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Title
Influences of Hillslope Biogeochemistry on Anaerobic Soil Organic Matter Decomposition in a Tundra Watershed

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1 **Influences of hillslope biogeochemistry on anaerobic soil organic matter decomposition in a**
2 **tundra watershed**
3

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12 **Key Points:**

13 • We compared CO_2 and CH_4 production in soils from two wetland areas along a tundra
14   hillslope gradient (toeslope and peat plateau).

15 • Production of both gasses was higher in the organic toeslope soils, while microbial N
16   limitation was higher in peat plateau soils.

17 • Downslope transport of N, DOM, and alkalinity increases greenhouse gas production in
18   the organic toeslope soils.
19

20   **Abstract**

21   We investigated rates and controls on greenhouse gas (CO_2 and CH_4) production in two

22   contrasting water-saturated tundra soils within a permafrost-affected watershed near Nome,

23   Alaska, United States. Three years of field sample analysis have shown that soil from a fen-like

24   area in the toeslope of the watershed had higher pH and higher porewater ion concentrations than

25   soil collected from a bog-like peat plateau at the top of the hillslope. The influence of these

26   contrasting geochemical and topographic environments on CO_2 and CH_4 production was tested

27   in soil microcosms by incubating both the organic- and mineral-layer soils anaerobically for 55

28   days. Nitrogen (as NH_4Cl) was added to half of the microcosms to test potential effects of N

29   limitation on microbial greenhouse gas production. We found that the organic toeslope soils

30   produced more CO_2 and CH_4, fueled by higher pH and higher concentrations of water-

31   extractable organic C (WEOC). Our results also indicate N limitation on CO_2 production in the

32   peat plateau soils, but not the toeslope soils. Together these results suggest that the weathering

33   and leaching of ions and nutrients from tundra hillslopes can increase the rate of anaerobic soil

34   organic matter decomposition in downslope soils by (1) increasing the pH of soil porewater; (2)

35   providing bioavailable WEOC and fermentation products such as acetate; and (3) relieving

36   microbial N limitation through nutrient runoff. We conclude that the soil geochemistry as

37   mediated by landscape position is an important factor influencing the rate and magnitude of

38   greenhouse gas production in tundra soils.

39   **1 Introduction**

40   Hillslope topography organizes the distribution of water, energy, and nutrients within

41   landscapes (Burt & Pinay, 2005). Erosion and selective leaching from areas with elevated

42   topography results in gradients of soil grain size, composition and chemistry (Milne, 1936;

43   Moore et al., 1993; Wang et al., 2009). This typically results in the accumulation of soil organic

44   matter (SOM), nutrients, and other soil-derived solutes in low-lying areas within watersheds

45   (Creed et al., 2002; Yoo et al., 2006), provided that the soils are hydrologically connected

46   (Hornberger et al., 1994; Stieglitz et al., 2003). In temperate watersheds, slope and elevation

47   have been successfully used to predict stocks of SOM, soil pH, and nutrient concentrations

48   (Creed et al., 2002; Hall & Olson, 1991; Moore et al., 1993). There is therefore growing interest

49   in representing hillslope processes as sub-grid cell heterogeneity in the next generation of Earth

50   system models (Fan et al., 2019).

51   Accurately representing these processes in the Arctic is particularly important because Arctic

52   soils contain about one-third of the total global C pool (Hugelius et al., 2014; Schuur et al., 2015)

53   and are severely under-studied compared to temperate soils (Metcalfe et al., 2018). There is

54   evidence that similar geochemical gradients exist on Arctic hillslopes, in that nutrients, soil

55   organic matter (SOM), and leachable cations accumulate in low-lying soils (Koch et al., 2014;

56   Lev & King, 1999; Stewart et al., 2014; Yano et al., 2010). However, the presence of permafrost

57   underlying many Arctic soils impedes drainage (Liljedahl et al., 2016). This isolates surface

58   water from deeper flow pathways and increases the hydrological connectivity of the surface

59   soils, potentially strengthening the relationship between the chemistry of the ridge and valley

60   surface soils (Bring et al., 2016). In addition, poor drainage results in saturated soils in upland

61   areas as well as lowlands. Therefore, we expect a trend of increasing pH and increasing nutrient

62   concentrations in low-lying soils compared to uplands due to erosion, selective leaching, and

63   weathering.

64   Previous studies suggest these environmental variables are important regulators of CO_2 and

65   CH_4 emission from SOM decomposition. For example, rates of anaerobic SOM decomposition in

66   boreal peatlands are consistently observed to increase along gradients of groundwater inputs,

67   from precipitation-fed bogs to mineral-rich fens (Keller et al., 2006; Thormann et al., 1999). This

68   pattern has been attributed both to higher alkalinity via weathering (Ye et al., 2012) and nutrient

69   availability (Bayley et al., 2005; Keller et al., 2006) provided by groundwater inputs. Studies of

70   tundra soils have also indicated that CH_4 production is sensitive to pH (Tang et al., 2016; Zheng

71   et al., 2019a), nutrients (Philben et al., 2019), and the concentration of labile organic matter

72   (Chen et al., 2018; Yang et al., 2016). Here, we test the hypothesis that the variability in pH and

73   nutrient availability over a permafrost-affected Arctic hillslope constitutes a landscape-level

74   influence over the rate and pathways of SOM decomposition.

75   We used laboratory microcosm incubations to investigate differences in potential anaerobic

76   SOM decomposition between two wetland areas within an Arctic watershed near Nome, Alaska.

77   Both are characterized by wet sedge tussock tundra plant community, organic-rich soils, and

78   water tables near the soil surface during the thaw season (Jafarov et al., 2018). However, field

79   observations revealed that their positions on opposite ends of the hillslope results in contrasting

80   porewater geochemistry. We hypothesized that these geochemical differences affect the rates and

81   pathways of anaerobic SOM decomposition, specifically that the transport of leached ions,

82   nutrients, and dissolved organic matter down the hillslope fuels greenhouse gas production in the

83   low-lying toeslope compared to elevated plateau.

84   **2 Materials and Methods**

85   2.1 Study site

86   Soil cores and porewater were collected from two locations within the same watershed in

87   the Teller Road mile 27 site of the Next Generation Ecosystem Experiment (NGEE)-Arctic

88   project (http://ngee-arctic.ornl.gov). The site is located in hilly terrain in the southern Seward

89   Peninsula, Alaska, on a southeast-facing hillslope (Jafarov et al., 2018). One site (hereafter

90   "Plateau") is located on the peat plateau on the top of the hillslope (N64.74512° W165.96668°,

91   WGS84 datum), and the other site ("Toeslope") from the wetland in the toeslope (N64.72946°

92   W165.94465°). Both sites are characterized by tussock tundra, sedge-dominated vegetation, and

93   a water table at or near the soil surface. The depth of the permafrost table varies with hillslope

94   position: the thick peat layers in the peat plateau insulate the soil, resulting in near-surface

95   permafrost, while the active layer is thicker on the hillslope due to greater snow accumulation

96   (Jafarov et al., 2018). The toeslope consists of a collapsed peat plateau wetland that lacks near-

97   surface permafrost (> 1 m thaw depth in August 2018). In August 2018 the surface water

98   temperature was 10 °C.

99

100   2.2 Soil and porewater collection

101   Porewater samples were collected from the two sites on five occasions over three years

102   (September 9 and 13, 2016; August 9, 2017; September 10, 2017; and August 22, 2018).

103   Samples were collected using PVC piezometers with slits cut into the sides 10–15 cm from the

104   bottom to allow soil porewater to percolate in while excluding soil particles. Piezometers were

105   installed at depths of approximately 35 and 70 cm to sample the organic and mineral soils,

106   respectively. pH was measured in the field using a Hanna Instruments portable pH meter.

