

1 **Effects of Disturbance on Seasonal CO₂ Dynamics in Two Boreal Forest Sites** 2 **Underlain by Permafrost**

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10 **Abstract.** Permafrost regions in subarctic and arctic areas harbor substantial carbon reserves, which are
11 becoming increasingly vulnerable to microbial decomposition as soils warm. As the seasonally thawed
12 active layer deepens and anthropogenic disturbances escalate, accurately predicting carbon fluxes from
13 disturbed environments underlain by permafrost requires a comprehensive understanding of soil
14 respiration dynamics. This study investigated the impact of surface disturbance on seasonal soil biological
15 properties in a boreal forest ecosystem near Fairbanks, Alaska. Further, we sought to identify the key
16 environmental and geochemical factors influencing soil biology in the undisturbed and disturbed soils.
17 Our results revealed a substantial rise in soil respiration at the disturbed boreal forest site, which exhibited
18 a 14.4% overall increase in CO₂ efflux compared to the undisturbed site. This effect was most pronounced
19 during the summer, when the increase in CO₂ efflux peaked at 20%. This heightened respiratory activity
20 was directly linked to significantly warmer soil conditions, with the mean annual soil temperature at the
21 disturbed site measuring 0.60 ± 0.16 °C, in stark contrast to the sub-zero temperatures of -0.37 ± 0.08 °C
22 at the undisturbed site. Furthermore, the disturbed site had 30% higher bacterial community richness, 1%
23 higher total mean C and 0.03% higher total mean N concentration levels, and 11.9% higher pH values in
24 the subsoil layer, as well as a 147% deeper maximum active thaw depth, suggesting potential controls
25 underlying the variation in CO₂ efflux. Our research underscores the essential importance of considering
26 the rise in carbon emissions from anthropogenically disturbed soils underlain by permafrost, which are
27 frequently neglected in assessments of the carbon cycle. This study contributes to a deeper understanding
28 of the complex interactions governing soil respiration in disturbed permafrost environments, ultimately
29 informing more accurate predictions of carbon fluxes in these ecosystems.

30 **1. Introduction**

31 Soil respiration, the process by which carbon dioxide (CO₂) is released from the soil surface to
32 the atmosphere, is a critical component of the global carbon cycle. This process encompasses the

33 microbial breakdown of organic material as well as the respiration of plant roots. Understanding soil
34 respiration dynamics is particularly crucial in boreal forests, as they comprise approximately 30% of
35 global forested area and play a vital role in global carbon sequestration (Bonan, 2008; Pan *et al.*, 2011;
36 Chi *et al.*, 2021). Recent studies indicate that increasing temperatures could lead to boreal forests
37 transitioning from functioning as carbon sinks to becoming carbon sources (Bond-Lamberty *et al.*, 2018;
38 Marty *et al.*, 2019, Harel *et al.*, 2023). In boreal forests, soil respiration is estimated to contribute up to
39 68% of the total ecosystem respiration (Parker *et al.*, 2020; Watts *et al.*, 2021) and is significantly
40 impacted by changes in soil temperature, soil moisture, the microbial community, and vegetation type
41 (Grace, 2004; Fekete *et al.*, 2014; Rodtassana *et al.*, 2021). Soil respiration in permafrost regions is also
42 influenced by the thickening of the active layer (the seasonally thawed soil surface layer) that occurs
43 when permafrost is degraded due to warmer temperatures (Koster *et al.*, 2017; Turetsky *et al.*, 2020; Watts
44 *et al.*, 2021).

45 Anthropogenic (e.g., trail development, firewood harvesting) and natural (e.g., wildfires, insects
46 and pathogens) disturbances fundamentally alter the landscape and soil properties, which in turn governs
47 soil respiration and the stability of massive permafrost carbon stores (Schepaschenko *et al.*, 2025; Miner
48 *et al.*, 2022). The primary disturbance agents—fire, timber harvesting, and insect outbreaks—have
49 intensified, impacting millions of hectares annually (Schepaschenko *et al.*, 2025). Wildfires are the
50 dominant natural disturbance in boreal forests, and their frequency and scale have grown significantly in
51 the recent years (Zhu *et al.*, 2022; Schepaschenko *et al.*, 2025). An intensified heat flux can thicken the
52 active layer (Yoshikawa *et al.*, 2002; Koster *et al.*, 2017 and 20187; Zhu *et al.*, 2022) to the point where it
53 no longer refreezes completely in the winter, leading to the formation of a talik, or a year-round unfrozen
54 layer of soil above the permafrost, making carbon available to microorganisms year round (Yoshikawa *et al.*
55 *et al.*, 2002; Zhu *et al.*, 2022).

56 Anthropogenic disturbances in boreal forests alter carbon cycling through distinct short- and
57 long-term phases that are primarily driven by impacts to the underlying permafrost. Initially, disturbances
58 like forest harvesting can cause a temporary decrease in total soil CO₂ efflux by eliminating root
59 (autotrophic) respiration (Akande *et al.*, 2023; Schepaschenko *et al.*, 2025). However, this short-term
60 effect is overshadowed by the dominant, long-term consequences of physical changes to the ground's
61 thermal regime. Activities such as land clearing for infrastructure and resource exploration remove the
62 insulating vegetation and surface organic layers, leading to warmer soil temperatures and a deeper active
63 layer (Forbes *et al.*, 2002; Foster *et al.*, 2022). This permafrost degradation exposes vast stores of
64 previously frozen organic carbon to microbial decomposition, transforming the disturbed landscape into a
65 persistent, long-term source of atmospheric CO₂ (Koster *et al.*, 2018; Miner *et al.*, 2022). As ecosystems

66 recover, the combination of this sustained decomposition with new root respiration can lead to total soil
67 efflux rates that ultimately exceed those of undisturbed forests (Akande et al., 2023). Consequently, these
68 human disturbances are a critical factor that accelerates the permafrost carbon feedback, significantly
69 impacting the overall carbon balance of the circumpolar north (Schepaschenko et al., 2025; Miner et al.,
70 2022).

71 Despite their importance, the cumulative impacts of these anthropogenic disturbances on
72 landscape and soil properties are not well understood, often being overshadowed by the more extensively
73 studied effects of wildfire (Foster et al., 2022). Therefore, investigating soil respiration in disturbed,
74 permafrost-affected environments is crucial for assessing the resilience and vulnerability of boreal
75 ecosystems. Under projected climate scenarios, the frequency and intensity of disturbances in these
76 regions are expected to increase, potentially leading to significant changes in soil carbon fluxes
77 (Schepaschenko et al., 2025). Further, insights gained from such studies can inform forest management
78 practices aimed at mitigating the impacts of disturbances and preserving the carbon sequestration
79 potential of boreal forests.

80 The aim of this study is to measure and compare the soil respiration rates in undisturbed and
81 disturbed sites in a subarctic boreal forest. The disturbance was due to historical activities related to
82 mining, such as trail development and firewood harvest. These disturbances took place in the early 1920s,
83 coinciding with the construction of a drainage ditch and an access trail to support mining operations in the
84 area. During the trail and draining ditch development, the groundcover vegetations and surface soils were
85 disturbed, and the trees were harvested. Currently, there is no active drainage at the research site, and the
86 trail is seldom used. We hypothesize that the disturbance had a lasting effect on soil activity and
87 properties and there will be a significant difference in the measurements between the undisturbed and
88 disturbed sites. Further, we hypothesize that this trend also occurs in winter, where measurements are
89 severely lacking, though soils are active.

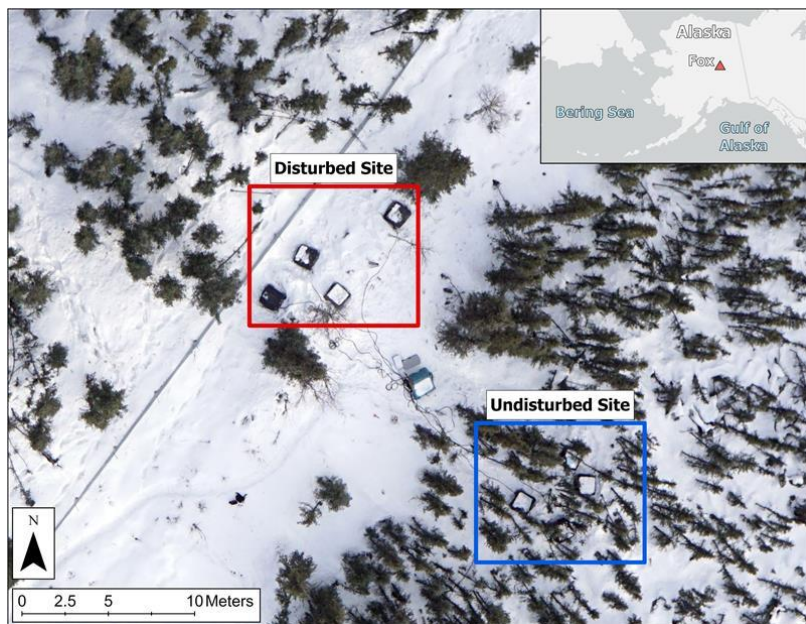
90 To reveal the fundamental controls driving variation in soil respiration within and between the
91 disturbed and undisturbed boreal forest sites, we examined seasonal soil CO₂ efflux alongside various
92 edaphic factors, including soil temperature, moisture content, soil organic matter (SOM), pH, and the
93 composition of microbial communities. To assess this relationship, a non-linear Random Forest Model
94 (RFM) was used in conjunction with regression analysis to analyze the time series data, which
95 encompassed variables such as soil respiration, temperature, moisture, and air temperature. Additionally,
96 R statistical ANOVA analysis was performed to evaluate soil characteristics, particularly pH and SOM, as
97 well as the composition of soil microbial communities. The emissions data and estimates of carbon fluxes
98 will be helpful to improve carbon modeling efforts. In the following sections, we detail the study site and

99 our methodological approach (Section 2), present the key findings related to soil conditions and
100 respiration (Section 3), and discuss the implications of these findings for understanding carbon cycling in
101 disturbed permafrost ecosystems (Section 4).

