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Controls on Carbon Gas Fluxes From a Temperate Forest Soil

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Controls on Carbon Gas Fluxes from a Temperate Forest Soil

Natalie White

Honors Senior Thesis 2022

Environmental Science: Ecosystems

Advisor: Ruth K. Varner

14 **Abstract**

15 Forest soils consume atmospheric methane (CH₄), serving as a major global CH₄ sink that
16 uptake an estimated 22 ± 12 Tg of CH₄ per year. Temperature and soil moisture have been
17 identified as key controls of the microbial consumption of CH₄ in forest soils. Climate-driven
18 warming and changing moisture regimes may impact forest soils' role in the carbon cycle, and
19 recent works suggests that forests could become weaker CH₄ sinks. Long-term monitoring sites
20 can capture these changes, leading to better predictions of CH₄ exchange between the
21 atmosphere and soils under climate change.

22 This study utilizes a long-term trace gas dataset from College Woods in Durham,
23 NH, USA to track both CH₄ and carbon dioxide (CO₂) fluxes between 1989 and 2021. Between
24 1989 and 2001 gas fluxes were measured approximately biweekly. In June 2021 the site was re-
25 established, and we collected weekly flux measurements at three collars on a hillslope and three
26 collars in a hollow. Flux measurements collected June-October 2021 in College Woods indicated
27 that average CH₄ uptake in these soils was 3.27 ± 1.16 mg m⁻² d⁻¹. This is consistent with the
28 3.35 ± 1.68 mg m⁻² d⁻¹ average uptake rate observed June-October 1989-2001. Average CO₂
29 emissions from June -August 2021 were 2.86 ± 0.91 μmol m²s⁻¹, also consistent with the $3.96 \pm$
30 2.36 μmol m²s⁻¹ average for 1989-2001. We did not observe a significant change in carbon
31 fluxes across the study period, in contrast with the recent studies suggesting the global forest soil
32 CH₄ sink is decreasing.

33

34 **1. Introduction**

35 Methane (CH₄) is a greenhouse gas with a warming potential 32 times that of carbon
36 dioxide (CO₂, Holmes et al., 2013). The amount of atmospheric CH₄ has been increasing from

37 pre-industrial levels around 715 ppb to the most recent estimate in early 2022 of 1909 ppb
38 (Dlugokencky, 2022). Sources of methane to the atmosphere are both naturally occurring and
39 human-caused; wetlands, burning of fossil fuels, and agriculture are the three largest sources
40 (Kirschke et al., 2013). Consumption of atmospheric CH₄ by soil accounts for the second largest
41 sink of CH₄ globally, with forest soils representing about 50% of this sink (Dutaur, 2007).
42 Between 2000-2009, the amount of CH₄ oxidized (consumed) by methane-oxidizing bacteria
43 (methanotrophs) in soils was estimated to be between 26 and 42 Tg yr⁻¹ (Kirschke et al., 2013).
44 Methanotrophy occurs in aerated soil environments where oxygen levels are sufficient for
45 methanotrophs, which are obligate aerobes, to oxidize CH₄ to CO₂. Forest soils are an aerobic
46 environment, therefore CH₄ oxidation is the primary methane cycling process present.

47 The primary controls on CH₄ uptake and release from soils include soil moisture, water
48 table depth, soil temperature, and vegetation (Topp and Pattey, 1997). Soil moisture has been
49 found to be the most influential factor impacting methane uptake rates. There is an optimal soil
50 moisture level where CH₄ uptake is maximized because there is enough water present to facilitate
51 biological activity, but not too much to either physically limit the amount of CH₄ that can enter
52 the soil through diffusion or create anaerobic conditions (Bowden et al., 1998; Curry et al., 2007;
53 Czepiel et al., 1995; Topp and Pattey, 1997). Temperature heavily influences CH₄ uptake as it
54 changes throughout the seasons. As temperatures increase, uptake rates increase because the
55 microbial community is more biologically active (Bowden et al., 1998; Price et al., 2004; Topp
56 and Pattey, 1997). Soil structure and texture class impacts CH₄ uptake as well; coarser soils allow
57 for more diffusion of atmospheric methane into the pore space (Verchot et al., 2000).

58 Climate change is altering the temperature and precipitation regimes created by large-
59 scale climate phenomena that control CH₄ uptake by forest soils. These large-scale changes

60 impact the soil properties that directly control CH₄ uptake (Blankinship et al., 2010). Warmer
61 average conditions may increase the ability of forest soils to consume methane because oxidation
62 rates increase with temperature (Bowden et al., 1998), and warmer conditions will cause soils to
63 dry out, opening pore space for increased diffusion rates of atmospheric CH₄ (Liu et al., 2019).
64 However, climate change isn't impacting the whole world in the same way; some areas are
65 getting wetter as they receive more rainfall on average. Campbell et al. (2007) recorded
66 increased precipitation in the United States over the past 50 years. Ni and Groffman (2018)
67 proposed that increasing precipitation was driving a decrease in CH₄ uptake across the US over
68 the past three decades when synthesizing over 300 CH₄ uptake studies. Several other studies
69 recorded an opposite trend wherein the CH₄ uptake rate has been increasing and strengthening
70 the forest soil sink of atmospheric CH₄ (Yu et al., 2017). Climate change is a complex process
71 with several interacting factors, thus it has been difficult to predict how CH₄ uptake rates will
72 respond to potentially synergistic environmental changes (Liu et al., 2019).

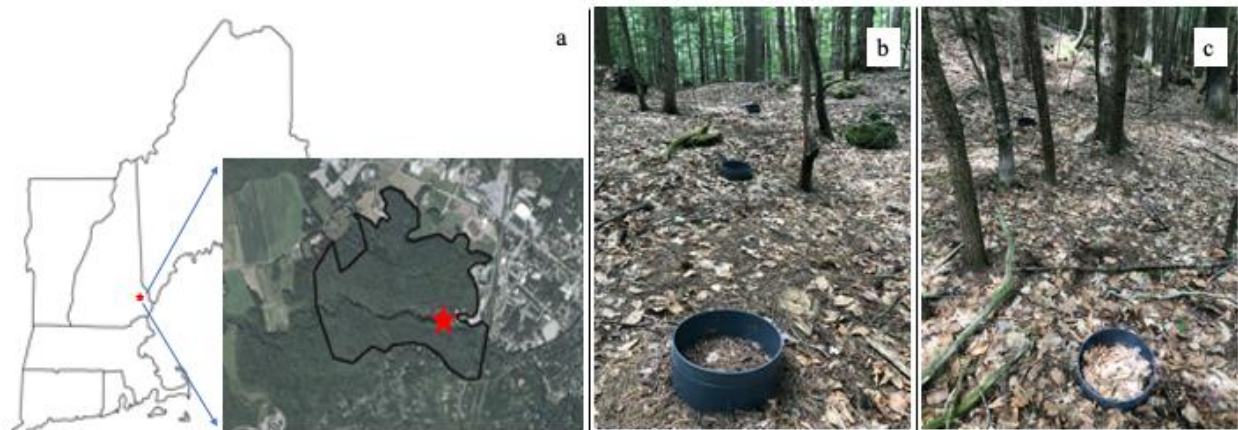
73 This study takes place in College Woods, a temperate forest located in Durham, NH. In
74 1989 the study site was established and CH₄ uptake and CO₂ emissions were measured through
75 2001. Crill (1991) analyzed the first 2 years of data and determined that CH₄ uptake here is
76 diffusionally controlled in the late spring, summer, and fall, and that uptake follows a seasonal
77 hysteresis. Soil CH₄ concentrations show that oxidation does not occur in the top organic layer of
78 soil, and that there is an optimal moisture level where oxidation reaches its maximum between 2
79 and 6 cm (Crill, 1991; Czepiel et al., 1995). This study sought to revisit the College Woods study
80 site and make the same measurements of carbon gas fluxes in order to compare contemporary
81 uptake and release rates to the historical rates. We aimed to understand if carbon gas fluxes at
82 this site are responding to changes in temperature and precipitation trends due to climate change.

83

84 2. Materials and Methods

85 2.1 Site Description

86 Measurements were conducted in College Woods (43.08 °N, -70.57 °E), on the campus of the
87 University of New Hampshire, Durham, NH, USA. College Woods is approximately 250 acres,
88 consisting of mixed hemlock-hardwood forest, the Oyster River, and the Oyster River Reservoir.
89 The dominant coniferous species in College Woods are Eastern Hemlock (*Tsuga canadensis*),
90 and White Pine (*Pinus strobus*). Dominant hardwood species include Red Oak (*Quercus rubra*),
91 Pin Cherry (*Prunus pennsylvanica*), Red Maple (*Acer rubrum*), Sugar Maple (*Acer saccharum*),
92 and Beech (*Fagus grandifolia*). There was no understory present at this site. The soil at the study
93 site is of the order Inceptisol which is a young, moderately acidic soil that drains well. The 30-
94 year normal maximum temperature in Durham, NH is 14.7°C and the minimum temperature is
95 2.7°C. The 30-year normal annual cumulative precipitation is 1151.9 mm (NOAA National
96 Centers for Environmental Information).



97
98 Figure 1: a) College Woods study site located on the campus of the University of New Hampshire, Durham, NH,
99 USA, 43.135 °N, -70.944 °E. b) Slope transect established in 2021 with three collars. c) Hollow transect established
100 in 2021 with three collars.

101 During a thaw in January 1989 two aluminum collars (0.397 m²) were cut into the soil in College
102 Woods with one on a slope side and the second in a hollow (Crill, 1991). Methane and CO₂ flux
103 measurements began the next month and continued through 2001 approximately every two
104 weeks. In the summer of 2021, we re-established the study site and collected measurements from
105 a total of six static flux chambers. Three circular collars (diameter 32 cm) were installed on a
106 transect following the downward slope of the hill the study site is on, and the other three collars
107 were installed along a transect of a hollow area on the hillside. The decision to install collars on
108 both a slope and in a hollow followed from the experimental design of the Crill (1991) study
109 which published the initial data from this site. The soil around each chamber was cut with a knife
110 to allow the collar to be pushed 5 centimeters into the soil. Once installed, the height of each
111 collar was measured four times around the collar and averaged to calculate the volume of the
112 collar with the equation $volume = 3.14 \times 16^2 \times h \times 0.001$.

