Effects of Disturbance on Seasonal CO_2 Dynamics in Two Boreal Forest Sites Underlain by Permafrost~~Effects of permafrost thaw on seasonal soil CO_2 efflux dynamics in a boreal forest site~~

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Abstract. Permafrost regions in subarctic and arctic areas harbor substantial carbon reserves, which are becoming increasingly vulnerable to microbial decomposition as soils warm. As the seasonally thawed active layer deepens and anthropogenic disturbances escalate, accurately predicting carbon fluxes from disturbed~~thawed~~ environments underlain by permafrost requires a comprehensive understanding of soil respiration dynamics. This study ~~aimed to~~ investigated the impact of surface disturbance on ~~year-round~~seasonal soil ~~respiration rates~~biological properties in a boreal forest ecosystem near Fairbanks, Alaska. Further, we sought to~~and~~ identify the key environmental and geochemical factors influencing ~~these processes~~soil biology in the undisturbed and disturbed soils ~~in a boreal forest ecosystem near Fairbanks, Alaska~~. Our results revealed a substantial rise in soil respiration at the disturbed boreal forest site, which exhibited a 14.4% overall increase in CO_2 efflux compared to the undisturbed site. This effect was most pronounced during the summer, when the increase in CO_2 efflux peaked at 20%. This heightened respiratory activity was directly linked to significantly warmer soil conditions, with the mean annual soil temperature at the disturbed site measuring 0.60 ± 0.16°C, in stark contrast to the sub-zero temperatures of -0.37 ± 0.08°C at the undisturbed site~~The disturbed site demonstrated an increase in mean annual soil temperatures, recorded at 0.60 ± 0.16°C, along with a 14.4% rise in mean annual microbial activity, which peaked at 20% during the summer, in contrast to the undisturbed site, which had a mean annual temperature of -0.37 ± 0.08°C. Furthermore, bacterial and fungal community composition differed significantly between the two sites, suggesting a potential mechanism underlying the variation in CO_2 efflux~~. Furthermore, the disturbed site had 30% higher bacterial community richness, 1% higher total mean C and 0.03% higher total mean N concentration levels, and 11.9% higher pH values in the subsoil layer, as well as a 147% deeper maximum active thaw depth, suggesting potential controls underlying the variation in CO_2 efflux. Our research underscores the essential importance of considering the rise in carbon emissions from anthropogenically disturbed soils ~~in disturbed areas~~ underlain by permafrost~~ areas~~, which are frequently neglected in assessments of the carbon cycle. This study contributes to a deeper

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understanding of the complex interactions governing soil respiration in disturbed permafrost environments, ultimately informing more accurate predictions of carbon fluxes in these ecosystems.

1. Introduction

Soil respiration, the process by which carbon dioxide (CO_2) is released from the soil surface to the atmosphere, is a critical component of the global carbon cycle. This process encompasses the microbial breakdown of organic material as well as the respiration of plant roots. Understanding soil respiration dynamics is particularly crucial in boreal forests, as they comprise approximately 30% of global forested area and play a vital role in global carbon sequestration (Bonan, 2008; Pan *et al*., 2011; Chi *et al*., 2021). Recent studies indicate that increasing temperatures could lead to boreal forests transitioning from functioning as carbon sinks to becoming carbon sources (Bond-Lamberty *et al*., 2018; Marty *et al*., 2019, Harel *et al*., 2023). In boreal forests, soil respiration is estimated to contribute up to 68% of the total ecosystem respiration (Parker *et al*., 2020; Watts *et al*., 2021) and is significantly impacted by changes in soil temperature, soil moisture, the microbial community, and vegetation type (Grace, 2004; Fekete *et al*., 2014; Rodtassana *et al*., 2021). Soil respiration in permafrost regions is also influenced by the thickening of the active layer (the seasonally thawed soil surface layer) that occurs when permafrost is degraded due to warmer temperatures (Koster et al., 2017; Turetsky *et al*., 2020; Watts *et al*., 2021).

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

Anthropogenic disturbances in boreal forests alter carbon cycling through distinct short- and long-term phases that are primarily driven by impacts to the underlying permafrost. Initially, disturbances like forest harvesting can cause a temporary decrease in total soil CO_2 efflux by eliminating root (autotrophic) respiration (Akande et al., 2023; Schepaschenko et al., 2025). However, this short-term effect is overshadowed by the dominant, long-term consequences of physical changes to the ground's thermal regime. Activities such as land clearing for infrastructure and resource exploration remove the insulating vegetation and surface organic layers, leading to warmer soil temperatures and a deeper active layer (Forbes et al., 2002; Foster et al., 2022). This permafrost degradation exposes vast stores of previously frozen organic carbon to microbial decomposition, transforming the disturbed landscape into a persistent, long-term source of atmospheric CO_2 (Koster et al., 2018; Miner et al., 2022). As ecosystems recover, the combination of this sustained decomposition with new root respiration can lead to total soil efflux rates that ultimately exceed those of undisturbed forests (Akande et al., 2023). Consequently, these human disturbances are a critical factor that accelerates the permafrost carbon feedback, significantly impacting the overall carbon balance of the circumpolar north (Schepaschenko et al., 2025; Miner et al., 2022).

Despite their importance, the cumulative impacts of these anthropogenic disturbances on landscape and soil properties are not well understood, often being overshadowed by the more extensively studied effects of wildfire (Foster et al., 2022). Therefore, investigating soil respiration in disturbed, permafrost-affected environments is crucial for assessing the resilience and vulnerability of boreal ecosystems.

