BURIED WOOD EFFECTS ON SOIL NUTRIENT SUPPLY AND MICROBIAL ACTIVITY IN DIFFERENT OIL SANDS RECLAMATION SOILS IN NORTHERN ALBERTA

by

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Abstract

Buried wood is an important component of natural and anthropogenic soils, yet it remains severely understudied. Nutrient immobilization as a response to wood addition in soils raises concerns from oil sands reclamation practitioners since the clear and grub procedures prior to soil salvaging tend to leave remains of unmerchantable wood that is salvaged with the soil and subsequently placed on reclamation landscapes as part of the cover soil. Additionally, there are no reports about how much buried wood is potentially added to reclamation soils in the area of Northern Alberta. This thesis aimed to investigate the impacts of buried wood on the nutrient supply and microbial communities in different soils used in oil sands reclamation and to determine how much buried wood is there in reclamation soils and how is this linked to the soil nutrient supply in the field. A 60-day incubation study was performed with different volumes and types of buried wood (0%-50%, aspen and pine wood), and four different soils (fine and coarse forest floor-mineral mix: fFFMM and cFFMM, peat-mineral mix: PMM, and Peat); analyses on soil nutrient supply rates, microbial biomass C and N, and Community Level Physiological Profiling were performed at the end of the incubation period, soil respiration was measured throughout the incubation. A complementary field study was performed in a 5-year old reclamation site with FFMM and PMM in Northern Alberta where buried wood sampling was performed and soil samples were collected for nutrient supply rate analysis. In the incubation study, responses varied depending on the soil type, but buried wood caused nitrogen immobilization in three out of the four soils at rates of 10% and above, due to an increase in the soil C:N ratio; soils with lower C:N ratio like fFFMM and PMM were more susceptible to nitrogen immobilization just after the smallest rate of 10%; buried wood increased the microbial activity but no significant changes in the soil metabolic profiles were noted. The field study

concluded that the average amount of buried wood is less than 1.5% or 34 m^3/ha in the top 20 cm of these cover soils and it was not linked to the soil nutrient profiles. The findings in this thesis suggest that although buried wood increases the soil C:N ratio and subsequently causes nitrogen immobilization, the amount present in reclamation soils is not a motive of concern for operational practices. However, supervision on how much buried wood is salvaged with soil is recommended to avoid nitrogen immobilization.

Preface

The following thesis is composed of original data obtained and analyzed by Laura Manchola Rojas. The author was responsible for research design, data collection, data analysis, and manuscript composition. Bradley D. Pinno was involved with research design, data analysis, and manuscript edits. M. Derek Mackenzie and Sebastian T. Dietrich contributed to research design, data analysis, and manuscript edits.

Partial data from Chapter II has been submitted for publication to the journal Forests as complementary data of a parallel study. Preliminary results of this thesis "Buried wood effects on nutrient supply in reclamation soils" were presented in a poster at the 85th Forest Industry Lecture Series Program from the University of Alberta.

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CHAPTER I: GENERAL INTRODUCTION

1.1 Buried wood concept

Downed wood is a key component in the dynamic and heterogeneous structure of a forest (Moroni et al. 2010). It is more common during the early-successional stages of a forest after disturbances like wildfires; pests, beetles; and wind, which result in fallen live and dead trees on the ground surface (Daust and Price 2013). During mid-successional stages, there is less downed wood as fewer trees die and fall. At the late-successional stages, and in old-growth forests, trees die of old age and fall, increasing and accumulating again downed wood on the ground (Zimmerman 2004). This woody material plays several roles in a natural forest including providing habitat for microbes, invertebrates, and some vertebrates like amphibians, reptiles, birds, and mammals; providing thermal refugia and hiding cover for animals; creating pools for aquatic organisms when fallen into water currents; absorbing and retaining water when highly decayed which helps seedlings survive drought; and decreasing fluctuations in soil temperature (Hart 1999; Kwak et al. 2015a, 2015b). For the soil, in particular, downed wood impacts geomorphic processes by reducing erosion and capturing sediments since downed logs act as a physical barrier to soil movement (Harmon et al. 2004). Also, as this wood decomposes it becomes a significant part of the soil organic matter, and functions as a slow-cycling pool of carbon (Moroni et al. 2010).

Downed wood can become buried wood as leaf litter accumulates and vegetation overgrows it, leading to a natural and gradual burial of the wood and eventually to its accumulation within the soil (Hagemann et al. 2010a). Another mechanism of wood burial in natural forests is a rapid burial resulting from catastrophic events like landslides and fluvial

deposition (Eden 1967). The resulting buried wood is defined as deadwood that is buried more than 50% by soil or litter (Moroni et al. 2015). Buried wood is more common in coniferous-dominated forests since these types of forests are associated with thicker litter layers and ground vegetation dominated by bryophytes and bushes, which help to accumulate, bury and preserve the downed wood (Hagemann et al. 2010a). In broadleaf forests the buried wood is limited, mainly because the ground is dominated by grasses and herbs, which die annually and decompose quickly; in consequence, downed wood is not able to be buried and it decomposes on the surface (Hobbie 1996; Prescott et al. 2000). Additionally, in broadleaf forests, decomposition is primarily by white-rot fungi which are faster than brown-rot fungi, common coniferous forest decomposers (Stokland 2012).

1.2 Buried wood in natural soils in Canada

Most of the buried wood studies done in Canada have occurred in post-fire forests with fewer studies after harvesting and insect outbreaks. In these studies, buried wood was found generally in the form of organic forest floor material and logs (Brais et al. n.d.; Manies et al. 2005; Moroni 2006; Hagemann et al. 2009; Moroni and Ryan 2010; Moroni et al. 2010). In 2015, Moroni et al. reviewed the literature and conducted a meta-analysis of the current buried wood data, and included new data from Canadian forests (Moroni et al. 2015). In terms of occurrence in a wide range of ecozones and ecoregions with different types of vegetation, forest structure, successional stages, and topographies, the highest occurrence was reported in British Columbia with buried wood presence in 22.8% of the plots sampled, followed by Ontario with 13.6%, whereas the lowest values were recorded in Nova Scotia with 7.1% and Alberta with 8.8%. In terms of volume, two paludified black spruce forests in Quebec and Labrador had the

largest volumes of buried wood with 935 m^3/ha and 487m^3/ha, respectively; while the smallest volume was 1.6 m^3/ha reported in a broadleaf forest in Nova Scotia.

1.3 Buried wood in reclamation soils in Canada

Buried wood is also a soil component in anthropogenic forests, but is severely understudied (Zeng et al. 2013; Moroni et al. 2015). In oil sands mining reclamation, the wood is buried by a rapid mechanical process that differs from natural forests. When starting the reclamation operations in a site (Alberta Environment and Water 2012), surface soils are salvaged from upland or lowland ecosystems to be used immediately as cover soil or stored in stockpiles for later use. Before soil salvaging, the merchantable timber is harvested and moved off-site, and the remaining slash and non-merchantable timber is coarse-mulched and left on site. Consequently, during soil salvaging operations this wood is also collected, mixed, and incorporated into the soil, which will be placed as cover soil (20 to 30 cm deep) on a reclamation site. Although the definition of buried wood has been established for natural landscapes and natural burial processes (Moroni et al. 2015), in this thesis, buried wood is considered the wood that has been incorporated to the soils as a result of reclamation operations. There is an estimation that the volume of wood that remains on the surface and is subsequently salvaged with the soil is approximately 20-50 m^3, resulting in a 1.5% of buried wood in a salvage depth of 30 cm (Robert Vassov, personal communication, Nov 11th 2021). However, this has not been properly quantified.

1.4 Soils in oil sands reclamation in Alberta

One of the goals of land reclamation in Alberta is that the resulting reclaimed land will have the ability to support a range of land uses similar to that prior to disturbance, but will not necessarily be identical. This is referred to as equivalent land capability and is a fundamental

goal of land reclamation in Alberta. Recreation, commercial forestry, grazing, agriculture, and industrial use are some examples of end land uses, with the most common being commercial forestry and Traditional Land Use (Alberta Environment 2010). The physical, chemical, and biological properties of the reclaimed land can determine which end land use is appropriate, and certainly, the soil is a key component taken into consideration. A soil that provides enough resources for vegetation growth and establishment, and other ecological services is crucial, and buried wood may be a critical, but understudied component of this reconstructed soil.

Soils used in oil sands mine reclamation in Alberta vary from upland to lowland origins. Forest floor-mineral mix (FFMM) is collected from upland forests and is a high-value material for use as a reclamation cover soil since it provides natural upland forest soil characteristics and, when directly placed, it provides seeds and propagules that support initial vegetation cover (McMillan et al. 2007; Brown and Naeth 2014). Although FFMM offers other several benefits like high organic matter content and an abundant source of macro and micro-nutrients, the upland territory has a limited extent when compared to the lowland or wetlands territory (Alberta Environment and Water 2012). In consequence, most of the cover soil used in reclamation is a mixture of peat and mineral material, known as peat-mineral mix (PMM). PMM is a mixture of peat or an organic horizon, with either underlying mineral material, subsoil from another site, or suitable overburden under the criteria of the Soil and Groundwater Remediation Guidelines (Alberta Environment 2009). This reclamation soil is also a successful material in upland reclamation, it has a high organic matter content linked to a high water-holding capacity, and has been shown to promote natural establishment on early-successional broadleaf trees like trembling aspen and balsam poplar (Errington and Pinno 2015; Pinno and Gupta 2018).

Additionally, the use of organic material or peat helps to compensate for volume when upland soils are restricted.

Several studies that have investigated FFMM and PMM characteristics provide a common ground about their main differences. Greater nitrogen availability is commonly observed in FFMM due to factors like a lower C:N ratio and greater enzymatic activity related to a greater organic matter decomposition and a higher nitrogen mineralization rate (McMillan et al. 2007; Mackenzie and Quideau 2012; Jamro et al. 2014). Similarly, phosphates and potassium availability also tend to be greater in FFMM, with reports of up to 16 times more P and 1.5 times more K when compared to PMM (Mackenzie and Quideau 2012; Brown and Naeth 2014; Pinno et al. 2014; Howell et al. 2017; Quideau et al. 2017). PMM is likely to have a higher pH than FFMM but lower than 8, up to 4 times more water-holding capacity, and 3 times more total carbon content (Mackenzie and Quideau 2012; Kwak et al. 2015b). Greater total nitrogen content is more occurrent in PMM (McMillan et al. 2007; Mackenzie and Quideau 2012; Kwak et al. 2015b) and it can be up to 7 times greater than in FFMM.

1.5 Impacts of buried wood on natural soils and reclamation soils

Buried wood represents an input of nutrients and organic matter to the soil thereby influencing the soil nutrient availability, both as a source and as a sink. When wood is incorporated into the soil, decomposing organisms, i.e. bacteria and fungi, start the process of decaying it (Swift 1977; Stokland 2012). However, wood decomposition is a process that can take prolonged periods (Swift 1977; Kirk and Cowling 1984; Tuomi et al. 2011), in temperate and boreal forests the decomposition of a tree typically takes 50-100 years (Stokland 2012). Coniferous wood usually takes longer to decompose than broadleaf wood due to several reasons: it has a higher lignin content that protects the wood from decomposition; the most dominant

wood-decomposers are brown-rot fungi which function at a slower rate than the white-rot fungi in broadleaf forests; and there is a tendency of coniferous wood being buried and preserved by bryophyte cover and overgrow, which provides a disadvantageous environment for decomposition (Hagemann et al. 2010a; Stokland 2012; Moroni et al. 2015).

Buried wood represents a high input of carbon, approximately 50% of the total wood mass (Pettersen 1984; Chandrasekaran et al. 2012), and wood decomposers need nutrients to carry out this extended task, especially nitrogen since it is necessary for enzymatic activity (Robertson and Groffman 2006). Therefore, the soil C:N ratio increases, and as a response the available nitrogen in the soil is consumed by the microbial communities to work towards decomposition (Moritsuka et al. 2004). This is known as nitrogen immobilization and results in nitrogen not being available for plant uptake (Swift 1977; Jansson 1982; Jonasson et al. 1996). As wood decomposition advances and the demand for nitrogen decreases, nitrogen is eventually released to the soil to be available for plant uptake (Boddy and Watkinson 1994).

There are studies about the effect of wood application on reclamation soils in the oil sands (Brown and Naeth 2014; Kwak et al. 2015a; Pinno and Gupta 2018), but all of them evaluated coarse woody debris, which is surface wood. The processes and conditions for this surface wood and soil interaction are different and may have different outcomes for soil nutrients and microbial communities. These studies have shown that surface-applied wood increases the soil bacterial biomass and functional group diversity (Kwak et al. 2015a), increases the soil water holding capacity and vegetation cover (Brown and Naeth 2014), supports native plants diversity, and reduces non-native species (Pinno and Gupta 2018). The impact of surface-wood application on nutrients is not clear. Brown and Naeth (2014) observed a decrease in soil available nitrate in sites with surface wood while Pinno and Das Gupta (2018) found that changes in the nutrient

supply rates were attributable to the difference in soil types and not to the application of surface wood. Additionally, Kwak et al (2015b), used trembling aspen wood extract in a laboratory incubation and found that nitrogen availability decreased in both FFMM and PMM as a response to the surface wood extract. Despite these studies, the buried wood component remains unexplored.

1.6 Study objectives

Buried wood is an understudied component of the soils used in oil sands reclamation in Alberta with the potential to impact soil nutrients and microbial activity. The main research questions are to quantify the amount of buried wood in reclamation soils and determine its impact on soil nutrients and microbial communities. The second chapter (Chapter II) of this document corresponds to a laboratory incubation study that aimed to observe the responses to different rates and types of buried wood in four different soil types commonly used in oil sands reclamation in terms of nutrient supply rates and microbial activity. The third chapter (Chapter III) of this document corresponds to a field study that aimed to quantify the amount of buried wood present in two different soils in a reclamation landscape and determine if this buried wood influenced the nutrient supply rates. The overall aim of this project is to gain further understanding of the coarse woody debris functioning as buried wood and as a reclamation material since it is added to potential cover soils, how is it impacting the soil and, in case of having detrimental effects, determine how much is the recommended application limit for operational practices.