107   Subsamples for cation analyses were returned to the laboratory and analyzed using inductively

108   coupled plasma mass spectrometry (ICP-MS). The full dataset from these field measurements

109   (Zheng et al., 2019b) is available in the NGEE-Arctic data portal (https://ngee-

110   arctic.ornl.gov/data).

111   Cores from the two sites were collected on June 1, 2017 prior to the thawing of the active

112   layer. Frozen cores were collected using an AMS Frozen Soil Powered Auger. Soil cores (5.1 cm

113   diameter) were removed from the unlined auger barrel, wrapped in plastic sleeve, and cooled

114   with freezer packs. The peat plateau core was drilled at location N64.74514° W165.96651° while

115   the toeslope core was from W64.729193°, W165.944072°. The core from the plateau was 76 cm

116   in length, and the toeslope core was 84 cm. The cores were shipped frozen to Oak Ridge

117   National Laboratory and stored frozen until the start of the incubation. The frozen cores were

118   transferred to an anaerobic chamber and separated into organic and mineral soil layers based on

119   visual inspection. The uppermost layers containing intact vegetation were removed. The 0-38 cm

120   and 0-34 cm intervals were characterized as organic for the toeslope and the plateau,

121   respectively. Core sections from 38-84 cm for the toeslope and 61-76 cm for the plateau were

122   used for the mineral soil.

123

124   2.3 Microcosm construction

125   The separated cores were cut into small (<0.5 cm^3) pieces using an oscillating cutting tool

126   and mixed with a spoon, creating four homogenized samples (organic and mineral soils for the

127   toeslope and the plateau). Soil microcosms were constructed by adding 7 g (wet soil) to 60 mL

128   serum bottles. 1 mL of either deionized water (control treatment) or NH_4Cl solution containing

129   32 mM N (+N treatment) was added to each microcosm, so that the amendment would increase

130   the concentration of inorganic N by approximately 10-fold, based on previous measurements of

131   N in the toeslope soil (Philben et al., 2019). Three replicate microcosms were prepared for the

132   control and +N treatments to be incubated at -2°C and 8°C for 55 days. In addition, three

133   replicates were constructed for destructive sampling and soil microbial analyses after 15 and 30

134   days for the 8°C treatment only. The microcosms were sealed with blue rubber septa, crimped

135   with aluminum caps, headspace flushed with N_2 for 10 minutes, and transferred to incubators at

136   the appropriate temperature.

137

138   2.4 Greenhouse gas and chemical analysis

139   Concentrations of CO_2 and CH_4 were measured in the headspace of the microcosms every

140   two days for the first two weeks, then every five days thereafter. On each sampling day, 0.5 mL

141   of the headspace was analyzed using gas chromatography, as previously described (Roy

142   Chowdhury et al., 2015). The microcosms incubated at -2°C were kept in a cooler filled with ice

143   packs during analysis to reduce temperature change during the incubation. Headspace CO_2 and

144   CH_4 concentrations were converted to total gas production using Henry's Law based on the

145   water content, temperature of incubation and measured soil pH (Sander, 2015). Gas production

146   was normalized to per g dry weight (gdw^{-1}) of the soil to compare between soils with variable

147   water contents.

148   Microcosms were destructively sampled after 15, 30, and 55 days of incubation. In an

149   anaerobic chamber, 2 g of each soil was extracted with 10 mL of degassed water or 0.1 M KCl in

150   a 15 mL plastic tube and placed on a reciprocal shaker for 90 minutes. The soil extracts were

151   centrifuged for 10 min and filtered through a 0.2 µm syringe filter. Aliquots of the KCl extracts

152   were analyzed immediately for pH and Fe(II) using the 1,10-phenanthroline method (Hach

153   method 8146). NH_4-N concentrations were also analyzed in the KCl extracts using the

154   colorimetric salicylate and cyanurate method (Hach method 10031).

155   The water extracts were analyzed for major anion content, low-molecular weight organic

156   acid concentration, UV-visible absorbance, and water-extractable organic C (WEOC). Samples

157   were either analyzed within three days of collection or frozen until analysis. Specifically, anions

158   (Cl^-, Br^-, NO_3^-, and SO_4^{2-}) and organic acids (formate, acetate, propionate, butyrate, and oxalate)

159   were analyzed in the water extracts using ion chromatography using previously established

160   methods (Herndon et al. 2015). The ions were separated using a 4 μm Dionex IonPac AS11-HC

161   column and gradient elution. The eluent was 1 mM KOH from 0-7 min, ramping to 15 mM from

162   7-16 min, 30 mM at 25 min, and 60 mM at 33 min. Ions were detected using a Dionex

163   suppressed conductivity detector.

164   Water-extractable organic carbon (WEOC) concentration in the soil extracts were

165   analyzed using a Shimadzu TOC-L analyzer after acidification with 0.1% HCl and purging to

166   remove inorganic C (CO_3^{2-} and HCO_3^-). Ultraviolet-visible (UV-Vis) spectroscopy was also

167   conducted on the water extracts in a 1 cm quartz cuvette over the range 200–800 nm on a

168   Hewlett-Packard 8453 spectrophotometer. Specific UV absorptivity at 254 nm ($SUVA_{254}$) was

169   calculated as:

$$SUVA_{254} = 100 \times \frac{A_{254}}{[WEOC]}$$

170   where A_{254} indicates the absorbance at 254 nm and [WEOC] is the WEOC concentration in mg C

171   L^{-1} (Weishaar et al., 2003).

172

173   2.5 FTICR-MS analysis of DOM molecular composition

174   Selected samples from Day 0 and Day 55 of the incubation were analyzed using Fourier

175   transform ion cyclotron resonance mass spectrometry (FTICR-MS). The WEOC fraction was

176   subjected to solid-phase extraction (SPE) using Bond Elut PPL cartridges (Agilent) following a

177   method developed by Dittmar et al. (2008). Sample volumes of approximately 5 mL at an

178   average DOC concentration of 35 ± 20 mg L^{-1} were acidified with HCl to pH ~2, and loaded

179   onto PPL cartridges (3 mL, 0.1 gram of resin), which were conditioned with LC-MS grade

180   methanol (Fisher Scientific) before use. PPL cartridges after desalting were dried by flushing

181   with ultra-purity nitrogen gas and DOM was eluted out with 1–2 mL of LC-MS grade methanol.

182   The eluate was then stored at -20 ℃ until FTICR-MS measurements. Samples were analyzed

183   using a Bruker Daltonics 12 Tesla Apex Qe FTICR-MS interfaced to an Apollo electrospray

184   ionization (ESI) source operating in the negative ion mode. Prior to analysis, DOM extracts and

185   PPL blanks were diluted with LC-MS grade water to a methanol:water ratio of 1:1. Samples

186   were injected into the ESI source at an infusion rate of 120 µL h^{-1}. The ESI spray current was

187   stable at approximately 20 nA for all sample runs. Ion accumulation in the hexapole was set to

188   3.0 seconds, and 300 transients were co-added and digitized with a 4 M Word data acquisition

189   size.