102 2. Materials and Methods

103 2.1 Site description

104 The study was conducted at two adjacent sites underlain by permafrost in a subarctic boreal
105 forest, of the discontinuous permafrost region, located at the U.S. Army Cold Regions Research and
106 Engineering Laboratory (CRREL) Permafrost Research Tunnel Facility in Fox, Alaska (64.9507 N -
107 147.6200 W, 248 m a.s.l.). The region experiences a continental climate, which is defined by an average
108 annual air temperature of $-2.4\text{ }^{\circ}\text{C}$, with average temperatures in July reaching $16\text{ }^{\circ}\text{C}$ and January
109 temperatures averaging $-21.9\text{ }^{\circ}\text{C}$; extreme temperatures throughout the year can range from $-51\text{ }^{\circ}\text{C}$ to
110 $38\text{ }^{\circ}\text{C}$ (Jorgenson et al. 2020). The two sites were situated approximately 10 m apart (Figure 1) and
111 exhibited comparable topography and parent material. The first site consists of an undisturbed black
112 spruce forest ecosystem (SI Figure S1); the second consists of an anthropogenically disturbed area where
113 trails were established, and black spruce tree cover was removed in firewood harvests during mining
114 activities in the region in the 1920s.



115

116 **Figure 1.** Aerial view of the study sites and experimental setup. The image displays the geographical
117 location and the layout of the sample plots at the undisturbed (blue rectangle) and disturbed (red

118 rectangle) study sites. The aerial image was captured on March 18, 2023. (Image credit: Dr. David
119 Brodylo).

120 The vegetation at the undisturbed site consists of small black spruce (*Picea mariana*) ranging
121 from densely distributed to tightly spaced. Understory canopy is dominated by marsh and bog Labrador
122 tea (*Rhododendron tomentosum*; *groenlandicum*). Forest floor cover is primarily mosses (i.e., feather
123 mosses and *Sphagnum spp.*) and small shrubs including lowbush cranberry (*Vaccinium vitis-idaea*). The
124 disturbed site is characterized by scattered birch (*Betula neolaskana*) and tall black spruce (*Picea*
125 *mariana*) cover. The understory canopy is primarily dwarf shrubs including marsh Labrador tea and bog
126 blueberry (*Vaccinium uliginosum*). The ground surface cover is dominated by grasses (*Poaceae*) and
127 sedges (*Cyperaceae*). The typical undisturbed soil profile at this site consists of a fibric organic layer of
128 variable thickness, which primarily contains undecayed and partially decayed moss and forest litter
129 materials (O horizon), underlain by an organic-rich mineral material layer containing hemic-sapric
130 organic fraction (A/B horizon), and thick accumulations of mineral material at the base (B/C horizon).
131 Disturbance seemingly impacts the thickness of the surface organic layer. Soil sampling at each site
132 targeted soil materials below the organic horizon. The sampled soil material at the two sites were
133 classified as mineral soil material (<20% organic matter [OM]; Soil Survey Staff, 2022) with a
134 subdivision of more organic-rich fractions (10-18% OM) comprising a “topsoil” layer (akin to an A
135 horizon) that starts below the organic layer (O horizon), and more mineral-rich material (<5% OM)
136 comprising a “subsoil” layer. The topsoil textures ranged from loam to silt loam, reflecting a higher
137 proportion of sand particles in the topsoil relative to the silt loam-textured subsoil (SI Table 1).

138 **2.2. Installation of soil and meteorological sensors**

139 Four plots were established at an undisturbed site, and the other four were located at an adjacent
140 disturbed site (Figure 1). At each site, sensors were distributed to measure total soil respiration
141 (autotrophic and heterotrophic), temperature, and volumetric water content (VWC) every 30 minutes from
142 November 4, 2022, to November 9, 2023. During the same period, air temperature and barometric
143 pressure were recorded every 15 minutes. Because some of the sensors have robotic arms to collect
144 measurements that would be impeded by snowfall in the winter, Costco folding tables (Columbus,
145 Indiana) with a surface of 91.4 cm × 91.4 cm were situated above the plots, during that period, to prevent
146 snow accumulation (Figure 2b). The folding tables were removed at the onset of the snow-free season to
147 reduce interference with natural environmental factors such as rain and solar radiation (Figure 2c).

148 Soil temperature, VWC, air temperature, and barometric pressure were measured using the
149 following Onset HOBO (Onset, Bourne, Massachusetts, USA) instrumentation: U30 USB Weather
150 Station, S-TMB-M002 12-Bit Temperature Smart Sensor, S-SMC-M005 EC5 Soil Moisture Smart

151 Sensor, S-THC-M002 Temperature/Relative Humidity Smart Sensor, and S-BPB-CM50 Smart
152 Barometric Pressure Sensor. Soil temperature and VWC were recorded in the topsoil and subsoil layers,
153 approximately 30 cm from each soil respiration sensor, at a depth of 18.5 ± 1.19 cm in the topsoil layer
154 and 34.5 ± 1.47 cm in the subsoil layer for the undisturbed site. At the disturbed site, the measurements
155 were taken at 13.5 ± 2.53 cm in the topsoil layer and 35.5 ± 1.85 cm in the subsoil layer. These depths
156 were measured from the top of the ground vegetation cover (the moss surface). At both sites, the
157 temperature and VWC sensors of the topsoil were installed below the fibric organic layer that had a
158 thickness of 16.5 ± 1.19 cm at the undisturbed site and 9.5 ± 0.87 cm at the disturbed site. The differences
159 in sensor placement depth were influenced by the variations in the thickness of the organic layer between
160 the two sites (SI Figure S2). VWC measurements were restricted to the summer and autumn seasons due
161 to the sensor's inability to measure below freezing temperatures. Air temperature and barometric pressure
162 were measured at 2 m above the substrate surface.

163 The depth of thaw in the active layer was assessed at ~ 10 cm from each of the eight chamber
164 plots during each site visit ($n = 160$) from 12 May to 3 Oct 2023. To determine this depth, a graduated
165 metal rod with a diameter of 1 cm (known as a frost probe) was inserted into the ground, at the same
166 location, until it met resistance, establishing the distance between the ground surface vegetation and the
167 top of the frozen soils (Shiklomanov *et al.* 2013).

