20 cm of the organic horizon closest to the soil surface, excluding the surface layer containing living plants. The total soil C content was 26.2% and 17.1% for the Teller and Council soils, respectively. The core segments were cut into 0.5 cm^3 pieces and thoroughly homogenized using a spoon. The homogenized soil

(3 g wet weight) was added to 26 mL serum bottles, and 0.2 mL of one of four amendments was applied: (1) degassed MilliQ water (control); (2) sodium acetate; (3) sodium acetate and 2-bromoethanesulfonate (BES); and (4) sodium acetate and sodium molybdate. Here, BES and molybdate were used as selective inhibitors of methanogenesis[27, 28] and sulfate reduction,[29, 30] respectively, to evaluate their relatively contributions to carbon mineralization. Additional details about the microcosms and concentrations of all amendments can be found in Table S1.

After the amendment, the microcosms were capped with a thick rubber septum and sealed with an aluminum crimp. The headspace was flushed with N_2 for 10 minutes, and microcosms were placed in an 8 °C incubator. For the Teller soils, three replicates were constructed for destructive sampling at seven time points, the control and control+acetate treatments, and three time points for the BES and molybdate inhibitor experiments (60 total microcosms). For the Council soils, three replicates were constructed for sampling at five time points for the control and control+acetate, and at four time points for treatments, BES and molybdate (69 total microcosms).

2.2 Greenhouse gas and chemical analysis

Concentrations of CO_2 and CH_4 in the headspace of the microcosms were analyzed prior to destructive sampling, using previously established methods.[17, 31, 32] 0.5 mL of the headspace was analyzed using an SRI 8610C gas chromatograph with a flame ionization detector (FID). CO_2 was converted to CH_4 using a methanizer for analysis by FID. Headspace CO_2 and CH_4 concentrations were converted to total gas production using Henry's Law based on the temperature of incubation and measured soil pH.[33] The limit of detection for CO_2 and CH_4 were 0.15 and 0.065 μmol g^{-1} dw, respectively.

Soils were destructively sampled in an anaerobic chamber, and 0.75 g of each soil was extracted with 10 mL of either degassed (anoxic) MilliQ water or 0.1 M KCl in a 15 mL plastic tube placed on a reciprocal shaker for 90 min. The soil extracts were centrifuged at 3000 RPM for 10 min and filtered through a 0.2-µm syringe filter. Aliquots of the KCl extracts were analyzed immediately for pH and Fe(II) using the 1,10-phenanthroline method (Hach method 8146).[34] The limit of detection for Fe(II) measurements was 0.25 µmol g^{-1} dw.

The water extracts were analyzed for major anion content, low-molecular-weight organic acid concentration, and water-extractable organic C (WEOC). Filtered samples were either refrigerated at 4°C and analyzed within three days of collection or frozen until analysis to minimize microbial activity in the extracts. Anions (NO_3^- and SO_4^{2-}) and organic acids (formate and acetate) were analyzed in the water extracts using ion chromatography, as previously described.[17, 31, 34] The ions were separated using a set of Dionex IonPac AS11-HC and AG11-HC columns and gradient elution. The eluent was 1 mM KOH from 0–7 min, ramping to 15 mM from 7–16 min, 30 mM at 25 min, and 60 mM at 33 min. Ions were detected using a Dionex suppressed conductivity detector. The acetate concentration of an experimental blank using the degassed water was 3.63 µM, and the corresponding detection limit was 1.03 µmol g^{-1} dw. The WEOC concentration in the soil extracts were analyzed using a Shimadzu TOC-L analyzed after acidification with HCl to pH < 1, and the detection limit was 0.31 µmol C g^{-1} dw.

2.3 Microbial community analysis

Total DNA was extracted from each sample by using 0.25 g of wet soil as input to the DNeasy PowerSoil Kit (Qiagen, Germantown, MD, USA) with minor modifications. All extractions were made for triplicate incubations per sampling point (n=3). Field collected soils were also extracted as triplicate for both locations (n=3). Prior to bead-beating, the samples were

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3 incubated in bead solution at 65 °C for 5 min to ensure that all buffer components (i.e. SDS) are in solution. Samples were disrupted by bead beating with a 1600 MiniG (SPEX Sample Prep, Metuchen, NJ, USA) at a setting of 1500 RPM for 60 s, and the DNA was further purified according to the kit protocol. 16S rRNA genes were amplified in PCR reactions using primers (F515/R806) that target the V4 region of the 16S rRNA gene where reverse PCR primer was barcoded with a 12-base Golay code.[35] The PCR reactions contained 2.5 µL Takara Ex Taq PCR buffer, 2 µL Takara dNTP mix, 0.7 µLRoche BSA (20 mg/mL), 0.5 µL each of the forward and reverse primers (10 µM final concentration), 0.125 µL Takara Ex Taq Hot Start DNA Polymerase (TaKaRa, Shiga, Japan), 1.0 µL genomic DNA (10 ng/reaction), and nuclease-free water in total volume of 25 µL. Reactions were held at 98 °C for 3 min to denature the DNA, followed by amplification for 25 cycles at 98 °C for 30 s, 52 °C for 30 s, 72 °C for 60 s, and a final extension of 12 min at 72 °C. Each sample was amplified in triplicate, combined, and purified using the Agencourt AMPure XP PCR purification system (Beckman Coulter, Brea, CA). The purified amplicons were quantified using the Qubit dsDNA HS assay (Invitrogen, Carlsbad, CA, USA). Amplicons were pooled (10 ng/sample) and sequenced on one lane of the Illumina Miseq platform (Illumina Inc, San Diego, CA). Sequence data is deposited at European Nucleotide Archive PRJEB37429.

Paired-end amplicon sequences were overlapped and merged using FLASH.[36] Quality filtering and demultiplexing were performed, as described previously.[37] Sequences were grouped into operational taxonomic units (OTUs) based on 97% sequence identity, and chimeric sequences were removed using UPARSE.[38] For 16S rRNA gene analysis, OTUs were given taxonomic assignments in QIIME [35] version 1.7.0 using the RDP classifier [39] and the SILVA database 132.[40] Phylogenetic trees were created using FastTree [41] under QIIME's default

parameters. All remaining analyses were performed in R version 3.5.1 [42, 43] via use of phyloseq,[44] vegan,[45] edgeR,[46] and ggstatsplot [47] packages. Amplicon data were proportionally rarified to the 90% of the minimum sample depth in the dataset without replacement. Data were normalized, and β-diversity was assessed using Bray-Curtis distance.[48] For multiple comparisons *p* values were adjusted via Bonferroni method. Differential changes in relative abundance of populations were calculated between biological replicates at the *Genus* level. edgeR was used to statistically compare differential OTU abundance between control and acetate amendments at each sampling point which resulted in detection of significantly enriched OTUs after accounting for false discover rates (FDR corrected P values, where $p < 0.05$).

2.4 Statistical analysis

Greenhouse gas production and porewater geochemistry data were analyzed by a linear mixed effects model using the package lme4 in R version 3.6.1.[42, 43] To test for differences between the two soils, region (Teller or Council) and day of incubation were used as the fixed factors, and microcosm replicate number was used as a random factor to account for the effects of repeated sampling. To test for the significance of treatment effects, treatment type and day of incubation were used as the fixed factors. Student's t-tests were also used to evaluate the significance of differences between individual time points. All statistical analyses used a significance threshold of p=0.05.

3. Results

3.1 CO_2 and CH_4 production

The rates of CO_2 production in the control incubations were similar in the Teller and Council soils (~ 0.15 µmol g^{-1} dry weight (dw) day^{-1} in the first 60 days; Fig. 1a,b). However, the

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3 rate of CH_4 production was higher in the Teller soil (p<0.001; Fig. 1c,d). This resulted in lower
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5 CO_2:CH_4 ratios in the Teller soils than the Council soils (1.2 and 4.4, respectively, after 60 days;
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8 p<0.001) (Fig. 2). The cumulative CO_2:CH_4 ratio in the Teller soils declined from 10 to 1.2 from
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10 the start of the incubation to 60 days (Fig. 2a). In contrast, the ratio was initially higher in the
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12 Council soils (19.2 after 4 days) and did not decline as extensively (Fig. 2b). The ratio was
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15 between 8 and 9 from 15 to 30 days and declined to 4.6 after 60 days of incubation.
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18 Changes in the concentration of potential TEAs were relatively small compared to total
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20 CO_2 and CH_4 production. Fe(II) concentrations increased by 2.5 and 3.1 µmol g^{-1} dw in 60 days
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22 during the incubation in the Teller and Council incubations (Fig. 3c,d), respectively. SO_4^{2-}
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25 concentrations declined in the Teller, but not the Council incubations (Fig. 3a,b), indicating that
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27 sulfate reduction occurred only in the Teller soils. SO_4^{2-} concentrations in the Teller soil were
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30 significantly lower at days 15, 30, and 60 compared to day 0 ($p < 0.05$). Nitrate (NO_3^-)
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32 concentrations did not change or slightly increased over the first 60 days of incubation in both
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34 soils, indicating minimal denitrification.
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37 3.2 Effects of acetate addition
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40 Acetate concentrations remained low (<2.5 µmol g^{-1} dw) during the incubations in both
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42 soils without amendments and did not accumulate (Fig. 4). The addition of acetate had little
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44 effect on CH_4 production in the first 60 days in the Teller soils. However, on day 180 the CH_4
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47 production in the Teller soils was higher than the control treatment (Fig. 1c). The final
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49 cumulative net CH_4 production was 2.5 times higher in the acetate addition treatment compared
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51 to control in the Teller soils (50.3 and 20.5 µmol g^{-1} dw, respectively, after 180 days), and the
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54 overall effect of acetate was significant (p=0.013). On the contrary, CH_4 in the acetate addition
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56 treatment was significantly lower than the control treatment in the Council soils (p<0.001; Fig.
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1d). These divergent trends became more apparent over the course of the experiment. The cumulative net production was 3.2 times higher in the control than the acetate addition treatment in the Council soils (2.1 and 0.7 μmol g^{-1} dw, respectively, after 60 days).

