BIOCHAR AS A SOIL AMENDMENT TO ENHANCE PETROLEUM HYDROCARBON DEGRADATION IN NORTHERN LANDFARMS

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ABSTRACT

Extreme environmental conditions in northern regions slows efforts to remediate petroleum hydrocarbon (PHC) contaminated soils through conventional landfarming practices. Thus, cost-effective soil remediation strategies that are active under frozen conditions and specific to cold regions are in strong demand. Biochar amendments have been added to soil to sequester carbon, immobilize organic contaminants, and improve long-term soil productivity; however, there is little information regarding their use and effectiveness under cold climate conditions. The purpose of this research was to determine if locally produced biochars can enhance PHC degradation at northern landfarms. This was accomplished by examining biochar additions to PHC contaminated soils under laboratory and field conditions. The degradation of F2 and F3-PHCs increased in bonemeal biochar-amended soils incubated under frozen conditions, but there was little difference in PHC degradation between biochar-amended and control soils under field conditions. Biochars selectively increased nutrient availabilities, but results varied between study location and year. Certain biochars increased liquid water content (θ_{liquid}), soil temperature, and pH, but this was dependent on factors such as incubation temperature, biochar feedstock, and application method. A combination of increased aromatic (*C2,3O* and *nahAc*) functional gene abundance and total PHC-degraders was linked to the reduction of F3-PHCs in bonemeal biochar-amended soil incubated under frozen conditions. Bone-derived biochars stimulated PHC degradation and influenced the physical, chemical, and biological properties of soil to a greater extent than wood-derived biochar, which was linked to inherent physicochemical properties of biochar such as ash content, pore size, pore volume, and surface area. Incorporated, rather than injected, fertilizer and/or biochar slurries effectively enhanced F2 and F3-PHC degradation, NO_3^--N availability, θ_{liquid}, and aromatic catabolic gene abundance in field studies. Charosphere soil, which immediately surrounds biochar particles, was identified as a spatially unique niche that supported higher ^{13}C-phenanthrene mineralization, aromatic catabolic gene abundance, and relative abundances of PHC degrading bacteria (i.e., *Bosea* and *Caulobacter*). This research provides evidence that bone-derived biochars selectively enhanced PHC degradation and improved several physical, chemical, and biological properties of northern soils.

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DEDICATION

This dissertation is dedicated to my Mom and Dad – I couldn't have done this without you.

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1. GENERAL INTRODUCTION

Petroleum hydrocarbons (PHCs) are commonly used by humans inhabiting northern regions for power generation, heating and transportation (Snape et al., 2001; Aislabie et al., 2006). Widespread PHC contamination can result from infrastructure failure, human error, or natural hazards (Snape et al., 2008). This is of concern on both local and global scales as PHC contamination can have a deleterious effect on human and ecological health. Human health is affected by PHCs in different ways; some compounds attack the central nervous system while others can affect the blood, immune system, liver, spleen, kidneys, and lungs (CCME, 2008). Certain PHCs can also be persistent, water soluble contaminants that are potentially toxic to terrestrial flora and fauna (Adam and Duncan, 1999; MacKinnon and Duncan, 2013). These types of adverse health and ecological effects are dependent on the types of chemicals present in PHCs, exposure time, and frequency of exposure. Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of degrading PHC compounds exist in the environment.

In northern regions, bioremediation can be a challenging process. Soils are often limited by water availability, nutrient availability, reduced microbial abundance and activity, and low organic matter content (Mohn and Stewart, 2000). Several psychrotolerant microbial species, indigenous to cold regions, have been isolated, identified, and shown to possess biodegradative function (Yu et al., 2000). The degradation of PHCs can be enhanced by understanding how the combination of specific soil properties and environmental conditions influence factors that regulate microbial activity. *Ex-situ* remediation techniques are used to manage PHC contaminated soils in harsh northern environments because they allow for the manipulation of soil properties that can be limiting factors for PHC degradation, including aeration, moisture content, temperature, and nutrient availability. Landfarming is a common, effective method used to remediate PHC contaminated soils in cold regions (Paudyn et al., 2008; Filler et al., 2009), but there are limited landfarming trials in northern regions and field trials have revealed conflicting results (McCarthy et al., 2004; McDonald and Knox, 2014). Beyond implementing soil turning

and fertilization, few researchers have attempted to optimize PHC degradation rates in northern landfarms using additional soil amendments.

Biochar is a soil amendment that is created through the pyrolysis of organic material such as wood, plant material, and to a lesser extent, bone (Suliman et al., 2016). Biochars can have many environmental benefits and have been extensively researched in tropical regions. The positive effects of biochars include improvements in soil fertility (Lehmann et al., 2006), water holding capacity (Głab et al., 2016; Omondi et al., 2016), cation exchange capacity (CEC) (Liang et al., 2006), and many other ecosystem services, including reducing the bioavailability of PHCs in soils (Rhodes et al., 2008; Koltowski et al., 2016) and increasing PHC degradation rates (Beesley et al., 2010; Bushnaf et al., 2011). Based on these observations, biochars have the potential to enhance bioremediation in northern landfarms; however, there are many challenges and uncertainties associated with the complex nature of biochar-soil interactions. Biochars vary greatly in their ability to enhance ecosystem services, based on the specific properties of biochar (i.e., feedstock, pyrolysis conditions, application rate), site-specific soil conditions (i.e., texture, CEC, nutrient availability, pH), and inherent environmental conditions. For example, contradictory results have been reported when measuring microbial biomass and activity in agricultural soils amended with biochar (Durenkamp et al., 2010; Zhang et al., 2014a). The complexity and variability of biochar applications to soil highlights the need for further research into its potential as a soil amendment to enhance PHC degradation rates in northern soils, and the need for identification of the underlying mechanisms driving positive effects in biochar-amended soils.

Biochar research is currently directed towards agricultural applications in tropical and temperate regions, with limited studies focusing on inorganic and organic contaminant removal from soil and water, and even fewer studies evaluating polycyclic aromatic hydrocarbon (PAH) and PHC degradation in contaminated soils. To the best of my knowledge, the effects of biochar have not been investigated under frozen conditions. Although an increasing number of studies show that biochar is beneficial to many aspects of the soil system, there is an alarming lack of consensus between findings. These inconsistent results reflect the lack of knowledge surrounding the mechanisms by which specific biochars can improve physical, chemical, and biological soil properties. This raises several interrelated questions associated with the potential for biochar applications at northern landfarms. Can biochar enhance PHC degradation? How

are the physical and chemical properties of soil influenced by biochar? What are the effects of biochar on the soil microbial community? Which feedstocks are most suitable for PHC degradation? What application method is most effective? Are microbial degradation and other soil properties spatially distributed in biochar-amended soils? Until these concerns are addressed, the potential of biochar to enhance PHC degradation in northern soils will not be realized.

The purpose of the research presented in this dissertation was to evaluate biochar as a soil amendment to enhance PHC degradation in northern landfarms and contribute to the understanding of specific soil-biochar interactions in northern soils. This was achieved by undertaking a combination of laboratory and field studies which were designed to address the following objectives and hypotheses:

Objective 1: Determine the effects of biochar on PHC degradation and associated soil properties under frozen conditions and estimate the degree of abiotic losses occurring from the soil-biochar mixture to confirm degradation (Chapter 3).

Hypothesis 1: Biochar stimulates PHC degradation by increasing θ_{liquid}, which also increases nutrient availability, and the abundance and activity of PHC-degrading soil microorganisms.

Objective 2: Determine the specificity of biochar and the most effective application method by evaluating PHC degradation and associated soil properties with different biochar formulations (Chapter 4).

Hypothesis 2: The physical and chemical properties of bone-derived biochars are more suitable for PHC degradation than wood-derived biochars, and homogenized biochar slurries are more effective than injected biochar slurries due to increased soil-biochar contact.

Objective 3: Determine if there is a spatial relationship between biochar particles and the surrounding soil, and if so, the influence on ^{13}C-phenanthrene mineralization and associated soil properties (Chapter 5).

Hypothesis 3: Biochar-induced changes in mineralization and soil properties occur predominantly in soils in direct contact with biochar particles but are dependent on biochar type.

A general introduction (Chapter 1) and literature review (Chapter 2) precede three research chapters (Chapters 3-5), which were written as stand-alone manuscripts for publication. These chapters are followed by a synthesis of the individual research studies (Chapter 6) and include overall conclusions and recommendations for future work. Literature cited throughout the dissertation is compiled in the Reference section (Chapter 7).

2. LITERATURE REVIEW

2.1 Petroleum Hydrocarbons in Soil

Petroleum hydrocarbons (PHCs) are one of the most widespread soil contaminants in Canada and consist of a wide range of organic compounds (CCME, 2008). Canada's Arctic has been identified as a region where ecosystem recovery is slower, and PHC contamination is more damaging (Snape et al., 2003). The number of contaminated sites in northern Canada is large; for instance, between 1971 and 2014, approximately 7,000,000 L of diesel fuel was spilled in Nunavut alone. Crude oil spills from ruptured pipelines are the largest source of terrestrial PHC contamination, but diesel fuels are also commonly spilled through infrastructure failure (i.e., leaking tanks), human error (i.e., fuel transfer), or natural hazards (i.e., extreme temperatures) (Snape et al., 2008). In Arctic, Antarctic, and alpine soils, PHC contamination can influence soil pH (Aislabie et al., 2004), moisture regimes (Siciliano et al., 2008), nutrient availability (Harvey et al., 2012), and microbial populations (Margesin et al., 2003). The process of cleaning up and monitoring contaminated sites is essential to protect the environment, as well as humans that reside near these sites.

2.1.1 Properties

Petroleum hydrocarbons consist of both aliphatic and aromatic hydrocarbons (CCME, 2008). Aliphatic hydrocarbons are non-aromatic, organic compounds containing hydrogen and carbon atoms. Aliphatic hydrocarbons are linear (*n*-alkanes), cyclic (*cyclo*-alkanes), or branched (*iso*-alkanes) compounds present as three structurally different groups: alkanes (C-C), alkenes (C=C), and alkynes (C≡C). Alkanes are the main components of fuels and oils, and are the least reactive class of organic molecules; this allows them to persist in the soil matrix and pose many ecological problems upon release (Wentzel et al., 2007). Aromatic hydrocarbons, commonly referred to as polycyclic aromatic hydrocarbons (PAHs), are heterocyclic structures composed of one or more benzene rings containing alkyl, halogen, nitro, and other functional groups (Table 2.1). Generally, PAHs are relatively stable and recalcitrant in soils, and thus, will degrade relatively slowly compared to other organic compounds like alkanes.

Table 2.1. Physiochemical properties of selected hydrocarbons (adapted from Stroud et al., 2007).

Hydrocarbon Group		Name	Formula	Molecular weight	Melting point	Boiling point	Solubility at 25°C	Log K_{ow}
				g mol^{-1}	°C	°C	mg L^{-1}	
Aliphatic	Alkane	Hexadecane	$C_{16}H_{34}$	226.44	18	287.0	0.0009	9.1
	Alkene	Hexadecene	$C_{16}H_{32}$	224.43	3-5	274.0	0.00123	NA
	Alkyne	Hexadecyne	$C_{16}H_{30}$	222.42	15	148.0	NA	NA
Aromatic	PAH	Naphthalene	$C_{10}H_8$	128.18	79-83	217.9	30.0	3.36
	PAH	Phenanthrene	$C_{14}H_{10}$	178.22	97-101	340.0	1.1	4.16
	PAH	Benzo(α)Pyrene	$C_{20}H_{12}$	252.31	175-179	495.0	0.0038	6.06

K_{ow}, octanol-water partition coefficient
NA, data not available

2.1.2 Fate and mobility

The physical and chemical properties of PHC compounds determine their fate and mobility in the environment (CCME, 2008). In the soil, PHCs may percolate into groundwater systems, volatilize into the atmosphere, and adsorb to soil particles, while others may be degraded by microbial populations. Volatility is dependent on carbon bonding and the number of carbon atoms within the molecule; therefore, aromatic hydrocarbons are less volatile than aliphatic hydrocarbons due to higher melting and boiling points. Generally, PHC compounds with single carbon bonds and low molecular weight are most likely to volatilize into the atmosphere. The structure of the PHC compound also determines its susceptibility to microbial attack. Aliphatic hydrocarbons are more prone to microbial degradation, while recalcitrant PHCs are usually PAHs composed of high molecular weight which are resistant to catabolic microbial activity (Margesin and Schinner, 1997).

The octanol-water partition coefficient (k_{ow}) describes the solubility and hydrophobicity of the compound and is used to predict the fate and transport of the compound in the environment. The non-polar nature of PHCs results in partitioning or sorption onto organic matter in soil; the extent of which is also described by the k_{ow} (Bressler and Gray, 2003). When a compound is sorbed strongly to soil organic matter and negatively charged soil particles, the bioavailability and migration tends to decline, impeding biodegradation (Yang et al., 2009). Chemical and biological availability, sequestration, biodegradation rate, and migration of PHC compounds are affected by k_{ow} and the amount of organic matter in the soil. Temperature also affects the physical nature and chemical composition of PHCs. The low ambient temperatures characteristic of cold environments increase viscosity, reduce evaporation, and decrease solubility of PHC compounds (Atlas, 1981). Petroleum hydrocarbons are excluded from ice as the soil freezes, resulting in an increased concentration of PHCs in soil pore water (Barnes et al., 2004).

2.1.3 Catabolic degradation pathways

Catabolic microorganisms are often found at higher concentrations at PHC contaminated sites than in pristine environments (Margesin et al., 2003), and the ubiquitous presence of PHCs has resulted in the maintenance of degradative potential in most populations (Johnsen and Karlson, 2005; Phillips et al., 2008). Bacteria that are capable of degrading PHC compounds have a diverse metabolism which enables them to use aliphatic and aromatic PHCs as a carbon

and energy source (Margesin et al., 2003; Obayori and Salam, 2010). These microorganisms have developed strategies involving specialized enzyme systems and metabolic pathways to access PHCs, degrade them, and convert them to easily metabolized substrates. The metabolism of PHCs is limited by their low water solubility, their tendency to accumulate in cell membranes, and the energy needed to activate the molecule (Rojo, 2009). Uptake mechanisms may vary depending on the bacterial species, the molecular weight of the compound, and the physical and chemical characteristics of the environment (Wentzel et al., 2007). To understand how remediation techniques may affect the degradative ability of soil microbial communities, these pathways must be understood.

Oxygen is required for aerobic degradation of PHCs by soil microorganisms. Soils with high water contents have limited gas diffusion and low oxygen levels, that can create anaerobic conditions and significantly reduce PHC degradation (Rayner et al., 2007). Under these conditions, aeration may be required to enhance degradation, or chemical amendments (i.e., peroxides) can be applied. In the absence of oxygen, PHC degradation can still proceed using alternative electron acceptors such as nitrate, manganese, iron, and sulphate (Eriksson et al., 2003; Powell et al., 2006b). Aerobic, rather than anaerobic, PHC bioremediation is preferred as the most common microbial degraders are aerobic (Bruce et al., 1992) and oxygen provides the greatest amount of energy per unit of PHC degraded (Menendez-Vega et al., 2007).

2.1.3.1 Aliphatic hydrocarbons

Aliphatic PHCs, such as short to medium chain alkanes (C_5-C_{16}), are initially metabolized by the terminal or sub-terminal incorporation of oxygen by a hydroxylase enzyme such as *alkB* (Van Beilen and Funhoff, 2007; Wentzel et al., 2007). Once oxidized to a primary alcohol, further oxidation by alcohol and aldehyde dehydrogenases convert the compounds to fatty acids that may be subsequently metabolized through the bacterial β-oxidation pathway (Van Hamme et al., 2003; Rojo, 2009). Cytochrome P450 (*CYP153*) is another common type of alkane hydroxylase, which is found in alkane-degrading bacteria which lack *alkB* (Van Beilen et al., 2006). Alkane degraders typically belong to the genera *Rhodococcus*, *Pseudomonas*, or *Acinetobacter* (MacCormack and Fraile, 1997; Whyte et al., 1997; Bej et al., 2000). Although many different alkane hydroxylase genotypes that metabolize alkanes of varying lengths are

present within hydrocarbon degraders (Van Beilen and Funhoff, 2007), *alkB* is commonly assessed in Arctic and Antarctic soils (Smits et al., 2002; Whyte et al., 2002).

2.1.3.2 Aromatic hydrocarbons

Aromatic hydrocarbons, such as naphthalene and phenanthrene, are metabolized by the incorporation of oxygen into the aromatic ring, which initiates ring fission (Cerniglia, 1992). Central intermediates such as catechols, protocatechuates, and gentisates are produced by the introduction of hydroxyl groups placed either adjacent (extradiol cleaving enzymes) or opposite to one another (intradiol cleaving dioxygenases) (Fuchs et al., 2011). These intermediate products are subject to oxygenolytic ring cleavage, followed by transfer of the ring cleavage products into the central metabolism (Díaz et al., 2013). Some aromatic hydrocarbons are metabolized through the coenzyme A (CoA) thioesters, which form non-aromatic epoxides by hydrolysis, rather than oxygenolytic ring cleavage (Pérez-Pantoja et al., 2010). Aromatic-degrading bacteria are common in the genera *Pseudomonas* or *Sphingomonas* (Aislabie et al., 2000). Naphthalene dioxygenase (*nahAc)* and catechol 2,3 dioxygenase (*C2,3O*) are well-studied enzymes that encode crucial first steps in aromatic PHC degradation pathways (Resnick et al., 1996; Van Hamme et al., 2003), and therefore, are primary target genes for studies assessing PAH degradation. Both genes have been identified in cold environments (Whyte et al., 1997; Margesin et al., 2003)

2.1.4 Bioremediation

Bioremediation uses biological processes to degrade, break down, transform, and remove organic contaminants in soil. Bioremediation can occur naturally or with the aid of amendments such as microbial inocula, nutrients, moisture, or oxygen. During bioremediation, microorganisms utilize contaminants in the soil as an energy source and metabolize the contaminant into a useable energy form through chemical specific oxidation-reduction reactions. Bioremediation has been proven successful for mitigation of petroleum hydrocarbons (Roy et al., 2014), halogenated compounds (Major et al., 2002), pesticides and herbicides (Kaake et al., 1992), nitrogen compounds (Ghoreishi et al., 2017), metals (Kang et al., 2016), and radionuclides (Law et al., 2010). There are two types of bioremediation techniques: (i) *in-situ*, which is carried out on-site without any soil removal; and (ii) *ex-situ*, which is carried out off-site and requires removal and transportation of contaminated material prior to treatment.

Generally, bioremediation requires less resource inputs than other techniques (i.e., chemical oxidation, soil washing), and is perceived as an environmentally friendly approach (O'Brien et al., 2017).

2.1.4.1 *In-situ* bioremediation

The advantages of *in-situ* bioremediation include lower costs and minimal environmental disturbance (Gruiz and Kriston, 1995). However, it can be challenging to deliver required amendments to remote or inaccessible sites, and environmental conditions, including temperature, moisture content, nutrient concentrations, and oxygen availability, are better controlled using *ex-situ* bioremediation techniques (Khan et al., 2004). Thus, *in-situ* bioremediation can be less reliable and take longer than *ex-situ* bioremediation. Bioventing (Rayner et al., 2007; King et al., 2014) and monitored natural attenuation (Delille and Pelletier, 2002) are common *in-situ* bioremediation technologies that have been successful in Arctic and Antarctic environments.

2.1.4.2 *Ex-situ* bioremediation

The advantages of *ex-situ* bioremediation include greater control over treatment parameters, higher efficiency, and quicker treatment. However, excavation activities can destroy existing soil structure and more land is required for storage of excavated soil (Lim et al., 2016). Excavated soils are also more susceptible to compaction by heavy equipment required for large-scale excavation. Biopiles (Sanscartier et al., 2009; McWatters et al., 2016) and landfarming (Paudyn et al., 2008) have been successful *ex-situ* bioremediation techniques in Arctic and Antarctic environments.

2.2 Bioremediation of Petroleum Hydrocarbons in Northern Soils

Natural attenuation of PHCs in cold climates is limited due to low ambient and soil temperatures, and deficiencies in available water and nutrients. Thus, terrestrial PHC contamination can be more persistent and environmentally damaging than in temperate climates (Mohn and Stewart, 2000). *In-situ* environmental conditions in cold regions are not ideal and allow PHCs to accumulate and persist in frozen soils, which reduce PHC degradation rates and overall bioremediation success. Therefore, *ex-situ* bioremediation (i.e., landfarming) has been identified as the most efficient method available for ameliorating and controlling the

factors limiting microbial activity (Aislabie et al., 2006; Delille et al., 2008; Paudyn et al., 2008). Landfarming generally refers to the process by which contaminated soils are spread out in a layer approximately 0.5 m thick and aerated and/or amended with nutrients and moisture to aid in volatilization and degradation.

2.2.1 Petroleum hydrocarbon degradation in frozen soils

The degradation of PHCs has been reported at low temperatures in Arctic (Whyte et al., 1999), alpine (Margesin et al., 2003), and Antarctic environments (Aislabie et al., 2004). Despite the activity of psychrotrophic microorganisms, northern soils have freezing soil temperatures, low moisture content, and limited nutrient bioavailability, which presents many challenges to effective and timely PHC bioremediation (Atlas, 1981; Whyte et al., 1999; Hoover and Pikuta, 2010). Bioremediation is a viable remediation strategy for PHC contaminated soil in northern regions due to the presence of cold-adapted, PHC-degrading microorganisms. Microbial respiration and PHC degradation occurs at sub-zero temperatures due to a portion of soil-water existing in the liquid phase at temperatures below 0 °C (liquid water; θ_{liquid}) during freeze-thaw periods, which allows significant degradation to occur (Chang et al., 2011). Deming (2002) proposed the new ecological group "eutectophiles", which includes unique microorganisms that take advantage of the eutectic interface between ice and water. This eutectic interface includes θ_{liquid}, which is a limiting factor for microbial activity, and the biodegradation of PHCs. Contaminated soils in frozen environments are commonly enriched with PHC-degrading microorganisms (Margesin and Schinner, 1999); however, the time it takes for microbial populations to recover from contamination events and degrade PHCs varies from site to site.

Soil properties are unique at every location; therefore, soils vary in their capacity to degrade PHCs, and there are vast differences in the mobility, fate, and persistence of PHCs in different soils due to the inherent variability of soils. Degradation is dependent on many soil properties and environmental conditions, including, but not limited to; temperature (Coulon et al., 2005), texture (Kogbara et al., 2015), moisture (Malina et al., 2002), oxygen content (Eriksson et al., 2003), pH (Leahy and Colwell, 1990), and nutrient availability (Chaineau et al., 2005). These soil properties directly influence soil microbial activity, which ultimately regulates the success and rate of PHC degradation. Using the common

properties of northern soils, a general approach to bioremediation in northern soils can be applied to site-specific remedial strategies.

2.2.2 Temperature

Bioremediation is most active during the summer months (2-4 months/year) when daily mean air temperatures generally range between 5-15 °C. In winter months (8-10 months/year), daily mean air temperatures can reach -30 °C, which limits microbial activity and bioremediation. Although some soil microorganisms are adapted to extreme heat or cold, temperatures at either extremity can hinder microbial growth and catabolic activity (Pietikäinen et al., 2005). Temperature also exerts a strong influence on the availability of water and nutrients in soil pores (Lahti et al., 2005; Harvey et al., 2012). The most limiting factor is the availability of water; water limits metabolic processes and retards nutrient and gas diffusion necessary for PHC degradation in frozen soils (Harvey et al., 2012). Furthermore, freezing temperatures may increase hydrocarbon viscosity, reduce evaporation of volatile components, and decrease water solubility (Filler et al., 2006). In northern soils, low temperatures also cause logistical challenges associated with soil manipulation.

2.2.3 Soil texture

Soil texture influences PHC degradation rates because pore size and particle size distribution affect the fate and availability of PHCs. For example, PHCs can leach or volatilize much more readily in a sandy soil, but can sorb tightly to soil particles in a predominantly clay soil (Kogbara et al., 2015). Studies have reported that bioremediation is more effective in predominantly sandy soils due to higher PHC bioavailability (Labud et al., 2007), but decreases in PHC concentrations were not separated based on volatilization and microbial degradation. Texture also influences soil properties responsible for the availability and mobility of water and nutrients, such as CEC and surface charge. A strong negatively charged soil (i.e., clay loam) will have a higher CEC and water holding capacity than a neutral soil (i.e., sand) (Senjobi and Ogunkunle, 2011). Generally, sandy soils are less fertile than clay soils, and soil microorganisms are less abundant and active (Shi and Marschner, 2014). The availability of water and nutrients directly affects soil microorganisms, which in turn, affects the catabolic processes associated with PHC degradation.

2.2.4 Water content

Water is arguably the most important soil constituent as it is essential for chemical reactions and microbial metabolism (i.e., diffusion of substrates, removal of waste products) to proceed. At temperatures below 0 °C, θ_{liquid} exists as thin layer surrounding soil particles, and the thickness of this layer is controlled by salinity, and adsorptive and capillary forces within the soil (Dash et al., 1999; Sparrman et al., 2004; Steven et al., 2006). The amount of θ_{liquid} is determined by ice crystal configuration, impurities, and pore size distribution (Reed et al., 1979). Microbial survival in frozen soil is dependent on θ_{liquid} and the diffusion of microbial substrates and waste products through the cell membrane (Ostroumov and Siegert, 1996). In frozen soils, θ_{liquid} has been positively correlated with elevated nutrient supply rates and gas diffusion (Rivkina et al., 2000; Harvey et al., 2012). Thus, manipulating factors that increase θ_{liquid} (i.e., surface area, porosity), could optimize PHC degradation in northern soils.

2.2.5 Oxygen content

Oxygen has been identified as a rate-limiting variable and an essential requirement for the oxidation of aliphatic and aromatic PHCs by soil microorganisms (Leahy and Colwell, 1990). Oxygen availability in soils is dependent on factors such as microbial oxygen consumption rate (Pinches and Pallent, 1986), soil type (Rayner et al., 2007), and soil moisture. Antarctic soils with sufficient aeration to prevent anaerobic conditions had gravimetric water contents between 3-11%, bulk densities between 1.0-1.2 g cm^{-3}, and air volume fractions between 40-50% (Aislabie et al., 2004; Ferguson et al., 2004; Powell et al., 2006b). Oxygen is not usually a limiting factor in most coarse textured soils, but could be limited in water logged Arctic soils (Rike et al., 2005).

2.2.6 pH

Optimal microbial activity and growth occurs between pH 6 and 9 (Vyas and Dave, 2007), although PHC degradation has occurred in acidic (Amadi et al., 1996) and alkaline (Kanekar et al., 1998) environments. Nutrient availability, which is controlled by sorption, speciation, and precipitation-dissolution reactions, is directly influenced by soil pH (Hinsinger, 2001). Generally, most nutrients are optimally available between pH 6.5-7.5, and nitrogen is less affected by soil pH than phosphorus. Various species of phosphate are distributed as a function of pH, and the charge of each species will determine the environmentally relevant

reactions (i.e., sorption/desorption) that will influence its mobility and distribution in the soil (Hanrahan et al., 2005). For example, phosphorus ions will precipitate as calcium phosphates in neutral to alkaline soils (pH > 7.5 to < 8.5) and iron and aluminum phosphates in acidic soils (pH < 5.5) (Hinsinger, 2001). During bioremediation, elemental sulphur can be used to decrease pH and lime ($CaCO_3$) can be used to increase pH.

2.2.7 Available nutrients

Microbial activity, and hence, bioremediation rates, are dependent on the bioavailability of substrates, electron acceptors, and nutrients (Yang et al., 2009). Petroleum hydrocarbon-contaminated soils with low levels of essential nutrients benefit from nutrient additions (i.e., fertilizers) to increase microbial biomass, which is required for significant degradation to occur (Ferguson et al., 2003). Nitrogen and phosphorus are important for microbial growth, with phosphate (PO_4^{3-}), nitrate (NO_3^-), and ammonium (NH_4^+) considered the most readily bioavailable forms. Fertilizers are commonly used to enhance bioremediation in northern soils, as these soils are usually deficient in both nitrogen and phosphorus (Margesin, 2000; Børresen and Rike, 2007). Excess nitrogen has been shown to decrease osmotic soil water potential, microbial activity, and PHC degradation rates in coarse-textured soils, so it is important to avoid over-fertilization (Walworth et al., 2007). Soil microorganisms have a relative balance of carbon (C) and nitrogen (N) in a ratio of 3:1 to 10:1 in their cells and require a C:N ratio of 24:1 in soil to maintain cellular processes. If the C:N ratio is too high, microorganisms must immobilize nutrients from the soil to fuel decomposition. As soil microorganisms metabolize carbon into biomass they require nitrogen and phosphorus in an approximate C_{TPH}:N:P ratio of 100:9:1 for optimum PHC degradation (Mohn and Stewart, 2000; Chang et al., 2010).

2.3 Biochar as a Soil Amendment

Biochar is organic biomass (i.e., wood, bone) that has been heated under low-oxygen conditions, also known as pyrolysis, to produce a carbon rich char. Biochars are modified by chemical transformations that are initiated by the instability of individual chemical bonds within the biomass (McLaughlin et al., 2009). For agricultural and environmental purposes, biochars are added to soils to sequester carbon, remediate organic contaminants, and improve long-term soil productivity. The addition of biochar to soils can increase water holding capacity (Karer et

al., 2013), improve aeration (Bushnaf et al., 2011), increase cation exchange capacity (CEC) (Liang et al., 2006), and stimulate the soil microbial community (Liao et al., 2016). Based on the positive effects observed from biochar additions to various soils, biochar could increase PHC degradation in northern soils under certain conditions. However, it is important to note that each biochar is unique due to variations in the feedstock source and production conditions.

2.3.1 Amendment formulation

The feedstocks used and the pyrolysis conditions by which the biochar is produced, can drastically affect its chemical properties, elemental composition, particle size distribution, and overall suitability as a soil amendment (Cetin et al., 2004; McLaughlin et al., 2009; Novak et al., 2009). The majority of the mineral content in the feedstock is carried over into the biochar, where it is concentrated by carbon, hydrogen, and oxygen during pyrolysis (Amonette and Joseph, 2009). Generally, wood-derived biochars have a higher carbon content, but lower ash, nitrogen, phosphorus, potassium, sulphur, and micronutrient contents than bone-derived biochars (Raveendran et al., 1995; Skodras et al., 2006; Amonette and Joseph, 2009). Bone-derived biochar can be a source of phosphorus which is immediately available upon application, and may also release nitrogen and phosphorus over a longer time period (Warren et al., 2009). Wood biochars also have low mineral matter contents while bonemeal biochars can contain up to 84% mineral matter (Purevsuren et al., 2004). Particle size distribution is also influenced by feedstock — during thermal decomposition of biomass, the porosity and structure of the biomass is retained and once organic volatiles are lost, an extensive pore network is formed from the creation of voids (Downie et al., 2009). There are advantages and disadvantages to all feedstocks, but the use of an effective local feedstock source which stimulates the economy while recycling waste materials is ideal.

2.3.2 Pyrolysis conditions

Biochar is produced under relatively low thermal conditions (250–700 °C), but the temperatures within this range produce biochars with drastically different properties. Biochars heated at high temperatures (400–700 °C) tend to have lower biochar mass recovery, higher surface area, and increased ash content (Singh et al., 2010). These characteristics may result from the dehydration and decarboxylation of functional groups or the removal of volatile

compounds, which decreases the total surface charge and results in biochar that contains a higher percentage of carbon, but less oxygen and hydrogen (Novak et al., 2009). By comparison, biochars produced at low temperatures (250–400 °C) generally exhibit higher biomass recovery and have more functional groups on exchange sites (Novak et al., 2009). In several studies, pyrolysis temperatures between 400 °C and 600 °C produced biochars with low hydrophobicity and high field capacity, which improves the ability of the soil to sorb water and resist erosion (Uzoma et al., 2011; Kinney et al., 2012).

2.3.3 Application method

Biochar studies often overlook the feasibility and practicality of mixing biochar into soil during large-scale field application (Edenborn et al., 2015). Arguably, the largest influence on the choice of application method is the availability of machinery (Blackwell et al., 2009). The density and fineness of biochar can also influence the method of application and incorporation. Biochars typically have low densities and a significant powder fraction that can become airborne in light wind during transport or application. For instance, during the establishment of a fine-grained biochar field trial, approximately 30% of the material was reportedly lost during loading, transport, and spreading (Husk and Major, 2010). Best management practices to reduce wind losses are to moisten biochar prior to field application, pellet the biochar, or mix the biochar with manures or composts (Blackwell et al., 2009). Methods of biochar application and incorporation in field studies include: broadcast (by hand or mechanically) and incorporate (Asai et al., 2009), traditional banding (Blackwell et al., 2007), subsurface banding (Sistani et al., 2004), incorporation with composts and manures (Yoshizawa et al., 2006), and top-dressing (Gathorne-Hardy et al., 2008).

2.3.4 Application rate

The rate at which biochars are applied also affects the degree of physical and chemical changes within the soil. With proper nutrient management, the positive effects of biochar on crop yield has been observed with rates of 5-50 tonnes (t) per hectare (ha) (Blackwell et al., 2007; Rondon et al., 2007). Higher biochar application rates are often more successful when a range of rates are used (Major et al., 2010). For example, a pot trial using four rates of biochar (0, 10, 25, and 50 t ha^{-1}) found that radish yield increased by 42% at 10 t ha^{-1} to 96% at 50 t ha^{-1} (Chan et al., 2008). However, decreasing yields were reported with higher biochar

application rates (Asai et al., 2009; Gaskin et al., 2010), while others reported that biochar application at 1-2 t ha^{-1} did not significantly affect plant growth or soil properties (Ahmed and Schoenau, 2015). The majority of biochar application rate studies assess crop growth and soil properties in agricultural soils, and few studies exist which evaluate the effect of biochar application rate on PHC degradation. At 2% (w/w) biochar amendment in laboratory-incubated soil, linear, cyclic, and branched alkanes degraded more rapidly than in an unamended control soil (Bushnaf et al., 2011). As of 2015, field tests with non-activated biochar for environmental remediation had not been identified (Hale et al., 2015). General recommendations for biochar application rates according to soil types and specific environmental applications are not available due to insufficient field data (Major, 2010).

2.3.5 Water holding capacity

Biochars have a highly porous structure and large surface area which controls aeration and hydrology within the soil (Atkinson et al., 2010). Water holding capacity, a measure of water stored in the soil, is typically measured to determine the effect of biochar on soil hydrology. Coarse particles like sand have a very low surface area, but when combined with biochar, the water holding capacity increases due to a net increase in surface area (Chan et al., 2007). The water holding capacity of loamy sand soils also increased after biochar additions, but different feedstocks produced biochars that were more effective than others at increasing water holding capacity (Yu et al., 2013, 2017a). Fewer studies exist which quantify the effects of biochar on plant-available water; i.e., the amount of water held loosely enough by soils that plants can access it. In sandy soils, plant-available water increased by 16% to 270% with biochar amendment rates ranging from 0.5-5% by mass (Briggs et al., 2012; Abel et al., 2013) and 5-15% by volume (Brockhoff et al., 2010; Briggs et al., 2012; Abel et al., 2013). In coarse-textured northern soils, biochar is expected to increase water availability — though results may vary based on biochar type and application rate.

2.3.6 Thermal properties

The thermal properties of soil influence microbial biomass and activity, nutrient mineralization, and soil water content. Due to the black color and unique physical and chemical properties of biochars, there is potential to change the soil temperature, albedo, and thermal conductivity. Research suggests that biochar has a moderating effect on soil temperature which

results from decreased reflectance and thermal conductivity (Zhang et al., 2013). Soil albedo decreased with increasing biochar application rate, but was dependent on soil moisture content, application method, and land use (Verheijen et al., 2013). Similarly, other studies demonstrated that surface albedo decreased up to 80% in biochar-amended soils when compared to a control (Meyer et al., 2012; Genesio et al., 2012; Zhang et al., 2017). A decrease in surface albedo may increase the soil temperature, which can increase soil respiration (Jiang et al., 2015). Soil surface temperature increased up to 4 °C in biochar and charcoal amended soils (Oguntunde et al., 2008; Vaccari et al., 2011). While surface temperature, albedo, and hydraulic conductivity appear to be affected by biochar applications, it is relatively unclear how this influences more complex processes such as crop growth and bioremediation.

2.3.7 pH and buffering capacity

The pH of both biochar and soil will determine the overall effect of biochar additions on soil pH. Biochars can have a large buffering capacity, depending on the production conditions and feedstock source, which can increase the pH of acidic soils (Shi et al., 2017; Yao et al., 2017). In general, increasing the pH of an acidic soil will promote a favorable environment for the survival and success of PHC degrading microbial communities. The addition of alkaline biochars to alkaline soils could have a negative effect on the availability of certain essential nutrients, depending on the factor by which pH increases. However, biochars have been reported to decrease the pH of calcareous soils (Liu and Zhang, 2012; Ippolito et al., 2016), due to the production of acidic functional groups during chemical and microbial oxidation of biochar particles (Cheng et al., 2006) and by increasing overall soil CEC (Laird et al., 2010; Fellet et al., 2011; Jones et al., 2011a; Peng et al., 2011). Clearly, biochar additions can increase, decrease, or maintain soil pH, so biochars must be matched carefully with soil to optimize bioremediation.