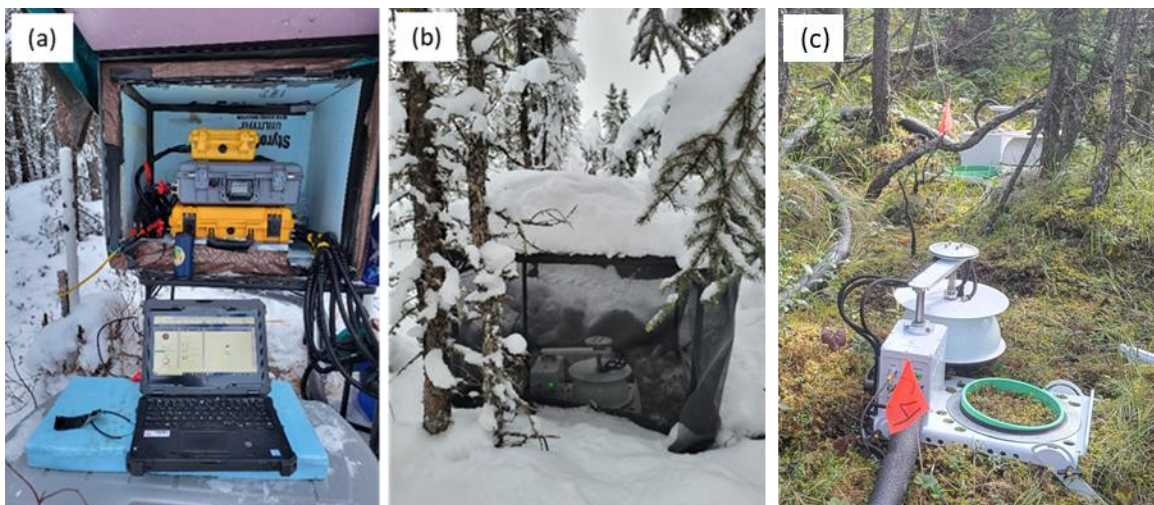
168 For the soil respiration measurements, which encompasses the overall release of CO_2 from the
169 soil into the atmosphere (including both autotrophic and heterotrophic processes), we installed 21.3 cm
170 diameter thick-walled polyvinyl chloride (PVC) collars (LI-COR inc., Lincoln, Nebraska, USA) in the
171 center of each plot. They were a height of 11.4 cm and were inserted 2-3 cm into the soil through the soil
172 vegetation cover. The collars spacing varied from two to four meters at the undisturbed site and from
173 three to eight meters at the disturbed site (Figure 1). We installed a 8200-104 Opaque Long-Term
174 Chamber (LI-COR inc., Lincoln, Nebraska, USA) above each collar, which yielded a total of eight. The
175 chambers were connected via 15 m long tubing and cable assembly to a LI-8250 multiplexer (LI-COR
176 inc., Lincoln, Nebraska, USA) that linked to a LI-870 $\text{CO}_2/\text{H}_2\text{O}$ gas analyzer (LI-COR inc., Lincoln,
177 Nebraska, USA). The cable and tubing assembly was wrapped in 1.3 cm thick tubular pipe insulation
178 foam to prevent internal clogging from freezing moisture to ensure data collection in winter.

179 **2.3 Respiration Data Collection**

180 The measurement process followed a closed-chamber dynamic methodology, which was
181 automated and controlled by the LI-8250 Multiplexer. At 30-minute intervals, the multiplexer would
182 initiate a measurement at one of the chamber locations. This involved the 8200-104 chamber

183 automatically rotating and lowering to seal onto the pre-installed PVC soil collar, creating a closed
184 headspace over the soil surface. Once the chamber was sealed, the LI-8250 directed a closed loop of air
185 from the chamber headspace to the LI-870 and LI-7810 analyzers and back. The gas analyzers measured
186 CO₂ and H₂O concentrations, for a period of 120 seconds, at a rate of 1 Hz. As CO₂ was respired, its
187 concentration within the closed system increased over time. The soil CO₂ flux was calculated from the
188 rate of this concentration increase. After each measurement cycle, the chamber automatically opened, and
189 the system proceeded to the next chamber in the sequence. A more detailed description of the soil gas flux
190 system's operation and these winter modifications can be found in Vas et al. (2023).

191 Throughout the year, we performed site visits on a near-weekly basis to download data and
192 maintain the equipment. Standard maintenance involved visually inspecting each chamber and clearing
193 the seals of any debris. During winter months, this was supplemented by ensuring the protective tables
194 remained free of deep snow, a necessary step to allow for sufficient air exchange to ensure the accuracy of
195 the measurements.



196

197 **Figure 2.** Custom modifications for LI-COR Soil gas flux system in cold climates. Enclosures were
198 designed to ensure optimal operating temperatures (a) (photo credits: Dragos A. Vas, 17 November 2023),
199 insulated tubing for instruments to prevent clogging, and long-term covers for chambers (b) (photo
200 credits: Dragos A. Vas, 13 January 2023) to inhibit snow accumulation or drifting on the chambers. Snow
201 free season photo of the chamber (c) (photo credits: Dragos A. Vas, 13 August 2023); the long-term
202 covers were removed at the beginning of the snow free season.

203 2.5 Soil collection and property analysis

204 Soil samples were collected from both the topsoil and subsoil layers across all plots in the autumn
205 (September 2022), winter (February 2023), and summer (June 2023) seasons to analyze potential
206 variations in microbial community composition among seasons, disturbance regimes, and soil layers. The

207 samples were collected at identical depths during each season, which coincided with the depth at which
208 the soil temperature and moisture probes were positioned; these depths varied for each plot according to
209 the organic layer thickness. The winter samples were acquired using a gas-powered SIPRE (Snow, Ice,
210 and Permafrost Research Establishment) corer (Jon's Machine Shop, Fairbanks, Alaska, USA). Nitrile
211 gloves were utilized to minimize any potential contamination to the cores. Furthermore, the SIPRE corer
212 and all associated tools were thoroughly sanitized with 70% isopropyl alcohol, DNA away, and RNase
213 away (Thermo Fisher Scientific in Waltham, MA, USA). The cores were then subsampled into
214 approximately 5 cm long cylinders using a sanitized hammer and chisel and were carefully placed into
215 sterile Nasco™ Whirl-pak bags (Thermo Fisher Scientific, Waltham, MA, USA); further information on
216 this sampling method can be found in Barbato *et al.* (2022). Summer and autumn soil samples were
217 gathered using a sanitized trowel with 70% isopropyl alcohol, DNA away, and RNase away. The samples,
218 approximately 5 cm thick, were placed in sterile Nasco™ Whirl-pak bags and immediately placed in a
219 cooler with frozen ice packs, then transferred to a freezer upon arrival at the Cold Regions Research and
220 Engineering Laboratory in Fairbanks, Alaska (CRREL-AK). All collected soil samples were kept at a
221 temperature of -25 °C until shipped to CRREL in Hanover, New Hampshire (CRREL-NH), where they
222 were stored at -20 °C until further processing. Deep freezing the soil samples immediately after collection
223 and maintaining this state until processing halts microbial growth and facilitates the preservation of the
224 microbial community structure at the moment of sampling (Baker et al., 2023; Doherty et al., 2020).

225 Loss on ignition (LOI) was measured as a proxy for SOM content (Storer, 1984) on all soil
226 samples. Here, LOI is the proportion of mass loss from oven-dried soil (dried at 105 °C for 24 hours)
227 following 2 hours at 360 °C in a muffle furnace. Soil total carbon and total nitrogen was measured via
228 combustion using a TruSpec C and N Analyzer (LECO, St. Joseph, MI, USA) at the University of
229 Wisconsin Soil and Forage Lab. Soil pH was measured from a 1:1 slurry of soil: CaCl₂ solution (0.01M)
230 using a pH probe (Hanna Instruments, Woonsocket, RI, USA) and a SevenEasy S20 pH meter (Mettler
231 Toledo, Columbus, OH, USA). Soil pH was converted to H⁺ concentration prior to taking an average or
232 statistical analysis. LOI total carbon, total nitrogen and soil pH were statistically analyzed using ANOVA
233 in R.

234 **2.6 Soil microbial DNA extraction, gene sequencing, and data analysis**

235 Soil was partially defrosted and homogenized in the sample bag prior to subsampling 250 mg into
236 bead beating tubes. Total genomic DNA was extracted using the DNeasy PowerSoil Pro Kit (Catalog No.
237 47014, Qiagen, Germantown, MD, USA), using a Precellys Evolution Touch homogenizer for the bead
238 beating step (Catalog number P002511-PEVT0-A.0, Bertin Technologies, Montigny-le-Bretonneux,

239 France). Automated DNA extraction was done with a QIAcube Connect (Catalog No. 9002864, Qiagen,
240 Germantown, MD, USA) and each extraction run included a blank. Extracted DNA was held at -20 °C.

241 Library preparation and sequencing was completed at Argonne National Laboratory (Lemont, IL,
242 USA), as follows. For bacterial analysis, the V4 region of the 16S rRNA gene was targeted for PCR
243 amplification with region-specific primers (forward primer 515F and reverse primer 806R); and for
244 fungal analysis, the ITS region was amplified using appropriate barcoded primers (Caporaso *et al.*, 2011;
245 Caporaso *et al.*, 2012; Apprill *et al.*, 2015; Parada *et al.*, 2016; Smith *et al.*, 2014; Walters *et al.*, 2016).
246 Each PCR reaction contained 1 µL template DNA, 12.5 µL AccuStart II PCR ToughMix (Quantabio,
247 Beverly, MA, USA), 1 µL forward primer with Golay barcode (5 µM concentration), 1 µL reverse primer
248 (5 µM concentration), and 9.5 µL DNA-free PCR water. PCR conditions were: 94 °C (3 minutes to
249 denature the DNA); 35 cycles of 94 °C (45 s), 50 °C (60 s), and 72 °C (90 s); final extension at 72 °C (10
250 minutes). PCR product was quantified using Quant-iT PicoGreen (P7589, Invitrogen, Waltham, MA,
251 USA). Equimolar amounts of amplicons were pooled, purified using AMPure XP Beads (A63881,
252 Beckman Coulter, Brea, CA, USA), quantified (Qubit, Invitrogen), and diluted to 6.75 pM using a 10%
253 PhiX spike. Paired-end 2 x 251 sequencing was done on a MiSeq (Illumina, San Diego, CA, USA).