CHAPTER II: INCUBATION STUDY

2.1 Introduction

2.1.1 Wood chemical composition and impact on soil nutrients and microbial communities

Buried wood represents an input of carbon in the form of carbohydrates (cellulose and hemicellulose, 65% to 85% of the total wood mass), and lignin, a complex organic polymer (Thomas n.d.; Pettersen 1984; Stokland 2012). Wood chemical composition by weight has been determined as 45% - 50% carbon, followed by oxygen with 40% - 45%, hydrogen at 6%, nitrogen at less than 1%, and several trace metal elements (Pettersen 1984; Chandrasekaran et al. 2012). When wood is added to the soil there is an increase in the soil C:N ratio that results in nitrogen immobilization by soil microorganisms (Moritsuka et al. 2004), due to a higher demand for this nutrient to increase microbial activity and work towards wood decomposition (Truong and Marschner 2018). Phosphorus has also been reported to limit wood-decomposers enzymatic production and thus is also immobilized (Sinsabaugh et al. 1993; Smyth et al. 2016). However, the rates of immobilization of P and N and their release as wood is decomposition progress have been linked to the initial soil C:N ratio and nutrient availability of the surface soil, with sooner decomposition and nutrient release in soils with lower C:N ratio (Bonanomi et al. n.d.; Bengtsson et al. 2003; Smyth et al. 2016). This evidences the influence of the soil properties in wood decomposition and the impact on the soil nutrients. Studies on coarse woody debris which is non-buried wood, show that in later decay stages (+15 years) carbon loss increases, and nitrogen is released back into the soil to be available for plant intake (Palviainen et al. 2010; Mukhortova 2012). Still, most of the research about forest surface wood and buried wood has been extensively focused on carbon storage and carbon sequestration potential (Hagemann et al.

2010b; Knicker 2011; Zeng et al. 2013; Adame et al. 2015; Moroni et al. 2015; Stokland et al. 2016; Dossa et al. 2018) and has left behind the impact on soil nutrients and how this can vary among different soil conditions.

It is important to consider that due to differences in cellulose, hemicellulose, and lignin contents in wood, the timelines for nutrient immobilization and release may vary (Pettersen 1984; Stokland 2012). For example, lignin is a complex structure that functions as the initial barrier to decomposition resisting microbial attack to protect the wood carbohydrates (Brais et al. n.d.; Kirk and Cowling 1984). This component is higher in coniferous wood compared to broadleaf wood, with 25-35% and 18-25%, respectively (Ulyshen and Šobotník 2018). Therefore, the changes in nutrients in terms of immobilization and release may also vary depending on the wood species.

In addition to nutrients, buried wood may also impact the soil microorganisms as these are the agents that carry out the wood decomposition and nutrient cycling processes. In a pine-wood decomposition study, it was found that bacterial community composition changed as a function of the wood properties rather than environmental conditions, and it was demonstrated that wood density (stage of decay) and wood chemical composition are factors that can select specific bacteria groups among the decay process (Kielak et al. 2016). Another study showed that the abundance of bacterial and archaeal genes increased as the wood decomposition progressed, and that community composition changed in response to wood density loss (Rinta-Kanto et al. 2016). A chronosequence study (Hu et al. 2017), linked microbial community composition to carbon loss during Chinese fir (*Cunninghamia lanceolate*) decomposition for 35 years, and found that in the early stages of decomposition (0-15 years) the dominant soil microbial communities were fungi, changing to bacterial communities in the late stages (15-35

years); fungi were associated with high-quality carbon and low wood/soil moisture (<20%), whereas bacteria were correlated with low-quality carbon and higher moisture, common in later stages of decay. Similarly, the soil properties have been shown to impact how the microbial communities respond to wood addition since different soil types provide different environmental conditions that influence the soil microbial activity. For example, moisture content is a beneficial factor for microbial activity and wood decomposition, but in the case of organic soils that usually have an elevated moisture content (Beckingham J.D. and Archibald 1996) soil temperature and oxygenation might decrease, causing declines in the microbial activity and the rate of wood decomposition (Hagemann et al. 2010a; Moroni et al. 2010). Contrarily, upland soils have more aeration, higher temperatures, and thus a higher microbial activity (Davidson and Janssens 2006). Several more factors like soil pH; phosphorus, potassium, and nitrogen availability have also been identified as limiting factors for microbial activity (Higashida et al. 1986; Cao et al. 2016). However, all these variables are potentially different among soils due to an inherent variability based on the soil nature and provenance (Howell et al. 2017).

2.1.2 Reclamation soils and buried wood

Soils used in oil sands mine reclamation in Alberta vary from upland to lowland origins. Forest floor-mineral mix (FFMM) is collected from upland forests and is a high-value material for use as a reclamation cover soil since it provides natural upland forest soil characteristics and, when directly placed, it provides seeds and propagules that support initial vegetation cover (McMillan et al. 2007; Brown and Naeth 2014). Although FFMM offers other several benefits like high organic matter content and an abundant source of macro and micro-nutrients, the upland territory has a limited extent when compared to the lowland or wetlands territory (Alberta Environment and Water 2012). In consequence, most of the cover soil used in reclamation is a

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evaluated coarse woody debris, which is surface wood. The processes and conditions for this surface wood and soil interaction are different and may have different outcomes for soil nutrients and microbial communities. These studies have shown that surface-applied wood increases the soil bacterial biomass and functional group diversity (Kwak et al. 2015a), increases the soil water holding capacity and vegetation cover (Brown and Naeth 2014), supports native plants diversity and reduces non-native species (Pinno and Gupta 2018). The impact of surface-wood application on nutrients is not clear. Brown and Naeth (2014) observed a decrease in soil available nitrate in sites with surface wood while Pinno and Das Gupta (2018) found that changes in the nutrient supply rates were attributable to the difference in soil types and not to the application of surface wood. Additionally, Kwak et al (2015b), used trembling aspen wood extract in a laboratory incubation and found that nitrogen availability decreased in both FFMM and PMM as a response to the surface wood extract. Despite these studies, the buried wood component remains unexplored.

2.1.3 Research questions, proposal and hypotheses

Consequently, the following research questions are proposed for this study:

- What are the impacts of buried wood on the soil nutrient supply rates and the microbial communities in reclamation soils?
- Do these impacts vary depending on the soil type and the wood type?

This incubation study will include four different types of soil commonly used in oil sands reclamation, fine forest floor-mineral mix (fFFMM), coarse forest floor-mineral mix (cFFMM), peat-mineral mix (PMM), and Peat; along with different buried wood treatments.

It is expected that buried wood will have an impact on the soil nutrient availability, especially nitrogen, considering that wood additions into the soil increase the demand for microbial activity to carry out the process of decomposition, and thus nitrogen is immobilized by these decomposer microorganisms. Furthermore, it is also expected that the responses in nutrients and microbial communities will vary depending on the soil type and on the initial proportions of carbon and nitrogen, in other words, the soil C:N ratio. This is expected because soils that have a lower carbon content might suffer a major alteration in their carbon proportion and the demand for nitrogen by wood-decomposing organisms will be greater than usual, resulting in a greater nitrogen immobilization and microbial activity. Another perspective is, soils with greater nitrogen availability will have a greater potential of nitrogen immobilization to deal with the new input of carbon, decreasing the nitrogen availability and increasing microbial activity.

In consequence, FFMM soils (cFFMM and fFFMM) are predicted to have a greater nutrient immobilization, more likely nitrogen immobilization since these soils are reported to have the greatest nutrient availability, microbial activity, and the lowest C:N ratio when compared to PMM and Peat (McMillan et al. 2007; Mackenzie and Quideau 2012; Errington and Pinno 2015). Followed by PMM which initially counts with a greater proportion of organic matter and hence a greater C:N ratio, wood addition will likely increase the C:N ratio but to a less extent. Therefore, nutrient immobilization is not expected to be as pronounced as in the FFMM soils. Lastly, Peat is predicted to have the least significant responses to buried wood considering it is the soil with the highest organic matter content and thus with the highest carbon content among all the soils used in this study, so no significant changes are expected.

2.2 Methods

A lab incubation was carried out following a multifactorial design with 4 types of soil, 2 types of wood shavings, and 4 amounts of wood shavings for a total of 32 treatments * 3 replicates = 96 samples. After the incubation period (day 60), analyses on nutrients and microbial activity were performed. Soil respiration was measured during the incubation period. Plant Root Simulator (PRS) probes (Western Ag Innovations, Saskatoon, SK, Canada) were used to determine the soil nutrient supply rates; Community Level Physiological Profile (CLPP) (Weber and Legge 2010) were performed to identify the different functional groups within the soil microbial communities, and soil respiration and microbial biomass C and N were measured as indicators of microbial activity.

2.2.1 Soils

Coarse forest floor-mineral mix (cFFMM), fine forest floor-mineral mix (fFFMM), Peat, and mineral sand were collected from different locations at an oil sands mine north of Fort McMurray. The fFFMM was collected from the cover soil (top 30 cm) of a 2-year-old reclamation site in late August of 2016 and remained cold-stored until use in this project in May of 2020. cFFMM, Peat, and underlying mineral sand were collected from pre-salvaged sites in May of 2020. Peat mineral mix (PMM) was prepared in the lab mixing Peat and underlying mineral sand to a volume ratio of 60:40 Peat to sand. All soils were sieved to remove large debris, including buried wood. A total of 250 L of each soil type was used for this study.

fFFMM was collected from a reclamation site and was characterized by a mesic moisture regime and a medium nutrient regime. Prior to its use in reclamation, this soil was salvaged from the top 30 cm (forest floor + upper mineral soil) of a fine-textured Gray Luvisol soil (“d” ecosite) (Beckingham J.D. and Archibald 1996), hand texturing in the lab identified a clay loam texture.

It is important to keep in mind that this soil was stored for several years in a barn. The temperature in the city of Edmonton varies from cold winters with a minimum average of -15°C to a maximum average temperature of 23.1°C during the summer (Government of Canada 2022). These factors of storage time and temperature can promote nitrogen mineralization and increase the nitrogen content in the soil after collection (Wu et al. 2018). In this case, since the fFFMM was stored at room temperature for an extended period, it is possible that nitrogen mineralization occurred and nitrogen content was higher than when the soil was initially collected. However, this effect was amended by pre-incubating the soil for a period of 14 days, which was reported to help soils gradually approach a nitrogen content similar to fresh soil samples (Wu et al. 2018).

cFFMM was collected from a pre-salvaged site and was characterized by a submesic moisture regime and a medium nutrient regime. This soil was salvaged from the top 30 cm (forest floor + upper mineral soil) of a "b" ecosite (Beckingham J.D. and Archibald 1996), hand texturing in the lab identified a loamy sand texture. Peat and underlying mineral sand were collected from a pre-salvaged "h" ecosite characterized by an hygric moisture regime (Beckingham J.D. and Archibald 1996). Peat was salvaged from approximately the top 1.2 m.

The highest buried wood content was found in cFFMM (21.24%) while fFFMM and Peat had less than 1% in volume (Table 1); Peat was the most acidic soil with a pH of 4.42 and was also the soil with the highest field capacity and electrical conductivity (194% and 1221 µS/cm, respectively), whereas the other soils had more neutral pH and field capacities from 15% to 30%. Peat had the highest concentration of total organic carbon (TC, 47.25%) and total nitrogen (TN, 2.21%).

Table 1. Physical and chemical characteristics of the soils used in the incubation study. Values are mean and standard errors in brackets. Letters indicate similarities among soils (Fisher's LSD test, $p<0.05$). Total nitrogen (TN), total organic carbon (TOC), electrical conductivity (EC), moisture content at field capacity (MC at FC), buried wood (BW). Soil types: Coarse forest floor-mineral mix (cFFMM), fine forest floor-mineral mix (fFFMM), peat-mineral mix (PMM).

Soil	TN (w/w%)	TOC (w/w%)	C/N ratio	pH	EC (µS/cm)	MC at FC (%)	BW (vol%)
cFFMM	**b** 0.20 (0.14)	**c** 4.27 (0.40)	21.35	**b** 6.32 (0.11)	**d** 161.60 (0.43)	**c** 15.11 (0.88)	21.24
fFFMM	**b** 0.19 (0.14)	**c** 3.55 (0.40)	18.68	**ab** 6.54 (0.49)	**c** 641.0 (0.0)	**b** 26.91 (0.32)	0.90
Peat	**a** 2.21 (0.14)	**a** 47.25 (0.40)	21.38	**c** 4.42 (0.014)	**a** 1221 (2.16)	**a** 194.38 (1.64)	0.026
PMM	**c** 0.04 (0.14)	**b** 8.92 (0.40)	18.20	**a** 6.84 (0.024)	**b** 764.67 (1.25)	**b** 30.47 (8.89)	-

2.2.2 Incubation

A multifactorial design was used for this incubation study including three factors: soil type (cFFMM, fFFMM, Peat and PMM), wood type (aspen and pine), and wood amount (0%, 10%, 20%, and 50% as volumetric percentage in sample). The aspen and pine wood were kiln-dried shavings commercially used as animal bedding; chemical analyses determined a TN concentration of 0.22% and a TC concentration of 48.34% for aspen, a TN of 0.16%, and TC of 48.28% for pine.

A total of 1 L was prepared for each treatment (e.g. Peat x Aspen x 50% = 500 mL Peat + 500 mL Aspen shavings) in reclosable bags; for the 0% treatments, the wood type factor was not considered. After mixing to homogenize the samples, three 500 mL Bernardin Mason Jars were filled with 250 mL of each treatment (See Table A1 for sample weights), for a total of 84 samples, each treatment in triplicate.

