Effects of Stockpiling and Organic Matter Addition on Nutrient Bioavailability in Reclamation Soils

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Nutrient bioavailability is crucial for vegetation establishment and organic matter cycling after major ecosystem disturbance such as open pit mining. In this study, the stockpiling and organic matter admixing effects on nutrient bioavailability were examined in soils used for reclaiming oil sands disturbed sites in northern Alberta, Canada. Stockpiled and directly salvaged peat mineral soil mix (PMM) and forest floor mineral soil mix (FFMM), the two main oil sands reclamation soils, and a nutrient poor mineral sub-soil (SS) were used in this experiment. Reclamation soils were inter-mixed at different ratios (PMM to FFMM or SS at 60:40, 80:20 and 90:10) to examine the organic matter admixing effects. Significant stockpiling effects on nutrient bioavailability and microbial functions were mostly observed in FFMM. Microbial biomass C was greater, and mineralization of lignin substrate was lower in both stockpiled PMM and FFMM soils compared to the directly salvaged soils. Significant fertility benefit was found in the FFMM-admixed SS and PMM soils through an increase in N and K availability. FFMM admixing also increased microbial functional diversity and assimilation rate compared to the non-admixed soils. Mineralization of polymeric substrates was the main driver of nutrient availability in stockpiled PMM, whereas carboxylic acids and carbohydrates were the major drivers in directly salvaged PMM, as indicated by the Random Forest models. The findings suggest that stockpiling effects are much stronger in FFMM than in PMM, and FFMM admixing to reclamation soils may provide nutritional and microbial functional benefits, especially in nutrient-poor soils.

Abbreviations: AOSR, Athabasca Oil Sands Region; AWCD, average well color development; CLPP, community level physiological profiling; FD, functional diversity; FFMM, forest floor mineral mix; OD, optical density; OM, organic matter; PMM, peat mineral mix; SIR, substrate induced respiration; SS, mineral sub-soil; TIN, total inorganic nitrogen.

Core Ideas

- **Stockpiling and organic matter admixing effects on reclamation soils were tested.**
- **Stockpiling effects were stronger on forest floor based reclamation soils.**
- **Organic matter admixing showed fertility and microbial benefits mostly in mineral sub-soils.**
- **A faster microbial assimilation was achieved in soils admixed with forest floor organic matter.**

Nutrient bioavailability plays a significant role in vegetation growth, organic matter (OM) cycling, and soil development after both anthropogenic and natural disturbances (Driscoll et al., 1999; Bendfeldt et al., 2001; Šourková et al., 2005; Macdonald et al., 2012). Disturbance effects on nutrient bioavailability depend very much on the scale and magnitude of disturbances. Natural disturbance, such as fire, alter the nutrient pool through direct changes in OM chemistry and indirect modification of soil structures and mineralization potentials (Fernández et al., 1997; Neff et al., 2005; Certini, 2005). The precise effect of anthropogenic disturbance on soil nutrients is, however, much more variable as it depends on the multidimensional nature of the disturbance and ecosystem properties (Maynard et al., 2014). For example, the severe disturbances associated with open-pit mining and subsequent reclamation activities have a significant effect on nearly all properties of soils. This has been reported in many studies related to physicochemical properties (Abdul-Kareem and McRae, 1984; Barbour et al., 2007), microbial community structure and functions (Stefani et al., 2018; Lewis et

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al., 2010, Béasse et al., 2015), and nutrient dynamics (Banning et al., 2011; MacKenzie and Quideau, 2012; Quideau et al., 2017).

Salvaging topsoil and immediately placing it on a reclamation ready site after mine disturbance are preferred treatments for economic and ecological reasons, but they are not always logistically feasible. So, these reclamation soils are generally stockpiled for later uses. Stockpiling topsoil can also potentially result in a change in many important edaphic properties. The major physicochemical changes in stockpiled soils can be associated with high compaction, low aggregate stability, high erosion potential, anaerobicity, low pH, low OM content and nutrient availability (Abdul-Kareem and McRae, 1984; Wick et al., 2008; Sheoran et al., 2010). Soil biological properties are the most adversely affected attributes due to stockpiling with a rapid reduction in viable seed propagules, microbial biomass, mycorrhizal population, decomposition potential, extracellular enzyme activities, and nutrient mineralization (Barkworth and Bateson 1964; Abdul-Kareem and McRae, 1984; Dickie et al., 1988; Harris and Birch, 1989; Visser et al., 1984; Ross and Cairns, 1981; Rives et al., 1980; Banning et al., 2008; Glen et al., 2008). These changes, however, depend very much on the nature of soil handling and stockpile management as well as on the inherent properties of the stockpiled soils (Anderson et al., 2008). Characterization of soil microbial community and functional biomarkers have been proven to be more powerful than other soil and aboveground site attributes in assessing the effects of operational management for reclamation and restoration (Harris, 2003).

Changes in OM quality during stockpiling is of particular concern for its direct relationship with nutrient availability mediated by soil microbial activities. Anaerobic conditions created by stockpiling, especially in deeper layers (i.e., greater than the rooting zone) can promote substantial thermo-chemical changes in OM quality which might lead to heavy leaching loss of essential plant nutrients such as N and P due to changes in mineralization (Vitousek et al., 1982; Cooke and Johnson, 2002; Grimm et al., 2003), accumulation of metals such as Fe, Mg, Zn and Cu (Abdul-Kareem and McRae, 1984; Ghose, 2004), enrichment of recalcitrant aromatic groups and reduction in labile, short-chain aliphatic groups (Béasse, 2012). Studies have shown that microbial communities in stockpiled and reclaimed soils are generally less efficient in mineralizing complex OM (e.g., phenolic complexes) compared to the communities that develop in natural forest floor (Visser et al., 1984; Banning et al., 2011). Although these effects generally aggravate with increasing soil depth in stockpiles with most detrimental effects observed at depth >1 m (Williamson and Johnson, 1990), significant changes can also take place near surface layer (0–50 cm) (Visser et al., 1984; Abdul-Kareem and McRae, 1984).