Under projected climate scenarios, the frequency and intensity of disturbances in these regions are expected to increase, potentially leading to significant changes in soil carbon fluxes (Schepaschenko et al., 2025).

Further, insights gained from such studies can inform forest management practices aimed at mitigating the impacts of disturbances and preserving the carbon sequestration potential of boreal forests.

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

To reveal the fundamental controls driving variation in soil respiration within and between the disturbed and undisturbed boreal forest sites, we examined seasonal soil CO_2 efflux alongside various edaphic factors, including soil temperature, moisture content, soil organic matter (SOM), pH, and the composition of microbial communities. To assess this relationship, a non-linear Random Forest Model (RFM) was used in conjunction with regression analysis to analyze the time series data, which encompassed variables such as soil respiration, temperature, moisture, and air temperature. Additionally, R statistical ANOVA analysis was performed to evaluate soil characteristics, particularly pH and SOM, as well as the composition of soil microbial communities. The emissions data and estimates of carbon fluxes will be helpful to improve carbon modeling efforts. In the following sections, we detail the study site and our methodological approach (Section 2), present the key findings related to soil conditions and respiration (Section 3), and discuss the implications of these findings for understanding carbon cycling in disturbed permafrost ecosystems (Section 4).

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2. Materials and Methods

2.1 Site description

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

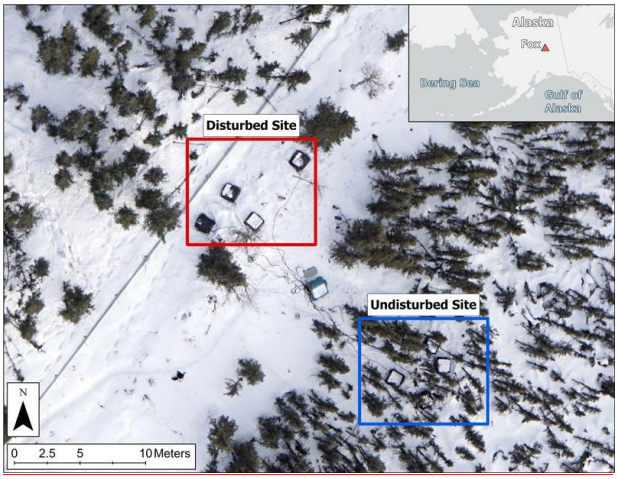

Figure 1. Aerial view of the study sites and experimental setup. The image displays the geographical location and the layout of the sample plots at the undisturbed (blue rectangle) and disturbed (red rectangle) study sites. The aerial image was captured on March 18, 2023. (Image credit: Dr. David Brodylo).

The vegetation at the undisturbed site consists of small black spruce (*Picea mariana*) ranging from densely distributed to tightly spaced. Understory canopy is dominated by marsh and bog Labrador tea (*Rhododendron tomentosum*; *groenlandicum*). Forest floor cover is primarily mosses (i.e., feather

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mosses and *Sphagnum spp.*) and small shrubs including lowbush cranberry (*Vaccinium vitis-idaea*). The disturbed site is characterized by scattered birch (*Betula neoalaskana*) and tall black spruce (*Picea mariana*) cover. The understory canopy is primarily dwarf shrubs including marsh Labrador tea and bog blueberry (*Vaccinium uliginosum*). The ground surface cover is dominated by grasses (*Poaceae*) and sedges (*Cyperaceae*). The typical undisturbed soil profile at this site consists of a fibric organic layer of variable thickness, which primarily contains undecayed and partially decayed moss and forest litter materials (O horizon), underlain by an organic-rich mineral material layer containing hemic-sapric organic fraction (A/B horizon), and thick accumulations of mineral material at the base (B/C horizon). Disturbance seemingly impacts the thickness of the surface organic layer. Soil sampling at each site targeted soil materials below the organic horizon. The sampled soil material at the two sites were classified as mineral soil material (<20% organic matter [OM]; Soil Survey Staff, 2022) with a subdivision of more organic-rich fractions (10-18% OM) comprising a "topsoil" layer (akin to an A horizon) that starts below the organic layer (O horizon), and more mineral-rich material (<5% OM) comprising a "subsoil" layer.
The topsoil textures ranged from loam to silt loam, reflecting a higher proportion of sand particles in the topsoil relative to the silt loam-textured subsoil (SI Table 1).

2.2. Installation of soil and meteorological sensors

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

Soil temperature, VWC, air temperature, and barometric pressure were measured using the following Onset HOBO (Onset, Bourne, Massachusetts, USA) instrumentation: U30 USB Weather Station, S-TMB-M002 12-Bit Temperature Smart Sensor, S-SMC-M005 EC5 Soil Moisture Smart Sensor, S-THC-M002 Temperature/Relative Humidity Smart Sensor, and S-BPB-CM50 Smart Barometric Pressure Sensor. Soil temperature and VWC were recorded in the topsoil and subsoil layers,

approximately 30 cm from each soil respiration sensor, at a depth of 18.5 ± 1.19 cm in the topsoil layer and 34.5 ± 1.47 cm in the subsoil layer for the undisturbed site. At the disturbed site, the measurements were taken at 13.5 ± 2.53 cm in the topsoil layer and 35.5 ± 1.85 cm in the subsoil layer. These depths were measured from the top of the ground vegetation cover (the moss surface). At both sites, the temperature and VWC sensors of the topsoil were installed below the fibric organic layer that had a thickness of 16.5 ± 1.19 cm at the undisturbed site and 9.5 ± 0.87 cm at the disturbed site. The differences in sensor placement depth were influenced by the variations in the thickness of the organic layer between the two sites (SI Figure S2). VWC measurements were restricted to the summer and autumn seasons due to the sensor's inability to measure below freezing temperatures. Air temperature and barometric pressure were measured at 2 m above the substrate surface.