To determine field capacity, the samples were poured into plastic rings and placed on ceramic pressure plates. The plates were then filled with Ultrapure water and the soils were allowed to absorb overnight until saturated. The excess water was removed gently and the plates were put into a 5 Bar Ceramic Plate Extractor (SoilMoisture Equipment Corp., Santa Barbara, CA), using compressed air as a pressure source the excess water was extracted under the constant pressure of 0.3 Bar for 24 h. Afterwards, the samples were removed from the plate extractor, weighed when wet, dried at 105°C for 24 h, and weighed one more time. The moisture content at field capacity was calculated using the dry and wet weights in the following equation:

$$\text{MC at FC} = \frac{\text{weight}_{\text{wet}} - \text{weight}_{\text{dry}}}{\text{weight}_{\text{dry}}} * 100 \quad (1)$$

The samples were kept at a moisture of 70% of FC with water added as required. Using the data of moisture content and field capacity of each sample, how much water was required to be added into each sample to achieve the 70% of moisture at field capacity was calculated with the following equations:

$$\text{MC at 70\% FC} = \text{FC} * 0.70 \quad (2)$$

$$\text{H}_2\text{O needed for 70\% FC}_{(\text{g})} = \frac{\text{MC at 70\% FC} * \text{weight}_{\text{fresh}}}{100} \quad (3)$$

$$\text{Additional H}_2\text{O for 70\% FC}_{(\text{g})} = \text{H}_2\text{O needed for 70\% FC}_{(\text{g})} - \text{Soil MC}_{(\text{H}_2\text{O g})} \quad (4)$$

The samples were wetted to reach 70% of moisture at field capacity and the final weight was recorded. During the incubation, samples were kept at a constant temperature of 25 °C in a Thermo Scientific™ Precision™ Low-Temperature BOD Refrigerated Incubator (Fisher Scientific). First, the samples had a pre-incubation period of 14 days, then the incubation continued for 60 more days. The samples were taken out of the incubator every two to three

days, aired out for 30 to 45 min to maintain an aerobic condition, then weighed and rewetted if needed to maintain the 70% of moisture at field capacity.

2.2.3 Soil analyses

After the incubation period was completed (day 60), a subsample of 10 g of each treatment was sieved (2 mm) and air-dried for 24 h. Then, pH and EC were measured using a SevenEasy pH meter and a FiveEasy Conductivity meter (© Mettler Toledo) respectively.

Soil nutrient supply rates: Plant Root Simulator (PRS) probes (Western Ag Innovations, Saskatoon, SK, Canada) were used to determine nutrient supply rates. At the end of the incubation, 100 mL of each sample was transferred into reclosable bags and wetted to FC; one pair of probes was inserted into the bags so that the soil completely covered the probes on both sides to a depth of at least 2 cm. The probes attract and adsorb ions through electrostatic attraction; each pair consists of a cation probe for ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{+2}); and an anion probe for nitrate (NO_3^-), sulphate (SO_4^{2-}) and phosphates ($H_2PO_4^-$, HPO_4^{2-}). The samples were incubated for 7 days at 25°C and aired out twice during the incubation to maintain aerobic conditions. After incubation, the probes were cleaned and rinsed thoroughly with ultrapure water, placed into reclosable bags, and sent to Western Ag. Innovations for analysis, which consisted of eluting the probes with 0.5 M HCl and determining inorganic nitrogen from the eluant through flow injection analysis, using a Skalar San++ Analyzer (Skalar Inc., Netherlands); the rest of the nutrients were measured using inductively coupled plasma (ICP) spectrometry (Optima ICP-OES 8300, PerkinElmer Inc., USA) (Western AG Innovations Inc. 2010).

Community-level physiological profile: Approximately 60 g of each sample was sieved (1 mm) and loaded into individual deep-wells that contained 15 different substrates and

deionized water as a control, so each deep-well had approximately 4 g of sample (Table 2). The deep-wells were clamped to indicator plates and incubated for a period of 6 h at 25 °C. This procedure intends that the plates absorbed CO_2 from soil respiration and change colour as a response to metabolic activity. The indicator plates were read before and after incubation (t0 and t6) using a Synergy HTX Multi-Mode Reader (BioTek Instruments Inc., Winooski, VT, USA), and Gen5 2.03 Microplate Reader and Imager Software to obtain the respiration rates or CO_2 production rates (µg CO_2-C/g/h) of each soil sample in response to each substrate through UV-Vis absorbance. Data was extracted and managed using Microsoft Excel and following the MicroResp™ Technical Manual (Campbell et al. 2015) for data transformation. Following this, the difference between CO_2 rate at t0 and t6 was calculated to obtain the net respiration rates.

Table 2. Carbon sources used as substrates in CLPP.

Substrate	Carbon type
N-acetyl glucosamine	Amino acid
L-alanine	
Ɣ-amino butyric acid	
L-arginine	
L-cysteine-HCl	
L-lysine-HCl	
L- (+)-arabinose	Carbohydrate
D- (-)-fructose	
D- (+)-galactose	
D- (+)-glucose	
D- (+)-trehalose	
Citric acid	Carboxylic acid
L-malic acid	
Oxalic acid	
α-ketoglutaric acid	
DI H_2O	None, basal respiration

Microbial biomass C and N (Chloroform fumigation with desiccator method): This technique measured microbial biomass C and N as the difference in total organic carbon (TOC) as non-purgeable organic carbon (NPOC) and total nitrogen (TN) in soil extracts from samples with and without fumigation. For each sample, 20 g were sieved (2 mm) and split with 10 g into a 50 mL beaker for fumigation and 10 g into a Nalgene bottle for direct extraction. Fumigation samples were placed into a vacuum desiccator with a beaker with chloroform in the middle of the samples. The desiccator was connected to a vacuum pump to extract the air until the chloroform boiled for 40 seconds; the vacuum was turned off and the desiccator was sealed and covered with a black plastic bag during a fumigation period of 24 h. Afterwards, the desiccator was opened and the chloroform beaker was removed, the desiccator was aired out for 1 h, and the remaining chloroform in gaseous form was extracted using the vacuum pump. Finally, the samples were transferred into Nalgene bottles for the extraction process. Once in the Nalgene bottles, fumigated and non-fumigated samples were treated with 40 mL of K_2SO_4, shaken horizontally for 30 min, and let to settle upright for 10 min. Using a vacuum manifold system with plastic funnels and 55 mm Whatman No. 42 filter papers, the samples were filtered into Falcon centrifuge tubes; then, the samples were diluted to 5:1 (25 mL of ultrapure water to 5mL of sample extract) and analyzed using a Shimadzu TOC-L CPH Model Total Organic Carbon Analyzer with an ASI-L and TNM-L (Shimadzu Corporation, Analytical & Measuring Instrument Division, Jiangsu, China). The method used for measuring TOC involves acidifying an aliquot of soil extract with 1 M HCl, sparging the sample to remove the purgeable organic and inorganic carbon, and combusting at 720 °C in a combustion tube, where the remaining sample is detected by a non-dispersive infrared detector for TOC. For TN, the soil sample was combusted to NO and NO_2, then reacted with ozone to form NO_2 in an excited state, and the resultant

photon emission was measured by a chemiluminescence detector (Williams 2000; Shimadzu Corporation 2001).

Soil respiration: As a measure of microbial activity, soil respiration was measured at days 0, 3, 7, 15, 30, 45, and 60 using an IRGA LI-8100A connected to a Multiplexer box LI-8150 and the LI-8100 Automated Soil CO2 Flux System (LI-COR Biosciences, Lincoln, NE, USA). The samples were connected to this multiplexed flask system, which pumped ambient air into the sample jars to mix the air inside and measured the change in CO_2 mole fraction during an observation length of 3 minutes per sample. Since the analysis is done under the same conditions of flask volume, pressure, and temperature for all the samples, the system multiplies this change of CO_2 concentration over time (dC/dt) by the ideal gas law to obtain a CO_2 flux.

2.2.4 Statistical analysis

All the data analysis was done using R software (Version 3.6.2, R Core Team, 2020) unless otherwise indicated. To test the significance of soil type, wood type, and wood amount, three-way ANOVAs were performed for nutrient supply rates, microbial biomass C and N, carbon source consumption, and mean respiration. The assumptions of normality and homogeneity of variance were tested with qqplots, fitted vs. residual plots, and Shapiro-Wilk tests; in the case of non-normal data but with homogeneous variances, log transformations were applied. Each soil respiration value was divided by the soil area (m^2) in the flasks and a general linear model was performed to the cumulative data. Model selection using the Akaike Information Criterion (AICc) was performed to select the most parsimonious model for the general linear model and the three-way ANOVAs. Fisher's Least Significant Difference Test was performed as a Post-hoc test to identify statistical differences similarities among treatments. For the CLPP data, an outlier test was performed for the CO_2 rates; the data was organized in two

matrixes, one with all the substrates and a second matrix with data grouped by carbon source (amino acids, carbohydrates, and carboxylic acids), along with data on pH, EC and nutrient supply rates. A Non-Metric Multidimensional Scaling (NMDS) ordination was conducted in PC-ORD followed by a Multiple Response Permutational Procedure (MRPP). Finally, a correlation matrix was created to calculate Spearman's correlation coefficients and test the strength and direction of the relationships among all the response variables and soil properties.

2.3 Results

Wood type (aspen and pine shavings) was not a significant factor ($p > 0.080$) for any response variable and therefore was excluded from further results.

2.3.1 Soil nutrients supply rates

For all nutrients and treatments, soil type had the largest impact ($p<0.001$) followed by wood amount ($p<0.020$), but the response varied by nutrient (buried wood*soil type interaction, $p<0.001$).

Among soils (Figure 1), TIN supply rate was the greatest in fFFMM controls (0% wood, 716 μg/10cm^2/7days) but this soil also had the largest decrease associated with buried wood; more than a 95% decrease in response to the smallest wood application (10%). As the buried wood application increased in fFFMM, TIN continued decreasing until reaching the minimum detection limit for the PRS probes (2 μg/10cm^2/7days) at the highest 50% wood application rate. PMM had the next highest supply rate of TIN in the control at 233 μg/10cm^2/7days with a decrease of 40% after a 10% addition of buried wood, and a 98% decrease with 50% addition of buried wood (4 μg/10cm^2/7days). Peat had a supply rate of 27 μg/10cm^2/7days in the control and only showed a decrease in TIN with a 20% wood application or above, at 50% of wood the

supply rate was lower than the detection limit. cFFMM had the lowest TIN levels in the control and there was no significant response to wood application ($p=0.744$).

For phosphates ($H_2PO_4^-$, HPO_4^{2-}) cFFMM had the highest supply rates with 29.9 $\mu g/10cm^2/7days$ at 0% buried wood, and decreased significantly as the buried wood increased to a supply rate of 20 $\mu g/10cm^2/7days$ at 50% buried wood ($p=0.002$). The other soils had supply rates equal or less than the detection limit across all treatments; there were no significant responses to buried wood application in any of these soils ($p>0.100$).

Potassium (K^+) supply rates had a different pattern in comparison to phosphates and TIN, since they increased as the buried wood amount increased ($p<0.001$) with exception of fFFMM that had no significant response throughout treatments ($p>0.100$). The soil with the highest supply rate in the controls was cFFMM (253 $\mu g/10cm^2/7days$, $p<0.001$) followed by fFFMM with 14.2 $\mu g/10cm^2/7days$. PMM and Peat had supply rates lower than the detection limit. At 50% buried wood, in cFFMM the supply rates increased more than double reaching 542 $\mu g/10cm^2/7days$; in PMM the supply rates were 22 times higher than at 0%; and, for Peat, the supply rates had the second-lowest values but still were 25 times higher than at 0% buried wood.

Calcium (Ca^{2+}) had the highest supply rates in PMM (2970 $\mu g/10cm^2/7days$, $p<0.001$) followed by Peat, fFFMM and cFFMM at 0% buried wood. At 10% buried wood, there was no significant response from any soil ($p=0.980$). For cFFMM, the supply rates were two times lower after 20% buried wood and above ($p<0.001$); in PMM the rates decreased 25% at 50% buried wood; Peat and fFFMM had no significant decrease or increase throughout the different buried wood amounts ($p>0.40$). Magnesium (Mg^{2+}) supply rates at 0% buried wood were the highest in fFFMM (382 $\mu g/10cm^2/7days$, $p<0.001$) followed by cFFMM, PMM, and Peat. fFFMM had no significant response to buried wood ($p=0.060$); For cFFMM, the supply rates decreased as the

buried wood amount increased, having a supply rate more than two times lower at 50% ($p<0.001$); PMM and Peat had no significant response to wood application ($p>0.100$). Finally, for sulphates (SO_4^{2-}), supply rates in the controls were higher for Peat (1722 µg/10cm^2/7days, $p<0.001$) followed by PMM, fFFMM, and cFFMM; sulphates in Peat decreased constantly after 20% buried wood; in PMM, supply rates increased constantly and started declining at 50%; and fFFMM had a significant increase of at least 45% as a response to a 10% buried wood application ($p<0.050$) but decreased constantly with higher buried wood amounts.