More than 900 km^2 of the Athabasca Oil Sands Region (AOSR) has been disturbed due to mining activities (Alberta Environment and Parks, 2018), and Alberta law requires mining companies to return all the disturbed areas to an equivalent land capability to pre-disturbed conditions (Government of Alberta, 2010). Available options for reclamation are limited to the use of overburden material and soils that are salvaged during mining activities. Much of the overburden materials are organic deficient mineral subsoils; thus, the OM-rich peat and upland boreal forest soils are of substantial value to reclamation efforts and are harvested and stockpiled separately. Approximately half of the future mine reclamation in the AOSR may be done using stockpiled materials. The process of salvaging and storing these soils involves some mixing with the lower mineral soils resulting in the two common reclamation soils in the AOSR viz. peat mineral soil mix (PMM) and forest floor mineral soil mix (FFMM). Although very different than PMM and FFMM in terms of texture and OM content, mineral subsoils have the potential to be used as topsoil if appropriately admixed with desirable OM and can support the nutritional demand for upland vegetation (Quideau et al., 2013a). Previous studies in the AOSR showed that FFMM and PMM soils have very different physicochemical and biological properties. FFMM soils in general have greater stock of viable seed propagules, microbial biomass and enzyme activities, and N, P, and K availability than the PMM soils which have greater S, Ca and Mg availability (McMillan et al., 2007; Mackenzie and Naeth 2010; MacKenzie and Quideau, 2012; Kwak et al., 2015; Howell et al., 2017; Howell and MacKenzie, 2017). The major differences in nutrient availability between these two soils were attributed to their OM quality and biogeochemical environments of the donor sites (Turcotte et al., 2009; Howell et al., 2017).

Peat is formed as a result of partial decomposition of OM in water-logged anoxic environment followed by a gradual build-up of recalcitrant organic complexes and humic substances over many years (Gorham, 1957; Chiou et al., 2000). Organic layers in natural forest soils, on the other hand, develop in well-drained and aerobic environments. FFMM soils, therefore, are characterized by more labile and simpler C substrates compared to PMM (Béasse et al., 2015). Admixing forest floor OM to PMM and SS could be a practical option to make the lowland and major plant nutrient deficient substrates more biogeochemically apt to support target upland vegetation and ecosystem functions specific to reclaimed forest sites in the AOSR. Given the extent of the disturbance in AOSR, the current supply of FFMM soils will not meet the reclamation need in the area and it would be appropriate to combine this with more abundant material such as peat and mineral sub-soils.

A better understanding of the relationships between organic matter chemistry, microbial functions, and nutrient bioavailability is needed for an ecologically justified use of reclamation soils and to ensure the long-term sustainability of the biogeochemical cycles in the reconstructed boreal ecosystems (Harris, 2003). Although several previous studies (e.g., MacKenzie and Quideau, 2012; Béasse et al., 2015) demonstrated the linkage between these processes in reclamation soils, there are still major unknowns as to what microbially mediated factors are responsible for changes in the nutrient supply rate under different storage and edaphic conditions. The current study investigated the stockpiling and OM admixing effects on the major microbial and OM quality drivers of nutrient

bioavailability in oil sands reclamation soils. We worked on three main hypotheses: (1) The stockpiled reclamation soils would exhibit lower microbial biomass, functional diversity and lower nutrient bioavailability compared to the directly salvaged soils, (2) organic matter admixing would increase microbial biomass, functional diversity, and nutrient bioavailability in reclamation soils. An increase in N availability in the FFMM-admixed soils and an increase in cation availability in the PMM-admixed soils were expected, and (3) drivers of nutrient bioavailability would vary between directly salvaged and stockpiled, and admixed and non-admixed soils. Nutrient bioavailability in FFMM and SS (i.e., soils developed in upland ecosystems) would be driven mostly by the mineralization of labile (short chain C) compounds, while in PMM (i.e., substrates developed in lowland and anoxic environments) would be driven mostly by the mineralization of recalcitrant (long chain C polymers) compounds.

METHODS

Experimental Design

Three reclamation soils commonly being used in the AOSR were used in the current experiment: (1) forest floor mineral soil mix (FFMM) salvaged from nearby upland forests, (2) peat mineral soil mix (PMM) salvaged from lowland peatbogs during oil sands mining, and (3) mineral sub-soil (SS) that is, the lower subsoils that get extracted before reaching the ore. Soil from a nearby post-fire stand (5-yr old) was used as benchmark to compare the processes between the reclaimed and natural soils as fire is the major natural disturbance in the area (Amiro et al., 2009; Deluca and Boisvenue, 2012).

Stockpiling Effects

Stockpiled soils (sFFMM and sPMM) were collected in 2014 from the surface layers (0–20 cm) of two stockpile dumps of approximately 5-yr old and stored in room temperature. Directly salvaged (hereafter, "fresh"; fFFMM and fPMM) soils were collected from the top 10 cm of a reclaimed site (57°20′59.02″ N, 111°49′36.26″ W) in 2015 and stored at 4°C several months until further processing.

Admixing Effects

Organic matter addition effects were tested by admixing sFFMM, sPMM, and SS at two different ratios (w/w dry weight basis). For PMM mixtures, sFFMM was admixed to sPMM at 10% (PF91) and 40% (PF64) ratios. The SS mixtures were made by admixing sPMM at 40% (SSP) and sFFMM at 20% (SSF) ratios.

The stockpiled soils were rewetted to 80% of maximum water holding capacity, and pre-incubated for 5 wk along with the fresh and natural soils prior to any further measurements. White sand (pH = 7) was used in the soil mixtures (10% w/w) to reduce compaction on rewetting and to create a homogenous pore distribution. Approximately 500 g of soil was incubated in 1-L Mason jar. The jars were weighed and vented two to three times a week to maintain an aerobic environment and constant moisture conditions. The soils were incubated at 22°C in dark and the experiment was run for 6 mo. A replication of six was used for all the treatments except for the fresh soils which had 12 replications.

Nutrient Bioavailability

Bioavailability of major plant nutrients was measured using plant root simulator probes (Western Ag Innovations, Saskatoon, SK, Canada). A pair of cation and anion resin membranes was buried in each microcosm and left for 8 wk after which they were extracted, washed with deionized water, and sent to Western Ag Innovations for nutrient analysis. Ammonium and NO_3–N were determined colorimetrically using an automated flow injection analyzer and all other nutrients (PO_4^{3-}, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}) were measured using inductively-coupled plasma spectrometry (Western Ag Innovations, Saskatoon, SK, Canada).

Soil Properties

Chemical Analyses

Soil pH and electric conductivity (EC) were measured in deionized water using a 1:10 solution for PMM and a 1:5 solution for FFMM and SS (Hendershot et al., 2007). Total C and total N were determined by dry combustion method using a CN analyzer (Costech Model EA 4010). Soil OM was measured using loss on ignition method (Lim and Jackson, 1982). Basic properties of the soils used in this study can be found in Table 1.

