Comparing the effect of organic matter quality on aerobic and anaerobic greenhouse gas production in a temperate peatland

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Abstract Peatlands have an outsized contribution to both carbon storage and methane (CH_4) emissions relative to their land surface area. The factors controlling these fluxes are uncertain, but critical to predicting the future trajectory of greenhouse gas emissions from peatlands. While the biochemical composition of organic matter (termed organic matter "quality") has been shown to be correlated with its future vulnerability to decomposition under oxic conditions, few experiments have assessed its impact on anaerobic respiration and CH_4 production. In this study, we assessed the relationship between environmental parameters, organic matter quality, and potential greenhouse gas production under both oxic and anoxic conditions. We used samples from multiple depths and microtopographies within a temperate bog to capture peats with a range of decomposition states and botanical origins. A multiple regression model successfully predicted approximately 65% of the variance in carbon dioxide (CO_2) production in oxic incubations. However, we found that the model was less successful in predicting anaerobic CO_2 and CH_4 production in anoxic incubations, explaining 31% and 42% of the variance in greenhouse gas production, respectively. These results suggest that biochemical composition has an important influence on potential CO_2 production under oxic conditions, but exerts less influence on rates of anaerobic respiration and CH_4 production. As climate change and drainage increase the oxygen exposure time of peats, organic matter quality will likely play an increasing role in determining the trajectory of CO_2 emissions from peatlands.

1. Introduction

Peatlands are among the largest terrestrial carbon (C) reservoirs, accounting for about one third of the world's soil organic C pool despite only occupying 2-3% of its land surface area [1,2]. The accumulation of C in these environments is due to plant productivity outpacing the very low rates of organic matter decomposition characteristic of peatlands [3]. Several environmental factors, including persistent anoxia, low pH, and cool temperatures, have been demonstrated to contribute to the stabilization of peat organic matter and its low rate of decomposition. In addition to these environmental factors, *Sphagnum* mosses, the dominant species in many northern peatlands, typically exhibit low litter quality and have been found to decompose slower than vascular plants even when incubated under the same environmental conditions [4,5].

While intact peatlands have functioned as net C sinks, their waterlogged and anoxic conditions also facilitate C loss through methane (CH_4) production via fermentation and methanogenesis [6]. Globally, wetlands represent the largest natural source of CH_4, producing 40% of the total CH_4 emitted to the atmosphere, with northern peatlands alone producing 10-13% of all CH_4 emissions [6–8]. While water table position and temperature have been demonstrated to be strong drivers of CH_4 emissions, large differences in CH_4 emissions have been observed among peatlands with similar climate and moisture balance [9]. Understanding current patterns of CH_4 emission and predicting their response to future climate change remain major challenges [10,11].

Differences in organic matter (OM) composition (also referred to as OM "quality") could potentially explain these discrepancies by playing an important role in regulating the future trajectory of decomposition and CH_4 emissions from peatlands. Such differences can arise from a combination of the botanical origin of the OM and selective microbial decomposition of specific biochemical groups [12]. Several studies have found that OM quality is correlated with peat decomposition rates under oxic conditions. For example, Hogg [13] found that potential respiration rates declined with depth in a peat profile, reflecting lower OM quality in deeper peat with a more advanced state of decomposition. A positive

relationship between O-alkyl C content (measured using ^{13}C-NMR) and potential respiration has been demonstrated [14,15], as well as a negative relationship between respiration and phenolic C [16]. This is significant because it suggests that lower quality OM can limit C mineralization when other stabilizing factors (e.g. anoxia or freezing temperatures) are lost, such as during peat drainage or permafrost thaw [17].

The extent to which OM quality affects anaerobic respiration (including both anaerobic carbon dioxide (CO_2) production and CH_4 production) is less understood [18] despite being identified as critical for predicting future emissions trajectories [19]. A limited number of studies have investigated the relationship, and have generally found that parameters associated with OM composition were only weakly correlated with respiration [7,11,20]. On the other hand, the botanical composition of peat has been shown to affect potential CH_4 production, although the specific biochemical parameters were not identified [4,21]. In addition, there is evidence that anaerobic respiration is substrate-limited and is stimulated by the addition of dissolved organic matter (DOM) and labile compounds [6,22]. Parameters related to the biochemical composition of the peat, such as the abundance and bioavailability of potentially fermentable sugars, are therefore hypothesized to be associated with anaerobic respiration, in addition to the previously established correlation with decomposition under oxic conditions.

Biochemical analyses such as the quantification of individual neutral sugars and amino acids could be a powerful tool for assessing organic matter quality. They have the advantage of molecular specificity, allowing the calculation of multiple indices associated with different aspects of decomposition. For example, a “degradation index” based on the change in the relative abundance of individual amino acids has been utilized to track the extent of decomposition of organic matter in a range of ecosystems [23–25], and the amino acid hydroxyproline has been used a biomarker of plant-derived nitrogen in soils and peats [26,27].

Hydrolyzable neutral sugars have also been used as an indicator of organic matter decomposition. Neutral sugars are primarily derived from carbohydrate polymers, which are abundant in plant biomass but are among the most labile fractions of organic matter [28]. Thus, the yield of hydrolyzable neutral sugars

declines as a fraction of total C as decomposition proceeds [29,30]. Neutral sugar analyses can also reveal insights about the cycling of specific carbohydrate classes. For example, rhamnose is a major constituent of "sphagnan", a pectin-like carbohydrate shown to contribute to the relative recalcitrance of *Sphagnum* [31,32], and can be used to track its fate in peat [33]. However, the potential utility of these analyses for evaluating the vulnerability of soil organic matter to future decomposition has not been assessed.

To address this, we evaluated the relationship between peat biochemical composition, environmental conditions (e.g. pH, depth, nutrient availability, etc.), and greenhouse gas (GHG) production under both oxic and anoxic conditions. We coupled microcosm incubation experiments and biochemical analyses for 60 unique peat samples taken from six different cores in a temperate peatland in southwestern Michigan. Despite their geographic proximity, the samples represent a gradient in redox conditions due to the hummock/hollow microtopography typical to bogs [21,34]. In addition, different species of *Sphagnum* and vascular plants inhabit hollow and hummocks, resulting in peats with contrasting biochemical composition [5,33,35]. Peats from contrasting depths within cores also represent a continuum of organic quality, as peat near the surface is typically less decomposed than peat buried deeper in the permanently anoxic layer [13,14,36].

Our overall goal was to determine the extent to which organic matter quality (as measured by biochemical composition) improves our ability to predict potential GHG production under both aerobic and anaerobic conditions. Thus, we hypothesized that (1) potential GHG production differs by depth and microtopography, reflecting differences in degree of decomposition and botanical origin; and (2) the inclusion of indices of biochemical composition will improve statistical models predicting GHG production under both aerobic and anaerobic conditions.