Acetate addition affected CO_2 production with patterns similar to the effects on CH_4 production. In the Teller soils, cumulative CO_2 production was equal or slightly lower in the acetate addition treatment compared to the control treatment for the first 60 days (Fig. 1a). However, it increased to 2.0 times higher than the control treatment by 180 days and was significantly higher overall (p=0.017). In the Council soils, the cumulative production was similar in the two treatments after 15 days, but after 60 days the CO_2 in the acetate addition soil incubations declined to 62% of the concentrations in the control incubations (p<0.001).

Water-extractable acetate concentrations did not change appreciably in acetate addition treatments of both soils during the first 15 days (Fig. 4), consistent with minimal microbial utilization over this period. After 15 days, acetate concentrations declined in the Teller soils, but not the Council soils. By the end of the incubation, the acetate concentration in the acetate addition treatment of the Teller soils was not significantly different from the concentration in the control soils (p = 0.682). In contrast, the final acetate concentration in the acetate addition treatment of the Council soils was not significantly different from the concentration at the start of the experiment (p = 0.575), indicating no net uptake or utilization occurred.

3.3 Effects of selective microbial inhibitors

In the Teller soils, the addition of BES and molybdate appeared to be very effective at suppressing methanogenesis and sulfate reduction, respectively, compared to the incubations with only acetate added (Fig. 1c, 3a). After 30 days of incubation with acetate and BES (the final

time point in the inhibitor incubations), CH_4 production was 5.7% of the production in the acetate addition incubation without BES ($p < 0.001$; Fig. 1c). In the molybdate incubations, the SO_4^{2-} concentrations did not decrease but slightly increased after 15 and 30 days compared to the concentration on day 0. This is in contrast to the decline in SO_4^{2-} concentrations to near the detection limit of 0.14 μmol g^{-1} dw over this period in the control incubations. Note that the increased SO_4^{2-} concentrations in the acetate+molybdate treatment at days 15 and 30 were attributed in part to the addition of a relatively high concentration of acetate (21 μmol g^{-1} dw), resulting in the desorption or anion exchange of SO_4^{2-} adsorbed on the soil. This phenomenon is known in soil science and ion-exchange literature.[49-51] Nevertheless, the results indicate that no net SO_4^{2-} reduction occurred in the molybdate incubations, consistent with suppression of sulfate-reducing bacteria.

BES also significantly inhibited CH_4 production in the Council soils, although the effect was smaller than in the Teller soils. The cumulative CH_4 production was 60% lower in the BES treatment compared to the acetate-only treatment ($p < 0.001$; Fig. 1c,d). The sulfate concentrations in the Council incubations did not indicate consistent sulfate reduction in any of the treatments (Fig. 3b), in part due to its low SO_4^{2-} concentration (near the detection limit), so molybdate did not have a significant effect on sulfate concentrations.

The inhibitors also affected the CO_2 production. CO_2 production was 33% lower in the BES+acetate treatment than the acetate-only treatment and 13% lower in the molybdate+acetate treatment after 30 days (Fig. 1a), although the difference was not significant in either case (p = 0.609 and 0.735, respectively). In Council soils, BES addition significantly decreased CO_2 production by 23% by 60 days (p=0.001; Fig. 1b). However, CO_2 production was not

significantly different between the molybdate+acetate incubation and the acetate-only incubation ($p = 0.206$).

3.4 Effects of incubation on microbial communities

The composition of the microbial communities before incubation differed between the two sites (Fig. 5). In Teller soils, *Acidobacteria* (12%), *Bacteriodetes* (14%), *Chloroflexi* (16%) and *Proteobacteria* (21%) were the major phyla (Fig. 5A, Fig. S1). In the Council soils, two bacterial phyla (*Acidobacteria* and *Proteobacteria*) accounted for ~50% of the total microbial community (Fig. 5A, Fig. S1). The composition of methanogenic archaea was also significantly different between the two sites. In the Teller soils, the hydrogenotrophic Rice Cluster II dominated the *Euryarchaeota*, accounting for 90% of the total *Euryarchaeota* (Fig. S2). Council soils, however, had multiple species of families *Methanobacteriaceae*, *Methanomassiliicoccaceae*, and *Methanosaetaceae* in addition to Rice Cluster II (Fig. S2).

Microbial communities in the Teller soils changed significantly over the course of the 180 days incubation (Fig. 5B). Ordination analysis could explain in total 57.2% of the observed variation which was mainly driven by incubation period ($F=80.27$, $p<0.001$) and acetate amendment ($F=34.5$, $p=0.045$). Overall community structure started to significantly change after 30 days (Acetate amendment: Control vs Treatment, $p = 0.021$) and continued to diverge during the course of the incubation (Acetate amendment Day 60: Control vs Treatment, $p < 0.001$; Acetate amendment Day 180: Control vs Treatment, $p = 0.016$). In the day 30 samples, acetate addition significantly ($p < 0.05$) increased the abundance of *Geobacter, Clostridium,* and *Acidobacteria* compared to the control incubations in the Teller soils. Using a negative binomial generalized linear model by EdgeR analysis, differentially abundant OTUs were identified. Significant increases (highest fold change, $p < 0.05$) in the abundance of several uncultured

Actinobacteria were observed by day 60 (Fig. 6, Fig. S3). From day 60 to 180, *Bacteroidetes* OTUs were enriched and became the dominant bacterial phylum. Finally, in day 180 samples, acetate addition significantly ($p < 0.05$) increased the abundance of *Methanosarcina,* a diverse clade of methanogenic archaea, as well as *Geobacter* and unknown phylum FCP246.

In the Council soils, the microbial community in the control incubations was much less dynamic (Fig. 5B), and the changes in community over time was insignificant. Acetate did not have a significant impact on the microbial communities in the Council soils (F=29.1, $p = 0.862$), which were mainly responsive to changes in formate concentrations (F=114.3, $p = 0.039$) However, acetate addition had a specific impact on several phyla. The relative abundance of *Firmicutes* OTUs from genera *Clostridium sensu stricto 11* and *Zymophilus* and *Bacteroidetes* OTUs from *Paludibacter* increased significantly over the course of the incubation (Fig. 6, Fig. S4).

The selective microbial inhibitors (BES and molybdate) had a relatively minor impact on the composition of the microbial communities despite mostly suppressing methanogenesis and sulfate reduction, respectively. In the Teller soils, BES did not significantly change the microbial community in the first 30 days (Fig. 5B). In the Council soils, BES addition appeared to reduce the relative abundance of most methanogenic archaea, although the effect was not significant (Fig. S5). However, one genus, *Methanomassiliicoccaceae*, shows the opposite trend and was higher in the BES treatment (Fig. S5). Molybdate also had no significant impact on the microbial community, and only the methanogen genus *Methanosaeta* had significantly lower abundance in the Council experiments compared to the acetate addition incubations.

4. Discussion

4.1 Quantifying pathways of anaerobic respiration

Comparing the changes in the concentrations of potential TEAs with the magnitudes of CO_2 and CH_4 production in the incubations allows a quantitative approximation of the amount of C respired using each pathway. We used the stoichiometry of anaerobic respiration reactions [52, 53] to estimate CO_2 produced from acetate via Fe(III) reduction, SO_4^{2-} reduction, and methanogenesis. Specifically, we assumed that one mole of CO_2 is produced for every four moles of Fe(II) produced during Fe(III) reduction; two moles of CO_2 are produced per mole of SO_4^{2-} reduced; and one mole of CO_2 produced per mole of CH_4 in methanogenesis. The results of these TEA budgets are shown in Table 1.