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

For the soil respiration measurements, which encompasses the overall release of CO_2 from the soil into the atmosphere (including both autotrophic and heterotrophic processes), we installed 21.3 cm diameter thick-walled polyvinyl chloride (PVC) collars (LI-COR inc., Lincoln, Nebraska, USA) in the center of each plot. They were a height of 11.4 cm and were inserted 2-3 cm into the soil through the soil vegetation cover. The collars spacing varied from two to four meters at the undisturbed site and from three to eight meters at the disturbed site. (Figure 1). We installed a 8200-104 Opaque Long-Term Chamber (LI-COR inc., Lincoln, Nebraska, USA) above each collar, which yielded a total of eight. The chambers were connected via 15 m long tubing and cable assembly to a LI-8250 multiplexer (LI-COR inc., Lincoln, Nebraska, USA) that linked to a LI-870 CO_2/H_20 gas analyzer (LI-COR inc., Lincoln, Nebraska, USA). The cable and tubing assembly was wrapped in 1.3 cm thick tubular pipe insulation foam to prevent internal clogging from freezing moisture to ensure data collection in winter.

2.3 Respiration Data Collection

The measurement process followed a closed-chamber dynamic methodology, which was automated and controlled by the LI-8250 Multiplexer. At 30 minute intervals, the multiplexer would initiate a measurement at one of the chamber locations. This involved the 8200-104 chamber automatically rotating and lowering to seal onto the pre-installed PVC soil collar, creating a closed headspace over the soil surface. Once the chamber was sealed, the LI-8250 directed a closed loop of air

from the chamber headspace to the LI-870 and LI-7810 analyzers and back. The gas analyzers measured CO_2 and H_2O concentrations, for a period of 120 seconds, at a rate of 1 Hz. As CO_2 was respired , its concentration within the closed system increased over time. The soil CO_2 flux was calculated from the rate of this concentration increase. After each measurement cycle, the chamber automatically opened, and the system proceeded to the next chamber in the sequence. A more detailed description of the soil gas flux system's operation and these winter modifications can be found in Vas et al. (2023).

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

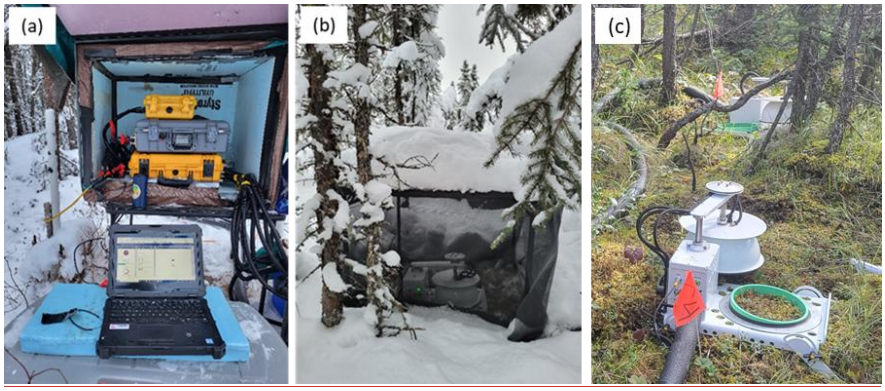

Figure 2. Custom modifications for LI-COR Soil gas flux system in cold climates. Enclosures were designed to ensure optimal operating temperatures (**a**) (photo credits: Dragos A. Vas, 17 November 2023), insulated tubing for instruments to prevent clogging, and long-term covers for chambers (**b**)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

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2.5 Soil collection and property analysis

Soil samples were collected from both the topsoil and subsoil layers across all plots in the autumn (September 2022), winter (February 2023), and summer (June 2023) seasons to analyze potential variations in microbial community composition among seasons, disturbance regimes, and soil layers. The samples were collected at identical depths during each season, which coincided with the depth at which the soil temperature and moisture probes were positioned; these depths varied for each plot according to

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

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Loss on ignition (LOI) was measured as a proxy for SOM content (Storer, 1984) on all soil samples. Here, LOI is the proportion of mass loss from oven-dried soil (dried at 105 °C for 24 hours) following 2 hours at 360 °C in a muffle furnace. Soil total carbon and total nitrogen was measured via combustion using a TruSpec C and N Analyzer (LECO, St. Joseph, MI, USA) at the University of Wisconsin Soil and Forage Lab. Soil pH was measured from a 1:1 slurry of soil:$CaCl_2$ solution (0.01M) using a pH probe (Hanna Instruments, Woonsocket, RI, USA) and a SevenEasy S20 pH meter (Mettler Toledo, Columbus, OH, USA). Soil pH was converted to H+ concentration prior to taking an average or statistical analysis. LOI total carbon, total nitrogen and soil pH were statistically analyzed using ANOVA in R.

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

Soil was partially defrosted and homogenized in the sample bag prior to subsampling 250 mg into bead beating tubes. Total genomic DNA was extracted using the DNeasy PowerSoil Pro Kit (Catalog No. 47014, Qiagen, Germantown, MD, USA), using a Precellys Evolution Touch homogenizer for the bead beating step (Catalog number P002511-PEVT0-A.0, Bertin Technologies, Montigny-le-Bretonneux, France). Automated DNA extraction was done with a QIAcube Connect (Catalog No. 9002864, Qiagen, Germantown, MD, USA) and each extraction run included a blank. Extracted DNA was held at -20 °C.