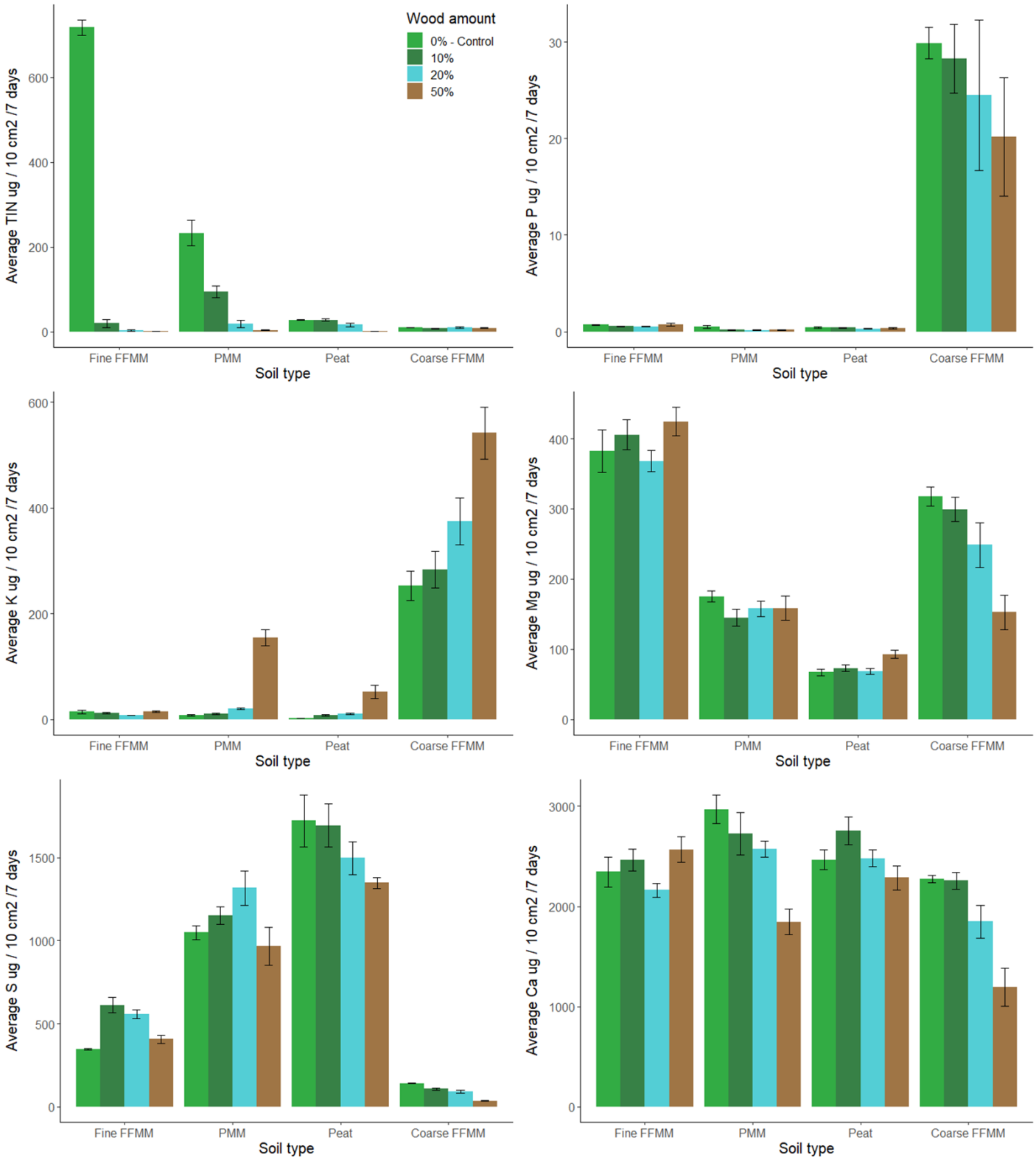

Figure 1. Soil nutrients supply rates for TIN ($NH_4^+ + NO_3^-$), phosphates ($H_2PO_4^-$, HPO_4^{2-}), potassium (K^+), magnesium (Mg^{+2}), sulphates (SO_4^{2-}), and calcium (Ca^{+2}) in different reclamation soils after a 60-days incubation different buried wood additions (%V).

2.3.2 Soil microbial communities (CLPP, respiration and biomass).

Buried wood addition increased carbon consumption rate (µg CO_2/g/h) in fFFMM, PMM, and Peat ($p<0.05$) but not cFFMM ($p>0.110$). PMM had the highest consumption rate of 82 µg Co_2/g/h followed by fFFMM with the highest report of 51 µg CO_2/g/h (Figure 2a). However, the overall carbon consumption rates between fFFMM and PMM were not significantly different ($p=0.042$). Peat and cFFMM had a carbon consumption rate approximately 10 times lower than fFFMM and PMM.

In fFFMM, amino and carboxylic acid consumption increased gradually and doubled at 50% of buried wood, while the carbohydrates consumption increased with 10% buried wood but remained constant with higher additions ($p>0.05$). In PMM, amino acid consumption increased after a 10% application, carboxylic acids usage increased 6.5 times in response to the 10% of buried wood addition, and carbohydrates consumption decreased with buried wood addition but no significant pattern was observed ($p<0.05$). In Peat, the consumption of all three carbon sources increased gradually after 10% of buried wood and were at least 2 times higher at 50% buried wood. Finally, in cFFMM amino acid and carbohydrates consumption rates increased with 10% buried wood while for carboxylic acids decreased, however, no clear pattern of response was observed.

Amino and carboxylic acids were the most consumed carbon sources (Figure 2b), accounting for 85% of the carbon consumption in fFFMM (43% and 42%, respectively), 94% in PMM (58% and 36%, respectively), and 71% in cFFMM (39% and 31%, respectively). Contrastingly, in Peat carbohydrates were the most consumed carbon sources followed by amino acids (37% and 30%, respectively).

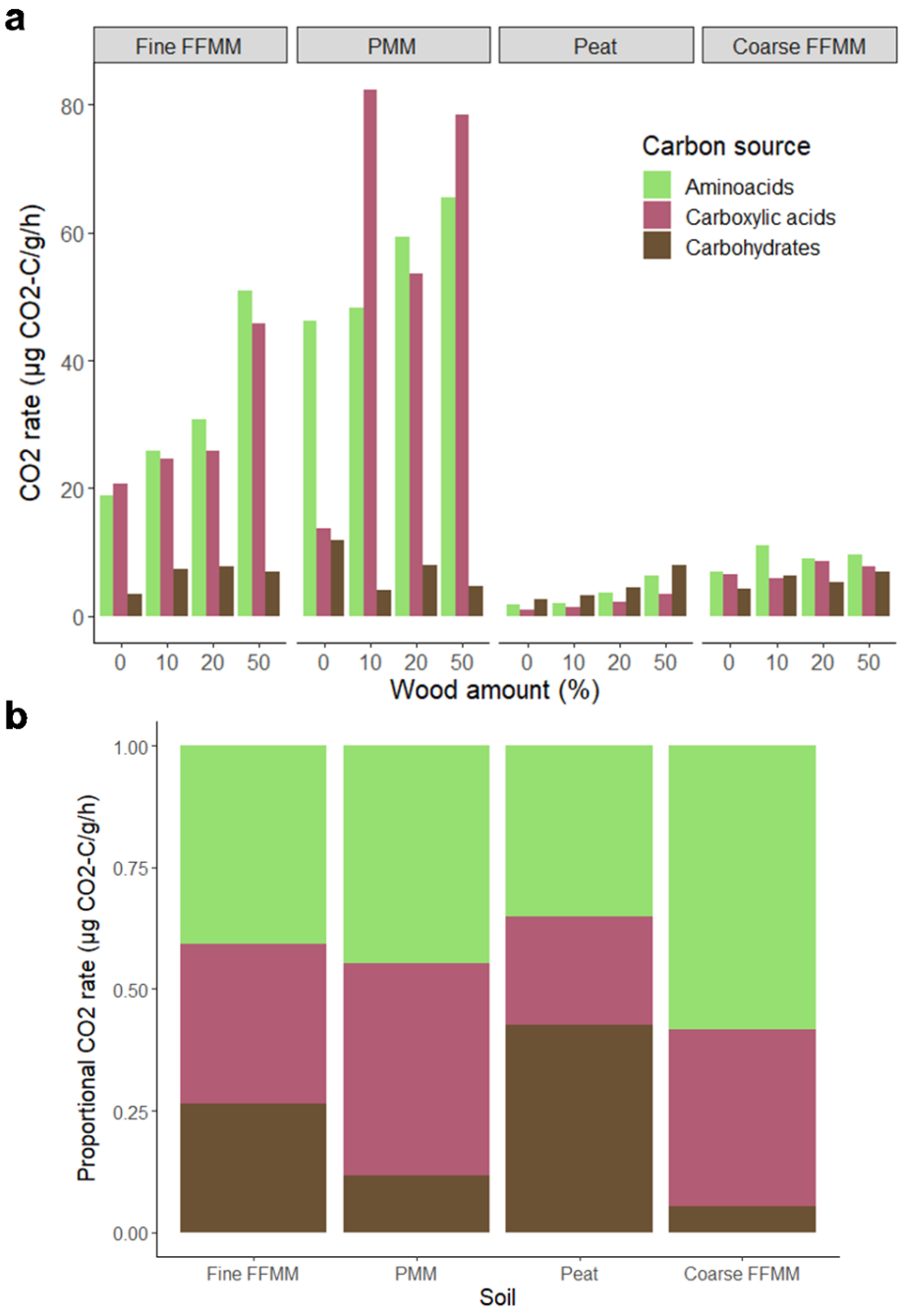

Figure 2 a. Consumption rates (CO_2 rate - µg CO_2/g/h) of different carbon substrates (amino acids, carboxylic acids, and carbohydrates) in fFFMM, PMM, Peat, and cFFMM as a response to buried wood addition after a 60-days incubation. **b.** Overall proportion of the different substrates consumption rate in the soils.

The variation in microbial communities among soils was significantly greater than the variation due to buried wood (Figure 3). Each soil had a significantly different microbial community activity ($p<0.005$) regardless of the amount of buried wood. However, the ordination plot shows that fFFMM and PMM are more similar. Peat had the greatest distance in the ordination from the other soils, likely indicating the most different microbial activity among the soils.

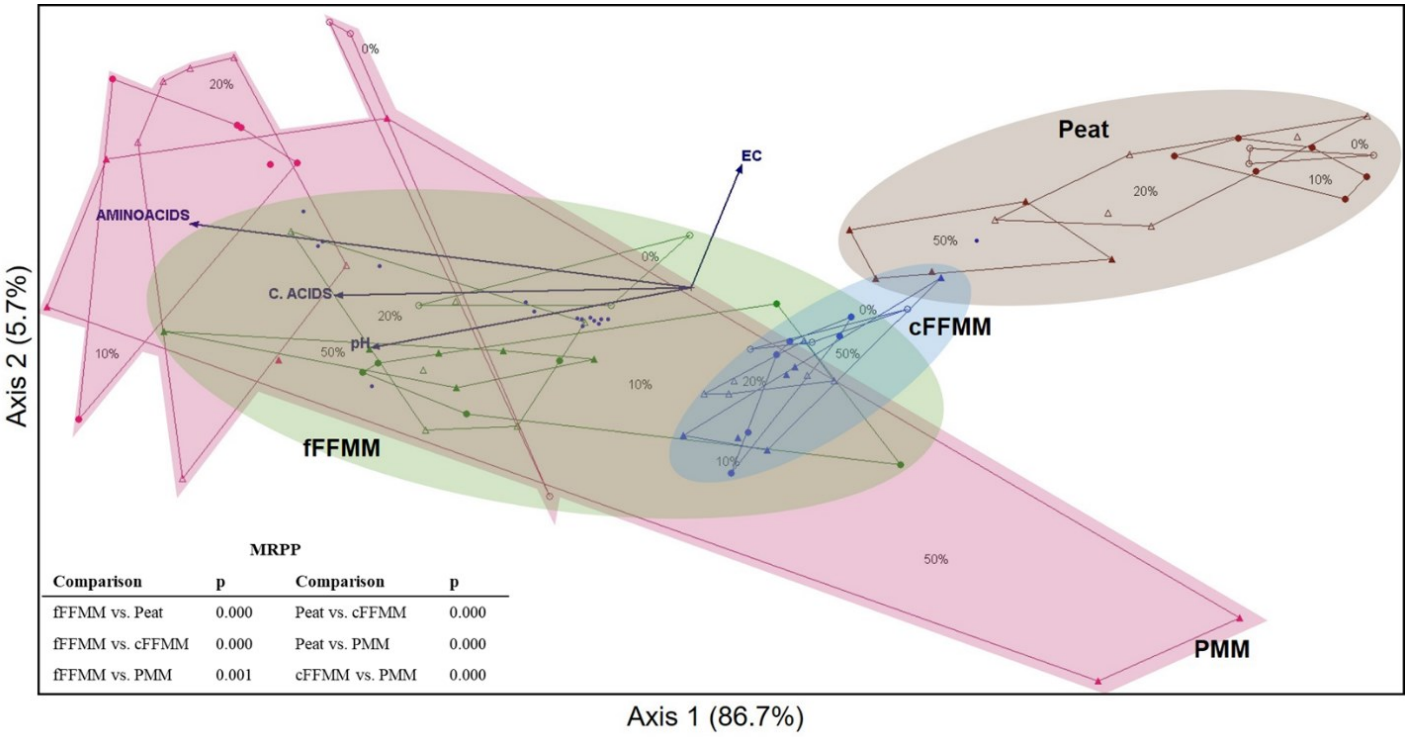

Figure 3. Non-metric Multidimensional Scaling (NMDS) ordination of fFFMM, cFFMM, PMM, and Peat with different buried wood amounts for Community-level Physiological Profile (stress= 6.23%) in response to carbon substrate (amino acids, carboxylic acids, carbohydrates, and water as blank), soil pH and EC, and soil nutrient supply rates. Multiple response permutational procedure (MRPP) shows all the soils have a significantly different microbial activity ($p<0.05$).

In terms of microbial biomass (organic C/MBC and organic N/MBN, Figure 4), in the controls, cFFMM had the highest organic carbon concentration (12.5 mg/L, $p<0.01$) followed by Peat, fFFMM, and PMM which had an initial organic C 4 times lower than fFFMM. In fFFMM,

MBC increased in response to the initial 10% of buried wood, but had no significant changes for greater buried wood additions ($p<0.05$). In PMM, MBC increased gradually with buried wood and had a peak at 50% buried wood ($p<0.05$), 4 times higher than the control. cFFMM had a decrease at 10% buried wood, recovered after 20% of buried wood but only increased MBC by 1% in comparison to the control. Peat had no significant response to wood addition ($p>0.05$). For MBN, fFFMM, PMM, and Peat had similar responses as observed for MBC with the exception that in the controls the MBN was below the detection limits, hence significant organic nitrogen concentrations were observed only after buried wood addition ($p<0.05$). On the contrary, in cFFMM the MBN had a decrease of 65% with 50% buried wood ($p<0.05$).