In the Teller soil incubations with no substrate additions, methanogenesis accounted for 83% of the cumulative CO_2 production in 60 days. The hydrogenotrophic Rice Cluster II were the predominant *Euryarchaeota* in these soils, so we assumed that the complete reaction produces CO_2 and CH_4 in a 1:1 ratio using syntrophic acetate oxidation with hydrogenotrophic methanogenesis. In contrast, methanogenesis in the Council soils contributed only 23% of the total CO_2 after 60 days. Council soils had a more diverse community of methanogens, including *Methanosaeta* that compete well at low acetate concentrations compared to *Methanosarcina* that grow best at high concentrations.[54]

Anaerobic respiration using alternative electron acceptors was relatively minor in both soils. Fe(III) reduction contributed 7 and 9% of the cumulative (60 day) CO_2 production in the Teller and Council soils, respectively, although our methodology may have underestimated Fe(III) reduction because solid-phase Fe(II) was not quantified but known to exist in these soils.[55] In the Teller soil, the significant decline in SO_4^{2-} in the first 15 days of incubation indicates that sulfate reduction account for 16% of CO_2 production over this interval, whereas no

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further sulfate reduction occurred after 15 days. In the Council soils, SO_4^{2-} concentrations did not decline, indicating negligible sulfate reduction occurred. Finally, there was no detectable changes in NO_3^- concentrations during incubations of either soil, indicating that denitrification was minimal. Overall, the total fraction of respiration in the control incubations from the combined Fe(III) and sulfate reduction was 13% and 8% in the Teller and Council soils, respectively, after 60 days, indicating low availability of alternative terminal electron acceptors in both sites compared to other tundra soils.[56, 57]

The fraction of CO_2 that could not be assigned to reduction of any of the measured TEAs could be attributed to carbohydrate fermentation to ethanol and CO_2 [17, 58] or reduction of natural organic matter moieties, as natural organic matter is capable of either accepting or donating electrons depending on its redox state.[53, 59, 60] Over the first 60 days of incubations, this fraction was 69% in the Council soils but only 4% in the Teller soils. This demonstrates that the Council soils had a much higher electron accepting capacity than the Teller soils, which were mainly methanogenic.

In the Teller soils, the predominant respiration pathways changed over time, evidenced by the decline of the CO_2:CH_4 ratio over the first 30 days of the incubation, from 10.0 on day 1 to 2.3 by day 30. Between days 60 and 180, CO_2 and CH_4 were produced at a nearly 1:1 ratio. This is the ratio that would be expected if syntrophic acetate or glucose oxidation with hydrogenotrophic methanogenesis was the only source of CO_2, with no contribution from the respiration of alternative TEAs. This is consistent with the depletion of potential TEAs in the Teller soils in the first 60 days, resulting in almost purely methanogenic conditions thereafter. In addition, the consumption of the added acetate in a 1:1 ratio indicates that the archaeal community is capable of high rates of acetate oxidation and methanogenesis, even though it is

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dominated by Rice Cluster II, a H_2-consuming clade. Alternatively, syntrophic acetate oxidation can decompose acetate to CH_4 and CO_2 through a strong syntrophy between acetate-consuming microbes and hydrogenotrophic methanogens.[61]

The results of the Teller acetate addition incubations also indicated the depletion of potential TEAs after 30 days in the incubations. By 180 days, nearly all of the added acetate was converted to CO_2 and CH_4 in an approximately 1:1 ratio, consistent with methanogenesis. In addition, the concentration of the dissolved ions in the microcosms indicated that other respiration pathways were not stimulated by the increase in acetate availability. Acetate addition showed negligible effects on the decline of SO_4^{2-} or the increase in Fe(II) concentrations compared to the control incubations, indicating that SO_4^{2-} and Fe(III) reduction were not stimulated. The low concentrations of both SO_4^{2-} and Fe(II) indicate limited potential for anaerobic respiration in these incubations, favoring methanogenesis. We note, however, that the measured Fe(II) concentrations in the KCl extract should be considered as a conservative estimate due to potential formation of the precipitated Fe(II) mineral phases, which were not accounted for during incubations.

The results of the inhibitor experiments are consistent with the above estimates of the fraction of C mineralized via methanogenesis and SO_4^{2-} reduction, although these experiments were only conducted for 30 days. If the C mineralized through a different pathway, then the total CO_2 produced in the incubation with inhibitors is expected to be lower than the acetate-only treatment by the same amount as the CO_2 produced via that pathway in the uninhibited experiment. Molybdate reduced the CO_2 production by approximately 13% in the Teller soils. This is similar to the estimate of C respired via SO_4^{2-} reduction based on changes in the

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3 concentration of SO_4^{2-} observed in the first 30 days of the control incubations (6%). Therefore, it
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5 appears that there was little substitution of other TEAs when SO_4^{2-} reduction was inhibited.
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8 Using the same approach, the BES inhibition of methanogenesis reduced CO_2 production
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10 by about 33% after 30 days compared to the acetate addition experiments. The decline in CO_2
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12 was smaller than the estimate of CO_2 produced via methanogenesis in the control incubations.
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14 Therefore, it appears that there was some increase of respiration via other pathways to
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16 compensate for the loss in respiration caused by the inhibition of methanogenesis. There was no
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18 evidence that either Fe(III) or SO_4^{2-} reduction was higher in the BES treatment incubations (Fig.
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20 3a,c); the additional respiration was likely resulting from organic matter reduction, as shown in
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22 some previous studies.[62, 63 64]
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25 The SOM decomposition pathways utilized by the Council soils differed greatly from the
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27 Teller soils. While the Teller soils were dominated by methanogenesis (especially later in the
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29 incubations), CH_4 production rates were low in Council soils. The addition of acetate inhibited
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31 methanogenesis compared to control incubations. Adding BES caused an additional 60%
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33 decrease in methanogenesis but did not completely abolish CH_4 production. The more diverse
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35 methanogen community in Council soil may not have been as susceptible to BES inhibition as
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37 the hydrogenotrophic methanogen population in the Teller soil.[28] This result could also be
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39 attributed to some methanogens, such as *Methanosarcina,* that are capable of anaerobic
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41 respiration using quinones as TEA when methanogenesis is suppressed by BES.[65] Supporting
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43 this idea, BES did not reduce CO_2 production over the first 30 days of these incubations,
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45 indicating that C respired via methanogenesis in the acetate addition incubations was instead
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47 respired using organic electron acceptors in the BES+acetate incubations. Instead of
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49 methanogenesis, the Council soils underwent more fermentation and respiration, as evidenced by
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higher CO_2:CH_4 ratios. The lack of change in Fe(II), SO_4^{2-}, and NO_3^- concentrations in the Council incubations (Fig. 3b,d) indicates that most CO_2 was produced by other uncharacterized pathways such as alcohol fermentation or respiration using soil organic matter as TEAs. The latter has been observed to be a dominant pathway for anaerobic respiration in many peatlands and Arctic wetland soils.[62636664] The strong contrast in mineralization pathways between the two sites is not unusual for Arctic wetlands, as CO_2:CH_4 ratios of anaerobic incubations can range from <1 to >1000.[12]

4.2 How will increasing labile substrate availability affect CH_4 emissions in tundra soils?

Thawing permafrost contains high concentrations of acetate and other labile C compounds. In addition, warming soils will increase the rate of microbial production of these compounds via fermentation. Current modeling frameworks indicate that the availability of labile substrates is a key bottleneck in anaerobic C mineralization.[10, 11] Increasing concentrations of labile C compounds such as acetate are therefore expected to result in increased CO_2 and CH_4 emissions from these soils. However, our results of acetate addition experiments demonstrate that increasing substrate availability will stimulate greenhouse gas emissions in some but not all tundra wetland soils.

Acetate addition clearly stimulated anaerobic respiration in the Teller soils, consistent with current models and expectations. The added acetate was utilized by the microbial community, first by consuming any available oxygen and TEAs, then through respiration via methanogenesis. This was supported by the decline in the CO_2:CH_4 ratio during the first 15 days of the experiment, and the observation that the ratio was consistently lower in the acetate addition microcosms than in the control incubations. The microbial community also responded in accordance with expectations. Upon incubation without amendment, the microbial community

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became enriched in fermenters such as *Firmicutes* and *Bacteroides*, consistent with the hypothesis that fermentation of labile substrates was the limiting step for SOM decomposition in these anaerobic soils. In incubations with acetate addition, these fermenting clades were not favored and did not increase in abundance over the course of the incubation. Instead, the microbes increasing in abundance during these incubations were microbial clades capable of utilizing the additional acetate. Microbial groups that increased significantly in the acetate addition incubations included the Fe(III)-reducing *Geobacter* and the methanogenic archaea *Methanosarcina*. As an alternative to Fe(III) reduction, *Geobacter metallireducens* and *Methanosarcina barkeri* cocultures have been shown to establish connections through pili and fuel methanogenesis via syntrophic oxidation using DIET.[13]

Both the biogeochemical and the microbial community composition data therefore demonstrate that the microbial community was able to exploit the increased acetate availability. This was evident in the increased CO_2 and CH_4 production as well as the observed shift in the composition of the microbial community favoring acetate-utilizing organisms.