2. Materials and Methods

2.1 Study Site and Sample Collection

Peat samples were collected from Miner Lake Bog in Allegan County, Michigan, USA (N 42.6074, W 86.0620). The site consists of a small bog area (approximately 50 m^2) with prominent leatherleaf

(*Chamaedaphne calyculata*) hummocks, surrounded by a poor-fen sedge meadow containing *Eriophorum* spp. and *Carex* spp. sedges. *Sphagnum* mosses are abundant within the bog, with *Sphagnum magellanicum* dominating in the hummock microtopography and *Sphagnum cuspidatum* dominating in the hollows. We collected a total of six peat cores (3 m in length) in May 2021 using a Russian-style peat borer (AMS, Idaho Falls, ID, USA). Two of the cores (Cores 1 and 2) were collected from hollow microtopographies within the bog, three (Cores 3, 5 and 6) were collected from hummocks in the bog, and one (Core 4) was collected from the sedge meadow. Cores were extracted in 50 cm segments, wrapped in plastic wrap immediately following collection, and stored at 4°C until further processing.

In the lab, 8 cm sections of peat were collected at intervals of 20 cm and homogenized by hand. Subsamples of peat were oven-dried at 70°C until constant mass to determine water content, then ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). C and N content was determined using an Elementar Isotope Cube elemental analyzer (Elementar, Langenselbold, Hesse, Germany). pH was measured using an additional subsample after mixing with $CaCl_2$ (2 mol L^{-1}) in a 1:10 (w/v) ratio using a Thermo Scientific Orion pH electrode (Thermo Scientific, Waltham, MA, USA).

2.2 Carbohydrate Analysis

Total non-cellulosic sugar content of these samples was reported in Bryan et al. (2024), and full methodological details can be found therein. Peat samples (20±1 mg dry weight) were hydrolyzed in 1 mL of 2 mol L^{-1} trifluoracetic acid (TFA) at 100°C for five hours [37,38]. Following hydrolysis, samples were centrifuged at 3000 RPM for five minutes. A 400 µL aliquot of the supernatant was transferred to a glass autosampler vial and evaporated under N_2 at 50°C using a Reacti-Therm heating block (Thermo Scientific, Waltham, MA, USA).

Sugar monomers were derivatized for gas chromatography/mass spectrometry (GC/MS) analysis following the method of Xia et al. (2018). Samples were first mixed with 100 µL of a 2:1 mixture of ethanethiol and TFA (13 mol L^{-1}) to prevent isomerization of the sugars and multiple peaks in the GC chromatograms [39]. Derivatization proceeded with the addition of 385 µL of pyridine, 75 µL of

hexamethyldisilazane, and 40 µL of chlorotrimethylsilane, heated to 70°C for 30 minutes. The organic phase was isolated using liquid-liquid extraction in chloroform and MilliQ water, dried over $MgSO_4$, and filtered through quartz wool. Samples were quantified using an Agilent 6890 GC with a Model 5973 Mass Selective Detector (Agilent Technologies, Santa Clara, CA, USA). The concentration of sugars in the peat samples was calculated using calibration curves derived from analysis of a standard mixture containing six sugar monomers (xylose, arabinose, rhamnose, galactose, glucose, and mannose).

The hydrolysis procedure was optimized for the recovery of sphagnan and hemicelluloses [33]. Optimizing the recovery of total sugars involves pretreatment of samples with concentrated H_2SO_4 for the digestion of cellulose, which can result in the degradation of other sugars [40,41]. The sugar yields from the TFA hydrolysis used in this study should therefore be interpreted as the total hemicellulose fraction, plus an unknown fraction of the cellulose.

2.3 Ion Analysis

An additional subsample of the peat was extracted for analysis of major cations and anions using ion chromatography (IC). 3±0.001 g of peat was weighed into 50 mL plastic centrifuge tubes with 30 mL of 10 mmol L^{-1} KCl. Samples were capped and shaken in a rotary shaker for one hour, then filtered using 0.45 µm syringe filters. Cation and anion analyses were performed simultaneously, using a Dionex Aquion system for cations and a Dionex Integrion system for anions. Both systems used suppressed conductivity detection. Cation analysis quantified Na^+, NH_4^+, Mg^{2+} and Ca^{2+} concentrations using a CS-12A column, while anion analyses quantified the organic acids acetate, propionate, formate, and butyrate as well as the inorganic ions NO_3^- and SO_4^- using an AS-12-HC column. The concentration of ions in each sample was calculated using calibration curves made from a mixed ion standard for each analyte. The total inorganic nitrogen (N) content was calculated as the sum of the measured NH_4^+ and NO_3^- concentrations for each sample. The total organic acid content was similarly determined as the sum of the observed acetate, propionate, formate, and butyrate concentrations for each sample.

148 *2.4 Total hydrolyzable amino acid analysis*

149 Total hydrolyzable amino acids (THAA) were analyzed using high performance liquid
150 chromatography (HPLC) following derivatization with ortho-phthaldehyde (OPA) and 9-
151 fluorenylmethylchloroformate (FMOC-Cl) reagents [42]. 25±1 mg of dried sample material were weighed
152 into 2 mL glass ampules with 1.5 mL of 6 M HCl. Ampules were flame-sealed and hand-shaken to distribute
153 the sample. Samples were then heated to 110°C in an oven for 20 hours for hydrolysis. Ampules were
154 cracked open, the contents transferred into 2 mL microcentrifuge tubes, and centrifuged at 3000 rpm for 5
155 minutes. 200 µL of supernatant were then transferred to 2 mL glass vials with 40 µL of internal standard (5
156 mmol L^{-1} norvaline). The vials were vortexed to mix prior to being evaporated under a gentle stream of N_2
157 gas. Samples were either frozen for later analysis (-20°C) or redissolved with 200 µL of 0.1 mol L^{-1} HCl
158 and vortexed to mix. Samples were analyzed with HPLC within 10 hours of having been thawed and diluted.

159 HPLC analysis was performed on an Agilent 1100 with a diode array detector (DAD) and an
160 Agilent AdvanceBio Amino Acid Analysis (AAA) column (Agilent). Derivatizations were performed
161 within the needle of the autosampler in a borate buffer (10.2 pH) prior to injecting sample into the column
162 during HPLC analysis to detect both primary and secondary amines. Primary amino acids were derivatized
163 with an OPA reagent and secondary amino acids were derivatized with a FMOC-Cl reagent. The mobile
164 phase for HPLC analysis consisted of an aqueous buffer (10 mM Na_2HPO_4 and 10 mM $Na_2B_4O_7$ at pH 8.2)
165 and acetonitrile:methanol:water (45:45:10, v:v:v). The flow rate was 1 mL min^{-1} and the eluent gradient
166 followed Henderson and Brooks (2010). OPA derivatives were detected at a wavelength of 338 nm with a
167 390 nm reference wavelength, while FMOC derivatives were detected at 262 nm with a 324 nm reference
168 wavelength. Samples were measured for the amino acids aspartic acid, glutamic acid, serine, histidine,
169 glycine, threonine, arginine, alanine, tyrosine, valine, phenylalanine, isoleucine, leucine, lysine, and
170 hydroxyproline, with norvaline as the internal standard. Methionine and tryptophan were destroyed in
171 hydrolysis. The different amino acid concentrations of each sample were calculated using calibration curves
172 made from a mixed amino acid standard of the aforementioned amino acids with a 1 mmol L^{-1} norvaline
173 internal standard. The full amino acid composition is provided in Online Resource 1.

