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**Organic capping type affected nitrogen availability and associated enzyme activities in reconstructed oil sands soils in Alberta, Canada**

G.M. Jamro1, 2, S.X. Chang1,\* and M.A. Naeth1

1 Department of Renewable Resources, University of Alberta, Edmonton, AB T6G 2E3,

Canada

2 Department of Soil Science, Sindh Agriculture University, Tandojam 70060, Sindh, Pakistan

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\*. Corresponding author: Email: [scott.chang@ualberta.ca](mailto:scott.chang@ualberta.ca), Phone: (780) 492-6375, Fax: (780) 492-1767

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**ABSTRACT**

Organic materials applied in land reclamation play a key role in the development of ecosystem properties and functions. Peat mineral soil mix (PMM) and LFH (identifiable litter (L), fragmented litter (F) and humus (H)) mineral soil mix (LFH) are commonly used organic amendments for oil sands reclamation in northern Alberta. These materials have contrasting soil properties, with organic matter in LFH more decomposed and having a lower carbon-to-nitrogen (C:N) ratio than that in PMM. We quantified the effects of LFH and PMM capping material on N availability and enzyme activities during early ecosystem development in the oil sands region. Monthly samples were taken from 0-10 and 10-20 cm layers from June through October in 2011 and 2012. The N availability and activities of soil enzymes including *β*-1,4-N-acetyl glucosaminidase (NAGase), urease, arylamidase and protease were measured. In-situ N availability was measured using plant root simulator (PRSTM) probes. Repeated measures ANOVA indicated that N availability and NAGase, arylamidase and protease activities were greater in LFH than in PMM and were affected by time of sampling. These differences were attributed to the lower C:N ratio in LFH than in PMM. We found greater N availability and enzyme activities in the fall than in the summer in both years. These differences were likely caused by fresh labile C inputs through root exudates and litter fall during fall that induced greater enzyme activities and led to greater N mineralization despite the potential limitation by the lower fall temperature. Overall, the greater N availabilities and enzyme activities in LFH suggest that LFH would be a better soil capping material than PMM for early ecosystem development in oil sands reclamation.

*Keywords:* Land reclamation, LFH capping, fluorimetric soil enzyme assay, PRS TM probe, ecosystem development

1. **Introduction**

Mining of oil sands in the Athabasca Oil Sands Region in northern Alberta is one of the largest anthropogenic disturbances of terrestrial ecosystems in the world (Alberta Government, 2010). Surface mining activities in this region have impacted approximately 767 km2 of land, representing 0.2% of the boreal forest (Alberta Government, 2013). Surface oil sands mining involves removal of several ecological layers including the vegetation, soil and geological material (Giesy et al., 2010). Current regulations require that oil sands companies reclaim disturbed areas to equivalent land capability after cessation of surface mining operations (CEMA, 2006), but given the magnitude of the disturbance, much research is needed to help restore disturbed ecosystems to pre-disturbance conditions.

Successful reclamation of disturbed oil sands areas requires a broad understanding of nutrient cycling and ecosystem development. Nitrogen (N) is often a limiting nutrient in boreal forest soils in the oil sands region (Cheng et al., 2011), particularly in newly reconstructed ecosystems in the oil sands, where native N inputs are often lacking (Bradshaw, 1987). Availability of N in reclaimed ecosystems is strongly regulated by organic matter (OM) decomposition, which is greatly influenced by soil enzyme activities (Sinsabaugh et al., 1991). Soil enzymes associated with N cycling include β-1, 4-N-acetylglucosaminidase (NAGase), which is involved in the degradation of chitin, a component of fungal cell walls (Ueno et al., 1991), protease, which catalyses protein hydrolysis to peptides and amino acids and may also supply a large part of the bioavailable N (Ladd and Butler, 1972), and arylamidase, which is involved in hydrolysis of N-terminal amino acid from peptides and amides in soils (Acosta-Martinez and Tabatabai, 2000). In contrast, urease plays a major role in the hydrolytic reaction of urea to form ammonium and carbon dioxide in the soil. Soil enzymes are highly sensitive to environmental changes and can therefore be used as indicators of functional processes related to vegetation establishment and soil quality. Thus, measurement of soil enzyme activities may provide an estimate of N cycling intensity (Dick et al., 1988).

A critical component of oil sands reclamation involves re-building the soil organic layer and accelerating soil profile development. This is often accomplished by large scale applications of organic matter. Two different types of organic matter commonly used as capping materials include the peat mineral soil mix (PMM) and LFH mineral soil mix (LFH), with the LFH including identifiable litter (L), fragmented and partially decomposed litter (F), and highly decomposed humus (H) material (MacKenzie and Naeth, 2007). The PMM is generally salvaged from lowlands within the mining footprint. The LFH is salvaged from upland boreal forest sites and typically includes Ae horizons from Luvisolic soils (Soil Classiﬁcation Working Group, 1998) and fine roots and tree stumps (MacKenzie and Naeth, 2007).

The two organic materials have differing nutrient availabilities. The PMM has a high total N and low available N due to its high content of more recalcitrant organic carbon (C) which mineralizes slowly and results in high C:N ratios (Hemstock et al., 2010), a widely accepted indicator of N release and substrate availability (Mohanty et al., 2013). The LFH has a lower C:N ratio and provides a rich source of labile C and nutrients (MacKenzie and Naeth, 2010). Despite the high nutrient availability in LFH and the potential use of LFH as a reclamation material, its role in oil sands reclamation has not been thoroughly investigated. Despite past research in the oil sands region, more research is needed to help us understand as to how these two different OM sources, PMM and LFH, will perform relative to each other as organic capping materials in reclamation applications. Although some small scale preliminary studies have been conducted on N cycling and enzyme activities in PMM and LFH materials (McMillan et al., 2007; Dimitriu et al., 2010; Mackenzie and Quideau, 2012), no rigorous large scale, field-based investigations comparing LFH and PMM as alternative organic capping materials for oil sands reclamation have been conducted (Naeth et al., 2013). Because of their contrasting biological properties and nutrient availabilities with a more decomposed OM in LFH, we hypothesized that N availability and associated enzyme activities will be greater in LFH than in PMM in reconstructed sites in oil sands reclamation. Findings from this study will help to establish effective reclamation materials for soil quality improvement during ecosystem development in large scale reclamation applications.