This response contrasted sharply with the Council soils, in which neither the biogeochemical data nor the microbial community composition analysis indicated a significant response to acetate addition. Unlike in the Teller experiments, there was no significant effect of acetate addition on CO_2 or CH_4 production in the Council soils, indicating that it did not stimulate microbial respiration. High acetate concentrations at the end of the incubation confirm that it was not utilized (Fig. 4b). Overall, there was little difference between the microbial community after incubation with added acetate and after the control incubation, consistent with the lack of acetate effect on the gas fluxes. Instead, our analysis found correlations between formate concentrations and changes in microbial community structure. The microbial genera that

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increases in abundance were both anaerobic fermenters, *Clostridium sensu stricto* 11 and *Paludibacter.* Despite the excess of acetate in these incubations, the Council soils became enriched in microbes that produce rather than consume labile substrates. This further demonstrates that the microbial community in the Council soils were unable to exploit the increased substrate availability provided by the added acetate, in contrast to the Teller soils.

Our results could not fully resolve the reasons for why the Council soils did not respond to acetate addition, and these mechanisms warrant further investigation. However, the geochemical differences between the sites and the responses of their respective microbial communities suggest some clues to the potential mechanisms. Specifically, the difference in pH between the two sites could cause the added acetate to inhibit microbes in the Council soils. The pKa of acetic acid is 4.8, and at the observed soil pH of 5.5 and the measured acetate concentrations, we would expect the concentration of protonated acetic acid to be 1.8 mM in the Council soils and 92 μM in the Teller soils. In its protonated form, acetic acid can diffuse through cell walls and become acutely toxic to microbes,[22] which could explain the decline in both CO_2 and CH_4 in the acetate addition experiments compared to the control (Fig. 1b). This is also supported by the unexpected increase in relative abundance in fermenting microorganisms despite the high availability of fermentation products, as these genera are likely capable of tolerating high concentrations of organic acids.

Differences in organic matter and electron acceptor availability between the sites (as described in section 4.1) could also contribute to the observed differences in response to acetate addition. Lower TEA availability such as in the Teller soils results in a higher fraction of acetate decomposition via methanogenesis rather than respiration, reducing the CO_2:CH_4 ratio. Other well-studied Tundra soils such as those at the Barrow Environmental Observatory (Utqiaġvik,

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Alaska) appear to have higher TEA availability, and specifically higher Fe(III) reduction than either of our study sites,[67, 68] which would increase the fraction of labile substrates respired to CO_2 rather than CH_4. However, it is not clear how differences in TEA availability would result in acetate reducing the CO_2 production in the Council soils compared to the control incubations because respiration via TEAs can also utilize acetate as an electron donor. Whatever the precise mechanism, our results demonstrate that while some tundra soils respond to increasing labile substrate availability with increased CO_2 and CH_4 production, this response is not universal.

5. Conclusions

Across the Arctic, ice-rich permafrost with labile C will thaw into anoxic environments, representing a large increase in the availability of substrates for anaerobic respiration and methanogenesis. Our results reveal that soil conditions and geochemistry could result in contrasting effects on the fate of these substrates, thereby influencing CO_2 and CH_4 production. In soils with circumneutral pH and few available electron acceptors, most of the labile C could be mineralized via methanogenesis, producing a greenhouse gas mixture with a higher radiative forcing compared to respiration. However, our Council results demonstrate that in more acidic soils, increased substrate availability may not stimulate greenhouse gas production and could even be inhibitory, in contrast with current models. We suggest that this is driven in part by the toxicity of acetic acid in low pH soils under low-flow or stagnant conditions, but further work is needed to confirm the responsible mechanism. Together, these observations demonstrate that divergent soil biogeochemical conditions will mediate a range of possible pathways and fates for the abundant labile C released during permafrost thaw and thus influence radiative forcing of tundra soils.

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Author contributions

MP, BG, and DEG conceived the idea. MP conducted the experiments, data synthesis, and wrote the paper. LZ and ZY contributed to the field sampling and incubation experiments. NT performed microbial measurements and data analyses, and all authors discussed and contributed to manuscript writing and interpretation. BG, DEG, and SDW oversaw the project.

Conflicts of Interest

There are not conflicts to declare.

Acknowledgments

We thank Xiangping Yin for technical support and Xujun Liang for assistance in constructing the soil microcosms. This research was part of the Next Generation Ecosystem Experiments (NGEE-Arctic) project supported by the Office of Biological and Environmental Research, Office of Science, in the Department of Energy (DOE). All data supporting the conclusions of this work can be found on the NGEE-Arctic Data Portal (https://ngee-arctic.ornl.gov/data, DOI: 10.5440/1529131). Oak Ridge National Laboratory is managed by UT-Battelle LLC for DOE under contract DE-AC05-00OR22725.

References

1. S. D. Bridgham, H. Cadillo-Quiroz, J. K. Keller and Q. Zhuang, Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales, *Global Change Biology*, 2013, **19**, 1325-1346.
2. W. J. Riley, Z. M. Subin, D. M. Lawrence, S. C. Swenson, M. S. Torn, L. Meng, N. M. Mahowald and P. Hess, Barriers to predicting changes in global terrestrial methane fluxes: analyses using CLM4Me, a methane biogeochemistry model integrated in CESM, *Biogeosciences*, 2011, **8**, 1925-1953.
3. R. L. Thompson, M. Sasakawa, T. Machida, T. Aalto, D. Worthy, J. V. Lavric, C. Lund Myhre and A. Stohl, Methane fluxes in the high northern latitudes for 2005–2013 estimated using a Bayesian atmospheric inversion, *Atmos. Chem. Phys.*, 2017, **17**, 3553-3572.

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4. C. Schädel, E. A. G. Schuur, R. Bracho, B. Elberling, C. Knoblauch, H. Lee, Y. Luo, G. R. Shaver and M. R. Turetsky, Circumpolar assessment of permafrost C quality and its vulnerability over time using long-term incubation data, *Global Change Biology*, 2014, **20**, 641-652.
5. E. A. G. Schuur, J. G. Vogel, K. G. Crummer, H. Lee, J. O. Sickman and T. E. Osterkamp, The effect of permafrost thaw on old carbon release and net carbon exchange from tundra, *Nature*, 2009, **459**, 556-559.
6. E. A. G. Schuur, A. D. McGuire, C. Schädel, G. Grosse, J. W. Harden, D. J. Hayes, G. Hugelius, C. D. Koven, P. Kuhry, D. M. Lawrence, S. M. Natali, D. Olefeldt, V. E. Romanovsky, K. Schaefer, M. R. Turetsky, C. C. Treat and J. E. Vonk, Climate change and the permafrost carbon feedback, *Nature*, 2015, **520**, 171-179.
7. W. R. Wieder, B. N. Sulman, M. D. Hartman, C. D. Koven and M. A. Bradford, Arctic soil governs whether climate change drives global losses or gains in soil carbon, *Geophysical Research Letters*, 2019, **46**, 14486-14495.
8. M. Etminan, G. Myhre, E. J. Highwood and K. P. Shine, Radiative forcing of carbon dioxide, methane, and nitrous oxide: A significant revision of the methane radiative forcing, *Geophysical Research Letters*, 2016, **43**, 12,614-612,623.
9. IPCC, *Climate Change 2013: The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Summary for Policy Makers*, Intergovernmental Panel on Climate Change, Cambridge, UK, 2013.
10. G. Tang, J. Zheng, X. Xu, Z. Yang, D. E. Graham, B. Gu, S. L. Painter and P. E. Thornton, Biogeochemical modeling of CO_2 and CH_4 production in anoxic Arctic soil microcosms, *Biogeosciences*, 2016, **13**, 5021-5041.
11. J. Zheng, P. E. Thornton, S. L. Painter, B. Gu, S. D. Wullschleger and D. E. Graham, Modeling anaerobic soil organic carbon decomposition in Arctic polygon tundra: insights into soil geochemical influences on carbon mineralization, *Biogeosciences*, 2019, **16**, 663-680.
12. C. C. Treat, S. M. Natali, J. Ernakovich, C. M. Iversen, M. Lupascu, A. D. McGuire, R. J. Norby, T. Roy Chowdhury, A. Richter, H. Šantrůčková, C. Schädel, E. A. G. Schuur, V. L. Sloan, M. R. Turetsky and M. P. Waldrop, A pan-Arctic synthesis of CH_4 and CO_2 production from anoxic soil incubations, *Global Change Biology*, 2015, **21**, 2787-2803.
13. A. E. Rotaru, P. M. Shrestha, F. Liu, B. Markovaite, S. Chen, K. P. Nevin and D. R. Lovley, Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*, *Appl Environ Microb*, 2014, **80**, 4599-4605.
14. D. W. Valentine, E. A. Holland and D. S. Schimel, Ecosystem and physiological controls over methane production in northern wetlands, *Journal of Geophysical Research: Atmospheres*, 1994, **99**, 1563-1571.
15. T. W. Drake, K. P. Wickland, R. G. M. Spencer, D. M. McKnight and R. G. Striegl, Ancient low–molecular-weight organic acids in permafrost fuel rapid carbon dioxide production upon thaw, *Proceedings of the National Academy of Sciences, USA*, 2015, **112**, 13946-13951.
16. J. Strauss, L. Schirrmeister, K. Mangelsdorf, L. Eichhorn, S. Wetterich and U. Herzschuh, Organic-matter quality of deep permafrost carbon – a study from Arctic Siberia, *Biogeosciences*, 2015, **12**, 2227-2245.