113 **2.2 Environmental Conditions- Temperature and Soil Moisture**

114 For all gas flux measurements, air and soil temperature measurements were collected in tandem
115 with soil gas flux measurements. Air temperature, soil surface temperature, and soil temperature
116 at 5 cm and 10 cm were measured adjacent to each flux chamber with a thermometer (Gilson
117 MA-118 Taylor 9878E Pocket Digital Thermometer). In 2021, soil moisture was also measured
118 at 3 and 10 cm in the soil adjacent to each flux chamber with a soil moisture probe (Campbell
119 Scientific HydroSense II). Precipitation data from a weather station in Durham, NH were

120 retrieved from the NOAA National Centers for Environmental Information for all months of the
121 year between 1989 and 2021 to provide cumulative monthly precipitation.

122 **2.3 CH₄ and CO₂ fluxes**

123 The initial study period (1989-2001) used the static chamber flux method to measure both CH₄
124 and CO₂ fluxes. The two aluminum collars were fitted with a flux chamber (0.152 m³) and sealed
125 by filling the groove of the collar with water. The chambers were dark, prohibiting
126 photosynthesis from taking place. As such, the CO₂ flux measurements represent net
127 (autotrophic and heterotrophic) soil respiration, not net ecosystem exchange (CO₂ uptake – net
128 respiration). Inside the chamber was a battery-operated fan to mix the headspace and a thermistor
129 for measuring the enclosed air temperature. Fluxes were measured by taking 60 ml samples of
130 the headspace at 4-minute intervals over a 20-minute period, with the first sample taken at
131 minute four. Polypropylene syringes with siliconized polypropylene plungers and
132 polycarbonate/nylon or polyethylene/nylon three-way stopcocks were used to draw out the
133 headspace samples. Headspace gas samples were equilibrated to room temperature in the lab for
134 two hours and analyzed within five hours after collection.

135 Throughout the growing season of 2021, June-October, the static chamber method was used
136 again for weekly CH₄ and CO₂ flux measurements. Collar lids were placed on the collars to seal
137 off air exchange, but the chambers were not equipped with a battery-operated fan. To ensure the
138 headspace was well-mixed, the syringe was used to pump the headspace air 10 times before
139 drawing the sample. The methods of Crill (1991) were followed with the addition of one 60 ml
140 sample of ambient air taken at each collar before flux measurements began. The headspace gas

141 samples were stored in their syringes and placed in a refrigerator for no more than 24 hours
142 before they were processed in the lab.

143 Headspace CH₄ concentrations were measured with a gas chromatograph equipped with a flame
144 ionization detector (GC-FID, Shimadzu GC-8A). The GC-FID was run with a 50°C column
145 temperature, 130 °C detector and injector temperature, and ultra-high purity nitrogen gas as the
146 carrier gas flowing at a rate of 30 mL per minute through a 2-m 1/8-inch o.d. stainless steel
147 packed column (HayeSepQ 100/120). Twenty mL samples of standard gas at 2.006 ppm CH₄
148 were used to calibrate the GC-FID. This breathing air cylinder standard was calibrated against a
149 standard from NOAA's Earth System Research Laboratory's Global Monitoring Division's
150 Carbon Cycle Greenhouse Gases Group. Each collar headspace sample was run twice and the
151 average CH₄ concentration was used for the final flux calculation with the equation: $\frac{mgCH_4}{m^2day} =$

$$152 \frac{\Delta ppm}{min} \times \frac{P}{RT} \times \frac{V_c}{A_c} \times \frac{1440min}{day} \times \frac{16molCH_4}{1g} \times \frac{1mg}{1000\mu g} \times \frac{1m^3}{1000L}$$

153 In this equation $\Delta ppm/min$ is the change in methane mixing ratio over the chamber closure
154 period calculated from a linear fit, P is pressure (atm), R is the gas constant (8.202^{-5}
155 $m^3atm/molK$), V_c is the chamber volume (L), and A_c is the chamber area (m^2). Precision for the
156 calculation of the fluxes with this equation was $\pm 0.5\%$. Samples were rejected if they did not fit
157 the 95% confidence interval with respect to the coefficient of determination: $n = 3$ ($r^2=0.95$), $n=4$
158 ($r^2= 0.87$), and $n = 5$ ($r^2= 0.75$). This calculation represents the slope of the linear regression of
159 the CH₄ concentration over the 20-minute sampling period. The soils in College Woods
160 predominantly consume methane, so the fluxes are written as negative numbers which represent
161 a loss of methane from the atmospheric pool.

162 Carbon dioxide concentrations were measured with an infrared gas analyzer (LI-COR IRGA). An
163 instrument response curve was generated by injecting two replicates of five volumes of standard
164 gas (Northeast Airgas, 980.9 ppmv): 1, 3, 5, 7, and 10 ml. A linear regression of the standards
165 indicated a slope and y-intercept. Two chamber headspace samples of 3 mL each were injected
166 into the IRGA, and the CO₂ concentrations were determined with the following equation:

$$167 \text{ CO}_2 \text{ concentration (ug ml}^{-1} \text{ CO}_2) = \text{Sample integrated area} \times m + b$$

168 where m is the slope and b is the y-intercept of the standard curve. A linear regression
169 determining CO₂ concentration change over time was calculated and used to calculate CO₂ flux
170 with the following equation:

$$171 \frac{\mu\text{mol CO}_2}{\text{m}^2\text{s}} = \Delta\text{CO}_2 \times \frac{V_c}{A_c} \times \frac{1 \text{ min}}{60\text{s}} \times \frac{1000 \text{ ml}}{\text{L}} \times \frac{1 \text{ mol CO}_2}{44 \text{ g CO}_2}$$

172 Where Δ CO₂ is the change in CO₂ concentration per minute.

173 **2.4 Soil poregas CH₄ and CO₂ concentrations**

174 Soil poregas samples were collected weekly between July 1st and October 13th 2021 on the same
175 dates that the static chamber fluxes were measured. Two sets of stainless-steel sippers with a 3
176 mm inner diameter were inserted into the soil adjacent to both the slope and hollow transect.
177 Each set of four sippers was inserted to depths of 2 cm, 6 cm, 11 cm, and 15 cm, following the
178 design of the Crill (1991) study. Polypropylene syringes equipped with three-way stopcocks
179 were used to pull a 50 mL sample from the sipper. Samples were stored in a refrigerator for no
180 more than 24 hours until they were processed in the lab. Analysis of poregas samples was

181 conducted on the GC-FID following the same process as described for the static chamber flux
182 measurements (See section 2.2).

183 **2.5 Statistics and Data Analysis**

184 A combination of Excel, R v.4.0.3, and JMP were used for statistical analysis. Data were cleaned
185 in the tidyverse (Wickham et al., 2021) and lubridate (Grolemund and Wickham, 2011) R
186 packages, with data visualization done in ggplot2 (Wickham, 2016). I performed a one-way
187 ANOVA to determine if there were differences in the environmental characteristics of the slope
188 and hollow sites. T-tests were used to determine if CH₄ and CO₂ fluxes between sites varied.
189 Linear regressions were used to assess if there were significant temporal trends in annual
190 temperatures and precipitation, as well as for both CH₄ and CO₂ fluxes over time. The confidence
191 intervals for each of these statistical tests was 95%.

192 Data was analyzed in four distinct groupings based on year and month. All data included
193 January-December 1989-2001 and June-October 2021. Growing season data included June-
194 October 1989-2001, and 2021. Historical data included January-December 1989-2001, and
195 contemporary data included June-October 2021.

196

197 **3. Results**

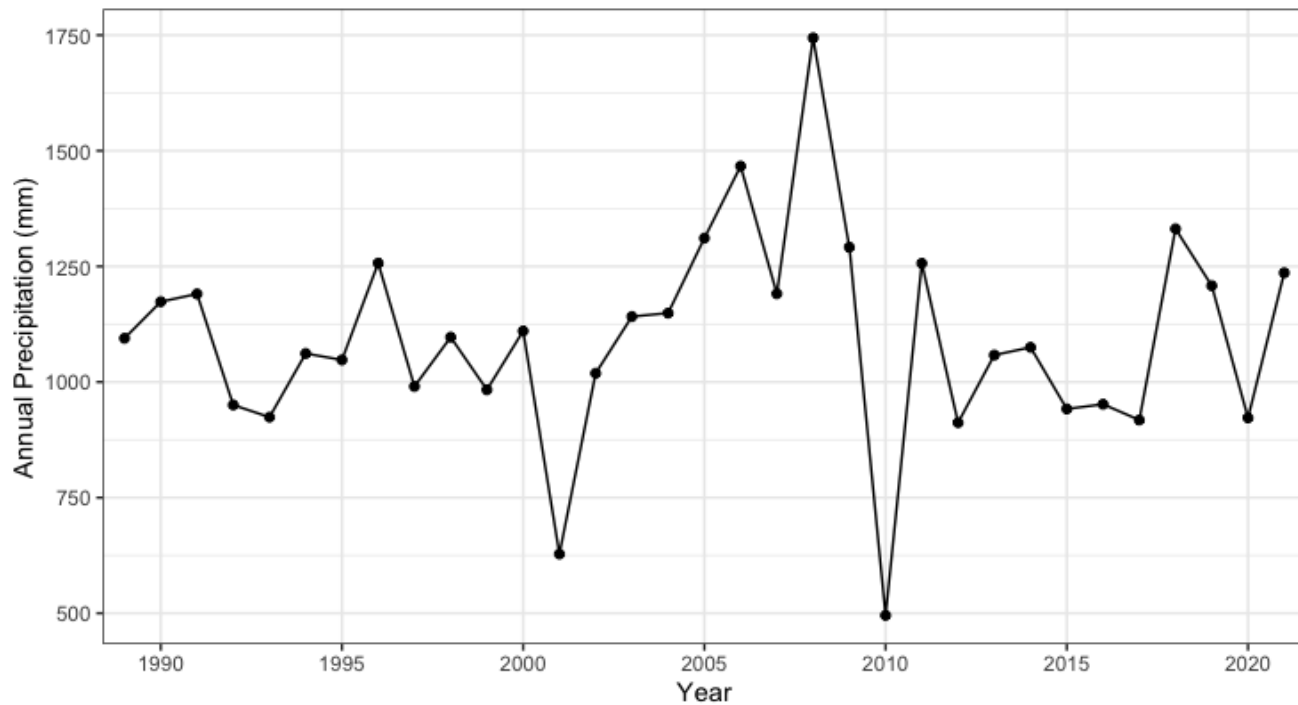
198 **3.1 Environmental Conditions – Temperature, Precipitation, and Soil Moisture**