1. **Materials and methods**
   1. *Research site*

The research was conducted on Suncor Energy Inc. Lease 86/17, located 24 km north of Fort McMurray, Alberta (56°39'N, 111° 39' W) in the central mixedwood natural subregion of the boreal forest region (Fung and Macyk, 2000). The area is characterized by long cold winters and short warm summers with a mean annual temperature of 0.7 °C from 1971 to 2000. Mean annual precipitation is 455 mm, which mostly falls as rain (342 mm) during summer (Environment Canada, 2003). Dominant tree species in natural forests in the region are trembling aspen (*Populus tremuloides* L.) and white spruce (*Picea glauca* L.)that exist in pure or mixed-wood stands (Fung and Macyk, 2000). The majority of soils have developed on glacial and glacial fluvial deposits. Gray Luvisolic soils (based on the Canadian system of soil classification, same below) are associated with till and lacustrine deposits, while Dystric Brunisols are associated with glaciofluvial outwash and eolian sands (Turchenek and Lindsay, 1982).

Air temperature and total precipitation data for the study period indicated that 2012 was slightly warmer and wetter than 2011. The mean average temperature was 14.6 °C in 2011 and 15.2 °C in 2012 during the sampling period (data not shown). The study site received 101 mm of precipitation in 2011 and 324 mm in 2012 during the sampling period (June-October) (Fig. 1) (O’Kane Consultants Inc.).

* 1. *Experimental design and plot establishment*

Research plots were established at Southeast Dump (56° 58' N, 111° 22' W) at Suncor Energy Inc. between November 2007 and February 2008 (Brown and Naeth, 2014). Two rows of plots were arranged along a slightly east-facing slope in a completely randomized block design. The plot size was 10 x 30 m. The plots were separated by a 5 m buffer. Half of the plots received freshly salvaged LFH and the other half received PMM following standard reclamation prescriptions. The LFH was applied at a depth of 20 cm, over 30 cm of mixed B and C horizon subsoil and 100 cm of clean overburden. The PMM was applied at a depth of 30 cm over 100 cm of clean overburden. The LFH plots had a greater vegetation cover (65%) of plant groups including forbs, grasses, sedges and woody species than the PMM plots (33%) in the second growing season in 2009 (Brown and Naeth, 2014). During the third (2010) and fourth (2011) growing seasons the density and cover of woody species were also greater in LFH than in PMM plots (Forsch and Naeth, unpublished; Naeth et al., 2013).

This study was conducted on three-year old established plots for two years using a 2 x 2 (two organic capping types and two sampling depth intervals of each organic capping type) completely randomized factorial design with six replications. Four 1 x 1 m quadrats were randomly established in each plot of organic capping (LFH or PMM) for monthly soil sampling from two depth intervals (0-10 and 10-20 cm) that represent a major part of the main rooting zone.

* 1. *Soil sample collection and analyses*

Soils were sampled monthly from the 0-10 and 10- 20 cm layers from June to August (summer) and September to October (fall) in 2011 and 2012 from each organic capping type. Four soil samples were randomly collected from each quadrat and bulked to form a composite sample of each organic material for each layer, with a total of 24 samples collected at each sampling. Soil samples were transported to the laboratory in a cooler containing ice packs. Fresh soil samples were homogenized, then sieved (2 mm) and stored at 4 °C until the analyses were complete in 7 days. A sub-sample of each sample was used for analysis of enzyme activities and microbial biomass C (MBC) and N (MBN). The remainder of each sample was air-dried at room temperature, ground, and used for measuring pH, electrical conductivity (EC) and available N (NH4+-N and NO3--N). A portion of the air-dried soil sample was ground with a ball mill (Mixer Mill MM200, Thomas Scientific, Swedesboro NJ) for 30 seconds and used for measurement of total C (TC) and total N (TN) as described below.

* 1. *Soil physical and chemical analyses*

Gravimetric water content in fresh soil samples was determined by drying the soil in an oven at 105 °C for 24 hours (Kalra and Maynard, 1991). Soil pH was measured in a 1:2 (m:v) soil:0.01 mol L-1 CaCl2 using a pH meter (Kalra and Maynard, 1991). Electrical conductivity was measured using an EC meter following an 1:5 soil:water extraction (m:v) (Kalra and Maynard, 1991). For NH4+-N and NO3- N analyses, soil samples were extracted using 2 mol L-1 KCl (Mulvaney, 1996). The extract was analyzed colorimetrically by the indophenol blue method for NH4+-N (Keeny and Nelson, 1982) and by the vanadium oxidation method for NO3--N (Miranda et al., 2001). The TOC and TN were analyzed by the dry combustion method using an automated elemental analyser (NA-1500 series, Carlo Erba, Milan, Italy).

* 1. *Soil microbial biomass measurement*

Soil MBC and MBN were measured using the chloroform fumigation-extraction method (Vance et al., 1987). All extractions were done within a week after sample collection. Fresh soil samples were fumigated with ethanol free chloroform for 24 hours in an evacuated desiccator. A 0.5 mol L−1 K2SO4 solution was used to extract C and N from fumigated and unfumigated samples at 1:5 (m:v) soil to solution ratio. After shaking for one hour on a shaker (Eberbach Corp., Michigan, U.S.A), the extracts were filtered using Fisher Q2 filter papers. Extractable C and N were analyzed using a TOC-VCSN analyzer (Shimadzu, Kyoto, Japan). The MBC and MBN were calculated by dividing the difference in extractable C or N between fumigated and unfumigated samples by a conversion factor of 0.45 for MBC and 0.54 for MBN (Joergensen, 1996).

* 1. *Soil enzyme assays*

Four extracellular enzymes involved in N cycling were measured, including N-acetyl-*β*-D- glucosaminidase (Enzyme classification (EC) number EC 3.2.1.14), urease (EC 3.5.1.5), protease (EC 3.4) and arylamidase (EC 3.4.11.2).