254 Sequencing data were processed in R (R-Core-Team, 2018), using a dada2 v1.18.0 pipeline
255 (Callahan *et al.*, 2016), as in Baker *et al.* (2023), implemented using Snakemake v7.25.0 (Mölder *et al.*,
256 2021). Taxonomy assignment was based on the SILVA 138.1 reference database for 16S sequences (Quast
257 *et al.*, 2013; Yilmaz *et al.*, 2013) and the UNITE database (release 25.07.2023) for ITS sequences (Kõljalg
258 *et al.*, 2013; Nilsson *et al.*, 2019). Chloroplasts and mitochondria were excluded from the dataset. A total
259 of 2836 fungal ASVs and 10,608 bacterial ASVs were identified (excluding extraction blanks). Amplicon
260 sequences are in the National Center for Biotechnology Information Sequence Read Archive (NCBI
261 SRA), accession PRJNA1178745. Though our bacterial primers targeted both bacterial and archaeal 16S
262 rRNA, we will refer simply to bacteria, which comprise 99.8% of total reads. Of archeal reads, 80%
263 represented the phylum *Crenarchaeota*. One sample was excluded from analysis because it was
264 mislabeled (2023_Feb, Chamber 3, Organic).

265 R software (R-Core-Team, 2018), and *ggplot2* (Wickham, 2016) were used for data analysis and
266 visualization; the bioinformatic approach followed West *et al.* (2022). Community composition was
267 visualized using principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities (Bray and Curtis,
268 1957) generated using *avgdist* from the R package *vegan* (Oksanen *et al.*, 2024) using rarefaction (999
269 iterations) to a sampling depth of 16,300 for 16S and 6150 for ITS. A significant effect ($p < 0.05$) of
270 disturbance, sampling date, and interaction of these factors on community composition was tested within
271 each soil layer (topsoil and subsoil), using permutational multivariate analysis of variance

272 (PERMANOVA; *adonis2* from *vegan*) (Anderson, 2001). Richness was evaluated using weighted linear
273 regression (*betta* function in *breakaway* R package) (Willis *et al.*, 2017), and a significant effect of
274 disturbance tested via ANOVA. Differential abundance (*differentialTest* in the *corncob* package) (Martin
275 *et al.*, 2021) was then used to identify significant enrichment or depletion of individual taxa due to
276 disturbance, after excluding taxa with mean relative abundance < 0.00001.

277 **2.7 Linear regression analysis**

278 For statistical analysis, data on soil respiration, soil temperature, and VWC, as well as air
279 temperature and barometric pressure from the eight chamber plots (four located at the disturbed site and
280 four at the undisturbed site), were averaged to obtain daily means (n=333 per variable) and seasonal
281 means (n=4 per variable). Seasons were defined according to observed efflux seasonality at the research
282 site, a classification consistent with previous research on boreal forest efflux (Wats *et al.*, 2021): winter
283 (November to March, n=123), spring (April to May, n=57), summer (June to August, n=92), and autumn
284 (September to October, n=61). Using these processed data, we performed a series of linear regression
285 analyses to determine the existence of distinct, linear trends between CO₂ efflux and each environmental
286 variable. This analysis was conducted separately for each site and season, and the coefficient of
287 determination (R²) was calculated to quantify the strength of each trend. Additionally, a one-way Analysis
288 of Variance (ANOVA) was used to test for statistically significant differences between the disturbed and
289 undisturbed sites. A p-value of less than 0.05 was considered significant for all tests. All statistical linear
290 regression analyses were conducted using Microsoft Excel.

291 **2.8 Random Forest modeling**

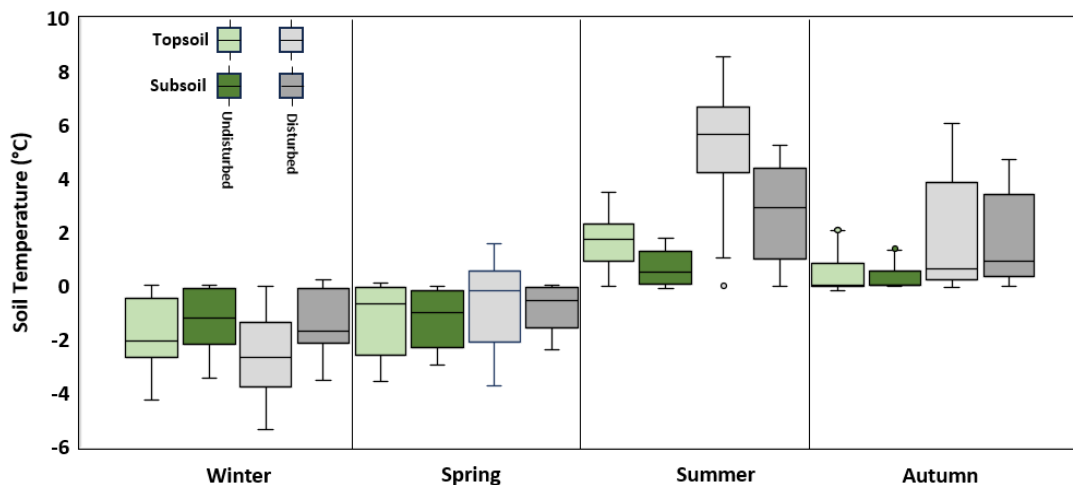
292 A regression-based Random Forest (RF) non-linear model developed in R was used to identify
293 the relative importance of the input variables to predict hourly and daily CO₂ concentrations. RF was
294 chosen over other algorithms due to its wide and successful application in determining variable
295 importance (Behnamian *et al.*, 2017; Lei *et al.*, 2024). In RF, the supervised non-linear algorithm can
296 combine predictions from hundreds or thousands of individual decision trees via bootstrap aggregation to
297 generate an ideal output (Schonlau and Zou, 2020). Compared with individual decision trees, this results
298 in an increase in generalization accuracy and a reduction in overfitting. A repeated k-fold cross-validation
299 technique was also employed. In this technique, data are randomly separated into *k* subsets with *k*-1 used
300 to train the model and the remainder to test the model, which is then repeated a specified amount. We
301 selected a value of 10 for *k* and a value of 5 for repetition. Input variables were the same for each instance
302 except barometric pressure being dropped for daily CO₂ concentrations due to poor importance values.
303 Thaw depth was static in the dataset that the model used from 4 October through 16 May due to the

304 presence of a frozen surface layer preventing thaw depth probing. Organic soil VWC and mineral soil
305 VWC from 1 November – 31 March and 1 April – 31 May were omitted for machine learning due to the
306 inability of the probes to function properly in subzero temperatures.

307 3. Results

308 3.1 Soil conditions

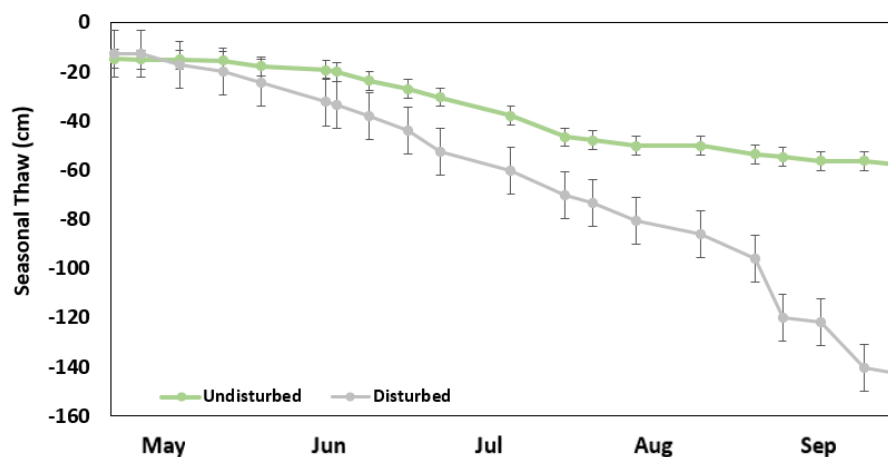
309 Soil temperature fluctuated by a factor of three at the disturbed site and by a factor of 1.9 at the
310 undisturbed site over the course of the year. The undisturbed and disturbed sites exhibited contrasting
311 thermal regimes. At the undisturbed site, the mean annual soil temperatures were below freezing,
312 exhibiting -0.33 ± 0.1 °C for topsoil and -0.41 ± 0.07 °C for subsoil (Figure 3). In contrast, the disturbed
313 site experienced positive mean annual soil temperatures, with 0.72 ± 0.2 °C for topsoil and 0.48 ± 0.13 °C
314 for subsoil. Winter was the only season with warmer topsoil and subsoil temperatures at the undisturbed
315 site. For both the undisturbed and disturbed sites, the subsoil layer was cooler than the topsoil layer in
316 terms of mean annual temperature. Significantly warmer soil temperatures were observed at the disturbed
317 site during the summer (4.04 ± 0.19 °C; $p < 0.001$, ANOVA) and autumn (1.88 ± 0.23 °C; $p < 0.001$,
318 ANOVA) in comparison to the temperatures recorded at the undisturbed site during the same seasons.
319 Conversely, the mean summer soil temperature at the undisturbed site was 1.15 ± 0.08 °C, while the mean
320 autumn soil temperature was 0.38 ± 0.07 °C (Figure 3). Soil temperatures in the shallower topsoil layer
321 exhibited greater variability throughout the year compared to the temperatures in the deeper subsoil layer
322 at both sites (Figure 3).