2.3.8 Nutrient status and transformations

Biochar additions contribute some nutrients to the soil depending on the feedstock utilized (DeLuca et al., 2009), but more importantly, they act as a driver for nutrient transformations in the soil (Glaser et al., 2002; Lehmann et al., 2003). Water holding capacity is enhanced with the addition of biochar, which can also increase the solubility and availability of micronutrients and macronutrients (Chan and Xu, 2009). Yeboah et al. (2009) demonstrated that nitrogen recovery (amount of nutrient in the crop as a ratio of the amount applied) in maize crops

could be improved through biochar application to a sandy loam soil, but not a silt loam soil, which suggests that the effectiveness of biochar is more strongly influenced by the indirect textural effect rather than direct nutrient additions. Other general mechanisms by which biochar influences nutrient turnover and transformations includes increasing the nutrient pool size and serving as a short term source of labile nutrients (Ducey et al., 2013), altering soil physical and chemical properties, and changing the structure and activity of microbial communities (DeLuca et al., 2015). The mechanisms driving nutrient transformations and additions to the soil are a complex interaction between the amendment formulation combined with physical, chemical, and biological characteristics of biochar and soil. Nutrients additions (i.e., fertilizers) can increase the positive response of biochar (Lehmann et al., 2006), but the direct influence of biochar additions on soil nutrient transformations are still not well understood. Coarse-textured northern soils are inherently nutrient poor so biochar additions could improve texture, water retention, and nutrient supplies to increase PHC degradation when soils are limited by these properties.

2.3.9 Soil microbial activity and community composition

Biochar has been considered as a recalcitrant carbon source that is capable of sequestering carbon in soils, but is largely unavailable to soil microorganisms (Baldock and Smernik, 2002; Skjemstad et al., 2002). However, microbial assimilation of biochars has been reported (Kuzyakov et al., 2009; Jones et al., 2011b), which suggests that the introduction of metabolically available volatile and labile compounds from biochar particles may significantly alter soil microbial activity (i.e., respiration), and result in positive benefits to bioremediation (Smith et al., 2010; Kolton et al., 2011). Several studies have demonstrated that biochar-amended soils affect the diversity, abundance, and distribution of soil microbial communities through greater microbial biomass (Thies and Suzuki, 2003; Zhang et al., 2014a), higher metabolic efficiency (Dil et al., 2014; Zheng et al., 2016), and greater abundance of hydrocarbon degrading genes (Germano et al., 2012; Dias et al., 2015). Moreover, the microbial community can shift in response to changes in physical, chemical, and biological soil properties induced by biochar additions. For example, studies report that biochar amendments have increased gram negative bacteria (Watzinger et al., 2014), increased bacterial 16S rRNA genes and decreased fungal 18S rRNA genes (Chen et al., 2013), and shifted dominant phylotypes (Han et al., 2017). In some cases, biochar additions can have neutral or negative effects on the soil microbial community due to direct toxicity from the biochar itself (Lyu et al.,

2016), inability to access tightly sorbed substrates (Prommer et al., 2014; Zhang et al., 2014b), or pore sizes that are too large or too small (Quilliam et al., 2013a). Although the effects of biochar on soil microbial community composition and activity have been evaluated across a range of biochar and soil types, results were variable and the mechanisms by the soil microbial community is affected are not well understood.

2.3.10 Microbial habitat

Biochar not only affects microbial community dynamics, but the porous structure of biochar can provide a suitable habitat for microorganisms by supplying nutrients (Angst and Sohi, 2013) and providing protection from desiccation (Zackrisson et al., 1996; Warnock et al., 2007). One study reported that under short term incubation (< 3 years), external and internal biochar surfaces are not a significant habitat for soil microorganisms but suggested that soil in the immediate vicinity had a greater influence on microbial structure and activity (Quilliam et al., 2013a). Other studies indicated that there was no effect on microbial biomass in biochar-amended soils, which suggested that biochar was unsuitable for microbial colonization (Chan et al., 2007; Durenkamp et al., 2010). Biochar macropores (> 200 mm) likely provide habitat for bacteria, which range in size from 0.3-3 µm, while micropores (< 2 nm) and mesopores (2-50 nm) could store water and dissolved nutrients and substrates required for microbial metabolism (Brewer and Brown, 2012; Quilliam et al., 2013a). Biochar feedstock and production temperature determine the size and abundance of pores. Other physical properties of biochar which may affect its suitability as a microbial habitat include surface area and thermal properties (Gul et al., 2015). Certain chemical properties, such as surface charge, nutrient concentration (Gul et al., 2015), chemi-sorbed gases (i.e., CO_2, O_2, N_2), and water availability (Pietikainen et al., 2000; Antal and Grønli, 2003), may influence biochar pores and attract soil microorganisms. There are many proposed mechanisms by which biochar can provide a habitat for soil microorganisms, but contradictory results have been reported due to the variability between biochars and soils, and the methods used to assess microbial colonization.

2.3.11 Bioremediation of organic contaminants

There are many ways in which biochars enhance the bioremediation of organic contaminants, including optimizing soil conditions to promote microbial degradation, immobilizing contaminants in the soil profile, and stimulating microbial activity and biomass

production. Several studies have reported successful degradation of PHCs (Bushnaf et al., 2011), PAHs (Liu et al., 2014, 2017), phenols (Yang et al., 2016a), pesticides (Qiu et al., 2009), and herbicides (Jones et al., 2011a) in biochar-amended soils. Nevertheless, biochars have been reported to reduce the bioavailability of hexachlorobenzene (Song et al., 2012) and isoproturon (Sopeña et al., 2012), and ultimately, lower their degradation rates. Few studies exist which monitor the effects of biochar on PHC degradation and results are often observational rather than mechanistic. As PHC degradation is a complex process that is highly dependent on soil properties and environmental conditions, PHC degradation rates are expected to be variable and inconsistent between locations. The process is further complicated by the variability and specificity of biochars, and with knowledge gaps in biochar-soil interactions.

2.3.12 Sorption of organic contaminants

Biochar additions can enhance the sorptive capabilities of soils and exert a strong influence on the toxicity, fate, and behavior of PHCs. Pyrogenic organic matter, such as biochar, and non-pyrogenic organic matter, such as plant material, differs in the concentration dependence of sorption, mechanism of sorption, and sorption reversibility. The sorption affinity of pyrogenic organic matter is usually non-linear as the sorption of a specific compound to biochar decreases with increasing concentration, indicating sorption to external or internal surfaces (Yang and Sheng, 2003). Generally, sorption to biochar is approximately one to two orders of magnitude greater than sorption to soil (Hale et al., 2015). Few studies exist that quantify the sorption of organic compounds to biochar in the presence of soil, due to the confounding effects of soil components such as clay and organic matter. The sorptive capacity of phenanthrene ranged from 1.98 to 4.53 log k_d at 1 mg L^{-1} in a pine needle biochar and soil mixture (Zhang et al., 2010) and from 3.77 to 5.12 log k_d at 1 mg L^{-1} with only pine needle biochar (Wang and Xing, 2007). The adsorption capacity of biochars have been linked to its organic carbon content, surface area, and porosity (Spokas et al., 2009; Dechene et al., 2014). In bioremediation studies, PHC degradation may be overestimated if biochar contributes to the removal of PHC via sorption, therefore, the degree of sorption by biochar must also be considered.

2.4 Conclusion

Bioremediation in northern Canada is a slow process limited by extreme environmental conditions. Conventional methods rely on fertilizer additions and soil turning to stimulate the

microbial community to catabolize organic contaminants. Biochar is a novel amendment that has received interest because of its positive effects on physical, chemical, and biological properties in agricultural soils. Biochar could have parallel effects on PHC contaminated soils and has the potential to increase bioremediation rates. Few, if any, studies have investigated biochar as a soil amendment to increase PHC degradation in soils that are periodically frozen, and only limited research exists in temperate regions. A knowledge gap exists as the physical, chemical, and biological mechanisms driving the positive effects of biochar are not well understood, especially in frozen soils.

Currently, research involving bioremediation and PHC degradation is targeted towards soils in tropical and temperate regions. Due to the vast differences in soil properties and environmental conditions between tropical, temperate, and northern regions, it is necessary to develop bioremediation techniques specific to northern regions. Through the development of optimal application rates and efficient application methods, biochar could enhance bioremediation rates and overall success across a range of contaminated sites in northern Canada. Furthermore, providing effective biochar formulations, specific to local remediation needs, could provide an accessible and affordable soil amendment, which makes use of by-products from other industries and stimulates the local economy. Working with local industry to develop and improve bioremediation technologies, methods, and protocols that are unique to northern soils is essential to alleviate current challenges to cost-effective remediation. However, further investigation is required prior to applying biochar as a soil amendment to enhance PHC degradation at northern landfarms.

3. PETROLEUM HYDROCARBON REMEDIATION IN FROZEN SOIL USING A MEAT AND BONEMEAL BIOCHAR PLUS FERTILIZER[1]

3.1 Preface

Petroleum hydrocarbon (PHC) remediation can be a slow and costly process in cold regions, due to short summer seasons and coarse, infertile soils. Landfarming is the most common and successful bioremediation method but can be ineffective in reducing PHC concentrations below remediation criteria in a timely manner. Biochar, an organic biomass created by pyrolysis, has enhanced soil properties and crop growth in agricultural soils and generated research interest for its use as a soil amendment. Few studies exist that use biochar as a soil amendment to increase PHC degradation, and even fewer have investigated biochar application to frozen soil. Biochar is a unique soil amendment that can be produced using local feedstocks with unsophisticated equipment. Currently, there are no studies that have attempted to increase PHC degradation in northern soils using biochar amendments. This laboratory study was carried out using fertilized, landfarmed soil from Iqaluit which was amended with different biochar and compost amendments. The overall objective of this study was to determine if meat and bonemeal biochar additions could further enhance PHC degradation rates in thawed and frozen soil.

[1] This chapter was published, with minor formatting differences, as Karppinen, E.M., R.E. Farrell, K.J. Stewart, and S.D. Siciliano. 2017. Petroleum hydrocarbon degradation in frozen soil using a meat and bonemeal biochar plus fertilizer. Chemosphere 173:330-339. Dr. Siciliano provided funding, developed the experimental design, and contributed his expertise in the expansion of the major ideas. Dr. Farrell assisted with establishing the schedule for data collection and analysis and provided editorial input. Dr. Stewart assisted with technical content and provided editorial input. I conducted laboratory analyses, completed the initial manuscript draft, prepared the figures and tables, and completed data analysis and interpretation.

3.2 Abstract

Petroleum hydrocarbon (PHC) degradation slows significantly during the winter, which substantially increases the time it takes to remediate soil in Arctic landfarms. The aim of this laboratory trial was to assess the potential of a meat and bonemeal (MBM) biochar to stimulate PHC degradation in contaminated soil collected from Iqaluit, Canada. Over 90 days, 3% (w/w) MBM biochar increased F3 (equivalent nC_{16}-C_{34}) -PHC degradation rate constants (k) in frozen soils when compared to the fertilizer (urea and monoammonium phosphate) control. Taking into consideration the extensive variability within treatments and negative k values, this difference may not reflect significant remediation. Decreasing C_{17}/Pr and C_{18}/Ph ratios in the frozen soil suggest that this reduction is a result of microbial degradation rather than volatilization. Amendment type and application rate affected the immediate abiotic losses of F2 and F3-PHC in sterile soils, with the greatest losses occurring in compost-amended treatments in the first 24 hours. In frozen soils, MBM biochar was found to increase liquid water content (θ_{liquid}), but not nutrient supply rates. Under frozen but not thawed conditions, genes for aromatic (*C2,3O* and *nahAc*) but not aliphatic (*alkB*) PHC degradation increased over time in both biochar-amended and control treatments but total viable PHC-degrading populations increased only in biochar-amended soils. Based on these results, it is possible that PHC degradation in biochar-amended soils is active and even enhanced under frozen conditions, but further investigation is required.

3.3 Introduction

Landfarming is the most common bioremediation method in cold regions but it is a slow and costly process that can be limited by extreme environmental conditions and remote locations (Mohn and Stewart, 2000). Conventional methods of petroleum hydrocarbon (PHC) remediation in landfarms rely on fertilizer additions and soil turning to stimulate the microbial community to catabolize organic contaminants; this approach, however, has yielded inconsistent results in cold environments (Powell et al., 2006a; Paudyn et al., 2008). Current bioremediation strategies are targeted toward the short summer months (2-4 months/year); but this is often an insufficient amount of time to meet soil remediation targets and environmental criteria (Mohn and Stewart, 2000). Substantial bioremediation can occur at sub-zero temperatures and extending microbial degradation of PHC further into the winter months could reduce the amount of time required to remediate landfarmed soil (Rike et al., 2003).

Biochar is a soil amendment that results from the heating of organic biomass under oxygen limited conditions, and has been used in environmental remediation to sorb organic pollutants and stimulate microbial degradation (Lehmann et al., 2011; Ogbonnaya and Semple, 2013). The type of feedstock and the conditions under which the pyrolysis is carried out, can drastically affect the chemical properties, elemental composition, and overall suitability of the resulting biochar as a soil amendment (Amonette and Joseph, 2009; McLaughlin et al., 2009). Typical feedstocks include wood products, leaves, grasses, manures, sludges, or crop residues. Biochars produced from meat and bonemeal (MBM) are less common but contain large amounts of calcium phosphate (Betts et al., 2013). Biochars derived from wood products generally have a higher carbon content, but lower ash, nitrogen, phosphorus, potassium, sulphur, and micronutrient contents than biochars derived from bonemeal (Amonette and Joseph, 2009). There are advantages and disadvantages to the use of all feedstocks; however, utilization of an effective local source of the feedstock is ideal in that it can stimulate the economy while recycling waste materials. In northern climates, wood stocks can be limited, but there are often local sources of meat and/or bone available for use as a feedstock for biochar production.

Whereas biochar additions may or may not contribute some nutrients to the soil (depending on the type of feedstock), a more important contribution is the ability of biochar to act as a driver for nutrient retention and transformation in the soil when supplemented with fertilizer (Glaser et al., 2002; Lehmann et al., 2003; Cantrell et al., 2012). The highly porous structure and large surface area of biochar alters nutrient retention, cation exchange capacity, aeration, and hydrology within the soil pores (Atkinson et al., 2010). For example, when sandy soils are combined with biochar, the water holding capacity and liquid water (θ_{liquid}) increases due to a net increase in surface area (Chan et al., 2007). Similarly, soil-biochar interactions increase the amount of θ_{liquid} in the soil, increasing nutrient supply rates (NSR), which in turn can increase degradation of PHCs (Harvey et al., 2012). In the presence of sufficient θ_{liquid}, microorganisms capable of living at the critical interface of water and ice (i.e., eutectophiles) catabolize PHCs under frozen conditions (Deming, 2002). These microorganisms remain viable and metabolically active at low temperatures because the θ_{liquid} allows mass transfer processes to proceed.

In addition to changing the soil environment, biochar may also provide unique habitats for specialized microorganisms to thrive in much the same way as organic matter additions (Rivkina

et al., 2000). The porous structure of biochar particles provides a suitable habitat for microorganisms by supplying nutrients and providing protection from desiccation. In turn, this promotes a larger microbial biomass, a greater abundance of culturable microorganisms and a higher metabolic efficiency relative to unamended soils (Thies and Grossman, 2006). Thus, there are two possible mechanisms by which biochar can increase microbial degradation in frozen soil; i) the biochar may alter nutrient and water diffusion rates and ii) the biochar may provide a suitable habitat for microorganisms that degrade PHCs (Thies and Rillig, 2009). Microbial colonization is dependent on pore size distribution within the biochar, as micropores are too small for most soil microorganisms, and habitable pores can become obstructed by soil or microbial components (Thies et al., 2015).

Few studies have been conducted on biochar-amended, PHC contaminated soils under frozen conditions; therefore, the site-specific effectiveness of using biochar as a soil amendment and the mechanisms driving PHC remediation are not well understood. The objectives of this study were to determine if MBM biochar additions could further enhance PHC degradation in fertilized, landfarmed soil from Iqaluit, Canada, and if so, to link this degradation to measurable chemical and microbial responses. I hypothesize that fertilizer plus the addition of MBM biochar has the potential to increase PHC remediation in cold regions by extending bioremediation rates into frozen months by manipulating θ_{liquid} to supply nutrients and stimulate microbial activity.

3.4 Materials and Methods

3.4.1 Soil and biochar characterization

Soil contaminated with PHCs from a landfarm in Iqaluit, Canada (63°45’N,68°31’W) was used in a bench-scale laboratory trial to assess the effectiveness of MBM biochar and fertilizer to enhance PHC degradation in northern soils under frozen and thawed conditions. Iqaluit is located on the shores of Frobisher Bay, and experiences a typical dry Arctic climate with average monthly temperatures below freezing for eight months of the year and approximately 400 mm of annual precipitation. This area contains igneous Canadian Shield bedrock overlain by continuous permafrost.

The soil used in this study was a mixture of weathered PHC contaminated material (i.e., P50 arctic grade diesel fuel, hydraulic, and heating oil) from Iqaluit and the

surrounding area, which had an average PHC content of 653 mg kg^{-1}. Total PHC breakdown was as follows; F1: 3 mg kg^{-1}, F2: 156 mg kg^{-1}, F3: 478 mg kg^{-1}, and F4: 16 mg kg^{-1}. Petroleum hydrocarbons were extracted using Accelerated Solvent Extraction (ASE) and Canadian Council of Ministers of the Environment (CCME) column clean-up (see Section 2.4). According to Tier 1 CCME Canada-wide standards (CWS) for residential land use and coarse-grained soils (Appendix A, Table A.1), F2-PHC concentrations already meet clean-up criteria (150 mg kg^{-1}) while F3-PHCs exceed criteria (300 mg kg^{-1}) by approximately 178 mg kg^{-1}.

The sandy soil (94% sand) had a near-neutral pH (7.5), was deficient in major nutrients (i.e., extractable NO_3^--N [1.43 mg kg^{-1}] and PO_4^{3-}-P [0.23 mg kg^{-1}]) and had low organic carbon (0.67%) and gravimetric moisture (9.81%) content. The MBM biochar (Titan Clean Energy Projects Corporation, Craik, SK) had a pH of 6.1, cation exchange capacity (CEC) of 35 $cmol_c$ kg^{-1}, Brunauer-Emmet-Teller (BET) surface area of 31 m^2 g^{-1}, average pore volume of 0.1003 cm^3 g^{-1}, and an average pore size of 11.83 nm (Betts et al., 2013). The major elements (present at concentrations >100 mg kg^{-1}) in the biochar were C (346 g kg^{-1}), Ca (135 g kg^{-1}), P (72.6 g kg^{-1}), K (18.2 g kg^{-1}), Na (10.7 g kg^{-1}), Fe (9.6 g kg^{-1}), Mg (53 g kg^{-1}), and Al (4.4 g kg^{-1}) (Betts et al., 2013). Composted sheep manure was locally sourced in Iqaluit and had a near neutral pH (7.4) and CEC of 90 $cmol_c$ kg^{-1}.

3.4.2 Experimental setup

Prior to start of the experiment, soil samples from various cells in the Iqaluit landfarm were sieved to remove large rocks, then combined and homogenized into the bulk starting material. Using a batch technique, the bulk soil was amended with urea (46-0-0) and/or monoammonium phosphate (MAP; 11-52-0) fertilizer and either compost or MBM biochar. Two levels were used for each amendment; compost at 5% or 10% (w/w) and biochar at 3% or 6% (w/w), to observe the effects at low and high amendment levels. Fertilizer additions (630 mg N kg^{-1} and 70 mg P kg^{-1} dry weight basis) were applied to maintain a C:N:P ratio of 100:9:1, which is considered optimal for Arctic sites (Chang et al., 2010). Whereas all treatments received nitrogen fertilizer, phosphorus fertilizer was only required for the control and compost treatments as biochar produced from bone is a potential source of calcium phosphate (i.e., hydroxyapatite)

and this particular MBM biochar contained enough residual phosphorus that extra fertilizer was not required (Warren et al., 2009).

After amending each batch, the soil was re-homogenized, weighed into 60 mL glass amber vials and plugged with sterile cotton balls to maintain aerobic conditions. Water was added to each vial to bring the soil to 60% of field capacity with the weight of each vial recorded; soil water content was monitored by regularly weighing the vials and adding water as needed to maintain the soil at 60% of field capacity. All soil treatments were incubated at 10 °C, in addition, the fertilizer control and 6% (w/w) MBM biochar were incubated at -5 °C. These two incubation temperatures were used to simulate summer and winter conditions in Arctic environments.

3.4.3 Soil treatments

Petroleum hydrocarbon degradation was examined under thawed conditions using five treatments, replicated eight times: (i) urea and MAP fertilizers; (ii) 3% (w/w) MBM biochar plus urea; (iii) 6% (w/w) MBM biochar plus urea; (iv) 5% (w/w) compost plus urea and MAP; and (v) 10% (w/w) compost plus urea and MAP (Table 3.1). The soils were incubated at 10 °C and destructively sampled mid-vial at 0, 30, 60, and 90 days. Day 0 treatments were incubated for 24 hours to allow the soil to reach the targeted incubation temperature but will be referred to as Day 0 for the remainder of the manuscript. Petroleum hydrocarbon degradation under frozen conditions was examined using two treatments, each with eight replicates: (i) urea and MAP fertilizer and (ii) 6% (w/w) MBM biochar plus urea (Table 3.1). The soils were incubated at -5 °C and destructively sampled mid-vial at 0, 30, 60, and 90 days.

Table 3.1. Biochar and compost treatments in thawed and frozen soils.

Incubation Temperature	Treatment
10 °C	Urea and MAP fertilizers
	3% (w/w) MBM biochar plus urea
	6% (w/w) MBM biochar plus urea
	5% (w/w) compost plus urea and MAP
	10% (w/w) compost plus urea and MAP
-5 °C	Urea and MAP fertilizers
	6% (w/w) MBM biochar plus urea

3.4.4 Petroleum hydrocarbon analysis

PHCs were extracted from soil samples using an ASE 200 System (Thermo Fisher Scientific, Burlington, ON). In summary, approximately 1 g of soil was extracted using 20 mL of hexane and acetone at 1:1 volume, under elevated temperature and pressure of 200 °C and 1500 psi, respectively. Preliminary analysis concluded that PHCs in the test soil were found almost exclusively in the F2 and F3-PHC fractions (see Section 3.4.1). Following ASE, the hydrocarbon extracts were passed through 50 cm columns containing sodium sulphate and silica gel to remove water and polar organic compounds, respectively (CCME, 2008). Quality control measures included duplicates, which were within 10-20 relative percent difference, matrix and method blanks, as well as spike recoveries (average F2 and F3-PHC spike recoveries were 81% and 87%, respectively). Both F2 and F3-PHC concentrations were quantified using a Varian CP3800 gas chromatograph fitted with a flame ionization detector (GC-FID) and chromatograms were analyzed using CompassCDS software (Varian, Santa Clarita, CA). Petroleum hydrocarbon data followed first-order kinetics, therefore, degradation rate constants (k) were derived as the slope of the natural log concentration (mg kg^{-1}) versus time (days) (Newell et al., 2002).

3.4.4.1 Degradation biomarkers

Two linear (*n*-) alkanes, heptadecane ($C_{17}H_{36}$; C_{17}) and octadecane ($C_{18}H_{38}$; C_{18}), and two branched/cyclic (*iso*-) alkanes, 2,6,10,14-tetramethylpentadecane ($C_{19}H_{40}$; pristine, Pr) and 2,6,10,14-tetramethylhexadecane ($C_{20}H_{42}$; phytane, Ph), were used to determine the extent of biodegradation for frozen treatments only. Standards were run to identify retention times in

chromatograms containing each compound. C_{17}/Pr and C_{18}/Ph ratios were calculated by dividing the area of the *n*-alkane by the area of the *iso*-alkane.

3.4.4.2 Abiotic loss estimation

Field-contaminated soil from Mestersvig, Greenland was used to estimate the degree of abiotic losses occurring as a result of the soil matrix and amendments. The sandy soil (88% sand) had a pH of 7.6 and low organic carbon (0.56%), which was similar to the Iqaluit soils, but F2 and F3-PHC concentrations were much higher, at 1731 mg kg^{-1} and 3944 mg kg^{-1}, respectively. The soil was air-dried and sieved to pass an 850 µm screen and then brought to 10% (w/w) water content, after which it was gamma irradiated to 30 kGy, which will sterilize most soils (McNamara et al., 2003). The following treatments, each with 8 replicates, were applied: (i) soil, (ii) soil plus 3% (w/w) MBM biochar, (iii) soil plus 6% (w/w) MBM biochar, (iv) soil plus 5% (w/w) compost, and (v) soil plus 10% (w/w) compost. Using a batch technique, the soil was homogenized and separated into amber glass vials containing zero headspace. Zero headspace was used to limit volatilization and provide estimation for the extent of other major abiotic losses, such as sorption. The vials were destructively sampled mid-vial for F2 and F3-PHC after 0 and 90 days. A different PHC extraction method was used due to ASE breakdown after extraction of the Iqaluit soils; however, both methods had similar F2 and F3-PHC spike recovery so the extraction methods provide comparable results. Petroleum hydrocarbons were extracted using a hexane-acetone extraction method, where 2.5 g soil plus 30 mL hexane and acetone (1:1) were combined with sodium sulphate and shaken overnight, centrifuged to remove the supernatant and evaporated to approximately 2 mL with nitrogen gas (Schwab et al., 1999). Quality control measures included duplicates, which were within 10-20 relative percent difference, matrix and method blanks, as well as spike recoveries (average F2 and F3-PHC spike recoveries were 82% and 87%, respectively).

3.4.5 Liquid water content

Volumetric water content was measured using time domain reflectometry (TDR), a technique that measures the dielectric constant of the medium. The dielectric constant of water ($K_{water} = 80$; $K_{ice} = 3.2$) is much higher than other soil constituents ($K_{air} = 1$; $2 \leq K_{soil} \leq 5$), thus, the dielectric constant of the medium is proportional to the amount of θ_{liquid} present (Topp et al., 1980). This technique is capable of measuring small quantities of θ_{liquid}; i.e., down to

0.05 m^3 H_2O m^{-3} soil (Siciliano et al., 2008), but requires a large volume of soil in order to generate waveforms without interference, thus, θ_{liquid} could not be measured directly from the experimental vials. An uncontaminated reference soil with similar texture (95% sand) was sieved (< 4.75 mm), brought to 16% (w/w) water holding capacity and packed to a bulk density of 1.4 Mg m^{-3} into 4 inch polyvinyl chloride (PVC) pipe with one end capped. Calibrated TDR probes were inserted and the pipes were frozen at -5 °C for 24 hours. Soil θ_{liquid} was measured with a Tektronix 1502B cable tester (Tektronix, Beaverton, OR) and quantified using Topp's equation (Topp et al., 1980). Two treatments were measured in triplicate: (i) fertilizer (urea and MAP) and (ii) 6% (w/w) MBM biochar plus urea. Soil texture, temperature, and bulk density are known to affect θ_{liquid} measurements when using TDR probes (Gong et al., 2003), therefore a sandy soil, incubated at the same temperature and packed to the same average bulk density of the Iqaluit soils was used to determine θ_{liquid}.

3.4.6 Nutrient supply rates

Samples from the 90 day incubation were processed for nutrient supply rates (NSR), with concentrations of the major nutrients (PO_4^{3-}-P, NO_3^--N, and NH_4^+-N) measured using anion and cation exchange resins (Western Ag Innovations, Saskatoon, SK). Resins were charged with 0.5 M $NaHCO_3$ and sandwiched between two Snapcap® 7 Dram vial lids packed with air dried soil brought to field capacity and wrapped in Parafilm® (Qian and Schoenau, 2002; Schafer et al., 2009). Thawed treatments were incubated at 10 °C while frozen treatments were incubated at -5 °C. After two weeks, the sandwiches were dissembled; the resins were rinsed with deionized water and shaken at 200 rpm for 1 hour in 0.5 M HCl. Nutrient concentrations were measured colorimetrically; NH_4^+-N was measured using a Smart Chem™ 200 discrete wet chemistry analyzer (Westco Scientific Instruments Inc., Brookfield, CT), while NO_3^--N and PO_4^{3-}-N were measured using a Technicon™ AutoAnalyzer™ II (SEAL Analytical, Mequon, WI).

3.4.7 Soil microbial community analysis

3.4.7.1 Functional gene abundance

Total community DNA was extracted from approximately 0.25 g of soil using the Powerlyzer™ Powersoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) to lyse cells and remove humic substances and other PCR inhibitors. Yield was quantified using a Qubit® Fluorometer (Thermo Fisher Scientific, Burlington, ON). Treatments were analyzed for the

presence of specific catabolic genes (Table 3.2) encoding enzymes involved in PHC degradation pathways: alkane monooxygenase (*alkB*) (Powell et al., 2006a), naphthalene dioxygenase (*nahAc*) (Whyte et al., 1997; Luz et al., 2004), and catechol 2,3 dioxygenase (*C2,3O*) (Baldwin et al., 2003). Standards were prepared by extracting the DNA of American Type Culture Collection (ATCC) standard organisms, followed by PCR amplification with the corresponding primer set and repeated gel purification.

Table 3.2. Primers and thermal conditions used for quantitative polymerase chain reaction (qPCR) amplification of alkane monooxygenase (*alkB*), catechol 2,3 dioxygenase (*C2,3O*), and naphthalene dioxygenase (*nahAc*).

Target gene Primer sequence†	**Ta‡** (°C)	**Primer** (µM)	**Expected size** (bp)	**Control Strain**
Alkane monooxygenase (*alkB*)	50.0	1.0	100	*P. putida*
F: 5'- AAC TAC ATC CGA GCA CTA CGG				ATCC 29347
R: 5'- TGA AGA TGT GGT TGC TGT TCC				
Catechol 2,3 dioxygenase (*C2,3O*)	65.6	1.0	406	*P. putida*
F: 5'- AGG TGC TCG GTT TCT ACC TGG CCGA				ATCC 29347
R: 5'- ACG GTC ATG AAT CGT TCG TTG AG				
Naphthalene dioxygenase (*nahAc*)	47.0	1.0	377	*P. putida*
F: 5'- CAA AAR CAC CTG ATT YAT GG				ATCC 17484
R: 5'- AYR CGR GSG ACT TCT TTC AA				

†Forward (F) and reverse (R) primers are indicated.

‡Ta = annealing temperatures used during real-time PCR.

For qPCR gene amplification, 4 µL of template DNA was added to a 15 µL final volume mixture containing QuantiTect SYBR® Green RT-PCR Kit (Qiagen, Valencia, CA) and 1.0 µM each of forward and reverse primers. After 10 minutes initial denaturing at 94 °C, amplification proceeded with 40 cycles of 1 minute denaturing at 94 °C, 30 seconds annealing at the appropriate temperature, 1 minute extension at 72 °C, followed by a melt curve analysis for 15 seconds at 95 °C, 1 minute at 60 °C, and 15 seconds at 95 °C (Table 3.1). Quality control measures included matrix blanks and a standard curve prepared in triplicate, ranging from 10^8-10^2 gene copies μL^{-1}. All PCR fragments were visualized on SYBR® Safe-stained 1.5% agarose gels to ensure target gene amplification.

3.4.7.2 Total heterotrophic PHC-degrading population

The most probable number (MPN) technique was used to enumerate culturable, aerobic PHC-degrading microbial populations using 96-well microtiter plates. Minimal salts medium (180 µL) was added to each well and supplemented with 3 µL of 5000 mg kg^{-1} F2-diesel fuel standard following serial dilution (Yergeau et al., 2009). After 90 days, soil from each treatment was diluted in a saline phosphate buffer solution (PBS); 1 g of soil was mixed with 9 mL of PBS to create a 1:10 dilution. The first row of wells was inoculated with 20 µL of the 1:10 dilution. The subsequent wells in each column were inoculated by transferring 20 µL from the previous well to create a dilution series ranging from 10^{-2}-10^{-7} gene copies μL^{-1}. The last row remained un-inoculated to serve as a sterile control. Following incubation at room temperature for three weeks, 50 µL of filter sterilized iodonitrotetrazolium (INT) violet (3 g L^{-1}) was added to identify positive wells (Haines et al., 1996). Red or pink positive wells were scored after an overnight, room temperature incubation with INT. Final count numbers were derived using an MPN calculator (Jarvis et al., 2010).

3.4.8 Data analysis

Data was tested for assumptions of normality and homogeneity of variance using the Shapiro-Wilk Test and Levene's Test, respectively. A student's t-test was used for frozen *k* values and MPN data in which only two treatments were compared. A one-way analysis of variance (ANOVA) was used for thawed *k* values, NSR, and MPN data, in which treatment was the only factor. A two-way ANOVA was used for isoprenoid ratios, abiotic loss estimation, and qPCR data, where treatment, time, and interaction effects were considered. Means separation

was performed using Tukey's multiple comparisons test. All data was processed in RStudio (Version 0.98.932) and visualized in Sigmaplot (Version 12.0).

3.5 Results and Discussion

3.5.1 Petroleum hydrocarbon degradation

Meat and bonemeal biochar additions influenced PHC concentrations in frozen soil by increasing F3-PHC, but not F2-PHC, removal over a 90 day period. In PHC contaminated soil incubated for 90 days at -5 °C, 3% MBM biochar reduced F3-PHC concentrations by 28% from 656 ± 83 mg kg^{-1} to 473 ± 36 mg kg^{-1}, while there was no significant change over time in the fertilizer control (Fig. 3.1). After 90 days, F2-PHC concentrations in frozen soil were reduced to 122 ± 14 mg kg^{-1} and 92 ± 17 mg kg^{-1} in 3% MBM biochar and the fertilizer control, respectively (Fig. 3.1). Frozen soil amended with 3% MBM biochar had a significantly higher F3-PHC *k* values than the fertilizer control at 0.0036 ± 0.0016 d^{-1} and -0.0007 ± 0.0015 d^{-1}, respectively; however, there were no significant differences between the fertilizer control and 3% MBM biochar-amended soil when comparing F2-PHC *k* values (Table 3.3, t-test, $P < 0.10$).

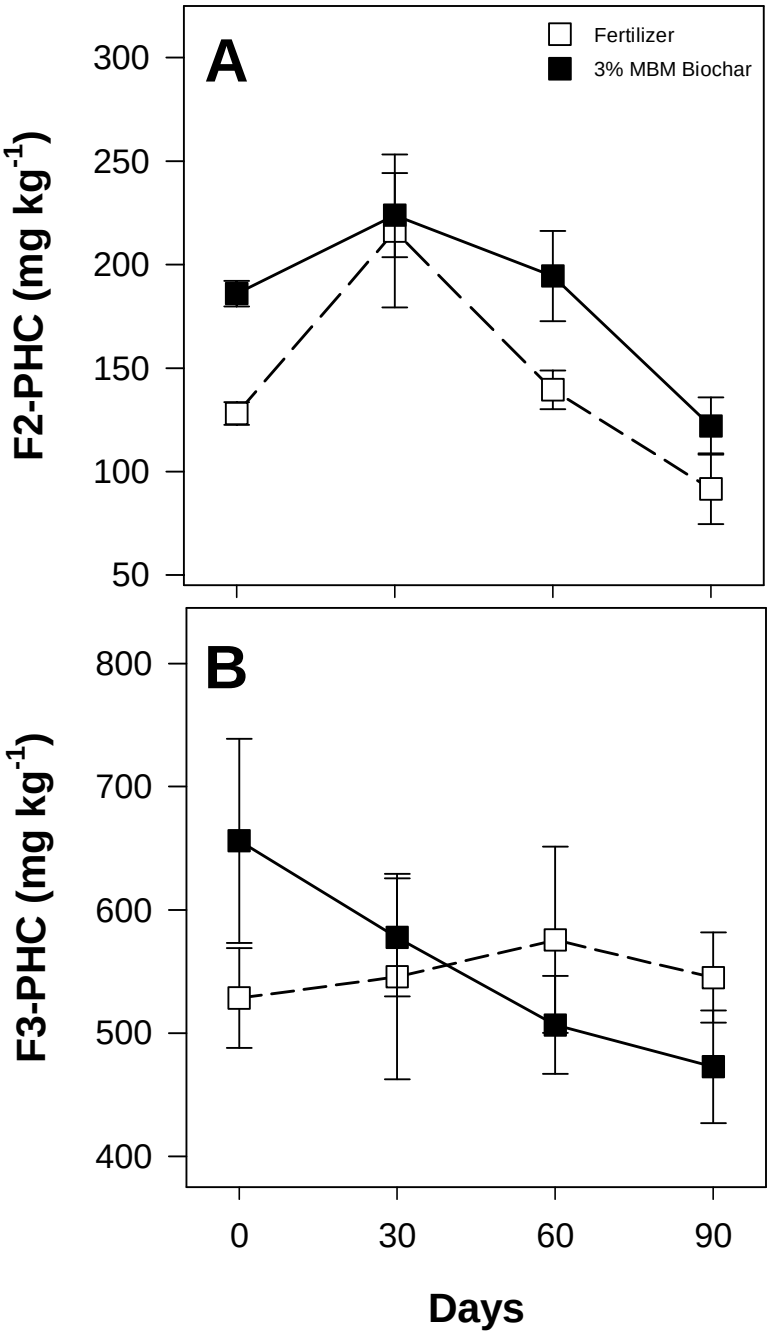

Fig. 3.1. Petroleum hydrocarbon (PHC) concentrations were measured in soil incubated under frozen conditions (-5 °C) over a 90 day period. Two treatments, fertilizer (□) and 3% (w/w) meat and bonemeal (MBM) biochar + fertilizer (■), were applied. Each point is the average concentration (n = 8) of (A) F2-PHC (C_{10}-C_{16}) and (B) F3-PHC (C_{16}-C_{34}), with standard error bars representing the standard error of the mean.