174 *2.5 Greenhouse Gas Production*

175 Two microcosms were constructed for each peat sample, with one for aerobic and one for anaerobic
176 incubation. For each microcosm, 8 g of field-moist homogenized peat were added to a 70 mL serum bottle
177 and wrapped in aluminum foil to exclude light. Microcosms for aerobic incubation were covered with
178 parafilm with holes to allow for gas exchange, pre-incubated for 7 to 10 days, then sealed with rubber septa.
179 The CO_2 concentration in the headspace was measured immediately after sealing and after 24 hours using
180 an Agilent 6890 gas chromatograph with a flame ionization detector (GC/FID) equipped with a methanizer
181 (Restek Corporation, Bellefonte, PA, USA). Aerobic CO_2 production was calculated as the change in CO_2
182 concentration over 24 hours.

183 Microcosms for anaerobic incubation were sealed with a rubber stopper immediately after adding
184 peat and the headspace flushed with N_2. These microcosms were incubated for 20-24 days, after which
185 cumulative CO_2 and CH_4 production was measured by analyzing the headspace with GC/FID. All
186 incubations were conducted at room temperature. Reported rates of CO_2 production in both the aerobic and
187 anaerobic incubations include production of dissolved inorganic C species calculated using Henry's Law
188 [43] and the measured pH of each sample.

189 *2.6 Data Analysis*

190 A degradation index based on the relative abundance of the individual observed amino acids was
191 constructed using principal components analysis (PCA) [23]. Hydroxyproline was excluded from this PCA
192 for consistency with previously published THAA degradation indices [23,25]. Amino acid degradation
193 index scores were calculated for each peat sample to represent the relative abundance of amino acids as a
194 whole in each peat sample. The parameter mole % of glycine was also calculated, representing the ratio of
195 glycine present in comparison to the other amino acids.

196 A second PCA consisting of all the measured parameters was also constructed in order to visualize
197 the relationship between the environmental conditions, organic matter quality, and the production of CO_2
198 and CH_4 in anaerobic and aerobic conditions. Chemical parameters used in this analysis were pH, C:N,

aerobic CO_2 production, anaerobic CO_2 production, CH_4 production, total organic acid content, total inorganic N content, % of total sugars as glucose, % of total sugars as rhamnose, % of total C as sugars, % of total C as amino acids, % of total N as amino acids, the amino acid degradation index, molar % of total amino acids as hydroxyproline, the molar % of glycine, and the C-normalized yield of hydroxyproline. The dataset used to generate the PCA is provided as Online Resource 2.

Multiple regression models were developed to determine the biochemical and environmental parameters that were significantly correlated with GHG production. Three separate models were constructed, using aerobic CO_2 production, anaerobic CO_2 production, and CH_4 production as the dependent variable, respectively. Each GHG production variable was log-transformed prior to applying the regression model. The resulting regression equations were used to calculate the "predicted" production of each GHG, which was compared to the measured production to evaluate the accuracy of the models. Additionally, two-way ANOVA tests were conducted to determine the impact of depth and microtopography on the production of each GHG measured, followed by Tukey's HSD (Honestly Significant Difference) post-hoc tests. All statistical analyses were performed in R (version 4.2.1; [44]).

3. Results

3.1 Greenhouse gas production in the peat profiles

Across all six cores and three microtopographies, aerobic CO_2 production was found to decrease with increasing depth (Fig. 1a). Generally, less CO_2 was produced at greater depths, and the highest rates of CO_2 production were found in the upper 1.5 m in all six cores. This was especially evident in Core 2 (collected from a hollow), in which the CO_2 production rate was about five-fold higher in the surface layers compared to the deepest layers. Aerobic CO_2 production was generally higher in the two hollow cores than in the other cores, regardless of depth. ANOVA analysis indicated that both depth ($F=35.88$; $p<0.001$) and microtopography ($F=25.40$; $p<0.001$) were significant indicators of aerobic CO_2 production. There was a significant difference between the hollow microtopography and either the hummock or sedge meadow

223 microtopographies ($p<0.001$), but there was no significant difference in production rates between the
224 hummock and the sedge meadow cores ($p=0.659$).
225
226 **Fig. 1** Depth profiles of CO_2 production in aerobic incubations (a), CO_2 production in anaerobic incubations
227 (b), and CH_4 production in anaerobic incubations (c). Samples are shown grouped by core (shape) and by
228 microtopography (color). ”gdw” indicates grams of dry weight of peat.
229
230 Rates of anaerobic CO_2 production were much lower than the aerobic rates in the same samples
231 (Figure 1b). CO_2 production varied significantly by depth ($F=13.813$, $p<0.001$), but not by microtopography
232 ($F=1.735$, $p=0.186$). Similar to the aerobic rates, CO_2 production was generally highest at the surface and
233 declined with depth (Fig. 1b). However, this was not observed in the core collected from the sedge meadow,
234 which had similarly low rates of CO_2 production at all depths.
235 A similar pattern was observed in the production of CH_4 at varying depths in the peat profile, and
236 the effect of depth was significant ($F=11.71$; $p=0.001$). Two cores (Cores 2 and 3) exhibited higher CH_4
237 production in surface peat samples (Fig. 1c), with deeper peat generally producing less CH_4. Below 70 cm,
238 CH_4 production was generally lower (<300 nmol g dry weight^{-1} day^{-1}), but did not exhibit a clear pattern of
239 change with depth. Both the hollow and hummock microtopographies produced weak, inverse correlations
240 between increasing depth and CH_4 production, generally producing less CH_4 at greater depths (Fig. 1c).
241 Two of the cores (1 and 3) exhibited a local maximum in CH_4 production between 1.5 and 2 m depth.
242 However, the sedge meadow microtopography produced very little CH_4 regardless of depth, and only two
243 of the samples from the sedge meadow core (70 and 90 cm) produced appreciable CH_4 fluxes (371.2 and
244 94.5 nmol g dry weight^{-1} day^{-1}, respectively). Microtopography was not found to have a significant effect
245 on CH_4 production ($F=0.60$; $p=0.552$).

246 *3.2 Relationship of greenhouse gas production to environmental and chemical parameters*

247 A principal components analysis of potentially significant biochemical parameters was conducted
248 in order to visualize the associations between organic matter quality, environmental conditions, and GHG
249 production of peat samples (Fig. 2). In general, we found that parameters with positive loadings on PC1 are
250 those typically associated with more degraded organic matter (lower OM quality) and lower rates of gas
251 production, whereas parameters on the negative axis of PC1 were most indicative of a less degraded state
252 (higher OM quality) and higher rates. Parameters with negative loadings on PC1 were C:N, anaerobic and
253 aerobic CO_2 production, CH_4 production, and % of total C as sugars. Notably, all three of the GHG
254 production parameters were positioned on the negative axis of PC1. Chemical parameters with positive
255 loadings on PC1 were pH, % of total sugars as rhamnose, % of total sugars as glucose, % of total C as
256 amino acids, the C-normalized yield of hydroxyproline, and total inorganic N content. Several amino acid
257 parameters (%N as THAA, %C as THAA, mole % hydroxyproline, and hydroxyproline yield) had negative
258 loadings on PC2, while the low-molecular weight organic acid concentration had the largest positive
259 loading on PC2.