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17. Z. Yang, S. D. Wullschleger, L. Liang, D. E. Graham and B. Gu, Effects of warming on the degradation and production of low-molecular-weight labile organic carbon in an Arctic tundra soil, *Soil Biology and Biochemistry*, 2016, **95**, 202-211.
18. B. H. Svensson, Different temperature optima for methane formation when enrichments from acid peat are supplemented with acetate or hydrogen, *Appl Environ Microb*, 1984, **48**, 389-394.
19. P. E. Galand, Y. K. and R. Conrad, Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems, *Biogeosciences*, 2010, **7**, 3893-3900.
20. S. Bonaiuti, C. Blodau and K.-H. Knorr, Transport, anoxia and end-product accumulation control carbon dioxide and methane production and release in peat soils, *Biogeochemistry*, 2017, **133**, 219-239.
21. K. N. Duddleston, M. A. Kinney, R. P. Kiene and M. E. Hines, Anaerobic microbial biogeochemistry in a northern bog: acetate as a dominant metabolic end product, *Global Biogeochem. Cycles*, 2002, **16**, 1063.
22. M. A. Horn, C. Matthies, K. Küsel, A. Schramm and H. L. Drake, Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat, *Appl Environ Microb*, 2003, **69**, 74-83.
23. J. B. Russell, Intracellular pH of acid-tolerant ruminal bacteria, *Appl Environ Microb*, 1991, **57**, 3383-3384.
24. P. K. Wüst, M. A. Horn and H. L. Drake, Trophic links between fermenters and methanogens in a moderately acidic fen soil, *Environmental Microbiology*, 2009, **11**, 1395-1409.
25. H. M. Kim, J. Y. Jung, E. Yergeau, C. Y. Hwang, L. Hinzman, S. Nam, S. G. Hong, O. S. Kim, J. Chun and Y. K. Lee, Bacterial community structure and soil properties of a subarctic tundra soil in Council, Alaska, *FEMS microbiology ecology*, 2014, **89**, 465-475.
26. M. Philben, N. Taş, H. Chen, S. D. Wullschleger, A. Kholodov, D. E. Graham and B. Gu, Influences of hillslope biogeochemistry on anaerobic soil organic matter decomposition in a tundra watershed, *Journal of Geophysical Research: Biogeosciences*, 2020, **125**, e2019JG005512.
27. R. Sparling and L. Daniels, The specificity of growth inhibition of methanogenic bacteria by bromoethanesulfonate, *Canadian Journal of Microbiology*, 1987, **33**, 1132-1136.
28. S. H. Zinder, T. Anguish and S. C. Cardwell, Selective inhibition by 2-bromoethanesulfonate of methanogenesis from acetate in a thermophilic anaerobic digestor, *Appl Environ Microb*, 1984, **47**, 1343-1345.
29. I. M. Banat, D. B. Nedwell and M. T. Balba, Stimulation of methanogenesis by slurries of saltmarsh sediment after the addition of molybdate to Inhibit sulphate-reducing bacteria, *Microbiology*, 1983, **129**, 123-129.
30. R. L. Smith and M. J. Klug, Electron donors utilized by sulfate-reducing bacteria in eutrophic lake sediments, *Appl Environ Microb*, 1981, **42**, 116-121.
31. E. M. Herndon, B. F. Mann, T. Roy Chowdhury, Z. Yang, S. D. Wullschleger, D. Graham, L. Liang and B. Gu, Pathways of anaerobic organic matter decomposition in tundra soils from Barrow, Alaska, *Journal of Geophysical Research-Biogeosciences*, 2015, **120**, 2345-2359.
32. T. Roy Chowdhury, E. M. Herndon, T. J. Phelps, D. A. Elias, B. Gu, L. Liang, S. D. Wullschleger and D. E. Graham, Stoichiometry and temperature sensitivity of

methanogenesis and CO_2 production from saturated polygonal tundra in Barrow, Alaska, *Global Change Biology*, 2015, **21**, 722-737.
33. R. Sander, Compilation of Henry's law constants (version 4.0) for water as solvent, *Atmos. Chem. Phys.*, 2015, **15**, 4399-4981.
34. M. Philben, J. Zheng, M. Bill, J. M. Heikoop, G. Perkins, Z. Yang, S. D. Wullschleger, D. E. Graham and B. Gu, Stimulation of anaerobic organic matter decomposition by subsurface organic N addition in tundra soils, *Soil Biology and Biochemistry*, 2019, **130**, 195-204.
35. J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight, QIIME allows analysis of high-throughput community sequencing data, *Nat Meth*, 2010, **7**, 335-336.
36. T. Magoč and S. L. Salzberg, FLASH: fast length adjustment of short reads to improve genome assemblies, *Bioinformatics*, 2011, **27**, 2957-2963.
37. N. A. Bokulich, S. Subramanian, J. J. Faith, D. Gevers, J. I. Gordon, R. Knight, D. A. Mills and J. G. Caporaso, Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing, *Nature Methods*, 2013, **10**, 57-59.
38. R. C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads, *Nature Methods*, 2013, **10**, 996.
39. Q. Wang, G. M. Garrity, J. M. Tiedje and J. R. Cole, Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, *Appl Environ Microb*, 2007, **73**, 5261-5267.
40. C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies and F. O. Glöckner, The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, *Nucleic Acids Research*, 2012, **41**, D590-D596.
41. M. N. Price, P. S. Dehal and A. P. Arkin, FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments, *PLOS ONE*, 2010, **5**, e9490.
42. R Core Team, R: A language and environment for statistical computing, (ver. 3.5.1), R Foundation for Statistical Computing, Vienna, Austria, 2018.
43. D. Bates, M. Mächler, B. Bolker and S. Walker, Fitting linear mixed-effects models Using lme4, *2015*, 2015, **67**, 48.
44. P. J. McMurdie and S. Holmes, phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data, *PLOS ONE*, 2013, **8**, e61217.
45. J. Oksanen, F. G. Blanchet, R. Kindt, P. Legendre, Peter R Minchin, R. O'Hara and P. S. Gavin L Simpson, M Henry H Stevens, Helene Wagner, Maintainer Jari Oksanen, Community ecology package, (ver.), 2013.
46. D. J. McCarthy, Y. Chen and G. K. Smyth, Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation, *Nucleic Acids Research*, 2012, **40**, 4288-4297.
47. I. Patil, C. Powell, W. Beasley, D. Heck and E. Hvitfeldt, ggstatsplot: Compatibility with `ggplot2 3.3.0`, (ver.), Zenodo, 2019.
48. J. R. Bray and J. T. Curtis, An Ordination of the Upland Forest Communities of Southern Wisconsin, *Ecological Monographs*, 1957, **27**, 325-349.