199 Cumulative annual precipitation and daily average precipitation in Durham, NH between 1989-
200 2021 had no significant temporal trend ($R^2 = 0.0016$ and 0.0098 , respectively; Fig. 3). However,

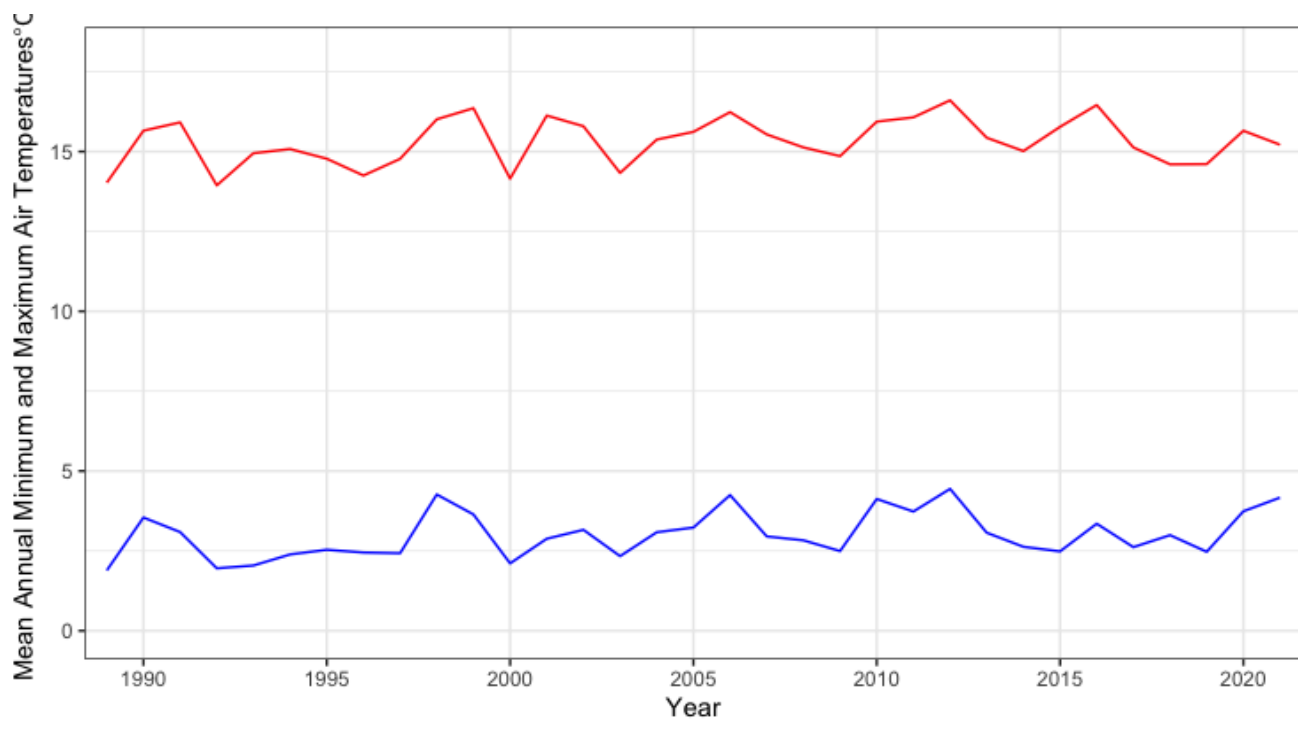
201 rainfall in July of 2021 (323.1 mm) was almost four times the average rainfall in July throughout
202 the whole 1989-2001 period (84.3 ± 44.6 mm), and precipitation for August, September, and
203 October of 2021 was higher than the historical average precipitation for these months (Table 1).
204 Mean annual maximum air temperatures revealed no significant change in temperature in
205 Durham, NH over the 32-year period ($R^2 = 0.03$, $p = 0.17$), while mean annual minimum air
206 temperatures slightly increased ($R^2 = 0.10$, $p = 0.04$).

207 Overall, temperature and moisture conditions across the slope and hollow transects were similar
208 in 2021. Air temperature ($p = 0.82$), soil surface temperature ($p = 0.74$), and soil temperature at
209 3 and 10 cm depth ($p = 0.47$ and 0.66 , respectively) all fell within the same ranges for the two
210 transects. At 3 cm deep, soil moisture was not significantly different in the two transects ($p =$

211 0.08). However, at 10 cm the soil in the hollow transect was slightly drier than in the slope
212 transect; mean soil moisture of $10.49 \pm 4.87\%$ and $13.13 \pm 6.26\%$, respectively ($p = 0.04$).



213



214

215 *Figure 2: A) Cumulative annual precipitation plotted over the 32-year period between 1989 and 2021. B) Annual*

216 *minimum and maximum air temperatures in Durham, NH between 1989-2001. Data from NOAA National Centers*
217 *for Environmental Information.*

218 Table 1: Precipitation in Durham, NH in 2021. Data retrieved from the NOAA Centers for
219 Environmental Information.

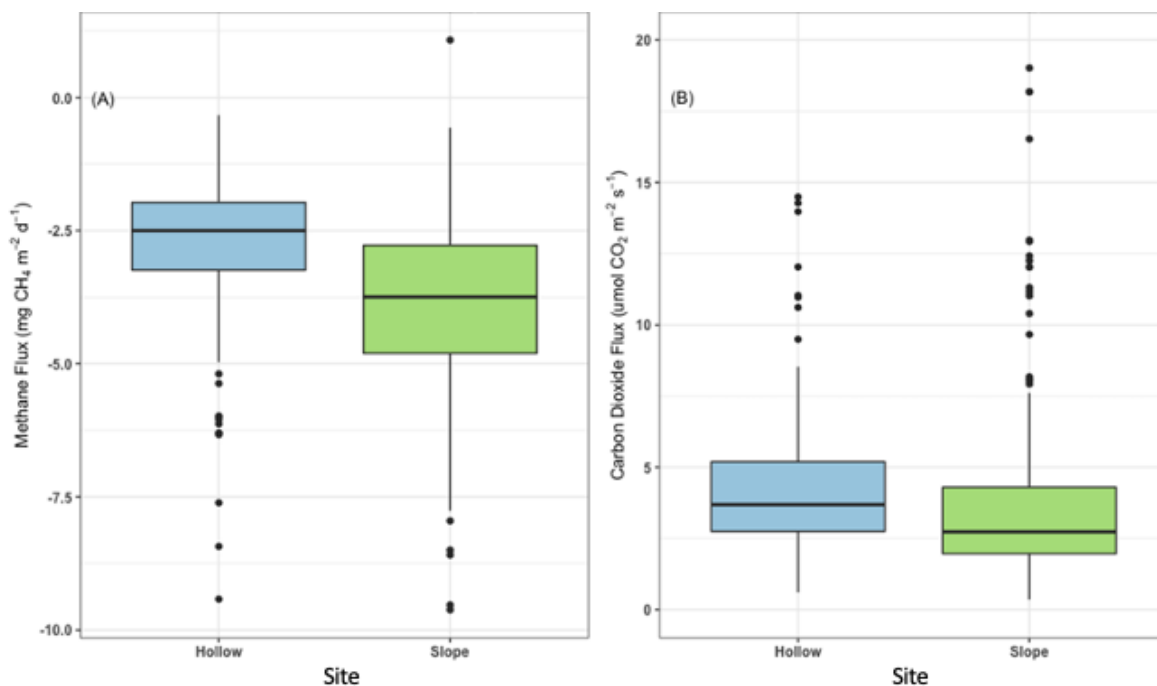
Month	Cumulative (mm)	Daily mean (mm)	Standard deviation (mm)
January	53.9	1.74	6.42
February	71.6	2.56	5.52
March	55	1.77	4.77
April	73.4	2.45	5.66
May	85.2	2.75	7.31
June	28.4	0.95	2.00
July	323	10.42	18.70
August	95.7	3.09	6.79
September	107.7	3.59	12.02
October	166.9	5.38	16.03
November	68.8	2.29	8.26
December	106.5	3.44	5.43

220

221 **3.2 Emissions and Soil Gas Concentrations Across Slope and Hollow**

222 When separated by landscape position, CH₄ uptake during the growing season (June-October)
223 was significantly lower in the hollow ($-2.77 \pm 1.233 \text{ mg m}^2\text{d}^{-1}$) than the slope ($-3.91 \pm 1.71 \text{ mg}$

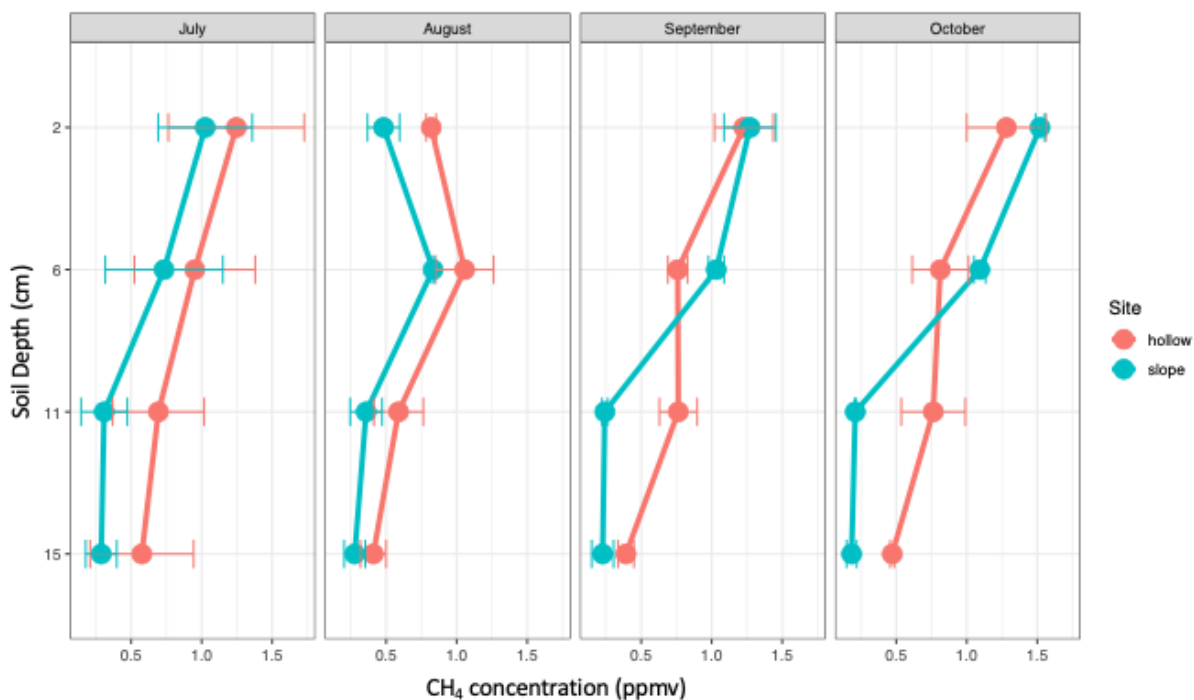
224 m^2d^{-1} , $p < 0.001$). Carbon dioxide emissions were not significantly different between the hollow
225 ($4.23 \pm 2.38 \mu\text{mol m}^2\text{s}^{-1}$) and slope ($3.78 \pm 3.21 \mu\text{mol m}^2\text{s}^{-1}$) transects ($p = 0.065$).