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

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

R software (R-Core-Team, 2018), and *ggplot2* (Wickham, 2016) were used for data analysis and visualization; the bioinformatic approach followed West et al. (2022). Community composition was visualized using principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities (Bray and Curtis, 1957) generated using *avgdist* from the R package *vegan* (Oksanen *et al*., 2024) using rarefaction (999 iterations) to a sampling depth of 16,300 for 16S and 6150 for ITS. A significant effect ($p < 0.05$) of disturbance, sampling date, and interaction of these factors on community composition was tested within each soil layer (topsoil and subsoil), using permutational multivariate analysis of variance (PERMANOVA; *adonis2* from *vegan*) (Anderson, 2001). Richness was evaluated using weighted linear regression (*betta* function in *breakaway* R package) (Willis *et al*., 2017), and a significant effect of

disturbance tested via ANOVA. Differential abundance (*differentialTest* in the *corncob* package) (Martin *et al*., 2021) was then used to identify significant enrichment or depletion of individual taxa due to disturbance, after excluding taxa with mean relative abundance < 0.00001.

2.7 Linear regression analysis

For statistical analysis, data on soil respiration, soil temperature, and VWC, as well as air temperature and barometric pressure from the eight chamber plots (four located at the disturbed site and four at the undisturbed site), were averaged to obtain daily means (n=333 per variable) and seasonal means (n=4 per variable). Seasons were defined according to observed efflux seasonality at the research site, a classification consistent with previous research on boreal forest efflux (Wats et al., 2021): winter (November to March, n=123), spring (April to May, n=57), summer (June to August, n=92), and autumn (September to October, n=61). Using these processed data, we performed a series of linear regression analyses to determine the existence of distinct, linear trends between CO_2 efflux and each environmental variable. This analysis was conducted separately for each site and season, and the coefficient of determination (R^2) was calculated to quantify the strength of each trend. Additionally, a one-way Analysis of Variance (ANOVA) was used to test for statistically significant differences between the disturbed and undisturbed sites. A p-value of less than 0.05 was considered significant for all tests. All statistical linear regression analyses were conducted using Microsoft Excel.

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2.8 Random Forest modeling

A regression-based Random Forest (RF) non-linear model developed in R was used to identify the relative importance of the input variables to predict hourly and daily CO_2 concentrations. RF was chosen over other algorithms due to its wide and successful application in determining variable importance (Behnamian *et al*., 2017; Lei *et al*., 2024). In RF, the supervised non-linear algorithm can combine predictions from hundreds or thousands of individual decision trees via bootstrap aggregation to generate an ideal output (Schonlau and Zou, 2020). Compared with individual decision trees, this results in an increase in generalization accuracy and a reduction in overfitting. A repeated k-fold cross-validation technique was also employed. In this technique, data are randomly separated into *k* subsets with *k*-1 used to train the model and the remainder to test the model, which is then repeated a specified amount. We selected a value of 10 for k and a value of 5 for repetition. Input variables were the same for each instance except barometric pressure being dropped for daily CO_2 concentrations due to poor importance values. Thaw depth was static in the dataset that the model used from 4 October through 16 May due to the presence of a frozen surface layer preventing thaw depth probing. Organic soil VWC and mineral soil

VWC from 1 November – 31 March and 1 April – 31 May were omitted for machine learning due to the inability of the probes to function properly in subzero temperatures.

3. Results

3.1 Soil conditions

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

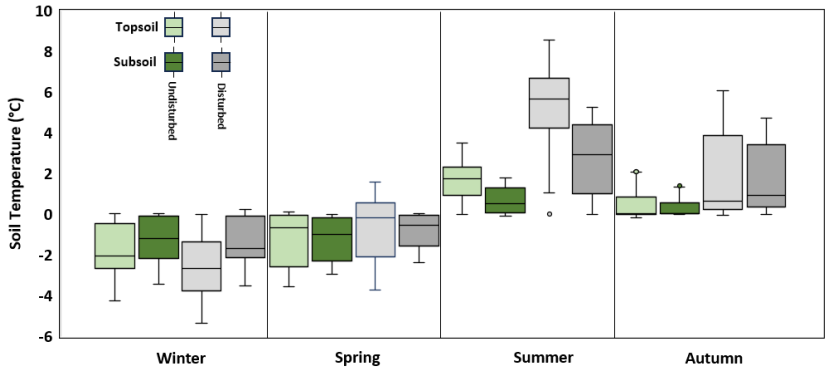

Figure 3. Seasonal soil temperature patterns. The seasons were delineated as winter (Nov–Mar), spring (Apr and May), summer (Jun–Aug), and autumn (Sep and Oct). Soil temperature are average daily values from the topsoil and subsoil layers at the 8 chamber plots: 4 at the undisturbed site and 4 at the disturbed site. The range of the boxplot represents the first and third quartiles, while the central line signifies the

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median. The whiskers of the box extend to the minimum and maximum values, with outliers represented by circles.