323
324 **Figure 3.** Seasonal soil temperature patterns. The seasons were delineated as winter (Nov–Mar), spring
325 (Apr and May), summer (Jun–Aug), and autumn (Sep and Oct). Soil temperature are average daily values

326 from the topsoil and subsoil layers at the 8 chamber plots: 4 at the undisturbed site and 4 at the disturbed
327 site. The range of the boxplot represents the first and third quartiles, while the central line signifies the
328 median. The whiskers of the box extend to the minimum and maximum values, with outliers represented
329 by circles.

330 VWC values ranged from $0.29 \pm 0.00 \text{ m}^3/\text{m}^3$ to $0.47 \pm 0.00 \text{ m}^3/\text{m}^3$ (1 Jun to 31 Oct 2024) (SI
331 Figure S3). Subsoil layer exhibited elevated mean seasonal VWC values, as compared to the topsoil
332 layer, at both locations (p values from 0.04 to < 0.001 , ANOVA). Average maximum seasonal thaw depth
333 exhibited significant differences between the two locations (p values 0.02, ANOVA), ranging from 58 ± 3
334 cm or 143 ± 29 cm at the undisturbed site or disturbed site, respectively (Figure 4). While the maximum
335 seasonal thaw depth remained relatively consistent across the undisturbed plots, ranging from 50 cm to 61
336 cm, the disturbed plot displayed a larger range in maximum thaw depth, varying from 82 cm to 204 cm.



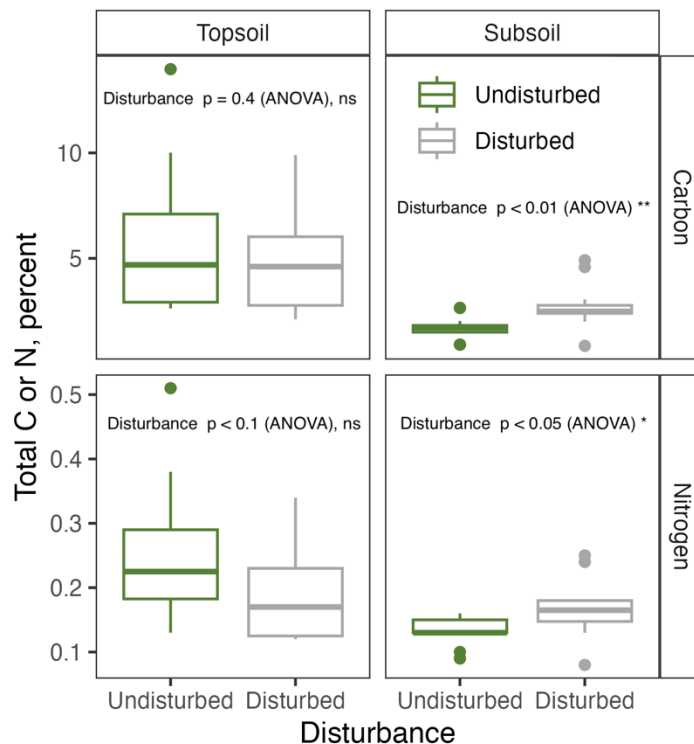
337
338 **Figure 4.** Average seasonal thaw depth at the undisturbed and disturbed sites measured using manual
339 frost probe measurements from 12 May to 3 Oct 2023. Error bars are standard error.

340 3.2 Soil properties

341 The disturbance had a significant effect on LOI (a proxy for SOM content) in the subsoil layer,
342 but not the topsoil layer (SI Figure S4). There was no significant effect of sampling date or significant
343 interaction of sampling date and disturbance on LOI. Mean total C concentration for subsoil at the
344 disturbed site was 2.7% (averaged across LiCor chamber plots and sampling dates), significantly greater
345 than that of the undisturbed site mean of 1.7% ($p < 0.01$; ANOVA). Mean LOI for the topsoil layer was
346 0.122 and 0.142 for disturbed and undisturbed, respectively (not significantly different).

347 Similarly, the disturbance was a significant factor for soil total C concentration and total N
348 concentration in the subsoil layer ($p < 0.01$ and $p < 0.05$, respectively; ANOVA), but not the topsoil layer
349 (Figure 5), and there was no significant effect of sampling date or significant interaction of sampling date

350 and disturbance on either C or N. Mean total C concentration for the topsoil layer was 4.7% and 5.8% for
 351 disturbed and undisturbed, respectively (not significantly different). Mean total N concentration for
 352 subsoil at the disturbed site was 0.16%, significantly greater than that of the undisturbed mean of 0.13%
 353 ($p < 0.01$; ANOVA). Mean total N concentration for the topsoil was 0.19% and 0.25% for disturbed and
 354 undisturbed, respectively (not significantly different).



355
 356 **Figure 5.** Soil total carbon and total nitrogen, at the disturbed and undisturbed sites. Boxplots represent
 357 all three sampling dates and four LiCor chamber plots, within each site.

358 Similar to the response of LOI, total C, and total N, soil pH was significantly different in the
 359 subsoil layer, depending on the site; the effect of disturbance on the topsoil layer was not significant (SI
 360 Figure S5). There was no significant effect of sampling date or significant interaction of sampling date
 361 and disturbance on soil pH. Mean pH for subsoil in the disturbed site was 4.63 (averaged across chambers
 362 and sampling dates), significantly greater than that of the undisturbed site mean of 4.11 ($p < 0.01$;
 363 ANOVA). Mean pH for the topsoil layer was 3.78 and 3.79 for disturbed and undisturbed, respectively
 364 (not significantly different).

365 **3.3 Soil microbial community composition and diversity**

366 We compared microbial community composition and diversity between the disturbed and
 367 undisturbed sites to enhance our understanding of the role that microbes play in soil respiration. By
 368 examining the effects of the site disturbance on microbial diversity—quantified through variations in
 369 species richness and community structure—we can ascertain which microbial groups exhibit the greatest
 370 sensitivity to environmental alterations and how their functional roles might adapt in response. The
 371 dominant bacterial phyla included *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, and
 372 *Chloroflexi*, which together comprised over 75% of relative abundance (Figure 6). For fungi, 54% of
 373 relative abundance was comprised of *Ascomycota* (mostly classes *Leotiomycetes* and
 374 *Archaeorhizomycetes*), and 40% was *Basidiomycota* (mostly class *Agaricomycetes*) (Figure 6).
 375 Differential abundance testing only identified several dozen taxa (after filtering for somewhat higher
 376 abundance taxa) that were either significantly enriched or depleted under disturbance (see SI Figures S6
 377 and S7). Notably, several Chloroflexi and Dormibacterota (a newly named phylum, previously identified
 378 as Chloroflexi) ASV's are depleted under disturbance relative to the undisturbed condition in subsoil,
 379 particularly at the February sampling date (SI Figure S7).



380
 381 **Figure 6.** Relative abundances of dominant bacterial phyla, faceted by sampling date and soil layer. Each
 382 plot is named for LiCor chamber (Ch1 through Ch8); Ch1, Ch2, Ch3, Ch4 are undisturbed plots, and Ch5,
 383 Ch6, Ch7, Ch8 are disturbed plots. One sample was excluded from analysis (2023_Feb, Chamber 3,
 384 Topsoil).