Microbial Analyses

Basal respiration was measured after 5 wk of pre-incubation. Gas samples were collected directly from the Mason jars using a gas tight syringe and analyzed in an infrared gas analyzer (EGM-5, PP Systems International). Microbial biomass C was determined using substrate induced respiration (SIR) (Anderson and Domsch, 1978) where D-glucose was used as a priming substrate. Substrate induced respiration based on L-alanine (Alfa Aesar; A15804-14) (SIR_{ala}) and hydroxycinnamic acid (Alfa Aesar; A15167-14) (SIR_{hcyn}) were also performed to assess the microbial mineralization efficiency of labile amino acid and recalcitrant lignin substrates in these soils. Substrate concentrations were standardized to achieve a 16 mg C mL^{-1} in the final solution (Degens, 1998). A saturated solution of hydroxycinnamic acid was used due to its low solubility. Briefly, 4 mL of L-alanine or hydroxycinnamic acid solution was added in 10 g (dry weight equivalent) soil and respiration measurements were taken at times of 2, 4, and 24 h using an infrared gas analyzer (EGM-5, PP Systems International).

Community level physiological profiling (CLPP) was conducted using the Biolog ecoplates (Biolog Inc., USA). Each ecoplate contains 31 different carbon substrates (Supplemental Table S1) commonly found in the soil and rhizosphere systems (Insam and Rangger, 1997). Briefly, a 10 g (dry weight) soil sample was mixed with 0.87% saline solution, shaken for 30 min and allowed to settle for 5 min. The suspension was then serially diluted to 10^{-3} concentration which was used for the final inoculation. A 150 μL suspension was inoculated in each well of the microplates. The microplates were then incubated at 22°C in the dark and color

Table 1. Mean (± SE) soil pH, electrical conductivity (EC), organic matter (OM), total nitrogen (TN), total carbon (TC), and micronutrients of the reclamation and natural soils used in the current study. Soils were stockpiled and fresh peat mineral soil mix (sPMM and fPMM), stockpiled and fresh forest floor mineral soil mix (sFFMM and fFFMM), and sub-soil (SS); soil were also mixed at different ratios (SSP is the SS and PMM mix; SSF is the SS and FFMM mix; PF64 is the PMM and FFMM mix at 60:40; PF91 is the PMM and FFMM mix at 90:10).

						Micronutrients		
Soil type	pH	EC	OM	TN	TC	Fe	Mn	Zn
		μS cm^{-1}	%			μg 10 cm^{-2} 8 wk^{-1}		
				Stockpiling				
fPMM	5.99 (0.34)	423.37 (111.9)	21.38 (5.14)	0.29 (0.05)	11.07 (2.71)	81.6 (19.65)	2.49 (0.92)	1.75 (0.33)
sPMM	4.53 (0.05)	269.83 (12.28)	25.50 (1.15)	0.50 (0.01)	13.63 (0.68)	105.33 (10.96)	44.25 (3.37)	1.56 (0.15)
fFFMM	6.45 (0.34)	609.90 (52.58)	5.38 (0.27)	1.03 (0.45)	2.54 (0.15)	36.10 (7.72)	1.48 (0.59)	1.21 (0.28)
sFFMM	5.85 (0.04)	492.33 (20.49)	8.90 (0.33)	0.26 (0.01)	3.99 (0.19)	50.83 (8.84)	1.48 (0.71)	3.16 (0.45)
				Admixing				
SS	7.64 (0.09)	1034.20 (58.99)	2.87 (0.03)	0.06 (0.00)	0.85 (0.01)	10.60 (0.87)	0.08 (0.03)	0.82 (0.22)
sPMM	4.53 (0.05)	269.83 (12.28)	25.50 (1.15)	0.50 (0.01)	13.63 (0.68)	105.33 (10.96)	44.25 (3.37)	1.56 (0.15)
sFFMM	5.85 (0.04)	492.33 (20.49)	8.90 (0.33)	0.26 (0.01)	3.99 (0.19)	50.83 (8.84)	1.48 (0.71)	3.16 (0.45)
SSP	6.35 (0.07)	871.80 (92.43)	12.02 (0.39)	0.23 (0.01)	5.75 (0.22)	102.20 (7.07)	4.54 (1.11)	1.18 (0.08)
SSF	7.41 (0.05)	799.0 (33.81)	4.47 (0.07)	0.11 (0.00)	1.67 (0.05)	19.33 (2.13)	0.06 (0.02)	0.70 (0.14)
PF64	4.65 (0.02)	399.80 (22.75)	19.58 (0.46)	0.38 (0.00)	10.08 (0.35)	78.80 (9.79)	25.32 (2.11)	2.32 (0.20)
PF91	4.61(0.07)	253.0 (29.36)	24.81(0.97)	0.46 (0.01)	12.76 (0.51)	91.40 (13.24)	44.22 (2.88)	1.82 (0.09)
Post-fire	5.63 (0.07)	194.80 (14.71)	3.29 (0.19)	0.11 (0.01)	1.57 (0.11)	188.40 (18.1)	27.02 (1.38)	1.14 (0.14)

development was measured at 590 nm every 24 h up to 10 d using a plate reader (Microlog MicroStation system). The optical density (OD) values were blank corrected by subtracting the OD of water cell prior to statistical analyses. The OD values were expressed as average well color development (AWCD) (Garland and Mills, 1991) using the following: AWCD = $\Sigma(OD_i/31)$, where OD_i is the optical density of each well after blank correction.

To compare metabolic preferences of microbial communities of different soil types, ecoplate substrates were grouped into five substrate guilds: carbohydrates (Car), carboxylic and ketonic acids (CKa), amino acids (Amn), amines and amides (Amd), and polymers (Pol) (Weber and Legge, 2009) (Supplemental Table S1). Based on the oxidative states, amino acid and carboxylic and ketonic acid substrates are the most labile, readily mineralizable substrates, whereas amines and amides and polymers are the most recalcitrant, energy expensive substrates (Nunan et al., 2015). Blank corrected OD values of each substrates in each guild were averaged and the changes in absorbance values were fitted over incubation time using a three-parameter sigmoidal equation:

$$y=\frac{a}{1+\exp\left[-\left(\frac{x-x_0}{b}\right)\right]}$$

where a is the maximum absorbance, x_0 is the time at which half of the maximum absorbance ($a/2$) is reached, and b is the rate of changes in absorbance (slope). Areas under curve (AUC) were also calculated for each guild using the trapezoidal estimation method (Hackett and Griffiths, 1997). The AUC is a measure of the total mineralization of a substrate by the microbial communities. Curve fitting and kinetic parameter estimation were done using the dynamic curve fitting option in Sigmaplot (v12.0).