Table 3.3. First-order rate constants for F2 and F3-petroleum hydrocarbon (PHC) degradation in thawed (10 °C) and frozen (-5 °C) soils.

Incubation Temperature	Treatment	Rate Constant (d^{-1})†	
		F2	F3
10 °C	Fertilizer	0.0064 (± 0.0019)	-0.0002 (± 0.0014)
	10% Compost	0.0047 (± 0.0028)	0.0006 (± 0.0024)
	5% Compost	0.0042 (± 0.0018)	0.0006 (± 0.0013)
	6% MBM Biochar	0.0028 (± 0.0015)	-0.0029 (± 0.0009)
	3% MBM Biochar	0.0007 (± 0.0019)	-0.0004 (± 0.0020)
-5 °C	Fertilizer	0.0056 (± 0.0019)	-0.0007 (± 0.0015)
	3% MBM Biochar	0.0062 (± 0.0011)	0.0036 (± 0.0016)

†(mean ± standard error)

Soils incubated under thawed conditions and amended with fertilizer alone or 5% compost exhibited increased F2-PHC removal when compared to soils amended with 10% compost, 3% MBM biochar or 6% MBM biochar (Appendix A, Fig. A.1). For example, F2-PHC concentrations decreased by 32% from 151 ± 10 mg kg^{-1} to 103 ± 15 mg kg^{-1} and by 12% from 162 ± 13 mg kg^{-1} to 143 ± 13 mg kg^{-1}, in 5% compost and 6% MBM biochar, respectively. Relative to soil amended with fertilizer alone, F3-PHC removal was greater in soils amended with 5% compost but lower in soils amended with 10% compost, 3% MBM biochar, or 6% MBM biochar (Appendix A, Fig. A.1). In thawed soils, there were no treatment differences for either F2 or F3-PHC *k* values, which ranged from 0.0007-0.0064 d^{-1} and -0.0029-0.0006 d^{-1}, respectively (Table 3.2, ANOVA, $P < 0.10$). Although treatment differences were insignificant, these trends suggest that the fertilizer only treatment was more successful in decreasing F2-PHC while 5% compost was more effective in decreasing both F2 and F3-PHCs.

From both the F2 and F3-PHC concentrations in the soil, and the calculated *k* values, it was established that there were no significant differences between treatments in thawed soils, which is likely due to the extensive variability within treatments (i.e., relative standard deviations of up to 52% and 28% in F2 and F3-PHCs). Variability at northern sites has been observed in phytotoxic responses to boric acid (Anaka et al., 2008), which is enhanced under low θ_{liquid} conditions (Schafer et al., 2009), and significant variation in relative standard deviations (up to 28%) of PHC concentrations has also been reported (Paudyn et al., 2008). Although treatment differences were expected, especially under thawed conditions, trends in the data still provide

insight into the suitability of compost and MBM biochar as soil amendments in northern environments but render any definitive conclusions difficult.

Under frozen, but not thawed, conditions, 3% MBM biochar successfully increased F3-PHC *k* values when compared to the fertilizer control but had little effect on F2-PHC. Due to extensive variability (i.e., relative standard deviations of up to 35% and 41% in F2 and F3-PHCs, respectively) and negative *k* values, which reflect an increase in PHCs over time, the differences specifically observed in frozen F3-PHCs may not be a reliable indication of significant remediation. In soils with adequate nutrients, temperature, and θ_{liquid}, there may be limited response from biochar applications because there are no environmental factors limiting soil physical, chemical, or biological processes (Lehmann, 2007). Although the frozen and thawed treatments had similar soil properties and fertilizer additions, frozen soils are limited by temperature, θ_{liquid}, nutrient availability, and gas exchange. These limiting conditions could contribute to the enhanced response of MBM biochar as its application altered the physical environment (i.e., θ_{liquid}), which in turn, alleviated some of these limitations. Petroleum hydrocarbons are separated into fractions based on equivalent straight-chain hydrocarbon boiling point ranges, so lighter F2-PHC (C_{10}-C_{16}) are generally catabolized by PHC-degrading microorganisms or volatilized before heavier F3-PHC (C_{16}-C_{34}). The observed reductions in F2 and F3-PHCs could be a result of varying degrees of abiotic processes such as volatilization and sorption-desorption, as well as, microbial catabolism. Thus, it was crucial to analyze isoprenoid ratios and estimate abiotic losses to identify the dominant mechanism driving PHC degradation in these frozen soils to facilitate the evaluation of PHC degradation.

3.5.1.1 Degradation biomarkers

Under frozen conditions, microbial degradation drove F2 and F3-PHC reductions in both the fertilizer control and MBM biochar-amended soils. The combination of both treatments yielded significantly lower C_{17}/Pr and C_{18}/Ph ratios from Day 0 (0.618 ± 0.080 and 0.719 ± 0.020, respectively) to Day 90 (0.558 ± 0.060 and 0.000 ± 0.000, respectively) (Fig. 3.2, ANOVA, $P < 0.10$). However, after Day 60, C_{18}/Ph was not detectable in chromatograms from either treatment. Generally, the chemical structure determines how PHCs will be preferentially degraded; *n*-alkanes > *iso*-alkanes > aromatics (Atlas, 1981). Under aerobic conditions, microbial catabolism of *n*-alkanes follows this general pattern and it is reflected by a decrease in

C_{17}/Pr and C_{18}/Ph ratios over time (Atlas, 1995). The *n*-alkane and corresponding *iso*-alkane volatilize at a similar rate; so, it is likely that a change in the isoprenoid ratios reflects biodegradation. Therefore, these results suggest that at -5 °C, aerobic microbial degradation is active and volatilization makes negligible contributions to PHC reductions in these soils.

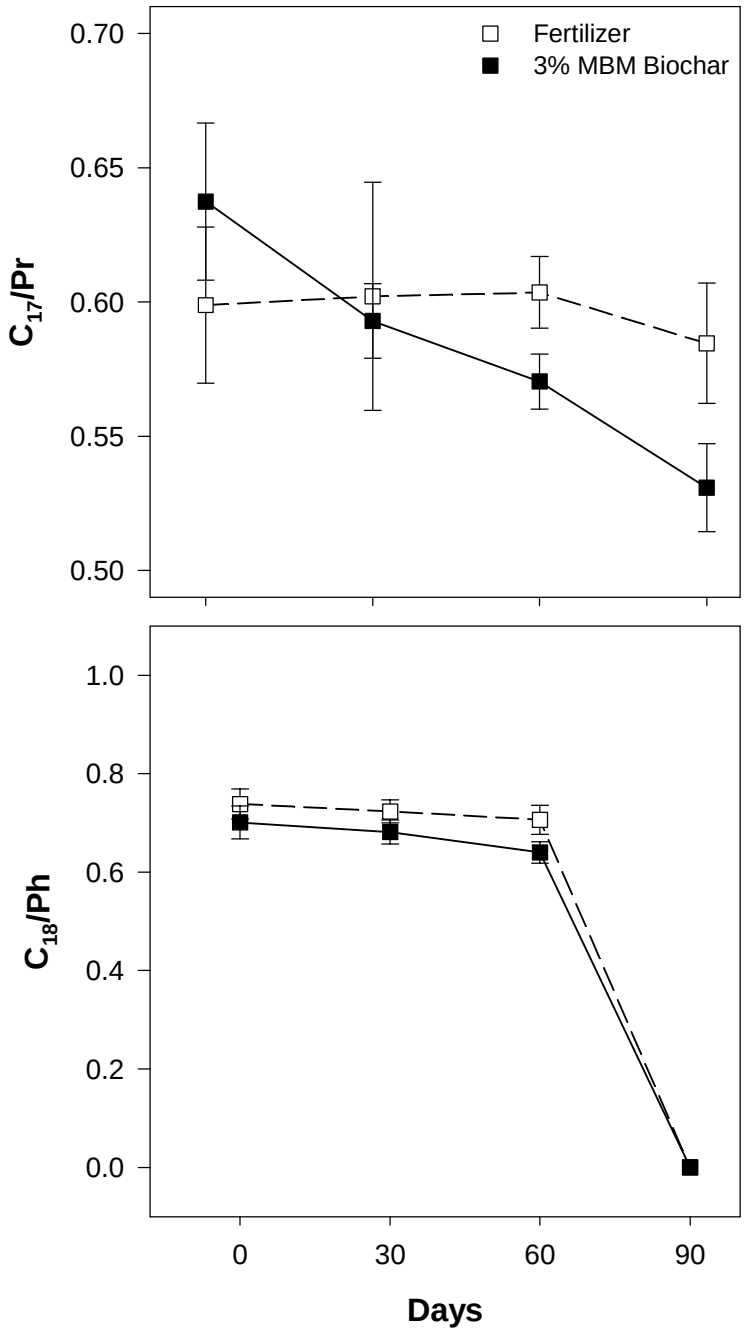

Fig. 3.2. Isoprenoid ratios C_{17}/Pr and C_{18}/Ph were measured in frozen treatments over 90 days. Each point is the average concentration (n = 8) of C_{17}/Pr and C_{18}/Ph, with standard error bars representing the standard error of the mean. In both fertilizer (□) and 3% MBM biochar-amended soils (■), the complete disappearance of C_{18} was evident by Day 90.

3.5.1.2 Abiotic loss estimation

Abiotic losses of F2 and F3-PHCs were dependent on amendment application rate, rather than time. Petroleum hydrocarbon concentrations in sterile soils were not significantly different at Day 0 and Day 90 in the control or amended soils, which indicate that the majority of abiotic losses occurred within 24 hours (ANOVA, $P < 0.05$). Significant differences were not evident when the control soil was compared to 3% MBM biochar (F2 and F3-PHC) and 6% MBM biochar (F2-PHC) (Fig. 3.3, ANOVA, $P < 0.05$). Conversely, 5% and 10% compost (F2 and F3-PHC) and 6% MBM biochar (F3-PHC) had significantly less PHC than the control soil (ANOVA, $P < 0.05$), which could be attributable to rapid adsorption of PHC to these amendments. Assuming there is no microbial degradation in sterilized soil and minimal volatilization in aged PHC contaminated soil in sealed vials, any differences in PHC concentrations when comparing the control soil to the amended soils might be attributed to sorption to the compost and biochar amendments. Pure MBM biochar and compost samples were also extracted to determine any co-extraction of organics which would overestimate PHC concentrations, but once extracted, these samples contained < 10 mg kg^{-1} F2 and F3-PHCs. The sorption affinity of pyrogenic organic matter, or biochar, is usually non-linear as the sorption of a specific compound to biochar decreases with increasing concentration, which indicates adsorption to external or internal surfaces (Yang and Sheng, 2003). Abiotic losses are much lower in field-contaminated soils and decrease with increasing concentration (Riser-Roberts, 1998), therefore, it is possible that the large difference between F2 and F3-PHC concentrations in the Greenland and Iqaluit soils would provide an underestimation of the degree of abiotic losses occurring in the Iqaluit soils. In summary, it is expected that sorption and other abiotic losses could affect initial PHC concentrations, specifically in compost-amended soils, with little or no subsequent effect over a 90 day period.

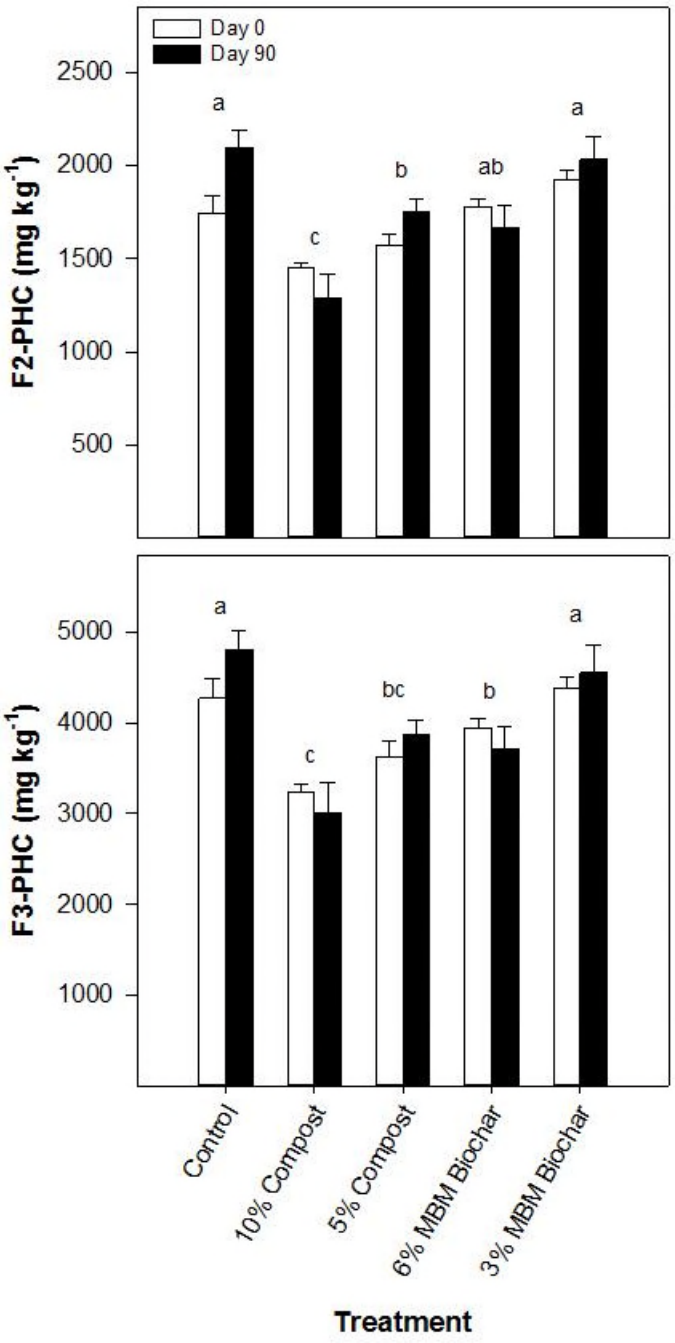

Fig. 3.3. The effects of meat and bonemeal (MBM) biochar and compost on abiotic loss of F2 and F3-petroleum hydrocarbons (PHCs). Field-contaminated soil was sterilized and amended with 10% and 5% (w/w) compost and 6% and 3% (w/w) MBM biochar; the soil contained approximately 6,500 mg kg^{-1} of total petroleum hydrocarbons (TPH) at Day 0. Each treatment was extracted for F2 and F3-PHCs to determine the extent of abiotic losses by each amendment. Each bar represents the average concentration (n = 8) for each treatment, with standard error bars representing the standard error of the mean. Within each treatment, Day 0 and 90 were not significantly different, thus, letters indicate significant differences between treatments (ANOVA, *P* < 0.05).

3.5.2 Liquid water content

Liquid water (θ_{liquid}) is present in soils frozen at -5 °C, ranging from 0.019-0.075 cm^3 cm^{-3}, and under frozen conditions, MBM biochar significantly increased θ_{liquid} compared to the fertilizer control (ANOVA, $P < 0.10$). Frozen soils amended with 3% MBM biochar had an average θ_{liquid} of 0.066 ± 0.005 cm^3 cm^{-3}, which is 40% higher than the θ_{liquid} of soils amended with fertilizer only (0.039 ± 0.011 cm^3 cm^{-3}). The results from this study are in accordance with a study that demonstrated switchgrass biochar pyrolyzed at 250 °C and 500 °C increased water storage relative to the control. It should be noted, however, these results were attained under thawed conditions using a plant-based biochar (Novak and Watts, 2013). Based on θ_{liquid} and pore size data of the MBM biochar used in this experiment, an increase in θ_{liquid} can be related to an increase in average pore volume and a decrease in average pore size (Harvey et al., 2012). Biochar application to soil decreases bulk density, and increases porosity and water holding capacity in sand and silt loam soils (Tian et al., 2015). The formation of ice is dependent on pore size; as pore size decreases, the freezing point of pore water decreases, which could allow more θ_{liquid} to exist in frozen soil amended with biochar. To my knowledge, this is the only θ_{liquid} measurement taken for MBM biochar-amended soils under frozen conditions. In this study, θ_{liquid} measurements were not taken in the same soils in which PHC degradation, NSR, or functional gene abundance were measured. Indeed, this soil had a similar texture as the Iqaluit soils, but any interpretation of the results is based on extrapolation to the Iqaluit soils, not direct θ_{liquid} measurements.

3.5.3 Nutrient supply rates

Under thawed conditions, the fertilized control soil had greater PO_4^{3-} and NH_4^+ availability when compared to soils with high and low application rates of MBM biochar and compost, while there was no difference between control and amended treatments under frozen conditions (Fig. 3.4). Several significant trends were detected under thawed conditions; i.e., compost had higher NH_4^+-N and NO_3^--N availability as compared to MBM biochar (Figs. 3.4B and C), whereas PO_4^{3-}-P availability was greater in MBM biochar treatments (Fig. 3.4A). Overall, lower NSRs occurred in the frozen treatments, although sufficient nutrients were still available to support microbial activity. This is supported by other research which demonstrates that bioremediation is a viable remediation strategy, even under frozen conditions (Harvey et al., 2012).

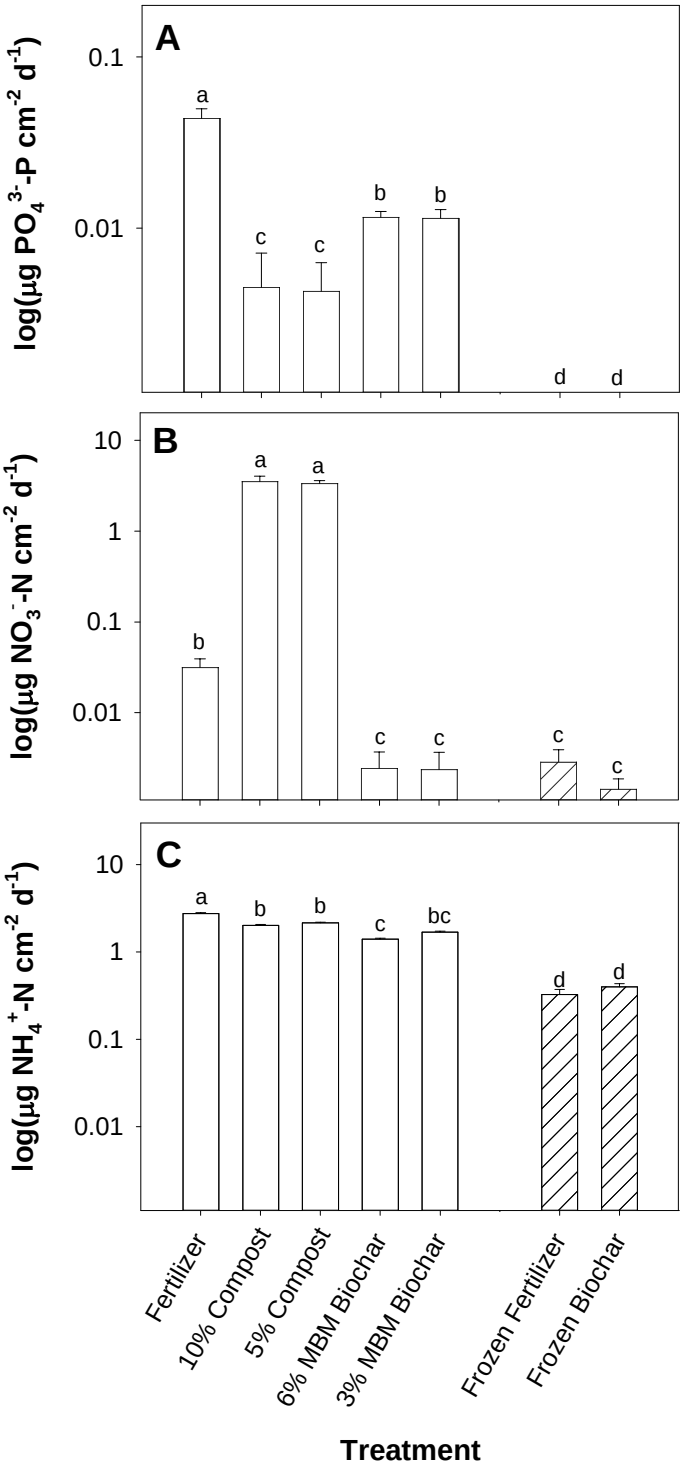

Fig. 3.4. Soil collected at 90 days was incubated for 3 weeks with anion or cation exchange resins and nutrient supply rates were measured for: (A) phosphate (PO_4^{3-}-P), (B) nitrate (NO_3^--N), and (C) ammonium (NH_4^+-N). Each bar represents the average concentration (n = 8) for each treatment, with standard error bars representing the standard error of the mean. Different letters represent significantly different nutrient supply rates (ANOVA, $P < 0.05$).

3.5.3.1 Phosphorus

Biochars derived from bone are known to have a liming effect on the soil, and P dissolution has been found to decrease with increasing pH (Warren et al., 2009; Parvage et al., 2013). Phosphate fixation occurs in alkaline soils (pH 6-8) as phosphate reacts with both the ionic and carbonate forms of Ca (i.e. the most predominant element in MBM biochar) to produce an insoluble mineral (Betts et al., 2013). Bonemeal biochar application can enhance P availability at low application rates (i.e., 0.5-1% [w/w]), but can also inhibit P availability at high application rates (i.e., 1-4% [w/w]) (Parvage et al., 2013). Both MBM biochar application rates (3% w/w and 6% w/w) used in this study would be considered 'high' so precipitation by calcium ions is likely limiting PO_4^{3-} availability under thawed conditions. Another method by which biochar can reduce P availability is through microbial stimulation and subsequent immobilization of available phosphate, but the mechanisms by which biochar stimulates microbial activity remain unclear (Powell et al., 2006a). Total phosphate and phosphate speciation influences the rate of PHC degradation (Siciliano et al., 2016), but under frozen conditions, PO_4^{3-}-P was below detection in both the control and MBM biochar-amended treatments (Fig. 3.4A), making it difficult to establish the role of phosphate in PHC degradation in frozen soil.

3.5.3.2 Nitrogen

Direct adsorption of positively charged cations to biochar occurs due to its high CEC and overall negative charge. Although dependent on nutrient and biochar type, adsorption isotherms demonstrate that NO_3^- and PO_4^{3-} are not readily adsorbed to biochar while NH_4^+ displays intermediate adsorption behavior (Yao et al., 2012). Under thawed conditions, MBM biochar-amended soils had lower NH_4^+-N and NO_3^--N availability (Fig. 3.4), which is likely due to direct sorption of NH_4^+ to the biochar and, in turn, a reduction in nitrification and subsequent NO_3^- availability (Ulyett et al., 2014). Under frozen conditions, there was no difference in NH_4^+-N and NO_3^--N availability between control and MBM biochar-amended treatments; this indicates that adsorption to biochar could be weaker in frozen soils, resulting in more available nitrogen.

3.5.4 Soil microbial community

3.5.4.1 PHC-degrading population

Soil collected at Day 90 was enumerated and total PHC-degrading populations were higher in 3% MBM biochar-amended soils compared to the fertilizer control. However, the difference was only significant in frozen soils which suggest a temperature-dependent microbial response (Table 3.4, ANOVA, $P < 0.05$). Under frozen conditions, MPN counts in the fertilizer control were significantly lower than 3% MBM biochar, at 4.19 ± 0.19 $_{log10}$MPN, and 4.63 ± 0.19 $_{log10}$MPN, respectively (t-test, $P < 0.05$). In thawed soils, there were no significant differences in MPN between treatments collected at 90 days (ANOVA, $P < 0.05$).

Table 3.4. Culturable, aerobic, petroleum hydrocarbon degrading microbial populations were enumerated from soils amended with 3% (w/w) meat and bonemeal (MBM) biochar and the fertilizer control treatment under thawed (10 °C) and frozen (-5 °C) conditions.

Incubation Temperature	Treatment	$_{log10}$(MPN)	Standard Deviation
10 °C	Fertilizer	4.12	0.19
	10% Compost	4.59	0.21
	5% Compost	4.59	0.21
	6% MBM Biochar	4.22	0.20
	3% MBM Biochar	4.33	0.20
-5 °C	Fertilizer	4.19	0.19
	3% MBM Biochar	4.63	0.19

3.5.4.2 Functional gene abundance

Following a 90 day incubation at -5 ºC, there was a significant increase in gene copy numbers for the functional genes *C2,3O* and *nahAc*; conversely, there was no change in *alkB* gene copy numbers (Fig. 3.5, ANOVA, $P < 0.05$). The following temporal trends occurred in thawed treatments, i) *nahAc* gene copy numbers significantly decreased in all treatments, ii) *C2,3O* gene copy numbers decreased in all treatments excluding the fertilizer control, and iii) *alkB* gene copy numbers decreased in all treatments, with the exception of 5% compost (ANOVA, $P < 0.05$).

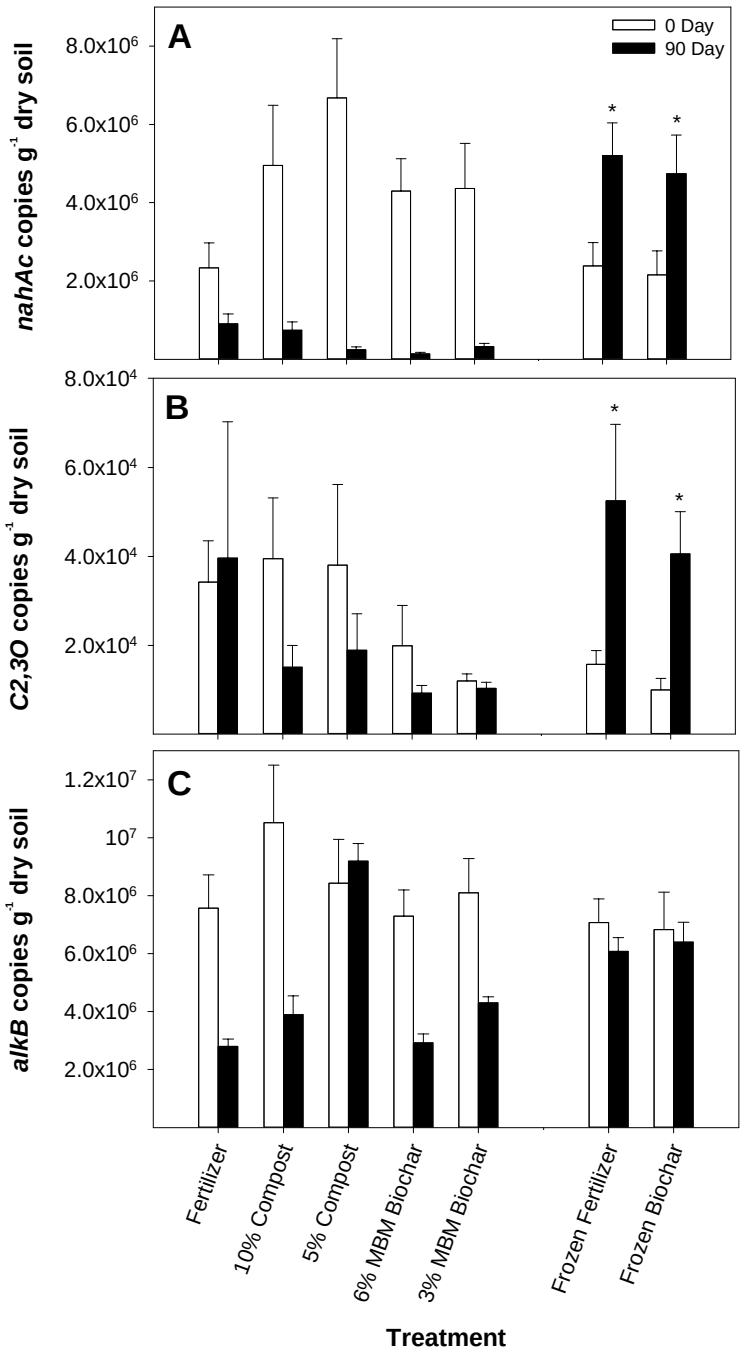

Fig. 3.5. Thawed and frozen treatments at 0 and 90 days were analyzed for the presence of the following catabolic genes: (A) naphthalene dioxygenase (*nahAc*), (B) catechol 2,3 dioxygenase (*C2,3O*) and (C) alkane monooxygenase (*alkB*), using quantitative polymerase chain reaction (qPCR). Each bar represents the average normalized concentration (n = 8) of gene copy numbers per gram of dry soil for each treatment and time period, with standard error bars representing the standard error of the mean. Asterisks (*) indicate that under frozen conditions, both fertilizer and 3% meat and bonemeal (MBM) biochar treatments significantly increased *C2,3O* and *nahAc* gene copy numbers after 90 days incubation (ANOVA, $P < 0.05$).

3.5.4.3 Effect of biochar on the soil microbial community

The degradation of short to medium *n*-alkanes are encoded by *alkB*, which uses hydroxylase to begin alkane metabolism, while *nahAc* and *C2,3O* both use enzymes to fissure aromatic PHC rings (Cerniglia, 1992; Van Beilen and Funhoff, 2007). Under thawed conditions, all three functional genes decreased in abundance over time in the majority of treatments, which suggests alkane and aromatic compounds had degraded, however, approximately 125 mg kg^{-1} of F2 and F3-PHCs remained in the soil after 90 days. Generally, an increase in gene copy number would precede aliphatic or aromatic hydrocarbon degradation, and a decrease would occur after those compounds had been degraded (Sei et al., 2003; Powell et al., 2006a). In contrast, increased copy numbers in aromatic functional genes (*nahAc* and *C2,3O*) were observed in the fertilizer and biochar-amended soils incubated at -5 °C, but only MBM biochar additions stimulated growth of total PHC-degrading populations. This combination of increased microbial activity unique to aromatic and total-PHC degraders appears linked to the reduction in F3-PHC concentrations under frozen conditions. Similar studies have found that biochar can stimulate polycyclic aromatic hydrocarbon (PAH)-metabolizing activity by increasing gene copy numbers associated with PAH degradation and by changing the structure of the microbial community (Liu et al., 2014), which supports the results obtained from frozen, but not thawed, incubations.

3.6 Conclusions

Although there were no clear treatment differences in *k* values in soils incubated at 10 °C for either F2 or F3-PHC, MBM biochar increased *k* values at -5 °C in F3, but not F2-PHC, when compared to the fertilizer control. Taking into consideration the large variation within treatment and negative *k* values calculated for F3-PHCs in both frozen and thawed soils, there is some evidence to suggest that MBM biochar additions will influence PHC degradation in northern landfarms but based on the limitations of this experiment, further studies are needed to confirm these results. Immediate abiotic losses were evident, especially with compost amendments, but did not appear to affect PHC concentrations after Day 0; therefore, reductions over time in F2 or F3-PHC in the experimental soils are not associated with abiotic losses, such as sorption by the amendments, but rather, microbial degradation. Isoprenoid ratios, compared only in frozen soils, also suggest that microbial catabolism is active.

Supporting increased F3-PHC degradation in frozen, biochar-amended soils were increases in total PHC-degrading microorganisms and θ_{liquid}. Measurements were taken in a reference soil, but due to the similar texture between soils, these results can be extrapolated. Under frozen conditions, functional genes encoding aromatic degradation pathways increased, but this occurred in both the fertilizer control and MBM biochar-amended soils. This may be attributable to differences in microbial community dynamics under different temperature regimes but is not clearly linked to PHC degradation in either thawed or frozen soils. There was no evidence that MBM biochar additions influenced nutrient availability under frozen conditions.

Whereas there is ample data concerning PHC remediation in Arctic soils, to my knowledge, there is little to no data available on biochar applications in PHC contaminated soils in cold regions. *Ex-situ* remediation is an established technique; however, protocols for reducing PHC contamination have yet to address the need for active remediation during the winter, which encompasses the majority of the year (8-10 months) in northern environments. Overall, the results of this laboratory study provide the basis for more in-depth investigation into the feasibility of large-scale biochar production, application, and resulting long-term effects on soil chemical, physical, and biological properties in northern environments.

4. APPLICATION METHOD AND BIOCHAR TYPE AFFECT PETROLEUM HYDROCARBON DEGRADATION IN NORTHERN LANDFARMS[2]

4.1 Preface

In frozen soils, meat and bonemeal biochar increased F3-PHC degradation rates but based on the limitations of a single laboratory study, further investigation was recommended. This prompted evaluation of how different biochar feedstocks and application methods can influence the suitability and efficacy of biochar applications to PHC contaminated soils in the laboratory and field. This study was carried out by evaluating PHC degradation in i) soils amended with different biochars in a laboratory study incubated under frozen conditions, ii) soils amended with different biochars and incubated for one year in the field, and iii) soils amended with different biochars using slurry and injection application methods and incubated for one year under field conditions. The overall objective of this study was to determine if biochar type and application method influence PHC degradation rates under field conditions.

[2] This chapter was published, with minor formatting differences, as Karppinen, E.M., S.D. Siciliano, and K.J. Stewart. 2017. Application method and biochar type affect petroleum hydrocarbon degradation in northern landfarms. J. Environ. Qual. 46:751–759. Dr. Stewart organized the Iqaluit and Whitehorse field trials, supervised the laboratory study, contributed funding for the purchase of supplies and critical equipment, and provided editorial input. Dr. Siciliano contributed funding for the purchase of supplies and critical equipment, assisted with technical content and interpretation, and provided editorial input. I assisted with experimental setup and design, conducted laboratory analyses, completed the initial manuscript draft, prepared the figures and tables, and completed data analysis and interpretation.

4.2 Abstract

Integrating biochar as a practical and successful remedial amendment at northern landfarms requires that its formulation and application be optimized for site-specific environmental conditions. Different biochar amendments were applied to petroleum hydrocarbon (PHC) contaminated soil at two northern field sites (Iqaluit and Whitehorse) and in a laboratory study at -5 °C, to determine the effects of application method (injection or incorporation) and biochar type (wood, fishmeal, bonemeal and/or compost) on PHC degradation and associated soil properties. Incorporation decreased F2 (equivalent nC$_{10}$-C$_{16}$) and F3 (equivalent nC$_{16}$-C$_{34}$) PHC concentrations in soil after 31 days, while injection did not decrease PHC concentrations until Day 334. Under controlled laboratory conditions, bonemeal-derived biochar selectively stimulated F3-PHC degradation in frozen soil over 90 days. In the field, there was little difference in PHC degradation between biochar types and the fertilizer control. Incorporation also increased NO_3^--N availability, and in field trials, all biochars increased NO_3^--N availability relative to the fertilized control, whereas the effects of biochars on NH_4^+-N and PO_4^{3-}-P were variable. Aromatic functional gene abundance was enhanced when treatments were incorporated, compared to when injected. In field trials, 6% Zakus wood plus fertilizer inhibited aliphatic and aromatic gene abundance. Liquid water content increased in incorporated treatments, specifically those amended with fishmeal biochar. Incorporation was the most successful application method for these northern soils, and while biochar amendments are not clearly effective in reducing PHC concentrations, there is evidence to suggest certain biochars can beneficially influence soil properties and PHC degradation under specific environmental conditions.

4.3 Introduction

Petroleum hydrocarbon (PHC) contaminated sites are found ubiquitously throughout northern Canada and landfarming, the process of excavating and spreading contaminated soils in layers or windrows, is often used as an *ex-situ* remediation strategy (Paudyn et al., 2008; Chang et al., 2010). In many cases, PHC-affected soil must be excavated because it is in close proximity to residential dwellings, a spill has occurred in a remote location, or environmental conditions (i.e., sub-zero temperatures, low moisture content, limited nutrient availability) do not readily support PHC degradation. Regardless of the situation, large amounts of PHC contaminated soil are received at landfarm facilities and remediated using fertilizers and

intermittent soil turning. This process can be lengthy due to a short thawed season (2-4 months/year) and extreme environmental conditions (Mohn and Stewart, 2000). Logistical challenges in northern Canada, including the cost and time involved with shipping large volumes of soil amendments, also contribute to delays in remediation.

In an effort to improve soil fertility and function, amendments such as compost, fertilizer, and more recently, biochar, have been evaluated for use in agronomic and environmental applications. The highly porous structure and large surface area of biochar can alter nutrient retention, cation exchange capacity (CEC), aeration, and hydrology within the soil pores (Atkinson et al., 2010). Biochar studies have yielded variable results but some positive outcomes include increased nutrient retention and water holding capacity (Karer et al., 2013), improvements in the sorption of organic contaminants, herbicides, and metals (Nartey and Zhao, 2014; Zhang et al., 2016; Szmigielski et al., 2017), and enhanced microbial activity (Zhang et al., 2014b). Based on these potential changes in soil properties, biochar could stimulate PHC degradation while utilizing waste products from local industries, however, its success and practicality under field conditions has not been well-studied, especially in northern environments (Hale et al., 2015). To my knowledge, there are a limited number of field studies involving biochar or activated carbon applications for PHC remediation (Cho et al., 2012; Hale et al., 2012; Meynet et al., 2012).

It is well established that the feedstock source and pyrolysis temperature have a large effect on the suitability of biochar as an agricultural or remedial amendment (Singh et al., 2010). Common feedstocks range from plant/woody material to animal rendering wastes, and pyrolysis temperatures of ≤ 500 °C produce biochars with ideal hydraulic properties and enhanced nutrient retention in sandy soils (Uzoma et al., 2011; Kinney et al., 2012), resulting from higher surface area and ash content (Singh et al., 2010). Although biochar itself can contribute some available nutrients, it is most effective when supplemented with fertilizer (Glaser et al., 2002; Lehmann et al., 2003; Cantrell et al., 2012). The accessibility of a local feedstock that is available in sufficient quantities is also an important factor to consider, especially for large-scale landfarm application in remote northern environments.

