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WILDFIRE IMPACT ON PERMAFROST: CHANGES IN MICROBIOAL COMMUNITY AND SOIL DECOMPOSITION

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Wildfire impact on permafrost: Changes in microbial community and soil decomposition

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ACADEMIC DISSERTATION

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Nothing is more fatal to the progress of the human mind than to presume that our views of science are ultimate, that our triumphs are complete, that there are no mysteries in nature, and that there are no new worlds to conquer.

Humphry Davy

CONTENTS

LIST OF ORIGINAL ARTICLES

This thesis is based on the three articles listed under below, which are referred to in text using their roman numerals.

I. **Zhou, X.**, Sun, H., Pumpanen, J., Sietiö, O.M., Heinonsalo, J., Köster, K., Berninger, F., 2019. The impact of wildfire on microbial C:N:P stoichiometry and the fungal-to-bacterial ratio in permafrost soil. Biogeochemistry 142, 1–17.

II. Aaltonen, H., Palviainen, M., **Zhou, X.**, Köster, E., Berninger, F., Pumpanen, J., Köster, K., 2019. Temperature sensitivity of soil organic matter decomposition after forest fire in Canadian permafrost region. Journal of Environmental Management 241, 637–644.

III. **Zhou, X.,** Sun, H., Sietiö, O.M., Pumpanen, J., Heinonsalo, J., Köster, K., Berninger F., 2020. Wildfire effects on soil bacterial community and its potential functions in a permafrost region of Canada. Applied Soil Ecology 156, 1-11.

Authors' contributions

Article	Study I	Study II	Study III
Original idea	FB, JP, **XZ**	JP, KK, HA	FB, JP, HS, **XZ**
Field work & experimental design	FB, JP, KK	FB, JP, KK, MP	FB, JP, KK
Experimental work & data collection	**XZ**, HS, OS	HA, **XZ**	HS
Statistical analyses	**XZ**	HA, **XZ**	**XZ**, OS
Drafting the manuscript	**XZ**	HA	**XZ**
Critical revision	JP, JH, FB	JP, KK, FB	JP, JH, OS, HS, FB

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ABSTRACT

Permafrost is prone to thawing under disturbances resulting from frequent wildfires in boreal forests due to climate change, increasing the risk of the release of carbon (C) from it. Although the decomposition of organic C is mainly determined by the activity of soil microorganisms, productions of pyrogenic material after fire may offset this process. To evaluate the C dynamics related to wildfire disturbance in permafrost regions, this study examined the postfire changes in soil organic matter (SOM) decomposition, and the microbial community composition and its potential functions during a > 100-year chronosequence of burnt boreal forests.

Based on the kinetic theory, the temperature sensitivity of slowly decomposing SOM tends to be higher than that of easily decomposing SOM. Consistently, we found the decomposition of SOM in burnt surface soils containing less-decomposable SOM generated by fire, was more sensitive to temperature than that in old-growth forests. Fire also decreased the microbial biomass and the fungal-to-bacterial ratio of the surface soils. Despite this, soil heterotrophic respiration and the microbial C:N:P ratio in burnt forests remained similar level to that in old-growth forests regardless of the changing SOM quality and quantity. This suggests the notion of a lower microbial C use efficiency following a fire. Unexpectedly, permafrost thaw did not alter the microbial biomass and the fungal-to-bacterial ratio, but increased the microbial metabolic quotient.

Illumina Miseq sequencing of bacterial 16S rDNA revealed that the bacterial community composition in recently burnt surface soils differed from it in old-growth forest soils. Permafrost thaw, however, showed little effect on the bacterial community composition. Bacterial communities of burnt surface-soil exhibited higher abundance of Ktedonobacteria (Chloroflexi) but lower abundance of Betaproteobacteria. Functional gene compositions (DNA-based) of the burnt surface soil differed from those of the unburnt ones; particularly for genes coding for C degradation and the nitrogen cycle. Yet, the difference in the frequency of genes responsible for C degradation between thawed and frozen permafrost was not statistically significant.

This thesis provides further evidence for effects of wildfire on the microbial biomass, microbial community composition, and its potential functions on the C and N cycles in boreal permafrost regions. To estimate the real pattern of soil C cycles following a fire, we must understand how fire affects the metabolic processes of soil microorganisms.

INTRODUCTION

Microorganisms are essential for regulating biogeochemical cycles and are sensitive to changing environments. The increasing frequency of wildfires, resulting from climate change, generally causes changes in vegetation coverage and soil characteristics, such as the soil carbon (C) and nutrient availability, pH, temperature, and moisture (reviewed by Certini 2005). The impact of fire on boreal forests appears more severe and persistent given the risk of permafrost thaw, since nearly 80% of the boreal forest is underlain by permafrost (Helbig et al., 2016). As the large amount of C is stored in the permafrost (Tamocai et al., 2009), increasing microbial activity is likely to result from the permafrost thaw, possibly accelerating soil organic matter (SOM) decomposition and the release of greenhouse gases (Davidson and Janssens, 2006; Pautler et al., 2010; Uhlířová et al., 2007). Nevertheless, wildfire also changes the structure of SOM, which becomes more recalcitrant to decomposition (Preston and Schmidt, 2006; Schmidt and Noack, 2000). Thus far, our understanding of the consequences of fire on climate change remains limited. Therefore, this thesis focuses on the long-term effects of wildfires on permafrost soils; in particular, I focus on SOM decomposition and microbial properties regulating the biogeochemical cycle of C.

1.1 Wildfire effect on permafrost

Boreal forests store nearly one-quarter of the global biomass C (Thurner *et al.* 2014; Saatchi *et al.* 2011), whilst about 80% of the boreal forest area is underlain by permafrost (Helbig et al., 2016). **Permafrost** is the soil that remains frozen at a temperature below the freezing point for water for at least two consecutive years. Permafrost covers about 24% of the land in the Northern Hemisphere (Zhang et al., 2003), and can be classified as continuous or discontinuous permafrost, depending on the proportion of permafrost underlies (Shur and Jorgenson, 2007). Because a cold temperature restricts the decomposition of litter-derived organic matter, permafrost ecosystems accumulate large amounts of soil organic C and nutrients. Soil layers above permafrost is known as **the active layer**, which thaws and freezes seasonally, and its thickness is primarily determined by the temperature difference between summer and winter (Kane et al., 1991; Zhang, 2005). Snow cover also affects the active layer thickness by insulating soil against the cold air (Yukon Ecoregions Working Group, 2004). In recent decades, however, the active layer thickness has increased in many regions because of global warming, which has increased the air temperature in the northern regions more drastically than elsewhere in the world (Overland et al., 2014).

Two of the main factors affecting the boreal forest C stock are the increasing fire frequency and fire areas resulting from a warming and drying climate (Kasischke and Turetsky, 2006; Kelly et al., 2013). Approximately 92% of the boreal forest area in Canada was burnt by large wildfires (>200 ha) between 1959 and 2007 (Mu et al., 2011). The most significant impact of wildfire on the soil is the loss of soil C stocks, not only in terms of quantity but also in their quality (Almendros and González-Vila, 2012; Certini, 2005). Wildfires also produce toxic compounds such as aromatic hydrocarbons (Abdel-Shafy and Mansour, 2016), which inhibit the growth of living

organisms. In addition, a burnt forest accumulates charcoal on the soil surface, enhancing the absorption of solar radiation, thereby continuously thawing the near-surface permafrost (Michaelides et al., 2019). Forest fires also increase the topsoil pH (Arocena and Opio, 2003; Ulery et al., 1993) and alter the biogeochemical cycle of nutrients, such as the soil nitrogen (N) (Prieto-Fernandez et al., 1993; Weston and Attiwill, 1990), phosphorus (P) (Cade-Menun et al., 2000), calcium (Ca), manganese (Mg), and potassium (K) (Simard et al., 2001; Tomkins et al., 1991).

1.1.1 Soil organic matter

Burning not only leads to the direct loss of SOM, but also degrades the SOM quality by producing charcoal that is derived from the incomplete combustion of the biomass (Knicker, 2007). Charcoal is often considered to be more resistant to decomposition (Lehmann et al., 2006; Schmidt et al., 2011). The decomposition rate of SOM primarily depend on the microbial activity and SOM quality. SOM fall roughly into two categories: easily decomposing substrates and slowly decomposing substrates. The former are mainly found in the organic soils of boreal forests, and consisting of plant residues that can easily decompose, whilst the latter forms the majority of SOM in mineral soils that hard to decompose (Karhu et al., 2010). The charcoal on the soil surface following a fire accumulates heat from the solar radiation given its low albedo. The subsequent increase in the soil temperature shortly following burning may accelerate SOM decomposition. Thus, the response of the decomposition rate of fire-affected SOM (charred SOM) and fire-unaffected SOM (original SOM) to the rising temperature is crucial when estimating the long-term response of soil C pools to wildfires.