Functional diversity (FD) was calculated using Shannon-Weaver diversity index (H) where $H = -\Sigma\, p_i\, (\ln p_i)$, where p_i is the ratio of the OD of a utilized substrate (OD_i) to the sum of the ODs of all utilized carbon substrates (ΣOD_i). Functional richness (R) was determined by the number of oxidized substrates above a threshold positive response; a blank corrected OD of 0.25 was used as threshold value (Garland, 1997). Optical densities at 120 h were used to calculate AWCD, R, and H, as this was the shortest incubation time which offered the best treatment sensitivity.

Statistical Analysis

Permutational analysis of variance (PERMANOVA) was used to test the stockpiling and admixing effects on soil properties (hypothesis 1 and 2). Bray-Curtis dissimilarity matrices were used for the multivariate analysis and Euclidean distance matrices were used for the univariate analysis (Anderson, 2001). One-way ANOVA was used to assess significant differences within soil groups.

Principal component analysis (PCA) was used to identify patterns of nutrient bioavailability and microbial functions between the soil types. Multi-variate differences among these patterns were tested using PERMANOVA using the Bray-Curtis distance matrix. A 9999 permutation was conducted for all the PERMANOVA analyses. Given the small number of replications per soil type and the variability within samples, a P value ≤ 0.10 was considered significant. PERMANOVA analyses were done using *adonis* function in *vegan* package (Oksanen et al., 2013) in R statistical software, version 3.4.2 (R Development Core team, 2017), and PCA analysis was done using PC-Ord (McCune and Mefford, 2011).

To further test the effects of OM admixing on substrate mineralization (hypothesis 2), gradual changes in FD values in different soils were modeled over the incubation period. Generalized additive modeling (GAM) was used to model the changes in FD over the incubation period. Generalized additive modeling was used for its flexibility in capturing dynamic

relationships between response and explanatory variables without the limitation of parametric assumptions (Guisan et al., 2002). Thin-plate regression spline and optimal effective degrees of freedom were used as GAM cross validation criteria (Wood, 2003, 2006). Generalized additive modeling analysis was performed in R using the *mgcv* package (Wood, 2006).

Random Forest modeling (Breiman, 2001) was used to determine the most important predictors of nutrient bioavailability in different soils (hypothesis 3). Random Forest is a modified classification and regression tree (CART) approach which uses a randomly selected subset of data (one third; termed as out-of-bag or OOB cases) to predict the fit of individual classification trees. Importance of variable in Random Forest model is measured by the mean decrease in prediction accuracy (increase in mean squared error or node impurity) when that specific variable is randomly permuted. Random Forest provides strong prediction as it is less susceptible to over-fitting and introduces randomness in the model by reiterative bootstrapping and random variable selection for each decision tree (Breiman, 2001). Availability of macronutrients was used as response variable and the soil and microbial properties were included as predictor variables in the models. Random Forest analysis was done in R using *randomForest* package (Liaw and Wiener, 2002) and the significance of the predictor variables and cross-validation of the models were assessed using the *A3* package (Fortmann-Roe, 2013).

RESULTS

Stockpiling Effects

Overall nutrient availability was significantly (P = 0.01) different between the fresh and stockpiled PMM and FFMM reclamation soils; however, stockpiling effects on individual nutrients was soil specific and did not follow any specific trend (Fig. 1a; Fig. 2a-b). Stockpiled PMM (sPMM) had lower total inorganic N (TIN) and Mg availability, and greater P availability than fPMM, and sFFMM had greater TIN and lower S availability than fFFMM (Fig. 2a-b). FFMM soils in general had more similar nutrient profile to post-fire soils (data not shown).

Significant differences were also observed in soil microbial properties between the fresh and stockpiled soils. Microbial biomass was greater and mineralization of lignin substrate (SIR_{hcin}) was lower in both sPMM and sFFMM compared to the fresh counterparts (Table 2). Basal respiration was greater in stockpiled than fPMM and mineralization of amino acid substrate (SIR_{ala}) was greater in stockpiled than fFFMM (Table 2). Stockpiling effects on the CLPPs were observed only in FFMM (Fig. 3a).

Admixing Effects

Effects of OM admixing on nutrient bioavailability was only observed for FFMM-admixing in SS and PMM (Fig. 1b). Individual macronutrients, however, showed variable responses to OM addition. Addition of FFMM to SS significantly (P = 0.0001) increased the availability of TIN but decreased S availability. A similar effect was also observed in PMM where addition of 40% FFMM significantly increased TIN and K availability but decreased P availability (Fig. 2c-d).

Microbial biomass and BR were significantly greater in the SS admixed with both PMM and FFMM. Addition of PMM to SS decreased SIR_{hcin} but increased SIR_{ala}, whereas the decrease in SIR_{hcin} in the FFMM-admixed SS was marginal compared to the PMM-admixed SS. Admixing FFMM to PMM did not have a significant effect on MBC but SIR_{ala} decreased in both PF64 and PF91 mixtures (Table 2). Admixing effects on the CLPPs were observed only in SS mixtures but not in the PMM mixtures (Fig. 3b).

Microbial FD gradually increased with time in all soils but showed variable patterns in PMM vs. FFMM-admixed soils. Functional diversity constantly increased during the first 110 h in SS, but this was achieved at 46 h in the FFMM-admixed SS and at 192 h in the PMM-admixed SS (Fig. 4a-c). A similar trend in FD was also observed in PMM where a maximum diversity in substrate utilization was achieved at much faster rate by adding as low as 10% FFMM (Fig. 4d-f).

Drivers of Nutrient Bioavailability

Availability of P, K and S in fPMM were controlled by carboxylic and ketonic acid (e.g., γ-hydroxybutyric acid and D-galacturonic acid) and carbohydrate substrates (e.g., D-cellobiose), whereas in sPMM these drivers were mostly polymers (e.g., α-cyclodextrin and glycogen) (Fig. 5; Supplemental Fig. S4). Drivers of TIN, however, was not greatly affected by stockpiling in PMM and Tween-40 (polymer) was the significant driver in both fresh and stockpiled soils. In fFFMM, availability of TIN, P, and S were mainly driven by carbohydrates (e.g., α-D-glucose-1-phosphate) and MBC, whereas these were mostly driven by C to N ratio and amino acids (e.g., L-Phenylalanine and L-arginine) in sFFMM (Fig. 5; Supplemental Fig. S4). Carboxylic and ketonic acids, and amino acids substrates were also the major drivers of nutrient bioavailability in post-fire soil. Overall, nutrient availability in stockpiled soils was driven by the joint control of several substrates (as indicated by the similar variable importance scores of the top variables), whereas importance of single drivers was much stronger in the fresh soils, and this was the case for both PMM and FFMM. A similar joint control of caboxylic and ketonic acids, carbohydrates and amino acids was also seen in the post-fire soil (Fig. 5).