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

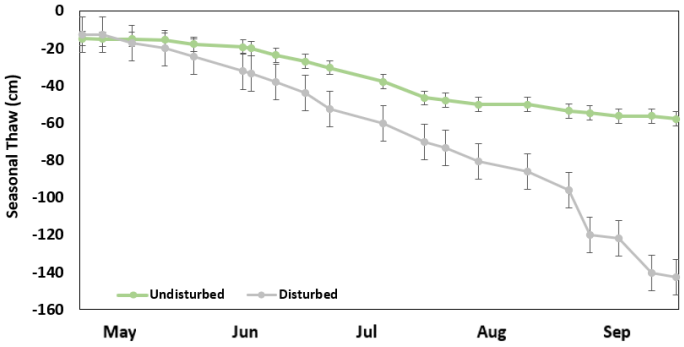

Figure 4. Average seasonal thaw depth at the undisturbed and disturbed sites measured using manual frost probe measurements from 12 May to 3 Oct 2023. Error bars are standard error.

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3.2 Soil properties

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

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

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

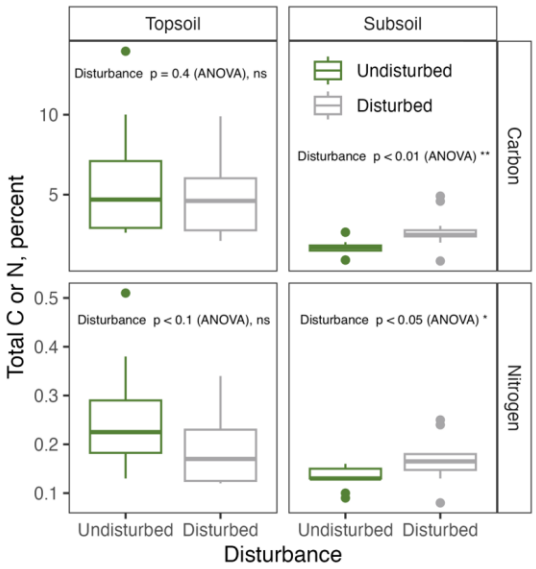

Figure 5. Soil total carbon and total nitrogen, at the disturbed and undisturbed sites. Boxplots represent all three sampling dates and four LiCor chamber plots, within each site.

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

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3.3 Soil microbial community composition and diversity

We compared microbial community composition and diversity between the disturbed and undisturbed sites to enhance our understanding of the role that microbes play in soil respiration. By examining the effects of the site disturbance on microbial diversity—quantified through variations in species richness and community structure—we can ascertain which microbial groups exhibit the greatest sensitivity to environmental alterations and how their functional roles might adapt in response.

The dominant bacterial phyla included *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*,

The dominant bacterial phyla included *Proteobacteria*,

Acidobacteria, *Actinobacteria*, *Verrucomicrobia*, and *Chloroflexi*, which together comprised over 75% of relative abundance (Figure 6). For fungi, 54% of relative abundance was comprised of *Ascomycota* (mostly classes *Leotiomycetes* and *Archaeorhizomycetes*), and 40% was *Basidiomycota* (mostly class *Agaricomycetes*) (Figure 6). Differential abundance testing only identified several dozen taxa (after filtering for somewhat higher abundance taxa) that were either significantly enriched or depleted under disturbance (see SI Figures S6 and S7). Notably, several Chloroflexi and Dormibacterota (a newly named phylum, previously identified as Chloroflexi) ASV's are depleted under disturbance relative to the undisturbed condition in subsoil, particularly at the February sampling date (SI Figure S7).

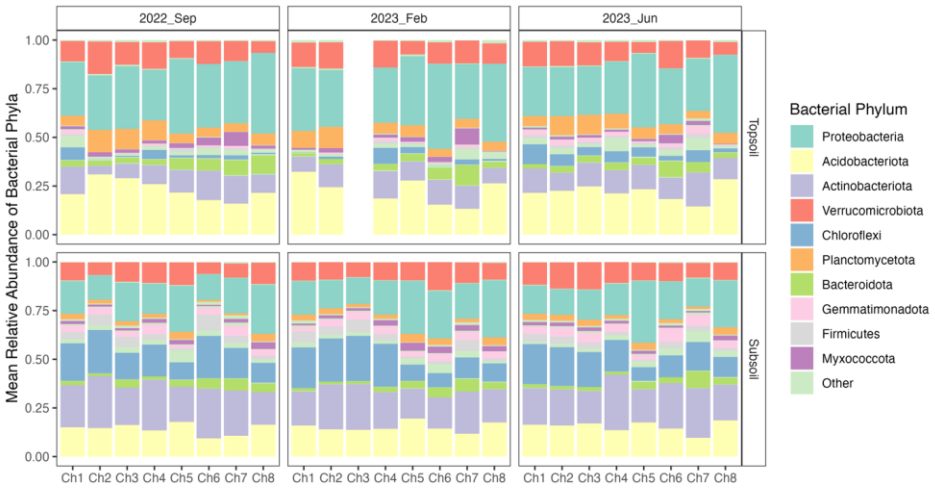

Figure 6. Relative abundances of dominant bacterial phyla, faceted by sampling date and soil layer. Each plot is named for LiCor chamber (Ch1 through Ch8); Ch1, Ch2, Ch3, Ch4 are undisturbed plots, and Ch5, Ch6, Ch7, Ch8 are disturbed plots. One sample was excluded from analysis (2023_Feb, Chamber 3,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