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49. G. R. Gobran, H. M. Selim, H. Hultberg and I. Andersson, Sulfate adsorption-desorption in a Swedish forest soil, *Water Air Soil Poll*, 1998, **108**, 411-424.
50. W. P. Inskeep, Adsorption of sulfate by kaolinite and amorphous iron-oxide in the presence of organic-ligands, *J Environ Qual*, 1989, **18**, 379-385.
51. H. Wijnja and C. P. Schulthess, Interaction of carbonate and organic anions with sulfate and selenate adsorption on an aluminum oxide, *Soil Sci Soc Am J*, 2000, **64**, 898-908.
52. C. M. Bethke, R. A. Sanford, M. F. Kirk, Q. Jin and T. M. Flynn, The thermodynamic ladder in geomicrobiology, *American Journal of Science*, 2011, **311**, 183-210.
53. J. D. Istok, M. Park, M. Michalsen, A. M. Spain, L. R. Krumholz, C. Liu, J. McKinley, P. Long, E. Roden, A. D. Peacock and B. Baldwin, A thermodynamically-based model for predicting microbial growth and community composition coupled to system geochemistry: Application to uranium bioreduction, *Journal of Contaminant Hydrology*, 2010, **112**, 1-14.
54. M. S. M. Jetten, A. J. M. Stams and A. J. B. Zehnder, Methanogenesis from acetate: a comparison of the acetate metabolism in Methanothrix soehngenii and Methanosarcina spp, *FEMS Microbiology Reviews*, 1992, **8**, 181-197.
55. A. M. Jubb, J. R. Eskelsen, X. Yin, J. Zheng, M. J. Philben, E. M. Pierce, D. E. Graham, S. D. Wullschleger and B. Gu, Characterization of iron oxide nanoparticle films at the air–water interface in Arctic tundra waters, *Science of The Total Environment*, 2018, **633**, 1460-1468.
56. D. A. Lipson, T. K. Raab, D. Goria and J. Zlamal, The contribution of Fe(III) and humic acid reduction to ecosystem respiration in drained thaw lake basins of the Arctic Coastal Plain, *Global Biogeochem Cy*, 2013, **27**, 399-409.
57. E. M. Herndon, Z. M. Yang, J. Bargar, N. Janot, T. Regier, D. Graham, S. Wullschleger, B. H. Gu and L. Y. Liang, Geochemical drivers of organic matter decomposition in arctic tundra soils, *Biogeochemistry*, 2015, **126**, 397-414.
58. M. Kramshøj, C. N. Albers, T. Holst, R. Holzinger, B. Elberling and R. Rinnan, Biogenic volatile release from permafrost thaw is determined by the soil microbial sink, *Nature Communications*, 2018, **9**, 3412.
59. D. R. Lovley, J. L. Fraga, J. D. Coates and E. L. Blunt-Harris, Humics as an electron donor for anaerobic respiration, *Environmental Microbiology*, 1999, **1**, 89-98.
60. Z. Struyk and G. Sposito, Redox properties of standard humic acids, *Geoderma*, 2001, **102**, 329-346.
61. S. Hattori, Syntrophic Acetate-Oxidizing Microbes in Methanogenic Environments, *Microbes and Environments*, 2008, **23**, 118-127.
62. T. Heitmann, T. Goldhammer, J. Beer and C. Blodau, Electron transfer of dissolved organic matter and its potential significance for anaerobic respiration in a northern bog, *Global Change Biology*, 2007, **13**, 1771-1785.
63. J. K. Keller, P. B. Weisenhorn and J. P. Megonigal, Humic acids as electron acceptors in wetland decomposition, *Soil Biology and Biochemistry*, 2009, **41**, 1518-1522.
64. R. M. Wilson, M. M. Tfaily, V. I. Rich, J. K. Keller, S. D. Bridgham, C. M. Zalman, L. Meredith, P. J. Hanson, M. Hines, L. Pfeifer-Meister, S. R. Saleska, P. Crill, W. T. Cooper, J. P. Chanton and J. E. Kostka, Hydrogenation of organic matter as a terminal electron sink sustains high CO2:CH4 production ratios during anaerobic decomposition, *Organic Geochemistry*, 2017, **112**, 22-32.

65. D. E. Holmes, T. Ueki, H.-Y. Tang, J. Zhou, J. A. Smith, G. Chaput and D. R. Lovley, A Membrane-bound cytochrome enables *Methanosarcina acetivorans* to conserve energy from extracellular electron transfer, *mBio*, 2019, **10**.
66. J. K. Keller and K. K. Takagi, Solid-phase organic matter reduction regulates anaerobic decomposition in bog soil, *Ecosphere*, 2013, **4**, art54.
67. D. A. Lipson, M. Jha, T. K. Raab and W. C. Oechel, Reduction of iron (III) and humic substances plays a major role in anaerobic respiration in an Arctic peat soil, *Journal of Geophysical Research*, 2010, **115**, G00I06.
68. K. E. Miller, C.-T. Lai, E. S. Friedman, L. T. Angenent and D. A. Lipson, Methane suppression by iron and humic acids in soils of the Arctic Coastal Plain, *Soil Biology and Biochemistry*, 2015, **83**, 176-183.

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Table 1. Summary of terminal electron acceptor (TEA) budgets during incubation experiments. TEA budgets are constructed assuming that one mole of CO_2 is produced for every four moles of Fe(II) produced, and two moles of CO_2 are produced per mole of SO_4^{2-} reduced.

Site	Treatment	Days	CO_2 produced μmol g^{-1} dw	CH_4 produced μmol g^{-1} dw	ΔFe(II)[a] μmol g^{-1} dw	ΔSO_4^{2-} μmol g^{-1} dw	TEAs % of CO_2	Methanogenesis[b] % of CO_2	Other % of CO_2
Teller	Control	15	3.68	1.11	0.58	0.29	19.8	30.0	50.2
		30	4.84	2.11	0.00	0.23	9.6	43.5	46.9
		60	9.06	7.51	2.51	0.29	13.4	82.8	3.8
	Acetate	15	2.83	1.49	1.30	0.28	30.9	52.5	16.6
		30	5.11	3.21	1.69	0.19	15.9	62.9	21.2
		60	10.92	6.86	1.94	0.25	9.0	62.8	28.2
	Acetate+BES	30	3.97	0.19	0	0.20	10.3	4.7	85.1
	Acetate+Molybdate	15	2.62	0.87	1.37	0	13.0	33.3	53.6
		30	4.53	1.93	0.30	0	1.7	42.7	55.6
Council	Control	15	4.50	0.55	2.18	0	12.1	12.2	75.7
		30	8.28	0.97	1.28	0	3.9	11.7	84.4
		60	9.28	2.13	3.14	0	8.5	22.9	68.6
	Acetate	15	3.79	0.41	1.90	0	12.5	10.8	76.7
		30	3.56	0.44	3.58	0	25.1	12.4	62.6
		60	5.68	0.67	3.59	0	15.8	11.7	72.5
	Acetate+BES	15	2.33	0.25	5.93	0	63.6	10.8	25.6
		30	3.00	0.19	3.84	0	31.9	6.4	61.6
		60	4.40	0.26	5.46	0	31.0	5.9	63.1
	Acetate+Molybdate	15	2.61	0.36	5.43	0	52.1	13.8	34.2
		30	3.75	0.34	5.32	0	35.5	9.1	55.5
		60	5.21	0.49	4.35	0	20.9	9.5	69.7

Notes: [a] Negative Δ values (decreasing Fe(II) or increasing SO_4^{2-}) are presented as 0 to indicate negligible Fe(III) or SO_4^{2-} reduction.

[b] One mole of CO_2 is produced per one mole CH_4.

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Figure Legends

Figure 1. Cumulative CO_2 and CH_4 production in the Teller and Council soil incubations. Symbols and error bars indicate mean ± 1 standard deviation of three replicate microcosms.

Figure 2. Ratio of cumulative CO_2 to CH_4 production in the Teller and Council soil incubations.

Figure 3. Time series of sulfate and ferrous iron concentrations in soil extracts over the course of the incubation experiments.

Figure 4. Time series of acetate concentrations in water extracts of Teller and Council soils over the course of incubation.

Figure 5. (A) Changes in relative abundance of microbial populations at Phylum level at different amendment conditions. NA: not assigned to a known phylum. (B) PCoA plot of total microbial community composition in samples based on Bray- Curtis distances. Axes show explained percent variation. Reference (red circle) represent samples collected at each study site prior to incubation. Control (dark goldenrod) represent soils incubated but not subjected to any amendment. Table shows correlations among ordination of samples with changes in soil chemistry during incubation. Significant correlations are in bold.

Figure 6. Bacterial OTUs that are differentially abundant at different sampling point. The y-axis represents Log fold changes (LogFC) of relative abundance calculated by EdgeR. Changes in OTU abundance was calculated between control and microbial abundances means from acetate amended Teller and Council soils at different dates of incubation. Positive LogFC values for each microbial taxa (at the genus level; $FDR < 0.05$) present significant increase.