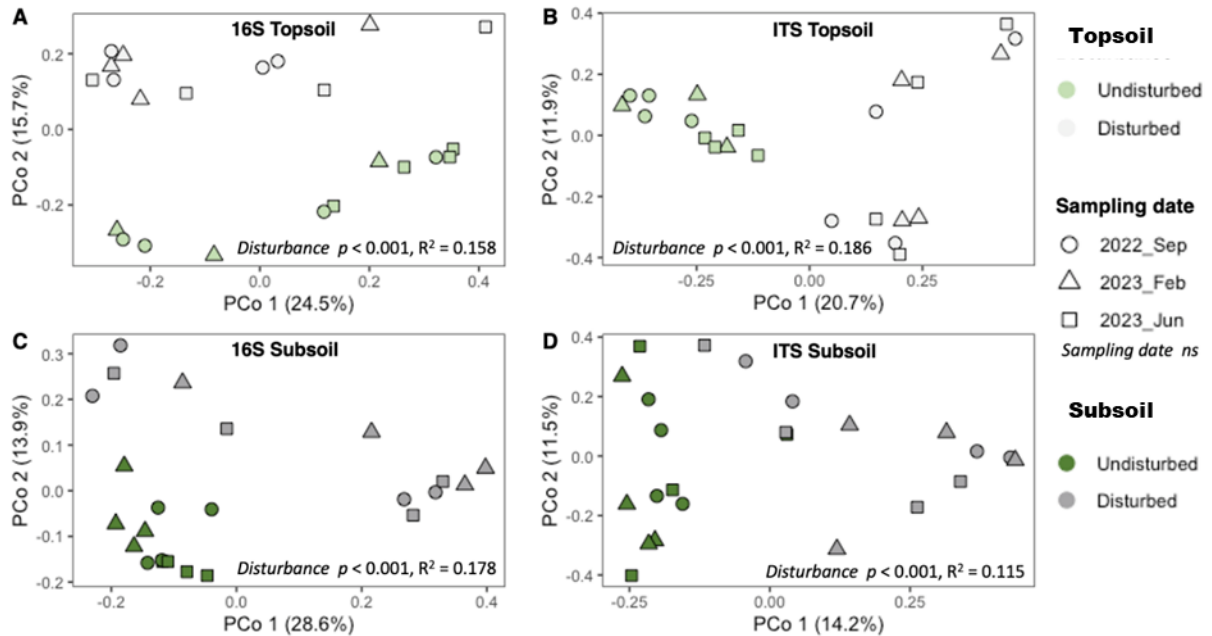


385

386 **Figure 7.** Relative abundances of dominant fungal classes, faceted by sampling date and soil layer. Each
 387 plot is named for LiCor chamber (Ch1 through Ch8); Ch1, Ch2, Ch3, Ch4 are undisturbed plots, and Ch5,
 388 Ch6, Ch7, Ch8 are disturbed plots. One sample was excluded from analysis (2023_Feb, Chamber 3,
 389 Topsoil).

390 Mean estimated bacterial richness across the dataset was 711.5 ASVs, with a significant
 391 differences between the two sites (SI Figure S8). Overall, the disturbed samples had 30% higher richness
 392 compared to the undisturbed samples ($p < 0.01$, ANOVA); within each date and soil layer combination,
 393 only the February 2023 subsoil samples demonstrated a significant effect of disturbance ($p < 0.01$,
 394 ANOVA). There was no effect of sampling date or soil layer, or interaction amongst the factors. Mean
 395 estimated fungal richness across the dataset was 165 ASVs; there was no effect of disturbance, sampling
 396 date, soil layer, or interaction amongst the factors (SI Figure S8).

397 Employing Bray-Curtis dissimilarities to assess beta diversity (Figure 8), we observed notable
 398 differences between the undisturbed and disturbed sites, concerning both bacterial and fungal
 399 communities in the topsoil and subsoil layers ($p < 0.001$ for all; $R^2 = 0.158, 0.178, 0.186, 0.115$ for
 400 bacterial topsoil, bacterial subsoil, fungal topsoil, and fungal subsoil communities, respectively;
 401 PERMANOVA). Sampling date did not have a significant effect.

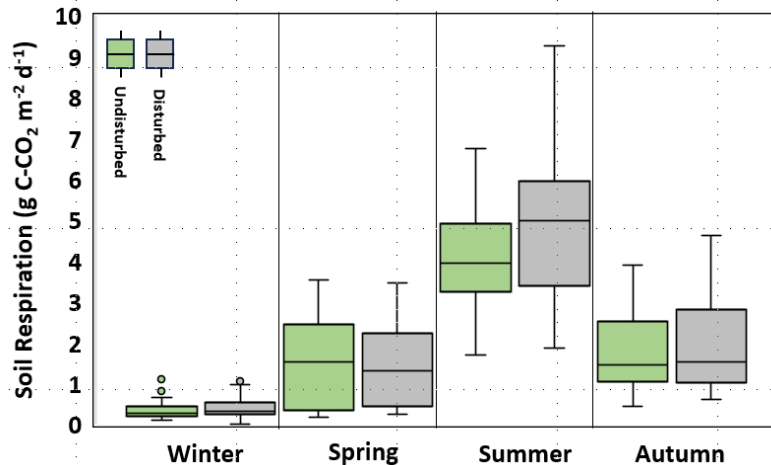


402

403 **Figure 8.** Principal coordinates analysis of Bray-Curtis dissimilarities of relative abundance data
 404 following rarefaction. Panels (A) and (C) represent the bacterial community (16S marker), and panels (B)
 405 and (D) represent the fungal community composition (ITS marker). The top panels (A & B; lighter
 406 shades) represent the topsoil layer community composition, while the bottom panels (C & D; darker
 407 shades) represent the subsoil community composition.

408 3.4 Soil Respiration

409 Total soil respiration rates showed distinct seasonal changes and were consistently higher, except
 410 during spring, at the disturbed site compared to the undisturbed one (Figure 9). The most significant
 411 activity occurred in the summer, with mean daily fluxes reaching 4.01 ± 0.12 g C-CO₂ m⁻² d⁻¹ at the
 412 undisturbed site and peaking at 4.81 ± 0.17 g C-CO₂ m⁻² d⁻¹ at the disturbed site, a statistically significant
 413 difference ($p < 0.0001$, ANOVA). Respiration rates declined in autumn, with mean daily fluxes of $1.79 \pm$
 414 0.13 g C-CO₂ m⁻² d⁻¹ (undisturbed) and 1.91 ± 0.15 g C-CO₂ m⁻² d⁻¹ (disturbed). The mean daily fluxes
 415 decreased further in spring, measuring 1.46 ± 0.14 g C-CO₂ m⁻² d⁻¹ (undisturbed) and 1.41 ± 0.13 g C-CO₂
 416 m⁻² d⁻¹ (disturbed); during these two shoulder seasons, the differences between the sites were not
 417 statistically significant. The lowest mean daily efflux was recorded during winter, with rates dropping to
 418 0.33 ± 0.01 g C-CO₂ m⁻² d⁻¹ at the undisturbed site and 0.39 ± 0.13 g C-CO₂ m⁻² d⁻¹ at the disturbed site,
 419 which, similar to the summer mean daily efflux, was also a statistically significant difference ($p < 0.01$,
 420 ANOVA). Annually, the disturbed site had a higher average soil efflux (2.07 ± 0.11 g C-CO₂ m⁻² d⁻¹) than
 421 the undisturbed site (1.81 ± 0.09 g C-CO₂ m⁻² d⁻¹), with the highest single mean daily flux of 9.27 g C-
 422 CO₂ m⁻² d⁻¹ recorded on July 20 at the disturbed site.



423

424 **Figure 9.** Seasonal soil respiration patterns observed at the undisturbed and disturbed sites. Soil
 425 respiration emissions are average diel fluxes from the 8 long-term chambers: 4 at undisturbed site and 4 at
 426 disturbed site. The range of the boxplot represents the first and third quartiles, while the central line
 427 signifies the median. The whiskers of the box extend to the minimum and maximum values, with outliers
 428 represented by circles.

429 3.5 Linear regression analysis to determine important variables contributing to soil efflux

430 A linear regression analysis was performed on the mean daily averages to investigate the seasonal
 431 correlation between soil efflux and various factors, including air temperature, barometric pressure,
 432 seasonal thaw depth, topsoil and subsoil temperature, and volumetric water content (VWC) at both
 433 undisturbed and disturbed sites. The analysis revealed that overall soil and air temperature exhibited the
 434 strongest correlation with soil respiration across all sites during different seasons. Soil and air
 435 temperatures showed the strongest correlations with soil efflux across all seasons. Seasonal R^2 values for
 436 soil temperatures ranged from 0.21–0.65 (winter), 0.87–0.92 (spring), 0.52–0.79 (summer), and 0.85–0.93
 437 (autumn). Air temperature also correlated strongly with soil efflux in spring ($R^2 = 0.74$ – 0.77) and autumn
 438 ($R^2 = 0.83$ – 0.87). Moderate correlations were observed between soil efflux and thaw depth at both sites,
 439 with R^2 values ranging from 0.39–0.50 in spring, 0.41–0.43 in summer, and 0.74 in autumn.