190   FTICR-MS was externally calibrated with a polyethylene glycol standard and internally

191   calibrated with naturally present homologous series of organic acids detected within samples

192   (Chen et al., 2018). Peaks detected in experimental blanks were removed from the DOM peak

193   list. Molecular formulas were assigned to peaks with a signal to noise (S/N) above 4, using the

194   molecular formula calculator from the National High Magnetic Field Laboratory (Molecular

195   Formula Calc v.1.0 © NHML, 1998). The criteria were set as $^{12}C_{2-50}$, $^{1}H_{2-120}$, $^{16}O_{0-30}$, $^{14}N_{0-10}$,

196    $^{32}S_{0-2}$, $^{34}P_{0-1}$, where the subscripts show the range of atoms allowed in each formula. The

197   majority (>95%) of the assigned formulas were within 0.5 ppm mass accuracy, and all formulas

198   were within 1.0 ppm mass accuracy. Molecules were categorized by compound class using

199   chemical composition metrics as described previously (Chen et al., 2018). Briefly, double bond

200   equivalent (DBE) values are calculated as $DBE = 1 + C - 0.5H + 0.5\,N + 0.5\,P$. The modified

201   aromaticity index (AI_{mod}) was calculated as $AI_{mod} = (1 + C - 0.5O - S - 0.5N - 0.5\,P - 0.5H)/(C$

202   $- 0.5O - N - S - P$), which indicates aromatics when the value is ≥ 0.5, or condensed aromatics

203   when the value is ≥ 0.67 (Koch & Dittmar, 2006).

204

205   2.6 Microbial community analysis

206   Total DNA was extracted from Day 0 and Day 55 microcosms by using 0.25 g of wet soil as

207   input to the DNeasy PowerSoil Kit (Qiagen, Germantown, MD, USA) with minor modifications.

208   Prior to bead-beating, the samples were incubated in bead-solution at 65 °C for 5 min. Samples

209   were disrupted by bead beating with a 1600 MiniG (SPEX Sample Prep, Metuchen, NJ, USA) at

210   a setting of 1500 rpm for 60 s and the DNA was further purified according to the kit protocol.

211   16S rRNA genes were amplified in PCR reactions using primers (F515/R806) that target the V4

212   region of the 16S rRNA gene where reverse PCR primer was barcoded with a 12-base Golay

213   code (Caporaso et al., 2010). The PCR reactions contained 2.5 μl Takara Ex Taq PCR buffer, 2

214   μl Takara dNTP mix, 0.7 μl Roche BSA (20 mg/ml), 0.5 μl each of the forward and reverse

215   primers (10 μM final concentration), 0.125 μl Takara Ex Taq Hot Start DNA Polymerase

216   (TaKaRa, Shiga, Japan), 1.0 μl genomic DNA (10 ng/reaction), and nuclease-free water in a total

217   volume of 25 μl. Reactions were held at 98 °C for 3 min to denature the DNA, followed by

218   amplification for 25 cycles at 98 °C for 30 s, 52 °C for 30 s, and 72 °C for 60 s, and a final

219   extension of 12 min at 72 °C. Each sample was amplified in triplicate, combined, and purified

220   using the Agencourt AMPure XP PCR purification system (Beckman Coulter, Brea, CA). The

221   purified amplicons were quantified using the Qubit dsDNA HS assay (Invitrogen, Carlsbad, CA,

222   USA). Amplicons were pooled (10 ng/sample) and sequenced on one lane of the Illumina Miseq

223   platform (Illumina Inc, San Diego, CA) resulting in 300 bp paired-end reads. Sequence data are

224   deposited at European Nucleotide Archive with accession PRJEB34184.

225   Paired-end amplicon sequences were overlapped and merged using FLASH (Magoč &

226   Salzberg, 2011). Quality filtering and demultiplexing were performed as described previously

227   (Bokulich et al., 2013). Sequences were grouped into operational taxonomic units (OTUs) based

228   on 97% sequence identity, and chimeric sequences were removed using UPARSE (Edgar, 2013).

229   For 16S rRNA gene analysis, OTUs were given taxonomic assignments in QIIME (Caporaso et

230   al., 2010) version 1.7.0 using the RDP classifier (Wang et al., 2007) and the SILVA database 132

231   (Quast et al., 2013). Phylogenetic trees were created using FastTree (Price et al., 2010) under

232   QIIME's default parameters. All remaining analysis were performed in R version 3.5.1 (Bates et

233   al., 2014; R Core Team, 2013) via use of phyloseq (McMurdie & Holmes, 2013), vegan

234   (Oksanen et al., 2013) and ggstatsplot (Patil et al., 2019) packages. Amplicon data were

235   proportionally normalized, and β-diversity was assessed by perMANOVA (Anderson & Walsh,

236   2013) using Bray-Curtis distance (Bray & Curtis, 1957). For multiple comparisons p values were

237   adjusted via Bonferroni method.

238

239   2.7 Statistical analysis

240   Porewater chemistry data were fit to a linear mixed effects model using the package lme4 in

241   R version 3.5.1 (Bates et al., 2014; R Core Team, 2013). Time of incubation (0, 30, or 55 days)

242   and treatment (control or +N) were used as fixed factors, and the microcosm replicate was used

243   as a random factor to account for the effects of repeated sampling. The treatment effect was

244   excluded from the model for the inorganic N ($NO_3^-+NH_4^+$) measurements so that temporal

245   changes in the control treatments were evaluated. For the CO_2 and CH_4 measurements, the

246   difference between concentrations at 29 and 50 days in the Control and +N treatments were

247   compared using the mixed effects model. Note that the days analyzed are different for the

248   greenhouse gas concentrations and porewater chemistry due to differences in their respective

249   sampling intervals. A separate ANOVA analysis was performed using the R base package to

250   evaluate differences in initial soil porewater chemistry among the four soils. A significance

251   threshold of p=0.05 was used for all statistical analyses.

252   **3 Results**

253   3.1 Porewater geochemistry of field samples

254   Comparison of the soil porewater geochemistry over five sampling time points indicated that

255   the average pH was approximately 1 pH unit higher in the toeslope soils than the plateau soils.

256   This was observed in both the organic soil (6.73 and 5.59, respectively; Fig. S1) and in the

257   mineral soils (6.62 and 5.86, respectively). The mean concentration of dissolved cations was also

258   higher and contained a higher proportion of alkali earth metals in the toeslope, consistent with

259   the input of weathering products from the hillslope. The mean molar ratio of alkali to alkali Earth

260   cations was 1.34 and 2.20 in the organic and mineral soils of the plateau, respectively, compared

261   to 0.11 and 0.08 in the organic and mineral soils of the toeslope (Fig. S1).

262

263   3.2 Rates and temperature sensitivity of greenhouse gas production

264   CO_2 production at both sites was higher in the organic soils than the mineral soils (Fig.

265   1a,b). It was higher in the organic soils of the toeslope than the organic soils of the plateau,

266   especially during the first 15 days of incubation (Fig. 1a; $p<0.001$). CO_2 production was not

267   significantly different between the two mineral soils. Similar to the patterns for CO_2 production,

268   CH_4 production was significantly higher in the toeslope soils than in the plateau soils in the

269   organic ($p=0.047$) but not the mineral soils (Fig. 1c,d), which produced low levels of CH_4.

270   N addition had contrasting effects on CO_2 and CH_4 production, as effects on CO_2 varied

271   by soil but CH_4 production was generally inhibited (Fig. 2). N addition had no effect on CO_2

272   production in either the organic or mineral soils from the toeslope. However, it significantly

273   increased CO_2 production in the organic plateau soils (p=0.010). N addition also increased CO_2

274   production in the plateau mineral soils, but the difference was not significant. In contrast, N

275   addition generally reduced CH_4 production in all soils except for the plateau mineral soil, which

276   exhibited a small but not significant increase. However, the effect on decreasing CH_4 production

277   was significant only for the toeslope soils (p=0.004 and 0.024 for the organic and mineral soils,