The temperature sensitivity of SOM decomposition, which is often described as Q_{10} or the response of the respiration rate (often measured using the CO_2 flux) to a 10°C temperature change, has been widely estimated from the incubation experiments. According to the kinetic theory, the temperature sensitivity of slowly decomposing substrates requiring high activation energies, is higher than that of easily decomposing substrates, unless other ecological factors suppress the decomposition process (reviewed by Conant *et al.* 2011). This theory, that the decomposition of less decomposable substrates is more sensitive to temperature, is supported by most empirical studies (Biasi et al., 2005; Bol et al., 2003; Curiel Yuste et al., 2007; Karhu et al., 2010; Malcolm et al., 2009). However, the temperature sensitivity of SOM can be increased by adding easily decomposable organic C, such as those from plant roots or fresh litter (Gershenson et al., 2009; Zhu and Cheng, 2011). Increases in the decomposition rates stemming from adding fresh litter are referred to as the priming effect, in which the increase in labile C input often stimulates the microbial decomposition of the slowly-decomposing SOM. Thus, in a fire-impacted boreal forest, SOM input from the regrowing plants may affect the temperature sensitivity of burnt SOM.

Organic matter in the deep soil layers is generally characterised by a longer turnover time than that in the organic layers (Tamocai et al., 2009). Aside from the low oxygen and cold environment that slow down the decomposition rate, deep soil layers are less affected by rhizosphere's 'priming effect' and the energy input of microorganisms remains limited. Due to the inhospitable environment with limited energy and nutrient supplies of deep soil layers, the microbial biomass appears to decrease with the soil depth (Fierer et al., 2003; Van Gestel et al., 1992). In permafrost, SOM is protected from decomposition by the low temperature. Therefore, permafrost soils may store large amounts of labile C. Following permafrost thaw that can be driven by fire-induced warming, microorganisms may cause SOM decompose faster (Schuur et al., 2009). Thus, over a

long time scale, a reduction in the amount of decomposable SOM or the sorption of SOM by mineral soils (Kawahigashi *et al.* 2006) may diminish the availability of SOM.

In addition to forest succession following a fire, the increasing litter input from overstorey vegetation increases the SOM and organic layer thicknesses (Jafarov et al., 2013; Shur and Jorgenson, 2007), as well as the thickness of the active layer above the permafrost table (Fig. 1). Thus, given the changing dynamics of forest floor C and other nutrient supplies, it is crucial to study the dynamics of SOM and soil microorganisms to understand the long-term effects of fire on biogeochemical cycles.

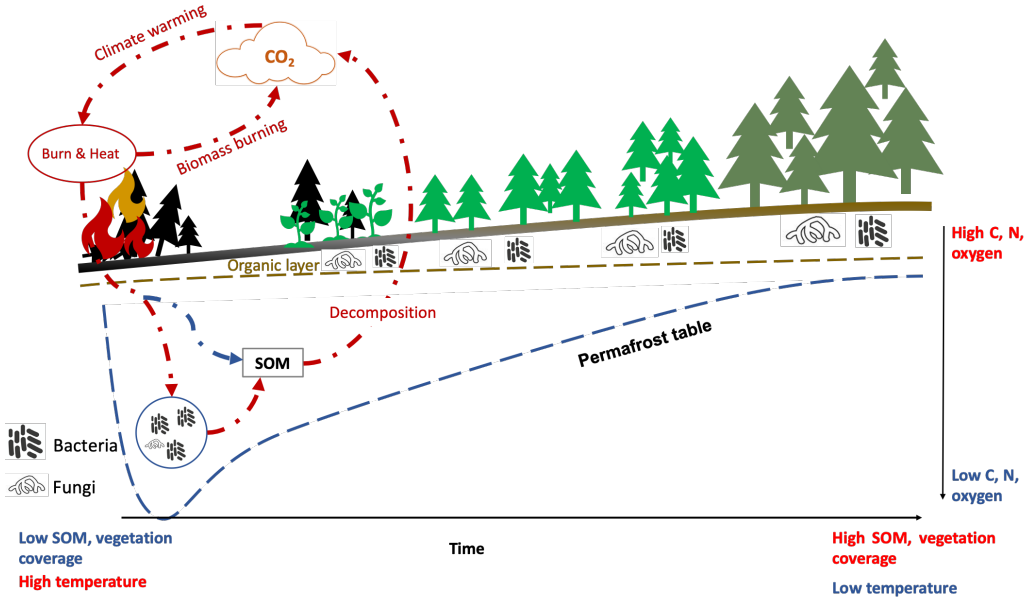

Figure 1| Post-wildfire permafrost features in the boreal forest. Permafrost thaws following a fire, gradually freezing again. The dark blue dashed line indicates the surface of the permafrost table. The organic layer is indicated by the area between the brown dashed line and the soil surface. Red dashed lines in the C cycle indicate the theoretical positive correlations, and the blue dashed line indicates the negative correlation. The bottom arrow indicates differences in the soil organic matter (SOM), vegetation coverage, temperature, and moisture developing over time. The right-side arrow shows the changes in the soil chemistry with depth.

1.2 Microbial ecology of permafrost

Microorganisms are the most phylogenetically diverse and most widespread group of organisms on Earth, inhabiting even the harshest environments such as permafrost (Mackelprang et al., 2011; Taş et al., 2014; Yergeau et al., 2010). In permafrost, microorganisms are less abundant, with a lower diversity compared to active-layer microorganisms (Yergeau et al., 2010). Permafrost thaw

may change the microbial community and its activity, thereby affecting the C cycle in boreal forests.

Apart from permafrost thaw, the direct impact of wildfire on microorganisms is a decrease in the microbial biomass (Dooley and Treseder, 2012), as well as a decrease in trees and ground vegetation, which provide energy and nutrients to soil microorganisms. In addition, burning also reduces and modifies the soil organic substrates and soil properties. All these processes challenge the survival of soil organisms. This raises the question of how and to what extent the microbial communities and their metabolic functions acclimate to changing environments. Will such changes apply to the thawing permafrost ecosystem?

1.2.1 Microbial stoichiometry and homeostasis

Ecological stoichiometry linked to biogeochemical cycles refers to the balance of multiple chemical elements — typically C, N, and P — in organisms and their environments (Redfield, 1958; Sterner and Elser, 2002). The consistent stoichiometric ratio (106:16:1) was first identified in marine plankton, which encouraged microbiologists to find a similar consistency (60:7:1) in soil microorganisms globally (Cleveland and Liptzin, 2007). Microbial stoichiometry is of interest because it regulates the C, N, and P cycles in soil ecosystems through immobilisation and mineralisation (Waring et al., 2013; Xu et al., 2013). These processes are strongly reflected in the stoichiometry of microorganisms and soil substrates (Mooshammer et al., 2012; Schimel and Bennett, 2004). For example, microorganisms tend to maintain their optimal ratios for growth and development either by respiring and excreting the excess elements (Tempest and Neijssel, 1992) or through absorbing elements deficient in the soil by using the extracellular enzymes (Mooshammer et al., 2014). These regulation processes that maintain the internal state of an organism are referred to as **homeostasis**. Organisms capable of maintaining their internal nutrient ratios are regarded as strict homeostatic organisms, whilst those incapable of doing so are non-homeostatic organisms.

The microbial stoichiometric ratio (i.e., the C:N:P ratio) can vary between microbial biomes for several possible reasons. First, microbial stoichiometric ratios strongly relate to shifts in the community structure (Mouginot et al., 2014). Although the C:N ratio varies with specific taxonomic clades, the fungal biomass on average has a higher C:N ratio than the bacterial biomass (Mouginot et al., 2014; Strickland and Rousk, 2010). Second, the microbial C:N and C:P ratios negatively correlate with the microbial growth rate because both N and P play a role in growth rate by generating proteins and nucleic acids (Sterner and Elser, 2002). Third, environmental factors such as temperature, pH, and nutrient availability can also alter the microbial C:N:P ratios (Chen et al., 2016). In this study, we attempt to illustrate these three assumptions through a series of fire-affected permafrost ecosystems by measuring the fungal-to-bacterial ratio, the soil respiration rate, and the soil C, N, and P contents.

Since variation in microbial stoichiometry often links to the growth rate, it also influences the **C use efficiency (CUE)**, typically defined as the ratio of net C assimilation and the total C uptake (Choudhury, 2000). A high CUE indicates a high growth rate in proportion to a low level of C released through respiration. In practice, however, measuring CUE is methodologically difficult. Thus, several parameters have been developed to estimate CUE, such as **the metabolic quotient** (**qCO_2**, the respiration rate per unit of biomass; Rutigliano *et al.* 2007). In this case, a high qCO_2 (a higher C release, but a less C stabilised in the microbial biomass) denotes a lower CUE of

microorganisms. Although multiple ecological factors may affect the microbial CUE, the most direct effects stem from the substrate composition (reviewed by Manzoni *et al.* 2012). Slowly decomposing substrates require more complicated metabolic processes to fully degrade, resulting in a high respiration per sequestrated C (van Hees et al., 2005). In addition, when microorganisms consume low-quality substrates such as those with high C:nutrient ratios, the excessive C is released through respiration or excretion to balance the microbial stoichiometry for an optimal growth rate (also described above as homeostatic regulation) (Manzoni and Porporato, 2009).