Biochar contains a significant powder fraction which can become airborne in light wind during transport or application. For example, during the establishment of a fine-grained biochar

field trial, approximately 30% of biochar was lost during loading, transport, and spreading (Husk and Major, 2011). Adding moisture to biochar can greatly reduce wind losses and Table B.1, in Appendix B, summarizes biochar applications as slurries that are injected, incorporated, or top-dressed. Top-dressed biochar, which is applied to the surface of the soil, can be eroded from steep slopes or even level areas after intense precipitation; therefore, the best way to mitigate water erosion is to apply biochar through injection, where biochar is applied below the soil surface at specific spacing and depth, or incorporation, where biochar is homogenized into the soil (Rumpel et al., 2006; Major et al., 2010). While incorporation is strictly an *ex-situ* remediation strategy, injection could be applied *in-situ* or possibly provide a less intensive *ex-situ* strategy that could reduce or eliminate the need for periodic soil turning.

A laboratory study and two years of field trials at two different northern landfarm facilities were used to evaluate the effects of application method and biochar type on indicators of PHC remediation. The objectives of this study were to determine if: i) application method of biochar and/or fertilizers will influence PHC degradation, ii) certain biochar feedstocks plus fertilizer increases PHC degradation significantly more than conventional amendments (fertilizer only), iii) the soil microbial community is affected by application methodology and biochar additions and, iv) there is any link between PHC degradation and physical and chemical soil properties.

4.4 Materials and Methods

4.4.1 Soil and biochar characterization

Landfarm field trials occurred simultaneously at two sites in northern Canada; one at a land treatment facility in Whitehorse, Yukon (60°42’34”N,135°04’07”W), and another at Nunatta Environmental Services in Iqaluit, Nunavut (63°45’00”N,68°33’00”W). These sites differ in terms of climate and geology, so the soils were characterized for various physical and chemical parameters (Appendix B, Table B.2). To aid visualization, a schematic summarizing site locations, average climatic conditions and field trials is available in Appendix B (Fig. B.1). Fishmeal biochar (Titan Clean Energy Projects, Craik; Fig. 4.1) and bonemeal and wood biochars (Zakus Farms, Whitehorse; Fig 4.1) were characterized for pyrolysis temperature, Brunauer-Emmet-Teller (BET) surface area, average pore volume, average pore size, pH, cation exchange capacity (CEC), and ash content (Appendix B, Table B.3). Two types of compost used in field trials were also characterized for pH and CEC (Appendix B, Table B.3).

Fig. 4.1. Fishmeal, bonemeal, and wood biochars used in the Biochar Type Trial and Biochar Application Trial.

4.4.2 Experimental setup

4.4.2.1 Field trials

For the field trials, F2 (equivalent *n*C_{10}-C_{16}) and F3 (equivalent *n*C_{16}-C_{34}) -PHC contaminated soils (i.e., contaminated through accidental spills in local northern environments) were collected from landfarm storage cells and amended with biochar and compost treatments. White polypropylene bulk bags (92 cm in length by 92 cm in width by 77 cm in height), containing 0.5 m^3 (both locations in the Biochar Application Trial) and 1.0 m^3 (both locations in the Biochar Type Trial) of amended soil, were used to contain experimental treatments at each landfarm. The bulk bags were randomly placed in storage cells and exposed to natural weather conditions for one year.

Slurries were prepared using biochar (at 6% v/v) and/or fertilizer plus 100 L of water. Injected and incorporated amendments were homogenized in pails with a cordless drill equipped with a paint mixer attachment, and constantly agitated. Injected amendment slurries were divided evenly into four pre-excavated holes on a 30 cm grid (Roberts et al., 1992), while incorporated slurries were mixed homogenously throughout the soil (Fig. 4.2).

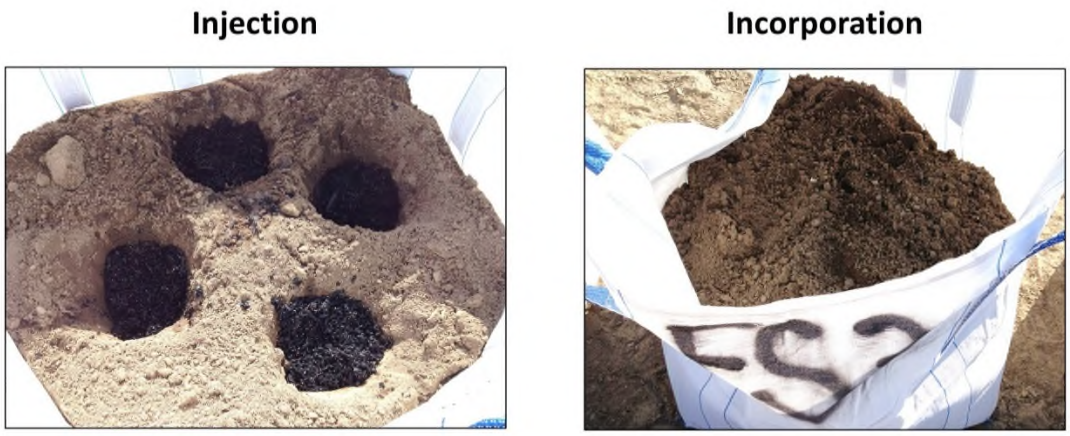

Fig. 4.2. Injected and incorporated amendment slurries applied to petroleum hydrocarbon (PHC) contaminated soil contained in mini bulk bags.

In all treatments, ammonium nitrate (34-0-0) and triple super phosphate (0-45-0) fertilizers were added to maintain a $C_{(TPH)}$:N:P ratio of 100:9:1 (Chang et al., 2010). In all studies, a fertilizer only treatment was used as a control as fertilization is currently the best management practice in northern landfarms, and many studies demonstrate that biochar alone cannot supply fertilizer requirements (Karer et al., 2013; Ahmed and Schoenau, 2015). All amendments (biochar and compost) were applied on a volume by volume (v/v) basis. Each bulk bag was subsampled monthly until soils were frozen and samples were stored at -20 °C in 120 mL amber glass jars until analysis. A soil auger was used to sample at a depth of 50 cm within each bulk bags.

4.4.2.2 Biochar application trial

In Iqaluit, PHC contaminated soil (400 mg kg^{-1} F2-PHCs and 100 mg kg^{-1} F3-PHCs) was loaded into the feed hopper of a rotary screening plant which removed stones and debris. Injected treatments did not require amendment homogenization so contaminated soil was moved by conveyer belt to fill each bulk bag with approximately 0.5 m^3 of material, after which, amendment slurries were added to four pre-excavated holes. Soil required for incorporated treatments was loaded into the feed hopper and amendment slurries were added. The feed hopper was allowed to turn with the gate closed until the material was evenly mixed, then allowed to move down the conveyer belt so that each bulk bag contained 0.5 m^3 of amended soil. In Whitehorse, residually contaminated soil (< 10 mg kg^{-1} F2 and F3-PHCs) from multiple locations at the landfarm were piled and homogenized using a skid steer equipped with a root

grapple bucket. For injected treatments, approximately 0.5 m^3 contaminated soil was separated into white polypropylene bulk bags using a small excavator, after which, amendment slurries were applied to four pre-excavated holes. Incorporated treatments were mixed in batches using a skid steer, and following homogenization, 0.5 m^3 was added to each bulk bag.

The following treatments were applied (Table 4.1) using both application methods (injected or incorporated), each with six replicates: (i) fertilizer (F), (ii) 6% Titan fishmeal biochar plus fertilizer (6TF), and (iii) 6% Zakus wood biochar plus fertilizer (6ZW). An additional treatment, 6% Zakus bonemeal biochar plus fertilizer (6ZB), was used in Whitehorse, but could not be applied in Iqaluit due to logistical restraints with biochar production.

Table 4.1. Treatments applied at Whitehorse and Iqaluit in the Biochar Application Trial.

Location	Treatment	Description
Whitehorse	F	Fertilizer
	6TF	6% Titan fishmeal biochar + fertilizer
	6ZW	6% Zakus wood biochar + fertilizer
	6ZB	6% Zakus bonemeal biochar + fertilizer
Iqaluit	F	Fertilizer
	6TF	6% Titan fishmeal biochar + fertilizer
	6ZW	6% Zakus wood biochar + fertilizer

4.4.2.3 Biochar type trial

In Iqaluit, soil contaminated with P-50 arctic grade diesel fuel (1500 mg kg^{-1} F2-PHCs and 200 mg kg^{-1} F3-PHCs) was collected from the Nunatta Environmental landfarm. Petroleum hydrocarbon contaminated soil plus amendments were loaded into the feed hopper of a rotary screening plant which removed stones and debris while homogenizing the soil and amendments. For each treatment, the amended soil was moved by conveyer belt to fill each bulk bag with approximately 1 m^3 of material. In Whitehorse, PHC contaminated soil (900 mg kg^{-1} F2-PHCs), was collected from storage cells in the landfarm. The soil plus amendments was separated into piles and homogenized using a skid steer equipped with a root grapple bucket. For each treatment, approximately 1 m^3 of amended soil was added to each bulk bag.

The following treatments (Table 4.2), each including five replicates, were used to amend PHC contaminated soil; (i) fertilizer (F), (ii) 3% Titan fishmeal biochar plus fertilizer (3TF), (iii) 6% Titan fishmeal biochar plus fertilizer (6TF), (iv) 6% Titan fishmeal biochar plus 5% compost plus fertilizer (6TFC), and (v) 6% Zakus wood biochar plus fertilizer (6ZW).

Table 4.2. Treatments applied at Whitehorse and Iqaluit in the Biochar Type Trial.

Location	Treatment	Description
Whitehorse	F	Fertilizer
	3TF	3% Titan fishmeal biochar + fertilizer
	6TF	6% Titan fishmeal biochar + fertilizer
	6TFC	6% Titan fishmeal biochar + 5% compost + fertilizer
	6ZW	6% Zakus wood biochar + fertilizer
Iqaluit	F	Fertilizer
	3TF	3% Titan fishmeal biochar + fertilizer
	6TF	6% Titan fishmeal biochar + fertilizer
	6TFC	6% Titan fishmeal biochar + 5% compost + fertilizer
	6ZW	6% Zakus wood biochar + fertilizer

4.4.2.4 Pilot study

Petroleum hydrocarbon contaminated soil (600 mg kg^{-1} F2-PHCs and 200 mg kg^{-1} F3-PHCs) from the Whitehorse landfarm was collected from below a 0.5 m layer of overburden material. The soil and biochar amendments were passed through a 4.75 mm sieve and homogenized in a stainless steel bowl. The following four treatments, each including five replicates, were used: (i) fertilizer (F), (ii) 6% Titan fishmeal biochar plus fertilizer (6TF), (iii) 6% Zakus bonemeal biochar plus fertilizer (6ZB), and (iv) 6% Zakus wood biochar plus fertilizer (6ZW) (Table 4.3). Following amendment and fertilizer additions, each soil treatment was divided into 60 mL glass amber vials containing approximately 45 g of soil and plugged with cotton balls. The vials were incubated at -5 °C and destructively sampled mid-vial at 0, 30, 60, and 90 days.

Table 4.3. Treatments applied in the Pilot Study.

Treatment	Description
F	Fertilizer
6TF	6% Titan fishmeal biochar plus fertilizer
6ZB	6% Zakus bonemeal biochar plus fertilizer
6ZW	6% Zakus wood biochar plus fertilizer

4.4.3 Petroleum hydrocarbon analysis

Mechanical shaking (Schwab et al., 1999) and hexane extraction (Mcintyre et al., 2007) methods were used to extract F2 and F3-PHCs from soil samples collected at Whitehorse and Iqaluit, respectively. The different extraction methods used reflect the laboratory capabilities at laboratories in Whitehorse, YT and Saskatoon, SK. Analytical and quality control procedures followed for each extraction method are described in Appendix B (Table B.4). Although both extraction methods yield similar F2 and F3-PHC extraction efficiencies, relative between-treatment differences are the primary focus for comparison. Petroleum hydrocarbons were quantified using a Varian CP3800 gas chromatograph fitted with a flame ionization detector and chromatograms were analyzed using CompassCDS software (Agilent Technologies, Santa Clara, CA). Decane ($C_{10}H_{22}$; C_{10}), hexadecane ($C_{16}H_{34}$; C_{16}) and tetratriacontane ($C_{34}H_{70}$; C_{34}) standards were used to create a curve to generate the area and concentration of F2 and F3-PHCs for each sample. Two linear alkanes, heptadecane ($C_{17}H_{36}$; C_{17}) and octadecane ($C_{18}H_{38}$; C_{18}), and two branched/cyclic alkanes, 2,6,10,14-tetramethylpentadecane ($C_{19}H_{40}$; pristine, Pr) and 2,6,10,14-tetramethylhexadecane ($C_{20}H_{42}$; phytane, Ph), were integrated from the chromatograms of soil samples in CompassCDS to determine the extent of biodegradation. The mass ratio for C_{17}/Pr and C_{18}/Ph was generated by dividing the area of the linear alkane by the area of the branched alkane. Pristine and phytane have similar boiling points to C_{17} and C_{18}, respectively, but the branched nature of the pristine and phytane compounds makes them resistant to degradation (Atlas, 1995).

4.4.4 Nutrient analysis

Bioavailable nitrate (NO_3^--N), ammonium (NH_4^+-N), and phosphate (PO_4^{3-}-P) were quantified from soil samples collected at the final sampling period using anion and cation exchange resins (Western Ag Solutions, Saskatoon, SK). Nutrient supply rates were measured using the “sandwich test”, a method by which an ion-exchange resin is sandwiched between soil

and exchanges counter ions for nutrient ions contained within the soil (Qian et al., 2007). NSR is calculated as

$$NSR = \frac{C\ x\ V}{S} / t$$

where C is the concentration of the adsorbed cation or anion (μg mL^{-1}) in HCl eluent, V is the volume of eluent (mL), S is the surface area of the membrane strip (cm^2), and t is the incubation period (d). Anion and cation exchange resins were regenerated by soaking in 0.5 M $NaHCO_3$, sandwiched between two Snapcap® 4 Dram vial lids packed with soil, and wrapped in Parafilm®. Following incubation at 4 °C for 2 weeks, resins were rinsed with deionized water and eluted in 0.5 M HCl. All nutrients were quantified colorimetrically using a Technicon™ AutoAnalyzer™ II (SEAL Analytical, Mequon, WI).

4.4.5 Functional gene analysis

Microbial DNA was extracted from approximately 0.25 g of soil from initial and final sampling periods using a Powerlyzer™ Powersoil® DNA Isolation Kit (MO BIO Laboratories) then quantified with a Qubit® Fluorometer (Thermo Fisher Scientific). Samples were analyzed for the presence of specific catabolic genes encoding enzymes involved in PHC degradation pathways: alkane monooxygenase (*alkB*), naphthalene dioxygenase (*nahAc*), and catechol 2,3 dioxygenase (*C2,3O*). Standards were prepared by extracting the DNA of American Type Culture Collection (ATCC) standard organisms, followed by polymerase chain reaction amplification with the corresponding primer set, and repeated gel purification. Further information regarding ATCC standard organisms and quantitative polymerase chain reaction (qPCR) and amplification conditions are presented in Appendix B (Table B.5).

4.4.6 Temperature and θ_{liquid} monitoring

Temperature was monitored during Whitehorse field trials using a thermocouple temperature sensor for soil and Type K thermocouples (Campbell Scientific). Volumetric water content was measured using time domain reflectometry, a technique that measures the dielectric constant of the medium. Probes were inserted 30 cm into each bulk bag and θ_{liquid} was measured with a Tektronix 1502B cable tester (Tektronix) and quantified using Topp's equation (Topp et al., 1980). Environmental data was not collected in Iqaluit or when air temperatures were below -20 °C in Whitehorse.

4.4.7 Statistical analysis

In the field trials (Biochar Application and Biochar Type trials) and Pilot Study, a one-way analysis of variance (ANOVA) was used to compare treatments analyzed for nutrient supply rates and θ_{liquid}. A two-way ANOVA was used for PHC concentration and functional gene abundance to compare treatments over time. Data was tested for assumptions of normality and homogeneity of variance using Shapiro-Wilk Test and Levene's Test and transformed if necessary. Multiple comparisons were made following a Tukey's post hoc analysis ($P < 0.05$). All data was processed in RStudio Version 3.3.2 (R Core Team, 2017) and visualized in SigmaPlot Version 12.

4.5 Results and Discussion

4.5.1 Petroleum hydrocarbon degradation

Across all biochar types and controls, incorporation caused a rapid (within 31 days) decrease in F2 and F3-PHC soil concentrations, whereas injection significantly decreased only at Day 334 (Figs. 4.3A-B, ANOVA, $P < 0.05$). For example, in incorporated treatments, average F2-PHCs reduced from 345 mg kg^{-1} (SE = 40) to 111 mg kg^{-1} (SE = 15), while injected treatments decreased from 406 mg kg^{-1} (SE = 35) to only 357 mg kg^{-1} (SE = 34), between Days 0 and 31. Based on changes in isoprenoid ratios, it was determined that any reduction in F2 and/or F3-PHCs resulted from microbial catabolism rather than volatilization (ANOVA, $P < 0.05$, Appendix B, Fig. B.2). Based on Canadian Council of Ministers of the Environment (CCME) F2-PHC clean-up criteria (150 mg kg^{-1}, fine-grained soil, agricultural land use, Appendix B, Table B.6), contaminated soil amended with incorporated treatments were below criteria before the winter season, while those amended with injected treatments required the winter and spring season to reduce concentrations below guidelines. Although initial F3-PHC concentrations, at approximately 50 mg kg^{-1}, were below CCME clean-up criteria (300 mg kg^{-1}, fine-grained soil, agricultural land use, Appendix B, Table B.6), incorporated treatments also decreased this low-level PHC contamination quicker than injected treatments.

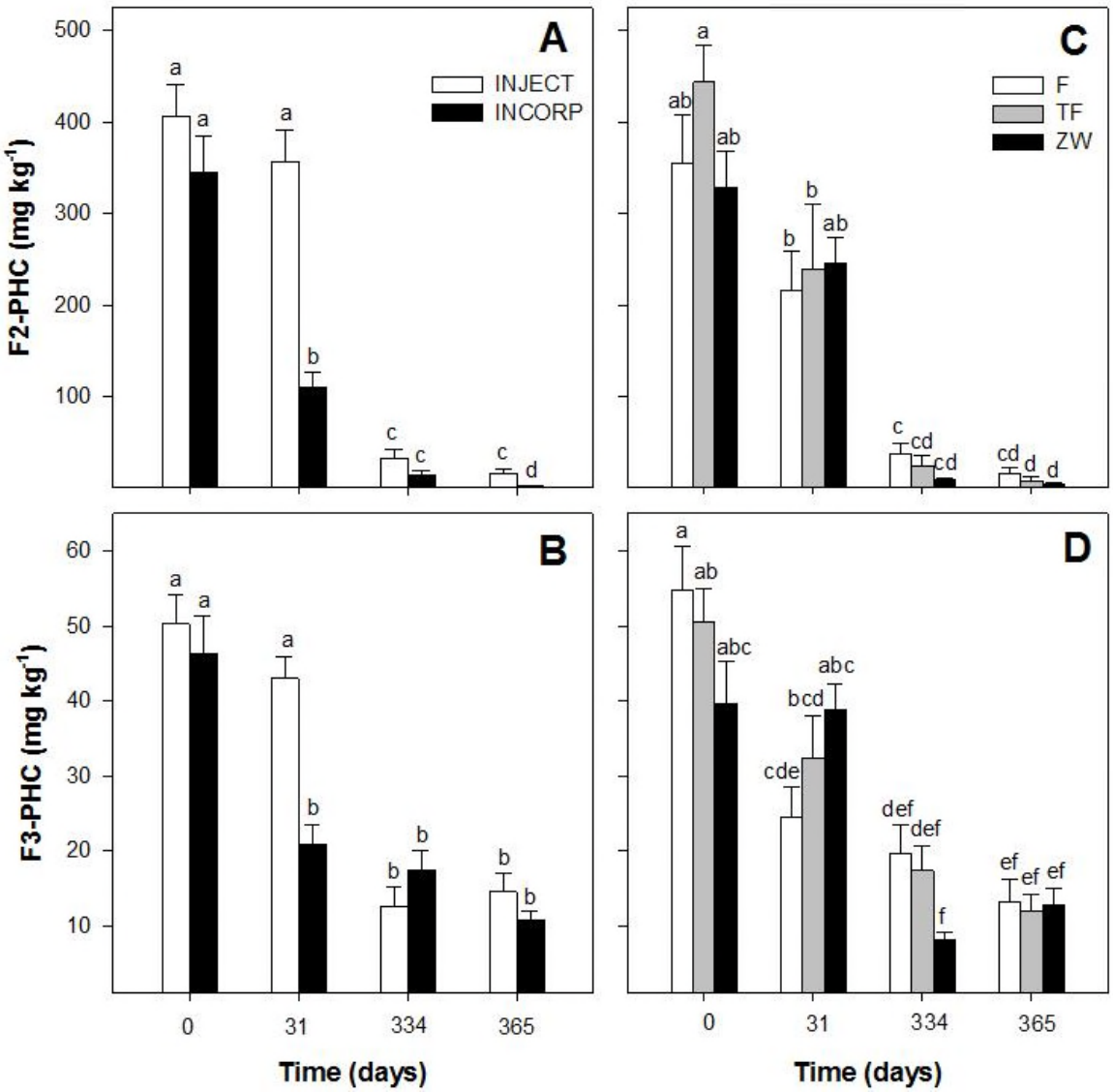

Fig. 4.3. In Iqaluit, F2 and F3-petroleum hydrocarbons (PHCs), in mg kg^{-1}, were measured in two different biochar application methods, injected (INJECT) or incorporated (INCORP), over 365 days (A and B). Concentrations of PHC were quantified by treatment, which included two biochars, 6% Titan fishmeal biochar plus fertilizer (TF) and 6% Zakus wood biochar plus fertilizer (ZW), and a fertilizer control (F) (C and D). Bars represent the mean (n = 6), with the error bars representing the standard error of the mean. Different letters indicate significantly different PHC concentrations (ANOVA, *P* < 0.05). The Whitehorse landfarm contained < 10 mg kg^{-1} of F2 and F3-PHCs during the field season, so PHC data were not available.

In the Biochar Application Trial, F2-PHC concentrations significantly decreased in soils amended with 6% Titan fishmeal biochar after 31 days, while 6% Zakus wood biochar and the fertilizer control decreased after 334 days (Fig 4.3C, ANOVA, $P < 0.05$). However, at 31 days, there was no difference in F2-PHC concentrations between treatments or the fertilizer control, with fertilizer soils containing 217 mg kg^{-1} (SE = 42), 6% Titan fishmeal plus fertilizer soils containing 240 mg kg^{-1} (SE = 70), and 6% Zakus wood plus fertilizer soils containing 246 mg kg^{-1} (SE = 28) (Table 4.4). Similarly, there was no difference in F3-PHC concentration at 31 days, but the fertilizer control significantly decreased after 31 days, while fishmeal and wood biochar-amended soils were reduced after 334 days (Fig. 4.3D, ANOVA, $P < 0.05$). Petroleum hydrocarbon degradation was evident over time in the Biochar Type field trials, but biochar type differences were only detected in F2-PHCs (Table 4.5, Appendix B, Fig. B.3, ANOVA, $P < 0.05$). In Whitehorse, F2-PHC concentrations were higher in soils amended with 6% Zakus wood biochar, when compared to the fertilizer control (Appendix B, Fig. B.3, ANOVA, $P < 0.05$). For example, average F2-PHC concentration over one year were 317 mg kg^{-1} (SE = 54) in the fertilizer control and 466 mg kg^{-1} (SE = 60) in wood biochar-amended soils. In Iqaluit, F2-PHC concentrations in biochar-amended soils were not different from the control soil.

Table 4.4. Analysis of variance (ANOVA) results for the Biochar Application Trial in which different biochars were injected or incorporated into bulk bags containing landfarm soil at Iqaluit, NU and Whitehorse, YT. Soil samples were analyzed for petroleum hydrocarbon (PHC) concentration, nutrient supply rates, functional gene abundance, and physical parameters.

Factor	PHC			Nutrient Supply Rate				Functional Gene				Physical Parameter		
	†df	F2	F3	df	NO_3^--N	NH_4^+-N	PO_4^{3-}-P	df	*alkB*	*C2,3O*	*nahAc*	df	θ_{liquid}	Temp
		----‡F-value----			----------§F-value----------				----------F-value----------				----‡F-value----	
Iqaluit														
¶AM	1	49.4	6.52	1	211	0.62	13.0	1	0.09	11.9	15.1		-	
#TR	2	1.42	0.81	2	49.7	4.05	12.9	2	0.98	3.45	1.33		-	
Time	3	293	70.3			-		1	11.9	1.09	0.00		-	
AM*TR	2	0.92	1.33	2	8.04	3.70	3.31	2	2.99	0.99	0.94		-	
AM*Time	3	7.33	8.44			-		1	0.10	5.53	0.00		-	
TR*Time	6	2.25	4.00			-		2	1.02	4.59	0.23		-	
AM*TR*Time	6	1.41	2.05			-		2	0.07	1.07	1.55		-	
Whitehorse														
AM		-		1	88.2	0.96	0.43	1	5.24	3.44	11.3	1	27.5	25.4
TR		-		3	5.75	0.06	7.72	3	5.33	0.13	2.01	3	30.5	1.95
Time		-				-		1	50.6	228	66.0	24	73.3	1070
AM*TR		-		3	36.1	1.51	5.56	3	0.05	2.96	1.04	3	29.3	0.79
AM*Time		-				-		1	4.26	10.3	16.5	24	0.71	5.64
TR*Time		-				-		3	1.13	0.18	0.41	72	0.68	1.38
AM*TR*Time		-				-		3	2.26	1.09	6.88	72	0.66	1.51

†df = degrees of freedom
‡F-values are not available for PHCs in Whitehorse or Physical Parameters in Iqaluit because these properties were not measured.
§F-values are not available for the factor Time, as nutrient supply rates were only measured at one time point.
¶AM = Application method
#TR = Treatment (fertilizer control and biochar types)
Underlined values indicate significance at $P \leq 0.05$.

Table 4.5. Analysis of variance (ANOVA) results for the Biochar Type Trial in which biochars were mixed into landfarm soil and incubated in 1 m^3 soil bags in Iqaluit, NU and Whitehorse, YT. Soil samples were analyzed for petroleum hydrocarbon (PHC) concentration, nutrient supply rates, functional gene abundance, and physical parameters.

Factor	PHC			Nutrient Supply Rate				Functional Gene				Physical Parameter		
	†df	F2	F3	df	NO_3^--N	NH_4^+-N	PO_4^{3-}-P	df	*alkB*	*C2,3O*	*nahAc*	df	θ_{liquid}	Temp
		----‡F-value----			----------§F-value----------				----------F-value----------				-----‡F-value----	
Iqaluit														
¶TR	4	2.83	0.44	4	1.86	2.14	1.96	4	6.06	4.39	5.50		-	
Time	4	210	121			-		1	23.5	0.04	0.04		-	
TR*Time	16	1.15	5.09			-		4	9.08	5.13	4.95		-	
Whitehorse														
TR†	4	8.77	-	4	7.05	1.01	0.28	4	1.05	3.29	8.83	4	6.88	29.2
Time	5	120	-			-		1	46.6	8.39	0.94	20	22.2	4630
TR*Time	20	1.65	-			-		4	2.17	2.83	0.38	80	0.56	1.97

†df = degrees of freedom

‡F-values are not available for F3-PHCs in Whitehorse or Physical Parameters in Iqaluit.

§F-values are not available for the factor Time, as nutrient supply rates were only measured at one time point.

¶TR = Treatment (fertilizer control and biochar types)

Underlined values indicate significance at $P \leq 0.05$.

When PHC contaminated soils were incubated at -5 °C in the laboratory Pilot Study, all biochar types had significantly less average F2-PHCs than the fertilizer control, but there were no differences between biochars (Appendix B, Fig. B.4, ANOVA, $P < 0.05$). While all biochar types had significantly less average F3-PHCs than the fertilizer control (Appendix B, Fig. B.4), only 6% Zakus bonemeal biochar plus fertilizer decreased F3-PHCs from 226 mg kg^{-1} (SE = 33) to 61 mg kg^{-1} (SE = 7) after 90 days (Fig. 4.4, ANOVA, $P < 0.05$). Supporting these results, a preliminary laboratory study found that F3, but not F2, -PHCs significantly decreased in soil amended with ca. 20% (v/v) bonemeal biochar, although a different bonemeal feedstock and application rate was used (Karppinen et al., 2017b). Biochar is most effective under limiting conditions (Lehmann, 2007), and it appears that certain biochars can selectively influence both F2 and F3-PHC degradation in frozen, laboratory incubated soils, but only F2-PHCs were affected in field trials, where results were variable between locations and experimental trials. Other studies have also demonstrated that positive short-term responses to biochar amendments (i.e., soil microbial activity) observed in the laboratory were not reflected in field studies as small, transient changes resulting from biochar additions are likely masked in inherently heterogeneous and environmentally variable field studies (Jones et al., 2012). Incorporation was the most successful application method for these northern soils, and while biochar amendments are not clearly effective in reducing PHC concentrations, there is evidence to suggest it can beneficially influence soil properties and microbial genes under specific environmental conditions.

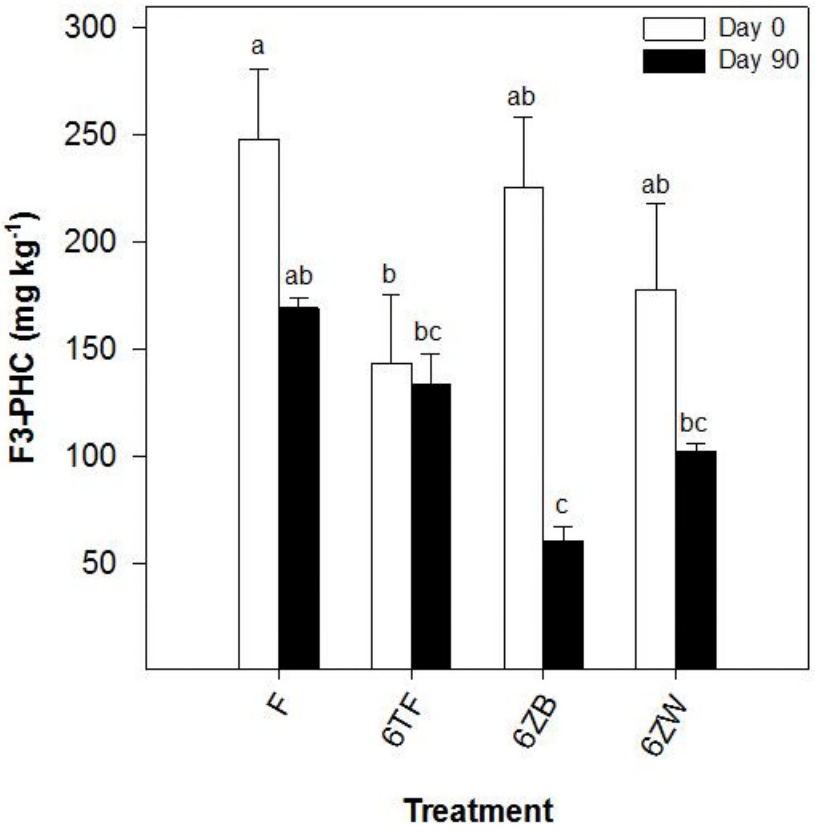

Fig. 4.4. In the laboratory study, F3-PHC concentrations, in mg kg^{-1}, were quantified in frozen soils on Days 0 and 90. Treatments included fertilizer (F), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Zakus bonemeal biochar plus fertilizer (6ZB), and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean (n = 5), with error bars representing the standard error of the mean. Different letters indicate significantly different F3-PHC concentrations (ANOVA, *P* < 0.05).

4.5.2 Nutrient availability

Incorporating biochar and fertilizers increased NO_3^--N availability in both locations, whereas injection of biochars and fertilizers was less consistent; only increasing NH_4^+-N availability in Whitehorse and PO_4^{3-}-P availability in Iqaluit (Fig. 4.5, ANOVA, $P < 0.05$). For example, NO_3^--N availability was 3.95 µg cm^{-2} d^{-1} (SE = 0.26) when incorporated, which was significantly higher than when injected (2.26 µg cm^{-2} d^{-1} [SE = 0.23]). During injection, soil disturbance is minimal and the process relies on the diffusion of amendments, resulting in conditions which do not stimulate the conversion of NH_4^+ to NO_3^- (i.e. nitrification). On the other hand, incorporation involves the mixing and homogenization of soil and amendments, which could aerate the soil while retaining sufficient soil moisture and facilitate conditions which increase NO_3^--N availability. Although NH_4^+-N and PO_4^{3-}-P availabilities were higher in injected treatments, there was no consistency between locations. This could result from local differences in soil properties between Iqaluit and Whitehorse, such as θ_{liquid} and the initial amount of PHC contamination (Appendix B, Table B.2), both of which can affect NH_4^+ (Harvey et al., 2012) and PO_4^{3-} (Mouat and Nes, 1985) availabilities.

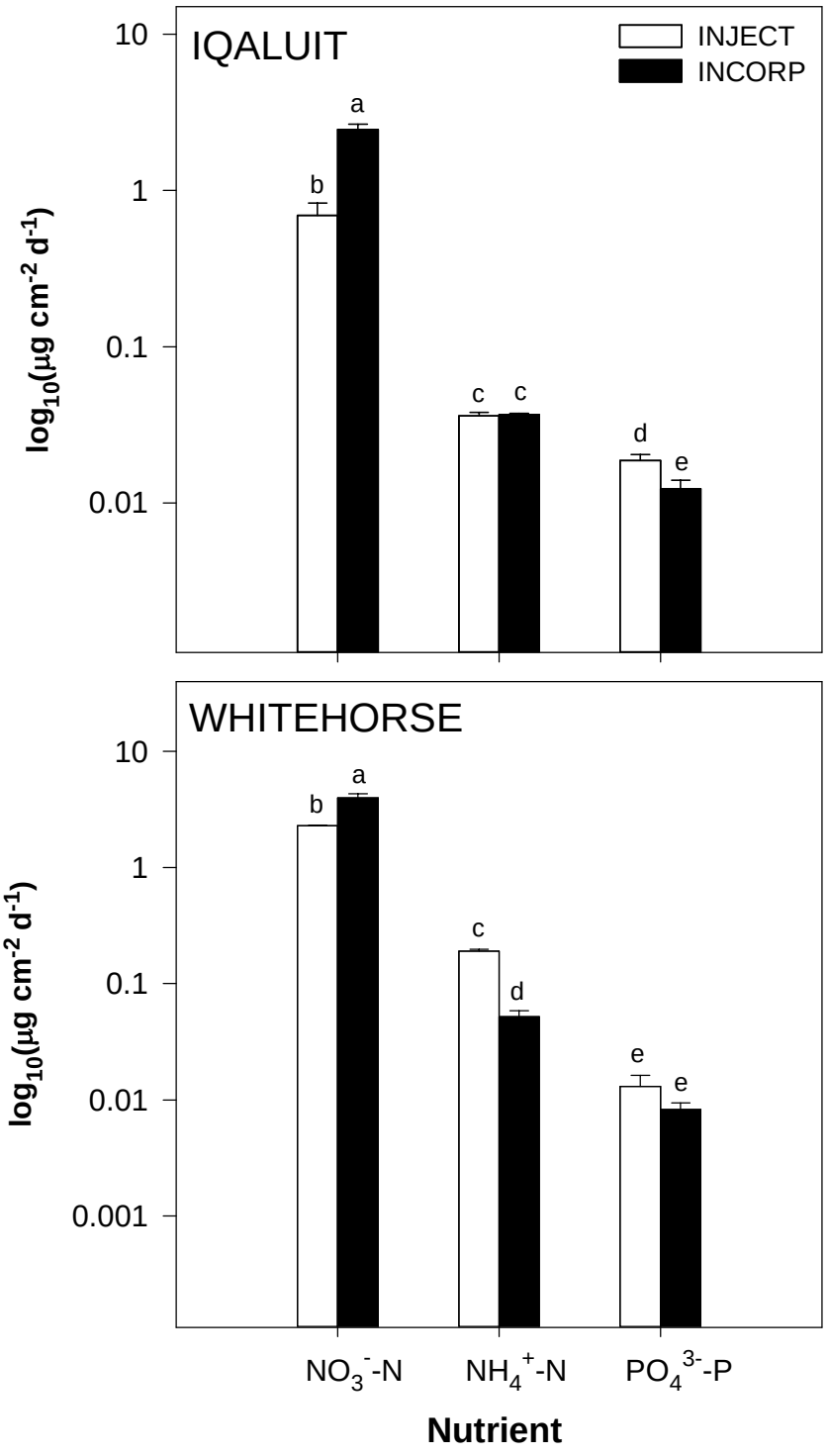

Fig. 4.5. Nitrate (NO^{3-}-N), ammonium (NH_4^+-N), and phosphate (PO_4^{3-}-P) availability, in log_{10}(µg cm^{-2} d^{-1}), was measured in injected (INJECT) and incorporated (INCORP) treatments in Iqaluit and Whitehorse. Samples were collected on Day 365. Bars represent the mean (n = 6), with the error bars representing the standard error of the mean. Different letters indicate significantly different nutrient availability between application methods (ANOVA, $P < 0.05$).