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Fig. 1

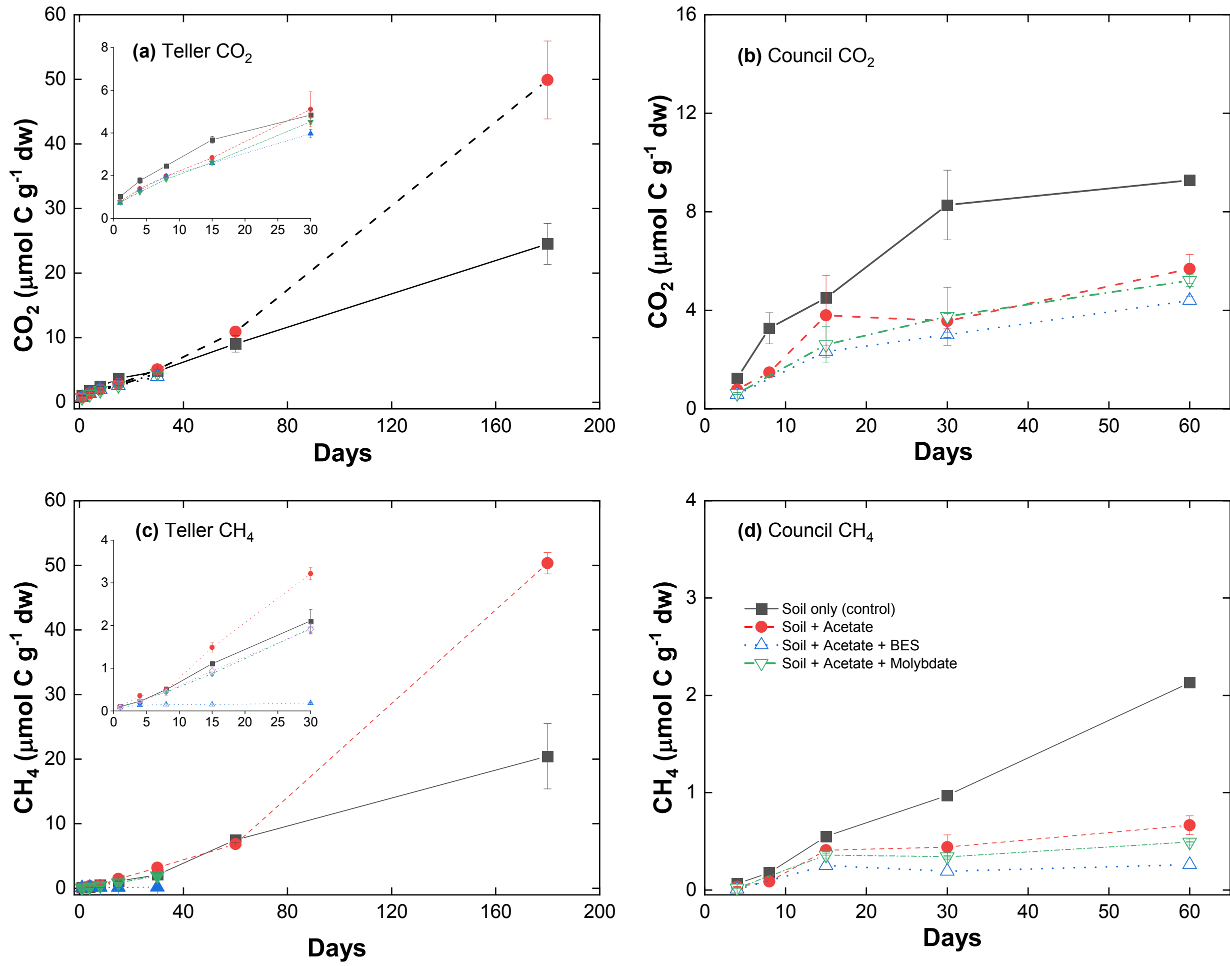

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Fig. 2

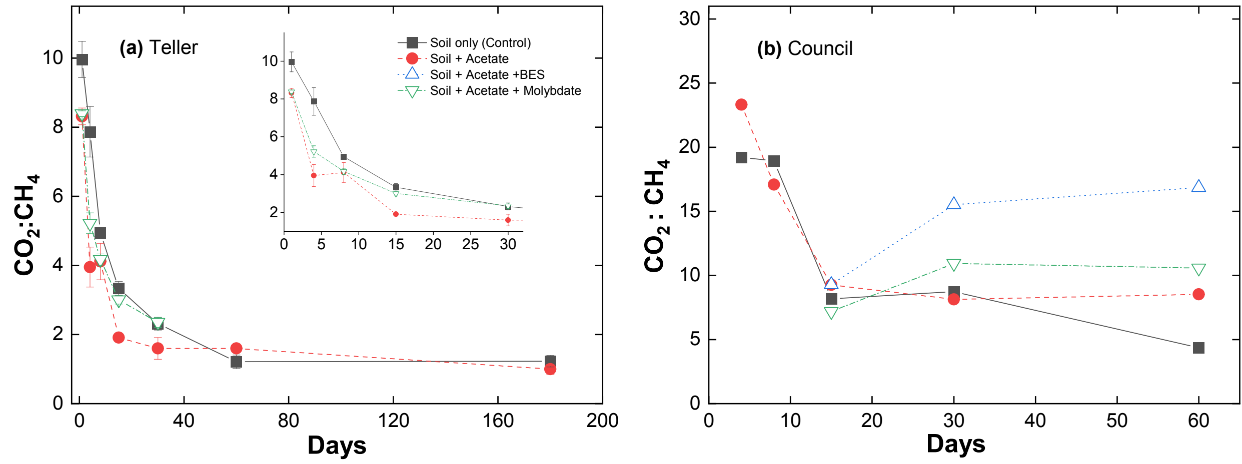

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Fig. 3

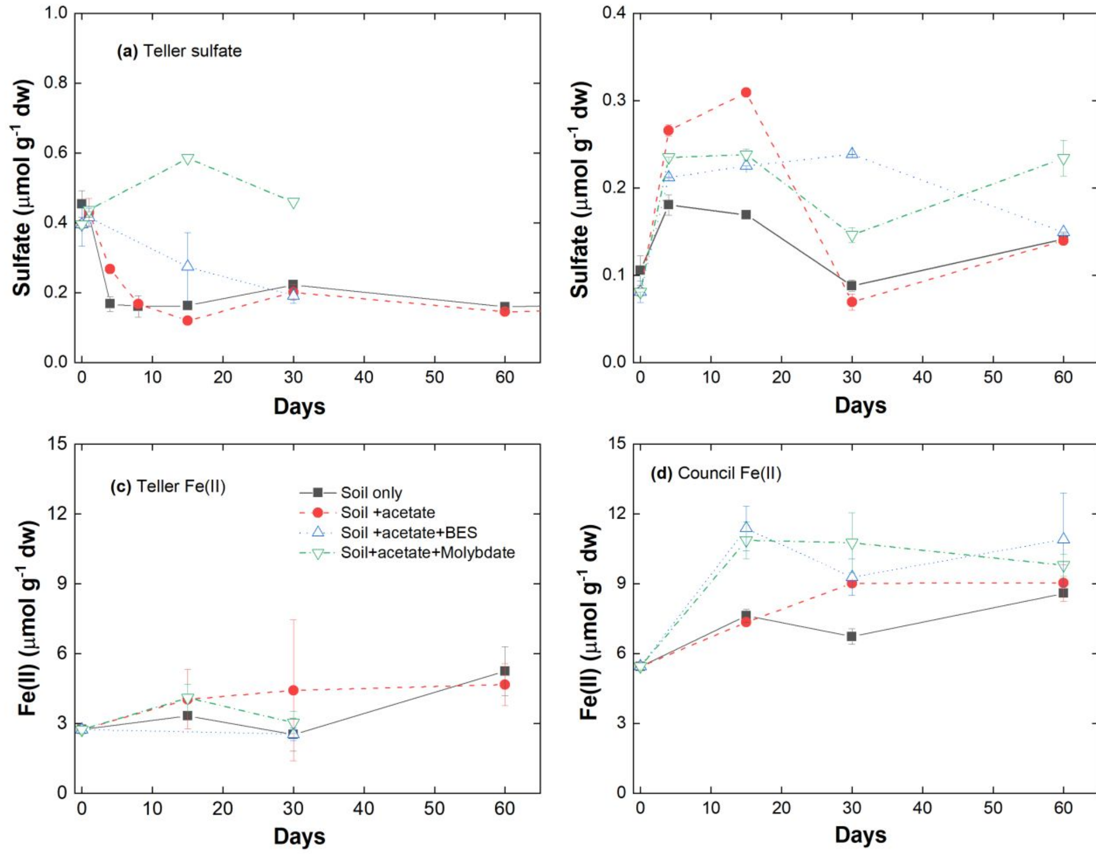

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Fig. 4

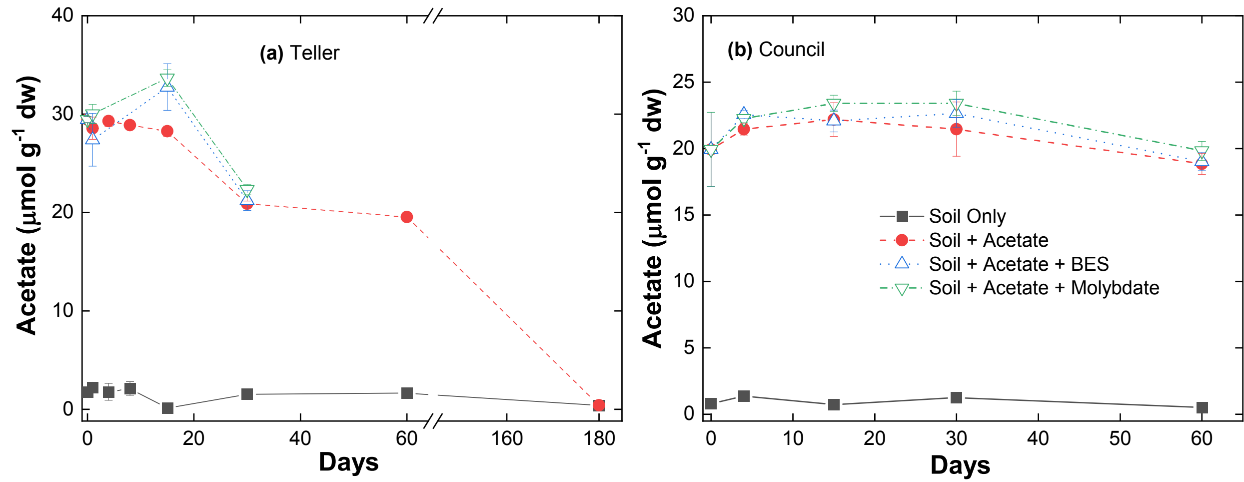
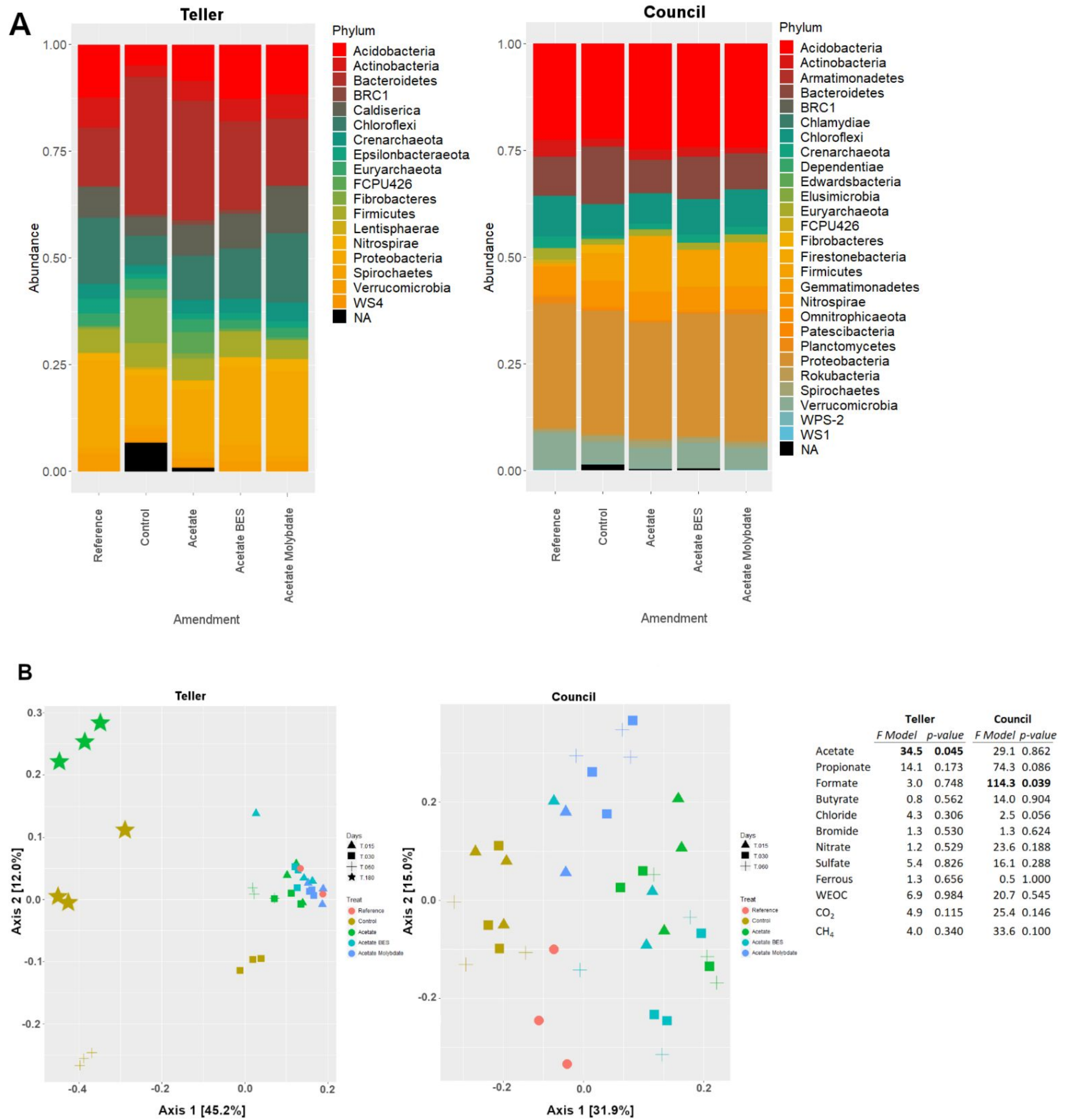


Fig. 5



	Teller		Council	
	F Model	*p-value*	*F Model*	*p-value*
Acetate	**34.5**	**0.045**	29.1	0.862
Propionate	14.1	0.173	74.3	0.086
Formate	3.0	0.748	**114.3**	**0.039**
Butyrate	0.8	0.562	14.0	0.904