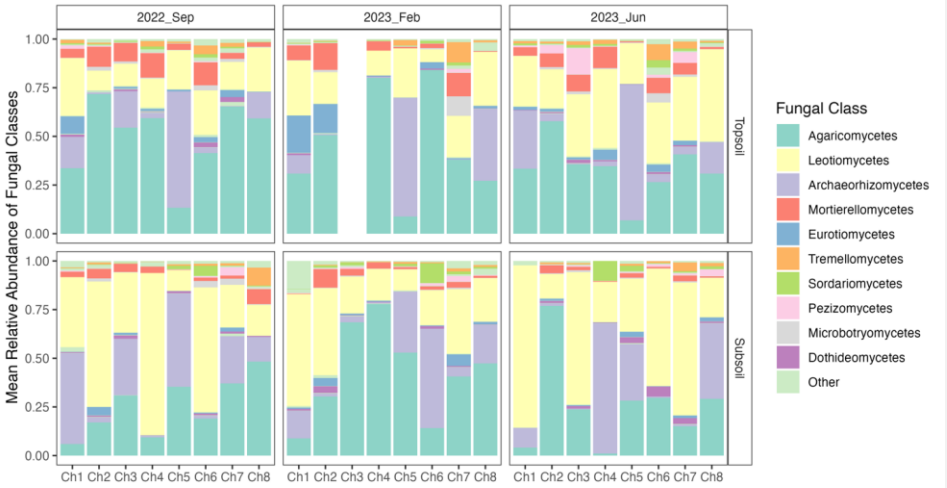

Figure 7. Relative abundances of dominant fungal classes, faceted by sampling date and soil layer. Each plot is named for LiCor chamber (Ch1 through Ch8); Ch1, Ch2, Ch3, Ch4 are undisturbed plots, and Ch5, Ch6, Ch7, Ch8 are disturbed plots. One sample was excluded from analysis (2023_Feb, Chamber 3,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

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

Employing Bray-Curtis dissimilarities to assess beta diversity (Figure 8), we observed notable differences between the undisturbed and disturbed sites, concerning both bacterial and fungal communities in the topsoil and subsoil layers ($p < 0.001$ for all; $R^2 = 0.158, 0.178, 0.186, 0.115$ for bacterial topsoil, bacterial subsoil, fungal topsoil,

($p < 0.001$ for all; $R^2 = 0.158, 0.178, 0.186, 0.115$ for bacterial topsoil,

bacterial subsoil, fungal topsoil, and fungal subsoil communities, respectively; PERMANOVA). Sampling date did not have a significant effect.

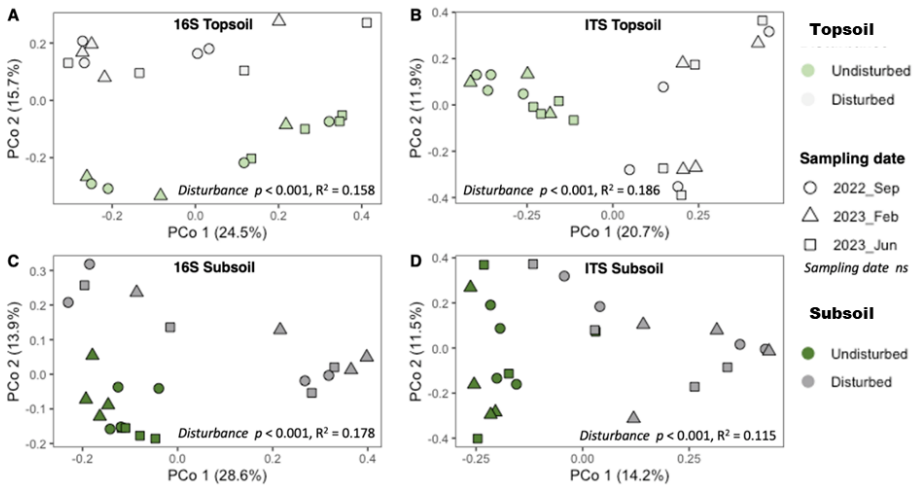

Figure 8. Principal coordinates analysis of Bray-Curtis dissimilarities of relative abundance data following rarefaction. Panels (A) and (C) represent the bacterial community (16S marker), and panels (B) and (D) represent the fungal community composition (ITS marker). The top panels (A & B; lighter shades)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

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3.4 Soil Respiration

Total soil respiration rates showed distinct seasonal changes and were consistently higher, except during spring, at the disturbed site compared to the undisturbed one (Figure 9). The most significant activity occurred in the summer, with mean daily fluxes reaching 4.01 ± 0.12 g C-CO_2 m^{-2} d^{-1} at the undisturbed site and peaking at 4.81 ± 0.17 g C-CO_2 m^{-2} d^{-1} at the disturbed site, a statistically significant difference ($p < 0.0001$, ANOVA). Respiration rates declined in autumn, with mean daily fluxes of 1.79 ± 0.13 g C-CO_2 m^{-2} d^{-1} (undisturbed) and 1.91 ± 0.15 g C-CO_2 m^{-2} d^{-1} (disturbed). The mean daily fluxes decreased further in spring, measuring 1.46 ± 0.14 g C-CO_2 m^{-2} d^{-1} (undisturbed) and 1.41 ± 0.13 g C-CO_2 m^{-2} d^{-1} (disturbed); during these two shoulder seasons, the differences between the sites were not statistically significant. The lowest mean daily efflux was recorded during winter, with rates dropping to 0.33 ± 0.01 g C-CO_2 m^{-2} d^{-1} at the undisturbed site and 0.39 ± 0.13 g C-CO_2 m^{-2} d^{-1} at the disturbed site, which, similar to the summer mean daily efflux, was also a statistically significant difference ($p < 0.01$, ANOVA). Annually, the disturbed site had a higher average soil efflux (2.07 ± 0.11 g C-CO_2 m^{-2} d^{-1}) than the undisturbed site (1.81 ± 0.09 g C-CO_2 m^{-2} d^{-1}), with the highest single mean daily flux of 9.27 g C-CO_2 m^{-2} d^{-1} recorded on July 20 at the disturbed site.