Overall, NO_3^--N availability was higher in biochar-amended soils than those amended with fertilizer only, but biochar type (i.e., wood, fishmeal, bonemeal) was not a significant factor (Figs. 4.6A and D, Appendix B, Fig. B.5, ANOVA, $P < 0.05$). In a few cases, all biochar feedstocks increased NO_3^--N availability, with the exception of 6% Titan fishmeal plus fertilizer. For example, in the Biochar Type Trial in Whitehorse, the fertilizer control supplied 1.43 µg NO_3^--N cm^{-2} d^{-1} (SE = 0.40) and 6% Titan fishmeal biochar plus fertilizer supplied 2.35 µg NO_3^--N cm^{-2} d^{-1} (SE = 0.56), which were significantly lower than 6% Zakus wood biochar plus fertilizer, which supplied 3.79 µg NO_3^--N cm^{-2} d^{-1} (SE = 0.16) (Fig. 4.6D). Studies show that biochar amendments have a stronger positive effect in soils where little to no net nitrification occurs, compared to soils which already exhibit high rates of net nitrification, where the effects of biochar are minimal or absent (Ducey et al., 2013). In these studies, coarse-textured, northern soils could be affected by biochar additions, due to inherently low net nitrification which increases in response to changes in the soil microbial community or θ_{liquid} dynamics.

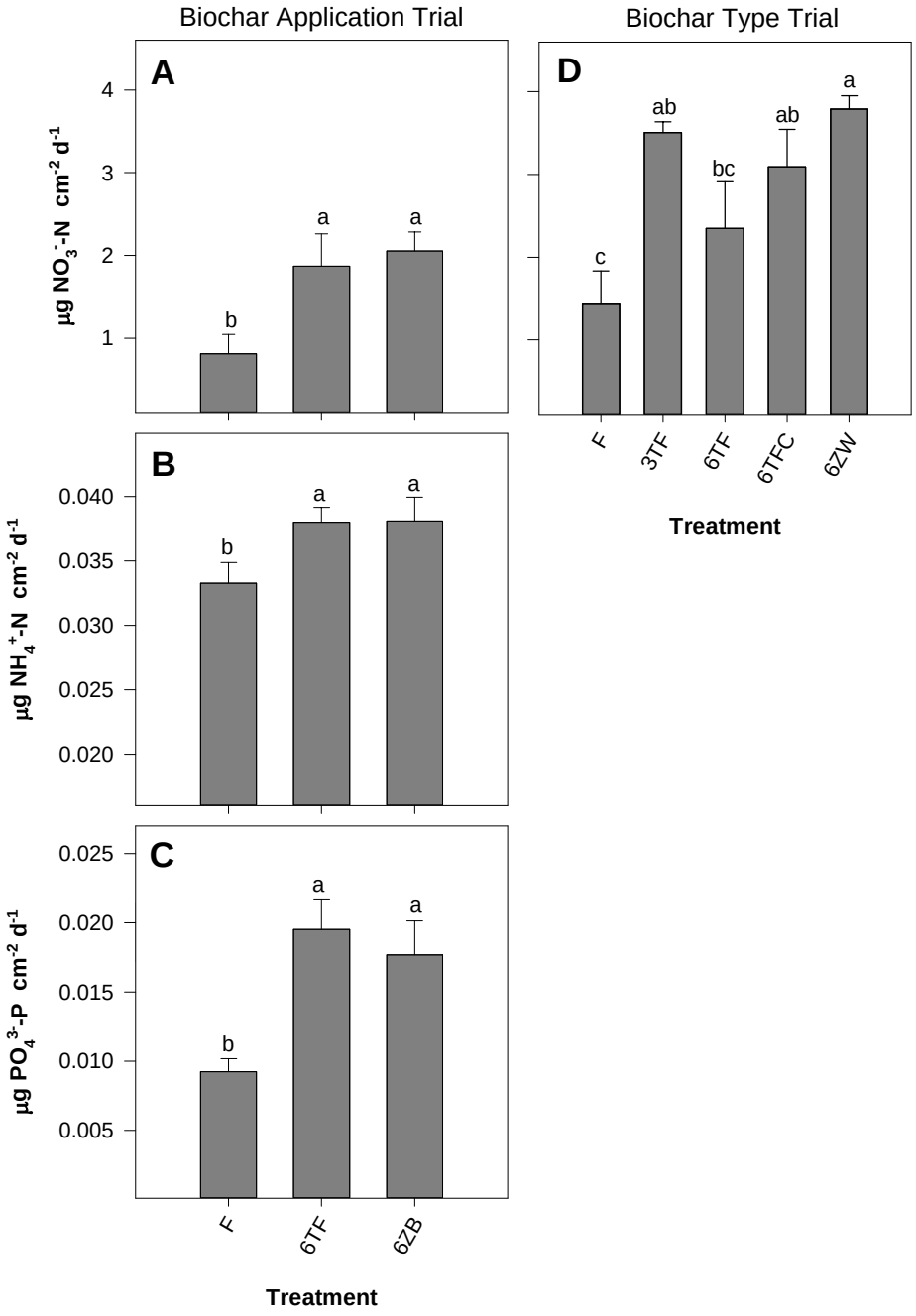

Fig. 4.6. Nitrate (NO^{3-}-N), ammonium (NH_4^+-N), and phosphate (PO_4^{3-}-P) availability, in µg cm^{-2} d^{-1}, was measured in the Biochar Application Trial in Iqaluit (Graphs A-C), while NO^{3-}-N availability, in µg cm^{-2} d^{-1}, was measured in the Biochar Type Trial in Whitehorse (Graph D). Treatments included fertilizer (F), 3% Titan fishmeal biochar plus fertilizer (3TF), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Titan fishmeal bonemeal biochar plus 5% compost plus fertilizer (6TFC), 6% Zakus bonemeal biochar plus fertilizer (6ZB), and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean with error bars representing the standard error of the mean. Different letters indicate significantly different nutrient availability between treatments (ANOVA, $P < 0.05$).

Biochar, regardless of feedstock, increased NH_4^+-N and PO_4^{3-}-P availability in PHC contaminated soil in Iqaluit. Biochars derived from wood or fishmeal had significantly higher NH_4^+-N and PO_4^{3-}-P availability than the fertilizer control in Iqaluit (Figs. 4.6B and C, ANOVA, $P < 0.05$), while only biochar derived from bonemeal increased PO_4^{3-}-P availability in Whitehorse (Appendix B, Fig. B.5, ANOVA, $P < 0.05$). More than likely, environmental conditions were too variable to identify similar comparisons between sites, or multiple mechanisms driving NH_4^+ and PO_4^{3-} availability were active. Although significant in a few cases, the effect of biochar on nutrient availabilities remains variable and inconclusive when both field trials are considered although NO_3^--N availability usually increased in biochar-amended soils when compared to the fertilizer control. Available NO_3^- is essential for microbial catabolism of PHCs in soils; therefore, biochar applications which increase NO_3^--N availability may also stimulate PHC degradation.

4.5.3 Functional gene abundance

In Iqaluit, where the soil was polluted with hydrocarbons, aromatic functional gene abundance (*C2,3O* and *nahAc*) was higher in incorporated treatments (Appendix B, Fig. B.6, ANOVA, $P < 0.05$), and *C2,3O* gene abundance decreased over time in injected treatments while there was no temporal change when biochars and the control were incorporated (Fig. 4.7B, ANOVA, $P < 0.05$). At Whitehorse, all functional genes were more abundant in injected treatments and increased over time in both application methods, but to a greater degree in injected treatments (Figs. 4.7D-F, ANOVA, $P < 0.05$). Increased *C2,3O* and *nahAc* gene abundance has been correlated with increased polycyclic aromatic hydrocarbon (PAH) mineralization (Phillips et al., 2008), which supports the finding that incorporated treatments increased F2 and F3-PHC degradation in Iqaluit. Population shifts can occur in response to changes in the chemical composition of PHCs; low molecular weight, aliphatic hydrocarbons generally disappear from PHC compounds first and selective pressure is exerted by the remaining aromatic PHC compounds (Sotsky et al., 1994; Liu et al., 2017). Once low molecular weight aliphatics are degraded their effect on the microbial community is limited, which could explain why application type did not influence *alkB* functional gene abundance in Iqaluit. Since the Whitehorse site was only residually contaminated, it is not surprising that microbial community dynamics differ from those at Iqaluit.

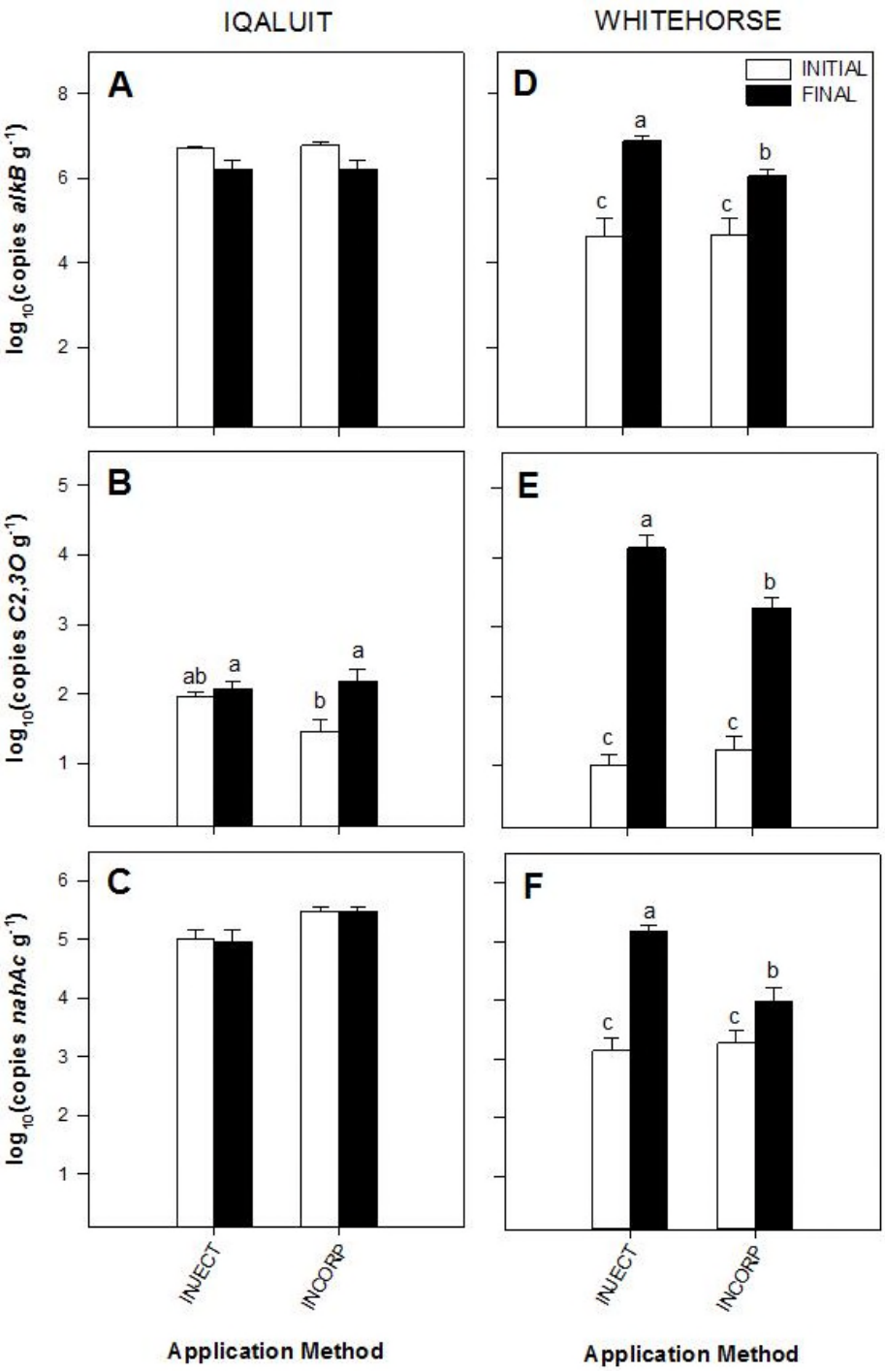

Fig. 4.7. Aliphatic (alkane monooxygenase; *alkB*) and aromatic (catechol 2,3 dioxygenase; *C2,3O* and naphthalene dioxygenase; *nahAc*) functional gene abundance, in $\log_{10}$(copies g^{-1}), was measured in injected (INJECT) and incorporated (INCORP) treatments at initial and final sampling periods in Iqaluit and Whitehorse. Bars represent the mean (n = 6) with the error bars representing the standard error of the mean. Different letters indicate significantly different gene abundance (ANOVA, $P < 0.05$).

In the Biochar Type Trial, aromatic functional gene abundance decreased in response to Zakus wood biochar additions (Fig. 4.8, ANOVA, $P < 0.05$). In the Biochar Application Trial, *alkB* and *C2,3O* gene abundance was affected by treatment, but Zakus wood biochar was equal to or greater than the fertilizer control (Appendix B, Fig. B.7, ANOVA, $P < 0.05$). While biochar additions did not influence PHC degradation more than the fertilizer control in the field, it appears that wood biochar had an inhibitory effect on functional gene abundance, at least during one year of field trials. Others found that rice hull biochar stimulated PAH-degradation by increasing gene copy numbers associated with PAH degradation and by changing the structure of the microbial community (Liu et al., 2014). However, Cao et al. (2016) found that wheat straw biochar inhibited the removal of phenanthrene due to adsorption and immobilization by the biochar, and decreased dehydrogenase activity in soil enzyme assays. While wood biochar appeared to inhibit genes encoding for aliphatic and aromatic degradation pathways, there was no indication that PHC degradation was affected, thus it is unclear if processes such as immobilization or sorption were driving microbial shifts. Compared to the other biochars used, Zakus wood biochar had a much smaller average pore size and volume, and lower CEC (Appendix B, Table B.3). It has been suggested that most bacterial and fungal hyphae cannot penetrate pores < 1000 nm in size (Quilliam et al., 2013a), and the wood biochar would contain a greater proportion of narrower pores which could reduce the abundance of microbial colonization.

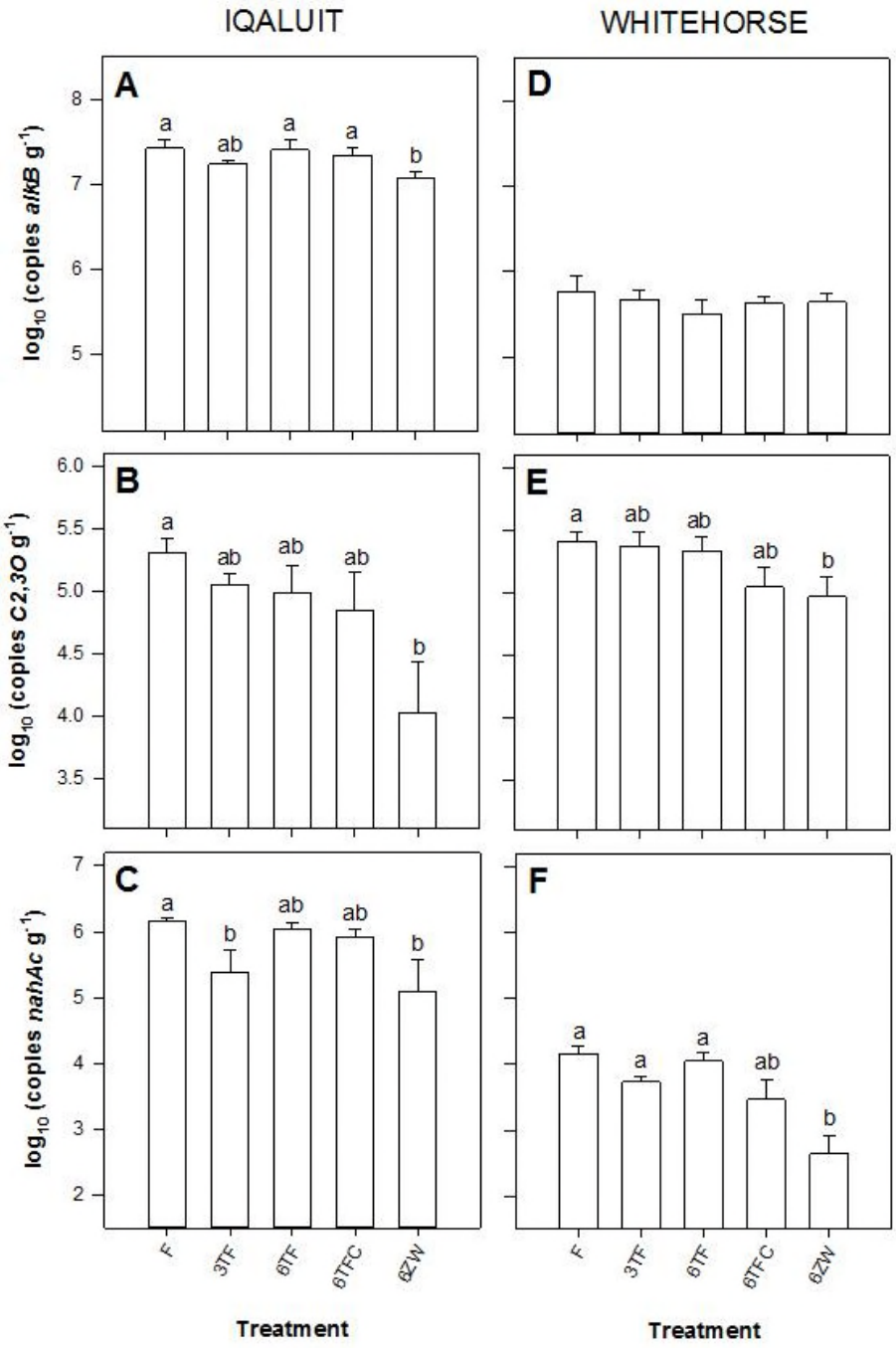

Fig. 4.8. Aliphatic (alkane monooxygenase; *alkB*) and aromatic (catechol 2,3 dioxygenase; *C2,3O* and naphthalene dioxygenase; *nahAc*), in $\log_{10}$(copies g^{-1}), in the Biochar Type Trial in Iqaluit (Graphs A-C) and Whitehorse (Graphs D-F). Treatments included fertilizer (F), 3% Titan fishmeal biochar plus fertilizer (3TF), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Titan bonemeal biochar plus 5% compost plus fertilizer (6TFC), and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean, with error bars representing the standard error of the mean. Different letters indicate significantly different functional gene abundance between treatments (ANOVA, $P < 0.05$).

4.5.4 Temperature and θ_{liquid}

In Whitehorse, average θ_{liquid} was higher in incorporated treatments but average soil temperature was higher injected treatments (ANOVA, $P < 0.05$). Average θ_{liquid} was 10.8% (SE = 0.21) and 9.8% (SE=0.22), while average temperature was 7.4 °C (SE = 0.22) and 7.7 °C (SE = 0.22), in incorporated and injected treatments, respectively. Incorporated treatments were homogenously mixed throughout the soil so an increase in θ_{liquid}, driven by biochar additions, was expected. However, within incorporated treatments, average θ_{liquid} was only higher in 6% Titan fishmeal biochar plus fertilizer when compared to other biochar types and the control but when injected, biochar type did not increase θ_{liquid} when compared to the fertilizer control (Appendix B, Table B.7, ANOVA, $P < 0.05$). Fishmeal-derived biochar increased the soils WHC and volumetric water content in treatments when incorporated, which suggests that this particular biochar has properties which positively impact soil water dynamics, and optimizes soil-biochar contact due to the contribution of intraparticle porosity within biochar particles in addition to interparticle porosity between biochar and soil particles (Masiello et al., 2015).

Biochar type only influenced θ_{liquid} in the Biochar Application Trial but affected both θ_{liquid} and soil temperature in the Biochar Type Trial (Tables 4.1 and 4.2). Across both trials, soil temperature and θ_{liquid} was highest in 6% Titan fishmeal plus fertilizer, when compared to the fertilizer control and other biochar types (i.e. 6% Zakus wood biochar plus fertilizer) (Appendix B, Fig. B.8, ANOVA, $P < 0.05$). Conflicting results have been reported in other field studies; biochar applied at 3-6% (w/w) increased water holding capacity of a sandy soil by up to 30% (Basso et al., 2013), while others showed no effect on water holding capacity for some soil-biochar mixtures (Kinney et al., 2012). At 6% (v/v), the application rates used in this study convert to < 0.5% (w/w), which is similar to other application rates that have increased soil water holding capacity in cool and temperate continental climates (Karhu et al., 2011; Streubel et al., 2011; Briggs et al., 2012).

4.6 Conclusions

When applying amendments to both northern landfarm sites, incorporation was the ideal method of application. Petroleum hydrocarbon degradation was linked to increases in multiple factors related to PHC degradation; aromatic PHC-degrading functional gene abundance, NO_3^- availability, and θ_{liquid}. Although injection resulted in a similar decrease in F2 and

F3-PHCs after one field season, degradation proceeded much slower, and other soil properties (catabolic functional gene abundance, θ_{liquid}, nutrient availability) were not clearly enhanced. Although biochar did not clearly increase PHC degradation relative to the fertilizer control, there was a small effect on F2-PHCs over time under field conditions. Available NO_3^- appeared to increase in soils amended with biochar, regardless of application method, but changes in NH_4^+ and PO_4^{3-} availability were less consistent. Functional gene abundance responded differently at each site, with treatment differences occurring, but inconsistently. Over both field seasons, θ_{liquid} was increased in 6% Titan fishmeal biochar plus fertilizer, but these measurements could only be taken in one location. With future investigation of the effects of biochar on PHC degradation across a range of northern field sites, the aim should be the identification of trends which could indicate which biochars might be more successful in soils with specific properties.

5. THE CHAROSPHERE PROMOTES MINERALIZATION OF ^{13}C-PHENANTHRENE BY PSYCHROTROPHIC MICROORGANISMS IN FROZEN, PETROLEUM HYDROCARBON CONTAMINATED SOIL[3]

5.1 Preface

The previous studies demonstrated that biochar can have positive effects on the soil microbial community, physical and chemical soil properties, and PHC degradation, but did not provide insight into the mechanisms driving these responses in biochar-amended soils. Other researchers identified a spatial effect, which indicated that soil immediately surrounding biochar particles, termed the 'charosphere', may be directly influenced by the physical and chemical properties of biochar, more so than bulk soil that is not in direct contact. Spatial niches, such as bulk soil, charosphere soil, and biochar particles, may control aspects of PHC degradation in frozen soils. This laboratory study was carried out using PHC contaminated soil from Greenland in which spatial sampling locations were created with mesh bags containing different biochars. The overall objective of this study was to determine if ^{13}C-phenanthrene mineralization and other soil properties are spatially distributed in frozen, biochar-amended soils.

[3] This chapter was submitted, with minor formatting differences, as Karppinen, E.M., S.D. Mamet, K.J. Stewart, and S.D. Siciliano. 2017. The charosphere promotes mineralization of ^{13}C-phenanthrene by psychrotrophic microorganisms in frozen, petroleum hydrocarbon contaminated soil. Soil Boil. Biochem. Dr. Siciliano contributed funding for the purchase of supplies and critical equipment, helped with experimental design, assisted with technical content and interpretation, and provided editorial input. Dr. Stewart assisted with technical content and experiment design, and provided editorial input. I completed experimental setup and design, conducted laboratory analyses, developed the initial manuscript draft, prepared the figures and tables, and completed data analysis and interpretation.

5.2 Abstract

Understanding the chemical and biological mechanisms by which biochar influences petroleum hydrocarbon (PHC) remediation in northern environments is critical before implementing large-scale commercial applications. To investigate microbial biodegradation activity under frozen conditions, spatial sampling locations were created with 60 µm mesh bags containing 6% (v/v) biochar made from either fishmeal, bonemeal, bone chip, or wood placed into jars. This experimental design created three soil niches: biochar particles, the charosphere (soil immediately surrounding biochar particles), and bulk soil located outside of the bags. After 90 days, ^{13}C-phenanthrene mineralization in charosphere soil reached 55% (SE = 16) in bonemeal biochar and 84% (SE = 8.2) in bone chip biochar treatments, compared to only 43% (SE = 4.8) in bulk soil and 13% (SE = 11) in bone chip particles. The pH of bone chip and bonemeal biochars remained near neutral, unlike wood biochar, which increased alkalinity and likely made phosphate unavailable for microorganisms. Charosphere soil had higher polyaromatic degradative gene abundances compared to bulk soil but gene abundance was not directly linked to ^{13}C-phenanthrene mineralization. In bone chip biochar-amended soils, phosphate successfully predicted microbial community composition. The relative abundances of *Bosea* and the psychrotrophic, hydrocarbon degrading *Caulobacter* increased in charosphere soil. The charosphere of bone-derived biochars enhances hydrocarbon degradation by maintaining phosphate solubility and promoting key psychrotrophic hydrocarbon degrading bacteria.

5.3 Introduction

Bioremediation is a viable option for managing petroleum hydrocarbon (PHC) contaminated sites across Canada, but the process is often slower and less successful in northern regions (Mohn and Stewart, 2000; Paudyn et al., 2008). Environmental and logistical challenges that can delay remediation include; relatively long winter and short summer seasons, predominantly sandy soils with low moisture and nutrient retention, and substantial costs associated with shipping fertilizer and fuel. In landfarms, where contaminated soil is spread in thin layers or windrows, fertilizer is added to stimulate microbial degradation and the soil is aerated with periodic tillage or turning. The application of compost, and to a lesser extent, biochar, has been evaluated for the potential to further stimulate PHC degradation rates under thawed conditions (Marchand et al., 2016; Yuan et al., 2009). In a previous study, Karppinen et

al. (2017b) found evidence to suggest that PHC degradation in biochar-amended soils was active and even enhanced under frozen, but not thawed, conditions.

Biochar has been rigorously studied as an agricultural soil amendment, due to its reported ability to increase nutrient retention and water holding capacity (Lehmann et al., 2003; Basso et al., 2013; Karer et al., 2013), alter the soils cation exchange capacity, aeration, and hydrology (Atkinson et al., 2010; Cornelissen et al., 2013), and stimulate microbial activity (Lehmann et al., 2011; Zhang et al., 2014b). Soil amendments, such as biochars produced from local feedstocks, increase PHC degradation in northern soils (Karppinen et al., 2017a). Although biochar studies can yield positive results, biochar applications can be inconsistent and vary depending on site-specific environmental and soil conditions, as well as on biochar production conditions, such as pyrolysis temperature and feedstock material (Amonette and Joseph, 2009; McLaughlin et al., 2009). A critical knowledge gap exists, for both temperate and northern regions, in understanding the mechanisms by which biochar might stimulate the microbial community to degrade PHCs, and how this may be related to changes in physical, chemical, and biological soil properties (Verheijen et al., 2009; Thies et al., 2015).

Researchers have speculated that microbes colonize the external and internal pore spaces of biochar because they provide microorganisms with protection from predation and desiccation, while supplying adsorbed water and nutrients (Thies and Rillig, 2009). However, studies show that microbial colonization of biochar surfaces can be difficult as pores < 1 µm are too small for microbial habitation, and organic materials (i.e., soil particles, oils, and waxes) can obstruct pores and deter colonization (Quilliam et al., 2013b; Thies et al., 2015). Biochars also, differ in pH, CEC, surface area, pore size distribution, and ash content (Singh et al., 2010; Cantrell et al., 2012), which can affect the suitability of biochar as a microhabitat for soil microorganisms (Lehmann et al., 2011) and influence physical and chemical soil properties (Chan et al., 2007; Atkinson et al., 2010). Therefore, a successful remediation approach must match biochar properties with microbial degrader niche requirements.

Biochars are often homogenized into soil in the laboratory or field, and only the net effect on the soil as a whole is considered. Soil immediately surrounding biochar particles, termed the ‘charosphere’, may be directly influenced by the physical and chemical properties of biochar, to a greater extent than bulk soil which is not in direct contact (Quilliam et al., 2013a). For

example, mineralization of labelled substrates has been used as a measure of degradative potential in many studies with biochar-amended soils (Ogbonnaya et al., 2014; Rhodes et al., 2010; Shan et al., 2015), and spatial differences were observed when comparing ^{14}C-labelled glucose mineralization between niches (Quilliam et al., 2013a). These spatial niches may become important when considering aspects of field application, such as feedstock, final particle size, and application rate. The intent would be to optimize the influence of a specific niche, whether it is the bulk soil, charosphere, or biochar particles, based on the desired benefits to agricultural or environmental application.

The objective of this study was to determine if there is a spatial relationship between biochar particles and the surrounding soil, and if so, the influence on ^{13}C-phenanthrene mineralization and other soil properties. I hypothesize that biochar-induced changes in mineralization and soil properties occur predominantly in soils in direct contact with biochar particles but is dependent on biochar type. Four different biochar types with differing properties were selected, based on feedstocks that could be practically generated in northern environments. It is expected that these analyses will contribute to the knowledge base of how biochar influences PHC degradation in frozen soils and provide insight into the mechanisms driving the response of soil microorganisms in biochar-amended soils.

5.4 Materials and Methods

5.4.1 Soil and treatment characterization

The soil used in this study was a mixture of weathered PHC contaminated material from Mestersvig, Greenland, which contained approximately 1,400 mg kg^{-1} F2 (equivalent *n*C_{10}-C_{16}) -PHCs and 600 mg kg^{-1} F3 (equivalent *n*C_{16}-C_{34}) -PHCs. The sandy soil (88% sand) had a near-neutral pH (7.5), was deficient in major nutrients (i.e., NH_4^+-N [7.64 µg g^{-1}], NO^{3-}-N [3.95 µg g^{-1}] and PO_4^{3-}-P [0.00 µg g^{-1}]) and had low organic carbon (0.56%) and gravimetric moisture (4.79%) content. Treatments (various biochars) were characterized for pH, pyrolysis temperature, bulk density, Brunauer-Emmet-Teller (BET) surface area, pore volume, pore size, cation exchange capacity (CEC), volatile matter, and ash content (Table 5.1).

Table 5.1. Characterization of biochar treatments for pH, pyrolysis temperature, bulk density, Brunauer-Emmet-Teller (BET) surface area, pore volume, average pore size, cation exchange capacity, volatile matter, and ash content.

Property	Biochar			
	Bonemeal	Bone Chip	Fishmeal	Wood
pH	9.6	8.5	9.0	8.9
Pyrolysis temperature (°C)	450	450	500	450
Bulk density (Mg m^{-3})	0.72	0.72	0.50	0.11
BET† surface area (m^2 g^{-1})	71	150	7.1	78
Average pore volume (cm^3 g^{-1})	0.22	0.38	0.03	0.01
Average pore size (nm)	11	9.8	13	2.2
CEC‡ ($cmol_c$ kg^{-1})	39	57	54	7
Moisture (%)	7	1	28	14
Volatile Matter (%)	14	15	40	32
Ash (%)	79	84	32	54

†BET = Brunauer-Emmet-Teller
‡CEC = cation exchange capacity

5.4.2 Experimental setup

In this laboratory study, contaminated soil was dried, brought to 10% gravimetric water content, and packed to the same bulk density into 250 mL amber glass jars. A 60 µm mesh nitex (nylon) bag (Dynamic Aqua-Supply, BC, CAN), which allows microbes to pass freely through the bag, was filled with either fishmeal, bonemeal, bone chip, or wood biochar and buried in the soil in order to create spatial sampling locations. An empty nitex bag was used in the control to establish any interactions between the soil and bags. The jars were sealed with Parafilm and left to incubate at room temperature for 3 weeks to establish microbial colonization, after which, the jars were incubated at -5 °C for 90 days to assess microbial activity under frozen conditions. Following incubation, each jar was sampled from three different locations; i) bulk soil, ii) charosphere, and iii) biochar particles. Bulk soil was sampled from soil approximately 1 cm from the nitex bag, the charosphere was sampled from soil contacting the nitex bag, while biochar particles were sampled directly from the nitex bag. The charosphere, defined as soil within 5 mm of the biochar particles, consisted of 20 g of soil. Biochar applied at 6% (v/v) was equal to 1.7 g to 11 g, depending on individual biochar densities.

5.4.3 Soil treatments

The four biochars used in this experiment were: fishmeal, bonemeal, bone chip and wood; and have been used in previous laboratory and field studies at a rate of 6% (v/v) in PHC contaminated soil (Karppinen et al., 2017a; b). An empty nitex bag was used in the control to establish any interactions between the bags and soil.

5.4.4 ^{13}C-phenanthrene mineralization assays

^{13}C-labelled phenanthrene (Sigma-Aldrich Corporation, MO, USA; 33% atom $C_2C_{12}H_{10}$) stock solution was spiked (0.1 mL at 1.5 g L^{-1} in toluene) into 0.25 g sterile sand in a 12 mL glass vial, evaporated in a fume hood, and mixed with 0.25 g of experimental soils. The process was repeated with unlabelled phenanthrene (Sigma-Aldrich Corporation, MO, USA; $C_{14}H_{10}$) stock solution. Following 14 days incubation at room temperature, CO_2 was analyzed on a Bruker 450 Gas Chromatograph (Bruker Corporation, MA, USA) and the delta ^{13}C (δ^{13}C) was measured on a Picarro G2201-i CO_2 Analyzer (Picarro Inc., CA, USA). By subtracting $^{13}CO_2$ production in unlabelled phenanthrene from $^{13}CO_2$ production in ^{13}C-labelled phenanthrene, substance mineralization was determined (Siciliano et al., 2016).

5.4.5 Petroleum hydrocarbon analysis

Both F2 and F3-PHCs were extracted using a hexane-acetone extraction method, where 2.5 g soil plus 30 mL hexane and acetone (1:1) were combined with sodium sulphate and shaken overnight, centrifuged to remove the supernatant and evaporated to approximately 2 mL with nitrogen gas (Schwab et al., 1999). Quality control measures included duplicates, which were within 10-20 relative percent difference of the original sample, method blanks, spike recoveries (average F2 and F3-PHC spike recoveries were 82% and 87%, respectively), and calibration to a standard curve ($R^2 > 0.99$). The F2 and F3-PHC concentrations were quantified using a Varian CP3800 gas chromatograph fitted with a flame ionization detector (GC-FID) and chromatograms were analyzed using CompassCDS software (Varian, CA, USA).

5.4.6 Chemical properties

pH was determined from a soil/biochar and 0.01 M $CaCl_2$ solution (2:1 w/v) using a pH meter (Mettler-Toledo, OH, USA). Both NH_4^+-N and NO^{3-}-N were extracted from 1 g moist soil/biochar with 10 mL of 2 M KCl (149 g L^{-1} potassium chloride), shaken for 30 mins at

160 strokes min^{-1}, and filtered through Whatman No. 42 filter paper. Phosphate (PO_4^{3-}-P) was extracted from 1 g moist soil or biochar with 10 mL of Kelowna solution (14 mL L^{-1} acetic acid; 19.25 g L^{-1} ammonium acetate; 0.56 g L^{-1} ammonium fluoride), shaken for 5 mins at 142 strokes min^{-1}, and filtered through ashless Whatman No. 40 filter paper. Nutrient concentrations were quantified using a Technicon™ AutoAnalyzer™ III (SEAL Analytical, WI, USA).

5.4.7 Soil microbial community analysis

5.4.7.1 Functional gene abundance

In this study, DNA was extracted using the Powerlyzer™ Powersoil® DNA Isolation Kit (MO BIO Laboratories, CA, USA) to lyse cells and remove humic substances and other PCR inhibitors. DNA yield was quantified using a Qubit® Fluorometer (Thermo Fisher Scientific, ON, CAN). Extracted DNA was analyzed for the presence of two catabolic genes, naphthalene dioxygenase (*nahAc*) (Whyte et al., 1997; Luz et al., 2004) and catechol 2,3 dioxygenase (*C2,3O*) (Baldwin et al., 2003), which encode enzymes involved in PHC degradation pathways. Standards were prepared by extracting the DNA of American Type Culture Collection (ATCC) standard organisms (ATCC 29347 for *C2,3O* and ATCC 17484 for *nahAc*), followed by polymerase chain reaction amplification with the corresponding primer set and repeated gel purification. Extracted DNA was also analyzed for the presence of RNA polymerase beta subunit (*rpoB*), which exists as a single copy in most bacteria (Dahllof et al., 2000). For *rpoB*, clone libraries were created with *E. coli* cells using the TOPO TA Cloning Kit (Invitrogen, CA, USA), followed by DNA extraction from clones. For qPCR gene amplification, 4 µL of template DNA was added to a 15 µL final volume mixture containing QuantiTect SYBR® Green RT-PCR Kit (Qiagen, CA, USA) and 1.0 µM each of forward and reverse primers. After 10 minutes initial denaturing at 94 °C, amplification proceeded with 40 cycles of 1 minute denaturing at 94 °C, 30 seconds annealing at the appropriate temperature, 1 minute extension at 72 °C, followed by a melt curve analysis for 15 seconds at 95 °C, 1 minute at 60 °C and 15 seconds at 95 °C (Table 5.2). Quality control measures included matrix blanks and a standard curve prepared in triplicate, ranging from 10^8-10^1 gene copies μL^{-1}. All PCR fragments were visualized on SYBR® Safe-stained 1.5% agarose gels to ensure target gene amplification.

Table 5.2. Primers and thermal conditions used for quantitative polymerase chain reaction (qPCR) amplification of catechol 2,3 dioxygenase (*C2,3O*), naphthalene dioxygenase (*nahAc*), and RNA polymerase beta subunit (*rpoB*).