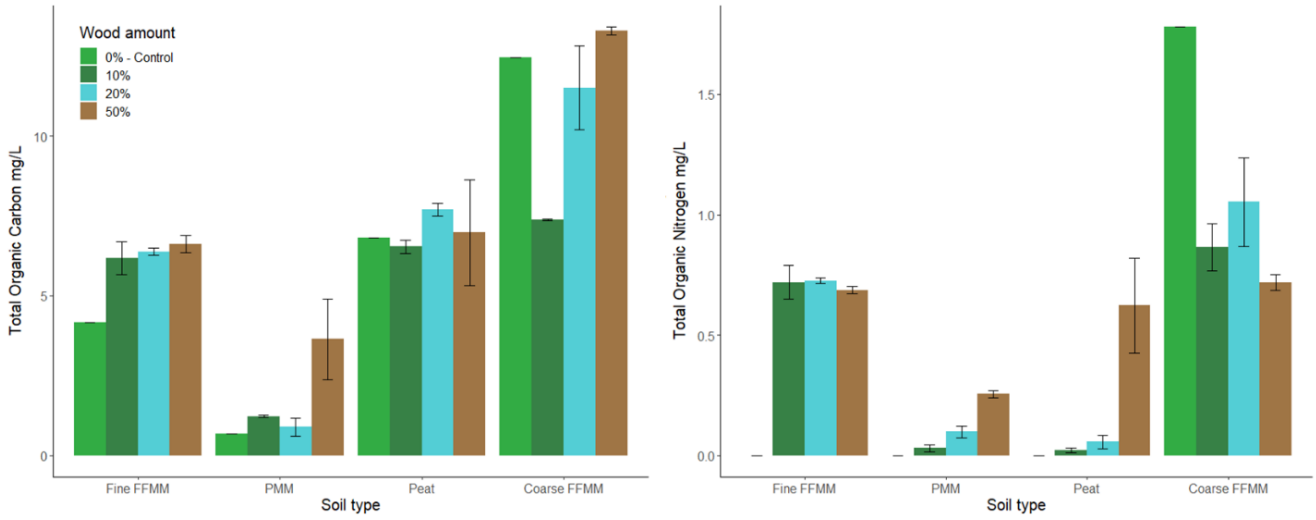

Figure 4. Soil microbial biomass (Total Organic Carbon-MBC and Total Nitrogen-MBN) in fFFMM, PMM, Peat, and cFFMM after a 60-days incubation with different buried wood additions (%V).

Mean soil respiration (Figure 5a) was the greatest in fFFMM for the controls (13.9 µmol $CO_2/m^2/s$) ($p<0.05$) followed by Peat, cFFMM, and PMM. In all soils except Peat, buried wood

addition increased soil respiration and the highest respiration rate was observed at 20% of buried wood, with an increase of 48% in fFFMM, 20% in cFFMM, and 16% in PMM. Peat had no significant response to buried wood until the 50% buried wood addition ($p<0.05$). Cumulative data (Figure 5b) shows that in fFFMM respiration increased gradually throughout the incubation period and at 20% the highest respiration was reported (139 µmol $CO_2/m^2/s$- Day60), a total increase of 1151% when compared to the respiration reported at Day 0 (12.07 µmol $CO_2/m^2/s$). For cFFMM and PMM, respiration in the controls had the lowest increase (1.13 µmol $CO_2/m^2/s$-Day0 to 9.43 µmol $CO_2/m^2/s$- Day60 in cFFMM, and 2.26 µmol $CO_2/m^2/s$-Day0 to 7.54 µmol $CO_2/m^2/s$- Day60 in PMM) in comparison to treatments with buried wood, which had an increase of at least 13%. Peat was the only soil with the highest respiration in the controls (53.94 µmol $CO_2/m^2/s$- Day60), buried wood addition decreased respiration in Peat up to 51%.

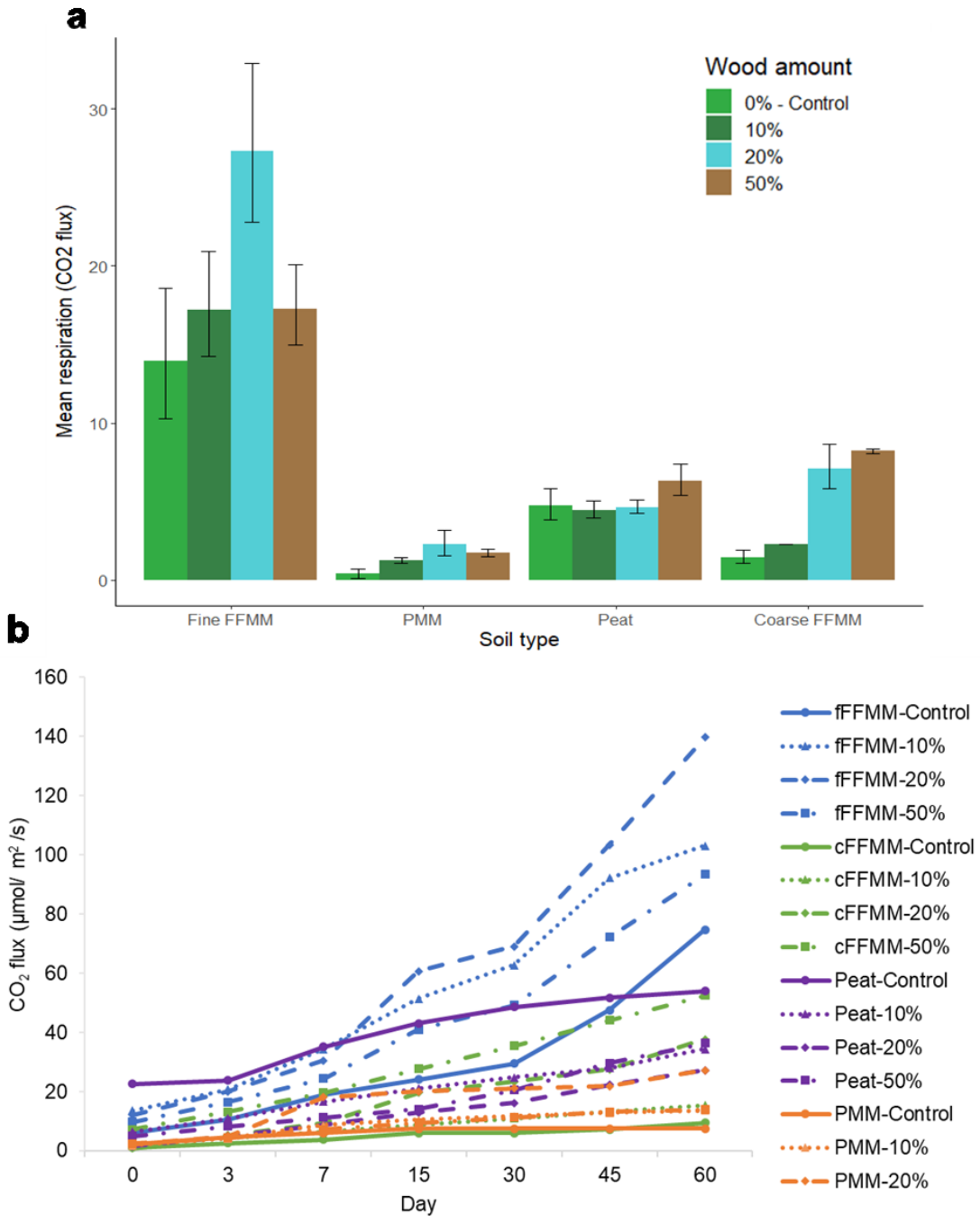

Figure 5. Soil respiration (CO_2 flux - µmol/m^2/s) in fFFMM, PMM, Peat, and cFFMM with different amounts of buried wood during a 60-day incubation. a. Mean soil respiration based on 2-weekly measurements (day 15, 30, 45, and 60). b. Cumulative soil respiration, fFFMM (blue), cFFMM (green), Peat (purple), and PMM (orange).

2.4 Discussion

Buried wood impacted the soil nutrients and microbial communities, mostly in fFFMM and PMM. Soil TIN was the most impacted nutrient by buried wood, while the other nutrients supply rates were dictated by soil type despite buried wood addition. Soil respiration, usage of amino acids and carboxylic acids, and microbial biomass C increased significantly as a response to buried wood addition. Peat and cFFMM had the least to not significant responses to buried wood. Regardless of the impact of the buried wood, all soils had contrasting responses, indicating that the main factor driving these responses was soil type, and only the changes within the soil were attributable to buried wood.

Wood addition to the soil resulted in a decrease of available nitrogen with the greatest impact on the lower C:N ratio soils. Overall, carbon consumption, microbial biomass C, and soil respiration were the lowest in the controls with no wood addition, and all increased with wood addition. As buried wood increases there is a higher demand for microbial activity to decompose the wood, resulting in an increment in soil microbial respiration, microbial biomass, and soil nitrogen usage. These findings coincide with studies that have found that microbial activity (respiration and biomass) increases and soil available nitrogen decreases with wood addition or when there is an increase in the soil C:N ratio (Moritsuka et al. 2004; Palviainen et al. 2010; Mukhortova 2012; Truong and Marschner 2018). Furthermore, this coincides with studies about surface wood on reclamation soils in Northern Alberta that reported decreases in available nitrogen in FFMM and PMM (Brown and Naeth 2014; Kwak et al. 2015b).

There was no significant difference in soil nutrients and microbial community responses between the aspen and pine wood addition. This could be mainly due to the disturbed lignin barrier in the wood shavings resulting from a physical disturbance like processing the wood into

shavings or grinding by insect borers in a natural context, which disrupts the lignin walls and exposes the cellulose and hemicellulose to decomposition (Kirk and Cowling 1984; Ulyshen and Šobotník 2018). Thus, there was no difference in resistance to breakdown between aspen and pine wood, resulting in a similar soil nutrient response. Another explanation could be that hemicellulose breakdown precedes lignin decomposition by white-rot fungi (Stokland 2012), so the lignin resistance is observed in a later decay stage again resulting in no difference among wood type in this short-term experiment.

Soil nutrient supply rates, carbon consumption, microbial biomass, and respiration varied among soil types with the greatest impact of buried wood on fFFMM and PMM. These soils with lower C:N ratio had a greater decrease of available nitrogen and a greater increase of soil respiration and microbial biomass with buried wood addition compared to cFFMM and Peat, soils with a higher C:N ratio. This suggests that, as expected, the increase in the soil C:N ratio in response to wood addition is the main factor driving the soil nutrients and microbial community responses, and this explains the different responses by soil. For example, fFFMM had the highest TIN supply rate without wood addition and the greatest decline just with the smallest wood application, simultaneously it also had a great increase in microbial biomass, carbon consumption, and respiration. This was likely because fFFMM has the highest nitrogen availability, microbial activity, and a lower C:N ratio in comparison to other soils used in reclamation like PMM and Peat (McMillan et al. 2007; Mackenzie and Quideau 2012; Jamro et al. 2014). Therefore, this soil had the greatest alteration on its C:N ratio, and consequently the most noticeable effect on microbial activity and TIN supply rates. PMM has a higher C:N ratio due to a higher proportion of organic matter and recalcitrant carbon (Hemstock et al. 2010), and has been reported to have lower microbial activity and nutrient content in comparison to fFFMM

(McMillan et al. 2007; Quideau et al. 2017), which also coincides with the results of this study. Thus, the responses were not as great since this soil already had a higher proportion of carbon and less available nitrogen. cFFMM had a higher C:N ratio compared to fFFMM and PMM, and the lowest nitrogen availability among all soils. Low nitrogen availability is typical in sandy soils (Carlyle et al. 1998; Erickson et al. 2005; Jalali and Merrikhpour 2008; Rees et al. 2020). Therefore, the increase in C:N ratio had no impact on nitrogen availability since this soil has an inherent low nitrogen content, but still was significant to increase microbial respiration and impact other nutrients. Finally, Peat had the highest C:N ratio and showed responses only with high additions of buried wood, since it already has an organic carbon content close to 50% as observed in this study (Table 1) and previous (Chambers et al. 2011). Therefore, this soil needed higher amounts of wood to have a significant change in the C:N ratio and had an impact on nutrients and microbial activity.

Regarding the other nutrients, phosphates ($H_2PO_4^-$, HPO_4^{2-}) had substantially low supply rates in all the soils (0.2 to 1.2 µg/10cm^2/7days) except for cFFMM with values up to 62 µg/10cm^2/7days and only cFFMM had a significant response to buried wood. Phosphorus immobilization has not been linked to a high C content or increases in the C:P ratio. Contrarily, studies have found that P immobilization is more related to a higher P content than to an increase in the C:P ratio (Enwezor 1976; Braakhekke et al. 1993; Bünemann et al. 2012). This still coincides with our findings since fFFMM, PMM, and Peat already had a low P content thus there was no potential for immobilization. However, the decrease of available P in cFFMM suggests that there was an impact from buried wood addition and the increase in the soil C:P ratio. Likely, the immobilization was mostly caused by the high P supply rates but yet this was related to the buried wood additions. Naeth et al. (2013) also found higher P availability in coarse mineral mix

soils than in other reclamation soils. The high supply rates in cFFMM could be because of the sandy texture of the soil and the high iron content inherent to Brunisolic soils (Smith et al. 2011). It is possible that due to the iron content in the Bm horizon and the aluminum present in the sand (McKeague and Day 1966), cFFMM has a high fixation capacity but the P is bonded and unavailable. However, this bonding is easily reversible and the neutral pH supports the solubilization and mineralization of P, making it available for plants (Richardson and Simpson 2011; Gustafsson et al. 2012). This agrees with a study that found that high P availability was linked to aluminum and iron bonding (Manimel Wadu et al. 2017). Another explanation could be that since sandy soils have high water percolation the P is prone to leaching (Djodjic et al. 2004), and because of the nature of this experiment the percolated water in the sample had no lower horizons to move into, resulting in a high P availability. This coincides with Quideau et al. (2017) who found that P release rates in FFMM and PMM depended solely on the volume of percolated water and that biological processes like microbial mineralization had no major participation in dictating P availability.

Buried wood had no impact on K supply rates in fFFMM, likely because K is immobile in soils with high clay content since it bonds strongly with the surface of the clay particles remaining fixed and unavailable (Lamp 1968; Mengel et al. 1976; Hoopen et al. 2010). Additionally, fFFMM had high rates of magnesium and calcium which can outcompete K for exchangeable sites on the soil particles (Lamp 1968; Reicks and Sciences Intern 2017), reducing, even more, the K availability. Ammonium has been reported to outcompete K (Hoopen et al. 2010; Bar Tal 2011) and this could explain why PMM and Peat had the highest K availability with the greatest buried wood amount and the less N availability since nitrogen immobilization reduced competition. Similarly, cFFMM had greater K availability as buried wood amount

increased, but in this case, it could be due to interactions with magnesium and calcium. K supply rates increased as Mg and Ca decreased so this could be also a case of competition between K and these nutrients.