Chloride	4.3	0.306	2.5	0.056
Bromide	1.3	0.530	1.3	0.624
Nitrate	1.2	0.529	23.6	0.188
Sulfate	5.4	0.826	16.1	0.288
Ferrous	1.3	0.656	0.5	1.000
WEOC	6.9	0.984	20.7	0.545
CO_2	4.9	0.115	25.4	0.146
CH_4	4.0	0.340	33.6	0.100

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Fig. 6

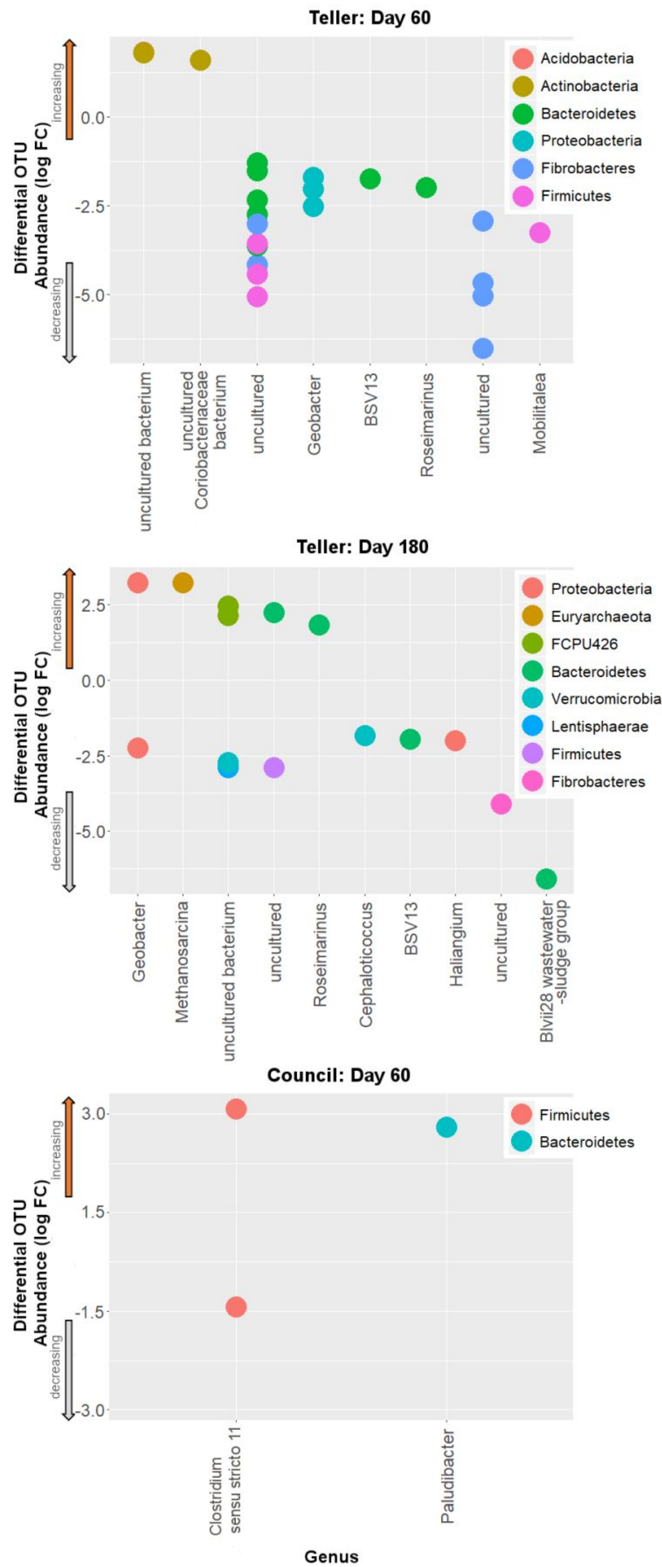

Table of contents entry

Divergent soil biogeochemical conditions will determine the fate and pathways of labile carbon released during permafrost thaw, thereby influencing the production of greenhouse gas mixtures and radiative forcing of tundra soils.

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