When compared to non-admixed SS, the major drivers of nutrients in the PMM-admixed SS did not shift at C-guild level except for K and S availability where the influence of long-chain polymer substrates, such as glycogen and Tween-80, was evident (Fig. 6; Supplemental Fig. S5), although the model predictability was very poor. Major drivers of nutrients in FFMM-admixed SS were carbohydrates and amino acids and also did not change in most cases.

Despite the PMM-FFMM mixtures being mostly PMM by mass, the nutrient availability was driven by the same C-guilds as in the FFMM soils; the carbohydrates and amino acids. For example, driver of TIN shifted from polymer based substrate to carbohydrates and soil chemical properties. Likewise P drivers

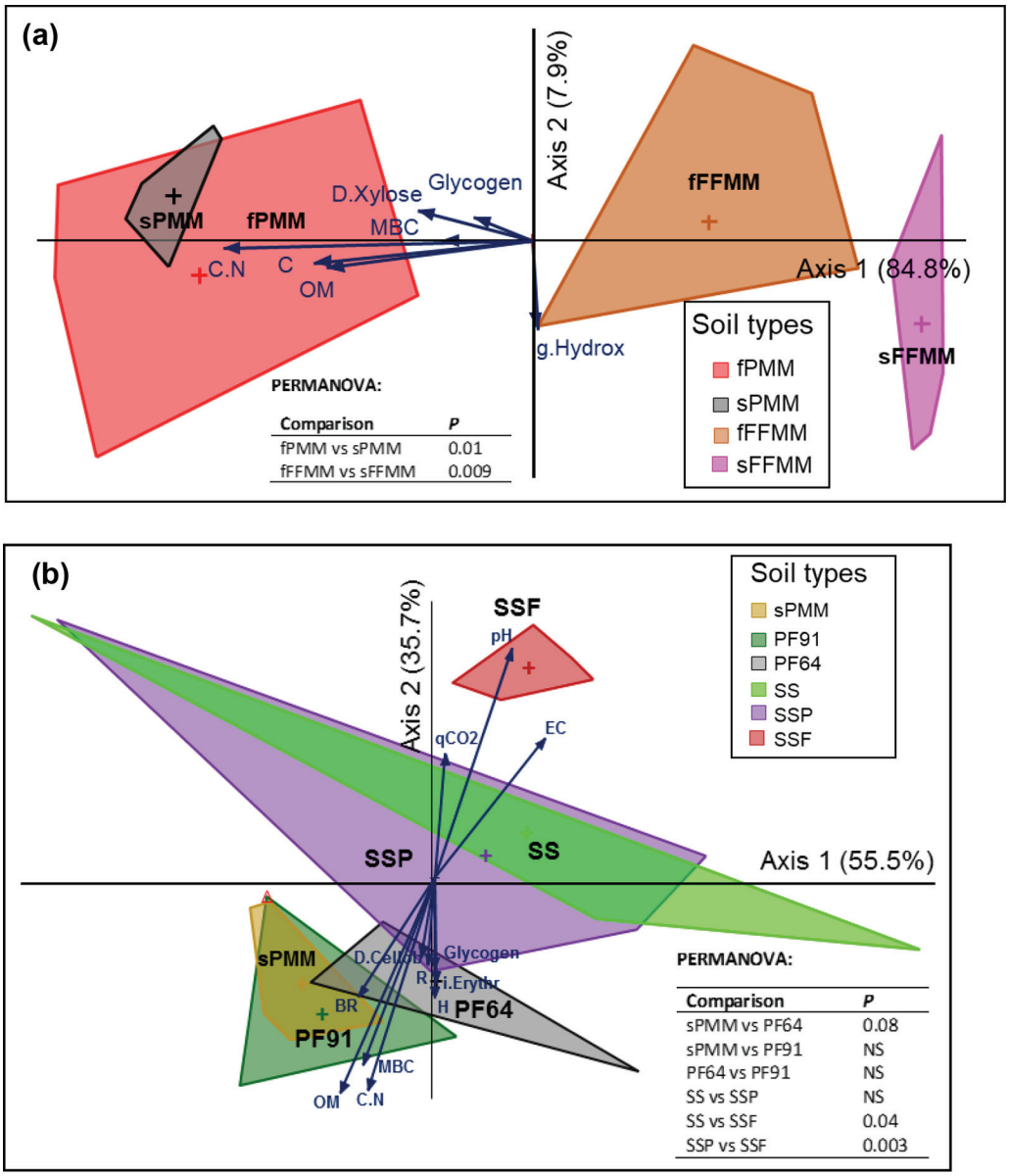

Fig. 1. Principal component analysis (PCA) showing stockpiling (a) and admixing (b) effects on nutrient availability in reclamation soils as measured by the plant root simulator probes. Multi-response permutation procedure analysis shows differences between soil groups. Stockpiled and fresh peat mineral soil mix (sPMM and fPMM), stockpiled and fresh forest floor mineral soil mix (sFFMM and fFFMM), and sub-soil (SS) were used and mixed at different ratios (SSP is the SS and PMM mix; SSF is the SS and FFMM mix; PF64 is the PMM and FFMM mix at 60:40; PF91 is the PMM and FFMM mix at 90:10). NS, not significant.

were shifted from carboxylic acid and amines to carbohydrates (Supplemental Fig. S5).