278   respectively).
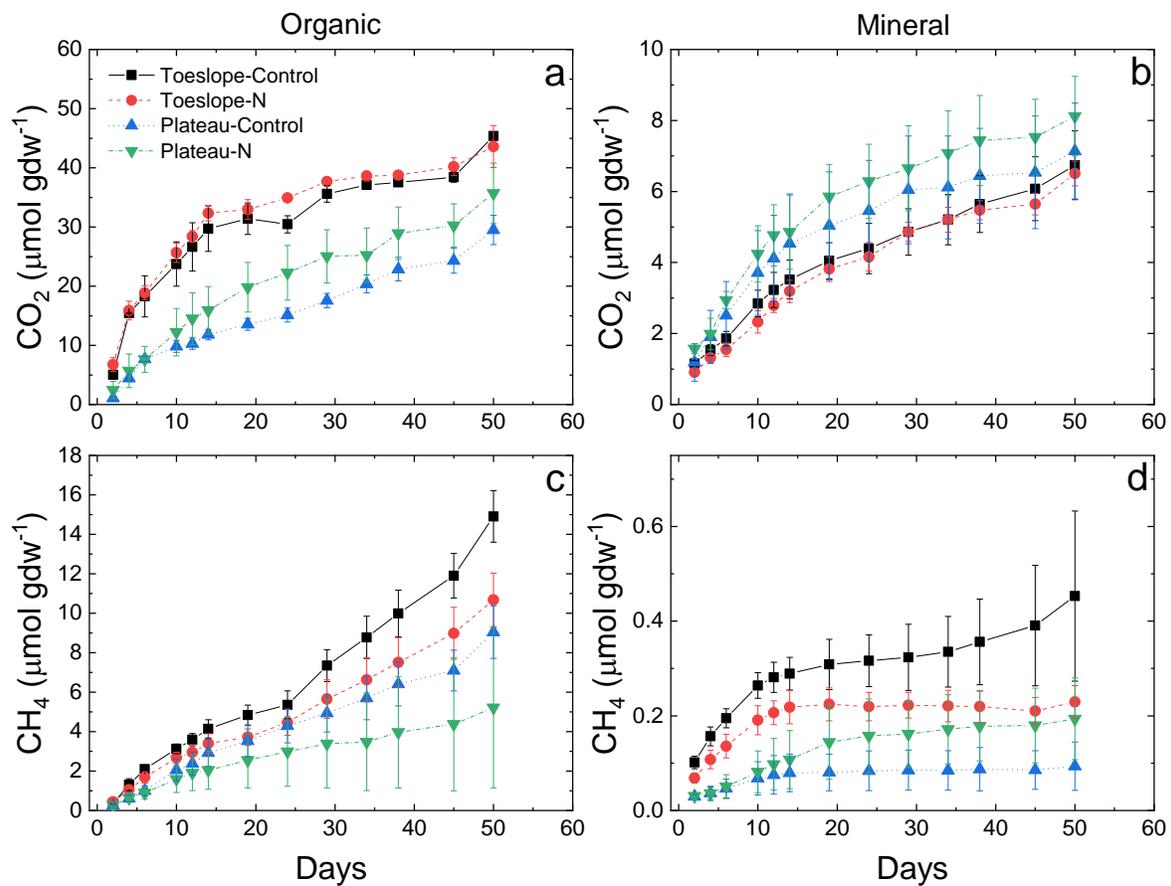
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280 **Figure 1**. Cumulative CO_2 (a,b) and CH_4 (c,d) production in the incubations of two contrasting
281 water-saturated tundra soils within a watershed near Nome, Alaska. Incubations were performed
282 with both the organic and mineral layer soils either with or without addition of inorganic N (as
283 NH_4Cl). Symbols indicate the mean of three replicates and error bars indicate one standard
284 deviation. Abbrviation "gdw" refers to g dry weight soil.

285
286 3.3 N dynamics

287 The extractable inorganic N content (the sum of independently measured NO_3^- and NH_4^+)

288 of the organic soil was higher in the toeslope than the plateau soils (0.96 and 0.63 µmol N gdw^{-1},

289 respectively; p=0.020; Fig. 2). The opposite pattern was observed in the mineral soils (p=0.001).

290 However, both plateau soils exhibited net N immobilization (i.e. the concentration of extractable

291 inorganic N significantly declined during the incubation; p < 0.001). The toeslope soils both

292 exhibited net N mineralization (increased extractable inorganic N) but the change was not

293 significant. Inorganic N concentrations in the +N treatment soils declined during incubation for

294 all four soils, indicating microbial uptake or gaseous loss. The decline in concentration was

295 similar for the two organic soils (4.4 and 4.2 µmol gdw^{-1} for the toeslope and plateau,

296 respectively), and was greater than that in the mineral soils (3.3 and 2.3 µmol gdw^{-1},

297 respectively).

298
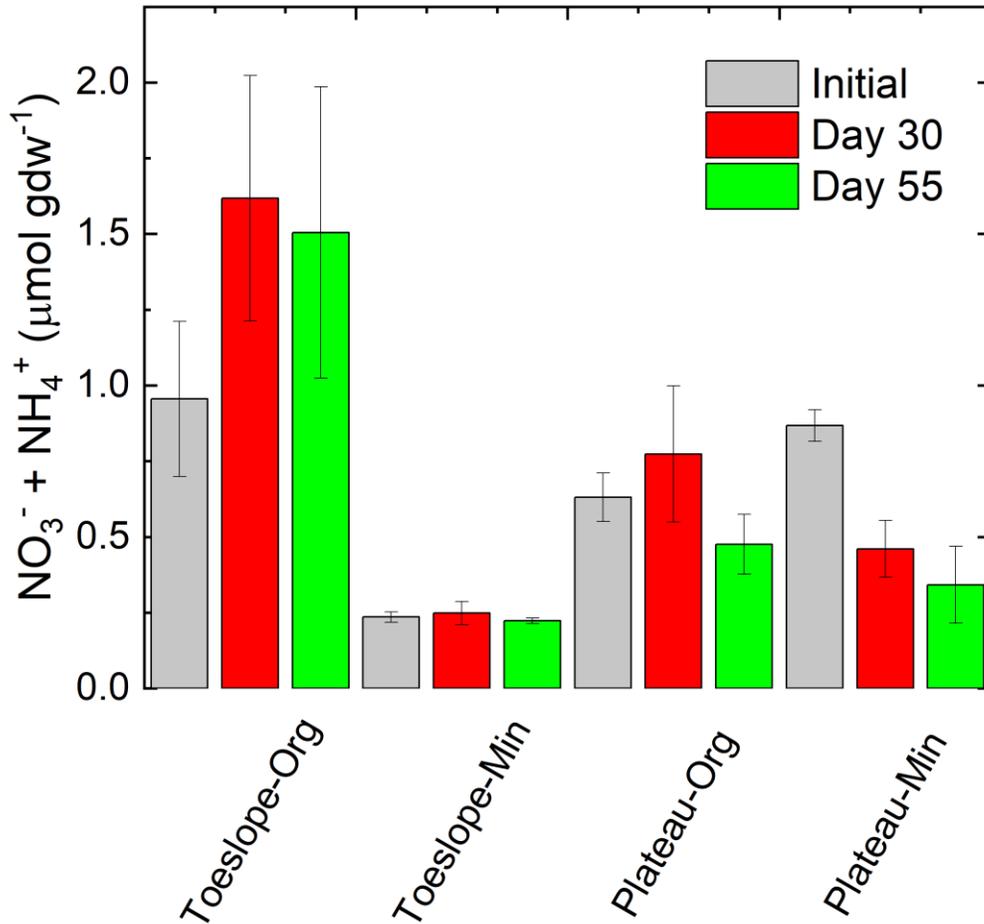
Figure 2. Total extractable inorganic N ($NH_4^+ + NO_3^-$) in the initial soils and after 30 and 55
days of incubation. The height of the bars indicate the mean of three replicates and the error bars
indicate one standard deviation.

302

303

304

3.4 WEOC quantity and composition

Initial concentrations of WEOC were higher in the toeslope than the plateau in both the

organic and mineral soils (Fig. 3a,b; $p < 0.001$). WEOC concentrations significantly declined

during incubation in all soils ($p < 0.001$), indicating decomposition and immobilization of

organic C was greater than the solubilization of solid-phase SOC. N addition significantly

increased the loss of WEOC after 55 days in all soils except for the toeslope organic soil.