For NAGase activity, soil sample suspensions were prepared by placing one gram of fresh soil in a 125 mL nalgene bottle. A 125 mL of sodium acetate buﬀer (50 mmol L-1, pH 5) was added to the bottle, and the suspension was homogenized using a magnetic stir plate until suspensions were transferred into black 96 well plates for determining NAGase activity (Sinsabaugh et al., 2003). A 200 µL soil suspension, and 50 µL of 200 µmol L-1 of substrate were pipetted onto the plate. Reference standards and quench controls were then added to each plate. The plates were placed in an incubator for three hours at 20 °C in the dark. A 20 µL of 0.5 mol L-1 NaOH was added to the plates using an auto dispenser to stop the enzymatic reaction. Fluorescence was measured at 360 nm excitation and 460 nm emissions using a Synergy HT multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT) and NAGase activity (µmol of substrate g-1 h-1) was calculated on an oven-dry mass basis.

Urease (amidohydrolase) activity was measured in clear 96 well plates (Sinsabaugh et al., 2000). Soil assay wells received 200 µL of soil suspension and 10 µL of 400 mmol L-1 urea substrate. A 200 µL of soil suspension and 10 µL of Milli-Q (Millipore, Bedford, MA) deionized water were pipetted into the negative control wells. Substrate control wells contained 200 µL of acetate buffer and 10 µL of urea substrate. Microplates were incubated for 18 hours at 20 °C. After incubation, ammonium concentration was quantiﬁed using colorimetric reagent packets including salicylate and cyanuarate from Hach (Loveland, CO 80539, U.S.A). Urease activity was calculated as nmol of ammonium released per gram of soil per hour (nmol NH4 g-1 h-1).

Protease activity was measured using a modified method of Ladd and Butler (1972). One gram of fresh soil was mixed with 2.5 mL of sodium caseinate (10 g mL-1) in 0.1 mol L-1 of tris–sodium borate buffer at pH 8.1. The mixture was incubated at 37 °C for 1 hour. The reaction was stopped with 2 mL of 17.5 % tricloracetic acid (TCA) and centrifuged. After centrifugation, 2 mLof the supernatant was mixed with 3 mL of 1.4 mol L-1 Na2CO3 and 1 mL of Folin-Ciocalteu reagent. Absorbance was recorded at 700 nm using a UV-spectrophotometer (Genesys 10-S, Rochester, NY).

Arylamidase activity was assayed according to Acosta-Martinez and Tabatabai (2000). One gram of fresh soil was incubated with 3 mL of 0.1 mmol L-1 tris (hydroxyl methyl) amino methane (THAM) buffer (pH 8.0) and 1 mL of 8.0 mmol L-1 l-leucine *β*-naphthylamide hydrochloride at 37 °C for 1 hour. The supernants were converted to an azo-compound by reacting with *p-*dimethylaminocinnamaldehyde. The absorbance was measured colorimetrically at 540 nm UV-spectrophotometer (Genesys 10-S, Rochester, NY).

* 1. *In-situ N availability measurement using* *plant root simulator probes*

Plant root simulator (PRSTM, Western Ag Innovations Inc. Saskatoon, SK, Canada) probes were used to measure soil N supply rates. PRSTM probes have an ion exchange membrane encapsulated in a plastic casing. The membrane captures cations and anions from soil solution and integrates the temperature and water effects on nutrient fluxes during incubation. Each probe has 10 cm2 area of membrane with an adsorbing surface area of 17. 5 cm2 (both sides). Four pairs of probes (cation and anion) were inserted in established quadrats of each amendment four times from June 2011 to July 2013 (June to September 2011, October 2011 to May 2012, June 2012 to October 2012, October 2012 to July 2013). At each sampling, probes were retrieved, washed with deionized water and sent to Western Ag Innovations Inc. Elution was conducted with 0.5 mol L-1 HCl solution and NO3-- N and NH4+-N were analyzed colorimetrically using an automated flow injection analysis (FIA) system (PRS Probe Operations Manual, 2010).

* 1. *Soil temperature and soil water content measurement*

HOBO micro station data loggers (Model H21-002; Onset Computer Corporation, Bourne, MA, U.S.A) connected with volumetric smart soil water sensors (Decagon Devices Inc., Pullman, WA, U.S.A.) and 12-bit smart temperature sensors (Onset Computer Corporation) were installed. Two sensors were installed at five cm depth in both amendment plots on relatively level ground in the bottom row of plots of each amendment (Brown and Naeth, 2013). The mean of sampling day data of soil temperature for each month of the sampling period of 2011 and 2012 were used for correlation analysis with N availability and soil enzyme activities.

*2.9 Statistical analyses*

A repeated measures analysis of variance (ANOVA) was used to assess capping material type and sampling depth effects over time on N availability, soil enzyme activities and MBC and MBN using the PROC MIXED model. The month of each sampling was considered the repeated measured variable for determining seasonal variation in 2011 and 2012. In this analysis, the output statistics passed tests for compound symmetry. Tukey’s HSD test was used to determine if there were significant differences between the capping treatments, depths, month of sampling and their interactions. A repeated measures ANOVA was also used to assess the capping type effects on N supply rate at different times of incubation of PRSTM probes. One-way ANOVA was used to determine the differences in the basic characteristics of caps. Correlation analysis was conducted to determine the relationship between N availability, soil enzyme activities, MBC, MBN, soil pH, soil temperature and soil water in each capping material separately. Linear regression was used to determine which enzyme activity had a strong relationship with N availability in each cap. All analyses were performed using the SAS 9.2 (SAS Institute Inc. USA) software.