DISCUSSION

Stockpiling Effects

Stockpiling soils has been known to have adverse impacts on nutrient availability (Abdul-Kareem and McRae, 1984; Harris and Birch, 1989). We expected to see a lower nutrient availability in the stockpiled soils compared to the fresh soils. The current study, however, showed variable effects of stockpiling on nutrient availability in the PMM and FFMM soils. The observed increase in N availability in sFFMM could be related to the aerobic conditions created by the mineral texture and the diverse microbiota of the soils (Stefani et al., 2018), whereas the decrease in N and increase in P availability in the sPMM could be due to the anaerobic conditions that might have resulted from the very high water holding capacity of this soil (Béasse et al., 2015). Ammonium ion concentration in the sPMM of the current study was found to be greater than the sFFMM. Studies by Harris and Birch (1989), Ross and Cairns (1981), and Sheoran et al. (2010) also reported a decrease in nutrient cycling (especially N) and an accumulation of ammonium in the stockpiled soils which was attributed to the anaerobic condition of the stockpile dump. Biologically reactive phosphorus can be highly solubilized under anaerobic and acidic conditions, especially through reduction and dissolution of iron complexes (Faulkner and Richardson, 1989; Wright et al., 2001) and both conditions exist in the sPMM soils with a high Fe and Mn ion concentrations.

Table 2. Mean (± SE) microbial and catabolic properties of the reclamation and natural soils. Soils were stockpiled and fresh peat mineral soil mix (sPMM and fPMM), stockpiled and fresh forest floor mineral soil mix (sFFMM and fFFMM), and sub-soil (SS); soil were also mixed at different ratios (SSP is the SS and PMM mix; SSF is the SS and FFMM mix; PF64 is the PMM and FFMM mix at 60:40; PF91 is the PMM and FFMM mix at 90:10).†

Soil type	MBC	BR	SIR_{ala}	SIR_{hcin}	H	AWCD
	mg C g soil^{-1}		μg CO_2–C g soil^{-1} h^{-1}			
Stockpiling						
fPMM	12.09 (3.00)a‡	0.13 (0.01)a	2.46 (0.26)a	3.32 (0.45)a	2.41 (0.07)	0.29 (0.01)
sPMM	23.06 (1.08)b	0.21 (0.01)b	2.25 (0.03)a	2.14 (0.04)b	2.42 (0.07)	0.28 (0.02)
fFFMM	6.45 (0.82)c	0.19 (0.03)b	1.61 (0.05)b	6.76 (1.81)c	2.42 (0.04)	0.31 (0.02)
sFFMM	13.69 (1.30)d	0.17 (0.01)b	1.96 (0.09)c	2.18 (0.08)b	2.27 (0.05)	0.29 (0.02)
Admixing						
SS	2.60 (0.20)a	0.01 (0.00)a	1.89 (0.25)a	6.77 (0.09)a	2.38 (0.08)a	0.21 (0.01)a
sPMM	23.06 (1.08)d	0.21 (0.01)e	2.25 (0.03)b	2.14 (0.04)b	2.42 (0.07)b	0.28 (0.02)b
sFFMM	13.69 (1.30)b	0.17 (0.01)e	1.96 (0.09)a	2.18 (0.08)b	2.27 (0.05)a	0.29 (0.02)b
SSP	16.86 (0.37)b	0.07 (0.00)b	2.25 (0.06)b	2.09 (0.04)b	2.36 (0.08)a	0.24 (0.02)b
SSF	4.32 (0.23)c	0.10 (0.01)c	1.53 (0.10)c	5.18 (0.17)c	2.24 (0.03)a	0.26 (0.02)b
PF64	23.74 (0.41)d	0.16 (0.01)d	1.79 (0.07)a	2.62 (0.05)d	2.50 (0.03)b	0.29 (0.01)be
PF91	21.18 (1.49)d	0.19 (0.01)de	1.91 (0.06)a	2.06 (0.06)b	2.45 (0.07)b	0.31 (0.01)ce
Post-fire	6.87 (0.58)ac	0.08 (0.00)c	1.45 (0.09)c	1.70 (0.07)b	2.28 (0.05)a	0.43 (0.03)d

† MBC, microbial biomass carbon; BR, basal respiration; SIR_{ala}, substrate induced respiration of L-alanine; SIR_{hcyn}, substrate induced respiration of hydroxycinnamic acid; H, microbial catabolic diversity at 120 h; AWCD, average well color development at 120 h.

‡ Different letters within group indicate significant differences ($P < 0.10$; one-way ANOVA).

A lower microbial biomass and activity were hypothesized in the stockpiled compared to the fresh soils, but this was not supported by the current study. Greater microbial biomass and basal respiration in the stockpiled soils were not surprising given that the soils were collected from the top 20 cm of the stockpile dumps. Williamson and Johnson (1990) reported a similar observation where they did find greater viable microbial biomass in the surface stockpiled soils (0–20 cm) compared to the deeper layers. Other stockpile studies, however, reported a decrease in microbial activities in the stockpiled soils collected from the surface of the mound (Abdul-Kareem and McRae, 1984; Harris et al., 1989; and Visser et al., 1984). The reduction of microbial biomass in the stockpiled soils has been attributed to decrease in organic C (Visser et al., 1984; Akala and Lal, 2001), anaerobicity (Williamson and Johnson, 1990; Ross and Cairns, 1981), pH change (Abdul-Kareem and McRae, 1984; Ghose, 2004), compaction and aggregate destabilization (Wick et al., 2008). Microbial biomass in our study was highly correlated with the OM content of the soils. The high MBC in the stockpiled soils might, therefore, be the result of greater available C and energy source compared to the fresh soils. While a greater microbial biomass was observed in the stockpiled soils, a significantly lower mineralization of lignin-based substrate in both sFFMM and sPMM (the only support of our hypothesis) may indicate a selective stress on special groups of microbes (e.g., fungal community) under stockpiled conditions. Fungi are the main decomposer of lignin (Kirk and Farrell, 1987; Hammel, 1997); however recent studies are showing some bacterial groups can degrade these recalcitrant substrates as well (Bugg et al., 2011; Brown and Chang, 2014). Harris et al. (1989) reported a relatively greater decline in fungal spores than in bacteria and found no sign of fungal recovery, while bacterial population replicated back to the pre-disturbed level on the surface of the stockpile. This is plausible given that fungi are truly aerobic and more prone to damages to their growth form due to disturbances involved in stockpiling processes (Stahl et al., 2003; Wick et al., 2008).