311   SUVA$_{254}$ in the WEOC was slightly higher in the toeslope than the plateau organic soils at the

312   beginning of the experiment (0.82 and 0.57, respectively; p = 0.014), indicating higher

313   aromaticity. SUVA$_{254}$ increased significantly during incubation in all soils except for the organic

314   plateau (Fig. 3c). In the mineral soils, the SUVA$_{254}$ of the WEOC was higher than in the organic

315   soils (2.35 and 1.04 in the toeslope and plateau, respectively; Fig. 3d; p = 0.014 and 0.019,

316   respectively). N addition resulted in a smaller increase of SUVA$_{254}$ in the mineral soil control

317   incubations (p < 0.001), but not in the organic soils of both sites.

318   This pattern was also observed in the concentrations of low-molecular weight organic

319   acids, primarily formate, acetate, and propionate. Concentrations were higher in the organic soils

320   of the toeslope than the plateau (Fig. 3e). Concentrations in the toeslope organic soil increased

321   throughout the incubation, indicating the production of organic acids via fermentation exceeded

322   their consumption. In the plateau organic soil, concentrations increased from 0 to 30 days, but

323   then declined to near the initial concentration after 55 days. Both mineral soils exhibited a net

324   decline in organic acid concentrations by the end of the experiment Fig. 3f). N addition appeared

325   to have little effect on the organic acid concentrations, and the final concentration was

326   significantly different only in the plateau organic soils (1.65 ± 0.20 and 0.55 ± 0.16 μmol gdw^{-1}

327   for control and +N treatments, respectively).

328

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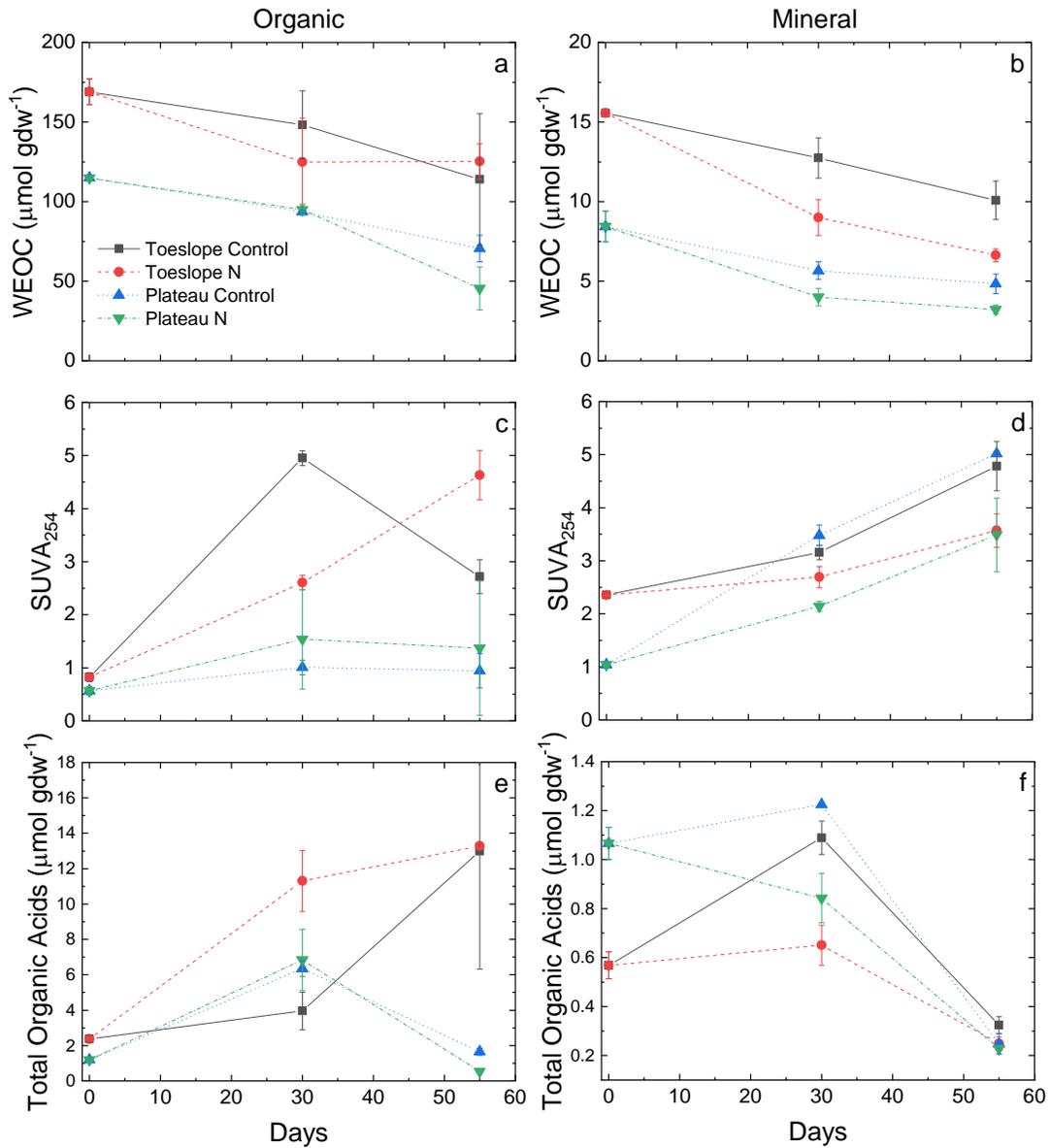
Figure 3. Water-extractable organic C (WEOC; a,b), specific ultraviolet absorbance at 254 nm (SUVA$_{254}$; c,d), and total extractable organic acids (sum of formate, acetate, propionate, and butyrate; e,f) in the initial soils and after 30 and 55 days of incubation. Symbols indicate the mean of three replicates and error bars indicate one standard deviation.