**3. Results**

*3.1 Basic characteristics of capping materials*

There were strong differences in soil properties between the capping material types. The TC was approximately two-fold greater in PMM than in LFH (Table 1). The TN ranged from 3.0 to 3.7g kg-1 in LFH and from 2.8 to 3.4 g kg-1 in PMM, with no significant differences between the two capsping materials. The C:N ratio of LFH and PMM caps did not differ significantly due to large variability in the dataset. The pH was higher in PMM than in LFH and higher in the 0-10 than in the 10-20 cm layer (Table 1). The EC was generally low in both LFH and PMM, indicating the non-saline nature of the LFH and PMM (Table 1). Between 2011 and 2012, soil water content (SWC) was similar in PMM and LFH. The SWC was greater in the PMM than in the LFH plots and ranged from 16.2 to 23.4% in the LFH and 28.8 to 29.5% in the PMM plots when the 0-10 and 10-20 cm layers were considered together (data not shown).

*3.2 Microbial biomass C and N*

Soil MBC was consistently the greatest in the 0-10 cm layer in LFH and the lowest in the 10-20 cm layer in PMM (Table 2). Soil MBC was significantly affected by capping material type, sampling depth and time, and their interactions (Table 3); the effect of time of sampling was greater in 2012 than in 2011 (Table 2). The MBC was consistently greater in the 0-10 cm than in the 10-20 cm layer.

Soil MBN response to the treatments was similar to that of MBC, but did not show as much variation among sampling months. The greatest MBN was found in the 0-10 cm layer in LFH in both 2011 and 2012 (4.4 mg N kg-1, on average, same below), followed by the 10-20 cm layer in LFH (2.9 mg N kg-1) and the 0-10 cm layer in PMM (2.7 mg N kg-1), and was consistently the lowest in the 10-20 cm layer in PMM (2.2 mg N kg-1).

*3.3 Available N*

Available N (NH4+-N and NO3--N) was significantly influenced by capping material type, and sampling depth and time (Tables 2 and 3). Concentrations of NH4+-N and NO3--N were generally the highest in the 0-10 cm layer of LFH and the lowest in the 10-20 cm layer of PMM (Table 2). Mean NH4+-N availability was 4.2 mg N kg-1 in 2011 and 4.8 mg N kg-1 in 2012. Mean NO3--N increased from 3.6 in 2011 to 6.3 mg N kg-1 in 2012. Changes in NO3--N of each capping material from 2011 to 2012 followed a pattern similar to that of NH4+-N. The magnitude of these changes, however, was greater for NO3--N than for NH4+-N (Table 2). Both NH4+-N and NO3--N concentrations were greater in the fall than in the summer.

The N supply measured using the PRS probes was approximately 50% greater in LFH than in PMM over the entire study (Table 4). The dominant form of N supply was NO3--N in both capping materials. During incubation from September 2011 to May 2012, the greatest NO3--N supply occurred in LFH. However, NH4+-N supply was below detection limit during incubation from September 2011 to May 2012.

*3.4 Soil enzyme activities*

Interactions between organic capping material types, sampling depths and sampling times significantly affected activities of NAGase, arylamidase, protease and urease in both 2011 and 2012 (Table 3). The NAGase activity was significantly greater in the 0-10 cm layer of LFH than in the other treatments. However, in most sampling times, differences between the 10-20 cm layer of LFH and both layers of PMM were non-significant. NAGase activity varied from 2011 to 2012 by 46, 29, 9 and -8% for LFH 0-10, LFH 10-20, PMM 0-10 and PMM 10-20 cm, respectively (Fig. 2a).

Arylamidase and protease activities followed a similar trend to that of NAGase activity (Fig. 2b). Both arylamidase and protease activities were greater in the fall than in the summer for all capping material type by depth combinations, particularly in 2012. The increase in arylamidase and protease activities from summer to fall in 2012 was twice as high in LFH as that in PMM. Urease activity declined from 2011 to 2012, with the greatest reduction in the 10-20 cm layer of LFH (17.8 %) and 0-10 cm layer of PMM (16.3 %) (Fig. 2d).

*3.5 Linkages among soil properties*

Many strong correlations were observed among SWC, pH, NH4+-N, NO3--N, activities of NAGase, protease, arylamidase and urease, MBC, and MBN (Table 5). The SWC, pH and soil enzyme activities in LFH were significantly correlated with response variables representing N availability. Similarly, NH4+-N and NO3--N were significantly correlated with soil enzyme activities in PMM. The NH4+-N and NO3 were significantly correlated with SWC in LFH, but not in PMM. Regression analysis indicated that available N was positively related with NAGase, arylamidase, protease and urease activities in both capping materials (Fig. 3).

1. **Discussion**

A key finding of our study is that the two most common organic capping materials used for oil sands reclamation in northern Alberta had contrasting soil properties (e.g., MBC, MBN, activities of NAGase, arylamidase, protease and urease, and N availability), consistent with previous findings in reclaimed soils in the area (McMillan et al., 2007; MacKenzie and Quideau, 2010). The differences in soil properties affected soil N availability and as such could influence early ecosystem development. The two capping materials differed in total C, MBC and MBN and, therefore, likely had different decomposition potentials. The narrower C:N ratio in LFH indicated more decomposed organic matter and a greater potential of N availability through organic matter mineralization relative to PMM. Greater microbial activity would result in greater organic matter decomposition and the lower organic matter C:N ratio in LFH supported higher MBC and MBN in LFH as compared with PMM (Hahn and Quideau, 2013).

Soil enzyme activities are considered soil quality indicators in the reclamation of disturbed ecosystems in the oil sands region (Dimitriu et al., 2010). The greater NAGase, arylamidase and protease activities in LFH than in PMM in the 0-10 cm layer in our study is consistent with the greater N availability in LFH than in PMM. The lower enzyme activities, and MBC and MBN in the 10-20 cm than in the 0-10 cm layer in both capping materials may be explained by the changes in nutrient levels, soil pH, SWC and O2 concentrations with depth (Ekenler and Tabatabai, 2004; Eilers et al., 2012). Among them, soil pH likely was the most influential factor (Table 1). Soil pH strongly affects abiotic and biotic factors such as C and nutrient cycling processes (Kemmitt et al., 2006) and microbial population composition (Will et al., 2010). Acidic soil pH favours fungal growth, whereas neutral or alkaline soil pH promotes bacterial growth (Will et al., 2010). Hence, changes in microbial population composition caused by soil pH may also influence soil enzyme activities since many enzymes exhibit pH optima (Baldrian et al., 2008). Thus, soil enzyme activities are affected by pH and the composition of the soil microbial community (e.g., enzymes originating from different organisms have different pH optima) and the location of the enzyme in the soil matrix (e.g., intracellular, free in solution, or adsorbed on solid surfaces) (Turner, 2010). As a result, soil enzyme activities in our study were different between 0-10 and 10-20 cm soil layers. However, it is difficult to pinpoint whether these changes were associated with soil pH alone or with other confounding factors since we have not determined the partition in fungal and bacterial biomass in this study (Rousk et al., 2009). Different soil enzyme activities between the 0-10 and 10-20 cm layers in this study might also be related to the higher aeration (Ellis and Atherton, 2003) and the greater amount of soluble C compounds added by plant roots in surface than in deeper soil layers (Will et al., 2010).