260

261 **Fig. 2** Loadings plot of the principal components analysis of GHG production rates, environmental
262 parameters, and biochemical parameters of the analyzed peat samples. Abbreviations for parameters are as
263 follows: aerobic CO_2 production ($AerCO_2$), anaerobic CO_2 production ($AnaCO_2$), CH_4 production (CH_4),
264 total organic acid content (TotalOrgAcids), total inorganic N content (TotalInorgN), molar % of total sugars
265 as glucose (%Glucose), % of total sugars as rhamnose (%Rhamnose), % of total C as sugars (%CSugars),
266 % of total C as amino acids (THAA%C), % of total N as amino acids (THAA%N), the amino acid
267 degradation index (AAIndex), molar % of total amino acids as hydroxyproline (%Hyp), the molar % of
268 glycine (%Glycine), and the C-normalized yield of hydroxyproline (HypC).

269

270 PCA scores were calculated for each peat sample and grouped by depth (Fig. 3a) and
271 microtopography (Fig. 3b). To facilitate this analysis, samples were grouped into bins, with depths of 0–1

m, 1–2 m, and 2–3 m below the peat surface. We found that PC1 was associated with depth, as samples from 2-3 m depth had consistently higher (more positive) PC1 scores than samples from closer to the surface. In contrast, the PC2 axis appeared to be more associated with microtopography. The hummock microtopography samples exhibited mostly negative PC2 scores, whereas the hollow microtopography had mostly positive PC2 scores. Sedge meadow samples had intermediate scores and were distributed around the y-axis of the scores plot (Fig. 3b).

Fig. 3 PC1 and PC2 scores for each peat sample grouped by depth (a) and microtopography (b).

3.3 Prediction of greenhouse gas production using multiple regression

A stepwise multiple regression model was constructed to evaluate the predictability of log-transformed aerobic and anaerobic CO_2 and CH_4 production based on the measured environmental and biochemical parameters. Our model for predicted CO_2 production under aerobic conditions identified pH, total organic acids, total inorganic N, mole % glucose, %N as THAA, mole % hydroxyproline, mole % glycine, and depth as significant parameters (Table 1). The multiple regression analysis for aerobic CO_2 production had the strongest fit among the three GHGs measured (adjusted $r^2 = 0.6487$; $p < 0.001$). There was also a relatively strong linear relationship between predicted and measured aerobic CO_2 production ($r^2 = 0.66$; $p < 0.001$; RMSE = 47.83; Fig. 4a).

Fig. 4 The relationship between measured GHG production and GHG production predicted by multiple regression analysis for aerobic CO_2 production (a), anaerobic CO_2 production (b), and CH_4 production (c). A line with a slope of 1, indicating perfect agreement, is provided for reference on each graph. RMSE indicates the root mean square error.

Table 1 Parameters and statistics from the multiple regression analysis

Model	Predictor	Estimate	SE	t-value	p
Aerobic CO_2 Production (adj. r-squared = 0.6487)					
	Intercept	1.819385	0.258929	7.027	1.8e-09
	pH	0.154359	0.040949	3.770	0.000363
	TotalOrgAcids	0.052000	0.009108	5.709	3.3e-07
	TotalInorganicN	-0.016851	0.005564	-3.029	0.003560
	%Glucose	-0.023644	0.009772	-2.420	0.018432
	%N as THAA	0.003104	0.002323	1.336	0.186348
	%Hyp	0.012211	0.005899	2.070	0.042560
	%Glycine	-0.018146	0.008094	-2.242	0.028498
	Depth	-0.121221	0.040233	-3.013	0.003722
Anaerobic CO_2 Production (adj. r-squared = 0.3058)					
	Intercept	0.196986	0.224888	0.876	0.38424
	CN	-0.020982	0.012984	-1.616	0.11088
	%N as THAA	0.019723	0.006259	3.151	0.00245
	%C as THAA	-0.074610	0.027023	-2.761	0.00745
	%Glycine	0.010603	0.006592	1.608	0.11250
	Depth	-0.051278	0.026871	-1.908	0.06070
CH_4 Production (adj. r-squared = 0.4178)					
	Intercept	2.84088	0.93239	3.047	0.003376
	CN	-0.12222	0.05933	-2.060	0.043536
	TotalOrgAcids	0.11211	0.03166	3.541	0.000755
	TotalInorganicN	-0.07179	0.01596	-4.498	3.02e-05
	%N as THAA	0.07653	0.02906	2.633	0.010622
	%C as THAA	-0.23782	0.11885	-2.001	0.049703

A second stepwise multiple regression model was constructed to evaluate predictors of anaerobic CO_2 production. N-related parameters (C:N, %N as THAA, %C as THAA, and mole % glycine) in addition to depth were significant parameters in this model, and the overall fit was weaker than for aerobic CO_2 production (adjusted $r^2 = 0.3058$; $p < 0.001$). The strength of fit between the predicted and measured results was similar ($r^2 = 0.39$; $p < 0.001$; RMSE=0.26; Fig. 4b). The multiple regression model for CH_4 production was similar in strength to the anaerobic CO_2 model (adjusted r^2=0.4178; $p < 0.001$), and C:N, total organic acids, total inorganic N, % Nas THAA, and %C as THAA were identified as significant. The strength of the relationship between the predicted and actual CH_4 production was also similar to the anaerobic CO_2 model ($r^2 = 0.49$; $p < 0.001$; RMSE = 98.72; Fig. 4c).

4. Discussion

4.1 Patterns of GHG production with depth and microtopography

We hypothesized GHG production would vary with depth and microtopography, reflecting differences in the extent of organic matter decomposition, botanical origin, and redox conditions. First, GHG production rates were hypothesized to decline with depth, as labile biochemicals are selectively removed from older peats as decomposition proceeds [13,14,45]. Second, samples from hummock microtopographies were hypothesized to have lower rates of CO_2 and CH_4 production than hollow or sedge meadow samples, reflecting the lower litter quality observed in the *Sphagnum* species typically inhabiting hummocks [46,47], as well as longer exposure to oxic decomposition before burial beneath the water table [27].

Both hypotheses were supported for the aerobic incubation experiments. Aerobic CO_2 production was highest in surface layers in all six cores, across all three microtopographies. This is consistent with previous studies, which have shown that labile substrates are generally depleted with increasing depth and age of the peat, resulting in lower rates of decomposition and CO_2 production [14]. We also observed that aerobic CO_2 production was higher in samples from the hollow cores throughout the peat profile, consistent with previous literature. *Sphagnum* mosses produce differing amounts of structural or metabolic

carbohydrates depending on their microtopography, with the hummock species spending more energy on the formation of structural carbohydrates, such as sphagnan, that are less-easily degraded than the metabolic carbohydrates associated with faster growth [46]. Since the hollow species are generally inundated and experience fewer seasonal and diurnal water table movements than hummocks, these species tend to devote more energy into the formation of relatively labile metabolic carbohydrates [48]. Accordingly, a decomposition experiment conducted at our field site found that *Sphagnum magellanicum* collected from the hummocks had higher sphagnan content and lower decomposition rates than *Sphagnum cuspidatum* collected from the hollows [33]. This is consistent with moss decomposition experiments from other peatlands [47]. Our results demonstrate that these differences in organic matter quality persist to the later stages of decomposition, as the hollow samples exhibited higher rates of aerobic CO_2 even in deep layers.