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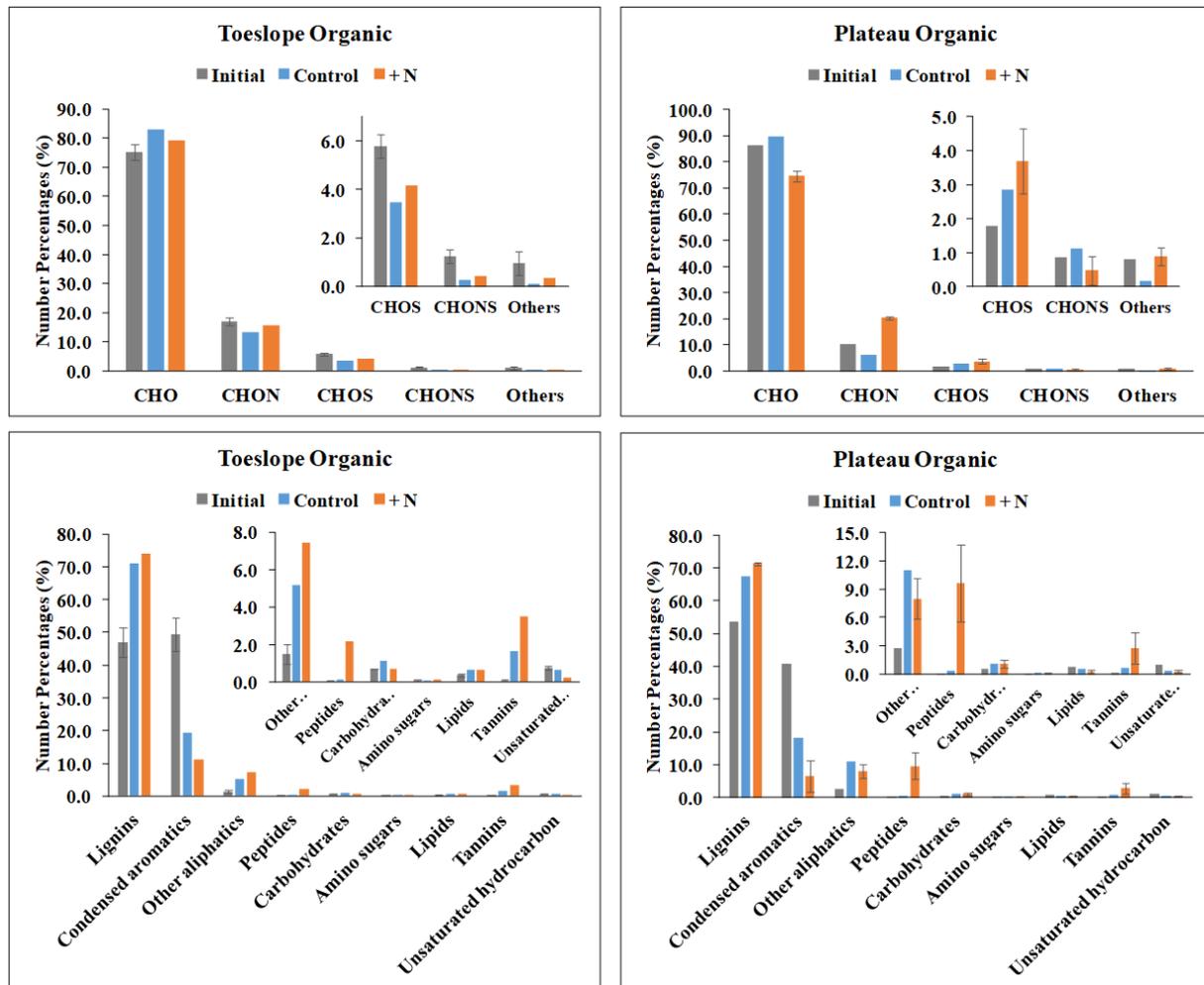
Figure 4. Relative abundance of molecular formulas from FTICR-MS analysis of the WEOC of the organic soils. Error bars indicate the deviation in results between sets of experimental replicates performed for selected samples.

FTICR-MS analysis of the WEOC indicated that formulas corresponding to lignin and condensed aromatic compounds were predominant in all porewater DOC samples (Fig. 4; Table S1). The number of lignin formulas increased in relative abundance during incubation, while condensed aromatics declined. The abundance of N-containing (CHON) and peptide formulas and their response to N addition varied by site. Between the organic soils, CHON formulas were more abundant in the toeslope than the plateau soils (16.9% and 10.4% of all formulas,

346 respectively; Fig. 4). Following N addition, the abundance of CHON formulas increased to

347 20.4% in the plateau soil, indicating incorporation of the added N into organic compounds, but

348 did not change in the toeslope soil. Peptide formulas also increased in abundance from <1% to

349 9.7% in the plateau N-addition incubation, but only increased to 2% in the toeslope soil

350 incubations.

351

352 3.5 Ion concentrations and pathways of anaerobic C decomposition

353 Changes of porewater ion concentrations during the incubations indicated a relatively

354 minor contribution of alternative electron acceptors to the total C mineralization. SO_4^{2-}

355 concentrations in the toeslope soils declined by 0.02-0.12 µmol gdw^{-1} (Fig. S2), indicating that

356 SO_4^{2-} reduction accounted for <1% of the total C mineralization. In the plateau soils, SO_4^{2-}

357 concentrations increased slightly during incubation, and SO_4^{2-} reduction was not detectable.

358 Denitrification was also minimal, as NO_3^- declined by <0.07 µmol gdw^{-1}, consistent with low

359 initial NO_3^- concentrations (data not shown). Reduction of Fe(III) was detectable in all soils

360 except for the plateau mineral soil, as measured by increasing Fe(II) concentrations in the

361 porewater (Fig. S2). The cumulative increase in extractable Fe(II) ranged from 0.4 to 1.4 µmol

362 gdw^{-1} in the control treatments and 0 to 2.9 µmol gdw^{-1} in the +N treatments, which were not

363 significantly different for any soil. However, Fe(III) reduction accounted for a small fraction of

364 C mineralization in all soils (<2%).

365

366 3.6 Microbial community composition

367 Microbial communities of toeslope and plateau soils were significantly different from

368 each other (p = 0.005) (Fig. S3). We also observed a strong effect (p = 0.001) where organic and

369   mineral soils in each location clustered separately indicating significant differences in each soil

370   compartment. *Proteobacteria*, *Bacteroides*, *Acidobacteria*, and *Chloroflexi* were abundant in all

371   soils (Fig. 5). Caldiserica were more abundant in the mineral soils than the organic soils.

372   Concentrations of WEOC ($p = 0.001$), propionate ($p = 0.001$), acetate ($p = 0.001$) and Fe(II) ($p =$

373   0.001) were significantly correlated with microbial community structure, especially in plateau

374   soils (Fig. S3). Soil pH overall showed a smaller correlation ($p = 0.006$) to the microbial

375   community structure. The effect of nitrogen addition on the microbial community was nuanced.

376   All soils clustered mainly based on their horizons ($p = 0.001$) where impact of nitrogen addition

377   was nested within each horizon ($p = 0.001$) and by itself was not a significant effect ($p = 0.881$).

378   Nitrogen addition effect was mainly observed as changes in microbial composition in

379   toeslope soils where *Firmicutes* significantly increased in both organic ($p = 0.035$) and mineral

380   ($p = 0.001$) soils with nitrogen addition (Fig. 5). Upon further analysis, we determined that

381   relative abundance of genus *Saccharofermentans* increased both in organic ($p = 0.025$) and

382   mineral ($p = 0.004$) soils in response to nitrogen addition (Fig. S5 and S6). Additionally, in

383   nitrogen-added mineral soils we detected significant increase in genus *Desulfosporosinus* ($p =$

384   0.045). In organic horizons of toeslope soils *Bacteroides* ($p=0.008$) and *Proteobacteria* ($p =$

385   0.039) also increased in their relative abundance but remained unchanged in mineral soils (Fig.

386   5). Plateau soils responded differently to nitrogen addition than toeslope soils where the largest

387   and most significant change observed was the decrease in *Bacteroidetes* both in organic ($p =$

388   0.010) and mineral ($p<0.001$) soils (Fig. 5). In both locations Archaeal populations was

389   dominated by methanogenic archaea of *Methanoflorentaceae* (former Rice Cluster II) (Adam et

390   al., 2017) (Fig. S8) which remained unchanged after nitrogen addition.