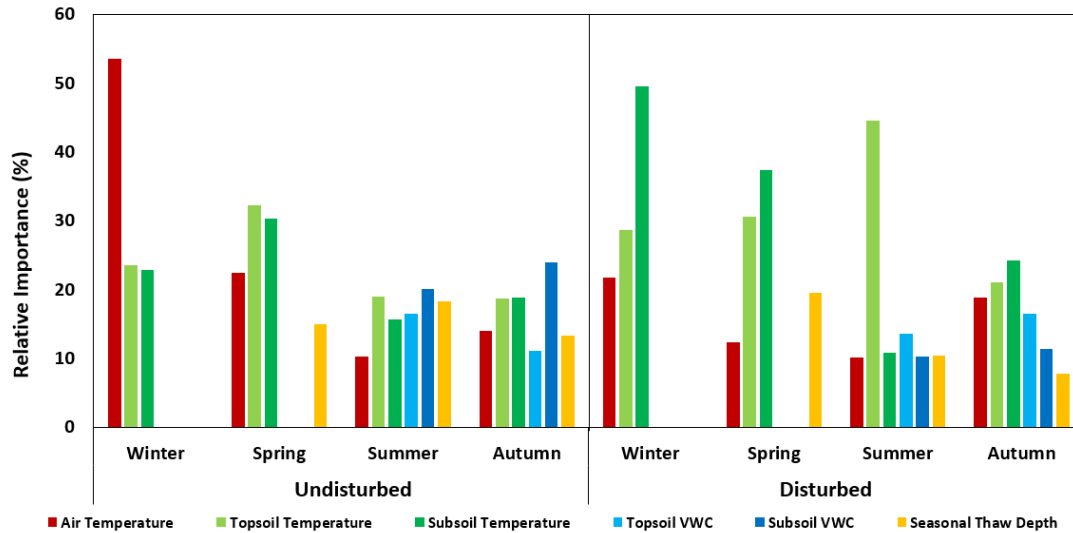
440 The correlation between soil efflux and topsoil and subsoil VWC varied during the summer and
 441 fall seasons, as well as across the two soil layers. At the undisturbed site, in summer, moderate correlation
 442 was found in the topsoil ($R^2 = 0.38$) and strong in the subsoil ($R^2 = 0.69$). In autumn, correlations were
 443 strong in the topsoil ($R^2 = 0.62$) but weaker in the subsoil ($R^2 = 0.22$). At the disturbed site, in summer, the
 444 correlation was weak in the topsoil ($R^2 = 0.09$) but strong in the subsoil ($R^2 = 0.64$). In autumn, weak
 445 correlations were observed in both layers ($R^2 = 0.28$ in topsoil and 0.10 in subsoil). Regression analysis
 446 showed weak to no correlation between soil efflux and barometric pressure during winter and autumn (R^2

447 = 0.02–0.12) and no correlation in spring and summer. A slightly higher correlation was noted in winter
448 when using hourly averages ($R^2 = 0.23$ undisturbed, 0.18 disturbed).

449 **3.6 Random forest efflux modeling**

450 The non-linear RF model effectively captured the impact of disturbance on the variation in soil
451 respiration at both locations, showing strong confidence levels with high R^2 and moderate to low mean
452 absolute error (MAE) values. Specifically, the model's R^2 values and corresponding MAE were 0.95 (0.14
453 MAE) for winter, 0.80 (0.38 MAE) for spring, 0.94 (0.16 MAE) for summer, and 0.82 (0.04 MAE) for
454 autumn at the undisturbed site, and 0.95 (0.12 MAE) for winter, 0.83 (0.52 MAE) for spring, 0.96 (0.13
455 MAE) for summer, and 0.84 (0.05 MAE) for autumn at the disturbed site. Skewness and kurtosis values
456 from the field data ranged from 0.04 - 1.24 and 1.61 - 4.56 at the disturbed site, and from 0.07 - 1.45 and
457 1.40 - 6.22 at the undisturbed site for all seasons, respectively. The ranges indicated that some instances
458 contained a more normal distribution while other instances, especially winter, were notably skewed and
459 tail-light and heavy.

460 The predictors' relative importance (RI) varied across seasons and sites (Figure 10). In the winter
461 model at the undisturbed site, air temperature emerged as the most influential predictor, accounting for
462 53.6% RI. Conversely, at the disturbed site, the subsoil temperature exhibited the highest predictive power
463 with 49.6% RI (figure 10). For the spring model, soil temperature was the best predictor at both sites, the
464 topsoil layer temperature exhibited a slightly greater predictive power at the undisturbed site, whereas the
465 subsoil layer temperature proved to be more influential at the disturbed site. In the undisturbed site's
466 summer model, the soil moisture and temperature as well as the seasonal thaw depth variables exhibit
467 similar RI values, ranging from 15.7 to 20.2%. Among these variables, air temperature has the lowest
468 predictive power, with an RI of 10.3%. Conversely, at the disturbed site, the topsoil layer temperature
469 stands out as the strongest predictor, with an RI of 44.6%. It is followed by the topsoil VWC, which has
470 an RI of 13.7%. The undisturbed site's autumn model indicates that subsoil layer VWC has the highest
471 predictive power at 24.3% RI, followed by subsoil layer temperature at 18.9% RI, and topsoil layer
472 temperature at 18.7% RI. However, the disturbed autumn model shows that subsoil layer temperature is
473 the most influential factor in predicting CO₂ efflux with 24.3% RI and topsoil layer (21.1% RI) and air
474 (18.9% RI) also playing significant roles.



475

476 **Figure 10.** Relative importance (%) of variables to predict CO₂ efflux. Missing or static input variables
 477 (e.g. winter VWC and seasonal thaw depth) were not measured for importance.

478 Throughout the different seasons, various factors contribute to the varying degrees of relative
 479 importance regarding soil respiration. These factors include air temperature, the temperature of both
 480 topsoil and subsoil layers, and volumetric water content, which pertains only to subsoil. This information
 481 is presented in Table 1.

482 **Table 1.** Tabulation of the most important predictor variables to explain variability in the soil efflux data
 483 between disturbed and undisturbed sites as a function of season.

Season	Plot	Highest Predictor	RI
Winter	Undisturbed	Air temperature	53.6
Winter	Disturbed	Soil layer temperature (subsoil)	49.6
Spring	Undisturbed	Soil layer temperature (topsoil)	32.2
Spring	Disturbed	Soil layer temperature (subsoil)	37.4
Summer	Undisturbed	Volumetric water content (topsoil)	20.2
Summer	Disturbed	Soil layer temperature (topsoil)	44.6
Autumn	Undisturbed	Volumetric water content (subsoil)	24.0
Autumn	Disturbed	Soil layer temperature (subsoil)	24.3

484

485 4. Discussion

486 The primary objective of this study was to determine if an anthropogenic disturbance from
 487 approximately a century ago has a lasting legacy on carbon efflux in a boreal forest ecosystem. We

488 hypothesized that the disturbance would result in significant, persistent differences in soil respiration and
489 edaphic properties between the disturbed and an adjacent undisturbed site. Crucially, we extended this
490 hypothesis to the understudied winter period, postulating that these differences in soil activity would
491 persist even under extreme cold temperatures. To elucidate the controls behind these variations, we
492 examined seasonal CO₂ efflux alongside a suite of controlling factors, including soil temperature,
493 moisture, soil organic matter, pH, seasonal thaw depth, and microbial community composition. In the
494 following sections, we discuss how these interconnected variables explain the observed respiration
495 patterns, providing insight into the long-term trajectory of carbon cycling following historical disturbance
496 in permafrost environments.

497 Our findings show that the temperatures of the active layer soil were markedly elevated at the
498 disturbed site in comparison to the undisturbed site (Figure 3). Previous research, conducted in similar
499 subarctic environments, has attributed this increase in temperature, in disturbed environments, to a
500 decrease in insulating organic matter (Gordon *et al.*, 1987; Amiro, 2001; Yoshikawa *et al.*, 2002; Koster *et*
501 *al.*, 2017 and 2018; Zhu *et al.*, 2022) and alterations in surface albedo resulting from disturbance (Amiro,
502 2001; Yoshikawa *et al.*, 2002; Koster *et al.*, 2018; Zhu *et al.*, 2022). The consequences of the modified
503 thermal conditions were found to be significant, as the heightened soil temperatures affected
504 biogeochemical cycles (Chatterjee *et al.*, 2008; Akande *et al.*, 2023) led to a deeper active layer (Forbes *et*
505 *al.*, 2002; Foster *et al.*, 2022). In the current study, winter was the only season during which soil
506 temperatures were elevated at the undisturbed site (Figure 3), as the unaltered surface vegetation provided
507 a layer of insulation against the low air temperature. Snow did not influence the thermal conditions at the
508 study locations, as both sites were devoid of snow during the winter to guarantee the proper functioning
509 of the long-term chambers.