Another important indicator for potential reclamation success in the oil sands region is soil N availability. We observed greater available N (NH4+-N and NO3- -N) in LFH than in PMM. The greater N availability indicates greater N mineralization in LFH than in PMM that was linked with the narrower C:N ratio in LFH (Brown and Naeth, 2013; Yan et al., 2012). The greater NO3- -N than NH4+-N in both LFH and PMM indicated that in those highly disturbed reclaimed soils nitrification activities were high (McMillan et al., 2007). This is in contrast with the low nitrification activities in natural forest soils in the vicinity of the study site (Yan et al., 2012). The low nitrification activities and the resultant low soil NO3--N concentrations in natural forests are typically affected by the low soil pH (Fisher and Binkley, 2000). For example, in the 21 stands studied in Yan et al. (2012), with the exception of two stands that had pH of 5.94 and 6.02, the other 19 stands had pH ranging between 3.67 and 4.95 (Chang et al., unpublished data). Because the LFH and PMM were mixed with mineral soil and the mixing with mineral soils increased the pH of those reclamation material (pH ranged from 5.70 to 7.50, Table 1), those higher pH and the physical disturbance likely encouraged soil nitrification activities (Kaur et al., 2010). Between the two amendment types, NO3--N was more predominant in PMM than in LFH, again likely attributable to the higher pH in PMM than in LFH. The higher pH in PMM than in LFH is consistent with earlier studies on reclaimed oil sands soils (Mackenzie and Naeth, 2010; MacKenzie and Quideau, 2012). The nitrification process is known to be more pH dependent than the ammonification process and nitrification activity can be inhibited at low pH (Myrold, 2005).

The strong positive correlations among soil enzyme activities, NH4+-N, NO3--N, SWC, pH, MBC, and MBN suggest that enzyme activities and N availability are affected by both biotic and abiotic factors (Tan et al., 2008). The stronger relationships between N availability, NAGase, arylamidase and protease in LFH than in PMM were similar to previous studies where it was shown that activities of NAGase (Andersson et al., 2004), arylamidase (Muruganandam et al., 2009) and protease (Lucas et al., 2008) have been associated with soil fungal biomass. Hence, we suggest that enzyme activities in this study may be dependent on mycorrhizal (part of the total fungal community) biomass, as indicated in Brown and Naeth (2014). Mycorrhizae fungi often dominate the microbial biomass in forest soils and litter and LFH material and many mycorrhizal fungi produce extracellular enzymes that catalyze C, N and phosphorus mineralization from OM and litter material (Smith and Read, 2008). Mycorrhizae fungi are also capable of utilizing some organic forms of N such as amino acids (Burke et al., 2011). The very low urease activity in our study is similar to the oil sands study of Dimitriu et al. (2010) and this might have been linked to the low availability of urease specific substrates in the studied soils. Different soil chemical properties such as soil pH of organic capping material may have influenced soil enzyme activities (Table 5). The lower enzyme activity in PMM than in LFH might be linked to the pH of PMM.

Seasonal changes in soil temperature, water content and pH typically affect substrate availability and soil microbial activities and processes (Baldrian et al., 2008). The seasonal changes may facilitate production of substrates for microbes, which in turn affect microbial processes. Thus, seasonality may affect microbial substrate availability, which is considered one of the main limiting factors for microbial activity and decomposition of soil organic matter (Corre et al., 2002). Fresh litter input to soils that mainly occur in the fall provides an important substrate and energy source for soil microorganisms that enhance microbial activities and SOM decomposition, in the form of positive priming effects (Kuzyakov et al., 2002). The higher MBC, MBN, soil enzyme activities and available N in the fall in this study were likely a result of the addition of labile C from fresh litter input, because soil temperature had no relationship with (and were thus not limiting) those studied parameters. Similarly, Baldrian et al. (2008) found that peak soil enzyme activities and MBC in October was associated with input of fresh litter in their study. The activities of NAGase (Burke et al., 2011), protease (Werdin-Pfisterer et al., 2009), arylamidase (Acosta-Martinez and Tabatabai, 2000) and urease (Cochran et al., 1989) can increase due to fresh litter input. Burke et al. (2011) found that decomposition of litter on the soil surface in late summer could have liberated and transported organic and inorganic compounds from litter into the soil which altered ectomycorrhizal and microbial activities, increasing N availability. The vegetation cover and soil water content in our study also changed from summer to fall and contributed to a greater change in substrate availability in LFH than in PMM. Changes in the composition of vegetation and soil water content between the study periods and sites with different organic capping materials could change the priming effect (Criquet et al., 2002; Schaaf et al., 2011). For example, in a study conducted on our site (Brown and Naeth, 2014) vegetation cover and species richness were greater in LFH than in PMM plots. Therefore, greater vegetation cover in LFH than in PMM would mean greater priming effect in the former than in the latter. Greater cover and species richness would contribute to greater and diverse litterfall to the soil that might have caused the greater effect in LFH than in PMM plots. However, it is not possible to directly quantify the effects of litter fall on N availability and associated enzyme activities in this study, since we have not measured the litter fall amount during the study period. In general, annually litter fall from herbaceous plants can comprise as much as 16% of forest litter fall (Gilliam, 2007). Waldrop and Firestone (2006) indicated that both seasonal soil water and C substrate differences were caused by differences in aboveground vegetation.