The effect of both depth and microtopography was smaller for anaerobic CO_2 and CH_4 production than for aerobic CO_2 production (Fig. 1b,c). While both were generally highest at the surface and declined with depth, the effect was smaller than in the aerobic experiments, and there was no significant difference among the three microtopographies. This suggests that the influence of biochemical composition arising from botanical origin and selective decomposition are less important for determining the rate of anaerobic compared to aerobic decomposition. The process of anaerobic respiration involves hydrolysis of complex biopolymers to monomeric DOM, fermentation to labile substrates such as organic acids, and finally respiration to CO_2 or CH_4 [34,49]. Organic matter composition would be expected to alter the availability of labile substrates for hydrolysis. However, the lack of difference among microtopographies suggests that respiration is ultimately limited by factors such as the availability of terminal electron acceptors rather than organic matter quality.

In terms of CH_4 emissions, the sedge meadow microtopography produced very little CH_4 across all depth profiles, compared to the hummock and hollow microtopographies (Fig. 1c). Fen peatlands generally produce more CH_4 than bog peatlands due to the presence of more labile C for microbial consumption originating from the non-*Sphagnum* plant species that dominate these environments [6]. However, the sedge

348 microtopography was observed to produce little to no CH_4 under anaerobic conditions. It is therefore
349 possible that methanogenesis was suppressed by the presence of an alternative electron acceptor in the
350 sedge meadow sampling site that represents a more energetically favorable reduction reaction than
351 methanogenesis. Oxygen transport to the rhizosphere through the aerenchyma in the sedges could result in
352 a higher degree of oxidation deeper in the peat profile compared to the bog [50]. Since the sedge meadow
353 microtopography is located in the more minerotrophic portion of the bog-fen gradient represented by this
354 study, alternative electron acceptors such as ferric iron or sulfates might originate from minerals present in
355 the groundwater feeding the fen peatland [51]. The low pH of the site would make ferrous iron reduction
356 particularly energetically favorable [52].

357 *4.2 Biochemical and environmental parameters associated with GHG production*

358 The suite of biochemical measurements collected alongside GHG production rates provides an
359 opportunity to identify the specific variables driving organic matter quality under both oxic and anoxic
360 conditions. Our PCA indicated that CO_2 production (both aerobic and anaerobic) and CH_4 production were
361 associated with C:N and % of C as sugars, with negative loadings on the PC1 axis (Fig. 2). pH, % of total
362 sugars as rhamnose, % of total sugars as glucose, % of total C as amino acids, and total inorganic N content
363 had positive loadings on PC1, indicating these parameters were associated with lower rates of GHG
364 production. We therefore interpreted peat with negative PC1 scores as indicative of higher bioavailability
365 and positive scores as representing more degraded peat. Consistent with this interpretation, PC1 scores of
366 peat samples were typically negative at the surface and positive deeper in the core (Fig. 3a).

367 The biochemical parameters associated with greenhouse gas production are largely consistent with
368 previous literature. Non-cellulosic sugars generally have high bioavailability, and high C:N is associated
369 with fresh, poorly decomposed peat [53,54]. Rhamnose is a major component of sphagnan, which
370 contributes to the relative recalcitrance of *Sphagnum*, and has been shown to be inversely correlated with
371 aerobic CO_2 production in these samples [32,33,47,55]. The positive PC1 loading of mole % glucose is
372 counterintuitive, as it is often associated with fresh, highly bioavailable peat [56,57]. However, our sugar

hydrolysis method was optimized for the maximization of sphagnan rather than total sugars, and cellulose recovery was likely poor [33]. The increase of glucose in deep peat layers associated with highly decomposed peat could reflect either a methodological artifact resulting from the partial breakdown of cellulose during hydrolysis method, or the accumulation of glucose-rich microbial exopolysaccharides in these layers [33,58].

While PC1 was most associated with variation in bioavailability and respiration rates, PC2 was most associated with microtopography (Fig. 3b). Hollow microtopography was associated with positive values on the PC2 axis, and hummock microtopography was associated with negative values. Sedge meadow samples were in the middle of the two, with the PC2 score of most samples clustered around 0. The loadings plot (Fig. 2) indicated that variables related to the amino acid composition (% of total N as amino acids, % of total C as amino acids, molar % of total amino acids as hydroxyproline, and C-normalized yield of hydroxyproline) were influential for PC2, and were specifically associated with the hummock microtopography.

The association of hydroxyproline with the hummock microtopography is consistent with differences in botanical composition across the microtopographies. Hydroxyproline is primarily found in hydroxyproline-rich glycoproteins (HRGPs) in plant cell walls, and lacks a major microbial source [26]. HRGPs produced by dicots are more extensively cross-linked than HRGPs in monocots, gynmosperms, or byrophytes [59], and are therefore more resistant to decomposition [26]. The hummocks in Miner Lake Bog are dominated by *Chamaedaphne calyculata* (a dicot) in addition to *Sphagnum,* but it is mostly absent in the hollows and sedge meadow. The higher yield and molar % of total amino acids as hydroxyproline in the hummocks is therefore consistent with the larger contribution of the more extensively cross-linked dicot HRGPs. However, this did not appear to affect the overall bioavailability of the peat, as neither hydroxyproline parameter had an appreciable loading on the PC1 axis (Fig. 2) and were significantly correlated only with CH_4 production in the multiple regression model (Table 1).

Our results indicate that a range of both environmental and biochemical parameters are significant predictors of decomposition, but that the important parameters are different for aerobic and anaerobic respiration. Consistent with previous studies, aerobic CO_2 production was best explained by a combination of depth, pH, total organic acids, and several biochemical indices reflective of organic matter composition (Table 1). As climate change increases transpiration and alters precipitation patterns, a net decline in peatland water table positions could increase the importance of organic matter composition in determining decomposition rates and the net C balance of peatland ecosystems.

In contrast, anaerobic respiration and CH_4 production were primarily associated with N and amino acid-related parameters, suggesting increased importance of N availability (Table 1). Specifically, both were negatively correlated with C:N, but positively correlated with %N as THAA. Low C:N and high %N as THAA are both associated with higher N availability, as the soil protein that comprises THAA is relatively labile and %N as THAA generally declines with decomposition [25,60]. On the other hand, CH_4 production (but not anaerobic CO_2 production) was negatively correlated with inorganic N. However, inorganic N has rapid turnover and is short-lived in soils, and is not likely to be representative of N availability over the timescale of our incubations. Previous research on the effect of N on CH_4 production is complex: while a metanalysis showed an overall increase in CH_4 emissions in response to N fertilization [61], several individual studies have found no effect [62–64]. Additionally, some of the stimulation of CH_4 production resulting from fertilization has been attributed to indirect effects of N addition, such as subsidence and the replacement of *Sphagnum* with aerenchyma-containing sedge vegetation [62,65]. The positive correlation observed between anaerobic respiration and N bioavailability (low C:N and high %N as THAA) are consistent with stimulation of respiration due to higher N availability. As peatlands experience increased N limitation in response to warming and C fertilization, the lower N availability could contribute to lower CH_4 emissions.