Stockpiling effect on microbial catabolic activities was only evident in FFMM and mainly through an increased activity of carbohydrate and amino acid substrates (Supplemental Fig. S2). Effects of storage condition on the functional abilities of soil microbial communities have been demonstrated in several previous studies. Goberna et al. (2005) reported an increase in carboxylic and amino acids utilization after short term (1 mo) storage of forest soils and the same study reported an increase in carbohydrate activities in soils stored for a year. Such an increase in the utilization of specific C-guilds in the stockpiled soils can be attributed to factors such as proliferation of stress-resistant microbes after an initial decrease in general groups (Pesaro et al., 2003), niche expansion of major microbial groups (Shishido and Chanway, 1998), and disruption of soil aggregates and subsequent release of occluded OM (Baldock and Skjemstad, 2000). The inherent biogeochemical properties of PMM soils may have contributed to the relatively weak sensitivity of microbial catabolic functions to stockpiling. The PMM soils were originally developed under anaerobic condition and any further anaerobicity under stockpiled condition might not have altered the microbial community structures and functions. This, however, needs to be confirmed with more systematic experimental approach.

Admixing Effects

Ensuring an adequate supply of quality top soils is a major challenge in reclaiming large scale disturbances such as oil sands mining in the AOSR. Use of OM-deficient soils in reclamation has environmental and ecological implications and may delay achieving the reclamation targets. Organic matter admixing has potential to alter nutrient supply rates in low quality soils to a

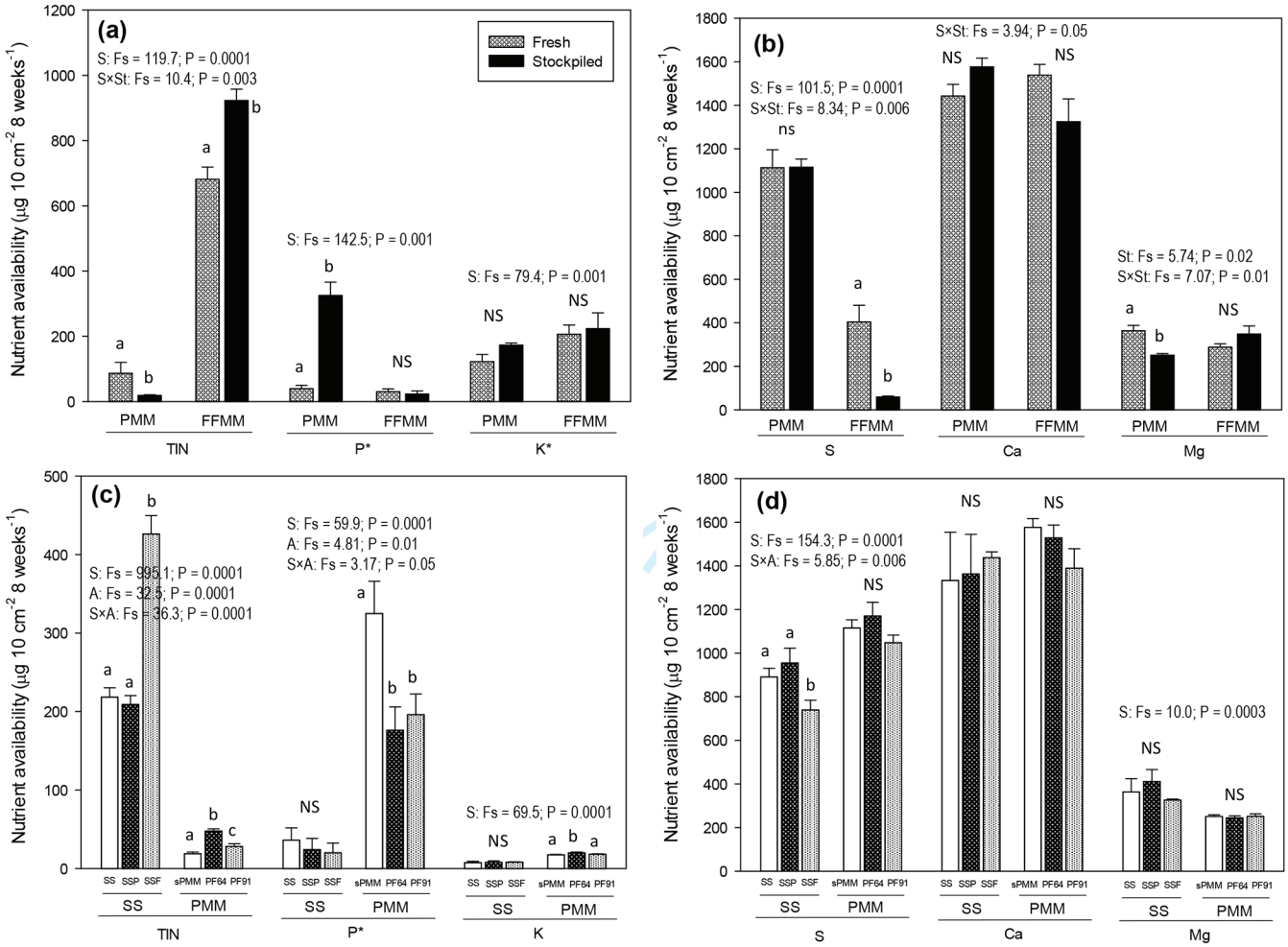

Fig. 2. Availability of major macronutrients in reclamation soils as measured by the plant root simulator probes. Significant effects of soil type (S), stockpiling (St; i.e., fresh vs. stockpiled), admixing (A; i.e., non-admixed vs. admixed) and interaction of these treatments (S × St; S × A) from a two-way permutational analysis of variance (PERMANOVA) are also shown for each nutrient. Different letters within soil group indicate significant differences. Values of P and K were rescaled with a factor of 100 (P* and K*, respectively). Stockpiled and fresh peat mineral soil mix (sPMM and fPMM), stockpiled and fresh forest floor mineral soil mix (sFFMM and fFFMM), and sub-soil (SS) were used and mixed at different ratios (SSP is the SS and PMM mix; SSF is the SS and FFMM mix; PF64 is the PMM and FFMM mix at 60:40; PF91 is the PMM and FFMM mix at 90:10). TIN, total inorganic nitrogen; Fs, pseudo F from PERMANOVA analysis; NS, not significant.

level that is optimum for sustaining plant growth (Larney and Angers, 2012). In reclamation context, admixing forest floor OM to peat and mineral subsoils may provide the benefit of native microbiota and seed propagules (Shrestha et al., 2009; Showalter et al., 2010; Béasse et al., 2015) while conserving the limited FFMM resources. Admixing peat-based reclamation soils can also be an alternative option to achieve a desired nutrient balance, especially in nutrient deficient mineral subsoils. The current study examined such possibilities by admixing forest floor and peat-based OM at two different ratios. However, the expected increase in nutrient availability was only observed in FFMM-admixed SS and PMM soils, mainly through the changes in N, P and S availability. Other studies conducted admixing experiments with similar soils reported variable results, although the physicochemical properties of soils and mixing ratios in those experiments were different than the current study. Dietrich et al. (2017) found that PMM admixing to SS decreased P but increased N, K, and S availability. MacKenzie and Quideau (2012) also reported an increase in N and K availability in FFMM-admixed PMM soils which corroborates our findings. McMillan et al. (2007), however, did not find any difference in N availability in between PMM and FFMM-overlaid PMM soils. The evidence from this experiment suggests that admixing FFMM at a rate of 20% to SS and a 40% to PMM may cause a noticeable shift in the availability of essential macronutrients, especially an increase in N and K availability.