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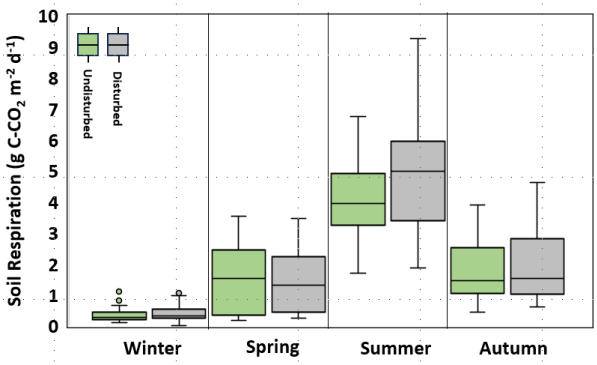

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463 **Figure 9.** Seasonal soil respiration patterns observed at the undisturbed and disturbed sites. Soil
464 respiration emissions are average diel fluxes from the 8 long-term chambers: 4 at undisturbed site
465 and 4 at disturbed site. The range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

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468 **3.5 Linear regression analysis to determine important variables contributing to soil efflux**

469 A linear regression analysis was performed on the mean daily averages to investigate the seasonal
470 correlation between soil efflux and various factors, including air temperature, barometric pressure,
471 seasonal thaw depth, topsoil and subsoil temperature, and volumetric water content (VWC) at both
472 undisturbed and disturbed sites.
473 The analysis revealed that overall soil and air temperature exhibited the strongest correlation with soil
474 The analysis revealed that overall soil and air temperature exhibited the strongest correlation with
475 soil respiration across all sites during different seasons. Soil and air temperatures showed the strongest
476 correlations with soil efflux across all seasons. Seasonal R^2 values for soil temperatures ranged from
477 0.21–0.65 (winter), 0.87–0.92 (spring), 0.52–0.79 (summer), and 0.85–0.93 (autumn). Air temperature
478 also correlated strongly with soil efflux in spring (R^2 = 0.74–0.77) and autumn (R^2 = 0.83–0.87).
479 Moderate correlations were observed between soil efflux and thaw depth at both sites, with R^2 values
480 ranging from 0.39–0.50 in spring, 0.41–0.43 in summer, and 0.74 in autumn.

481 The correlation between soil efflux and topsoil and subsoil VWC varied during the summer and
482 fall seasons, as well as across the two soil layers. At the undisturbed site, in summer, moderate correlation
483 was found in the topsoil (R^2 = 0.38) and strong in the subsoil (R^2 = 0.69). In autumn, correlations were
484 strong in the topsoil (R^2 = 0.62) but weaker in the subsoil (R^2 = 0.22). At the disturbed site, in summer, the
485 correlation was weak in the topsoil (R^2 = 0.09) but strong in the subsoil (R^2 = 0.64). In autumn, weak

correlations were observed in both layers (R^2 = 0.28 in topsoil and 0.10 in subsoil). Regression analysis showed weak to no correlation between soil efflux and barometric pressure during winter and autumn (R^2 = 0.02–0.12) and no correlation in spring and summer. A slightly higher correlation was noted in winter when using hourly averages (R^2 = 0.23 undisturbed, 0.18 disturbed).

3.6 Random forest efflux modeling

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

The predictors' relative importance (RI) varied across seasons and sites (Figure 10). In the winter model at the undisturbed site, air temperature emerged as the most influential predictor, accounting for 53.6% RI. Conversely, at the disturbed site, the subsoil temperature exhibited the highest predictive power with 49.6% RI (figure 10). For the spring model, soil temperature was the best predictor at both sites, the topsoil layer temperature exhibited a slightly greater predictive power at the undisturbed site, whereas the subsoil layer temperature proved to be more influential at the disturbed site. In the undisturbed site's summer model, the soil moisture and temperature as well as the seasonal thaw depth variables exhibit similar RI values, ranging from 15.7 to 20.2%. Among these variables, air temperature has the lowest predictive power, with an RI of 10.3%. Conversely, at the disturbed site, the topsoil layer temperature stands out as the strongest predictor, with an RI of 44.6%. It is followed by the topsoil VWC, which has an RI of 13.7%. The undisturbed site's autumn model indicates that subsoil layer VWC has the highest predictive power at 24.3% RI, followed by subsoil layer temperature at 18.9% RI, and topsoil layer temperature at 18.7% RI. However, the disturbed autumn model shows that subsoil layer temperature is the most influential factor in predicting CO_2 efflux with 24.3% RI and topsoil layer (21.1% RI) and air (18.9% RI) also playing significant roles.