391
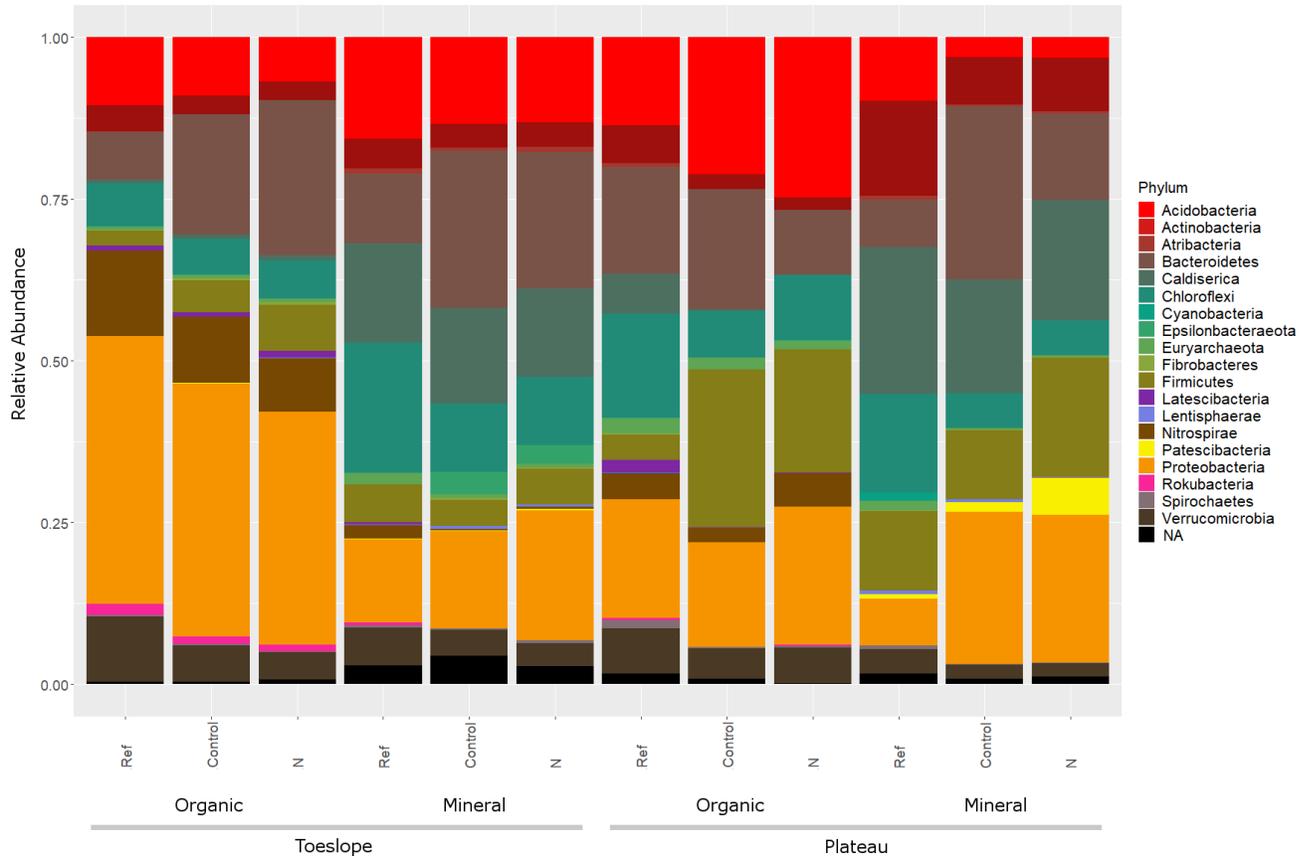
Figure 5. Relative abundance of microbial phyla extracted from the soils before and after incubation. NA: not assigned to a known phylum.

393

394

4 Discussion

4.1 Effects of topography on rates of SOM decomposition

Higher exchangeable aluminum and organic acid concentrations reduce the pH in the peat plateau soil compared to toeslope soils. Preliminary speciation calculations indicate that most of the aluminum, iron and alkaline earth metal ions in these pore waters are complexed by DOC. Therefore, the cation exchange capacities of DOC and SOM provide an important pH buffer to the soils. Similar precipitation-fed wetlands with no mineral inputs are often acidic due to the accumulation of organic acids resulting from fermentation (Gorham & Janssens, 1992). Synoptic sampling and analysis of the soil pore waters over three years have indicated that the soils from

404   the plateau have sodium bicarbonate-type pore waters, while the toeslope soil pore waters have a

405   calcium-magnesium bicarbonate chemistry with a lower ionic strength. These differences in pH

406   and geochemical environment are consistent with advanced mineral weathering and high cation

407   exchange capacity of peat in the plateau. At the base of the hillslope, however, runoff containing

408   leached alkaline earth metals floods tussock graminoid tundra. Therefore, the difference in pH

409   between the sites is associated with hillslope position, vegetation, and geomorphology.

410   Overall, our results show that both CO_2 and CH_4 production were higher in the organic

411   toeslope soils from the base of the hillslope than in the organic soils from the peat plateau. This

412   is likely in part due to the higher pH in the toeslope soils, which is a key control on anaerobic

413   SOM decomposition. Empirical estimates of the pH optimum for methanogenesis range from 6.2

414   to 7.5 (Cao et al., 1995; Meng et al., 2012; Tang et al., 2016). Observations of CO_2 and CH_4

415   emission also vary along natural pH gradients in peatlands (Moore & Knowles, 1990; Zalman et

416   al., 2018), and laboratory incubations show that buffering soils at a higher pH increases

417   production of both gasses, especially CH_4 (Dunfield et al., 1993; Valentine et al., 1994; Ye et al.,

418   2012). This is consistent with our observations of higher rates of CO_2 and CH_4 production in the

419   circumneutral toeslope soils. However, we observed that the hillslope position affected CO_2

420   much more than CH_4 production, resulting in lower CO_2:CH_4 ratios in the plateau soils than at

421   the base of the hillslope. This result contrasts with observations that methanogenesis is more

422   sensitive to pH than other pathways of anaerobic C mineralization, and suggests that other

423   hillslope differences such as differences in DOC concentration and composition also contribute

424   to the differences in greenhouse gas production.

425   Porewater DOC represents the fraction of C directly available to soil microorganisms,

426   and as such is the most important in shaping SOC decomposition dynamics (Chen et al., 2018;

427   Yang et al., 2016). For example, radiocarbon measurements of CH_4 in peatlands reflect the

428   young age of the DOC pool rather than the old age of the much larger SOC pool (Chanton et al.,

429   1995, 2008). Accordingly, some biogeochemical models estimate methanogenesis in part as a

430   function of DOC concentration (Cao et al., 1995, 1998; Tian et al., 2010; Xu & Tian, 2012).

431   WEOC represented a small fraction of the total SOC in both soils (0.33 and 0.24% in the organic

432   soils of the toeslope and plateau, respectively), but the concentrations were higher in the toeslope

433   soils. The higher concentrations of WEOC indicate a larger pool of potentially fermentable

434   substrates and likely contributes to the higher CO_2 and CH_4 production from these soils.

435   The patterns of WEOC concentrations during the incubations also suggest limitation of

436   labile C in the plateau soils. There was a net loss of WEOC during all incubations, indicating that

437   the degradation of DOC by microorganisms (or other uptake/immobilization) exceeded the

438   production of new DOC through the solubilization of solid-phase SOC. Consistent with previous

439   studies (Chen et al., 2018; Yang et al., 2016), the degradation of DOC was selective, as $SUVA_{254}$

440   values increased during the incubation. High $SUVA_{254}$ values are correlated with the abundance

441   of aromatic DOC and are associated with lower CH_4 production (D'Andrilli et al., 2010;

442   Hodgkins et al., 2016). The inhibitory effect of highly aromatic DOC on C mineralization could

443   be higher in these anaerobic incubations due to the low activity of phenol oxidase in the absence

444   of oxygen (Freeman et al., 2004; Pind et al., 1994). Therefore, our results indicate selective

445   decomposition of the bioavailable and easily fermentable DOC pool, and accumulation of the

446   aromatic fraction. This effect was more pronounced in the plateau soils where initial DOC

447   concentrations were lower. The depletion of easily fermentable C likely contributes to the lower

448   CO_2 and CH_4 production in the organic soils.

449   Limitation of bioavailable DOC was also apparent in the concentrations of low molecular

450   weight organic acids, the immediate substrates for methanogenesis as well as Fe(III) and SO_4^{2-}

451   reduction in these soils (Bethke et al., 2011). Models (Tang et al., 2016; Zheng et al., 2019a),

452   observations (Christensen et al., 2003; Valentine et al., 1994), and experiments (Herndon et al.,