Buried wood increased microbial activity in general supporting our hypothesis since the new input of wood demanded the microbial communities to work towards the process of decomposition (Moritsuka et al. 2004; Truong and Marschner 2018). Findings in the carbon source consumption showed how the microbial communities used carbon within a diverse set of metabolic pathways, supporting different anabolic and catabolic processes (Jones et al. 2018). For example, in fFFMM, all carbon substrates were more consumed as a response to buried wood, but amino acids consumption was slightly higher than carboxylic acids and the pathways supported by carbohydrates remained as the least used. fFFMM had the highest respiration among soils, other studies have also reported that respiration in FFMM is higher than in other reclamation soils like PMM and Peat (Naeth et al. 2013; Mackenzie et al. 2014), being indicative of high microbial activity. PMM was the soil with the greatest metabolic change. Buried wood provoked a remarkable increase in the consumption of carboxylic acids, implying that this carbon source was crucial to support the metabolic activity of wood decomposers, and carbohydrates remained as the carbon source with the least metabolic demand, as in fFFMM. In Peat and cFFMM, microorganisms consumed more carbon in response to wood addition but no change in the metabolic diversity was observed. It is possible that Peat had the lowest carbon consumption due to the nature of the microbial communities, considering that this soil was not collected from the top but a depth of 0.3 to 1.3 m and that Peat is usually under water-saturated conditions, it is likely that the microorganisms in this soil were anaerobic. Thus, an aerobic incubation is a different condition that could affect the performance and activity of the

communities. Peat was the only soil in which carbohydrates were the most used carbon source, this could be explained since several studies have found that carbohydrate consumption is linked to the proliferation of stress-resistant groups, breakage of soil particles, and release of organic matter (Baldock and Skjemstad 2000; Pesaro et al. 2003), which is expected since aerobic conditions increase decomposition activity and this is also supported by the increase in microbial nitrogen, possible evidence of more enzymatic activity. Finally, cFFMM had no clear response to buried wood in terms of carbon consumption and microbial biomass and held a low respiration and carbon consumption possibly because of the low nitrogen, organic matter content, and water holding capacity, crucial factors for supporting microbial activity (Higashida et al. 1986; Cao et al. 2016). Although buried wood caused changes within the microbial communities, each soil had a different physiological profile regardless of the wood addition.

Overall, fFFMM and PMM had similar responses to buried wood and also had similarities among the controls. The lowest C:N ratios, and similar pH and moisture content at field capacity were also shared as base characteristics before the incubation (Table 1). This similarity is likely because the addition of mineral material to peat results in a soil mixture with chemical and physical properties that function more similar to an upland surface soil, in this case, fFFMM, which is why PMM is a widely used material for oil sands reclamation and it is preferred over pure peat (Alberta Environment and Water 2012). Nonetheless, it is important to consider the nature of the mineral material, in this study PMM had a coarse mineral proportion (sand). This mineral itself when compared to fFFMM has different characteristics like a lower total cation exchange capacity (Shepherd and Bennett 2008), lower water retention, and nutrient availability (Erickson et al. 2005; Jalali and Merrikhpour 2008; Rees et al. 2020). However, when mixed with peat, a material high in organic matter and with a high water-holding capacity

(Mackenzie and Quideau 2012; Quideau et al. 2017), the interaction between these two materials results in a PMM that resembles the fFFMM. This study provides evidence that this resemblance also applies to how the soils respond to buried wood in terms of nutrient supply rates and the soil microbial communities. Yet, it would be important to investigate how a PMM with a fine mineral proportion would respond to buried wood addition.

On the opposite, Peat was the most different soil (Figure 3). Peat is the less preferred material to be used as cover soil in oil sands reclamation since it is not similar to upland soils due to the absence of a mineral component (Alberta Environment and Water 2012). From the initial characterization (Table 1), Peat was the most different soil with the lowest pH, twice the salinity, a higher C:N ratio, and approximately 5 times more moisture content when compared to fFFMM and PMM. A pH far from neutral and high moisture content are known to decrease microbial enzymatic activity, breakdown of organic matter, and thus the soil nutrient availability (Burns et al. 2013). This explains why this soil was the most different when considering all the response variables and the soil properties together, as this soil was already different from the starting point the responses to the same treatment, in this case, the buried wood addition, were also different. Finally, cFFMM had a similar C:N ratio with Peat and was also less similar to PMM and fFFMM when responding to buried wood and even among the controls. It could be said that this sandy soil, in contrast to Peat, was lacking an organic component since the low moisture content (Table 1), low organic matter content, and low water-holding capacity seemed to be the factors that caused the different responses in terms of microbial activity and nutrient supply rates.

CHAPTER III: FIELD STUDY

3.1 Introduction

Buried wood is defined as wood that is buried more than 50% by soil or litter (Moroni et al. 2015). In a natural landscape, it can be buried gradually by litter accumulation and vegetation overgrow, or rapidly by catastrophic events like landslides and fluvial deposition (Eden 1967; Hagemann et al. 2010a). This soil component is more common in coniferous forests since these ecosystems are associated with ground vegetation dominated by bryophytes, which functions as a thermal layer that traps moisture in the soil (Hagemann et al. 2010a). This results in high moisture content and low temperatures in the soil, decreasing the rate of wood decomposition and leading to wood preservation and accumulation (Moroni et al. 2015). In contrast, buried wood in broadleaf forests is less abundant due to ground vegetation that decomposes quickly and a more aerated soil with higher temperatures, which favors wood decomposition (Hobbie 1996; Prescott et al. 2000; Stokland 2012).

Studies about buried wood in Canada have occurred mostly in post-fire forests with fewer studies after harvesting and insect outbreaks. In these studies, buried wood was found generally in the form of organic forest floor material and logs (Brais et al. n.d.; Manies et al. 2005; Moroni 2006; Hagemann et al. 2009; Moroni and Ryan 2010; Moroni et al. 2010). In 2015, Moroni et al. reviewed the literature and conducted a meta-analysis of the current buried wood data, and included new data from Canadian forests (Moroni et al. 2015). In terms of occurrence in a wide range of ecozones and ecoregions with different types of vegetation, forest structure, successional stages, and topographies, the highest occurrence was reported in British Columbia with buried wood presence in 22.8% of the plots sampled, followed by Ontario with 13.6%,

whereas the lowest values were recorded in Nova Scotia with 7.1% and Alberta with 8.8%. In terms of volume, two paludified black spruce forests in Quebec and Labrador had the largest volumes of buried wood with 935 m^3/ha and 487m^3/ha, respectively; while the smallest volume was 1.6 m^3/ha reported in a broadleaf forest in Nova Scotia.

However, buried wood is also a soil component in anthropogenic forests, but is roughly understudied (Zeng et al. 2013; Moroni et al. 2015). In oil sands mining reclamation, the wood is buried by a rapid mechanical process that differs from natural forests. When starting the reclamation operations in a site (Alberta Environment and Water 2012), surface soils are collected from upland or lowland ecosystems to be used immediately as cover soil or stored in stockpiles for later use. Before soil salvaging, the merchantable timber is harvested and moved off-site, and the remaining slash and non-merchantable timber is coarse-mulched and left on site. Consequently, during soil salvaging operations this wood is also collected, mixed, and incorporated into the soil, which will be placed as cover soil (20 to 30 cm deep) on a reclamation site.

Nutrient immobilization, especially nitrogen immobilization as a response to wood addition in soils raises concerns in oil sands reclamation practices, since when wood is incorporated into the soil, decomposing organisms, i.e. bacteria and fungi, start the process of decaying it (Swift 1977; Stokland 2012). But, wood decomposition is a process that can take prolonged periods (Swift 1977; Kirk and Cowling 1984; Tuomi et al. 2011), in temperate and boreal forests the decomposition of a tree typically takes 50-100 years (Stokland 2012). Buried wood represents a high input of carbon, approximately 50% of the total wood mass (Pettersen 1984; Chandrasekaran et al. 2012), and wood decomposers need nutrients to carry out this extended task, especially nitrogen since it is necessary for enzymatic activity (Robertson and

Groffman 2006). Therefore, the soil C:N ratio increases, and as a response the available nitrogen in the soil is consumed by the microbial communities to work towards decomposition (Moritsuka et al. 2004). This is known as nitrogen immobilization and results in nitrogen not being available for plant uptake (Swift 1977; Jansson 1982; Jonasson et al. 1996). As wood decomposition advances and the demand for nitrogen decreases, nitrogen is eventually released to the soil to be available for plant uptake (Boddy and Watkinson 1994). There is an estimation that the volume of wood that remains on the surface and is subsequently salvaged with the soil is approximately 20-50 m^3, resulting in a 1.5% of buried wood in a salvage depth of 30 cm (Robert Vassov, personal communication, Nov 11th 2021). However, this has not been properly quantified.

There are studies about the effect of wood application on reclamation soils in the oil sands (Brown and Naeth 2014; Kwak et al. 2015a; Pinno and Gupta 2018), but all of them evaluated coarse woody debris, which is surface wood. The processes and conditions for this surface wood and soil interaction are different and may have different outcomes for soil nutrients and microbial communities. These studies have shown that surface-applied wood increases the soil bacterial biomass and functional group diversity (Kwak et al. 2015a), increases the soil water holding capacity and vegetation cover (Brown and Naeth 2014), supports native plants diversity, and reduces non-native species (Pinno and Gupta 2018). The impact of surface-wood application on nutrients is not clear. Brown and Naeth (2014) observed a decrease in soil available nitrate in sites with surface wood while Pinno and Das Gupta (2018) found that changes in the nutrient supply rates were attributable to the difference in soil types and not to the application of surface wood. Additionally, Kwak et al (2015b), used trembling aspen wood extract in a laboratory incubation and found that nitrogen availability decreased in both FFMM and PMM as a response

to the surface wood extract. Despite these studies, the buried wood component remains unexplored.

Consequently, the following research questions are proposed for this study:

- How much buried wood is there in reclamation soils?
- Are the soil nutrient supplies being impacted by the amount of buried wood present in the soils?

It is expected that the PMM soil will have the largest amount of buried wood than FFMM according to the previously reported in the literature (Moroni et al. 2015). However, as an alternative hypothesis, it could also be expected that FFMM has the largest amount of buried wood since broadleaf forests have more woody species, higher tree productivity, and thus more wood biomass production. Despite the merchantable timber being harvested and removed from the broadleaf forest, there may be remains from the harvesting process like branches, twigs, and other pieces of wood will represent a larger input than in the lowland forest, where there are fewer woody species and the plant productivity is lower due to the poorer nutrient and moisture regime (Beckingham J.D. and Archibald 1996).

In terms of nutrients being linked to the buried wood, it is expected that larger amounts of buried wood will be linked to nitrogen immobilization, especially in FFMM. Since FFMM soils have a lower C:N ratio than PMM soils, the presence of buried wood increases the carbon content in the soil, and thus the demand for nitrogen by the microbial communities will be higher. PMM is expected to be less impacted by buried wood since this soil already has a higher proportion of organic matter and hence a greater C:N ratio, so buried wood presence might alter the C:N ratio to a less extent.

3.2 Methods

3.2.1 Study area

Sampling for this study took place in July 2020 on an operational 5-year-old reclamation site at an oil sands mine 75 km Northwest north of Fort McMurray, Alberta, Canada (57.3377 ˚N -111.7552 ˚W). Located in the Central Mixedwood natural subregion, the natural ecosystem of this area is characterized by mixtures of upland forest and vast extensions of wetlands (Beckingham J.D. and Archibald 1996; Natural Regions Committee 2006). This reclamation site was created in the Spring of 2015 over a tailings dyke built with mine overburden material, 1 m of clay subsoil was placed on top of the overburden; then the site was capped with patches of forest floor-mineral mix (FFMM) surrounded by peat mineral mix (PMM) at a depth of 0.30 m (Debortoli et al. 2019; Trepanier et al. 2021). The FFMM was originally collected from an upland forest area in a fine-textured "d" ecosite, from the top 15 to 30 cm of an undisturbed Gray Luvisol (forest floor + upper mineral) (Beckingham J.D. and Archibald 1996). Peat and underlying mineral soil for the PMM were collected from a cleared lowland, characterized by an hygric to subhydric moisture regime and a poor to rich nutrient regime (Beckingham and Archibald 1996).

3.2.2 Buried wood and soil sampling

Thirty plots were selected randomly in each soil type throughout the reclamation site for a total of 60 plots. Two sampling spots were selected randomly from within 3 m of the plot centre for buried wood sampling. A pit was dug to a depth of 25 cm and a volume of 37 l of soil was collected. (61cm x 41cm x 23cm tote bin used as a guide). The volumetric samples were sieved in the field by hand and all the buried wood pieces were collected in reclosable bags. Additionally, a soil sample without buried wood of 1 L was collected from each plot for

laboratory analysis. The samples were collected on the last day of fieldwork and stored in a refrigerator, then stored in a freezer at -20 °C in the laboratory.

Soil volumetric water content (VWC) and temperature were measured for each plot in the field with a FieldScout TDR Soil Moisture Meter (FieldScout TDR 300, Spectrum Technologies Inc, Aurora, IL) and a digital thermometer (Taylor, Oak Brook, IL, USA).

Once in the laboratory, wood volume and size were measured for the collected buried wood. Wood volume was measured through water displacement, using a plastic container and wire mesh to hold down the wood; the volume of water displaced by the wood was considered the wood volume. In the reclamation literature, for woody material the concept of CWD is utilized and generally considers woody material greater than 8 cm in diameter (Alberta Environment and Water 2012); however, smaller woody material is also present in the reclamation soils. This is why we have decided to include all the woody material as buried wood, and establish different wood size classes based on length, diameter, and overall shape (Table 3): fine (narrow wood pieces when comparing diameter to length, generally include twigs and small branches, usually no greater than 1.5 cm in diameter), chips (chip-shaped or flatted pieces, usually no longer than 20 cm), and coarse (bulky and rounded pieces that seem like they were part of big branches or the trunk, usually greater than 8 cm in diameter). In addition to the wood volume in each soil sample, each wood piece was assigned into one size class and the volume of wood per class was measured. Furthermore, wood pieces were classified into one of the five decay classes established for hardwood logs and CWD by Pyle and Brown (1998, 1999).

Table 3. Size-class and decay-class system for buried wood.