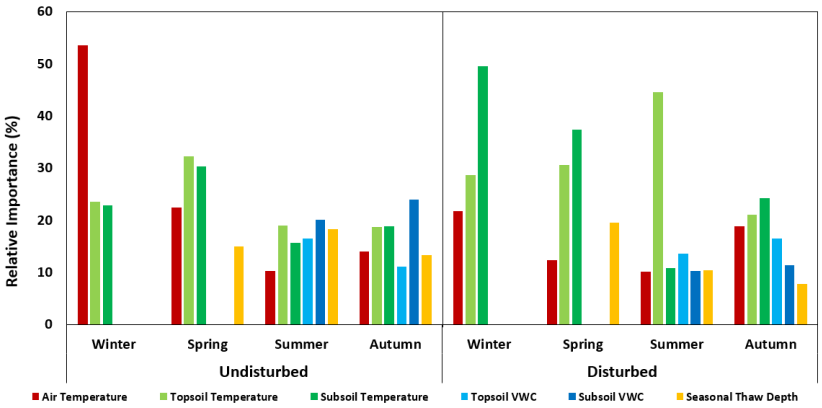

Figure 10. Relative importance (%) of variables to predict CO_2 efflux. Missing or static input variables (e.g. winter VWC and seasonal thaw depth) were not measured for importance.

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

Table 1. Tabulation of the most important predictor variables to explain variability in the soil efflux data 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

4. Discussion

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

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hypothesized that the disturbance would result in significant, persistent differences in soil respiration and edaphic properties between the disturbed and an adjacent undisturbed site. Crucially, we extended this hypothesis to the understudied winter period, postulating that these differences in soil activity would persist even under extreme cold temperatures. To elucidate the controls behind these variations, we examined seasonal CO_2 efflux alongside a suite of controlling factors, including soil temperature, moisture, soil organic matter, pH, seasonal thaw depth, and microbial community composition. In the following sections, we discuss how these interconnected variables explain the observed respiration patterns, providing insight into the long-term trajectory of carbon cycling following historical disturbance in permafrost environments.

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

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

undisturbed site.

Our results reveal a clear and persistent legacy of historical disturbance on soil carbon efflux, with the disturbed site exhibiting significantly higher overall respiration rates than the adjacent undisturbed site (Figure 9). This difference was most pronounced during the peak of the growing season, where summer CO_2 fluxes at the disturbed site were nearly 20% higher, a statistically significant margin ($p < 0.0001$). This finding strongly suggests that the initial disturbance—likely leading to warmer soils and a deeper active layer—has created conditions that continue to favor more rapid decomposition a century later. Interestingly, while respiration rates declined expectedly during the autumn and spring shoulder seasons, the differences between the two sites became statistically insignificant. This may indicate that during these transitional periods, other environmental factors, such as soil moisture levels) or temperature thresholds, become more dominant controllers of microbial activity (Kim et al., 2012), temporarily masking the underlying differences in soil properties. Crucially, our data demonstrate that the elevated carbon loss at the disturbed site persists even into the winter. Although absolute fluxes were at their lowest, the wintertime respiration at the disturbed site was still significantly higher than at the undisturbed site ($p < 0.01$). This confirms that the ground remains a dynamic system and that the historical disturbance has altered the soil's thermal regime or substrate availability in a way that sustains higher microbial activity year-round, reinforcing the notion that winter is a critical (Natali et al., 2019; Miner et al., 2022), and often overlooked, period for carbon cycling in these ecosystems.

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

Our random forest model analysis found that key abiotic factors drive distinct seasonal patterns in soil efflux between the disturbed and undisturbed sites (Figure 10)

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. In winter, air temperature emerged as the most significant predictor for the undisturbed sites, likely due to its direct impact on microbial activity and respiration rates. In contrast, the disturbed site during the same season showed a stronger correlation with the subsoil layer temperature, suggesting that disturbances may alter the thermal dynamics of the soil profile, making soil temperature a more crucial determinant (Table 1). Moreover, given that both locations experienced snow denial, the more substantial ground vegetation cover at the undisturbed site offered superior thermal insulation to the soil compared to the thinner ground cover vegetation at the disturbed site. Furthermore, it is very likely that winter soil respiration rates were higher at both sites where snow was not cleared, than at the chamber sites, as deep snow can provide an insulating effect as found by Welker et al. (2000) and Miner et al. (2022). During spring, topsoil layer temperature was the primary predictor for the undisturbed site, highlighting the importance of organic matter decomposition driven by temperature changes. For the disturbed site, the subsoil layer temperature remained the dominant factor, indicating that disturbances may have disrupted the organic layer, shifting the focus to the subsoil layer's thermal conditions.

In the summer months, the volumetric water content in the subsoil layer emerged as the principal predictor for the undisturbed site (Table 1), highlighting the essential role of moisture availability in influencing microbial activity and respiration during this warmer period. Conversely, the disturbed site exhibited a more pronounced correlation with the temperature of the topsoil layer (Table 1), indicating that disturbances may have modified the soil's hydrological characteristics by increasing thaw depth and enhancing drainage, which resulted in a reduced volumetric water content and rendered temperature a more significant factor than moisture. As autumn approached, the volumetric water content in the subsoil layer reestablished itself as the key predictor for the undisturbed site (Table 1), reaffirming the ongoing significance of moisture for microbial functions as temperatures began to decline. In contrast, for the disturbed site, the temperature of the subsoil layer continued to be the prevailing influence, underscoring the enduring effects of disturbances on the thermal dynamics of the soil. Other permafrost study sites in the Arctic have shown a reliance of soil efflux on soil moisture and temperature and suggest the drying out of soil regimes and subsequent infiltration of snowmelt may also be a contributing factor for predicting soil efflux values (Welker et al., 2000) and therefore annual data collection may likely be necessary to determine long-term trends at sites where different soil types and disturbances exist.

Conclusion

Permafrost-affected ecosystems are experiencing dramatic change from anthropogenic causes, both in terms of warming and physical disturbance to the soils. Active layers are deepening, unlocking carbon that was previously inaccessible. Here, we sought to measure soil respiration in undisturbed and

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

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

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

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

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