1.2.2 Bacterial community composition and functions

Microorganisms in permafrost are considered psychrotolerant — that is, they can grow and reproduce in a subzero environment (Wilhelm et al., 2012). In recent decades, high-throughput DNA sequencing data from soil microorganisms of the permafrost region have become available (Mackelprang et al., 2011; Taş et al., 2014; Yergeau et al., 2010). Surviving at a low temperature is challenging for bacteria. Therefore, they have developed a set of specific proteins to adapt to the cold, such as cold-shock proteins and proteins regulating membrane fluidity (reviewed by Jansson & Taş 2014). Nonetheless, microbial abundance and diversity in permafrost remain lower than those in the active layer (Steven et al., 2007; Wilhelm et al., 2011). Thus, permafrost thaw may increase microbial abundance and reshape the microbial community and functions. In addition, vegetation removal following a fire can considerably restructure the microbial community composition, selecting taxa clades better adapted to energy-limited environments (Fierer et al., 2007). Although the response of the microbial community to fire and warming has garnered considerable interest (Day et al., 2019; Taş et al., 2014; Woodcroft et al., 2018; Yuan et al., 2018), knowledge of the impact of fire on permafrost microbial communities remains scarce given the heterogeneity of permafrost and the complexity of diverse fire-induced environments (Carson and Zeglin, 2018; Sun et al., 2014).

Researchers, however, observed that bacterial diversity is higher than the diversity of fungi and archaea in permafrost soils (Steven et al., 2007; Taş et al., 2014). Thus, understanding postfire changes in the bacterial functions is important for estimating the soil C fluxes. To date, genomic studies of permafrost using metagenomics or gene-chip approaches have emerged (Sun et al., 2016; Taş et al., 2014; Yergeau et al., 2010). These approaches can detect numerous genes for various functions, the most discussed of which within permafrost studies consists of the genes involved in the C and N cycles. In the permafrost layers, particularly those with high water contents, the anaerobic respiration pathways are essential for further C degradation through methanogenesis (Mackelprang et al., 2011; Taş et al., 2014). Moreover, although genes coding for ammonification and denitrification are also found in the permafrost, their abundances are too low to denitrify nitrate to N_2. Therefore, those microorganisms in the permafrost are more likely to produce N_2O instead (Mackelprang et al., 2011).

Microbial functions primarily driven by a limited set of metabolic pathways can be presented in various taxonomic clades, that is referred to as functional redundancy. Many studies across types of environments reveal that specific microbial metabolic functions largely correlate with environmental factors, although they are less likely correlate with shifts in the abundance of specific species (Burke et al., 2011; Louca et al., 2017). Thus, instead of merely studying the shifts in the microbial community structure, it is necessary to identify the change in the microbial metabolic functions reflecting the ultimate question regarding how wildfire affects the soil ecosystem functions and biogeochemical cycles.

AIMS AND HYPOTHESES

The main aim of this thesis is to estimate how wildfires affect soil microbial properties and how these changes affect microbial respiration and SOM decomposition in a permafrost region of Northern Canada. First, we identified the long-term effects of fire on the soil properties and vegetation, and tested how these variables explain the changes in the microbial biomass, the fungal-to-bacterial ratio, and the microbial C:N:P stoichiometric ratio of the active layer and permafrost table (study I). Second, to understand how wildfire affects SOM availability and microbial respiration, we measured the temperature sensitivity of SOM decomposition and the microbial metabolic quotient (qCO_2) (study II). Furthermore, to estimate the soil ecosystem functions following a fire, we identified and discussed the shifts in the bacterial community composition and the functional gene structure (study III).

Hypotheses

1 Fire decreases the soil microbial biomass and alters compositions of fungal and bacterial communities due to the vegetation removal (study I).

2 Fire transforms boreal forest SOM to slowly decomposing SOM (primarily charcoal), in which contains less available C to microorganisms.

   a) Limited soil C supply may lead to a lower microbial C:N ratio (study I).
   b) Slowly decomposing SOM would result in a lower heterotrophic respiration of soil (study II).
   c) Decomposition of the slowly decomposing SOM is more sensitive to the temperature than that of the original SOM according to the kinetic theory (study II).

3 Microbial community composition and its potential functions may shift in order to acclimatize to rapid changes in the microclimate caused by fire (study III).

4 Permafrost thaw is more likely to increase the microbial biomass and SOM decomposition rate, reshapes the bacterial communities, and stimulates their potential functions on the C and N cycles (studies I, II, and III).

MATERIALS AND METHODS

A detailed description of the material and methods can be found in the manuscripts appearing at the end of this dissertation (studies I–III). Table 1 lists all of methods used in this dissertation research.

3.1 Site description and sampling

The study areas were located in the continuous permafrost zone in the Yukon and the North West Territories (66°22′ N - 67°26′ N, 136°43′ W - 133°45′ W), Canada. The soils in Northern Canada fall within the Cryosolic order (Stanek, 1982). The bedrock from the study areas consists of Cretaceous sandstones covered by ice-rich fluvial and clay-rich colluvial deposits, with continuous permafrost (90-100%) underneath (Hadlari, 2006). Black spruce (*Picea mariana* (Mill.) Britton, Sterns and Poggenburg) and white spruce (*Picea glauca* (Moench) Voss) represent the dominant tree species in these areas, with lingonberry (*Vaccinium vitis-idaea* L.), cloudberry (*Rubus chamaemorus* L.), bog bilberry (*Vaccinium uliginosum* L.), and *Rhododendron groenlandicum* Oeder as dominant dwarf shrubs.

We studied the effect of fire on permafrost thawing and their consequent effects on the soil microorganisms using a fire chronosequence approach, whereby we selected study sites in areas with similar soil types, vegetation types, and climates, but with different postfire time scales. The fire chronosequence was selected along the Dempster Highway in forest stands where the last forest fires occurred 3 ($Fire_3$), 25 ($Fire_{25}$), 46 ($Fire_{46}$), and >100 years ago ($Fire_{100}$, set as the control). In each age area, three 150-m-long lines (replicates) were established at least 200-m apart. $Fire_{100}$ lines were placed in forest stands not affected by a fire for at least 100 years. Three lines of $Fire_{100}$ were located adjacent to the three fire-affected areas — $Fire_3$, $Fire_{25}$, and $Fire_{46}$, respectively (Fig. 2). Along each line, three 400-m² plots (pseudo-replicates) were placed at least 50-m away from the other. Soil samples were taken from each plot at depths of 5, 10, and 30 cm. In total, we collected 107 samples (4 time point after a fire × 3 sites (lines) × 3 plots per site × 3 layers per plot, with one sample missing) for the molecular biology studies in microcentrifuge tubes (2-ml in volume), transported in a Dryshipper filled with liquid nitrogen, and stored at -80 °C before analysis. Soil samples for the microbial biomass and the incubation experiments were transported in an icebox and kept at -20 °C until analysis. We recorded the active layer thickness and the organic layer depths across all areas to compare the changes in permafrost at different stages of recovery following a fire (Table 1). In addition, postfire microclimates, such as vegetation coverage and soil properties were measured or estimated across all areas.

3.2 Microbial stoichiometry and carbon use efficiency (study I & II)

The soil samples (n = 107) used for the microbial biomass measurements and the incubation experiment were sieved through 2-mm mesh after the removal of visible roots. Prior to measurement and incubation, samples were pre-incubated at 4°C for 1 and 3 weeks, respectively.