226
227 *Figure 3: Methane and carbon dioxide fluxes separated by hollow versus slope transect. Calculated with growing*
228 *season measurements from all studied years. Within each box, black horizontal lines represent median values, and*
229 *the upper and lower bounds represent the 25th and 75th percentiles.*

230 Soil poregas measurements made during the 2021 growing season indicate that CH₄
231 concentrations are higher through the whole depth gradient in the hollow transect ($p = 0.042$). At
232 2 cm the hollow averaged 1.2 ppmv while the slope averaged 1.12 ppmv, at 6 cm the hollow
233 averaged 0.90 ppmv while the slope averaged 0.88 ppmv, at 11 cm the hollow averaged 0.71
234 ppmv while the slope averaged 0.28 ppmv, and at 15 cm the hollow averaged 0.50 ppmv while
235 the slope averaged 0.25 ppmv. The largest decrease in soil gas CH₄ concentration, and therefore
236 the most CH₄ oxidation, occurs between 6 and 11 cm for all observed months. On the slope in
237 July and August the CH₄ concentration is lower than in the hollow at all depths, yet in September

238 and October the pattern changes and the CH₄ concentration at 2 and 6 cm deep on the slope
 239 increases above the concentration in the hollow for the respective depths. August exhibits a
 240 pattern of increasing CH₄ concentration between 2 and 6 cm, whereas all other months show a
 241 decreasing CH₄ concentration with depth. August is the only month that does not have the
 242 highest CH₄ concentration at the shallowest depth. Carbon dioxide concentrations in the poregas
 243 increased with depth and did not show any significant differences between the sites (Appendix
 244 Fig. 1).



245
 246 *Figure 4: Soil profile concentrations of CH₄ and CO₂ in 2021. Each point represents the monthly mean value for soil*
 247 *gas CH₄ concentration at each depth, with error bars showing ± one standard deviation of the mean.*

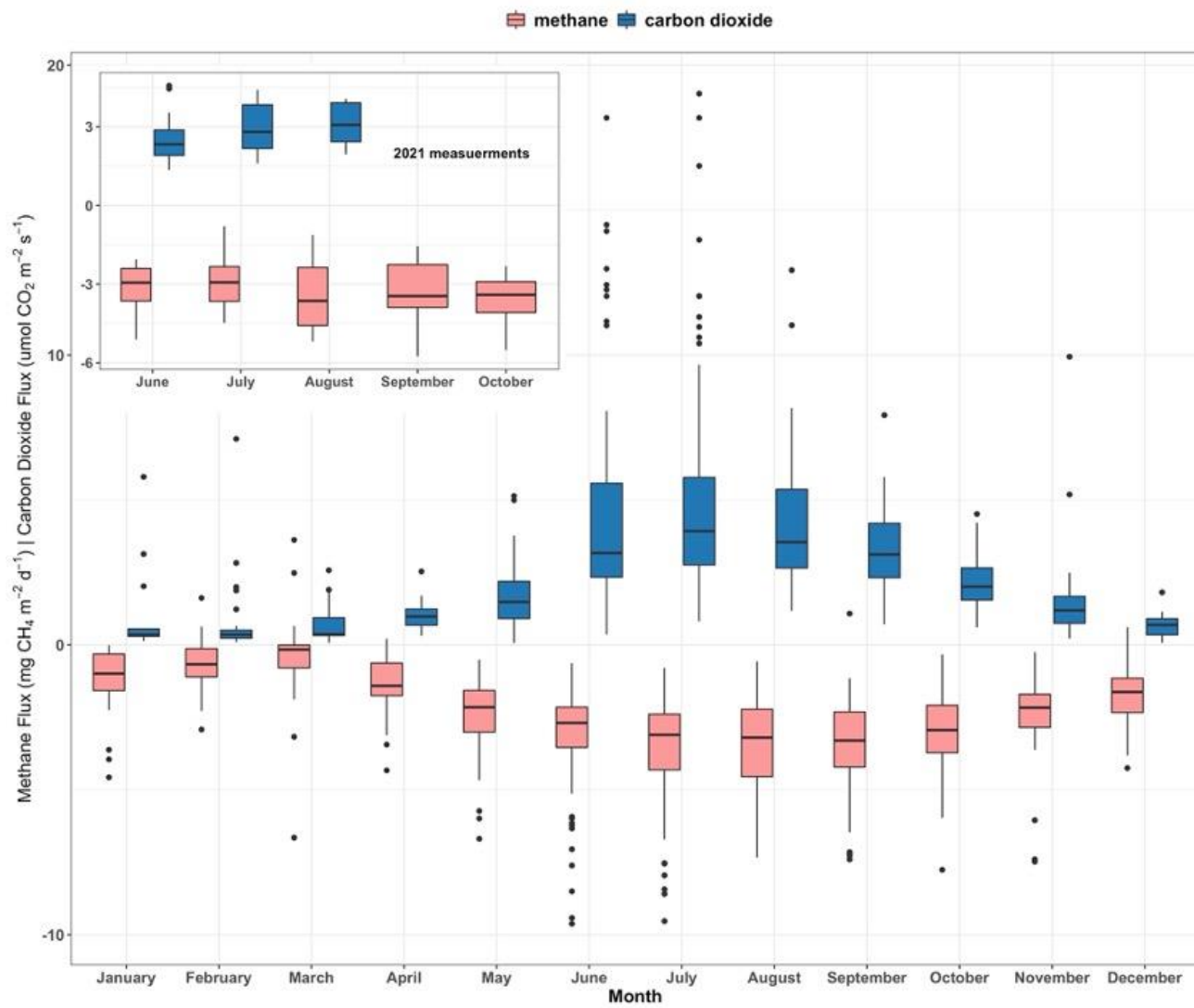
248 3.3 Seasonal and Interannual Trends in CO₂ and CH₄ Fluxes

249 We observed methane uptake for the majority of measurements (n = 649), with -9.62 mg CH₄
 250 m²d⁻¹ on June 23, 1999 being the largest uptake observed. There were few measurements when

251 the soil emitted methane (n=18, < 3% of all measurements), most of which occurred either in
252 winter or spring. Figure 4 depicts the seasonality of methane uptake; maximum uptake is reached
253 in the warmest months of the year while uptake is lowest in the winter months. Over the course
254 of the initial study period from 1989-2001 the average annual CH₄ uptake was -2.90 ± 1.58 mg
255 m²d⁻¹. The growing season average uptake throughout this period, defined as June through
256 October, is -3.42 ± 1.34 mg m²d⁻¹. Measurements in 2021 were only made June through October,
257 resulting in an average growing season CH₄ uptake of -3.27 ± 1.16 mg m²d⁻¹.

258 Carbon dioxide flux measurements (n = 606) indicated that the soil in College Woods was a net
259 source of CO₂ to the atmosphere, aside from occasions of winter freeze when soil respiration was
260 not taking place. Average annual CO₂ emissions between 1989-2001 was 3.37 ± 2.23 μmol m²s⁻¹
261 , while the growing season average was 4.10 ± 1.42 μmol m²s⁻¹. In 2021, the growing season
262 average CO₂ emissions (only measured June – August) were 2.86 ± 0.91 μmol m²s⁻¹. The

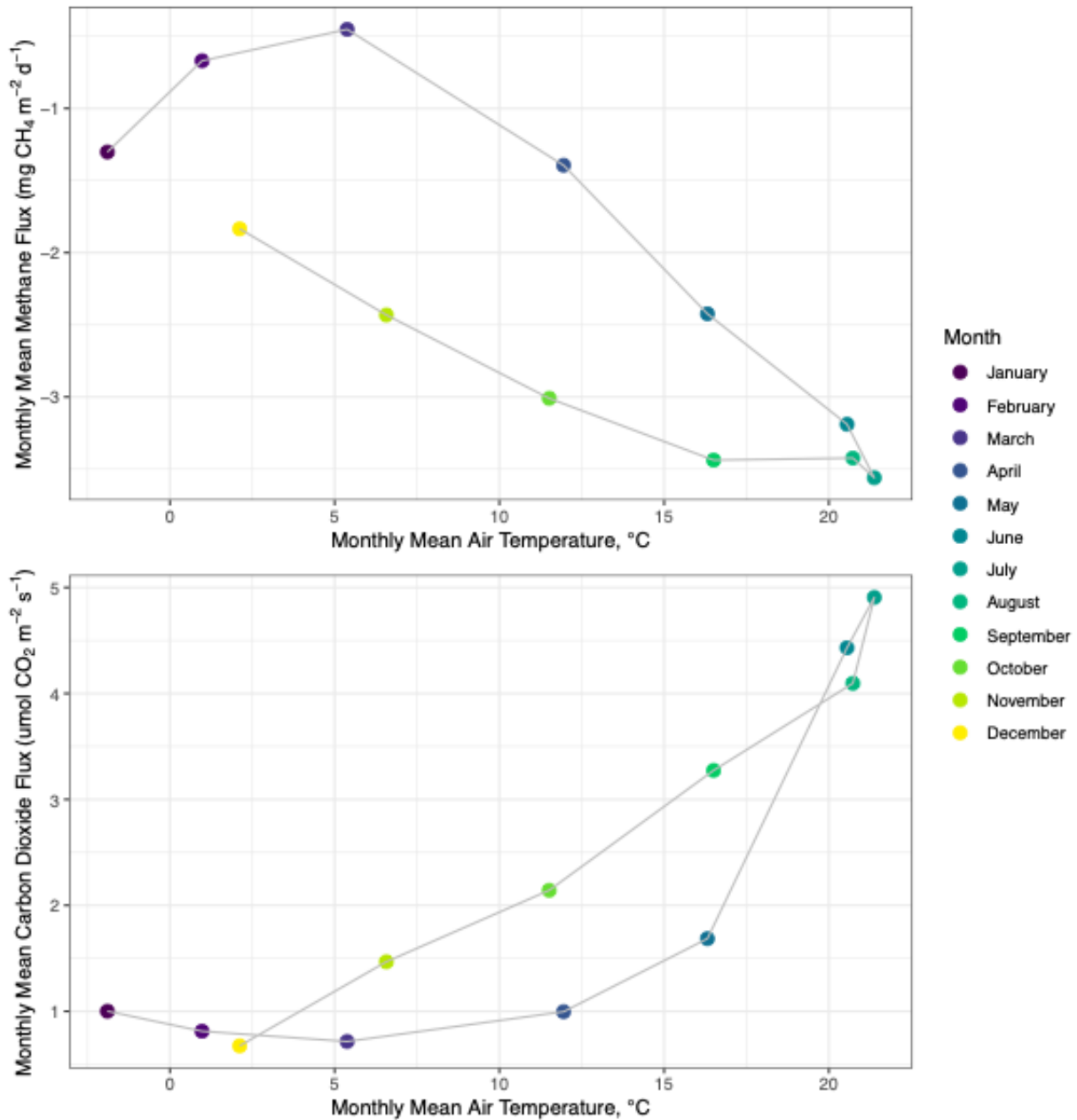
263 seasonal variation in CO₂ emissions is visible in Figure 5, showing a similar trend to CH₄ as the
264 size of the flux increases during the warm months.