420 *4.3 Predicting GHG production with multiple regression models*

421 Stepwise multiple regression models were used to predict GHG production rates in each of the
422 three microtopographies. Our model's predicted CO_2 production rates displayed a relatively strong linear
423 relationship with the experimentally observed production rates (R^2=0.66; Fig. 4a). Several parameters
424 associated with biochemical composition, including mole % glucose, mole % hydroxyproline, and mole %
425 glycine were identified as significant in the stepwise regression model, indicating that organic matter
426 composition affects the vulnerability of peat organic matter to decomposition under oxic conditions. These
427 results support our hypothesis that inclusion of biochemical parameters improves the predictability of
428 potential greenhouse gas production in addition to parameters such as depth and soil pH.

429 The multiple regression models for the anaerobic experiments explained a somewhat smaller
430 fraction of the variance of both CO_2 and CH_4 production (31% and 42%, respectively). While no sugar-
431 related parameters improved the model according to the stepwise regression procedure, amino acid-related
432 parameters were found to be significant. These results suggest that consideration of N bioavailability,
433 including biochemical analysis of hydrolyzable amino acids, can improve the prediction of anaerobic GHG
434 emissions. Although this hypothesis was supported, models accounted for less than half of the variance in
435 both anaerobic CO_2 and CH_4 production. It is clear that other unmeasured variables such as availability of
436 inorganic and organic terminal electron acceptors [66,67], DOC availability [34,49], and differences in
437 communities of methanogenic archaea and methanotrophic bacteria [68,69] also play a role in determining
438 rates of anaerobic respiration. However, understanding of the biochemical composition of the peat does
439 improve the skill of the models in predicting GHG production.

440 **5. Conclusions**

441 We hypothesized that analysis of biochemical composition as a measure of organic matter quality
442 would improve our ability to predict potential greenhouse gas production in peat soils. Our hypothesis was
443 supported most strongly for oxic incubation experiments, in which multiple parameters associated with the
444 carbohydrate and amino acid composition significantly improved our multiple regression model. This is

consistent with previous studies demonstrating a link between organic matter quality and vulnerability to future decomposition. These results are important because they support the evidence that differences in botanic origin and decomposition history will play an important role in determining the future trajectory of GHG emissions from peatlands in response to increasing evapotranspiration and increasing fluctuation of the water table driven by climate change.

Biochemical analyses (particularly hydrolyzable amino acid analysis) also improved stepwise regression models for the prediction of anaerobic CO_2 and CH_4 production, although a smaller fraction of the variance was explained than in the aerobic models. Notably, we found that low C:N and high %N as THAA were correlated with both higher anaerobic CO_2 and higher CH_4 production. As both of these parameters are associated with higher N bioavailability, these results suggest N limitation of anaerobic respiration in this temperate peatland. Overall, our results illustrate the utility of including consideration of organic matter quality in predictions of future GHG feedbacks of temperate peatlands.

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689

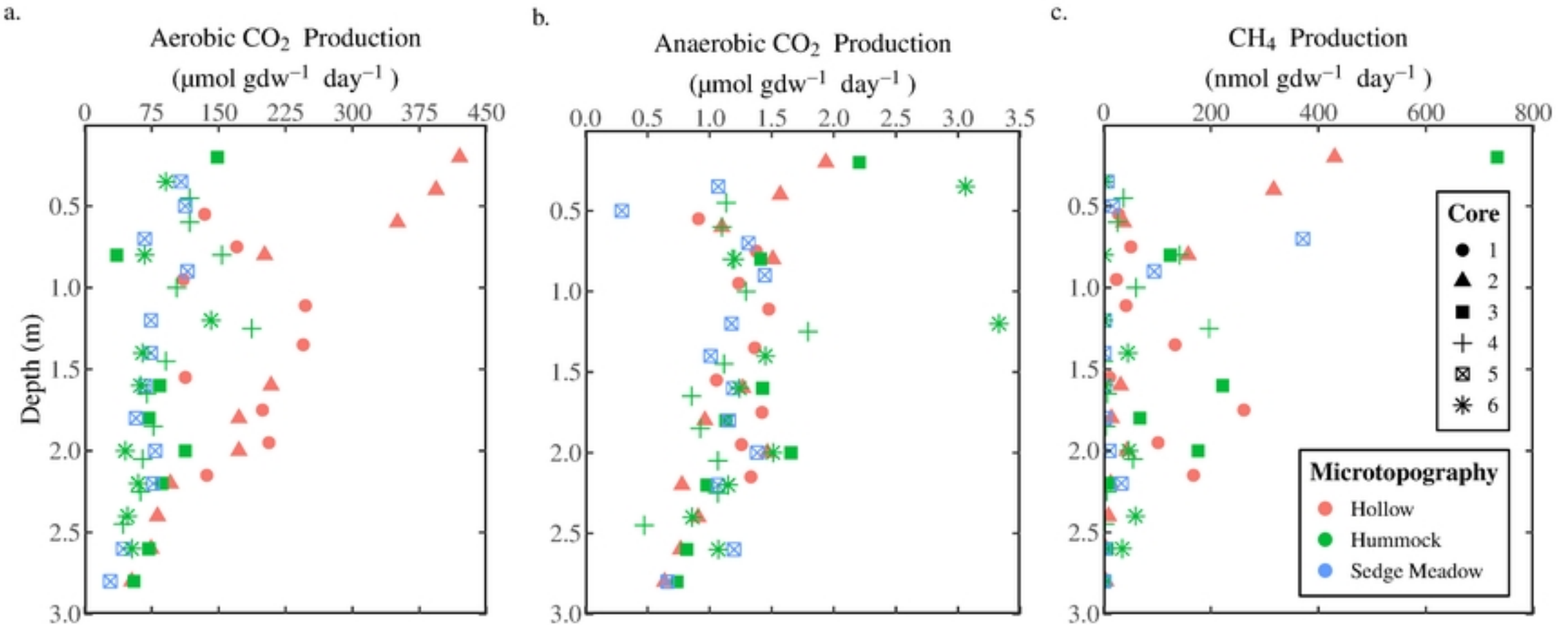

a.
Aerobic CO_2 Production
(μmol gdw^{-1} day^{-1})
0 75 150 225 300 375 450
Depth (m)
0.5 1.0 1.5 2.0 2.5 3.0
b.
Anaerobic CO_2 Production
(μmol gdw^{-1} day^{-1})
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
c.
CH_4 Production
(nmol gdw^{-1} day^{-1})
0 200 400 600 800
Core
1
2
3
4
5
6
Microtopography
Hollow
Hummock
Sedge Meadow