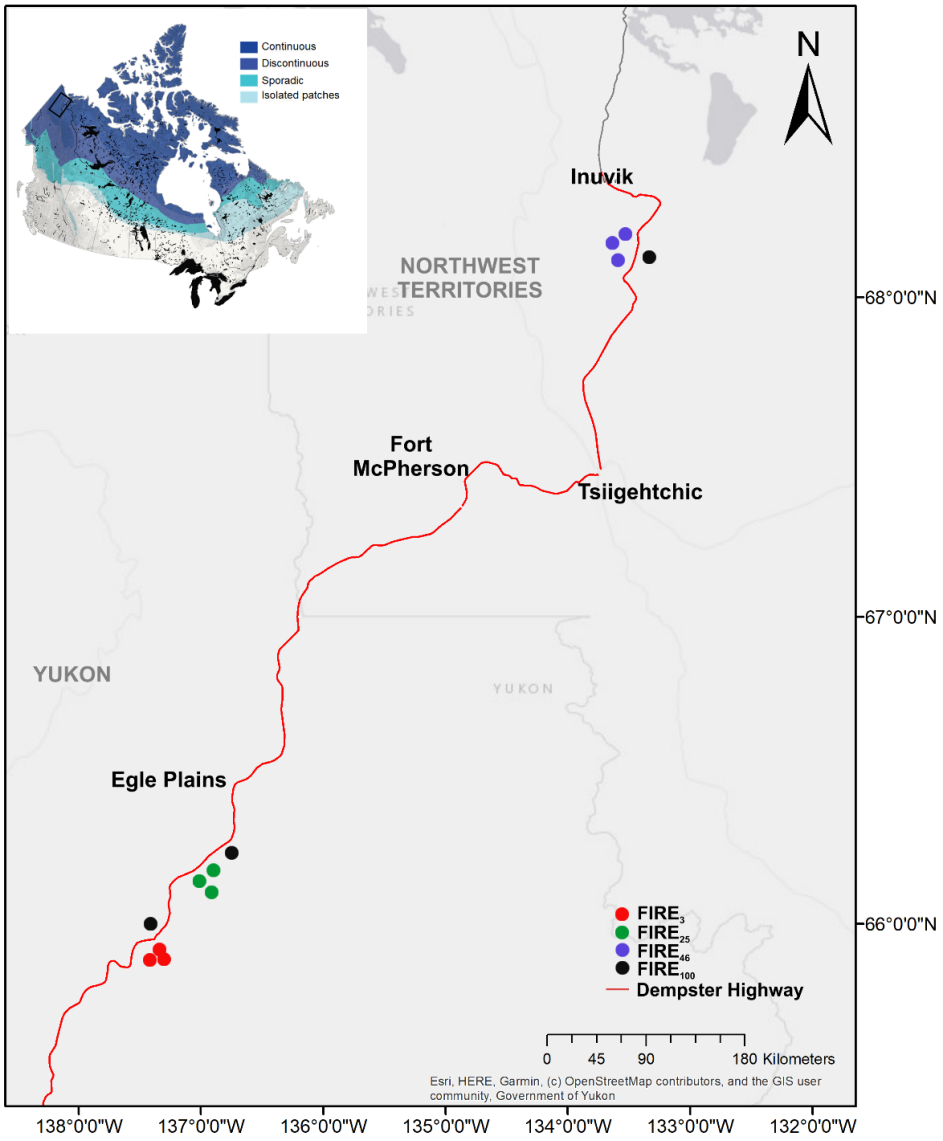

Figure 2| Soil sampling locations for four fire classes and 12 sampling lines in Canadian boreal forests along the Dempster Highway.

3.2.1 Stoichiometry and the fungal-to-bacterial ratio (study I)

Soil microbial biomass was estimated using chloroform fumigation extraction method. Briefly, C and N were extracted using K_2SO_4, while we extracted P using $NaHCO_3$ before and after chloroform fumigation (Beck et al., 1997). Extracts were filtered and quantified using a total organic C analyser (Vario MAX C&N analyser, Elementar Ltd., UK) for C and N, and the ammonium molybdate-malachite green method for P (D'Angelo et al., 2001). The differences between fumigated and nonfumigated extracts indicated the microbial biomass. Contents of C, N, and P determined from nonfumigated soil indicated the soil extractable C, N, and P, respectively. In addition, soil DNA was extracted and purified, and fungal 18S ribosomal DNA (rRNA) and 16S rDNA gene copy numbers were further determined through real-time quantitative PCR (Helin *et al.* 2017). The fungal-to-bacterial ratios (F:B ratio) were estimated using the ratio between 18S rDNA and 16S rDNA gene copy numbers.

Our sampling design risks violating the assumption of the independence of observations for logistical reasons (sample plots were located 50-m from each other along lines). Therefore, as an alternative to the analysis of variance and general linear models, we applied mixed-effect models. To explain the variation in the microbial C, N, and P, the F:B ratio, and the microbial qCO_2 across different fire areas, we fit the mixed-effect models using the observed environmental variables as the explanatory variables (fixed effects) and the sampling lines as the random effects, the details of which appear in the attached manuscripts (studies I and II).

To calculate the degree of homeostasis, we used the classical method by fitting the data to the homeostatic model (Sterner and Elser, 2002) as follows:

$$\log(y) = c + \frac{1}{H}\log(x_{ij}) + \beta_i + \varepsilon_{ij}$$

where y is the microbial element content or the molar ratio, x is the extractable soil element content or ratio, and c is a constant intercept. H represents the degree of homeostasis, β is the random effect for each sample line, and ε is the error. The higher H is, the stronger the microbial homeostatic regulation against the soil nutrient supply.

3.2.2 Carbon use efficiency and SOM temperature sensitivity (study II)

To determine the soil respiration, we used the following methods: approximately 25-ml soil samples were weighed and added to a 500-ml incubation bottles placed in climate chambers (WEISS WKII 340, Weiss Klimatechnik, Germany) at 1, 7, 13, and 19 °C, respectively, for 24 h before gas sampling. Carbon dioxide (CO_2) concentrations were then analysed in an Agilent Gas Chromatograph (GC 7890A Agilent Technologies, USA). The subduction of CO_2 fluxes between bottles containing soil and the blank bottles led to the CO_2 respiration rate per unit of organic matter ($\mu gCO_2\ gC^{-1}\ h^{-1}$). The microbial metabolic quotient (qCO_2) was calculated as the ratio of CO_2 production to the microbial biomass.

In addition, Q_{10} value was used to quantify the temperature sensitivity of SOM decomposition. Its value was derived from the exponential model below:

$$R_h = R_{ref} Q_{10}^{\frac{T - T_{ref}}{10}}$$

where R_h is the observed microbial respiration rate ($\mu g CO_2\ gC^{-1}\ h^{-1}$) at corresponding temperature T (°C), T_{ref} is the reference temperature (10 °C), and R_{ref} is the respiration rate at the reference temperature.

3.3 Bacterial community and the functional gene structure (study III)

3.3.1 Community composition

DNA was extracted from a 0.25-g (fresh weight) soil sample using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel GmbH & Co) applying modifications as described in Timonen et al. (2017). DNA was further purified using PowerClean Pro DNA Clean-Up Kit according to the manufacturer's instructions (MoBio Laboratories). We quantified DNA concentrations using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and diluted the samples with nuclease-free water to 10 ng μl^{-1}.

In order to identify shifts in the bacterial community composition, we performed direct PCR amplification and fingerprinting of the specific profile of the bacterial 16S DNA v3-v4 region (for 107 soil samples). The final PCR amplicons were purified and sequenced using the pair-ended (PE-300) Illumina MiSeq Platform. The sequence data were filtered, de-noised, and clustered into operational taxonomic units (OTUs) using mothur [version 1.39, (Schloss et al. 2009)] following the standard operating procedure (Kozich et al. 2013, accessed 15 January 2017). Sequences were clustered into 26160 OTUs. To avoid taxa that seldomly appeared among samples, OTUs that had < 5 reads globally in the dataset were removed (Callahan et al., 2016). The original sequences are available in the NCBI database under Bioproject number PRJNA514982.

The dissimilarities in the bacterial community structure between different age areas were tested through a permutational multivariate analysis of variance (PERMANOVA). Differential gene expression analysis (DEA) based on the negative binomial distribution was applied to test for differences in the taxonomic amount at the phylum and class levels between recently burnt areas and old-growth areas. Canonical correspondence analysis (CCA) was conducted to test the contribution of the environmental variables (including the abiotic and biotic variables described in Table 1) to the variation of microbial community assemblages across different fire areas. The effects of the abiotic variables and biotic variables were interpreted using variance partitioning analysis.

3.3.2 GeoChip 5.0K microarray

For the functional gene structure, we measured 24 DNA samples using the GeoChip 5.0K microarray from two layers (5-cm and 30-cm layers) across the four age areas using 3 replicates in each area. The replicates were divided from a pooled DNA sample from 3 sample lines in each age area. Briefly, the genomic DNA was mixed with random primers and then labelled with 15-µl labelling master mix, after which the labelled genomic DNA was purified, dried, and hybridised in the GeoChip 5.0K microarray as described in He *et al.* (2007).

The GeoChip 5.0K contains 167 044 predetermined probes detect bacterial functional genes. The genes used in this dissertation were from 6 gene categories (i.e., the C, N, and P cycles, stress response, organic remediation, and metal homeostasis), and 50 gene subcategories fell within

these 6 categories. Dissimilarities in the overall gene structures were examined using PERMANOVA with the Bray–Curtis dissimilarity indices. Differences in the gene frequency of each gene subcategory between the fire areas were tested using the nonparametric Kruskal–Wallis test. Canonical Correspondence Analysis (CCA) was implemented to understand the relationship between the functional gene structures with environmental variables. We also analysed the variance partitioning linking the bacterial community assemblages (at the phylum level), biotic variables, and abiotic variables to the functional gene composition (Oksanen *et al.* 2018).

Table 1| Summary of the analyses and statistics used in the studies

ANALYSES	STUDIES
Abiotic environmental variables	
Soil pH and electrical conductivity (EC)	I, II, & III
Soil temperature and moisture	I, II, & III
Active layer and organic layer thickness	I, II, & III
Soil organic C, N, and P content	I, II, & III
Soil organic matter	II
Biotic environmental variables	
Tree and ground vegetation biomass	I
Hypotheses 1 & 4	
Chloroform fumigation extraction of the microbial biomass	I
DNA extraction	I
PCR	
Fungal 18S rRNA amplification	I
Bacterial 16S rRNA amplification	I & III
Real-time quantitative PCR (qPCR) of the fungal-to-bacterial ratio	I
Hypotheses 2 & 4	
Microbial C:N:P ratio and homeostasis	I
Temperature sensitivity (Q_{10})	II
Metabolic quotient (qCO_2) for CUE	II
Incubation CO_2 fluxes from each soil layer	II
Hypothesis 3 & 4	
MiSeq sequencing of the bacterial community structure	
Bacterial 16S rDNA v3–v4 region	III
Sequence data processing with mothur in the CSC environment	III
GeoChip 5.0K of the bacterial functional gene composition	III
STATISTICS (R environment)	
Linear mixed–effect model	I & II
Model comparison (Akaike Information Criterion (AIC))	I
Permutational multivariate analysis (PERMANOVA)	III
Multivariate analyses (CCA and CA)	III
Correlation analysis	III
Variance partitioning analysis	III
Analysis of variance (ANOVA)	III
Paired t-tests	III

RESULTS AND DISCUSSION

Table 2 summarises the results from the tests of our primary hypotheses. Detailed results and the accompanying discussion appear in the individual manuscripts (studies I–III). This chapter provides highlights and briefly introduces the main results and findings from each manuscript.