265
266 *Figure 5: Timeseries showing monthly methane (n = 649) and carbon dioxide (n = 606) fluxes. The boxplot includes*

267 *both historical data (1989 - 2001) and data from 2021 (in inset). Within each box, black horizontal lines represent*
268 *median values, and the upper and lower bounds represent the 25th and 75th percentiles.*

269 Both CH₄ and CO₂ fluxes followed seasonal patterns dictated heavily, but not solely, by
270 temperature. Figure 6 plots the fluxes of both gases in relation to air temperature throughout the
271 year.



272

273 *Figure 6: Hysteresis of monthly mean methane and carbon dioxide fluxes by monthly mean air temperature*

274 *throughout the year. Calculated with all data from all study years.*

275

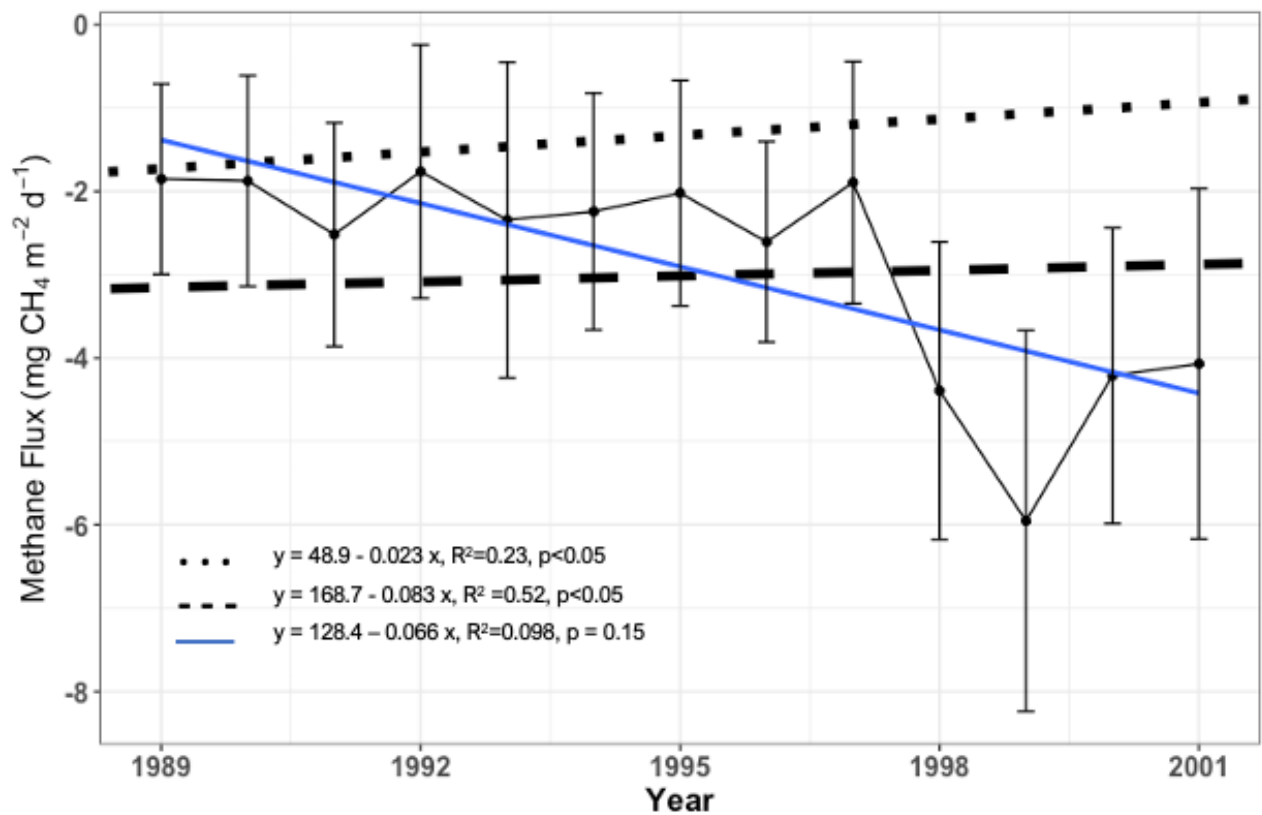
276 On the interannual timescale, the data collected in this study does not show a significant change

277 in CH₄ or CO₂ fluxes over time. In Figure 7, the blue trendline is calculated from this study's

278 data and shows that year does not explain significant variance in the amounts of CH₄ uptake (R^2

279 = 0.098, $p = 0.15$). Both lines imposed on top of this study's data are adapted from Ni and
 280 Groffman (2018). The dotted line represents the linear regression of global CH_4 soil uptake
 281 between 30-60°N from 1987-2016, and the dashed line is the CH_4 uptake in Hubbard Brook, NH
 282 from 2002-2015. Both datasets show significant decreases in CH_4 uptake over their respective
 283 time period.

284

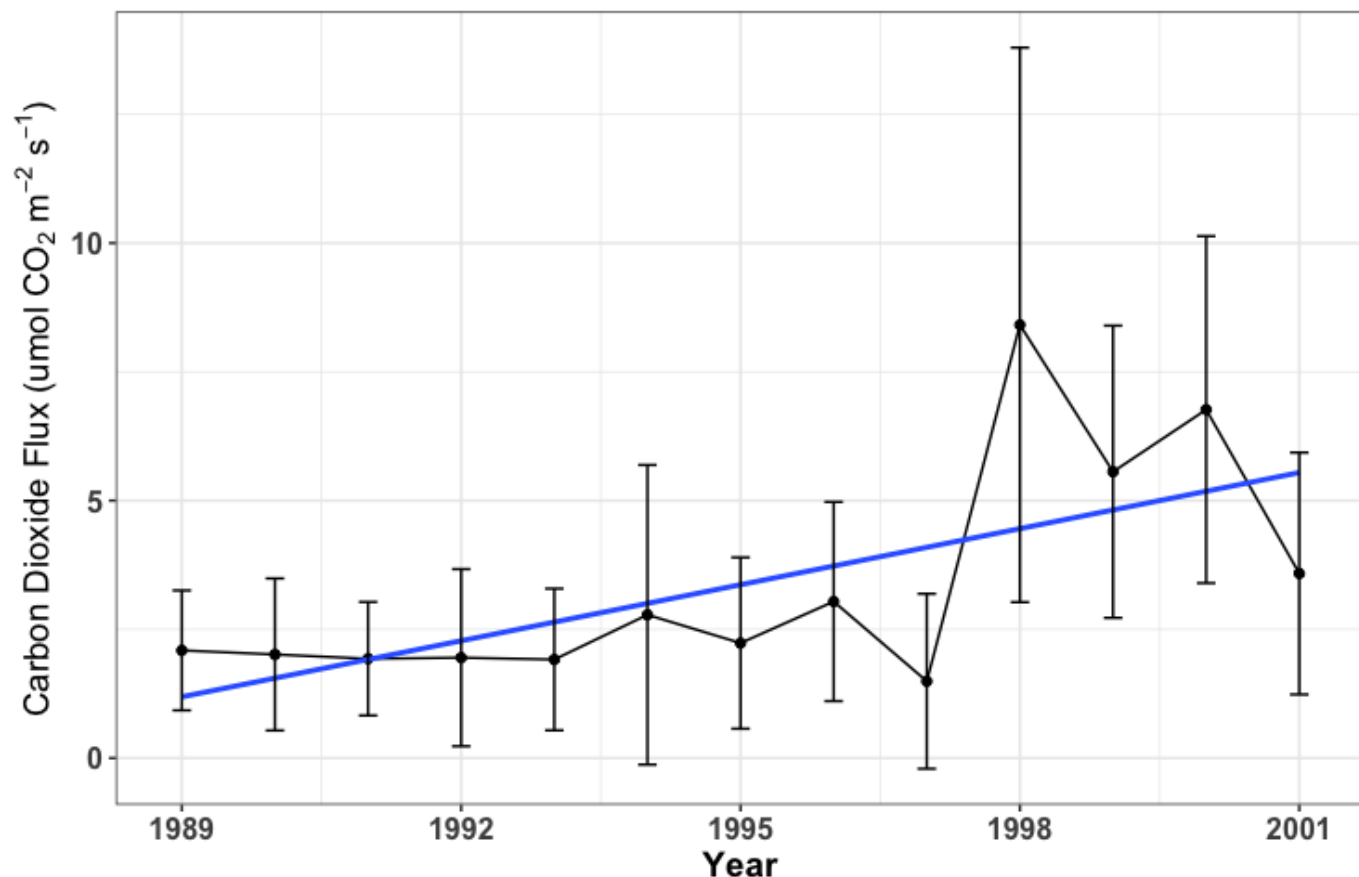


285

286 *Figure 7: Timeseries graph of CH_4 soil uptake between 1989-2001. Data points represent annual mean CH_4 uptake,*
 287 *with error bars showing \pm one standard deviation of the mean. The blue line is the trendline calculated from this*
 288 *study's data ($R^2=0.098$, $p = 0.15$). The dotted line represents the linear regression of global CH_4 soil uptake*
 289 *between 30-60°N from 1987-2016, and the dashed line is the CH_4 uptake in Hubbard Brook from 2002-2015 (Ni*
 290 *and Groffman, 2018).*

291

292 The time series of CO₂ emissions between 1989-2001 also shows no significant temporal trend in
293 forest soil C emissions ($R^2 = 0.06$, $p = 0.396$).