453   2015; Hershey et al., 2014) indicate that the availability of these substrates limits rates of

454   methanogenesis and anaerobic C mineralization. The increasing organic acid concentrations in

455   the organic toeslope soils indicated that their production via fermentation exceeded their

456   utilization, and they were therefore not limiting for anaerobic respiration. This was supported by

457   the stable microbial community structure in soils with prevalence of carbohydrate-fermenting

458   populations such as *Firmicutes* and *Bacteroidetes*. In contrast, the pattern of organic acid

459   concentrations and microbial community composition in the mineral horizons suggests substrate

460   limitation of respiration. In the toeslope organic soil, increases in the carbohydrate-fermenting

461   genus *Saccharofermentans* and *Desulfosporosinus*, which includes bacteria capable of anaerobic

462   respiration using sulfate, Fe (III) or fumarate as electron acceptors, suggests close coupling of

463   organic acid fermentation and anaerobic respiration (Chen, 2017; Nixon et al., 2017). In plateau

464   mineral soil horizons, observed increases in Group I clostridia (*Clostridium sensu stricto*) (Gupta

465   & Gao, 2009) signals the emergence of fermentative pathways leading to alcohol, butyrate,

466   acetate and hydrogen production. In both locations and soil horizons relative abundance of the

467   dominant methanogenic lineage *Methanoflorentaceae* (former Rice Cluster II) showed no

468   significant change with incubation, yet we observed different CH_4 production rates. The

469   hydrogenotrophic methanogen candidatus *Methanoflorens stordalenmirensis* was identified as

470   the predominant methanogen in a northern Sweden permafrost thaw site (Mondav et al., 2014).

471   The strong correlation of WEOC and organic acid concentrations with the overall microbial

472   community composition (Fig. S3) provides other lines of evidence that substrate availability was

473   a key control on microbial growth. Therefore, we conclude that continued bioavailability of

474   WEOC (Fig. 3) in the toeslope supported a higher rate of fermentation, in turn fueling higher

475   rates of CO_2 and CH_4 production.

476

477   4.2 Greater microbial N limitation in the plateau soils

478   Several lines of evidence indicate that the plateau soils were more N-limited than the

479   toeslope soils, especially in the organic soil. First, the concentration of extractable inorganic N

480   (NO_3^-+NH_4^+) was higher in the organic soils of the toeslope than the plateau. Second, extractable

481   inorganic N increased during the incubation of the toeslope organic control soils, indicating net

482   N mineralization. This is consistent with previous observations that net N mineralization is

483   generally higher in soils in lower topographic positions (Giblin et al., 1991; Nadelhoffer et al.,

484   1991). In contrast to the toeslope organic soil, both plateau soils exhibited declining inorganic N

485   concentrations during incubation, indicating either net N immobilization or gaseous N losses.

486   This observation is consistent with greater N availability in the toeslope soils. The increase in

487   CHON and peptide formulas following N addition in the plateau but not the toeslope organic

488   soils by FTICR-MS analysis also demonstrates that the added N was assimilated to a greater

489   degree in the plateau soils, consistent with greater microbial N limitation. Finally, N addition

490   increased CO_2 production in the organic plateau soils but not the toeslope soils, further

491   demonstrating that SOM decomposition was N-limited in the former but not the latter.

492   Although we do not have direct evidence of the sources of N to the toeslope soils, these

493   patterns are consistent with leaching and downslope transport of inorganic and labile organic N

494   down the hillslope. The differences in pH, ion composition, and alkali-to-alkaline earth metal

495   ratio of the porewater demonstrate distinct sources of water inputs, indicating the water in the

496   toeslope soils is derived from hillslope runoff. Previous studies have demonstrated that nutrients

497   (including N) are transported downslope and accumulate in low-lying areas (Stewart et al.,

498   2014). Our results extend these findings to tundra landscapes and demonstrate that they cause

499   associated differences in anaerobic SOM decomposition. The hillslope gradient in decomposition

500   is similar to the hydrological fen-bog gradient in peatlands, in which wetlands with higher pH

501   and nutrient availability are associated with higher rates of anaerobic SOM decomposition,

502   especially CH_4 production (Keller et al., 2006; Thormann et al., 1999; Ye et al., 2012).

503   Our experiments utilized cores collected in early June, before the Arctic growing season.

504   N limitation in tundra soils is observed to be highest at the peak of the growing season, when

505   plants and microorganisms are competing for available N (Melle et al., 2015). These microcosm

506   incubation experiments are therefore conservative estimates of the potential N limitation of

507   SOM decomposition; the lack of effect of N addition on CO_2 production in the toeslope soils

508   does not preclude N limitation during the summer, as has been observed previously (Philben et

509   al., 2019). However, our results are consistent with relatively greater N limitation in the plateau

510   soils compared to the toeslope.

511   In contrast to the organic soils, there was no evidence that N availability in the mineral

512   soils differed between the plateau and the toeslope. There was no difference in initial extractable

513   N content. No net N mineralization was observed in the toeslope soils, and N addition did not

514   significantly increase CO_2 production. This indicates that the apparent microbial N limitation

515   was limited to the organic plateau soil. Mineral soils could therefore experience a smaller

516   increase in CO_2 production in response to increased N availability.

517   While N addition stimulated CO_2 production in the organic plateau soil, it consistently

518   decreased CH_4 production in all but one soil (the plateau mineral soil). This likely results from

519   suppression of methanogenesis due to reduced pH. The pH optimum for methanogenesis is

520   between 6 and 7, and the slope of the response function appears to be steeper than that of CO_2

521   production due to the specificity of the microbes and enzymes involved (Ye et al., 2012). The pH

522   of the KCl extracts following incubation was consistently lower in the microcosms with added

523   NH_4Cl compared to the control microcosms. This was in part due to the weakly acidic nature of

524   the NH_4Cl solution (pH ~5.7), but also likely due to the effects of increased proton production

525   via fermentation, especially in the plateau soils. The lower pH in the N-fertilized treatments

526   could result in reduced CH_4 production despite other indicators of microbial N limitation,

527   emphasizing the complex feedbacks regulating CH_4 production.

528   **5 Conclusions**

529   We observed major differences in the rates of greenhouse gas production in two wetland

530   areas within the same watershed. Despite surface similarities between the wetland areas, the

531   toeslope organic soil produced significantly more CO_2 and CH_4 than the plateau soil due to

532   higher availability of labile DOC and higher pH than the plateau soils during anaerobic

533   incubation. Our results also indicate that inorganic N concentrations were lower and soil C

534   decomposition was more N-limited in the plateau soils than the toeslope soils, which exhibited

535   net N mineralization while the plateau soils had net N immobilization. Examination of the

536   changes in porewater geochemistry, soil microbial community and response to N addition

537   indicate that the differences are due to a combination of (1) higher pH due to leaching of mineral

538   weathering products from the hillslope; (2) higher N availability for microorganisms; and (3)

539   higher availability of fermentable DOC, which provided an ample supply of labile organic

540   substrates throughout the incubations in the toeslope soils. These results demonstrate that

541   topographic position, geochemical environment, and hydrologic flow are important

542   considerations to predict the fate of thawing permafrost.

Acknowledgments and data availability

544   The authors declare no financial conflicts of interest. All data supporting the conclusions of

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803
804

Figure 1.

Organic | Mineral

a
- Toeslope-Control
- Toeslope-N
- Plateau-Control
- Plateau-N

CO_2 ($\mu mol\ gdw^{-1}$)

b

CO_2 ($\mu mol\ gdw^{-1}$)

c

CH_4 ($\mu mol\ gdw^{-1}$)

Days

d

CH_4 ($\mu mol\ gdw^{-1}$)

Days

Figure 2.

Figure 3.

Organic

Mineral

Figure 4.

Figure 5.