Soil pH and EC in both capping treatments were within the acceptable range according to Alberta Tier 1 guidelines (Alberta Environmental Protection, 1994) for the oil sands region. The higher pH and EC in PMM may be due to the alkaline nature of salvaged mineral material in the oil sands region (Fung and Macyk, 2000). In general, we considered LFH as a better soil material than PMM because most of the studied soil properties for LFH were better suited (such as having higher N availabilities) for vegetation development than those of PMM. The LFH material may also provide more native seeds and propagules for revegetation than PMM (Mackenzie and Naeth, 2007) that can help to expedite ecosystem development in oil sands reclamation (Naeth et al., 2013). However, the availability of LFH for reclamation is generally more limited (Naeth et al., 2013). In addition, the long-term effect of LFH and PMM application for land reclamation is not very well understood and should be further studied in the future.

In conclusion, the two capping materials commonly used for land reclamation in the oil sands region had contrasting properties. The LFH-mineral soil mix was a capping material that had a better quality than the peat-mineral soil mix for land reclamation in the oil sands region in Alberta, based on its greater N availability and its ability to support greater enzyme activities. The N availability and enzyme activities were highly related and greater N availability in the capping material will reduce the risk of N deficiencies and minimize the need for N fertilization in developing ecosystems after land reclamation. This calls for reclamation policies that promote careful planning for salvaging LFH materials and using them for land reclamation in the oil sands region, to take advantage of the existence of LFH materials that are considered superior than peat materials.

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**Table 1**

Selected properties of LFH mineral soil mix (LFH) and peat mineral soil mix (PMM) organic caps used for oil sands reclamation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Cap type  and depth (cm) | pH | ECa  (dS m-1) | TCb  (g kg-1) | T Nc  (g kg-1) | C:Nd |
| LFH 0- 10 | 6.55b† | 0.02a | 56.8b | 3.7a | 17.0a |
| LFH 10-20 | 5.70c | 0.03a | 54.2b | 3.0a | 21.5a |
| PMM 0-10 | 7.50a | 0.06a | 102.0a | 2.8a | 37.7a |
| PMM 10-20 | 6.60b | 0.04a | 107.0a | 3.4a | 46.5a |
| **Two-way ANOVA** |  |  |  |  |  |
| Caps | \*\*\* | ns | \*\* | ns | ns |
| Depth | \*\*\* | ns | ns | ns | ns |
| Caps\* depth | ns | ns | ns | ns | ns |

†Means with different lowercase letters indicate significant difference between organic caps and their depths in each column *p* < 0.05; \*\*, *p* < 0.01; \*\*\*, *p* < 0.001; and ns, not significant

Abbreviations: aEC = electrical conductivity, bTC = total C, cTN = total N, and dC:N = C to N ratio

**Table 2**

Effects of organic cap types on selected soil properties in organic caps used for oil sands reclamation.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil  Property a | Cap type  and depth  (cm) | 2011 | | | | |  | 2012 | | | | |
| Jun. | Jul. | Aug. | Sept. | Oct. |  | Jun. | Jul. | Aug. | Sept. | Oct. |
| MBC (mg C  kg-1) | LFH 0-10 | 19.7a† | 15.8a | 20.0a | 12.5a | 18.3a |  | 33.7a | 24.3a | 9.6a | 31.1a | 35.2a |
| LFH 10-20 | 8.4b | 6.3b | 10.3b | 7.9ab | 8.7b |  | 9.9b | 16.2b | 6.2bc | 18.6b | 20.2b |
| PMM 0-10 | 7.7b | 8.6b | 9.6b | 6.7ab | 11.9ab |  | 27.0a | 13.6bc | 9.0ab | 15.3b | 17.1b |
| PMM 10-20 | 5.5b | 4.3b | 4.1b | 4.1b | 4.0b |  | 6.1b | 6.3c | 4.3c | 7.1c | 7.9c |
| MBN (mg N  kg-1) | LFH 0-10 | 4.6a | 4.0a | 4.9a | 3.1a | 4.3a |  | 5.7a | 4.5a | 1.9a | 5.0a | 4.5a |
| LFH 10-20 | 2.7ab | 2.3b | 3.8ab | 2.4ab | 2.2b |  | 3.0ab | 3.2a | 1.2ab | 3.8ab | 3.8ab |
| PMM 0-10 | 3.0ab | 2.6b | 3.0b | 2.3ab | 3.9a |  | 4.2ab | 3.7a | 1.5ab | 3.1ab | 1.5b |
| PMM 10-20 | 2.4b | 1.9b | 2.7b | 1.3b | 2.8ab |  | 2.2b | 3.0a | 1.0ab | 2.2b | 2.0ab |
| NH4+-N (mg N  kg-1) | LFH 0-10 | 2.6a | 3.9a | 7.5a | 3.6a | 7.6a |  | 4.3a | 6.1a | 6.5a | 6.6a | 7.6a |
| LFH 10-20 | 1.9b | 2.5b | 6.4b | 2.8b | 5.7ab |  | 2.4ab | 5.4a | 6.4a | 6.5a | 6.4ab |
| PMM 0-10 | 2.4ab | 3.2b | 7.3ab | 2.6b | 6.4a |  | 2.8ab | 4.1a | 5.4ab | 5.2ab | 5.9ab |
| PMM 10-20 | 1.9b | 2.6b | 5.0c | 3.1a | 4.2b |  | 1.7b | 2.6b | 2.8b | 3.9b | 4.0b |
| NO3--N (mg N  kg-1) | LFH 0-10 | 3.0a | 4.9a | 5.4a | 6.2a | 8.0a |  | 5.5a | 6.4a | 6.7ab | 9.8ab | 10.8ab |
| LFH 10-20 | 1.3c | 1.6c | 3.4b | 3.5b | 4.8bc |  | 2.8b | 3.4b | 4.8b | 5.7b | 6.0b |
| PMM 0-10 | 2.1b | 2.9b | 3.6b | 3.8b | 6.5ab |  | 4.9a | 7.5a | 8.8a | 10.5a | 11.2a |
| PMM 10-20 | 1.2c | 1.7c | 1.9c | 2.5c | 4.4c |  | 2.2b | 3.7b | 5.1b | 5.6b | 5.6b |