Our results supported most but not all, of the hypotheses we presented (Table 2). Whilst forest fires increased the soil temperature and thawed permafrost, the effects on C release remained modest. Reductions in the quality of the soil organic matter as well as reductions in the microbial biomass potentially contributed to an increase in the microbial activity (interpreted as qCO_2, the bacterial potential functions on C degradation) due to the increasing temperature.

Contrary to our hypotheses, microbial stoichiometry remains stable, although the available C and nutrients of SOM changed. In addition, the opposing hypothesis, where no effects from permafrost thaw on the microbial biomass, communities, and their potential functions, was supported.

Table 2| Results from tests of the main hypotheses in this dissertation

HYPOTHESES	RESULTS
1. Fire decreases the soil microbial biomass and alters compositions of fungal and bacterial communities due to the vegetation removal.	Supported. The surface microbial biomass and fungal-to-bacterial ratio in $Fire_3$ were significantly lower than those in $Fire_{100}$. However, these differences no longer existed between $Fire_{25}$ and $Fire_{100}$ (study I).
2. Fire transforms boreal forest SOM to slowly decomposing SOM (primarily charcoal), in which contains less available C to microorganisms.	Supported. The available soil C and N were the lowest in $Fire_3$ amongst the four study areas (study I).
a) Limited soil C supply may lead to a lower microbial C:N ratio.	Rejected. Despite the dramatic change in the soil available C and N contents, the microbial C:N ratio remained nearly constant (study I).
b) Slowly decomposing SOM would result in a lower heterotrophic respiration of soil.	Rejected. Fire did not change the microbial respiration (study II). Along with a decrease in the microbial biomass after a fire (study I), the metabolic quotient, that is, the soil respiration per unit of microbial biomass, increased (study II).
c) Decomposition of the slowly decomposing SOM is more sensitive to the temperature than that of the original SOM according to the kinetic theory.	Supported. The temperature sensitivity of the SOM decomposition in $Fire_3$, particularly those from the 5- and 30-cm layers, was significantly higher compared to that in $Fire_{100}$ (study II).
3. Microbial community composition and its potential functions may shift in order to acclimatize to rapid changes in the microclimate caused by fire.	Supported. The abundance of some bacterial clades changed following a fire, although for many clades it remained unchanged. Fire decreases the potential functions in surface soils, such as through C degradation and N fixation (study III).
4. Fire-induced permafrost thaw increases the microbial biomass and SOM decomposition rate, and reshapes the bacterial communities and stimulates their potential functions, especially for the C and N cycles.	Rejected. Although differences in these indices between frozen and melted permafrost were not significant, permafrost thaw can increase the temperature in surface soils causing the changes listed above (studies I, II, & III).

4.1 Microclimate caused by fire (studies I, II, & III)

The burning of forest biomasses results in a large release of large C into the atmosphere (Dixon and Krankina, 1993; French et al., 2000). Postfire changes such as the rise in soil temperature will continuously affect the soil C release, particularly in permafrost regions. To understand the response of microorganisms to fire on a longer time scale, we determined SOM quality, microbial biomass, fungal-to-bacterial ratio, microbial metabolic quotient (qCO_2), and bacterial community composition and its functions along a fire chronosequence.

Recently burnt sites were generally warmer, drier, and featured less vegetation coverage on the forest floor than the old-growth forest sites (Table 3). Fire causes an increase in the amount of solar radiation reaching to the soil surface by the removal of mosses and vegetation. Therefore, it raises the soil temperature and increases the thickness of biologically active layer above permafrost (Certini, 2005). The following section briefly summarises the main results of this PhD related research. Details can be found in the individual manuscripts (studies I–III).

Table 3| Observed environmental factors across fire-affected and fire-unaffected forest stands. Arrows represent the significant differences between values in burnt forest classes and the control ($Fire_{100}$) at $p < 0.05$.

Environmental factors	5 cm				10 cm				30 cm			
	$Fire_3$	$Fire_{25}$	$Fire_{46}$	$Fire_{100}$	$Fire_3$	$Fire_{25}$	$Fire_{46}$	$Fire_{100}$	$Fire_3$	$Fire_{25}$	$Fire_{46}$	$Fire_{100}$
Abiotic variables												
Act. Lay. Thick. (cm)	**101**↑	**88**↑	**65**↑	29	–	–	–	–	–	–	–	–
Org. Lay. Thick. (cm)	**5.34**↓	**10.20**↓	14.11	16.00	–	–	–	–	–	–	–	–
Soil Type	Org.	Org.	Org.	Org.	**Min.**	Org.	Org.	Org.	Min.	Min.	Min.	Min.
pH	4.54	4.81	6.64	4.77	4.70	5.16	6.56	5.00	5.15	5.42	7.02	5.68
Temp (°C)	**7.20**↑	**7.05**↑	**8.89**↑	6.86	**5.18**↑	**5.72**↑	**4.87**↑	3.16	**4.01**↑	**3.64**↑	**2.80**↑	-0.06
Moisture (%)	**35.71**↓	**37.46**↓	52.33	55.63	38.62	43.17	45.83	54.36	35.71	38.08	52.33	55.37
C_{ext} (mg g-1)	1.95	1.58	5.51	9.73	0.30	1.03	0.73	3.62	0.51	0.30	0.43	0.96
N_{ext} (mg g-1)	0.42	0.29	0.26	0.48	0.04	0.19	0.15	0.27	0.05	0.05	0.02	0.10
Biotic variables												
C_{mic} (mg g-1)	3.49	3.01	5.87	10.16	0.13	3.10	1.32	3.38	0.88	0.18	0.19	1.36
N_{mic} (mg g-1)	0.26	0.31	0.45	0.80	0.01	0.05	0.13	0.50	0.09	0.01	0.01	0.15
P_{mic} (mg g-1)	0.09	0.15	0.16	0.37	0.01	0.07	0.01	0.23	0.00	0.02	0.00	0.02
F:B ratio	0.07	0.22	0.08	0.07	0.08	0.04	0.21	0.01	0.09	0.04	0.03	0.12
Vegetation. Coverage (%)	15.5	33.4	47.2	37.4	–	–	–	–	–	–	–	–
Foliage biomass (kg m^{-2})	0	0	682.6	1265.8	–	–	–	–	–	–	–	–

* Act. Lay. Thick., active layer thickness; Org. Lay. Thick., organic layer thickness; C_{ext} and N_{ext}, extractable C and extractable N contents, respectively; C_{mic}, N_{mic}, and P_{mic}, the microbial biomass C, N, and P, respectively.

4.2 Postfire decomposition of the SOM (study II)

Our incubation experiment showed that the difference in the microbial respiration between recently burnt soils and old-growth soils was not significant (study II, Fig. 2). Decomposition of surface layer SOM (5 cm) of $Fire_3$ appeared more sensitive to temperature – higher in Q_{10} value – than it of $Fire_{25}$ ($p < 0.05$) and $Fire_{100}$ ($p = 0.06$). This pattern was more evident in the 30-cm layer ($p < 0.05$), but not at 10 cm (Fig. 3). We found no difference in Q_{10} value between $Fire_{25}$ and $Fire_{100}$ (study II).

Fire typically converts boreal forest SOM to pyrogenic material (primarily charcoal), which is more resistant to decomposition (Knicker, 2007). This is consistent with our previous study showed that SOM in $Fire_3$ contained less labile SOM than $Fire_{100}$ (Aaltonen et al., 2019). Thus, the increasing temperature sensitivity amongst SOM decomposition in $Fire_3$ adds further evidence that slowly decomposing SOM is more sensitive to the rising temperature than that of labile SOM (Biasi et al., 2005; Bol et al., 2003; Curiel Yuste et al., 2007; Karhu et al., 2010; Malcolm et al., 2009). This is because fire transforms boreal forest SOM into pyrogenic material (charcoal), which is far more resistant to decomposition (Knicker, 2007). Surprisingly, the temperature sensitivity of SOM in $Fire_3$ at depth of 30 cm was also the highest amongst the areas studied, although soil heating even in intense fire rarely reaches > 20 cm (Neary et al., 1999). Thus, the higher temperature sensitivity in deep-layer SOM in a recently burnt area presumably results from the translocation of pyrogenic materials from the topsoil. However, additional input from easily decomposable SOM can be expected from regrowing plants, which may enhance the decomposition of SOM at the early recover stage from fire (Gershenson et al., 2009; Zhu and Cheng, 2011). Yet, the temperature sensitivity of SOM in $Fire_{25}$ was lower than that in $Fire_3$, although rather similar to that in $Fire_{100}$. This likely results from the SOM composition in $Fire_{25}$, which may already have developed a similar status to SOM in $Fire_{100}$ (Fig. 3).