Size class	Description	Decay class*	Description
Fine	Narrow and thin pieces; generally, twigs and small branches; no greater than 1.5 cm in diameter.	I	Bark sound and firmly attached; no stains by weathering
Chips	Chip-shaped or flatted pieces; usually no longer than 20 cm.	II	Bark, if present, not firmly attached; generally solid wood; surface does not flake off when kicked.
Coarse	Bulky and rounded pieces, seem to be part of big branches or the trunk of a tree.	III	Bark generally absent; firm when kicked; surface flakes off or has a shredded appearance; wet wood will compress like a sponge and bounce back; generally soft wood.
		IV	Will crush into pieces when kicked; very spongy wood or powder wood
		V	Predominantly powder wood.

* Adapted from Cogent characteristics of log decay classes I-V (Pyle and Brown 1998, 1999).

3.2.3 Soil analyses

Soil samples were placed on a laboratory shelf to reacclimate to room temperature from the storage conditions (-20°C), the bags remained closed to keep the field moisture. Afterwards, a subsample of approximately 15 g from each bag was air-dried completely, then ground and analyzed for total carbon (TC), total nitrogen (TN), and total organic carbon (TOC). To determine the weight concentration (w/w%) of TC and TN, the samples are completely combusted with chromium (III) oxide and silvered cobaltous oxide catalysts, purified oxygen is added to the reaction to increase the temperature from 1020°C to 1800-2000°C, causing the carbon to convert to CO_2 and the nitrogen to N_2 and NO_x. These combustion gases are carried to a reduction furnace to reduce all the NO_x species to N_2, and then through a sorbent trap to absorb water. The final N_2 and CO_2 gases are detected quantitatively by a Thermal Conductivity

Detector, the peak signals are proportional to the quantity of C and N present in the sample. For total organic carbon (TOC), the samples were acidified with 1M HCl to remove the inorganic carbon, then the samples are oven-dried at 70°C and analyzed by combustion analysis as described above. A second subsample of 10 g was sieved (2 mm) and air-dried for 24 h for pH and EC measuring following the method of Kalra and Maynard (1991) with deionized water, using a SevenEasy pH meter and a FiveEasy Conductivity meter (© Mettler Toledo).

Nutrient supply rates: In order to obtain results that are comparable with previous studies in oil sands reclamation soils, Plant Root Simulator (PRS) probes (Western Ag Innovations, Saskatoon, SK, Canada) were be used to determine the soil nutrient supply rates. 100 mL of each sample was transferred into reclosable bags and wetted to field capacity; the samples were pre-incubated for two weeks before probe insertion. After the pre-incubation period, one pair of probes was inserted into the bags so that the soil completely covered the probes at least 2cm. The probes attract and adsorb ions through electrostatic attraction; each pair consists of a cation probe for ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{+2}); and an anion probe for nitrate (NO_3^-), sulphate (SO_4^{2-}) and phosphates ($H_2PO_4^-$, HPO_4^{2-}). The samples were incubated for 7 days at 25°C and aired out every two to three days to maintain the aerobic conditions. After incubation, the probes were cleaned and rinsed thoroughly with ultrapure water, placed into reclosable bags, and sent to Western Ag. Innovations for analysis, which consisted of eluting the probes with 0.5 M HCl and determining inorganic nitrogen from the eluant through flow injection analysis, using a Skalar San++ Analyzer (Skalar Inc., Netherlands); the rest of the nutrients were measured using inductively coupled plasma (ICP) spectrometry (Optima ICP-OES 8300, PerkinElmer Inc., USA), (Western AG Innovations Inc. 2010).

3.2.4 Statistical analysis

All the data analysis was done using R software (Version 3.6.2, R Core Team, 2020) unless otherwise indicated. Buried wood volume data was normal for both soils (Shapiro-Wilk test $p=0.580$ for FFMM and $p=0.618$ for PMM) but did not fit the assumption of homogeneity of variances (Fligner-Killeen test $p<0.001$); therefore, two-sample t-tests for unequal variances were performed to determine significant difference. To test differences among buried wood size and decay classes, Welsch's ANOVAs were performed. A Non-Metric Multidimensional Scaling (NMDS) ordination was performed in PC-ORD to analyze the nutrient supply rates, buried wood content, and soil characteristics (pH, EC, TOC, VWC, and C:N ratio), followed by a Multiple Response Permutational Procedure (MRPP). Finally, a correlation matrix was created to calculate Pearson's correlation coefficients and test the strength and direction of the relationships among the buried wood and all the response variables and soil properties.

3.3 Results

3.3.1 Buried wood in reclamation soils

FFMM had more buried wood (27.8 m^3/ha – 1.24%) than PMM (9.58 m^3/ha – 0.42%, $p=0.017$). Coarse and chips were the most abundant sizes of wood ($p<0.001$), occupying at least 75% of the total buried wood in both soils (Figure 7a). In terms of decay (Figure 7b), the most abundant decay class was III (absent bark and firm wood) for both soils ($p<0.001$) occupying 64.6% and 44.8% of the total buried wood in FFMM and PMM, respectively. In FFMM, class III (bark absent but solid wood) was followed by class IV (spongy wood), class II (bark present and signs of weathering), and class I (bark present and solid wood) occupying the less volume (3.07%). In PMM, class III was followed by class II, class I, and class IV with less volume (13.4%). The only class that was not observed in either soil was class V, highly decayed powder

wood.

Figure 6. Proportional buried wood (m^3/ha) found in FFMM and PMM in a 6-years old reclamation site. a. Composition of size classes, b. Composition of decay classes.

3.3.2 Soil nutrients and properties in relation to buried wood

Nutrient supply rates varied by soil type but were not affected by buried wood amount in either soil ($p>0.60$). Buried wood amount was not significantly correlated to any nutrient supply rate nor any soil parameter ($p>0.05$, Table 4). TIN supply rates were different between FFMM and PMM (145.93 µg/10cm^2/7days and 97.19 µg/10cm^2/7days, respectively, $p=0.007$). Phosphates and K supply rates were significantly lower in PMM ($p<0.05$, Table 5) while sulphates supply rates were almost 2.6 times higher in PMM ($p<0.001$). pH, EC, and volumetric

water content (VWC) were similar in both soils ($p>0.09$). Total organic carbon (TOC) was 42% higher in PMM (11.58%, $p<0.001$), and the C:N ratio was also higher in PMM ($p<0.001$). Despite the differences in terms of individual nutrients, buried wood content, and the C:N ratio, the overall soil profile was not significantly different between FFMM and PMM (Figure 8, stress=7.43%, $p=0.248$).

Table 4. Correlation between buried wood amount (BW, m3/ha), nutrient supply rates (µg/10cm2/7days), and soil parameters for FFMM and PMM. Values indicate Spearman's correlation coefficients, no correlation was significant ($p>0.05$). TIN: Total inorganic nitrogen, EC: Electrical conductivity, VWC: Volumetric water content, T: Temperature, TOC: Total organic carbon.

Buried Wood in	TIN supply rates	Phosphates supply rates	K supply rates	pH	EC (µS/cm)	VWC	T (°C)	TOC (%)
FFMM	-0.05	0.04	-0.15	-0.12	0.06	0.08	0.26	-0.09
PMM	0.24	0.07	-0.17	0.3	-0.01	-0.1	0.01	0.24

Table 5. Soil nutrient supply rates, buried wood content, and physicochemical characteristics of FFMM and PMM in a 5-years old reclamation site. Values indicate means, p: Two-sample t-test p value, *indicates significance ($p<0.05$).

	Soil type		
Soil characteristic	**FFMM**	**PMM**	**p**
Buried wood (m^3/ha)	27.8	9.58	0.000*
TIN supply rates (µg/10cm^2/7days)	145.93	97.19	0.0076*
Phosphates supply rates (µg/10cm^2/7days)	2.67	1.12	0.017*
K supply rates (µg/10cm^2/7days)	30.87	18.70	0.025*
Ca supply rates (µg/10cm^2/7days)	2367.22	2649.46	0.0086*
Mg supply rates (µg/10cm^2/7days)	460.65	405.73	0.052
S supply rates (µg/10cm^2/7days)	276.86	727.31	0.000*
pH	6.93	6.70	0.096
EC (µS/cm)	497.94	492.23	0.948
TOC (w/w%)	4.92	11.58	0.000*
C:N ratio	21.14	26.03	0.000*
VWC	34.95	48.06	0.150

Temperature (°C)	15.0	15.94	0.037*

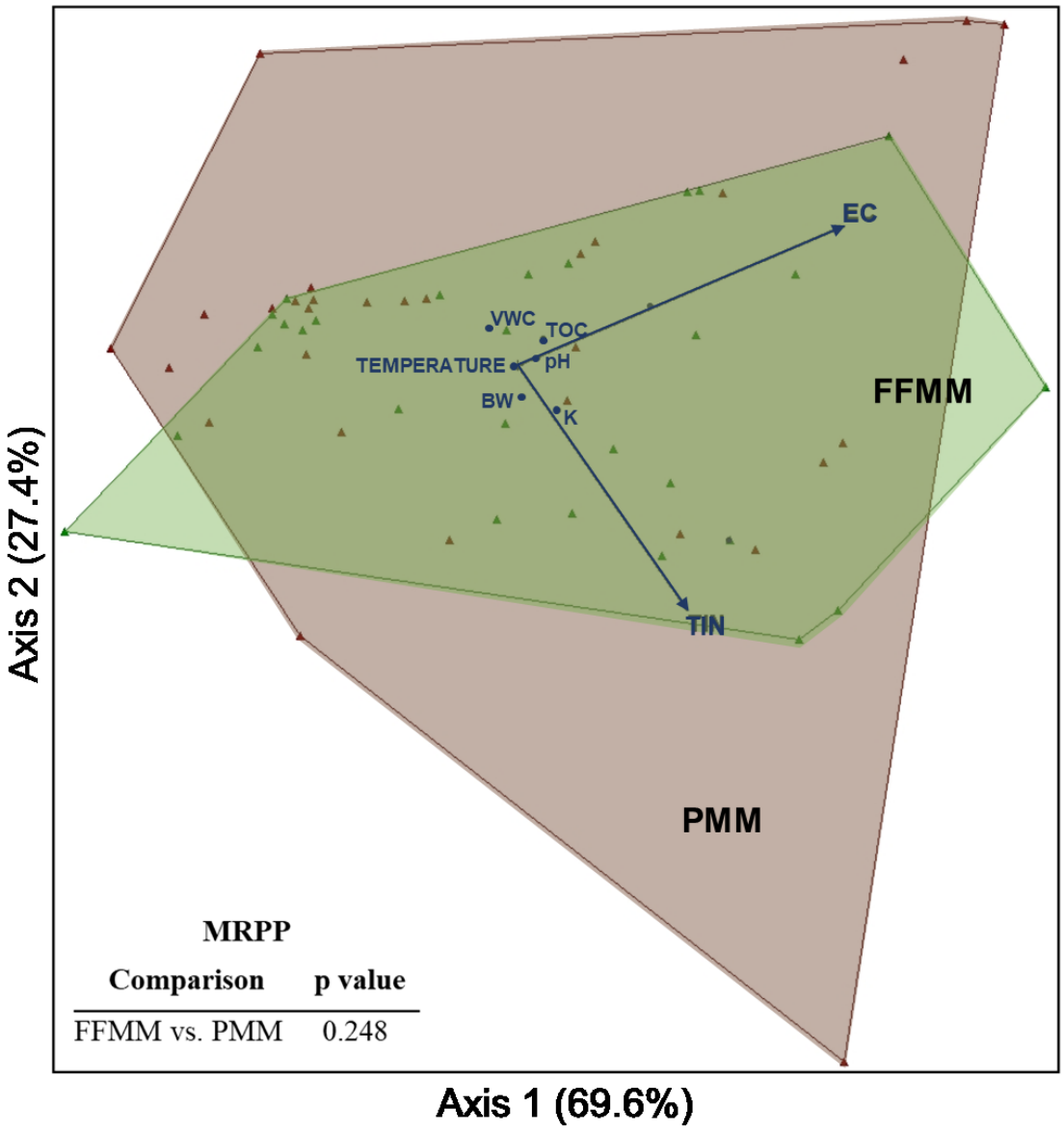

Figure 7. Non-metric Multidimensional Scaling ordination (stress=7.43%) of FFMM and PMM in a 6-years old reclamation site for soil buried wood (BW), TIN, P, and K supply rates, pH, EC, temperature, volumetric water content (VWC), total organic carbon (TOC), and C:N ratio. Multiple response permutational procedure (MRPP) shows that there is no significant difference between soils (p=0.248).

3.4 Discussion

PMM soil had significantly less buried wood than the FFMM. This is likely because the source of this soil, lowland forests, have a vegetation predominantly of non-woody plants like shrubs and mosses; additionally, tree productivity is lower than in upland forests due to the poor drainage and nutrient regime of lowland soils (Beckingham J.D. and Archibald 1996), decreasing, even more, the potential of wood biomass. In contrast, upland forests are more physically structured, meaning they have more horizontal and vertical layers compound by different types of vegetations that form a canopy, an understory, a shrub layer, and ground cover (Perry et al. 2008). This results in more abundance of woody material that will potentially be salvaged and incorporated into the soil as buried wood. This also explains the difference in size class composition, as coarse woody material like big branches and trunk pieces are more likely to come from an upland forest and fine woody material like twigs and small branches are more representative of lowland vegetation. Initial decay stages (Class I and II) were more observed in PMM, this was probably because during the salvaging process buried wood that was already in the soil was also collected along with the wood coming from the clear-cutting operations. Thus, this wood had been protected from decomposition by the low oxygenation and low temperatures common in lowland landscapes (Hagemann et al. 2010a; Moroni et al. 2010).