†Means with different lowercase letters indicate significant difference between organic caps and their depths in each column

a soil property: MBC: microbial biomass C, MBN: microbial biomass N, NH4+-N: 2 mol L-1 KCl extracted ammonium, NO3--N: 2 mol L-1 KCl extracted nitrate

**Table 3**

Effects of organic caps, depth, time and their interactions on soil properties in oil sands reclamation.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil property a | Cap type | | Depth | | Cap type × Depth | | Time | | Cap type × Time | | Depth × Time | | Cap type × Depth × Time | |
| F value | *p* value | F value | *p* value | F value | *p* value | F value | *p* value | F value | *p* value | F value | *p* value | F value | *p* value |
|  |  |  |  |  |  |  | 2011 |  |  |  |  |  |  |  |
| MBC | 83.4 | <0.001 | 104.8 | <0.001 | 32.8 | <0.001 | 23.4 | <0.001 | 7.8 | <0.001 | 1.6 | 0.188 | 1.4 | 0.238 |
| MBN | 28.4 | <0.001 | 66.8 | <0.001 | 7.0 | <0.015 | 15.2 | <0.001 | 3.6 | 0.002 | 8.7 | <0.001 | 1.3 | 0.290 |
| NH4-N | 18.8 | <0.001 | 32.5 | <0.001 | 0.1 | 0.739 | 74.1 | <0.001 | 2.1 | 0.019 | 1.4 | 0.249 | 2.4 | 0.057 |
| NO3-N | 84.8 | <0.001 | 169.2 | <0.001 | 12.9 | 0.001 | 82.1 | <0.001 | 1.9 | 0.044 | 2.3 | 0.068 | 1.1 | 0.382 |
| NAGase | 60.8 | <0.001 | 28.8 | <0.001 | 14.3 | 0.001 | 18.4 | <0.001 | 3.7 | <0.001 | 0.7 | 0.594 | 0.5 | 0.715 |
| UR | 31.7 | <0.001 | 66.4 | <0.001 | 2.7 | 0.110 | 114.5 | <0.001 | 2.3 | 0.013 | 4.6 | 0.002 | 0.3 | 0.881 |
| PRT | 165.0 | <0.001 | 125 | <0.001 | 2.9 | 0.104 | 21.3 | <0.001 | 7.1 | <0.001 | 9.0 | <0.001 | 4.4 | 0.003 |
| ARA | 345.7 | <0.001 | 296.3 | <0.001 | 14.3 | 0.001 | 53.9 | <0.001 | 6.8 | <0.001 | 18.9 | <0.001 | 0.3 | 0.877 |
| 2012 | | | | | | | | | | | | | | |
| MBC | 107.7 | <0.001 | 118.7 | <0.001 | 1.5 | 0.241 | 32.8 | <0.001 | 8.4 | <0.001 | 17.1 | <0.001 | 0.7 | 0.583 |
| MBN | 16.4 | <0.001 | 12.8 | 0.001 | 0.9 | 0.362 | 14.2 | <0.001 | 1.8 | 0.055 | 3.3 | 0.014 | 0.3 | 0.850 |
| NH4-N | 13.6 | <0.001 | 21.2 | 0.002 | 0.8 | 0.371 | 21.3 | <0.001 | 0.6 | 0.799 | 0.5 | 0.741 | 0.3 | 0.860 |
| NO3-N | 39.6 | <0.001 | 60.6 | <0.001 | 2.4 | 0.139 | 32.3 | <0.001 | 2.0 | 0.028 | 5.3 | 0.001 | 0.2 | 0.913 |
| NAGase | 121.1 | <0.001 | 38.1 | <0.001 | 25.6 | <0.001 | 36.9 | <0.001 | 10.0 | <0.001 | 6.6 | 0.001 | 3.6 | 0.061 |
| UR | 140.4 | <0.001 | 231.9 | <0.001 | 16.7 | 0.006 | 185.7 | <0.001 | 10.0 | <0.001 | 24.4 | <0.001 | 9.7 | 0.081 |
| PRT | 112.0 | <0.001 | 89.7 | <0.001 | 9.3 | 0.01 | 16.6 | <0.001 | 4.1 | <0.001 | 8.5 | <0.001 | 1.9 | 0.125 |
| ARA | 394.1 | <0.001 | 346.5 | <0.001 | 19.1 | <0.001 | 126.6 | <0.001 | 27.1 | <0.001 | 46.9 | <0.001 | 9.4 | 0.058 |

a Soil property: MBC = microbial biomass C, MBN = microbial biomass N, NAGase = β-1, 4-N-acetylglucosaminidase, UR = Urease, PRT = Protease, ARA = Arylamidase

**Table 4**

Soil N supply (µg N per 10 cm2), measured using Plant Root Simulator (PRS TM) probes, in LFH and PMM organic capping materials used for oil sands reclamation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Incubation period | NO3‑-N | |  | NH4+-N | | Mineral N |  |
| LFH | PMM |  | LFH | PMM | LFH | PMM |
| Jun. to Sept. 2011 | 4.4b | 0.6b |  | 1.7bc | 0.4c | 6.1abc | 1.0c |
| Sept. 2011 to May 2012 | 10.9a | 3.9b |  | ND | ND | 11.0a | 4.0bc |
| May to Oct. 2012 | 2.9b | 2.4b |  | 1.4bc | 1.1bc | 4.3bc | 3.4bc |
| Oct. 2012 to Jul. 2013 | 3.7b | 4.2b |  | 3.4a | 2.1b | 7.0ab | 6.2abc |
| Total | 22.1a | 11.1b |  | 6.4a | 3.5b | 28.5a | 14.6a |

|  |  |  |  |
| --- | --- | --- | --- |
| **Repeated Measures ANOVA** | |  |  |
|  |  |  |  |
| Caps | \*\* | \*\* | \*\* |
| Time | \*\* | \*\* | \*\* |
| Caps\* Time | \* | ns | \* |