4.3 Postfire microbial stoichiometry of the soil (study I)

The microbial biomass (I) of surface soils (5 and 10 cm) was significantly lower in $Fire_3$ than in $Fire_{100}$ (Fig. 3A), findings consistent with previous study showed that soil microbial biomass declined following fires in boreal forests (Dooley & Treseder 2012). When measuring the fungi and bacteria separately (study I), fungal gene copy number negatively responded to fire, unlike bacterial gene copy number, which in agreement with earlier studies (Dooley and Treseder, 2012; Hamman et al., 2007). Thus, the decline in the fungal-to-bacterial ratios following a fire definitely resulted from a reduction in the abundance of fungi (Fig. 3). However, contrary to our hypothesis, permafrost thaw did not affect the microbial biomass or the fungal-to-bacterial ratio. The mixed–effect models showed that fire-induced changes in soil-extractable C, P, and vegetation biomass explained 76% of the microbial biomass variation (study I, Table 1).

The microbial C:N and N:P ratios remained constant across all age classes regardless of changes to the soil C, N, and P supplies (study I, Table 3). This suggests that constraint homeostasis regulates nutrient stoichiometry within microorganisms (Cleveland and Liptzin, 2007; Xu et al., 2015). The two primary causes for the change in the microbial stoichiometry are the shift in the fungal and bacterial proportions (study I) and the change in the microbial qCO_2 with different SOM qualities (study II).

4.3.1 Links between the microbial metabolic quotient and microbial stoichiometry (studies I & II)

The microbial respiration rate per unit microbial biomass (qCO_2, metabolic quotient) was higher in fire-affected soils ($Fire_3$ and $Fire_{25}$) compared to the soil in old-growth forests ($Fire_{100}$) across all soil layers (study II, Fig. 3). This fire-induced increase in the microbial qCO_2 is consistent with previous studies (De Marco et al., 2005; Fritze et al., 1993; Rutigliano et al., 2007). In addition, we observed a surprisingly constant microbial C:N ratio across all fire areas, although the C:P and N:P ratios varied (study I, Table 3). This suggests that, in terms of C and N, soil microorganisms tended to maintain stable stoichiometric ratios through homeostatic regulation (Cleveland and Liptzin, 2007; Xu et al., 2015). In fact, the increase in qCO_2 following a fire supports the constant microbial C:N ratio based on the CUE theory. Furthermore, our results showed a significantly lower qCO_2 in $Fire_{46}$ than the remaining areas (Fig. 3B). This likely resulted from the higher soil pH in $Fire_{46}$ (Table 3), possibly inhibiting the respiration of soil microorganisms. Amongst our study areas, soil pH did not change over time since a fire likely resulting from differences in the parent materials.

In severely restricted C and nutrients, microorganisms can adjust their CUE to maintain their stoichiometry by releasing any excess elements (Mooshammer et al., 2014; Russell and Cook, 1995; Spohn and Widdig, 2017). One theory explaining CUE argues that microorganisms burn more energy (C) to gain N from the recalcitrant organic matter when facing nutrient limitations (Craine et al., 2007). Furthermore, a so-called 'overflow respiration' enables microorganisms respiring excess C to maintain the stoichiometric ratio of themselves in soils with high C:nutrient ratios. In fact, multiple incubation studies observed overflow respiration in microbial communities (Moorhead et al., 2012; Tempest and Neijssel, 1992). Other researchers observed that qCO_2 tends to increase with an increasing temperature or declining moisture (reviewed by Manzoni *et al.* 2012). However, such overflow respiration assumptions are questioned by the debate surrounding metabolic processes for C respiration requiring N-rich proteins to complete the reaction (Hessen and Anderson, 2008). In this sense, the higher C respiration should have stemmed from a higher N availability. This contradicts assumptions regarding the leading cause of overflow respiration. Thus, whilst overflow respiration can perfectly explain the observed C flow, the mechanisms within the 'black box' remain the topic of debate.

4.3.2 Links between the fungal-to-bacterial ratio and microbial stoichiometry (study I)

In the surface layers (5 and 10 cm), the fungal-to-bacterial (F:B) ratios were lower in $Fire_3$ compared to $Fire_{100}$, whilst we detected no significant difference of F:B-ratios in the permafrost layer between fire areas (study I, Fig. 3). However, changes in F:B-ratios resulted from shifts in fungal gene copy numbers across the four age areas, whilst bacterial gene copy numbers remained relatively constant across the four forest classes (study I, Fig. 3). This agrees with a previous study showing that fungi are less heat tolerant (>200 °C) than bacteria (Bárcenas-Moreno and Bååth, 2009). Although it appears that the bacterial gene copy numbers were unlikely affected by fires, a molecular level analysis is required to discover the effect of fire on the bacterial community composition (see section 4.4).

Theoretically, the fungal biomass possesses a higher biomass C:N ratio (on average 15:1) than the bacterial biomass C:N ratio (6:1) (Waring et al., 2013). Shifts in the microbial C:N ratio should increase with increasing F:B ratios. However, whilst we observed an increase in the F:B ratios over time following a fire, the microbial C:N ratio remained relatively stable (12–14:1).

Shifts in the F:B ratio alongside the environmental variables in our study remained consistent with patterns observed using a phospholipid fatty acid (PLFA) method (Waring 2013). In this study, however, the F:B ratios were based on the gene copy number ratio instead of the biomass ratio used previously. Thus far, the F:B biomass ratio remained underestimated regardless which methods being applied, including the qPCR approach used in our study, microscopy, and PLFAs (Joergensen & Wichern 2008).

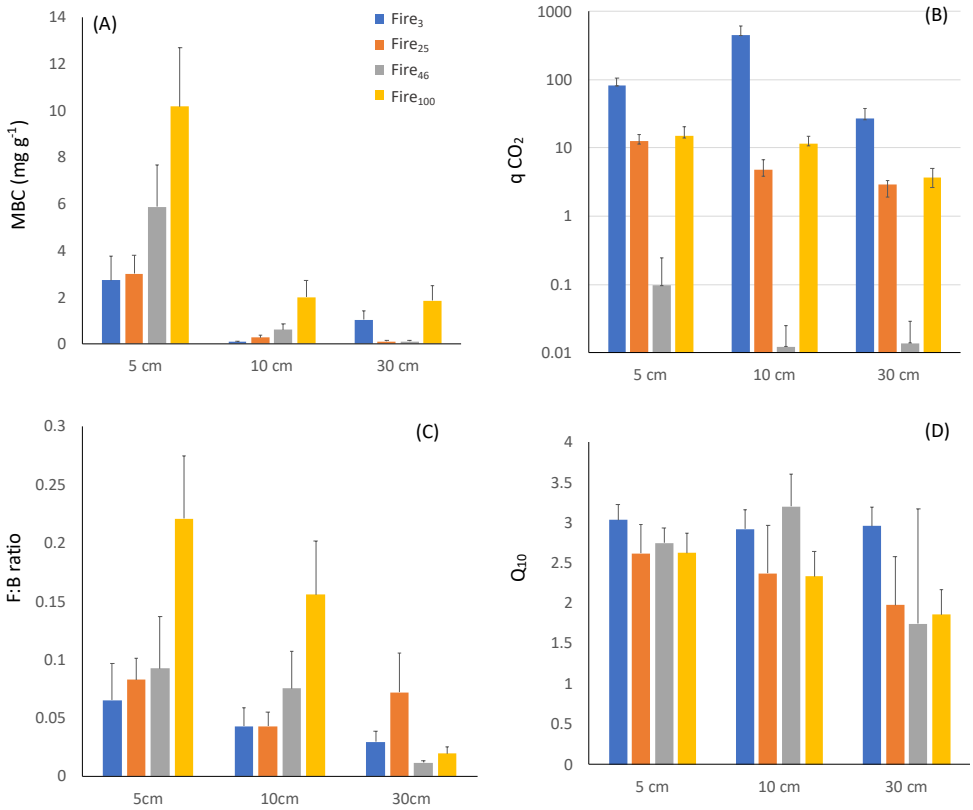

Figure 3| Mean (+ standard error) of the soil microbial biomass (A), microbial metabolic quotient observed from soils incubated at 19°C (B), fungal-to-bacterial ratio (C), and the temperature sensitivity (D). Samples were collected from three soil depths (5, 10, and 30 cm) across the four forest stands. Statistically significant differences ($p < 0.05$) compared within each soil layer are denoted using different letters above the bars.

Table 4| Relative abundance (%) of bacterial classes (average abundance; n = 9) in four forest areas. Phyla and classes that were significantly more abundant (↑) or less abundant (↓) between burnt forests ($Fire_3$, $Fire_{25}$ and $Fire_{46}$, respectively) and $Fire_{100}$. are labelled with corresponding arrows.