Figure 1

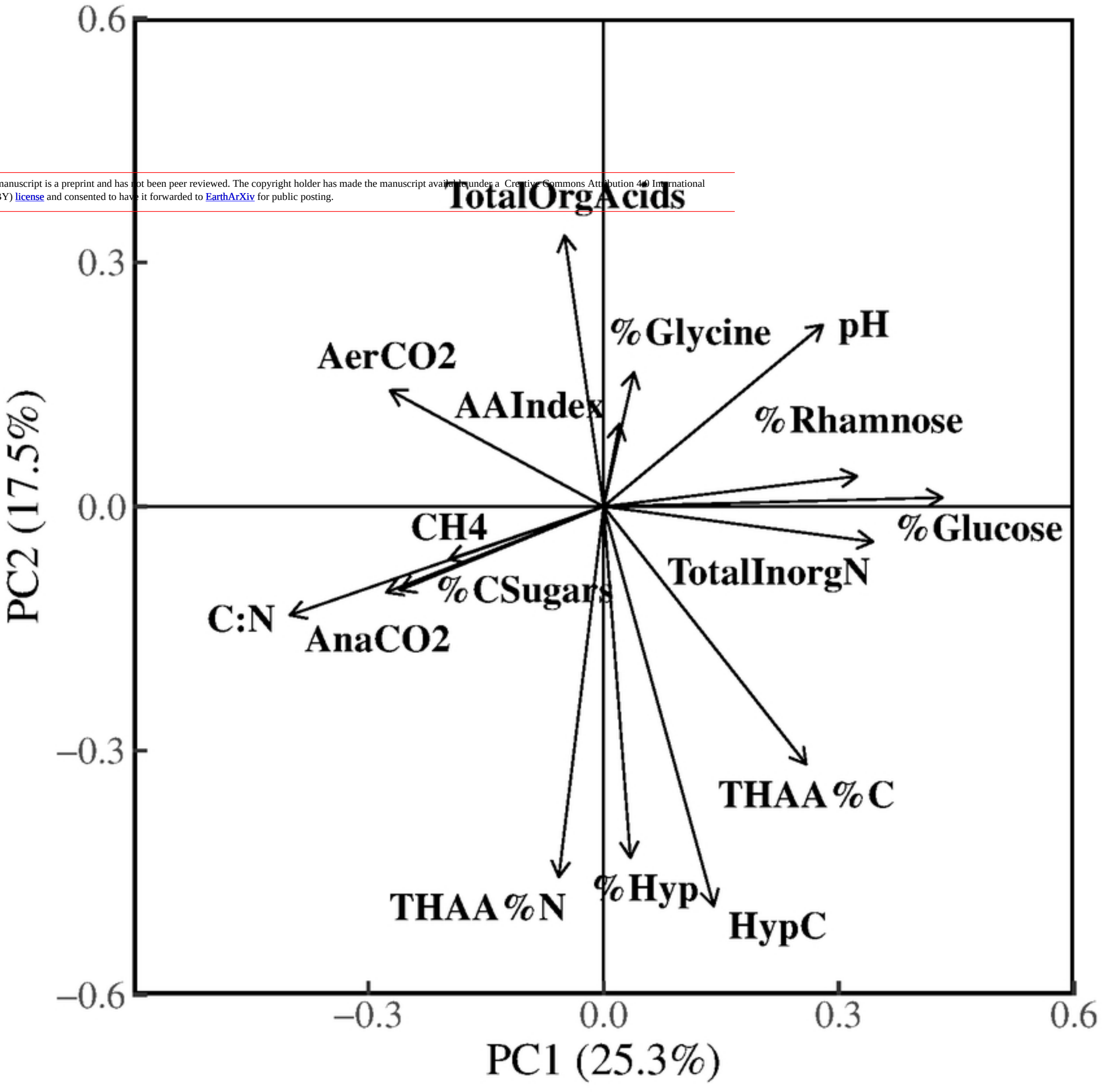
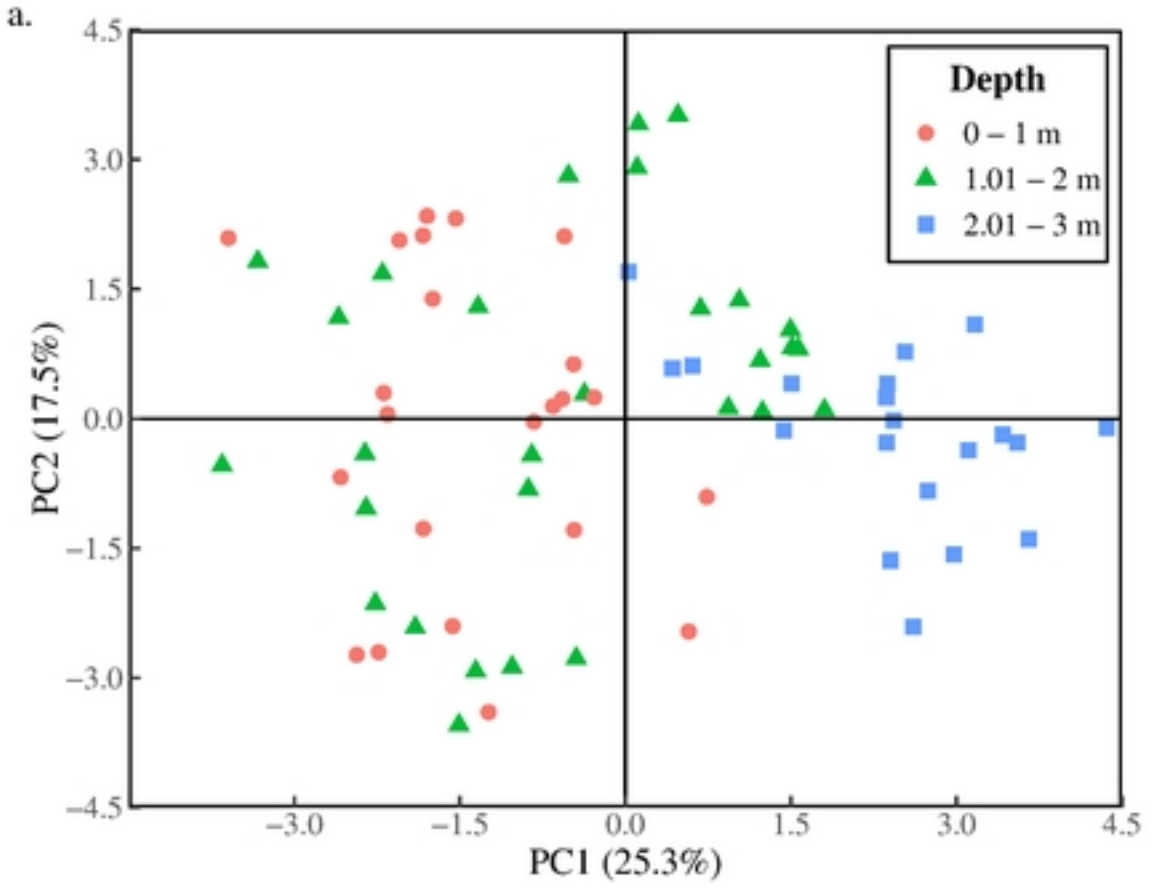


Figure 2


a.
Depth
0 – 1 m
1.01 – 2 m
2.01 – 3 m
PC2 (17.5%)
PC1 (25.3%)
4.5
3.0
1.5
0.0
−1.5
−3.0
−4.5
−3.0
−1.5
0.0
1.5
3.0
4.5