Target gene Primer sequence[†]	**Ta**[‡] (°C)	**Primer** (µM)	**Expected size** (bp)	**Control Strain**
Catechol 2,3 dioxygenase (*C2,3O*)	65.6	1.0	406	*P. putida*
F: 5'- AGG TGC TCG GTT TCT ACC TGG CCGA				ATCC 29347
R: 5'- ACG GTC ATG AAT CGT TCG TTG AG				
Naphthalene dioxygenase (*nahAc*)	47.0	1.0	377	*P. putida*
F: 5'- CAA AAR CAC CTG ATT YAT GG				ATCC 17484
R: 5'- AYR CGR GSG ACT TCT TTC AA				
RNA polymerase beta subunit (*rpoB*)	68.0	1.0	398	*E. coli*
F: 5'- CAA CAT CGG TTT GAT CAA C				
R: 5'- CGT TGC ATG TTG GTA CCC AT				

[†]Forward (F) and reverse (R) primers are indicated.

[‡]Ta = annealing temperatures used during real-time PCR.

5.4.7.2 Bioinformatics processing

Bacterial community composition was assessed by high-throughput amplicon library sequencing as described in Lamb et al. (2016). Prior to PCR reaction, *A. fischeri* (ATCC 14546) was spiked at 1% (0.1 ng) and used as an internal standard (Smets et al., 2016). Primers contained an Illumina adapter and a unique barcode sequence for sample pooling. Samples were amplified with the universal 16S rRNA primer set 515F/806R, which amplifies a 291 bp fragment near the bacterial v4 region (Caporaso et al., 2010), and purified using Beckman Coulter AmPure XP Beads on a magnetic stand. The Illumina indexes/adapters were added in the next PCR and samples were purified again using the AmPure XP Beads. Following pooling, the samples were sequenced on an Illumina MiSeq using a V3 chemistry kit (600 cycles). European Bioinformatics Institute (EBI) Metagenomics was used as a platform to analyze Illumina MiSeq sequence data (Mitchell et al., 2016). Sequencing data have been deposited in the European Molecular Biology Laboratory (EMBL) with accession number ERP019994.

5.4.8 Statistical analysis

All statistical analyses were executed using RStudio Version 3.3.2 (R Core Team, 2017). A two-way Analysis of Variance (ANOVA) was used to assess the effects of treatment and sampling location on mineralization, pH, nutrients, and functional gene abundance. Data was tested for assumptions of normality using Shapiro-Wilks test and homogeneity of variance using Bartlett's test, and means separation was performed using Fisher's Least Significant Difference (LSD) multiple comparisons test. Bar graphs were created in SigmaPlot Version 12. Prior to bacterial community analysis, operational taxonomic unit (OTU) counts were normalized to *A. fischeri* and DNA concentration then transformed with DESeq2 in RStudio (Love et al., 2014). Principal coordinates analysis (PCoA) was used to identify differences in microbial community composition in bone chip biochar-amended soils, while distance-based redundancy analysis (db-RDA) with Bray-Curtis distance was used to determine the relationship between environmental variables and bacterial community. Bacterial community composition data and graphs were processed in RStudio, using the *vegan* Version 2.4.4 (Oksanen et al., 2017), *ggplot2* Version 2.2.1 (Wickham, 2009), and *ggtree* Version 1.10.0 (Yu et al., 2017b) packages.

5.5 Results and Discussion

5.5.1 ^{13}C-phenanthrene mineralization

Significant spatial patterns were evident in soils amended with bonemeal and bone chip biochar, with the greatest ^{13}C-phenanthrene mineralization occurring in the charosphere soil (Fig. 5.1). For example, in soils amended with bone chip biochar, ^{13}C-phenanthrene mineralization in charosphere soil (84% [SE = 8.2]) was significantly higher when compared to bulk soil (43% [SE = 4.8]) and bone chip biochar particles (13% [SE = 11]) ($P < 0.05$). In soils amended with bonemeal biochar, ^{13}C-phenanthrene mineralization was significantly higher in charosphere soil when compared to bonemeal biochar particles, but not bulk soil ($P < 0.05$). On average, ^{13}C-phenanthrene mineralization in biochar particles was low (17% [SE = 6.7]), however, enhanced mineralization occurred in wood biochar particles (63% [SE = 15]). The heterogeneity of these spatial niches was highlighted by variable ^{13}C-phenanthrene mineralization rates in biochar particles and charosphere soil (i.e., relative standard deviations ranging from 40-200% in biochar particles and 22-137% in charosphere soil), compared to the bulk soil (i.e., relative standard deviations ranging from 18-32%).

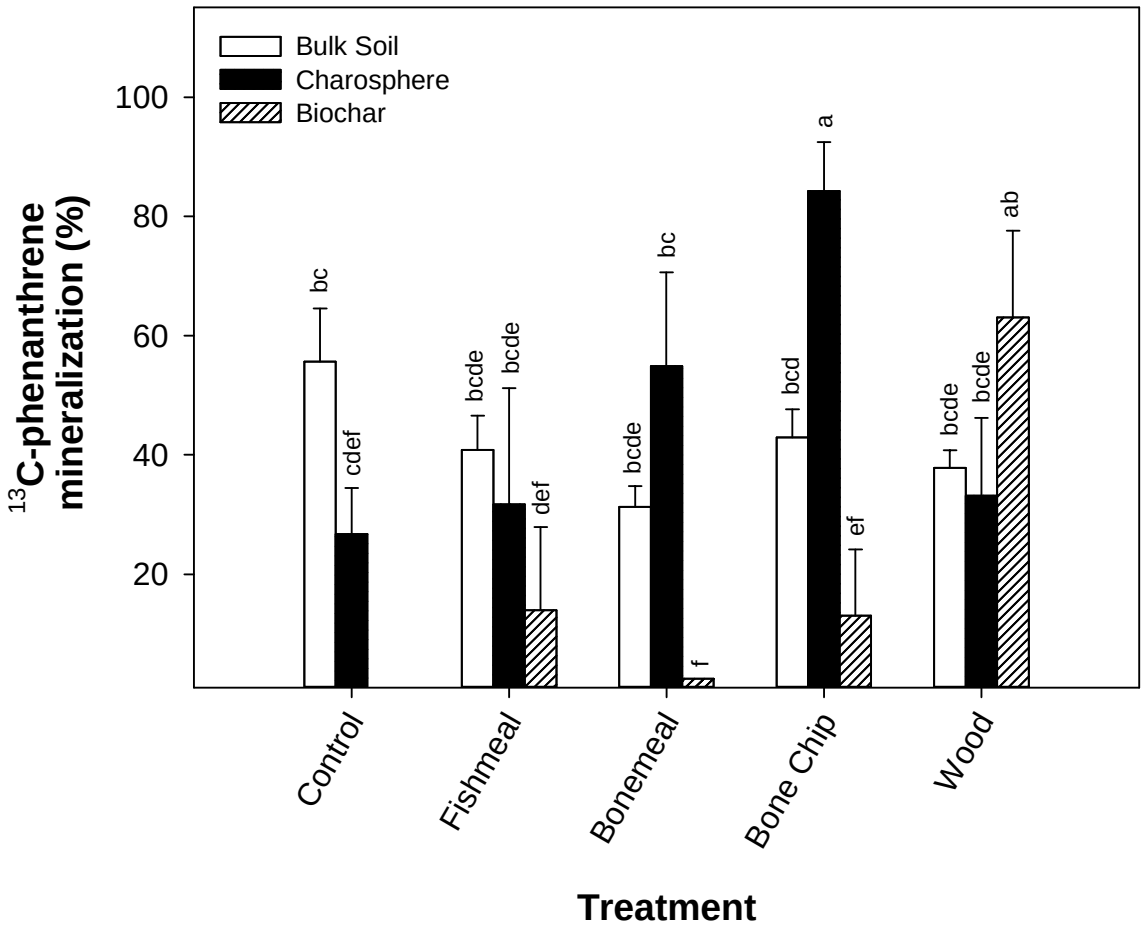

Fig. 5.1. ^{13}C-phenanthrene mineralization (%) was measured in bulk soil, charosphere soil, and biochar particles of a control and bonemeal, bone chip, fishmeal, and wood biochar-amended soils. Bars represent the mean (n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different ^{13}C-phenanthrene mineralization (ANOVA, $P < 0.05$).

Previous studies that separated charosphere soil from biochar particles and the bulk soil concluded that biochar altered physicochemical properties of charosphere soil and influenced soil microbial activity and structure in agricultural soil (Quilliam et al., 2013a) or cadmium transport and transformation in metal contaminated soil (Wang et al., 2017). Of the four biochars used in this experiment, those derived from bone (bonemeal and bone chip) had the highest ash content and pore volume, and bone chip biochar had the highest BET surface area (Table 5.1). Although biochar particles were generally unsuitable sites for mineralization, charosphere soil is under the direct influence of biochar particles, which suggests that a combination of one or more of the aforementioned properties increased ^{13}C-phenanthrene mineralization in the charosphere. For example, greater surface area and porosity can influence water and nutrient retention (Atkinson et al., 2010; Lehmann et al., 2011) and stimulate microbial colonization (Jaafar et al., 2015). In this study, related chemical and microbial changes, which may influence ^{13}C-phenanthrene mineralization in the charosphere of bone derived biochars, were observed in subsequent analyses.

5.5.2 pH

Compared to the control soil, the pH of both soil and biochar in bone-derived treatments were similar or lower, while pH was elevated in all spatial niches of wood biochar treatments and in the bulk soil of fishmeal biochar treatments (Fig. 5.2, ANOVA, $P < 0.05$). For instance, compared to the pH of the unamended control soil (7.52 [SE = 0.03]), the pH of bone chip biochar particles was significantly lower (7.12 [SE = 0.01]). There were no significant differences between sampling location in bonemeal biochar treatments. The pH of all biochars decreased from initial values (Table 5.1), with an average difference of 2.1 and 1.4 units in bonemeal and bone chip biochars, respectively.

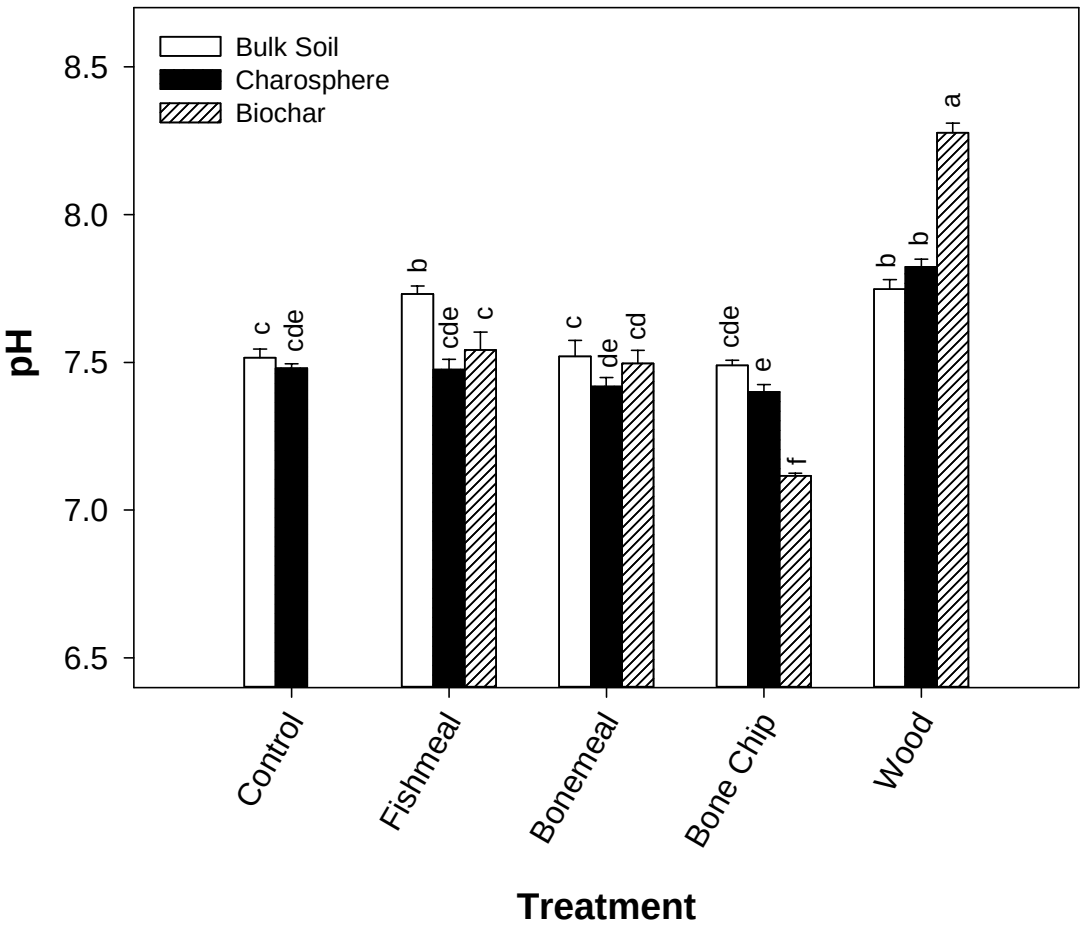

Fig. 5.2. After 90 days incubation at -5 °C, pH was quantified in bulk soil, charosphere soil, and biochar particles of a control and bonemeal, bone chip, fishmeal, and wood biochars. Bars represent the mean n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different pH (ANOVA, *P* < 0.05).

In this study, average pH increased in a neutral soil (pH = 7.5) when amended with fishmeal and wood biochars but remained neutral with bonemeal and bone chip biochars. Biochar can increase the pH of acidic soils (Yao et al., 2017) and decrease the pH of calcareous soils (Liu and Zhang, 2012; Ippolito et al., 2016), but this is dependent on the pH of both the biochar and soil. Biochars are thought to decrease pH by altering soil buffering capacity due to the production of acidic functional groups during chemical and microbial oxidation of biochar particles (Cheng et al., 2006) and by increasing overall soil CEC with the addition of biochars with high CEC (Laird et al., 2010; Fellet et al., 2011; Jones et al., 2011a; Peng et al., 2011). The bone-derived biochars used in this study had high CEC, relative to wood biochar (Table 5.1), which may have buffered potential pH changes from the bone-derived biochars compared to the large increase seen with wood biochar.

5.5.3 Extractable nutrients

In biochar particles, NH_4^+-N and NO_3^--N concentrations were higher in bonemeal biochar, while the concentration of PO_4^{3-}-P was higher in bone chip biochar (Fig. 5.3, ANOVA, $P < 0.05$). For example, PO_4^{3-}-P concentrations were 4,177 μg g^{-1} (SE = 444) in bone chip biochar and 450 μg g^{-1} (SE = 95) in bonemeal biochar. In general, nutrient concentrations in the charosphere and bulk soils were comparable to the control and elevated to varying degrees in biochar particles (Appendix C, Fig. C.1, ANOVA, $P < 0.05$). However, average NO_3^--N concentrations were similar between biochar particles and charosphere soil in bone chip biochar treatments (Fig 5.3B, ANOVA, $P = 0.95$).

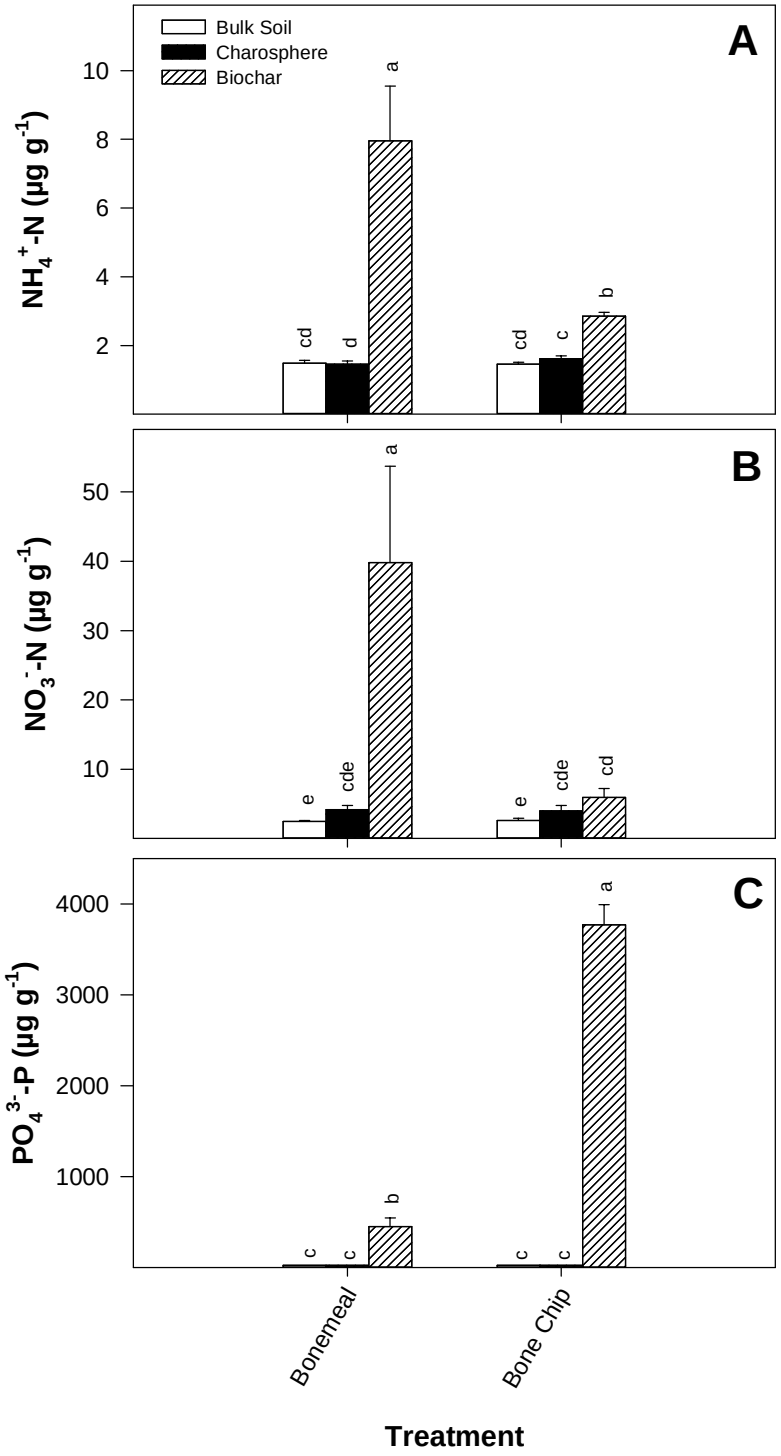

Fig. 5.3. After 90 days incubation at -5 °C, the following extractable nutrients: (A) ammonium (NH_4^+-N), (B) nitrate (NO_3^--N), and (C) phosphate (PO_4^{3-}-P), in µg g^{-1}, were quantified in the bulk soil, charosphere soil, and biochar particles of bonemeal and bone chip biochar-amended soils. Bars represent the mean (n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different nutrient concentrations (ANOVA, $P < 0.05$).

In a study using giant reed biochar, the release of PO_4^{3-} and NH_4^+ decreased with increasing pH, while NO_3^- was not dependent on pH (Zheng et al., 2013). The nutrients PO_4^{3-} and NH_4^+ are likely more soluble and therefore bioavailable in bonemeal and bone chip biochar-amended soil, specifically in bone chip biochar particles which had a significantly lower pH than the control soil (Germano et al., 2012). Recent studies suggest that biochar promotes plant growth by capturing and slowly releasing nitrate (Haider et al., 2016). In temperate field trials, up to 60% of nitrate was released from co-composted biochar-amended soils that underwent additional 2 M KCl extractions, which indicates that total nitrate may be underestimated as nitrate is captured and slowly released from biochar (Kammann et al., 2015; Hagemann et al., 2017). Similarly, in this study, less NO_3^--N may have been extracted from bone chip biochar particles due to its ability to capture and retain nitrate. Alternatively, it is possible that fewer nutrients were extracted from the bulk soil and charosphere due to immobilization by soil microorganisms, which is supported by generally higher ^{13}C-phenanthrene mineralization rates in bulk and charosphere soils. Nutrient transformations, including immobilization, have been linked to biochar-induced changes in microbial processes (Gul and Whalen, 2016).

5.5.4 Functional gene abundance

After 90 days, functional gene abundance responded to biochar type and sampling location in frozen, PHC contaminated soils (Appendix C, Fig. C.2, ANOVA, $P < 0.05$). Both biochar particles and charosphere soil had higher *C2,3O* gene abundance than the bulk soil (Fig. 5.4A, ANOVA, $P < 0.05$); *nahAc* gene abundance was greater in charosphere soil when compared to the bulk soil, but not biochar particles (Fig. 5.4B, ANOVA, $P < 0.05$), and; there were no differences in *rpoB* gene abundance between sampling locations (Fig. 5.4C, ANOVA, $P < 0.05$). For example, *C2,3O* abundance was 6.01 $\log_{10}$(copies g^{-1}) (SE = 0.31) in bone chip biochar particles, 5.99 $\log_{10}$(copies g^{-1}) (SE = 0.19) in the charosphere soil, and 4.68 $\log_{10}$(copies g^{-1}) (SE = 0.05) in the bulk soil. However, in bonemeal biochar-amended soil, all functional gene abundances were higher in biochar particles when compared to bulk and charosphere soils (Fig. 5.4A-C, ANOVA, $P < 0.05$). Results from the control treatment, fishmeal biochar, and wood biochar are provided in Appendix C (Fig. C.2).

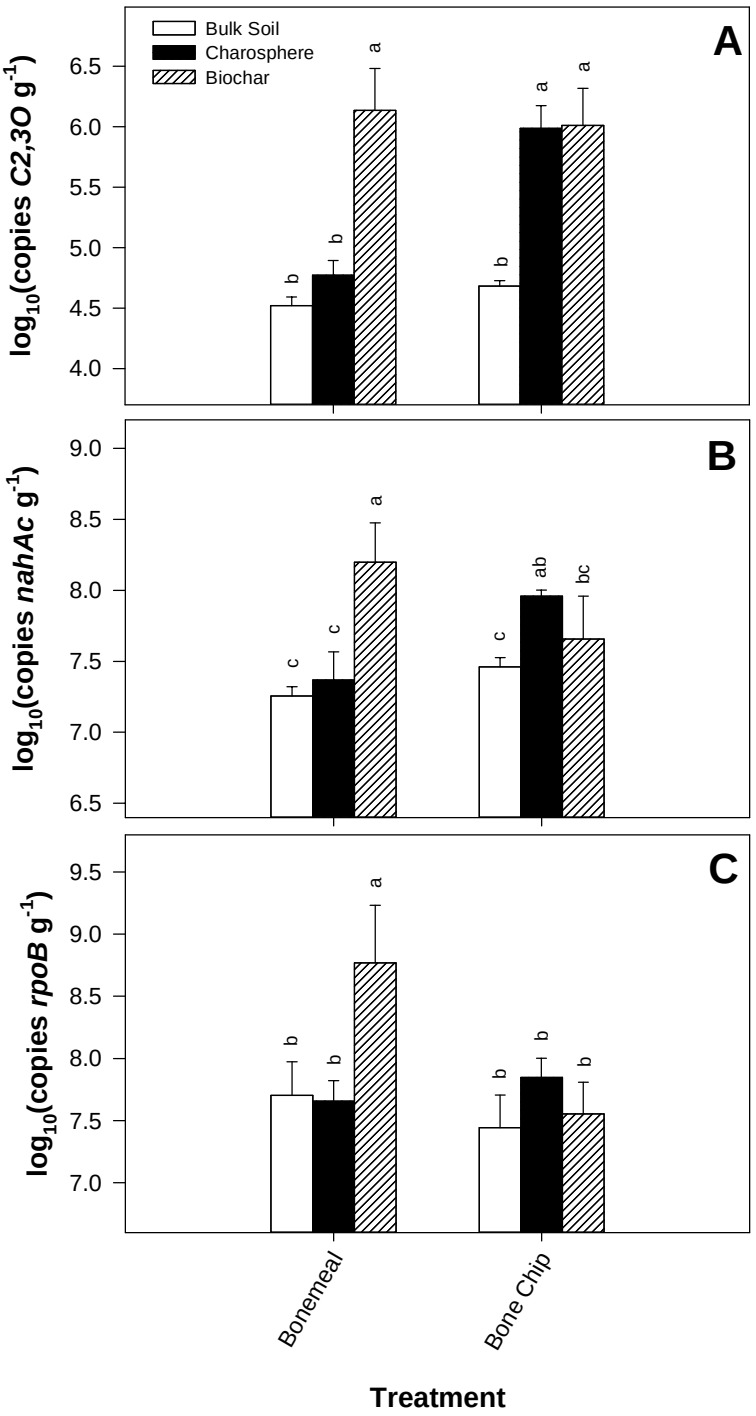

Fig. 5.4. After 90 days incubation at -5 °C, functional gene abundance for the following genes: (A) catechol 2,3 dioxygenase (*C2,3O*), (B) naphthalene dioxygenase (*nahAc*), and (C) RNA polymerase beta subunit (*rpoB*), in $\log_{10}$(copies g^{-1}), were quantified in the bulk soil, charosphere soil, and biochar particles of a control and the bonemeal and bone chip biochar treatments. Bars represent the mean (n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different functional gene abundance (ANOVA, $P < 0.05$).

In a laboratory study that evaluated meat and bonemeal biochar amendments to frozen PHC contaminated soil, *C2,3O* and *nahAc* functional gene abundance increased over 90 days but there was no difference between the control and biochar treatments in homogenized soils (Karppinen et al., 2017b). However, this study did not include sampling location as a factor while the present study highlights that biochar effects are localized in specific spatial niches of the soil. Increased *C2,3O* and *nahAc* gene abundances correlate with increased polycyclic aromatic hydrocarbon (PAH) mineralization (Phillips et al., 2008). In this study, both *C2,3O* and *nahAc* gene abundance and ^{13}C-phenanthrene mineralization were elevated in the charosphere of bone chip biochar. However, for treatments in which ^{13}C-phenanthrene mineralization was negligible (i.e., 0-13%) such as bonemeal and bone chip biochar particles there was not a clear link to functional genes. There are many steps involved in PAH degradation (Liang et al., 2016), and *nahAc* may not be the limiting gene under frozen conditions.

5.5.5 Soil microbial community analysis

5.5.5.1 Community-level

Phosphate concentration in soil successfully predicted ($P < 0.10$) microbial community composition (Fig. 5.5). The distance-based redundancy analysis (db-RDA) explained 86% of the total variance and in contrast an unconstrained ordination explained 77% of the variance. Other soil factors such as ^{13}C-phenanthrene mineralization ($P = 0.65$) and changes in pH ($P = 0.88$) were not significant predictors of community composition. Similarly, average diversity indices, which have been normalized to *A. fischeri* and rarefied, showed no significant differences between treatments or sampling locations (Appendix C, Table C.1, ANOVA, $P > 0.05$).

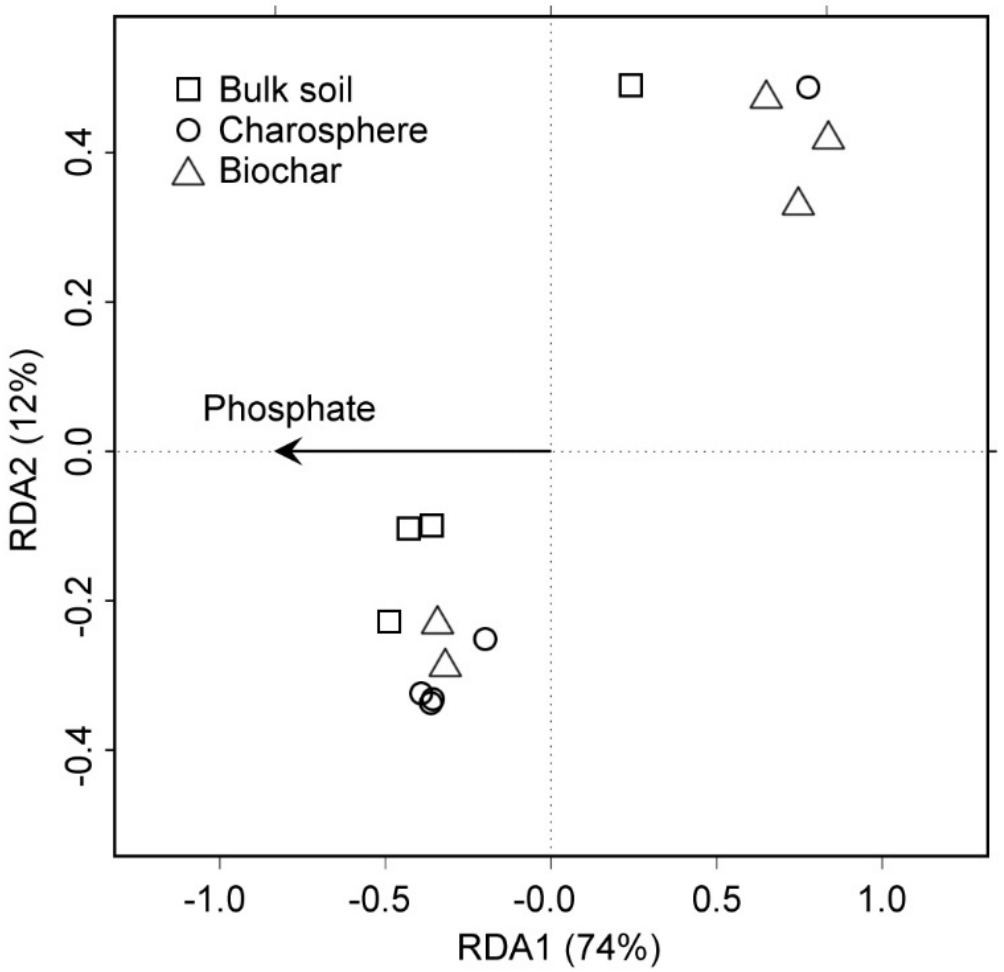

Fig. 5.5. Distance-based redundancy analysis (db-RDA) of bacterial community composition in bone chip biochar-amended soils, with environmental vectors. Symbols represent replicates of the bacterial communities present in each sampling location; bulk soil (□), charosphere (○), and biochar particles (△). Arrows indicate correlation between the environmental parameters and community structure of samples.

Supporting the results of this db-RDA analysis, PO_4^{3-} concentrations were orders of magnitude greater in bone chip biochar particles when compared to charosphere and bulk soils (ANOVA, $P < 0.05$). Two distinct spatial clusters are visible with the upper right cluster dominated by biochar particles, which might indicate that these communities are different but that this incubation temperature and time, i.e., -5 °C for 90 days, did not allow the microbial community to fully differentiate based on spatial sampling locations (Fig. 5.5). The relative percentages of phosphate minerals and available organic phosphorus influence microbial community composition in PHC contaminated and forest soils (DeForest and Scott, 2010; Siciliano et al., 2016). Combined with the observation that PO_4^{3-} absorption to biochar is linear (Sarkhot et al., 2013), it is possible that the spatial distribution of phosphate is driving a shift in microbial community in bone chip biochar particles.

5.5.5.2 Individual-level

In bone chip biochar-amended soil, seven genera showed significant differences between sampling locations (Fig. 5.6, Poisson Regression, $P < 0.05$). The relative abundances of *Mesorhizobium*, *Janthinobacterium*, *Pseudomonas*, and *Arthrobacter* decreased in both charosphere soil and biochar particles when compared to the bulk soil. However, the relative abundance of *Agrobacterium* increased in biochar particles while the relative abundances of *Caulobacter* and *Bosea* increased in charosphere soil. In all sampling locations, *Pseudomonas* was the most abundant genus, followed by *Agrobacterium*, *Bosea*, and *Arthrobacter*.

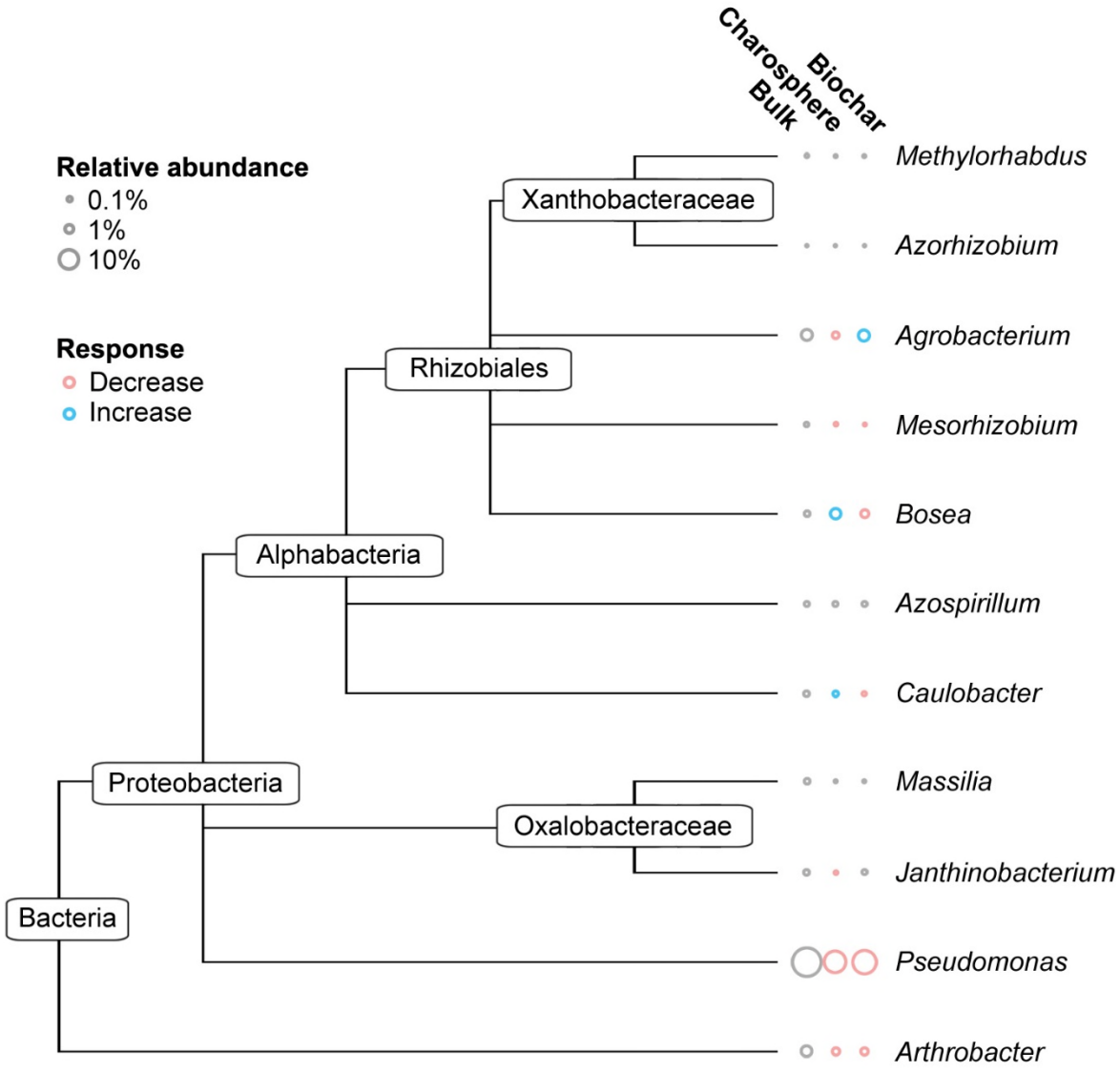

Fig. 5.6. Phylogeny and relative abundances of dominant (> 0.1%) OTUs in microbial consortia from bulk soil, charosphere soil, and biochar particles in bone chip biochar-amended soils. Circle colors indicate significantly greater (light blue) or lower (pink) abundances, respectively, in spatial sampling locations (Poisson Regression, $P < 0.05$).

Petroleum hydrocarbon contamination in soils can cause a reduction in species richness and shifts in the dominant phylotypes, and the addition of biochar also has been shown to affect microbial community composition (Taketani et al., 2013; Yang et al., 2016b). In this study, *Bosea* and *Caulobacter* are both gram-negative bacterium from the phylum *Proteobacteria* whose relative abundance increased in charosphere soil and decreased in biochar particles of bone chip biochar-amended soils. *Bosea* was identified as an inorganic sulfur-oxidizing bacterium able to fix nitrogen and degrade crude oil and other petroleum hydrocarbon compounds (Das et al., 1996; Seo et al., 2007; Jiao et al., 2016). *Caulobacter* has been isolated from Canadian artic soils contaminated with diesel fuel and shown to degrade alkane hydrocarbons and PAHs, even at low temperatures (Nierman et al., 2001; Mazzon et al., 2008; Bell et al., 2011; Yergeau et al., 2012). Furthermore, *Caulobacter* is characterized by its ability to elongate its stalk in order to increase phosphate uptake in nutrient-poor soils (Ong et al., 1990; Gonin et al., 2000). These results suggest that environmental conditions in the charosphere are unique and select for microorganisms that degrade petroleum hydrocarbons and increase nutrient bioavailability.