294
295 *Figure 8: Timeseries graph of CO₂ soil emissions between 1989-2001, calculated with data collected by the Trace*
296 *Gas Biogeochemistry lab. Data points represent annual mean CO₂ emissions, with error bars showing ± one*
297 *standard deviation of the mean.*

298 **4. Discussion**

299 **4.1 Spatial Variation of CH₄ and CO₂ Fluxes**

300 On the small spatial scale of this study, few differences were observed between the fluxes of
301 carbon gasses in the slope and hollow chambers. Methane uptake on the slope was slightly
302 higher than in the hollow on average for all months of all study years (Figure 3). The

303 hypothesized differences between the slope and the hollow were that the slope would have
304 slightly drier soil as precipitation would run off downhill and drain through the soil, whereas
305 rainfall would collect more easily in the hollow and drain more slowly, therefore influencing
306 CH₄ uptake through differences in soil moisture conditions. However, our observations of the
307 two landscape positions found the only significant difference in soil moisture between the two
308 sites to be that at 10 cm deep the hollow had drier soil. Drier soil is more conducive to CH₄
309 oxidation because the open pore spaces allow diffusion of atmospheric gases into the soil, which
310 is the primary mechanism by which CH₄ enters the soil and is oxidized. Soil gas profiles indicate
311 that the most CH₄ oxidation occurs between 6 and 11 cm. Thus, drier soils below 10 cm would
312 not be impacting the soil depth at which the most oxidation is occurring.

313 In their 1997 laboratory study, Bowden et al. found that maximum CH₄ uptake occurred when
314 around 60% of the pore space was filled with water (WFPS). This optimal WFPS finding was in
315 line with the results of another laboratory study, which found that optimal WFPS was between
316 50-70% (Nesbit and Breitenbeck 1992). Methane uptake increased as the soil moisture increased
317 from 0-60%, peaked between 60-70%, and then began decreasing after 70%. Soils in College
318 Woods have been found to have optimal soil moisture levels as well. Czepiel et al. (1995) used
319 incubation experiments to observe CH₄ oxidation in the soils of College Woods in relation to soil
320 moisture. Optimal uptake was found to be at moisture levels between 18-33% in grassy areas and
321 between 30-51% in wooded areas. During the 2021 growing season the hollow averaged a
322 moisture content of $10.49 \pm 4.87\%$, while the slope averaged $13.13 \pm 6.26\%$. The slope exhibited
323 more CH₄ uptake throughout the season with a slightly higher soil moisture content. In the
324 context of the optimal soil moisture levels found by Czepiel, this higher moisture content
325 corresponds to the higher CH₄ uptake level. It is important to note that although the moisture

326 levels at 10 cm between the slope and hollow in 2021 were significantly different, the difference
327 is a small percentage and thus is unlikely to significantly impact CH₄ uptake rates.

328 Soil gas profiles between the two sites showed clearer differences than the gas fluxes between
329 sites. On average, soil CH₄ concentration in the hollow was higher than on the slope. In July and
330 August the CH₄ concentration in the hollow was higher at all depths, then in September and
331 October the CH₄ concentration on the slope increased above the hollow concentrations for the
332 two shallowest depths (Figure 4). The pattern of soil CH₄ concentrations in August 2021 differ
333 slightly than the three other observed months; as the depth increases from 2 to 6 cm the CH₄
334 concentration increases as well. This is the opposite pattern of the decreasing concentrations with
335 depth that every other month and depth range exhibited. A large rain event that caused
336 precipitation to reside in the top ~5 cm layer of soil could help explain why little CH₄ was able to
337 diffuse into the soil, yet the soil moisture at 3 cm recorded in August was $8.83 \pm 3.32\%$ in the
338 hollow and $6.00 \pm 1.2\%$ in the slope – both within the typical range of moisture levels seen at
339 each site.

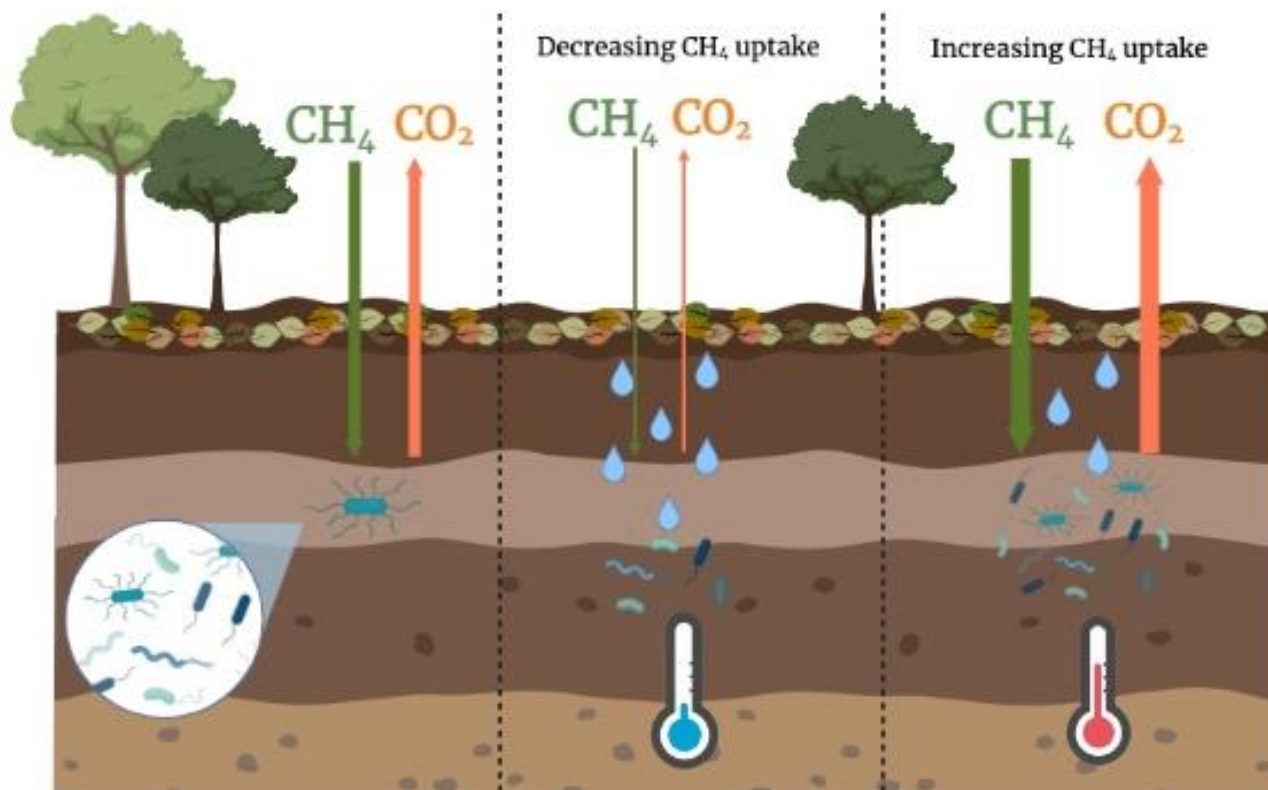
340 On the slope, the most oxidation in all four months occurred between 6 and 11 cm. This depth
341 has been found to be the typical range at which CH₄ oxidation primarily occurs (Price et al,
342 2004). Yet in the hollow, Figure 4 illustrates that the most oxidation occurs between 6-11 cm in
343 July and August but switches to between 2-6 cm in September and October. This was an
344 unexpected finding, as little to no methanotrophic activity occurs in the organic layer (Price et
345 al., 2004). Changes in soil moisture throughout the season could shift the depth of CH₄ oxidation.
346 Despite September and October receiving less precipitation than July which caused the soil at the

347 whole study site to be drier, the slope site did not experience this same change in oxidation
348 depth.

349 **4.2 Seasonality of CH₄ and CO₂ Fluxes**

350 The change in fluxes throughout the year are driven by seasonal differences in temperature and
351 precipitation that Durham, NH experiences due to its location along climatic gradients. Figure 5
352 illustrates the yearly pattern of changing CH₄ and CO₂ fluxes for both the total historical data and
353 a separate inset of this year's collected data. The warmest months in Durham are June, July, and
354 August where the temperature averages $20.7 \pm 3.9^{\circ}\text{C}$. These three months experience the highest
355 CH₄ uptake rates as well. The consistency of the CH₄ uptake being around $-3.00 \text{ mg m}^{-2} \text{ d}^{-1}$ in

356 the warmest months during both the historical and contemporary measurements supports that this
357 site is influenced by long-term, recurring climatic patterns.



358
359 *Figure 9: Conceptual model of a temperate forest system depicting the factors that affect rates of CH₄ uptake and*
360 *CO₂ emissions. Created in Biorender.*

361 During the winter in Durham, NH the temperature drops below freezing and snow and ice are
362 present at the study site. Methane uptake at this time of year drops because atmospheric gases are
363 not diffusing into the soil as easily, and the cold temperatures decrease the activity of the
364 methanotrophs (Crill, 1991). When there is little snowfall, and the soil is exposed to cold
365 temperatures it will likely freeze. In these instances where the soil freezes, the methane uptake
366 stops because the microbes are not biologically active. Both Figures 5 and 6 pinpoint March as
367 the month experiencing the least CH₄ uptake at $-0.45 \pm 1.70 \text{ mg/m}^2\text{d}^{-1}$, while February and

368 January follow behind as the months with the second and third least amount of CH₄ uptake, –
369 $0.67 \pm 0.88 \text{ mg/m}^2\text{d}^{-1}$ and $-1.30 \pm 1.29 \text{ mg/m}^2\text{d}^{-1}$ respectively. As Figure 9 demonstrates, cold
370 temperatures decrease the ability of a soil to take up CH₄.