		5 cm				10 cm				30 cm			
Phylum	Class	$Fire_3$	$Fire_{25}$	$Fire_{46}$	$Fire_{100}$	$Fire_3$	$Fire_{25}$	$Fire_{46}$	$Fire_{100}$	$Fire_3$	$Fire_{25}$	$Fire_{46}$	$Fire_{100}$
Acidobacteria	**Acidobacteria**	11.78	12.45	1.64 ↓	10.15	7.29 ↓	8.19	0.94	12.56	3.8	3.32	0.25↓	3.69
	Acidimicrobiia	4.56	3.78	4.23	2.43	4.05	3.79	4.93	4.68	3.08	2.85	3.93	3.9
	Holophagae	0.22	0.22	0.6 ↓	0.13	1.23	1.09	1.93	0.56	1.96	1.51	3.34↑	1.89
	Solibacteres	4.07	5.14	1.35 ↓	2.31	4.8 ↑	5.37	1.62	2.55	3.25	3.36	1.11	2.84
	Actinobacteria	8.52	6.84	10.61	7.38	5.91	6.29	5.11	7.41	5.77	3.67	3.37	5.5
	Blastocatellia	0.01 ↓	0.09	1.44 ↑	0.24	0.22	0.29	2.17 ↑	0.64	0.5	0.54	3.2↑	1.13
	Coriobacteriia	0	0	0	0	0.01	0.03	0.01	0	0.17	0.03	0.04	0.14
	Thermoleophilia	3.34	2.44	7.3 ↑	2.22	4.2	3.84	12.12↑	3.73	4.6	4.02	15.75	6.64
Bacteroidetes	Sphingobacteriia	2.67	2.86	3.36	2.86	2.2 ↓	2.38	2.3	3.38	1.94	1.91	1.45	1.98
	Cytophagia	0.06 ↓	0.14	0.68	0.22	0.07	0.19	0.19	0.29	0.04	0.09	0.11	0.12
	Bacteroidia	0	0	0	0	0.01	0.01	0	0.01	0.6	0.04	0↓	0.19
Chloroflexi ↑	Ktedonobacteria	2.2 ↑	0.69	0.07	0.11	3.28 ↑	2.81	0.1	0.63	4.83	5.12	0.12↓	1.92
	Chloroflexia	0 ↓	0.01	0.26	0.07	0 ↓	0.01 ↓	0.47	0.12	0.02	0.09	0.48↑	0.09
	Anaerolineae	0.01 ↓	0.04	0.21	0.11	0.08	0.07	0.23	0.13	0.2	0.22	0.24	0.21
	Thermomicrobia	0	0.01	0.1 ↑	0.02	0.01	0.02	0.1 ↑	0.03	0.03	0.05	0.15 ↑	0.07
Firmicutes	Clostridia	0.84	1.48	0.33 ↓	0.71	0.98	1.17	0.22 ↓	0.65	1.07	0.5	0.27	0.87
	Bacilli	0.03	0.03	0.85 ↑	0.05	0.1	0.03	0.62 ↑	0.06	0.1	0.07	1.44 ↑	0.19
Gemmatimonadetes	Gemmatimonadetes	0.46	0.37	2.79 ↑	0.24	2.08	1.93	4.32 ↑	1.29	3.09	3.72	6.59 ↑	4.21
Parcubacteria	Nomurabacteria	0.05	0.08	0.17	0.04	0.08	0.07	0.16	0.13	0.46	0.15	0.2	0.64
	Moranbacteria	0	0	0.03	0	0.01	0	0.08	0.01	0.07	0.02	0.09	0.28
	Azambacteria	0.05	0.04	0.04	0.02	0.05	0.03	0.04	0.04	0.1	0.06	0.03	0.16
Planctomycetes	Planctomycetacia	3.35	3.02	2.13	2.1	2.5	1.93 ↓	1.25 ↓	2.38	1.37	1.17	0.61	1.16
	Phycisphaerae	1.83	1.77	0.83 ↓	1.11	1.33	1.33	0.74	1.16	0.75	0.83	0.44	0.62
Proteobacteria ↓	Alphaproteobacteria	17.54↓	20.17	25.29	18.64	17.57↓	16.02↓	14.94↓	18.46	10.63	9.82	6.68	11.84
	Betaproteobacteria	1.92↓	3.33	5.05	3.69	4.93	4.04	7.69	4.86	6.86	6.33	9.09	9.36
	Gammaproteobacteria	5.28	6.67	4.34	5.51	3.08 ↓	3.79 ↓	1.4	6.94	1.36	1.22	0.45	1.68
	Deltaproteobacteria	3.99	3.33	2.52	2.49	2.88	2.65	3.02 ↑	2.98	2.02	2.45	2.88↑	2.27
Saccharibacteria	Saccharibacteria_class	0.47	0.49	0.83	0.48	0.38	0.59	0.35	0.75	0.51	0.4	0.2	0.71
Verrucomicrobia	Opitutae	1.57	0.93	0.16	0.59	0.65	0.67	0.12↓	0.81	0.48	0.31	0.14	0.39
	Spartobacteria	1.84	1.89	2.88	1.39	4.3	2.85	3.22↑	2.18	3.36	3.02	2.33	2.33

The intensity of the colour represents the relative abundance of classes in soils of each area. Results of significance tests can be found in study III, Table A.3.

4.4 Bacterial community composition responses to wildfires (study III)

A permutational analysis of variance (PERMANOVA) indicated that the overall bacterial communities (at the OTU level) in $Fire_3$ and $Fire_{25}$ significantly differed from those in $Fire_{100}$ ($p < 0.05$; study III, Table 3). In the surface soils, Proteobacteria (Alphaproteobacteria and Betaproteobacteria) were less abundant in $Fire_3$ than in $Fire_{100}$ ($p < 0.05$). This result agrees with findings from Taş *et al.* (2014), who noted that fire decreases the abundance of Proteobacteria. Our result also partly agrees with a study on Canadian forest soils, finding that Alphaproteobacteria decreased following a fire, whilst Betaproteobacteria increased (Smith et al., 2008). An abundance of evidence supports the assumption that Proteobacteria is more abundant in soils with higher organic C contents (Fierer et al., 2007; Goldfarb et al., 2011), the likely explanation for why Proteobacteria was less abundant in burnt soils that contain less labile SOM.

In addition, in our study, the abundance of Chloroflexi, of which mainly class Ktedonobacteria was more abundant in $Fire_3$ than in $Fire_{100}$ ($p < 0.05$; Table 4), while the abundance of other classes in Chloroflexi, such as Chloroflexia and Anaerolineae, either remained constant or decreased following a fire (Table 4). This likely results from Ktedonobacteria forming branched mycelia with spores (Cavaletti et al., 2006; Yabe et al., 2010) that can survive in harsh environments, whilst Anaerolineae cannot since it is a non-spore-forming bacteria (Yamada et al., 2006). However, the bacterial community composition varies dramatically across different ecosystems, given that previous studies found that fire causes either an increase or decrease in the abundance of Actinobacteria and Firmicutes or a reduction in Chloroflexi (Ferrenberg et al., 2013; Smith et al., 2008; Taş et al., 2014). These inconsistencies from previous studies likely result from the difference in time since the fire, which varies from several weeks to 7 years. Some bacterial clades favour postfire conditions (Pérez-Valera et al., 2019) which usually feature a higher nutrient availability. However, the advantages of any postfire nutrient release declines within a few months (reviewed by Certini 2005), possibly favouring species better adapted to low-nutrient soils.

In addition, surface soils of $Fire_{46}$ contained the most unique bacterial communities amongst the four study areas, with a higher abundance of Gemmatimonadetes and a lower abundance of Acidobacteria. This likely results from the soil pH (see study III, Fig. 3A) which was higher in $Fire_{46}$ (pH = 6.6) compared with the remaining areas (pH < 5; Table 3). Since Acidobacteria prefers acidic soils (Baldrian et al., 2012; Jones et al., 2009) and Actinobacteria is more abundant in alkaline soils (Jeanbille et al., 2016; Lauber et al., 2008), the differences in bacterial community composition in $Fire_{46}$ likely stem from the difference in the soil pH. The importance of the soil pH in structuring the bacterial community has been widely documented (Bru et al., 2011; Fierer and Jackson, 2006; Hartman et al., 2008). Variance partitioning also confirmed that the soil pH was the primary determinant of community composition of bacteria in $Fire_{46}$ (study III, Fig. 3).

Our statistical analysis showed that fires did not affect the abundance of the primary bacterial clades (at the phylum and class levels) in the permafrost layer (30-cm layer) when comparing each clade between thawed permafrost (in $Fire_3$ and $Fire_{25}$) and frozen permafrost ($Fire_{100}$). Taş *et al.* (2014) reported a similar result, finding that most taxonomic clades were unaffected by permafrost thaw apart from an increase in an undescribed phylum AD3 in the thawed permafrost table. Whilst MacKelprang *et al*. (2011) found permafrost thaw can shift the microbial community composition similar to that in the active layer, C contents in permafrost of their study were four to ten times higher than of ours. Thus, the lack of a response from bacterial communities to permafrost thaw in our study likely stems from the presence of microorganisms in C limited permafrost in our study areas. However, bacterial community in $Fire_{46}$ significantly differed from

the remaining study areas, featuring a higher abundance of Actinobacteria and a lower abundance of Acidobacteria. Similar to the surface soil, the soil pH in the permafrost table in $Fire_{46}$ (pH = 7.0) was the highest amongst the four areas studied (pH < 5.7).