5.6 Conclusions

In this study, it was demonstrated that biochar creates a spatial distribution in the soil, due to the influence of biochar particles on charosphere soil. Traditional sampling methods, which consider the effects of biochar in homogenized soils, may be overlooking significant results which are masked when spatial sampling locations are not separated. In accordance with many biochar studies, the effects of chemical and biological properties of the charosphere soil are dependent on biochar type. In these soils, bone-derived biochars, specifically bone chip biochar, were most successful in stimulating ^{13}C-phenanthrene mineralization in frozen soils. The bone-derived biochars appear to be acting through a phosphate pathway, as it was only these biochars that kept the soil in the optimum phosphate solubility range (i.e., pH < 7.5). In the bone chip biochar-amended soils, phosphate was a significant determinant of the overall microbial communities and the increase by biochars occurred in the charosphere and corresponded to the enhancement of *Caulobacter*, a well-known psychrotrophic bacterium well suited to scavenging phosphate in nutrient poor soils. This research contributes to the knowledge base surrounding biochar applications to enhance PHC degradation in frozen soils and suggests that optimizing the charosphere in bone-derived biochars may increase remediation success in northern regions.

6. SYNTHESIS AND CONCLUSIONS

Currently, biochar research is focused in tropical and temperate regions; as well, laboratory and field studies that examine the effect of biochar on PHC degradation in soil are limited. While biochar has been proven an effective soil amendment for many ecosystem services, there is an alarming lack of consensus between findings and proposed mechanisms of action. The primary goal of these studies was to address several knowledge gaps surrounding biochar applications to enhance PHC degradation in northern soils. First, laboratory studies confirmed that biochar enhanced F3-PHC degradation in frozen soils by increasing θ_{liquid}, total PHC-degrading microorganisms, and aromatic functional gene abundance. Second, field studies determined that bone-derived biochars which are applied as incorporated slurries are the most effective at promoting PHC degradation in northern soils. Finally, a laboratory study identified that ^{13}C-phenathrene mineralization and other soil properties are spatially distributed in biochar-amended soils, and bone-derived biochars successfully enhanced mineralization and pH. These studies demonstrate that bone-derived biochars may be suitable amendments for PHC degradation in northern soils, but further evaluation is required to develop general recommendations for northern applications.

6.1 Summary of Findings

6.1.1 Petroleum hydrocarbon degradation

Biochar can enhance PHC degradation under frozen conditions. For instance, F3-PHC concentrations decreased by up to 28% and *k* values were significantly greater in meat and bonemeal biochar-amended soil than the control (Chapter 3). Meat and bonemeal biochar had no effect on F2 or F3-PHC degradation under thawed conditions, which prompted further investigation into biochar effects in frozen soils. Regardless of feedstock, frozen soil with biochar additions had significantly less F2-PHCs on Day 90, but F3-PHCs only decreased over 90 days in bonemeal biochar-amended soils (Chapter 4). Under frozen conditions with limiting environmental conditions (i.e., θ_{liquid}, nutrient availability, and gas exchange), positive effects were more pronounced than in thawed soils.

In both years of field trials, differences in F2 or F3-PHC degradation were minimal when comparing biochar-amended soils and control soils (Chapter 4). For example, from Day 0 to Day 31, F2-PHCs significantly decreased in fishmeal biochar-amended soils, but on Day 31 there was no difference in F2-PHC concentration between soil amended with fishmeal biochar and the control. These results suggest that small, transient changes in soil properties and PHC degradation resulting from biochar additions are likely masked by the inability to control inherently heterogeneous environmental conditions. Across all biochar types and controls, incorporation caused a rapid (within 31 days) decrease in F2 and F3-PHC soil concentrations, whereas injection exhibited a delayed decrease (up to 334 days). Incorporated treatments resulted in soil which was below concentration guidelines before the winter season, which is important in northern regions with short growing seasons.

6.1.2 Physical and chemical soil properties

Biochars can contain a large supply of nutrients and selectively increase nutrient availabilities. Available NH_4^+-N, NO_3^--N, and PO_4^{3-}-P did not increase in biochar-amended soils under thawed or frozen conditions, but meat and bonemeal biochar supplied more PO_4^{3-}-P than compost in thawed soils (Chapter 3). In both years of field trials, NH_4^+-N, NO_3^--N, and PO_4^{3-}-P availabilities increased in some biochar-amended soils, but results varied between location and study year (Chapter 4). High extractable nutrient concentrations in biochar particles demonstrated that biochars can potentially supply significant amounts of nutrients (Chapter 5). In thawed soils, biochar-amended soils contained more NH_4^+-N, NO_3^--N, and PO_4^{3-}-P than the control, which was attributed to a concentration of nutrients, specifically PO_4^{3-}-P, in biochar particles.

Biochar can increase θ_{liquid}, soil temperature, and pH. Under frozen conditions, θ_{liquid} was higher in meat and bonemeal biochar-amended soil than the control (Chapter 3). In both years of field trials, θ_{liquid} was higher in fishmeal biochar-amended soils than controls, but soil temperature only increased in the first year of field trials (Chapter 4). Fishmeal and wood biochars increased pH in frozen soils (Chapter 5). To my knowledge, this is the first time θ_{liquid} has been measured in frozen, biochar-amended soils, and these results demonstrate that biochars can enhance certain physical and chemical soil properties under both thawed and frozen conditions.

6.1.3 Soil microbial community

Biochar can influence individual soil microorganisms and alter the soil microbial community. In frozen soils, aromatic (*C2,3O* and *nahAc*) functional gene abundance increased over time in both bonemeal biochar-amended and control treatments, whereas total PHC-degrading populations increased only in bonemeal biochar-amended soils (Chapter 3). This combination of increased microbial activity unique to aromatic and total PHC-degraders was linked to the reduction in F3-PHC concentrations under frozen conditions. Similarly, aromatic functional gene abundance (*C2,3O* and *nahAc*) was higher in frozen, PHC contaminated soil with bone-derived biochar additions (Chapter 5). However, this increase in aromatic functional gene abundance was not linked to increased ^{13}C-phenanthrene mineralization. In the first year of field trials, wood biochar inhibited both aliphatic (*alkB*) and aromatic (*C2,3O* and *nahAc*) functional gene abundance, but there was no indication that PHC degradation was affected (Chapter 4). Although the soil microbial community was sensitive to biochar applications, this was not clearly linked to PHC degradation.

6.1.4 Biochar feedstock

Bone-derived biochars were more effective in stimulating PHC degradation than those derived from wood. In Chapter 3, meat and bonemeal biochar increased F3-PHC degradation rates, θ_{liquid}, aromatic functional gene abundance, and total PHC degraders in frozen soil. In Chapter 4, fishmeal biochar influenced F2-PHC degradation under field conditions and bonemeal biochar increased F3-PHC degradation in frozen soil. In field trials, nutrient availabilities increased regardless of feedstock, but θ_{liquid} and soil temperature increased only in soils amended with fishmeal biochar. In several chapters, the effectiveness of bone-derived biochars was linked to its inherent physicochemical properties such as ash content, pore size, surface area, and CEC, which are determined by feedstock source and pyrolysis conditions.

6.1.5 Application method

Homogenous incorporated slurries were the most effective biochar application method in northern landfarms. In Chapter 4, incorporated biochar slurries decreased F2 and F3-PHC concentrations faster than injected biochar slurries. Incorporated biochar slurries also had greater NO_3^- availability, θ_{liquid}, and aromatic functional gene abundance. The process of homogenization, which aerates the soil and promotes soil-biochar contact, was thought to

stimulate PHC degradation and enhance NO_3^- availability and θ_{liquid}. Chapter 5 confirmed that the spatial distribution between biochar particles and surrounding soil dictates the response of many soil properties related to PHC degradation.

6.1.6 Biochar-soil spatial relationship

Microbial degradation is influenced by the spatial relationship between biochar particles and the surrounding soil. In Chapter 5, ^{13}C-phenanthrene mineralization increased in the charosphere of bonemeal and bone chip biochar-amended soils. In bone chip biochar treatments, the charosphere soil had higher aromatic catabolic functional gene abundance than the bulk soil or biochar particles. Soil pH remained near neutral after bone chip biochar additions, and the relative abundances of *Bosea* and *Caulobacter* increased in charosphere soil. Furthermore, PO_4^{3-} concentration successfully predicted microbial community abundance, which may be linked to pH and phosphate solubility. These results demonstrate that the spatial distribution of biochar in soil is an important mechanism driving PHC degradation and improved soil properties in northern soils, and the charosphere has unique conditions which select for microorganisms that degrade PHCs and increase phosphate availability.

6.2 Conclusions

The overall goal of these studies was to evaluate biochar as a soil amendment to enhance PHC degradation in northern landfarms and contribute to the understanding of specific soil-biochar interactions in northern soils. The degradation of PHCs was influenced by many interrelated factors, including the inherent properties of soil and biochar, environmental conditions, application method, and soil-biochar contact. The biochars assessed in these studies were incubated under comparable environmental conditions in the laboratory and field yet facilitated different amounts of PHC degradation and altered the physical, chemical, and biological properties of soil to varying degrees. Most notably, biochar increased F3-PHC degradation and θ_{liquid} in frozen soils, bone-derived biochars applied as incorporated slurries were the most effective at promoting PHC degradation under field conditions, and biochar created a spatial distribution in the soil due to the influence of biochar particles on charosphere soil. Other results indicated that biochar had a positive effect on PHC degradation rates and certain soil properties but were highly variable and it was often difficult to draw strong conclusions. Collectively, these individual studies demonstrate that bone-derived biochars have significant

potential to increase PHC degradation rates and enhance the physical, chemical, and biological properties of northern soils, but also highlight the need for further research into methods to provide a more spatially consistent beneficial effect. To the best of my knowledge, this is the first study to investigate the use of biochar as a soil amendment in frozen soils and to enhance PHC degradation in northern landfarms.

6.3 Significance

The significance of this research lies in the knowledge gained concerning PHC degradation and biochar application in northern landfarms. The successful implementation of *ex-situ* remediation technologies that are tailored for northern landfarms results in increased PHC degradation rates and soil turnover. The cost of remediating contaminated soil in northern Canada is often a limiting factor, and by optimizing environmental conditions to improve PHC degradation rates using low maintenance remediation technologies, more contaminated soil can be processed in less time with less cost. There is also an opportunity to reduce local waste by creating biochars with appropriately selected waste material. Technology transfer between, and increased capacity of northern biochar producers will improve the likelihood for effective remediation but will also provide economic benefit for companies operating in northern regions due to reduced northern logistics and shipping costs. However, several issues with the practicality of producing and applying biochars in northern environments were identified, including; difficulties associated with producing biochars with consistent properties using small-scale burning units, biochar's significant powder fraction that can become airborne in light wind during transport or application, and high application rates (i.e., 6% [v/v] converts to 7-43 t ha^{-1} depending on the biochar's bulk density). Overall, working with local industry to develop and improve remediation technologies, methods and protocols that work in northern soils is essential to alleviate current challenges to cost-effective remediation.

6.4 Future Research

This dissertation highlights that further studies are required to (i) understand how biochar applications can benefit PHC remediation in northern landfarms and (ii) determine how practical the production and application of biochars are in northern locations. Rather than simply quantifying PHC degradation and the associated physical, chemical, and biological soil properties, there is also a need to identify the specific mechanisms driving the positive effects of

biochar in northern soils. Based on the results obtained from laboratory and field studies, several knowledge gaps prompted potential areas of future research.

6.4.1 Field studies

Northern soils are unique and there have been few studies which measure the response of F2 and F3-PHCs and associated soil properties under field conditions. Several studies have indicated that positive responses to biochar amendments observed in short-term laboratory experiments were not paralleled in field studies (Jones et al., 2012). In field studies, certain physical, chemical, and biological soil properties, including θ_{liquid}, NO_3^- availability, and functional gene abundance, were enhanced, but results were inconsistent between locations (Chapter 4). There was little difference in PHC degradation in biochar-amended and control soils at both locations and F2 and F3-PHC concentrations were relatively low. To confidently recommend the use of biochar as a soil amendment in northern landfarms, field studies conducted over a range of PHC concentrations and on sites with varying soil and climactic condition should be evaluated. Although enhancing soil properties is important for the microbial community and overall soil productivity, this must be reflected by enhanced degradation rates to become a practical and economical soil amendment for northern landfarms.

6.4.2 Linking biochar, phosphate availability, and PHC degradation

Nutrient availabilities, specifically N and P, are limiting factors for PHC degradation in northern soils (Mohn and Stewart, 2000). Studies have shown that total phosphate and phosphate mineralogy are linked to PHC degradation rates and microbial community composition in calcareous soils (Siciliano et al., 2016). Biochar may also directly and indirectly influence P availability in soil by altering soil pH (Ippolito et al., 2016) and by changing nutrient sorption and leaching dynamics (Yao et al., 2012). Although biochar additions can increase PO_4^{3-} availability (Chapter 4) and certain biochars contain a large supply of extractable PO_4^{3-} (Chapter 5), observations were inconsistent and a direct link between increased PO_4^{3-} availability in biochar-amended soils and enhanced PHC degradation was not established. Upon further investigation, extractable PO_4^{3-} successfully predicted microbial community composition, and increased the relative abundance of *Caulobacter*, a psychrotrophic bacterium capable of accessing PO_4^{3-} in the charosphere of nutrient poor soils (Chapter 5). These results suggest that P availability could be an important mechanism driving PHC degradation in

northern soils, and future research should attempt to characterize the link between P availability and biochar additions and indicate how this influences soil microorganisms and PHC degradation.

6.4.3 Further evaluation of the charosphere

The charosphere is described as the soil immediately surrounding and influenced by biochar particles, and has been identified as a separate spatial niche that has unique properties from biochar particles and bulk soil (Quilliam et al., 2013a; Wang et al., 2017). Charosphere soil had higher ^{13}C-phenanthrene mineralization, aromatic catabolic gene abundance, and relative abundances of PHC-degrading bacteria, but this was dependent on biochar feedstock (Chapter 5). This study was limited by the amount of charosphere soil that was available for laboratory analysis. Further evaluation of the role of the charosphere in laboratory and field soils could reveal other soil properties that are spatially distributed in biochar-amended soils. For example, biochar additions influenced θ_{liquid}, pH, and F2 and F3-PHC concentrations in frozen soils (Chapter 3; Chapter 4), but these responses might have been masked by the homogenization of spatial sampling locations. Additional data which is separated into specific spatial niches (i.e., bulk soil, charosphere soil, biochar particles) would be useful for determining the role of the charosphere in enhancing PHC degradation in northern soils.

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APPENDIX A. SUPPLEMENTAL MATERIAL (CHAPTER 3)

A.1 CCME CWS for Petroleum Hydrocarbons in Soil

Petroleum hydrocarbons (PHCs) are separated into fractions based on their relevant physical and chemical properties and toxicological characteristics. For the purposes of the Canadian Council of Ministers of the Environment (CCME) Canada-wide standards (CWS), aliphatic and aromatic PHCs are grouped according to normal straight chain hydrocarbon boiling point ranges; Fraction 1 (F1): C_6-C_{10}; Fraction 2 (F2): > C_{10}-C_{16}; Fraction 3 (F3): > C_{16}-C_{34}, and; Fraction 4 (F4): > C_{34} (CCME, 2008). Generally, F1 and F2-PHCs contain low molecular weight compounds which are more soluble and volatile, while F3 and F4-PHCs contain high molecular weight compounds, which immobilize and persist in the environment. The Government of Nunavut's remedial guidelines are based on the CCME CWS for PHCs in soil. The guidelines were developed for four generic land uses; residential (residential or recreational activity), agricultural (producing crops or tending livestock), commercial (free access to members of the public) and industrial (production, manufacture or construction of goods), and two soil textures; coarse (> 75 μm) and fine (≤ 75 μm) -grained soils (CCME, 2008). For each land use and soil texture, separate, conservative guidelines for acceptable contamination levels have been established for F1-F4 PHCs (Table A.1). The most relevant guidelines are residential land use, coarse-grained soils as remediated soil is often returned to residential areas and soils are coarse-textured (> 90% sand).

Table A.1 A summary of Tier 1 petroleum hydrocarbon (PHC) standards, in mg kg^{-1}, for surface soil, by land use and soil texture (adapted from CCME, 2008).

Land Use	Soil Texture	CCME Fraction			
		F1	F2	F3	F4
		---------- mg kg^{-1} ----------			
Agricultural	Coarse-grained	30	150	300	2,800
	Fine-grained	210	150	1,300	5,600
Residential	Coarse-grained	30	150	300	2,800
	Fine-grained	210	150	1,300	5,600
Commercial	Coarse-grained	320	260	1,700	3,300
	Fine-grained	320	260	2,500	6,600
Industrial	Coarse-grained	320	260	1,700	3,300
	Fine-grained	320	260	2,500	6,600

A.2 Petroleum Hydrocarbon Degradation in Thawed Soils

In thawed soils, there was no significant F2 or F3-PHC degradation over time (Fig. A.1, ANOVA, $P > 0.05$). Although insignificant, it appears that fertilizer and 5% compost had the greatest F2-PHC removal while 5% compost had the greatest F3-PHC removal. Relative standard deviations in each treatment were very high (up to 52% and 28% for F2 and F3-PHCs, respectively).

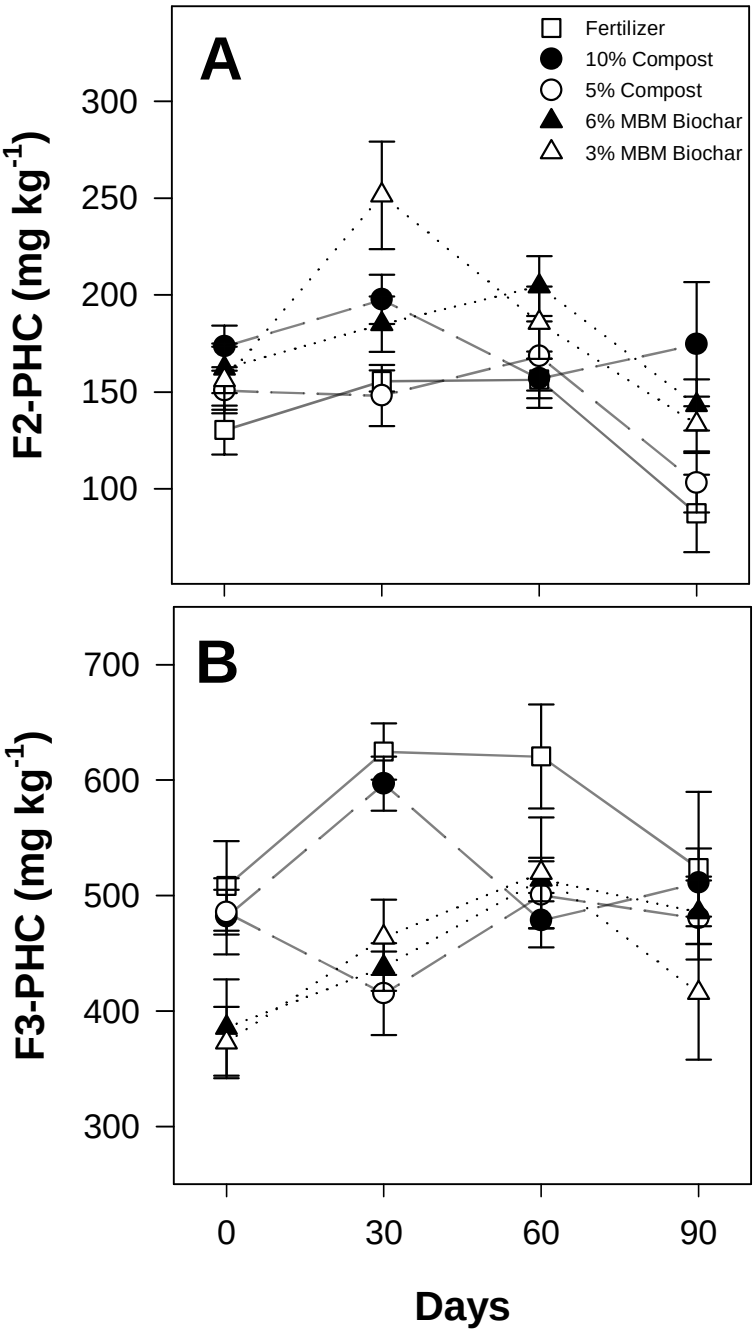

Fig. A.1 Petroleum hydrocarbon (PHC) concentrations, in mg kg^{-1}, were measured in soil incubated under thawed conditions (10 °C) over a 90 day period. Five treatments, fertilizer, 5% (w/w) compost + fertilizer, 10% (w/w) compost + fertilizer, 6% (w/w) meat and bonemeal (MBM) + fertilizer, and 3% (w/w) MBM biochar + fertilizer, were applied. Each point is the average concentration (n = 8) of (A) F2-PHCs (C_{10}-C_{16}) and (B) F3-PHCs (C_{16}-C_{34}), with standard error bars representing the standard error of the mean.

APPENDIX B. SUPPLEMENTAL MATERIAL (CHAPTER 4)

B.1 Biochar Application Methods

Biochar applied as slurries that are injected, incorporated, or top-dressed are summarized in Table B.1. Practically, only injected and incorporated slurries were used for field experiments, as these application methods were most logistically suited for the two northern locations.

Table B.1 A comparison of various application technologies used to apply biochar to soil.

Method	Technique	Advantages	Disadvantages	References
Incorporation (Neat)	Biochar is applied neat (without additional water) and homogenized into the soil.	Biochar can be applied using a variety of equipment and a water source is not required.	Powder fraction can be lost during transport or application and soil must be turned periodically.	Husk and Major, 2011.
Point Injection	Inject biochar slurry into soil; it should diffuse throughout the soil.	Eliminates need for mixing and turning. Biochar can be applied *in-situ*.	Biochar is concentrated in one area and could have limited diffusion from the injection point. Flow blockages can occur if biochar particles are too large or application rate is too high.	Janzen and Lindwall, 1989; Major, 2010.
Incorporation (Slurry)	Biochar slurry is homogenized into the soil for even distribution of biochar.	Viable application method for *ex-situ* operations.	Soil must be turned periodically.	Major, 2010.
Top-dressing	Biochar slurry is applied to the surface of the soil, even on sloping terrain.	Eliminates mixing and turning while supplying moisture. Biochar can be applied *in-situ*.	Intense precipitation can erode biochar from steep slopes or even level surfaces.	Major, 2010; Blackwell, 2009; Lehmann, 2009; Stewart, 2013.

B.2 Soil and Biochar Characteristics

Soil properties from landfarms in Whitehorse, YT and Iqaluit, NU (Table B.2) and biochar and compost amendment properties (Table B.3) were measured. Those measured include texture, pH, total organic carbon, and available nutrients (NO_3^--N and PO_4^{3-}-P), while amendment properties include pH, pyrolysis temperature, Brunauer-Emmet-Teller surface area, average pore volume, average pore size, cation exchange capacity, and ash content.

Table B.2 Soil characteristics at Whitehorse, YT and Iqaluit, NU.

Soil Property	Whitehorse	Iqaluit
Texture	SL	S
pH	8.28	8.11
TOC† (%)	0.32	0.38
NO_3^--N‡ (mg kg^{-1})	3.4	1.9
PO_4^{3-}-P (mg kg^{-1})	2.0	6.2

†Total organic carbon
‡Available NO_3^--N was extracted using calcium chloride; available PO_4^{3-}-P was extracted using modified Kelowna

Table B.3 Characterization of biochar and compost amendments used in laboratory and field studies.

Amendment Property	Biochar Amendments			Compost Amendments	
	Titan Fishmeal	Zakus Bonemeal[£]	Zakus Wood	Boreal Compost[§]	Iqaluit Compost[§]
pH	9.0	9.5	8.9	8.2	7.4
Pyrolysis temperature (°C)	500	450	450	-	-
BET surface area[†] ($m^2\ g^{-1}$)	7.1	110	78	-	-
Pore volume ($cm^3\ g^{-1}$)	0.025	0.299	0.006	-	-
Pore size (nm)	13.4	10.6	2.2	-	-
CEC[‡] ($cmol_c\ kg^{-1}$)	54	48	7	90	90
Ash (%)	32	82	54	-	-

[†]BET = Brunauer-Emmet-Teller
[‡]CEC = cation exchange capacity
[£]Average of two different feedstocks used over the course of laboratory and field studies.
[§]Only pH and CEC were measured in compost amendments.

B.3 Field Trial Schematic

To aid visualization, a schematic summarizing site locations, average climatic conditions, and field trials is presented (Fig. B.1).

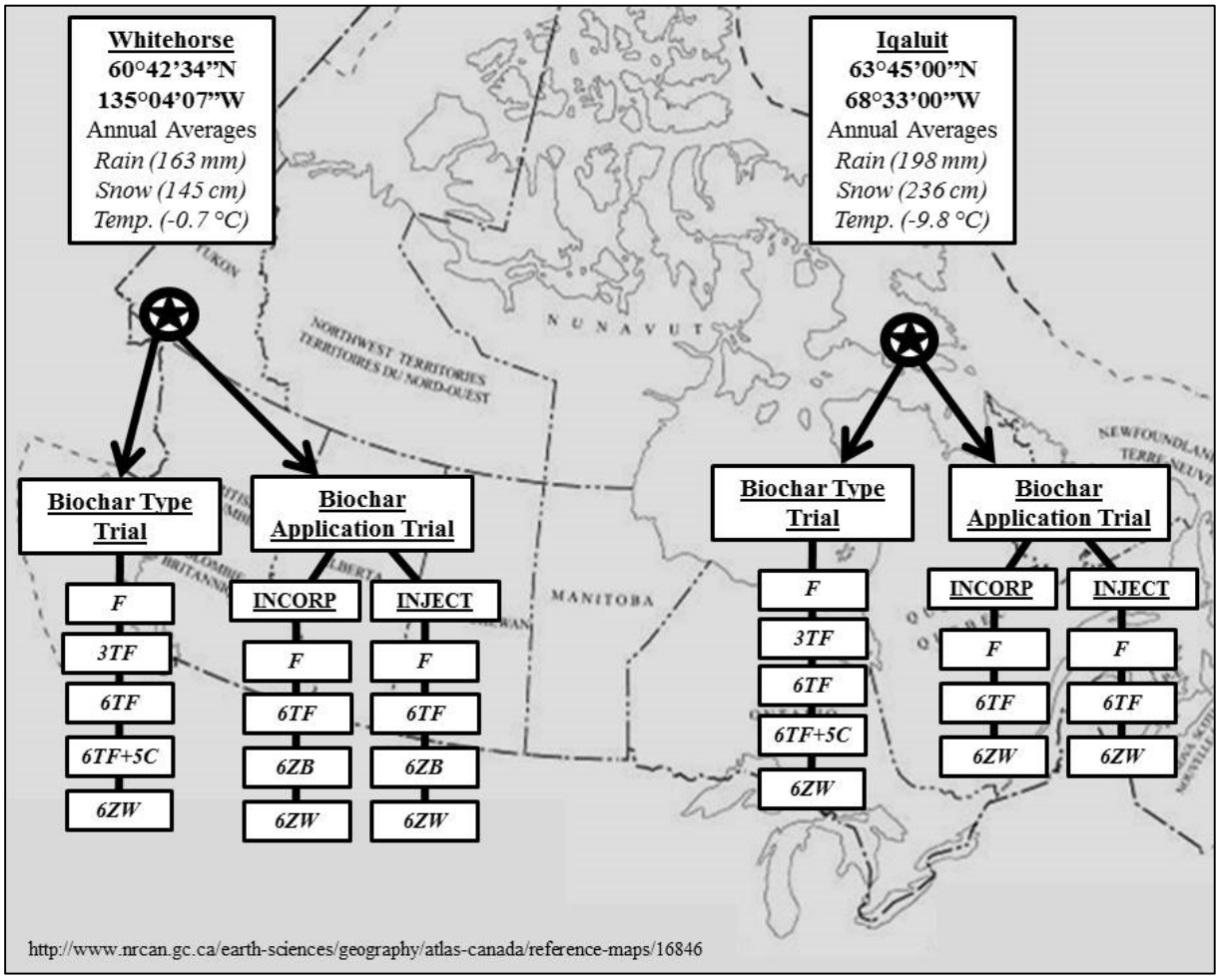

Fig. B.1 A summary schematic summarizing site locations, average climatic conditions, and field trials. Treatments are abbreviated as: fertilizer (F), 3% Titan fishmeal biochar + fertilizer (3TF), 6% Titan fishmeal biochar + fertilizer (6TF), 6% Titan fishmeal biochar + 5% compost + fertilizer (6TF+5C), 6% Zakus bonemeal biochar + fertilizer (6ZB), and 6% Zakus wood biochar + fertilizer (6ZW), while application methods are abbreviated as: incorporation (INCORP) and injection (INJECT). Map and average climatic condition were obtained from Government of Canada online resources.

B.4 Petroleum Hydrocarbon Analytical and Quality Control Procedures

Two different PHC extraction methods were utilized during the completion of the laboratory and field trials. Petroleum hydrocarbons were extracted from Whitehorse soil samples at the Yukon Research Center using a mechanical shaking method (Schwab et al., 1999), while a hexane shaking method was used at the University of Saskatchewan to extract PHC from Iqaluit soil samples (Mcintyre et al., 2007). Average C_{10}, C_{16}, and C_{34} spike recoveries from both methods are presented in Table B.4.

Table B.4 Comparison of average spike recoveries from sterile quartz sand spiked with C_{10}, C_{16}, and C_{34} petroleum hydrocarbon (PHC) standards, using mechanical and hexane shaking methods.

Method	Average Spike Recovery		
	C_{10}	C_{16}	C_{34}
	---------- % ----------		
Mechanical Shaking	96.3	94.7	68.9
Hexane Shaking	92.3	93.3	69.0

B.4.1 Mechanical Shaking Method

Approximately 2 g of field moist soil was weighed into a glass scintillation vial, acetone (10 mL) was added to each vial and it was placed on an Eberbach E-6010 fixed-speed reciprocal shaker (Eberbach™ Corporation, Ann Arbor, MI) for 1 hr at 120 cycles min^{-1}. The contents were then transferred to a 15 mL Falcon® tube and centrifuged for 10 min at 1500 rpm. The extract was decanted and another 10 mL of acetone was added and the shaking process repeated. To remove water and polar organic compounds from the sample, a PHC cleanup procedure outlined by CCME (2008) was followed. First, glass wool was inserted into the tip of a 50 cm glass column and 8 g anhydrous sodium sulphate was added. The PHC extract was then added to the column and eluted with 10 mL of hexane which was collected in a round bottom flask. Approximately 1.8 mL of toluene was added to the eluted material in preparation to evaporate the sample using a Buchi® R-210 Rotavapor® (Buchi Corporation., New Castle, DE). To further purify the sample, glass wool was inserted into the tip of a 50 cm glass column and filled with 5 g of silica gel (previously baked at 100°C for 24 hrs and stored in a desiccator) overlain by 2.5 g of anhydrous sodium sulphate. The sample was then transferred to the column, eluted with 40 mL of hexane and collected in a round bottom flask. Finally, the eluted material

was evaporated to 1.0-1.8 mL and transferred to a 2 mL GC vial. To ensure quality control, a blank and reference spike, were extracted every 20 samples.

B.4.2 Hexane Shaking Method

Approximately 10 g of field moist soils were mixed with 10 mL each of water and hexane in 30 mL Teflon® tubes, shaken at 200 rpm overnight on a Barnstead-Labline rotator (Thermo Scientific, Lenexa, KS) and centrifuged for 10 mins at 1500 rpm. An aliquot of the hexane layer was pipetted from the tube and transferred to a 2 mL gas chromatography (GC) vial. To ensure quality control, a blank and reference spike were extracted every 20 samples.

B.5 qPCR Reaction and Amplification Conditions

Functional gene abundance was quantified for alkane monooxygenase (*alkB*) (Powell et al., 2006a), catechol 2,3 dioxygenase (*C2,3O*) (Baldwin et al., 2003), and naphthalene dioxygenase (*nahAc*) (Whyte et al., 1997; Luz et al., 2004). To generate a quantitative polymerase chain reaction (qPCR) gene reaction mix, 4 µL template DNA was added to a 15 µL final volume mixture containing QuantiTect SYBR® Green RT-PCR Kit (Qiagen, Valencia, CA), betaine or bovine serum albumin (BSA) and 1 µM each of forward and reverse primers. After 10 minutes initial denaturing at 94 °C, amplification proceeded with 40 cycles of 1 minute denaturing at 94 °C, 30 seconds annealing at the appropriate temperature, 1 minute extension at 72 °C, followed by a melt curve analysis for 15 seconds at 95 °C, 1 minute at 60 °C and 15 seconds at 95 °C (Table B.5). Both *C2,3O* and *nahAc* genes required touchdown qPCR from the appropriate annealing temperature. Quality control measures included matrix blanks and a standard curve prepared in triplicate, ranging from 10^8 to 10^1 gene copies μL^{-1}. All PCR fragments were visualized on SYBR® Safe-stained 1.5% agarose gels to ensure target gene amplification.

Table B.5 Primers and amplification conditions used for quantitative polymerase chain reaction (qPCR) amplification of alkane monooxygenase (*alkB*), catechol 2,3 dioxygenase (*C2,3O*), and naphthalene dioxygenase (*nahAc*).

Target gene †Primer sequence	‡Ta (°C)	Primer (µM)	Expected size (bp)	Control Strain
Alkane monooxygenase (*alkB*)	50.0	1.0	100	*P. putida*
F: 5'- AAC TAC ATC CGA GCA CTA CGG				ATCC 29347
R: 5'- TGA AGA TGT GGT TGC TGT TCC				
Catechol 2,3 dioxygenase (*C2,3O*)	65.6	1.0	406	*P. putida*
F: 5'- AGG TGC TCG GTT TCT ACC TGG CCGA				ATCC 29347
R: 5'- ACG GTC ATG AAT CGT TCG TTG AG				
Naphthalene dioxygenase (*nahAc*)	47.0	1.0	377	*P. putida*
F: 5'- CAA AAR CAC CTG ATT YAT GG				ATCC 17484
R: 5'- AYR CGR GSG ACT TCT TTC AA				

†Forward (F) and reverse (R) primers are indicated.

‡Ta = annealing temperatures used during real-time PCR.

B.6 CCME CWS for Petroleum Hydrocarbons in Soil

For the purposes of the Canadian Council of Ministers of the Environment (CCME) Canada-wide standards (CWS), aliphatic and aromatic petroleum hydrocarbons (PHCs) are grouped according to normal straight chain hydrocarbon boiling point ranges; Fraction 1 (F1): C_6-C_{10}; Fraction 2 (F2): > C_{10}-C_{16}; Fraction 3 (F3): > C_{16}-C_{34} and Fraction 4 (F4): > C_{34} (CCME, 2008). The guidelines were developed for four generic land uses; residential (residential or recreational activity), agricultural (producing crops or tending livestock), commercial (free access to members of the public), and industrial (production, manufacture or construction of goods), and two soil textures; coarse (> 75 μm) and fine (≤ 75 μm) (CCME, 2008). For each land use and soil texture, separate, conservative guidelines for acceptable contamination levels have been established for F1-F4 PHCs (Table B.6). The most relevant guidelines are residential land use and coarse-grained soils as remediated soil is often returned to residential areas and experimental soils are coarse-textured (> 90% sand).

Table B.6 A summary of Tier 1 petroleum hydrocarbon (PHC) standards, in mg kg^{-1}, for surface soil, by land use and soil texture (adapted from CCME, 2008).

Land Use	Soil Texture	CCME Fraction			
		F1	F2	F3	F4
		---------- mg kg^{-1} ----------			
Agricultural	Coarse-grained	30	150	300	2,800
	Fine-grained	210	150	1,300	5,600
Residential	Coarse-grained	30	150	300	2,800
	Fine-grained	210	150	1,300	5,600
Commercial	Coarse-grained	320	260	1,700	3,300
	Fine-grained	320	260	2,500	6,600
Industrial	Coarse-grained	320	260	1,700	3,300
	Fine-grained	320	260	2,500	6,600

B.7 Biomarker Analysis

Over time, PHC reductions in soil were a result of microbial catabolism, rather than volatilization, regardless of application method or treatment. For example, in the Biochar Application Trial, C_{17}/Pr ratios showed a significant decreasing trend in all treatments, with an average of 1.283 at Day 0 to 0.678 at Day 304 (Fig. B.2; ANOVA, $P < 0.05$). Similarly, C_{18}/Ph ratios also significantly decreased in all treatments from an average of 1.243 at Day 0 to 0.469 at Day 304 (Fig. B.2, ANOVA, $P < 0.05$). The complete removal of *iso*-alkanes, pristane and phytane, by Day 304 occurred in all but one incorporated treatment whereas only one injected treatment exhibited this behavior. The disappearance of these compounds combined with significant reduction in PHC concentrations in soil by Day 31 suggests that microbial degradation proceeded more quickly in incorporated treatments. It is well established that *ex-situ* bioremediation is a viable remediation strategy in harsh, northern environments and that fertilization of PHC contaminated soil can successfully increase bioremediation rates. Paudyn et al. (2008) monitored C_{17}/Pr in aerated trial landfarm plots in Iqaluit and found that isoprenoid ratios decreased in fertilized plots but not in unfertilized plots. Similarly, Ferguson et al. (2003) found that low nutrient concentration rather than water was the main limiting factor for PHC degradation in Antarctic soil incubated at 10 °C. All soils contain the same amount of fertilizer, so it is expected that nutrients will not be a limiting factor, rather, that either application method or biochar additions would change isoprenoid ratios over time, but these factors were not significant.