510 Disturbance had a significant impact on total carbon, total nitrogen (Figure 5), and soil pH in the
511 subsoil layer (SI Figure S5). In the subsoil, the average total carbon concentration at the disturbed site
512 was markedly higher than that at the undisturbed site (Figure 5). A comparable significant rise was noted
513 for total nitrogen in the subsoil of the disturbed site when contrasted with the undisturbed site (Figure 5).
514 Prior research has indicated that elevated carbon and nitrogen levels in the soil enhance soil respiration
515 (Oertel *et al.*, 2016, Akande *et al.*, 2023, Schepaschenko *et al.*, 2025). Nevertheless, in the topsoil layer,
516 these effects were not statistically significant, implying that the increase in soil respiration was likely
517 derived from the subsoil layer. Following this trend, soil pH in the subsoil was also significantly
518 impacted, with the mean pH in the disturbed site being significantly higher than the mean at the
519 undisturbed site.

520 Our results reveal a clear and persistent legacy of historical disturbance on soil carbon efflux,
521 with the disturbed site exhibiting significantly higher overall respiration rates than the adjacent
522 undisturbed site (Figure 9). This difference was most pronounced during the peak of the growing season,
523 where summer CO₂ fluxes at the disturbed site were nearly 20% higher, a statistically significant margin
524 ($p < 0.0001$). This finding strongly suggests that the initial disturbance—likely leading to warmer soils
525 and a deeper active layer—has created conditions that continue to favor more rapid decomposition a
526 century later. Interestingly, while respiration rates declined expectedly during the autumn and spring
527 shoulder seasons, the differences between the two sites became statistically insignificant. This may
528 indicate that during these transitional periods, other environmental factors, such as soil moisture levels or
529 temperature thresholds, become more dominant controllers of microbial activity (Kim et al., 2012),
530 temporarily masking the underlying differences in soil properties. Crucially, our data demonstrate that the
531 elevated carbon loss at the disturbed site persists even into the winter. Although absolute fluxes were at
532 their lowest, the wintertime respiration at the disturbed site was still significantly higher than at the
533 undisturbed site ($p < 0.01$). This confirms that the ground remains a dynamic system and that the
534 historical disturbance has altered the soil's thermal regime or substrate availability in a way that sustains
535 higher microbial activity year-round, reinforcing the notion that winter is a critical (Natali et al., 2019;
536 Miner et al., 2022), and often overlooked, period for carbon cycling in these ecosystems.

537 Beyond the direct physical and chemical changes, the historical disturbance also left a significant
538 imprint on the soil's biological community. The effects of disturbance on microbial community structure
539 were substantial, with significant alterations noted in both bacterial and fungal compositions (Figure 8).
540 Notably, the date of sampling did not appear to influence community structure, indicating that the
541 disturbance itself serves as the principal factor driving these changes. This finding is consistent with
542 earlier studies that suggest soil disturbances can disrupt microbial habitats, resulting in a reorganization of
543 community dynamics (Chatterjee *et al.*, 2008; Dimitriu et al., 2010). Such changes can lead to cascading
544 effects on ecosystem functions, including nutrient cycling and the decomposition of organic matter (Peng
545 et al., 2008). We found that the disturbance-driven changes led to higher average soil temperatures and
546 increased concentrations of mean SOM (LOI) (SI Figure S4), mean total carbon, and mean total nitrogen
547 (Figure 5) in the subsoil, which positively influenced the mean annual soil respiration rates, resulting in
548 an overall 14.4% increase when compared to the undisturbed site.

549 Our random forest model analysis found that key abiotic factors drive distinct seasonal patterns in
550 soil efflux between the disturbed and undisturbed sites (Figure 10). In winter, air temperature emerged as
551 the most significant predictor for the undisturbed sites, likely due to its direct impact on microbial activity
552 and respiration rates. In contrast, the disturbed site during the same season showed a stronger correlation

553 with the subsoil layer temperature, suggesting that disturbances may alter the thermal dynamics of the soil
554 profile, making soil temperature a more crucial determinant (Table 1). Moreover, given that both locations
555 experienced snow denial, the more substantial ground vegetation cover at the undisturbed site offered
556 superior thermal insulation to the soil, compared to the thinner ground cover vegetation at the disturbed
557 site. Furthermore, it is very likely that winter soil respiration rates were higher at both sites where snow
558 was not cleared, than at the chamber sites, as deep snow can provide an insulating effect as found by
559 Welker et al. (2000) and Miner et al. (2022). During spring, topsoil layer temperature was the primary
560 predictor for the undisturbed site, highlighting the importance of organic matter decomposition driven by
561 temperature changes. For the disturbed site, the subsoil layer temperature remained the dominant factor,
562 indicating that disturbances may have disrupted the organic layer, shifting the focus to the subsoil layer's
563 thermal conditions.

564 In the summer months, the volumetric water content in the subsoil layer emerged as the principal
565 predictor for the undisturbed site (Table 1), highlighting the essential role of moisture availability in
566 influencing microbial activity and respiration during this warmer period. Conversely, the disturbed site
567 exhibited a more pronounced correlation with the temperature of the topsoil layer (Table 1), indicating
568 that disturbances may have modified the soil's hydrological characteristics by increasing thaw depth and
569 enhancing drainage, which resulted in a reduced volumetric water content and rendered temperature a
570 more significant factor than moisture. As autumn approached, the volumetric water content in the subsoil
571 layer reestablished itself as the key predictor for the undisturbed site (Table 1), reaffirming the ongoing
572 significance of moisture for microbial functions as temperatures began to decline. In contrast, for the
573 disturbed site, the temperature of the subsoil layer continued to be the prevailing influence, underscoring
574 the enduring effects of disturbances on the thermal dynamics of the soil. Other permafrost study sites in
575 the arctic have shown a reliance of soil efflux on soil moisture and temperature and suggest the drying out
576 of soil regimes and subsequent infiltration of snowmelt may also be a contributing factor for predicting
577 soil efflux values (Welker et al., 2000) and therefore annual data collection may likely be necessary to
578 determine long-term trends at sites where different soil types and disturbances exist. Overall, the observed
579 patterns indicate that soil disturbances fundamentally alter the relationships between abiotic factors and
580 CO₂ efflux. By reshaping thermal profiles and hydrological properties, disturbances intensify microbial
581 activity across seasons, thereby increasing CO₂ emissions.

582 **Conclusion**

583 Permafrost-affected ecosystems are experiencing dramatic change from anthropogenic causes,
584 both in terms of warming and physical disturbance to the soils. Active layers are deepening, unlocking

585 carbon that was previously inaccessible. Here, we sought to measure soil respiration in undisturbed and
586 disturbed boreal forest sites to examine patterns of seasonality, the effect of legacy disturbance on soil
587 system, and the influence of soil abiotic properties on respiration. This is particularly timely because our
588 findings indicate that a historic disturbance in subarctic soils can have a lasting effect on the microbial
589 community composition, but less so on the seasonal activity of the soils. The discovery of persistent
590 microbial activity throughout the winter is critically significant, as future warming may prevent the active
591 layer from fully refreezing, thereby intensifying year-round decomposition and carbon release. There
592 were notable changes in soil temperature (162% warmer overall), the composition of bacterial
593 communities (30% higher richness overall), total mean C (1% higher) and N (0.03% higher)
594 concentration levels and pH (11.9% higher) values in the subsoil layer, and the depth of the maximum
595 active thaw depth (147% deeper) as a result of disturbances, which subsequently led to 14.4% higher soil
596 respiration rates. Soil respiration was primarily regulated by temperature, (air and soil) while factors such
597 as soil volumetric water content and the depth of the active layer also contributed, with their relative
598 importance varying throughout the different seasons. These findings underscore the complex interplay
599 between seasonal variations, long lasting effect of soil and vegetation disturbance, and abiotic factors in
600 determining soil respiration rates. Understanding these relationships is essential for accurate modeling of
601 carbon cycling and for developing effective strategies to mitigate the impacts of soil disturbances on
602 ecosystem functions. Both natural and anthropogenic disturbances can lead to a marked rise in the
603 emission of carbon dioxide and other greenhouse gases into the atmosphere. Neglecting to account for
604 these disturbances may result in a considerable underestimation of the role of soils in global carbon
605 cycles. Future investigations should concentrate on the long-term consequences of these dynamics,
606 especially considering ongoing warming change and its impact on permafrost regions.

607 *Data availability.* The datasets produced and/or examined in the present study can be obtained from the
608 corresponding author upon reasonable request.

609 *Author Contributions.* DAV conceptualized the study and managed the data collection, RAB provided
610 funding and insight on microbial data analysis, DAV, AJB, and WBB collected the soil samples, DAV and
611 DB performed the soil respiration analysis, JRW performed the soil property and microbial community
612 analyses. DAV drafted the initial manuscript and all authors contributed to revisions.

613 *Competing Interests.* The authors have no relevant financial or non-financial interests to disclose.

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617 properties.

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619 Platform Against Extreme Cold Weather’.

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