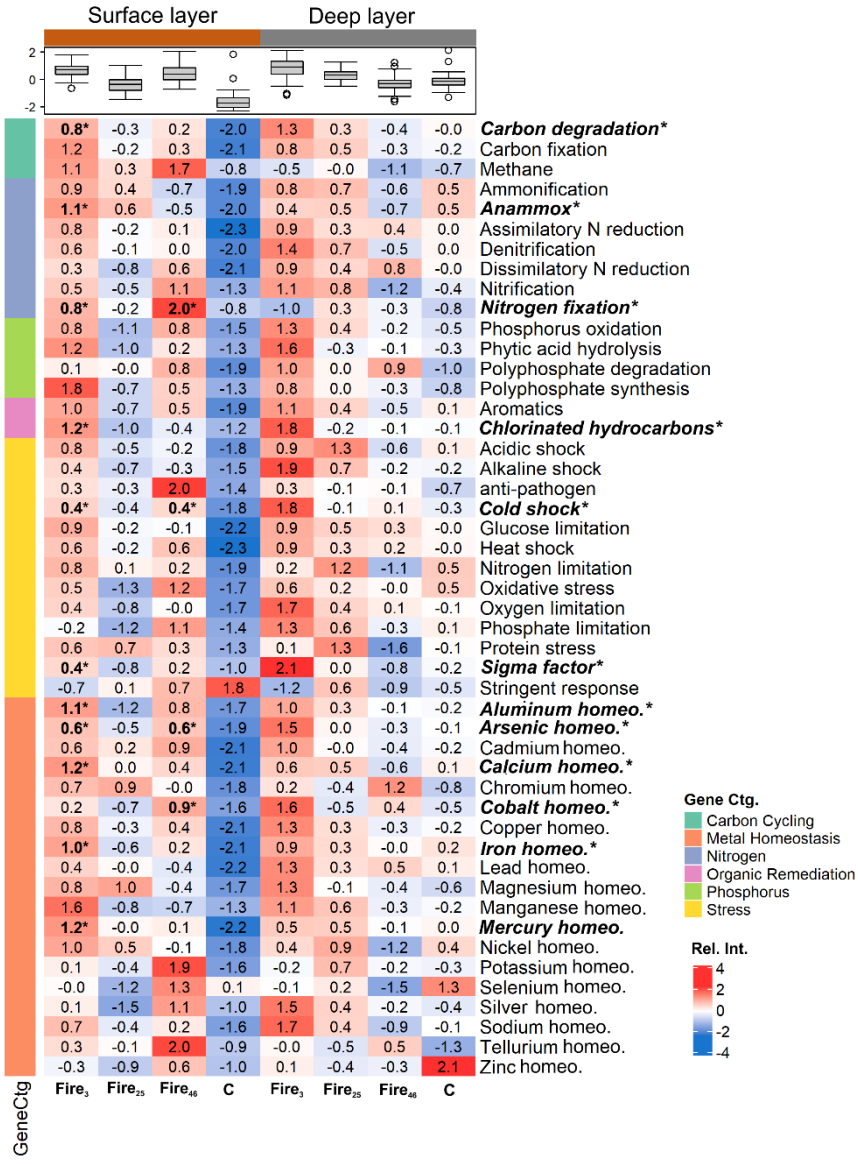

Figure 4| Heatmap of gene categories (subcategory 1 in Geochip 5.0K) showing the changes in the relative intensities across the four forest stands. Significant differences in the functional gene frequencies between fire-affected soils and the control appear in bold, with an asterisk indicating a significance of $p < 0.05$).

4.5 Bacterial functional gene composition responses to wildfires (study III)

Taking the overall gene frequencies into account, recently burnt surface soils ($Fire_3$) contained higher functional gene frequencies compared with surface soils of the old-growth forests (PERMANOVA, $p < 0.05$). Yet, we observed no significant difference of functional gene frequencies between $Fire_{25}$ and $Fire_{100}$ (study III). In the surface layer, most gene families (genes coding for the same metabolic pathway) were more frequent in $Fire_3$ than in $Fire_{100}$, particularly those coding for C degradation and the anaerobic ammonium oxidation (anammox) pathways ($p < 0.05$; Fig. 4). These results add to the increasing evidence indicating that rising temperatures increases the microbial functional gene abundances and diversity (Coolen et al., 2011; Mackelprang et al., 2011), and not necessarily changing the microbial biomass or the microbial community structure. For instance, a wildfire study in a mixed conifer forest showed that the number of N-fixing and ammonia-oxidising genes was higher in burnt soils, although the abundance of the according species decreased (Yeager et al., 2005). In a burn experiment in a Alaskan boreal forest, microbial activity increased with an increasing soil temperature, whilst the microbial biomass remained constant (Bergner et al., 2004).

Most genes coding for organic remediation and metal homeostasis remained consistently more frequent in $Fire_3$ compared with $Fire_{100}$ ($p < 0.05$; Fig. 4). Amongst the stress response genes, the genes coding for a cold-shock protein were more abundant in recently burnt soils compared with old-growth forest soils. This is probably because soil frost more likely penetrates into fire-affected soils due to the diminished vegetation coverage, canopy interception, and lack of snow cover following a fire. Thus, bacterial communities on the surface soils develop cold-resistant functions to overcome the cold (Fig. 4). Bacterial clades capable of resisting high heavy metal contents and toxic compounds more likely survive, owing to the specific heavy metal contents such as Mn, Mg, Fe, and Pb, as well as hydrocarbons found to increase following a fire (Certini, 2005; Gonzalez Parra et al., 1996; Koh et al., 2004).

In addition to the postfire changes in bacterial communities and the functional gene structure of the surface layer, we expected similar effects from fires on the deep soil layers. However, we found no significant differences in functional gene frequencies between thawed and frozen permafrost, although the gene frequencies tended to slightly increase following a fire (Fig. 4). By contrast, earlier studies showed that the C degradation and nitrification processes were higher in thawed permafrost than in frozen permafrost (Mackelprang et al., 2011; Taş et al., 2014; Yuan et al., 2018). However, the permafrost C content in our study was two to four times smaller than that found in previous studies. Thus, the C limitation likely explains why the bacterial community and functional genes fail to respond to permafrost thaw.

In our study, the functional gene composition we observed were DNA based, merely reflecting potential functions of bacterial communities instead of the real metabolic activity. In current study, although we found the functional gene frequencies in thawed permafrost did not differ with those in frozen ones, the genes observed in the frozen permafrost may largely inactive because of the frozen condition. Thus, we suggest that future studies combine DNA- and RNA-based approaches to explore activated microbial functions.

CONCLUSIONS

Wildfires in the boreal forest permafrost zone have become more frequent in recent decades (Kasischke and Turetsky, 2006; Kelly et al., 2013), thereby increasing the risk of C release from permafrost. In this study, apart from biomass burning, fire also cause vegetation removal and a decrease in the amount of extractable organic C on the forest floor. Moreover, postfire increases in black carbon continuously absorb heat from the solar radiation which eventually melts permafrost over 1-m deep. These postfire changes reduce the surface soil microbial biomass and fungal-to-bacterial ratio, as well as the microbial C use efficiency. The effect of fire on soil respiration, however, were negligible. Moreover, fire increased the temperature sensitivity of SOM decomposition, since it converted labile SOM to more recalcitrant pyrogenic material requiring a higher activation energy for decomposition (Aaltonen et al., 2019). Despite the lower SOM quality in recently burnt soils, microorganisms nevertheless retained a constant C:N ratio. We interpret this constant stoichiometric ratio as a microbial strategy which maintains optimal growth.

Although wildfire did not change bacterial biomass (interpreted using the bacterial gene copy number), fires reshaped the bacterial community composition in the surface layer. Burnt soils featured a higher abundance of Ketdonobacteria (Chloroflexi) yet a lower abundance of Alphaproteobacteria and Betaproteobacteria (Proteobacteria). These changes were only found in the surface layer and appear primarily driven by the changes in abiotic variables. However, no significant changes in community composition were discovered in the deep soil layer even after experiencing a change from frozen to thawed. The overall functional gene composition measured by GeoChip 5.0K in this study showed a clear increase following a fire. Postfire increases in the bacterial potential functions of C degradation and the N cycle (N fixation and annamox) add to the evidence provided by the increase in the microbial qCO_2 following a fire shown above.

However, our results show that permafrost thaw following a fire did not affect soil respiration, the size of soil microbial biomass, or the composition of bacterial communities (at the class and phylum levels) or their functional potentials. Nevertheless, we still found a higher metabolic quotient (qCO_2) in thawed permafrost versus frozen permafrost, possibly reflecting a higher soil microbial activity after permafrost thaw.

This dissertation illustrates that wildfire can affect the SOM decomposition by influencing microbial metabolic activities on the C and N cycles. However, additional studies aimed at identifying the effect of wildfire on various genes or enzymes regulating microbial activities, or studies on a broader spatial scale with shorter postfire time intervals remain necessary. Here, we relied a DNA-based method to analyse the functional gene composition. Future studies should employ a combination of both DNA- and RNA-based approaches to improve our understanding of the substantial response of microbial functions in postfire ecosystems.

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