371 Although the same pattern of increasing CH₄ uptake during warm months and decreasing CH₄
372 uptake during cold months is observed at this site every year, Figure 6 indicates that the
373 temperature change by season is not the only driving force behind these changing fluxes. Both
374 CH₄ and CO₂ fluxes show substantial seasonal hysteresis between C gas fluxes and air
375 temperature, suggesting that the temperature sensitivity of gas fluxes varies across the year.
376 Months in the fall with average air temperatures similar to those of spring months exhibit more
377 CH₄ uptake. This is due to CH₄ uptake being physically limited by diffusion in the early spring.
378 Spring snowmelt contributes to muddy soil conditions in the early spring which fills the pore
379 space of the soil with water, limiting the amount of atmospheric gas exchange that can occur
380 between the atmosphere and soil (Bowden et al, 1998). Seasonal changes involve interactions
381 between temperature and moisture, forcing soil CH₄ uptake to respond along both gradients
382 (Bowden et al, 1998). As the soil dries over the season, temperatures warm, and vegetation
383 begins to grow, there is more available pore space for atmospheric CH₄ to move into (Castaldi
384 and Fierro, 2005). As a result, more soil CH₄ uptake occurs. The physical limitations of soil pore
385 space, as determined by moisture levels, result in less CH₄ uptake in the spring than in the fall
386 despite the average monthly air temperatures being similar. Methane uptake at this site in 1989
387 and 1990 exhibited the same seasonal pattern where CH₄ uptake increased in the fall relative to
388 spring uptake levels, despite having the same average monthly air temperature (Crill, 1991).

389 The hysteresis of CO₂ in Figure 6 shows a similar pattern to that of CH₄ where emissions are
390 highest in the three warmest months (June, July, and August), and lowest in the cold months. The

391 largest increase in CO₂ emissions occurs between May and June, jumping from a monthly
392 average of $1.69 \pm 1.08 \mu\text{mol}/\text{m}^2\text{s}^{-1}$ to $4.643 \pm 3.46 \mu\text{mol}/\text{m}^2\text{s}^{-1}$. After this increase, the emissions
393 levels remain higher throughout the fall despite monthly average air temperatures being
394 comparable to spring temperatures. 1989 and 1990 data from this site follow the same shape as
395 the hysteresis in Figure 6; the largest increase in monthly average CO₂ emissions occurred
396 between May and June, with emissions maximizing in July (Crill, 1991). The driving forces
397 behind this phenomenon include the accumulation of soil organic matter, the growth of the
398 microbial communities performing heterotrophic respiration, and phenological changes in the
399 rhizosphere over the season (Lloyd and Taylor, 1994). As the number of microbes performing
400 decomposition increases along with the amount of material available to be decomposed, CO₂
401 emissions in this system increase (Aronson et al. 2010). Temperature and soil moisture are
402 essential in this process because they facilitate the other changes that result from seasonality.

403 **4.3 Change Over Time**

404 Average annual precipitation in College Woods has not significantly changed since this study
405 began in 1989 (Figure 2A). Average daily maximum and minimum temperatures have not
406 significantly changed over this time period either (Figure 2B). Therefore, any variation in annual
407 CH₄ uptake between the beginning of this study and the most recent measurements cannot be
408 explained by these two factors alone.

409 Cumulative precipitation in 1989 was 1094.5 mm and in 2021 it was 1236.1 mm, with respective
410 growing season average CH₄ uptake rates of $-2.47 \pm 0.35 \text{ mg m}^2\text{d}^{-1}$ and $-3.27 \pm 0.27 \text{ mg m}^2\text{d}^{-1}$.
411 Neither the annual precipitation sums, nor the annual average CH₄ uptake rates have changed
412 significantly between these two years, indicating that any change in the CH₄ uptake rate is not

413 fully explained by the change in precipitation. However, increased soil moisture resulting from
414 increased precipitation does impact the ability of a soil to consume CH₄ (Bowden et al. 1998).
415 July 2021 received heavy rainfall, totaling 323.0 mm. This is more than three times the average
416 rainfall total for July 1989-2001 of 101.3 mm. The CH₄ soil uptake of July 2021 was $-2.87 \pm$
417 $1.02 \text{ mg m}^2\text{d}^{-1}$ compared to the $-3.77 \pm 1.88 \text{ mg m}^2\text{d}^{-1}$ average uptake in July from all other years
418 included in this study. Decreased CH₄ uptake in July of 2021 as compared to July in every other
419 year of the study can reasonably be attributed to this large quantity of precipitation that the study
420 site received, due to the relationships between soil moisture, diffusion, and CH₄ uptake described
421 in the previous section.

422 Figure 7 plots the trend of CH₄ uptake throughout this study and compares it to a synthesis of
423 over 300 studies reporting on CH₄ uptake rates. This synthesis found significant decreases in
424 CH₄ uptake rates over the past three decades both globally between 30-60 degrees North and at
425 two sites: one in Baltimore and one nearby in NH – Hubbard Brook (Ni and Groffman 2018). In
426 contrast, College Woods did not exhibit a significant decrease in CH₄ uptake between 1989 and
427 today, and there is an insignificant increasing trend in CH₄ uptake in the 1989 – 2001 data from
428 College Woods. Ni and Groffman (2018) found that increases in soil moisture were underlying
429 the decreases in CH₄ uptake at Hubbard Brook, as the site has experienced increasing soil
430 moisture over the past 14 years due to the trend of increasing precipitation in the United States
431 (Groffman et al., 2012). In the analysis of College Woods data, precipitation was used as a proxy
432 for soil moisture. However, no such significant increase in precipitation was observed at this site.

433 No significant changes in CO₂ release from College Woods have been recorded over the 1989-
434 2001 period. As soil respiration is primarily controlled by temperature and moisture, significant
435 temporal changes of these two factors are expected for there to be significant change in CO₂

436 emissions. Lloyd and Taylor (1994) conclude that although increases in temperature will
437 decrease the activation energy for respiration, warming temperatures will have an unknown
438 effect on the size of the soil carbon pool. As a result, predictions of change in soil respiration in
439 response to warming temperatures cannot accurately be made.

440 **5. Conclusions**

441 Measurements of CH₄ uptake and CO₂ emissions in College Woods between June and October
442 2021 are consistent with the same flux measurements made June-October 1989-2001 at this site,
443 suggesting that carbon gas cycling is controlled by recurring and long-term climatic trends.
444 Unlike several of the studies synthesized by Ni and Groffman (2018), annual cumulative
445 precipitation and average annual maximum temperatures did not significantly change between
446 1989-2021. Perhaps then, it is the changing length of the seasons that alters CH₄ uptake and CO₂
447 emissions under climate change, not strictly the annual temperature and precipitation averages.

448

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452 the 2021 measurements I would like to thank the Hamel Center for Undergraduate research for
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Table 1: Monthly Averages for 1989-2001 and 2021.

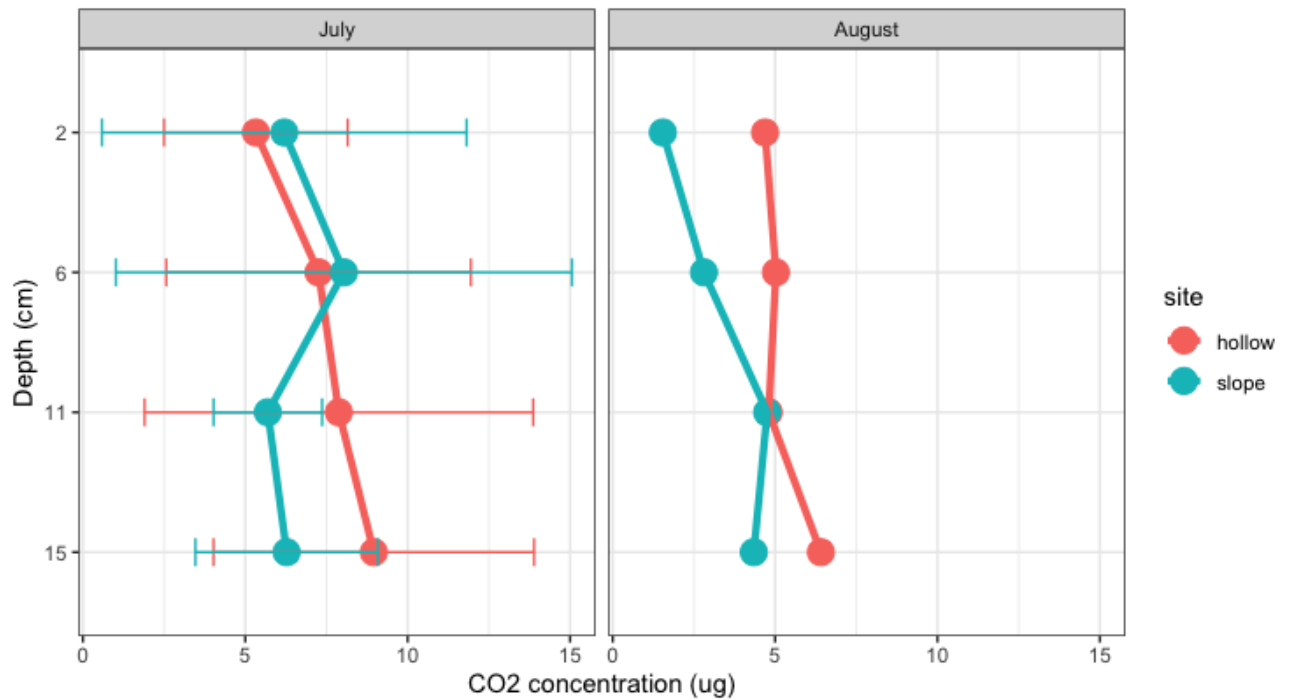
Year	Month	CH ₄ mg m ² d ⁻¹	CH ₄ SD	CO ₂ μmol/m ² s ⁻¹	CO ₂ SD	Daily precip. (mm)	Daily precip. SD
1989	February	0.0375	0.26056	NA	NA	2.4321429	5.19865
1989	March	-0.04	0.1249	NA	NA	1.9483871	4.983564
1989	April	-1.1625	0.692382	NA	NA	3.0066667	6.505326
1989	May	-1.89	0.40025	NA	NA	4.7064516	10.30825
1989	June	-2.05143	0.868877	3.190795	0.657404651	4.0633333	8.144661
1989	July	-2.1775	1.393542	3.211119	0.701858804	3.4548387	6.624995
1989	August	-2.74667	1.32278	2.979736	0.522584346	2.7290323	4.831024
1989	September	-2.515	0.476519	2.356635	1.342468	3.8333333	8.604623
1989	October	-2.84667	1.01964	1.784251	0.715886015	4.0354839	7.562696
1989	November	-2.056	0.542468	0.91527	0.5609618	4.1766667	7.115388
1990	January	-0.13	0.014142	0.138021	NA	1.783871	2.885261
1990	February	-0.225	0.714178	0.240777	NA	2.4571429	4.775356
1990	March	-0.23	0.255995	0.931167	0.226849131	1.2387097	3.04507
1990	April	-0.80714	0.914362	1.009851	0.437640748	4.21	9.692989
1990	May	-1.41667	0.641176	1.368506	0.425882302	5.0483871	8.2299
1990	June	-2.695	0.439735	2.398005	0.972515032	1.8233333	3.572373
1990	July	-2.9225	0.102103	4.608566	1.606113471	2.1483871	6.214868
1990	August	-2.73	1.129779	3.442186	1.241424396	4.6225806	10.69158
1990	September	-3.505	1.491995	3.629508	1.452082571	1.4833333	3.337259
1990	October	-2.54167	0.60496	2.145386	0.899470593	6.7451613	14.47204
1990	November	-2.2025	0.909299	1.392827	0.690386688	3.7066667	10.84003
1990	December	-2.352	1.371831	0.737017	0.186507056	3.1870968	9.763461
1991	February	-0.31	0.028284	0.373285	0.030882743	2.0214286	7.241514
1991	March	-1.5375	0.321079	0.292304	0.150653033	3.2064516	6.414044
1991	April	-1.855	0.855798	1.067414	0.351861709	2.99	9.710405
1991	May	-2.14125	1.069291	1.610063	0.770265456	2.6032258	7.282741
1991	June	-2.57625	0.787871	2.688225	0.880916185	1.9	3.471559
1991	July	-4.051	1.094942	2.961695	1.111760812	2.3387097	5.787727
1991	August	-2.63333	1.371111	2.887474	0.337401488	7.6354839	25.31735
1991	September	-3.315	1.678145	2.632532	0.946281368	5.3533333	13.29814
1991	October	-2.82778	1.234704	2.116942	0.707435382	2.5387097	5.041804
1991	November	-2.44375	0.883757	1.448224	0.49925161	3.7366667	9.579665
1991	December	-1.06	1.164446	0.688796	0.751654781	2.3645161	4.809473