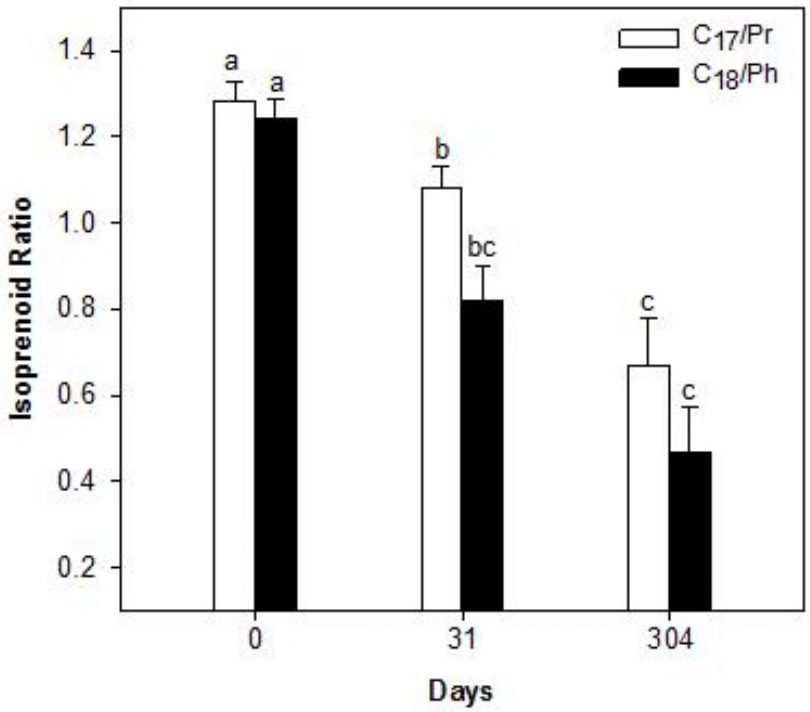

Fig. B.2 Isoprenoid ratios C_{17}/Pr and C_{18}/Ph measured in Iqaluit over 304 days. Symbols represent the mean (n = 6), with the error bars representing the standard error of the mean. Different letters indicate significantly lower C_{17}/Pr and C_{18}/Ph isoprenoid ratios over time (ANOVA, $P < 0.05$).

B.8 Petroleum Hydrocarbon Degradation

B.8.1 Biochar Type Trial

PHC degradation was evident over time in the Biochar Type field trials, but biochar type differences were only detected in F2-PHCs (Fig. B.3). For example, average F2-PHCs in Iqaluit were the same in the fertilizer control and biochar types, but biochar type differences resulted between 6% Titan fishmeal plus fertilizer (454 mg kg^{-1} [SE = 111]) and 3% Titan fishmeal plus fertilizer (575 mg kg^{-1} [SE = 129]) (ANOVA, $P < 0.05$).

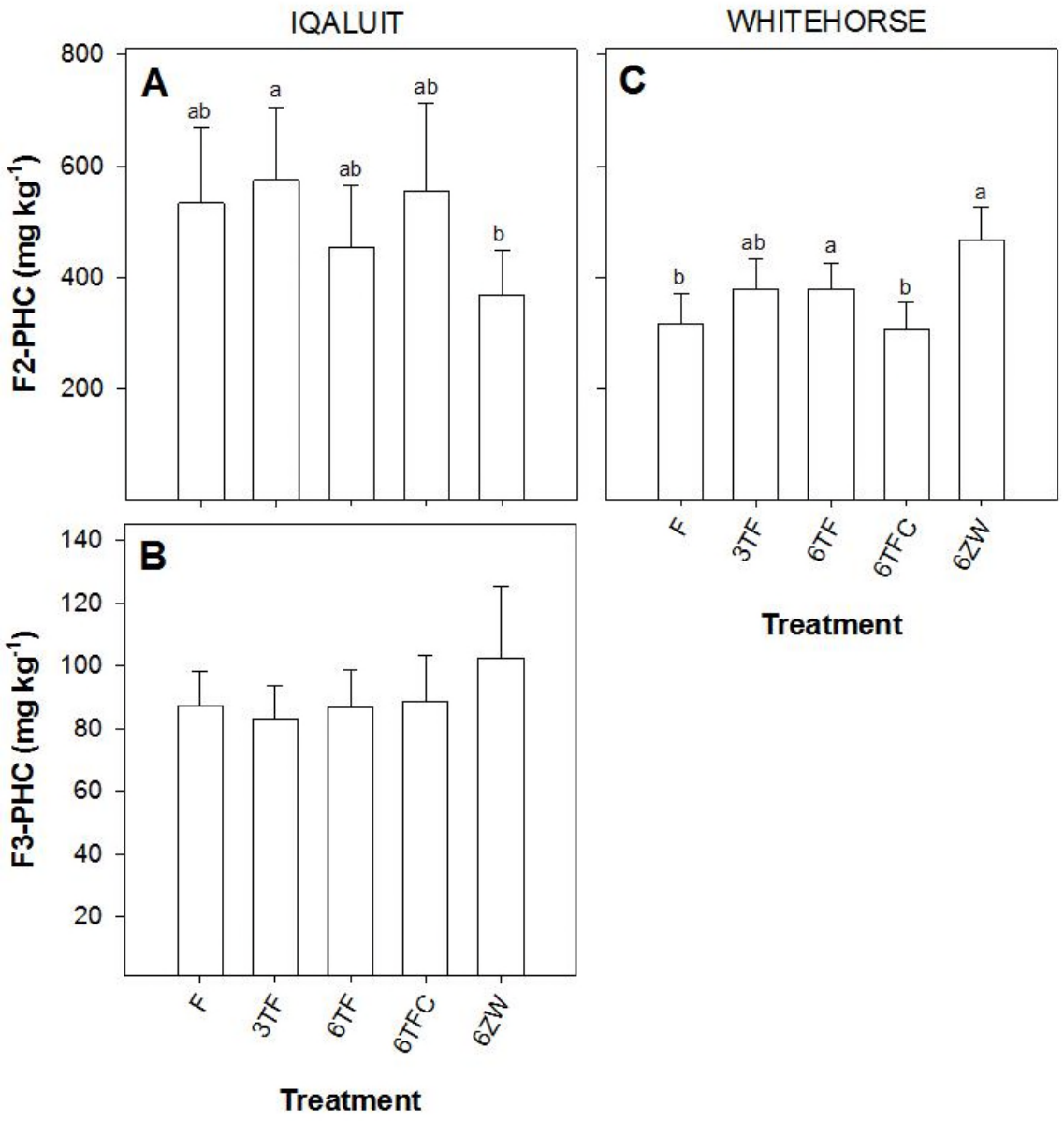

Fig. B.3 In the Biochar Type Trial, F2 and F3-petroleum hydrocarbon (PHC) concentration in soil were measured in Iqaluit (Graphs A-B) and Whitehorse (Graph C). Treatments included fertilizer (F), 3% Titan fishmeal biochar plus fertilizer (3TF), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Titan fishmeal biochar plus 5% compost plus fertilizer (6TFC), and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean (n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different F2 and F3-PHC concentrations between treatments (ANOVA, *P* < 0.05).

B.8.2 Pilot Study

All biochar types had less average F2 and F3-PHCs in soil when compared to the fertilizer control (Fig. B.4, ANOVA, $P < 0.05$). For example, the fertilizer control had an average of 721 mg kg^{-1} (SE = 56) F2-PHCs, while 6% Titan fishmeal biochar plus fertilizer, 6% Zakus bonemeal biochar plus fertilizer and 6% Zakus wood biochar plus fertilizer had an average of 511 (SE = 31), 466 (SE = 39) and 536 (SE = 35) mg kg^{-1} F2-PHCs, respectively.

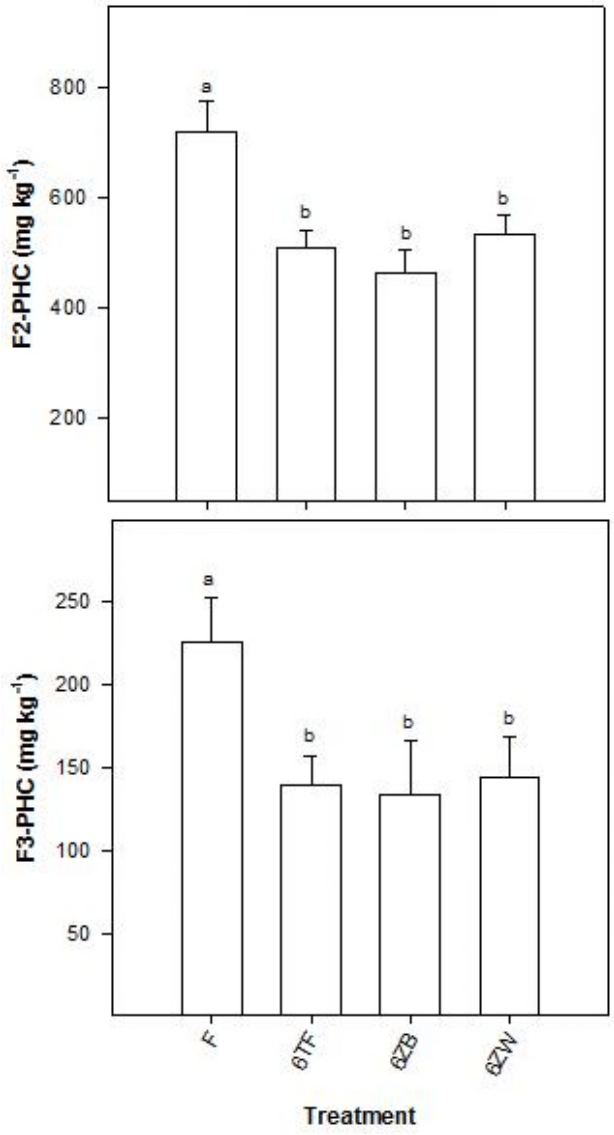

Fig. B.4 Average F2 and F3-petroleum hydrocarbon (PHC) concentration in biochar plus fertilizer-amended soils and the fertilizer control under frozen conditions. Treatments included fertilizer (F), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Zakus bonemeal biochar plus fertilizer (6ZB) and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean (n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different average F2 and F3-PHC concentrations between treatments (ANOVA, $P < 0.05$).

B.9 Nutrient Supply Rates

In the Biochar Application Trial in Whitehorse, NO^{3-}-N and PO_4^{3-}-P availability differed significantly between treatments (ANOVA, $P < 0.05$). The availability of NO^{3-}-N was greater in 6% Zakus bonemeal and wood biochars plus fertilizer than the fertilizer control, but 6% Titan fishmeal biochar plus fertilizer was not different (Fig. B.5). The availability of PO_4^{3-}-P was significantly higher in 6% Titan fishmeal biochar plus fertilizer when compared to fishmeal-derived biochar and the fertilizer control but was not different than wood-derived biochar (ANOVA, $P < 0.05$).

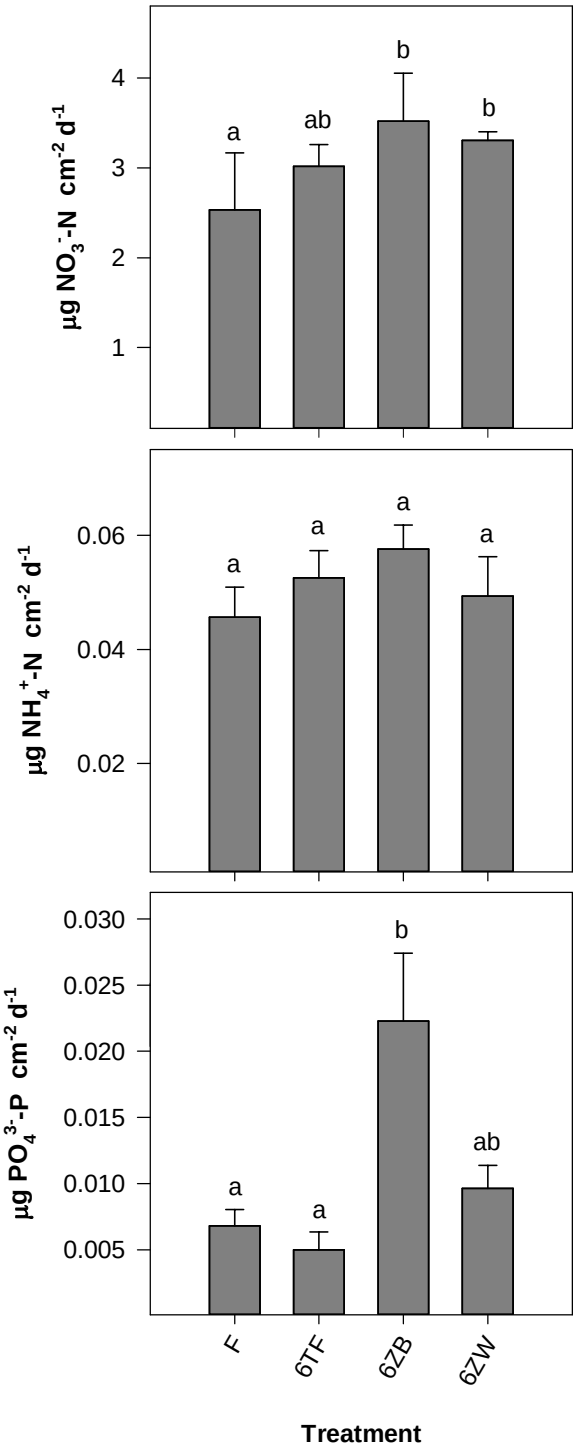

Fig. B.5 Nutrient supply rates in Whitehorse in the Biochar Application Trial. Treatments included fertilizer (F), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Zakus bonemeal biochar plus fertilizer (6ZB), and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean, with error bars representing the standard error of the mean. Different letters indicate significantly different nutrient availabilities between treatments (ANOVA, $P < 0.05$).

B.10 Functional Gene Abundance

In the Biochar Application Trial, average aromatic (*C2,3O* and *nahAc*) functional gene abundance was higher in incorporated treatments in Iqaluit, while *alkB* and *nahAc* were higher in injected treatments in Whitehorse (Fig. B.6). Treatment significantly affected *C2,3O* and *alkB* in Iqaluit and Whitehorse, respectively (Fig. B.7, ANOVA, $P < 0.05$). In Iqaluit, 6% Zakus wood biochar plus fertilizer had greater *C2,3O* abundance than the fertilizer control, but not more than 6% Titan fishmeal biochar plus fertilizer (Fig. B.7A). In Whitehorse, there was no difference in *alkB* abundance when comparing biochar types and the fertilizer control, but 6% Zakus bonemeal biochar plus fertilizer contained lower abundance than 6% Titan fishmeal biochar plus fertilizer, but not 6% Zakus wood biochar plus fertilizer (Fig. B.7B). All other genes, *alkB* and *nahAc* in Iqaluit, and *C2,3O* and *nahAc* in Whitehorse, did not differ significantly between treatments (ANOVA, $P > 0.05$). It was expected to observe different trends between locations as the soils were contaminated in Iqaluit, but not in Whitehorse.

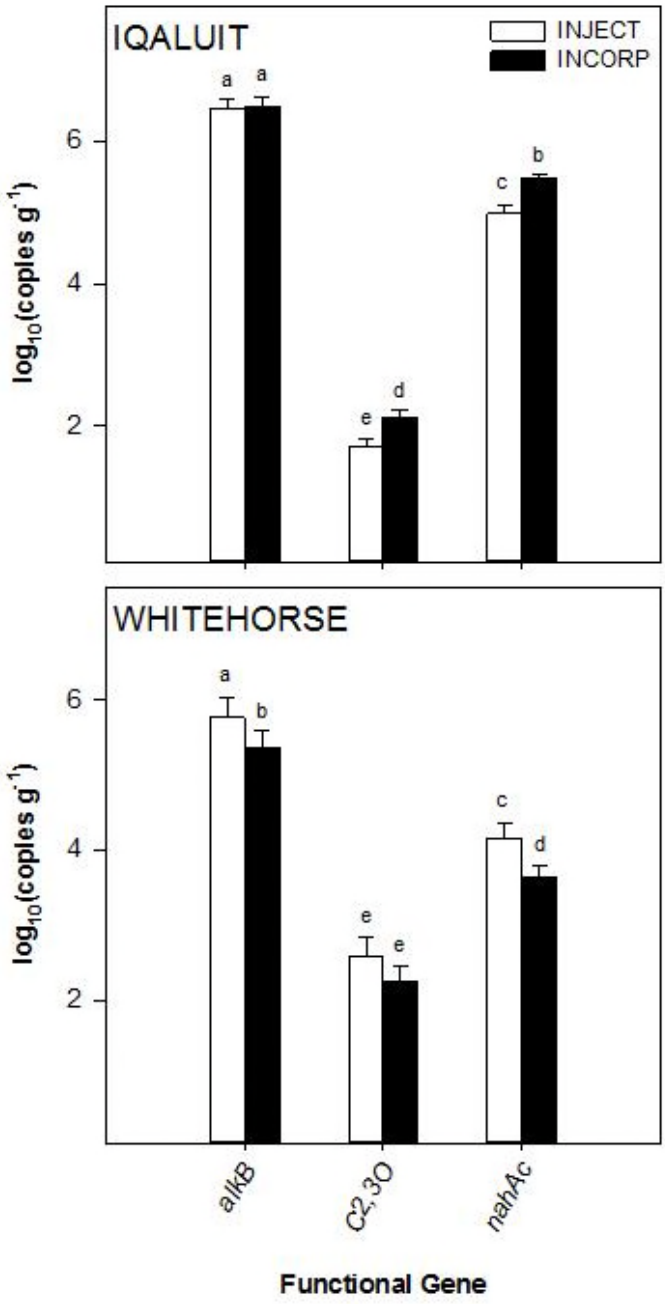

Fig. B.6 In the Biochar Application Trials, aliphatic and aromatic functional gene abundance was compared between two application methods, injection (INJECT) and incorporation (INCORP), in Iqaluit and Whitehorse. The following functional genes were quantified: alkane monooxygenase (*alkB*), catechol 2,3 dioxygenase (*C2,3O*), and naphthalene dioxygenase (*nahAc*). Bars represent the mean, with error bars representing the standard error of the mean. Different letters indicate significantly different functional gene abundance between application methods (ANOVA, $P < 0.05$).

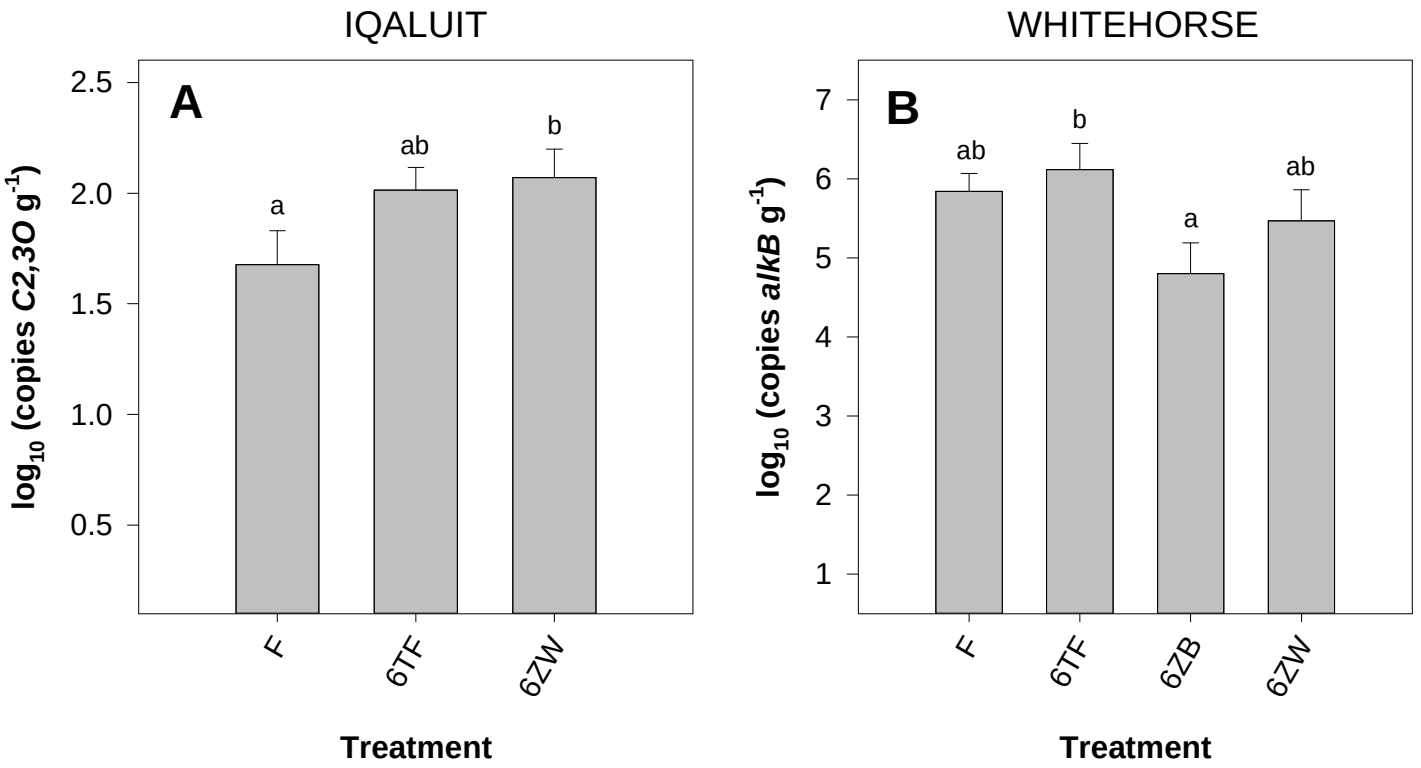

Fig. B.7 Alkane monooxygenase (*alkB*) and catechol 2,3 dioxygenase (*C2,3O*) functional gene abundance was quantified in the Biochar Application Trial in (A) Iqaluit and (B) Whitehorse. Treatments included fertilizer (F), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Zakus bonemeal biochar plus fertilizer (6ZB), and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean, with error bars representing the standard error of the mean. Different letters indicate significantly different functional gene abundance between treatments (ANOVA, $P < 0.05$).

B.11 Temperature and Liquid Water Content

In Whitehorse, liquid water content (θ_{liquid}) was highest when 6% Titan fishmeal biochar plus fertilizer was incorporated (Table B.7, ANOVA, $P > 0.05$). Treatment differences were evident in both the Biochar Application Trial and Biochar Type Trial when measuring soil temperature and θ_{liquid} (Fig. B.8). Temperature and θ_{liquid} measurements were only taken in uncontaminated soil at the Whitehorse site due to personnel and technological restraints in Iqaluit.

Table B.7 Average liquid water content (θ_{liquid}), in %, in injected (INJECT) and incorporated (INCORP) treatments across one field season in the Biochar Application Trial. Different letters indicate significantly different θ_{liquid} between treatments and application methods (ANOVA, $P < 0.05$).

Treatment	θ_{liquid}	
	INJECT	INCORP
	---------- % ---------	
Fertilizer	11.2[b]	9.7[b]
6% Titan Fishmeal biochar plus fertilizer	9.6[b]	13.8[a]
6% Zakus bonemeal biochar plus fertilizer	9.2[b]	9.3[b]
6% Zakus wood biochar plus fertilizer	9.1[b]	10.0[b]

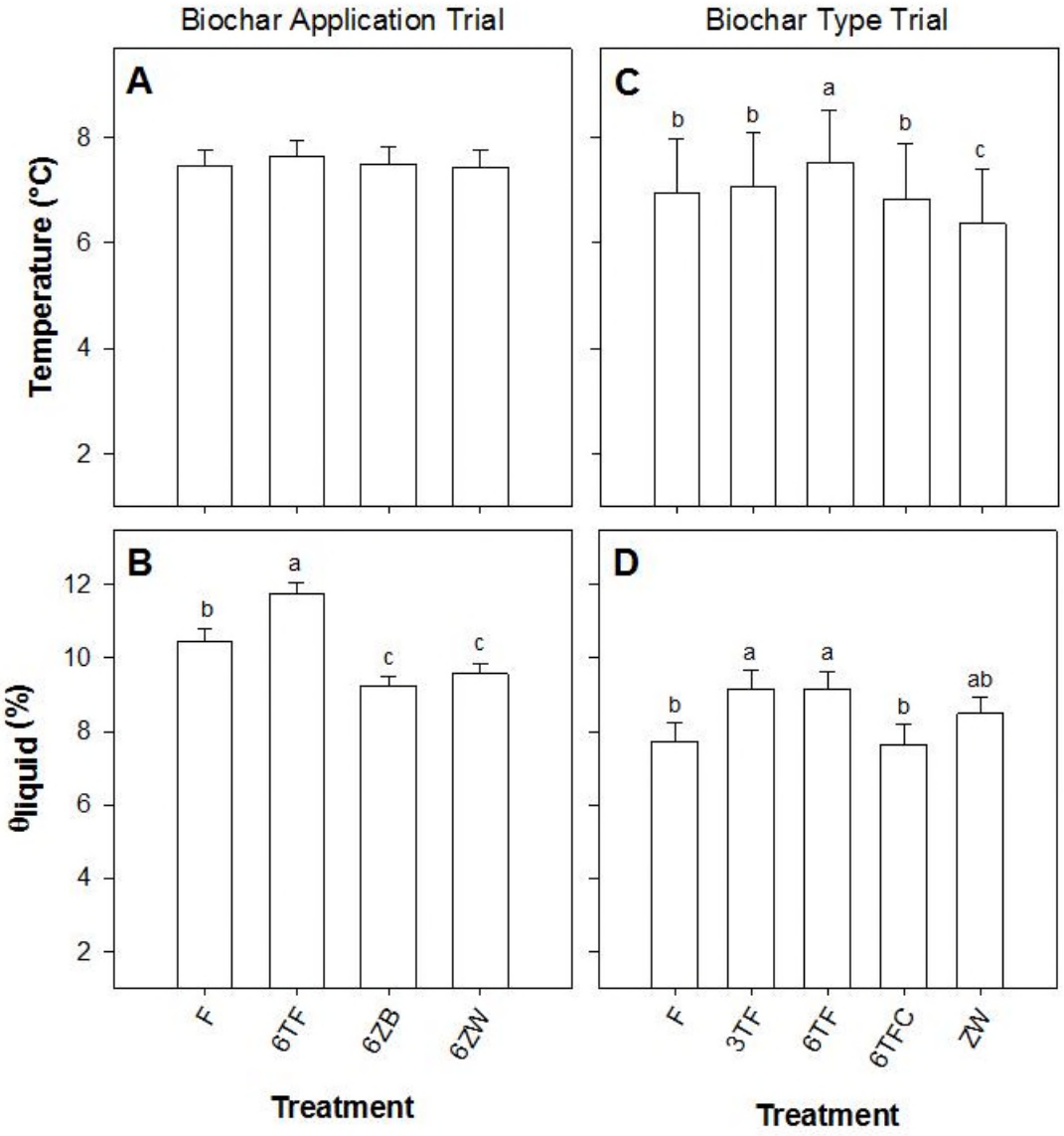

Fig. B.8 Average temperature and liquid water content (θ_{liquid}) by treatment, across one field season in the Biochar Application Trial (Graphs A-B) and Biochar Type Trial (Graphs C-D). Treatments included fertilizer (F), 3% Titan fishmeal biochar plus fertilizer (3TF), 6% Titan fishmeal biochar plus fertilizer (6TF), 6% Titan fishmeal biochar plus 5% compost plus fertilizer (6TFC), 6% Zakus bonemeal biochar plus fertilizer (6ZB), and 6% Zakus wood biochar plus fertilizer (6ZW). Bars represent the mean, with error bars representing the standard error of the mean. Different letters indicate significantly different temperature and/or θ_{liquid} between treatments (ANOVA, $P < 0.05$).

APPENDIX C. SUPPLEMENTAL MATERIAL (CHAPTER 5)

C.1 Nutrients

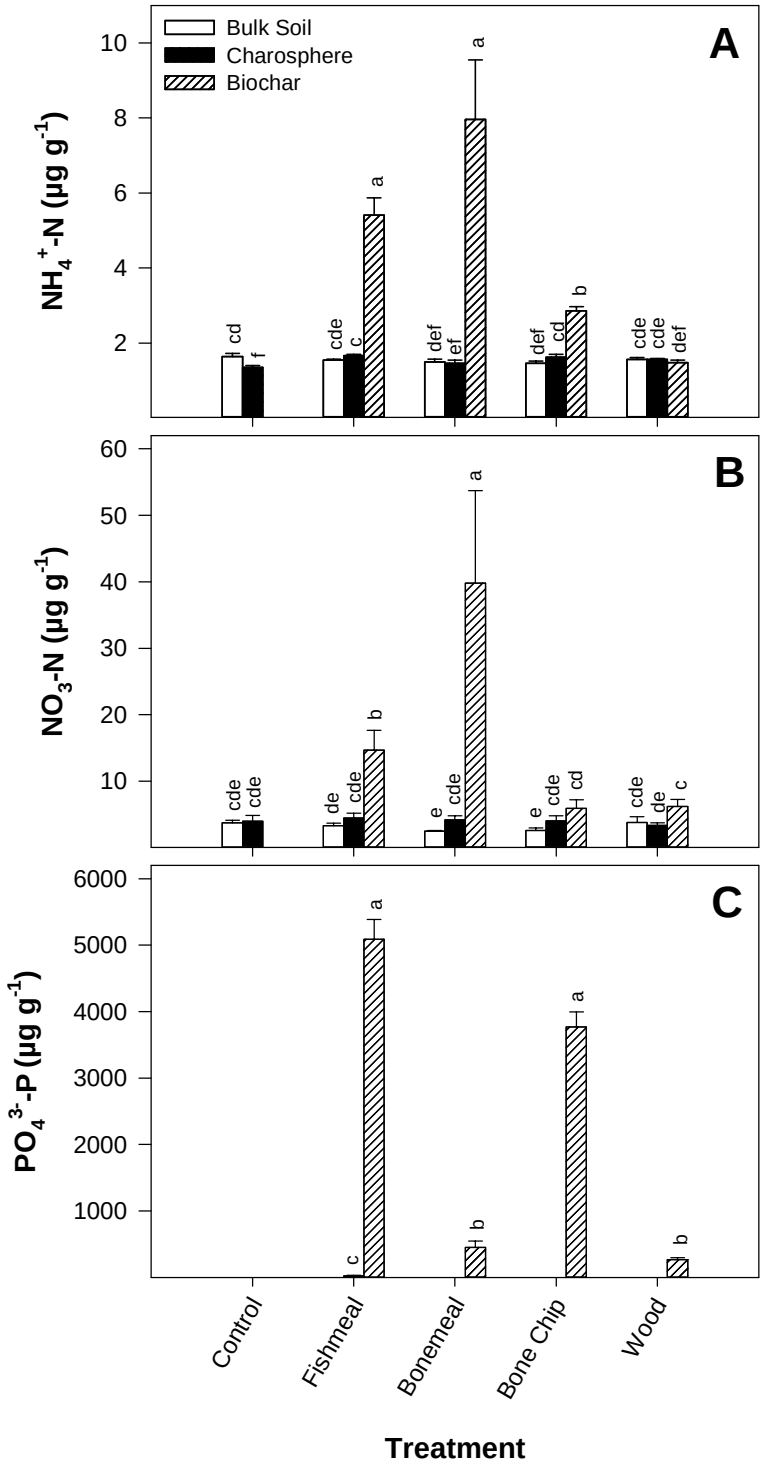

Fig. C.1 After 90 days incubation at -5 °C, the following extractable nutrients: (A) ammonium (NH_4^+-N), (B) nitrate (NO^{3-}-N), and (C) phosphate (PO_4^{3-}-P), in µg g^{-1}, were quantified in the bulk soil, charosphere soil, and biochar particles of a control and biochar treatments. Bars represent the mean (n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different nutrient concentrations (ANOVA, $P < 0.05$).

C.2 Functional Gene Abundance

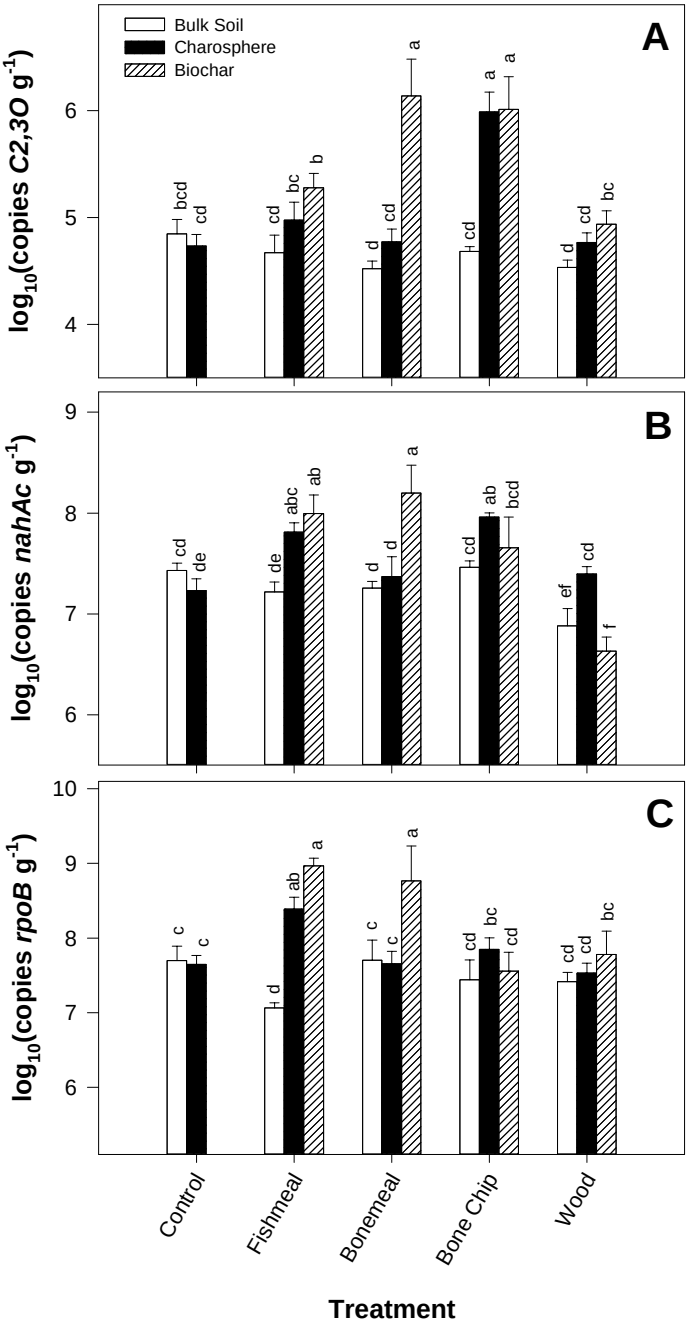

Fig. C.2 After 90 days incubation at -5 °C, functional gene abundance for the following genes: (A) catechol 2,3 dioxygenase (*C2,3O*), (B) naphthalene dioxygenase (*nahAc*), and (C) RNA polymerase beta subunit (*rpoB*), in $\log_{10}$(copies g^{-1}), was quantified in the bulk soil, charosphere, and biochar particles of a control and biochar treatments. Bars represent the mean (n = 5), with the error bars representing the standard error of the mean. Different letters indicate significantly different functional gene abundance (ANOVA, $P < 0.05$).

C.3 Diversity Indices

Table C.1 Average (mean ± standard error) diversity indices, which were normalized to *A. fischeri* and rarefied. There were no significant differences between treatments and/or sampling locations (ANOVA, $P > 0.05$).

Treatment	n	Chao Richness	Simpson's Diversity	Shannon's Diversity	J-Evenness	E-Evenness
---------- Bulk Soil ----------						
Control	5	615 ± 25	0.81 ± 0.09	2.73 ± 0.44	0.54 ± 0.07	0.12 ± 0.02
Fishmeal	5	547 ± 66	0.89 ± 0.01	3.07 ± 0.05	0.58 ± 0.01	0.11 ± 0.01
Bonemeal	5	602 ± 65	0.87 ± 0.03	2.85 ± 0.25	0.56 ± 0.04	0.12 ± 0.02
Bone Chip	5	556 ± 79	0.81 ± 0.08	2.64 ± 0.33	0.50 ± 0.06	0.08 ± 0.01
Wood	5	621 ± 24	0.90 ± 0.01	3.07 ± 0.09	0.59 ± 0.03	0.13 ± 0.03
---------- Charosphere ----------						
Control	5	543 ± 55	0.73 ± 0.11	2.30 ± 0.52	0.47 ± 0.08	0.10 ± 0.02
Fishmeal	5	582 ± 49	0.85 ± 0.03	2.81 ± 0.15	0.56 ± 0.02	0.11 ± 0.01
Bonemeal	5	460 ± 80	0.88 ± 0.02	2.87 ± 0.14	0.55 ± 0.02	0.10 ± 0.01
Bone Chip	5	563 ± 82	0.89 ± 0.01	3.00 ± 0.07	0.57 ± 0.01	0.11 ± 0.01
Wood	5	657 ± 18	0.90 ± 0.03	3.16 ± 0.26	0.63 ± 0.04	0.17 ± 0.03
---------- Biochar ----------						
Control	NA	NA	NA	NA	NA	NA
Fishmeal	5	608 ± 36	0.73 ± 0.10	2.36 ± 0.45	0.47 ± 0.07	0.09 ± 0.02
Bonemeal	5	524 ± 76	0.87 ± 0.02	2.81 ± 0.13	0.56 ± 0.02	0.11 ± 0.01
Bone Chip	5	568 ± 35	0.70 ± 0.13	2.27 ± 0.51	0.45 ± 0.09	0.09 ± 0.03
Wood	5	574 ± 41	0.73 ± 0.09	2.25 ± 0.31	0.47 ± 0.06	0.09 ± 0.03

n = number of samples
NA = not applicable