*p* < 0.05; \*\*, *p* < 0.01; \*\*\*,; and ns, not significant

†Means with different lowercase letters indicate significant difference between organic caps a Abbreviations: ND = not detected

**Table 5**

Pearson correlation coefficient (*r* -value) and significance+ among soil variables in LFH and PMM organic caps used for oil sands reclamation (n=120).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variablea | SWC | MBC | | MBN | | NH4+ | | NO3- | | AN | | NAGase | | UR | | PRT | | ARA | | Stemp | | pH | |
|  | LFH |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| MBC | 0.48\*\* |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| MBN | 0.35\*\* | 0.52\*\* | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| NH4+-N | 0.31\*\* | 0.49\*\* | | 0.15 | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| NO3--N | 0.31\*\* | 0.58\*\* | | 0.21\* | | 0.72\*\* | |  | |  | |  | |  | |  | |  | |  | |  | |
| AN | 0.33\*\* | 0.57\*\* | | 0.19\* | | 0.94\*\* | | 0.91\*\* | |  | |  | |  | |  | |  | |  | |  | |
| NAGase | 0.50\*\* | 0.45\*\* | | 0.21\* | | 0.58\*\* | | 0.71\*\* | | 0.69\*\* | |  | |  | |  | |  | |  | |  | |
| UR | 0.34\*\* | 0.23\*\* | | 0.07 | | 0.52\*\* | | 0.63\*\* | | 0.61\*\* | | 0.71\*\* | |  | |  | |  | |  | |  | |
| PRT | 0.45\*\* | 0.59\*\* | | 0.26\*\* | | 0.63\*\* | | 0.75\*\* | | 0.74\*\* | | 0.75\*\* | | 0.73\*\* | |  | |  | |  | |  | |
| ARA | 0.49\*\* | 0.61\*\* | | 0.27\*\* | | 0.75\*\* | | 0.84\*\* | | 0.86\*\* | | 0.81\*\* | | 0.71\*\* | | 0.88\*\* | |  | |  | |  | |
| Stemp | -0.29 | -0.13 | | 0.05 | | -0.43 | | -0.39 | | -0.45 | | -0.52 | | -0.46 | | -0.35 | | -0.39 | |  | |  | |
| pH | 0.25\*\* | 0.51\*\* | | 0.40\*\* | | 0.24\*\* | | 0.43\*\* | | 0.35\*\* | | 0.39\*\* | | 0.44\*\* | | 0.60\*\* | | 0.48\*\* | | 0.02 | |  | |
| EC | -0.07 | -0.06 | | -0.01 | | -0.01 | | 0.10 | | -0.01 | | 0.04 | | 0.02 | | -0.02 | | 0.05 | | -0.01 | | -0.08 | |
|  | PMM | | | | | | | | | | | | | | | | | | | | | | | |
| MBC | 0.19\* | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| MBN | 0.05 | | 0.41\*\* | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| NH4+-N | 0.10 | | 0.35 | | 0.01 | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| NO3--N | 0.09 | | 0.46\*\* | | 0.02 | | 0.60\*\* | |  | |  | |  | |  | |  | |  | |  | |  | |
| AN | 0.11 | | 0.46\*\* | | -0.01 | | 0.85\*\* | | 0.93\*\* | |  | |  | |  | |  | |  | |  | |  | |
| NAGase | 0.04 | | 0.25\*\* | | 0.01 | | 0.32\*\* | | 0.42\*\* | | 0.43\*\* | |  | |  | |  | |  | |  | |  | |
| UR | 0.20\* | | 0.17 | | 0.05 | | 0.49\*\* | | 0.53\*\* | | 0.57\*\* | | 0.42\*\* | |  | |  | |  | |  | |  | |
| PRT | 0.16 | | 0.49\*\* | | 0.12 | | 0.43\*\* | | 0.65\*\* | | 0.62\*\* | | 0.38\*\* | | 0.53\*\* | |  | |  | |  | |  | |
| ARA | 0.08 | | 0.42\*\* | | 0.08 | | 0.51\*\* | | 0.71\*\* | | 0.67\*\* | | 0.45\*\* | | 0.63\*\* | | 0.88\*\* | |  | |  | |  | |
| Stemp | -0.36 | | -0.27 | | -0.04 | | -0.29 | | -0.37 | | -0.37 | | -0.36 | | -0.46 | | -0.31 | | -0.28 | |  | |  | |
| pH | -0.02 | | 0.47 | | 0.23\*\* | | 0.27\*\* | | 0.36\*\* | | 0.34\*\* | | 0.17 | | 0.28\*\* | | 0.69\*\* | | 0.66\*\* | | 0.03 | |  | |
| EC | -0.21 | | 0.07 | | 0.06 | | -0.01 | | 0.04 | | 0.03 | | 0.04 | | 0.11 | | 0.21 | | 0.10 | | -0.02 | | 0.22 | |
| +\*Significant at the P < 0.05 level \*\* Significant at the P <0.01 level | | | | | | | | | | | | | | | | | | | | | | | | |

a Variables: SWC: soil water content, MBC: microbial biomass C, MBN: microbial biomass N, NH4+: ammonium, NO3-: nitrate, AN: available N, NAGase: β- 1, 4-N- acetylglucosaminidase, UR: urease, PRT: protease, ARA: arylamidase, Stemp: soil temperature EC: electrical conductivity, pH

**Figure captions**

**Fig. 1.** Monthly precipitation (bars) and mean monthly air temperature (line) during sampling periods.

**Fig. 2.** Changes in (a) β-1, 4-N-acetylglucosaminidase, (b) arylamidase, (c) protease and (d) urease activities in LFH and PMM from June to September in both 2011 and 2012 in oil sands reclamation. Error bars indicate standard errors (n=6).

**Fig. 3.** Relationships between N availability and (a) β-1, 4-N-acetylglucosaminidase (b) arylamidase, (c) protease, and (d) urease activities in LFH and PMM.

**2012**

**2011**

Fig. 1.

Fig. 2.

Fig. 3.