1992	January	-0.6825	0.723389	0.359139	0.014472883	1.9935484	5.296536
1992	February	0.4	0.757661	0.329549	0.144490965	1.8655172	6.505507
1992	March	-0.005	0.205994	0.343734	0.037612	2.6677419	6.075546
1992	April	-1.36571	0.915639	0.83192	0.375001925	2.02	7.094213
1992	May	-1.6325	0.703675	0.980025	0.415669049	0.9741935	2.72641
1992	June	-2.26286	0.656194	2.251858	0.829165628	3.8533333	11.88325
1992	July	-3.145	2.523671	4.963612	0.870164848	3.3677419	6.681287
1992	August	-2.18375	1.459079	4.09126	0.842192694	2.9322581	7.030144
1992	September	-2.94875	1.045076	3.175324	1.231401231	2.32	5.294526
1992	October	-2.23625	1.059001	1.559583	0.562409846	2.7774194	8.183834
1992	November	-1.455	0.829861	0.474156	0.209289103	4.4	7.459685
1992	December	-0.978	0.364925	0.448232	0.341424861	2.0096774	3.932883
1993	January	-1.935	2.38295	2.962932	4.00777786	1.3258065	2.9759
1993	February	-0.775	0.547996	0.187662	0.048854156	2.5142857	6.248301
1993	March	1.65	1.713651	0.935422	NA	3.3107143	6.922927
1993	April	-0.43	NA	0.799945	0.348405001	4.06	6.528854
1993	May	-2.7	0.933809	1.291629	0.545251649	0.6	1.852566
1993	June	-2.622	0.52756	2.66569	0.973564683	2.0666667	4.694482
1993	July	-3.17667	0.4121	2.158508	1.317372348	1.2225806	3.127375
1993	August	-3.913	0.974133	2.618022	1.099871803	1.8096774	5.34555
1994	January	-1.885	1.821529	1.699935	2.030204781	3.3354839	8.139719
1994	February	-1.27333	0.877515	1.267241	0.865655247	1.4535714	4.686741
1994	March	-6.65	NA	1.900501	0.95329641	4.4741935	11.01148
1994	May	NA	NA	1.268962	0.713315459	3.2935484	7.163888
1994	June	-1.91714	0.857763	6.622719	5.450988796	1.4633333	3.044439
1994	July	-2.5675	1.079703	2.929273	2.003876545	1.8258065	4.621397
1994	August	-3.33667	2.438059	2.482294	1.521335924	3.3193548	8.830758
1994	October	-1.4825	0.326943	1.283369	0.679655358	0.1548387	0.616354
1994	November	-2.685	0.558614	1.082633	NA	2.4366667	6.803421
1994	December	-2.1825	0.421298	NA	NA	4.5451613	12.90023
1995	January	-0.88	0.575674	NA	NA	3.6451613	7.370429
1995	February	-1.42167	0.878258	0.831666	0.653980624	2.5	5.686827
1995	March	-1.1125	1.41394	1.222998	0.775734581	1.5322581	3.119336
1995	April	-1.1875	0.38187	0.893952	0.31157878	1.57	3.691477
1995	May	-1.5925	0.324487	1.91139	1.56047786	2.2451613	3.897421
1995	June	-1.775	0.776681	3.246671	0.865745087	1.5833333	3.385373
1995	July	-3.84	1.901911	4.09981	1.616196258	3.1096774	6.424918
1995	August	-2.84	0.212132	6.378853	1.230203508	2.2258065	5.957515
1995	September	-3.6275	1.653972	1.965458	1.027273779	2.36	5.928633

1995	October	-3.11	0.937408	3.096709	0.828347537	5.3516129	13.4586
1995	November	-1.745	0.900907	1.797399	0.382224365	6.2766667	15.42914
1995	December	-1.62	0.127279	0.874509	NA	2.0193548	3.851962
1996	January	NA	NA	0.442898	0.162719951	2.2419355	3.742617
1996	February	-0.98	0.254558	3.681421	4.842945654	1.3931034	3.284045
1996	April	-2.76	2.220315	1.404805	0.007371624	5.63	11.19622
1996	May	-1.895	1.859691	1.94609	NA	3.3129032	5.807968
1996	June	-2.42875	0.955173	4.013209	2.24262316	1.5733333	3.406478
1996	July	-2.877	1.282948	4.118529	1.325513747	5.7	14.80861
1996	August	-3.01833	0.827126	3.870806	1.643502578	0.6903226	3.23031
1996	September	-2.52	1.66319	3.862869	1.123072182	3.05	6.454816
1996	October	-3.63	1.007472	2.79048	0.395563409	9.7935484	32.16386
1996	November	-2.355	0.580026	1.242397	0.598539571	1.6233333	5.175651
1996	December	-2.25	1.241639	0.870957	0.20703692	4.6344828	9.348311
1997	January	-1.1975	0.860518	0.361025	0.12949858	3.1322581	7.725473
1997	February	-1.43	0.569298	0.198115	0.117041341	1.9464286	5.251348
1997	March	-0.3375	0.302366	0.245408	0.127548334	3.662069	6.443236
1997	April	-0.44	0.098995	1.534992	1.412535273	5.01	12.82071
1997	May	-1.835	0.827315	1.535235	1.26767426	2.0064516	3.679759
1997	August	-4.025	0.190919	5.235967	0.852818719	3.0580645	7.79516
1997	September	-3.315	1.718269	4.165456	0.663852056	1.76	5.243097
1997	October	-3.55	1.004092	2.70386	0.901412557	0.6709677	2.109849
1997	November	-2.36	1.25865	1.298292	0.065781022	5.6133333	16.67643
1997	December	-2.92	1.880904	0.667542	0.672592645	2.6258065	6.734487
1998	April	-2.195	1.760696	0.711163	0.094210069	1.72	5.246963
1998	June	-4.24	1.661525	11.02045	5.426735258	10.1785714	24.47158
1998	July	-4.493	1.674827	13.1625	3.646705706	1.6967742	3.921265
1998	August	-4.67	1.288798	6.451802	1.603137552	2.2258065	5.665272
1998	September	-5.38	2.856711	4.008105	0.152216691	1.62	4.786576
1998	October	-4.21	2.489016	4.111915	0.572209411	4.1903226	9.702967
1998	November	-4.74	3.761808	1.714682	1.090431578	1.81	4.924488
1999	May	-3.275	0.629325	5.065525	0.100525921	2.6967742	5.496149
1999	June	-6.729	2.185622	6.387628	3.783039809	0.76	2.525812
1999	July	-6.74833	1.262734	6.245494	1.629037959	1.9806452	5.067769
1999	September	-4.5225	2.733354	4.504467	0.913226602	7.8833333	21.08873
1999	October	-5.225	3.585031	1.965197	0.190357834	3.1516129	7.343608
2000	May	-4.22	3.493108	1.490399	1.068800029	2.862069	6.49029
2000	June	-3.58	1.578647	7.363118	4.110051852	5.2526316	11.77414
2000	July	-3.79	1.591639	7.697246	2.639432365	4.0258065	10.21427

2000	August	-4.106	1.64687	7.292967	2.791502558	2.0258065	5.943791
2000	September	-5.045	1.775339	4.331621	2.564833434	2.8066667	6.961418
2000	November	-6.765	1.011163	7.563427	3.361322828	4.0433333	8.488153
2001	January	-2.965	1.393	1.250528	1.092403363	0.4068966	1.496169
2001	February	-0.74	0.141421	1.527112	1.838066781	0.8307692	2.533104
2001	May	-4.54333	1.17449	2.385948	0.79490355	1.016129	3.025018
2001	June	-3.7325	1.60367	6.58646	1.059329825	4.48	9.766177
2001	July	-4.7675	3.390874	4.963875	0.604774332	2.5903226	4.872806
2001	August	-5.58333	0.992891	NA	NA	1.0645161	2.728681
2021	June	-3.16389	0.923511	2.535705	0.933778357	0.9466667	1.997711
2021	July	-2.86833	1.016159	2.965024	0.892544416	10.4193548	18.70167
2021	August	-3.40333	1.575991	3.091798	0.899509237	3.0870968	6.792042
2021	September	-3.34667	1.255173	NA	NA	3.59	12.01942
2021	October	-3.5875	1.051468	NA	NA	5.383871	16.02881

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534 Figure 1: Soil gas profile of CO₂ concentrations. Each point represents the monthly mean value for soil gas CO₂

535 concentration at each depth, with error bars showing ± one standard deviation of the mean.