Greater nitrogen availability in FFMM compared to PMM is consistent with previous findings in reclamation soils of this area (McMillan et al. 2007; Mackenzie and Quideau 2012; Jamro et al. 2014). This difference is attributed to different factors like the lower C:N ratio and greater enzyme activity, organic matter decomposition, and nitrogen mineralization in FFMM. Phosphates and K availability also tend to be higher in FFMM in accordance with previous studies that have found up to 16 times more P and 1.5 times more K in FFMM compared to

PMM (Mackenzie and Quideau 2012; Brown and Naeth 2014; Pinno et al. 2014; Howell et al. 2017; Quideau et al. 2017). The difference in P availability is attributed to biotic and abiotic processes, like a higher P mineralization rate and desorption or dissolution of mineral phosphates (Bünemann et al. 2012). Additionally, peat is likely to have P deficiency due to the high C content (Mcgill and Cole 1981; Wan et al. 2015), which potentially favors P immobilization in this PMM soil. Potassium availability is likely related to the higher total cation exchange capacity of FFMM due to the higher clay content (Tahir and Marschner 2017). Findings in calcium and sulphates availability are consistent with a previous study that compared the biogeochemistry of these two soils (Mackenzie and Quideau 2012), where PMM had up to 3 times more Ca and 3.7 more times sulphates than FFMM. Organic soils in fens tend to have higher calcium content since the groundwater tends to be rich in calcium and magnesium (Golder Associates Ltd. 2007). Similarly, sulphates are linked to decomposed organic matter (Eriksen and Schnug 1998), characteristic of organic soils and this study also found a higher concentration of TOC in PMM.

Despite these differences, FFMM and PMM had no difference in their overall soil profile when considering all the characteristics together (Figure 8). This could be attributed to the similarity in pH, VWC, and temperature. The fact that both soils were under similar environmental conditions is possibly mitigating the individual differences, since these environmental factors have been reported to limit the performance of soils and are strongly linked to enzymatic activity, thus to nutrient release and availability (Bhattarai et al. 2015). Previous studies have reported that FFMM and PMM differ in physical and chemical properties and nutrient dynamics (McMillan et al. 2007; Mackenzie and Quideau 2012; Quideau et al. 2017). However, in one of these studies (Mackenzie and Quideau 2012) the samples were

directly collected from the natural forests, whereas in this study the samples were collected from soils that had been already disturbed by the process of salvaging and placement, and had been in the same reclamation landscape for 5 years. In other studies, is very common that the factors were tested individually or per category and no multivariate analysis was performed to test the overall performance of the soils considering nutrient availability and physical and chemical properties altogether. But, it is important to keep in mind that the findings of this field study are limited to the reclamation site since as mentioned before by Howell et al. (2017) the differences among studies in this same region highlight the variation inherent to the soil characteristics and quality based on their provenance. Furthermore, it has been reported that when soils are moved from a natural landscape and placed in a reclamation setting, with time, these are less similar to the ecosystem of origin and may become novel ecosystems (Rowland et al. 2009). This is a possibility for these two soils that have been removed from their natural landscapes and endured the same environmental conditions for 5 years, there is more similarity between them but it is outside the scope of this study to determine if they are becoming more distant from their original ecosystems.

The buried wood volume found in both soils did not surpass the 1.5% estimated in oil sands reclamation soils for this area. These values were too low in comparison to the buried wood amounts used in the incubation study (0%, 10%, 20%, 50%), which allowed to see differences in nitrogen availability. In the field, there was not enough variation of buried wood within each soil that allowed to identify different levels and observe associated differences in nitrogen availability. The low proportions that the buried wood is occupying in both cover soils (FFMM: 1.24% and PMM: 0.42%) limits the impact this material can have on the soil nutrients and the microbial communities, like a significant increase in the soil C:N ratio and subsequently

nitrogen immobilization due to a higher demand of nitrogen by microorganisms to perform wood decomposition (Moritsuka et al. 2004; Truong and Marschner 2018). However, this does not mean that the buried wood is not affecting reclamation soils, it means that at these levels of application this material represents a low proportion of the soil, and then it is not enough to alter the soil C:N ratio and cascade changes in microbial activity and nutrient availability. If a higher variation of buried wood were present in the soils, the largest volumes of buried wood would be related to nitrogen immobilization, additionally, the soil with more buried wood would also be the soil with less nitrogen availability. In this case, FFMM had the largest volume of wood (1.24%) and the greatest nitrogen availability at the same time (146 µg/10cm^2/7days), yet these two parameters were not correlated, since nitrogen availability was a function of other factors, as previously discussed.

CHAPTER IV: CONCLUSIONS AND MANAGEMENT IMPLICATIONS

4.1 Study implications and limitations

The findings of this research provide a further understanding of how buried wood can potentially impact the soil nutrient availability in different soil types used in oil sands reclamation in Northern Alberta, along with some insight for future reclamation practices. Yet, it is important to consider the associated limitations.

The incubation study found that buried wood addition can cause nitrogen immobilization due to an alteration in the soil C:N ratio and an increase in the demand for nitrogen by the soil microbial communities. This could imply a detrimental impact of buried wood on the soil fertility and thus on the plant growth in reclamation settings. However, the ratios used in the incubation study are not representative of the operational field applications, which are estimated to be less than 1.5% and this was confirmed by the field study. Therefore, one of the implications of this study is that the amount of buried wood present in reclamation soils in the area of study is not large enough to cause concerns about soil nutrient availability, specifically, nitrogen availability.

Another implication for reclamation practices is, soils with a lower C:N ratio are more susceptible to nitrogen immobilization as a response to buried wood addition. This means that when salvaging soils like fFFMM (upland FFMM with a fine mineral component) and PMM it is recommended to monitor the amount of wood that is incorporated into the soil.

Both studies in this research evidenced the resemblance between fFFMM and PMM, and how Peat was the most different soil. These findings support the utilization of PMM in upland reclamation as the mixing of peat and mineral material results in soil with similar characteristics of an upland soil, in this study the fFFMM. However, the findings in this study are limited to PMM soils with a coarse mineral component (sand), since a PMM with a fine mineral component might have different responses to buried wood and probably a different degree of similarity to upland soils. Peat was the most different soil when compared to fFFMM and PMM, however, this is a suitable material for reclamation of wetlands or transitional sites that require higher moisture and higher organic content, and it can also be used in upland reclamation as a layer within different profiles. Although the cFFMM had lower nitrogen availability and microbial activity, this study does not place cFFMM as a less recommended soil for reclamation practices, as different reclamation objectives have different soil requirements and coarse soils may be suitable for the establishment and development of sites with drier/poorer and moisture and nutrient regimes. These implications are in agreeance with the currently accepted practices in oil sands reclamation in Northern Alberta (Alberta Environment and Water 2012).

Additional limitations of this study are the use of wood shavings in the incubation study since the field applications of buried wood are in form of coarse-mulched material, which could mean a different response in the field. Also, both the incubation study and the field study are short-term studies considering the period of incubation (60) and the age of the reclamation site (5 years) after wood application. It is outside the scope of this study to determine the long-term impact of buried wood on soil nutrient availability, however, it is expected that as wood reaches higher stages of decomposition, the demand for nitrogen will decrease and it will be released back to the soil to be available for plant uptake, as long as the soil conditions are favorable.

4.3 Future research and recommendations

Research that investigates the impact of lower amounts of buried wood is recommended as the applications used in this study were significantly larger than the observed in the field. A greenhouse study that investigates the soil nutrient availability and the plant growth of species of interest in oil sands reclamation in response to levels of 0%, 2%, 6%, 8%, and 10% of buried wood would be interesting to perform, as the soil responses with applications below 10% are still unknown, and these levels of buried wood are closer to the actual field applications. Additionally, the use of coarse mulch in buried wood studies in oil sands reclamation soils is suggested instead of shavings. If possible, it would be better to use the buried wood from the same site of soil collection to include the particular types of wood present in each ecosystem.

The fact that Peat was the most different soil in the incubation study in terms of nutrient availability and microbial activity, invites to investigate the performance of this material in a field setting. What differences can be observed when comparing Peat with other reclamation soils in the field and what possible advantages or disadvantages does Peat provide when used as a reclamation soil?

Research that investigates the differences between PMM-fine mineral and PMM-coarse mineral in terms of nutrient availability, plant growth, and overall properties would be interesting to carry out. This would provide a better insight into the variations within this type of reclamation soil, how different mixes can be more similar to upland soils, and how beneficial they are for plant growth depending on the species of study. Also, the interaction between different mineral and organic components when doing mixes to create reclamation soils should be investigated, and how these different mixes can impact moisture retention, nutrient availability, and plant growth.

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APPENDIX

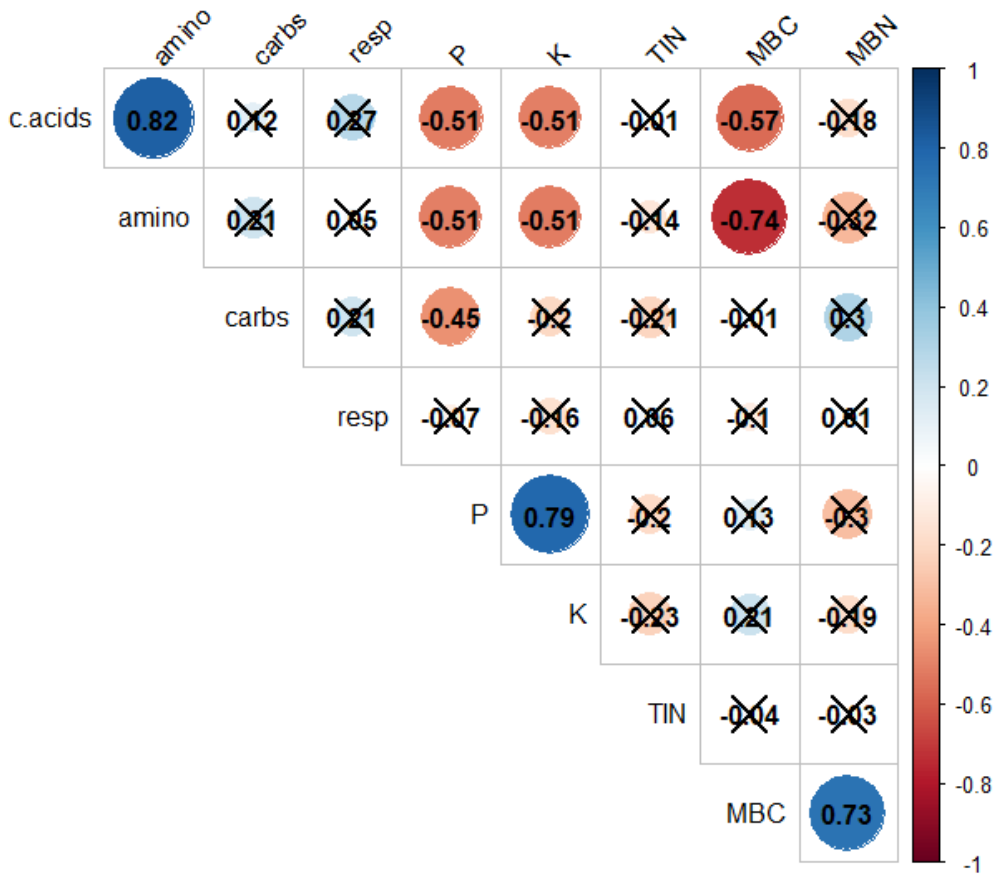

Figure A1. Correlation plot with Spearman's correlation coefficients for the response variables in the incubation study. Amino acids, carboxylic acids, and carbohydrates consumption; soil respiration; TIN, P, and K supply rates; and microbial biomass C and N. Values indicate rho coefficients, crossed cells indicate no significant correlation ($p<0.05$).

Table A1. Treatments and sample weights used in the incubation study. Water-filled pore space: WFPS.

Treatment	Sample ID	Total jar weight (g)	WFPS (%)
Fine FFMM 0%	1	494.52	19.63
	2	499.04	21.06
	3	499.42	20.37
Fine FFMM Aw 10%	4	490.17	19.65
	5	485.92	17.76
	6	501.89	20.19
Fine FFMM Aw 20%	7	475.98	17.49
	8	473.92	18.03
	9	474.29	17.16
Fine FFMM Aw 50%	10	411.26	10.91
	11	438.13	16.55
	12	409.08	11.06
Fine FFMM Pine 10%	13	483.39	18.13
	14	502.43	21.29
	15	485.99	19.08
Fine FFMM Pine 20%	16	458.24	15.09
	17	461.17	16.39
	18	458.03	16.49
Fine FFMM Pine 50%	19	402.52	10.88
	20	430.4	12.90
	21	380.89	9.83
Peat 0%	22	389.41	27.08
	23	387.73	23.96
	24	394.74	25.81
Peat Aw 10%	25	366.11	23.11
	26	366.53	22.95
	27	372.68	24.10
Peat Aw 20%	28	363.97	20.05
	29	365.09	21.64
	30	379.62	21.99
Peat Aw 50%	31	353.36	13.14
	32	337.7	12.93
	33	338.3	12.53
Peat Pine 10%	34	389.73	24.56
	35	382.78	23.43
	36	369.4	23.33
Peat Pine 20%	37	366.84	17.74
	38	354.49	16.55
	39	376.69	21.16

Peat Pine 50%	40	353.82	13.50
	41	345.72	16.54
	42	341.24	12.64
Coarse FFMM 0%	43	443	8.77
	44	431	8.96
	45	444	8.02
Coarse FFMM Aw 10%	46	433.1	9.22
	47	441.89	9.82
	48	433.62	11.00
Coarse FFMM Aw 20%	49	427.22	9.77
	50	405.91	8.80
	51	424.75	8.34
Coarse FFMM Aw 50%	52	364.32	6.00
	53	371.16	4.69
	54	370.09	5.65
Coarse FFMM Pine 10%	55	431.42	10.35
	56	438.81	10.56
	57	444.76	10.30
Coarse FFMM Pine 20%	58	430.9	10.74
	59	426.03	12.17
	60	419.6	9.36
Coarse FFMM Pine 50%	61	363.76	7.29
	62	363.43	6.84
	63	386.16	8.52
PMM 0%	64	449.19	11.34
	65	456.52	22.34
	66	442.28	22.19
PMM Aw 10%	67	436.5	21.27
	68	458.86	17.36
	69	454.01	16.71
PMM Aw 20%	70	412.4	13.84
	71	431.61	14.66
	72	443.05	21.82
PMM Aw 50%	73	370.87	11.49
	74	372.01	7.96
	75	378.5	10.86
PMM Pine 10%	76	439.23	17.71
	77	470.17	21.65
	78	450.25	16.79
PMM Pine 20%	79	425.53	14.01
	80	433.81	17.02
	81	427.12	20.87

	82	366.98	10.15
PMM Pine 50%	83	366.41	11.57
	84	361.6	9.65