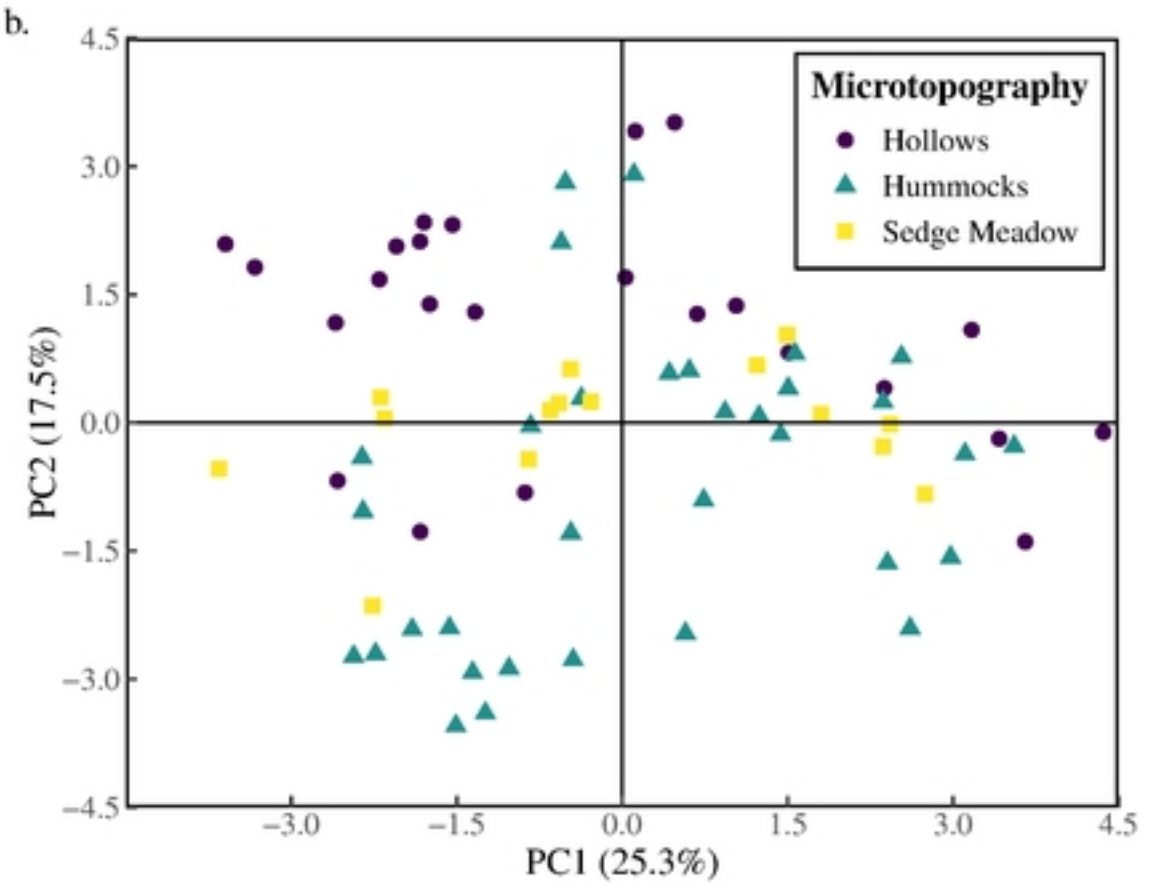

b.
Microtopography
Hollows
Hummocks
Sedge Meadow
PC2 (17.5%)
PC1 (25.3%)
4.5
3.0
1.5
0.0
−1.5
−3.0
−4.5
−3.0
−1.5
0.0
1.5
3.0
4.5

Figure 3

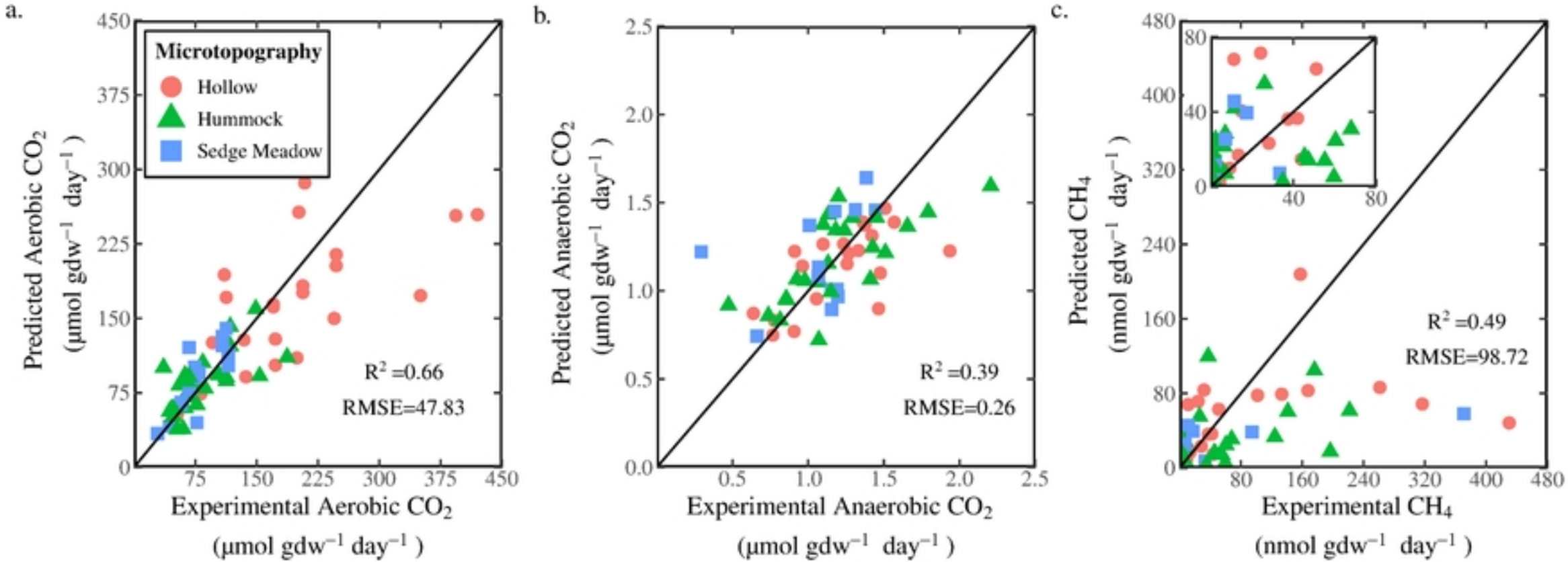

a.
Microtopography
Hollow
Hummock
Sedge Meadow
Predicted Aerobic CO_2 (μmol gdw^{-1} day^{-1})
Experimental Aerobic CO_2 (μmol gdw^{-1} day^{-1})
R^2 =0.66
RMSE=47.83
b.
Predicted Anaerobic CO_2 (μmol gdw^{-1} day^{-1})
Experimental Anaerobic CO_2 (μmol gdw^{-1} day^{-1})
R^2 =0.39
RMSE=0.26
c.
Predicted CH_4 (nmol gdw^{-1} day^{-1})
Experimental CH_4 (nmol gdw^{-1} day^{-1})
R^2 =0.49
RMSE=98.72

Figure 4