The hypothesized change in the microbial properties due to OM addition was only observed in SS. Addition of both PMM and FFMM to SS showed improvement in microbial activities; however, considering the nutrient bioavailability, microbial metabolic profiles and functional diversity, admixing FFMM seems more beneficial and ecologically appropriate. Some of the observed nutritional benefit in admixed SS might have resulted due to the changes in carbohydrate and polymer mineralization rates. Organic amendments and fertilization have been shown to have positive effects on the nutrient status and microbial functions in low quality soils. Quideau et al. (2013a) in a greenhouse study with reclamation soils found that nutrient

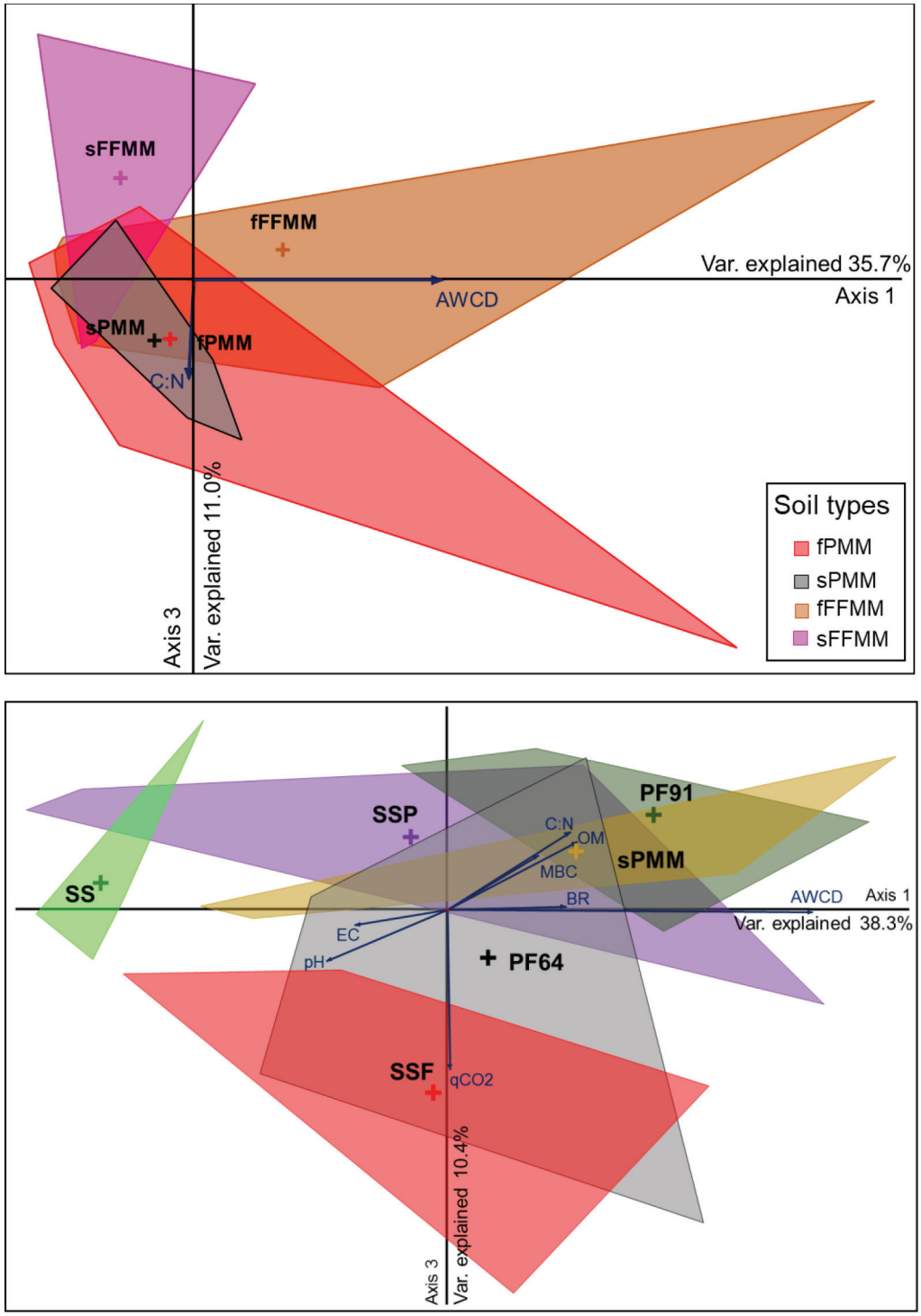

Fig. 3. Principal component analysis (PCA) showing stockpiling (a) and admixing (b) effects on microbial functional profiles in reclamation soils as measured by Biolog ecoplate. The PERMANOVA analysis shows differences between soil groups. Stockpiled and fresh peat mineral soil mix (sPMM and fPMM), stockpiled and fresh forest floor mineral soil mix (sFFMM and fFFMM), and sub-soil (SS) were used and mixed at different ratios (SSP is the SS and PMM mix; SSF is the SS and FFMM mix; PF64 is the PMM and FFMM mix at 60:40; PF91 is the PMM and FFMM mix at 90:10). NS, not significant.

addition only affected the microbial functions in nutrient poor B-horizon and marginal soils. The nutrient induced changes in microbial activity has often been attributed to the changes in chemical environment and C availability mediated through OM or fertilizer addition (Ramirez et al., 2010; Belyaeva and Haynes, 2009; Larney and Angers, 2012). Along with increasing C availability, OM addition also introduces microbial strains to the system which are capable of degrading variable chemical compounds unique to the host system (Pérez-Piqueres et al., 2006; Bastida et al., 2008). For instance, mineralization rate of lignin-based substrate (SIR_{hcin}) was an order of magnitude greater than the basal respiration rate in SS which may indicate the presence of special microbial groups that can break down complex, energy expensive substrates. A similar level of

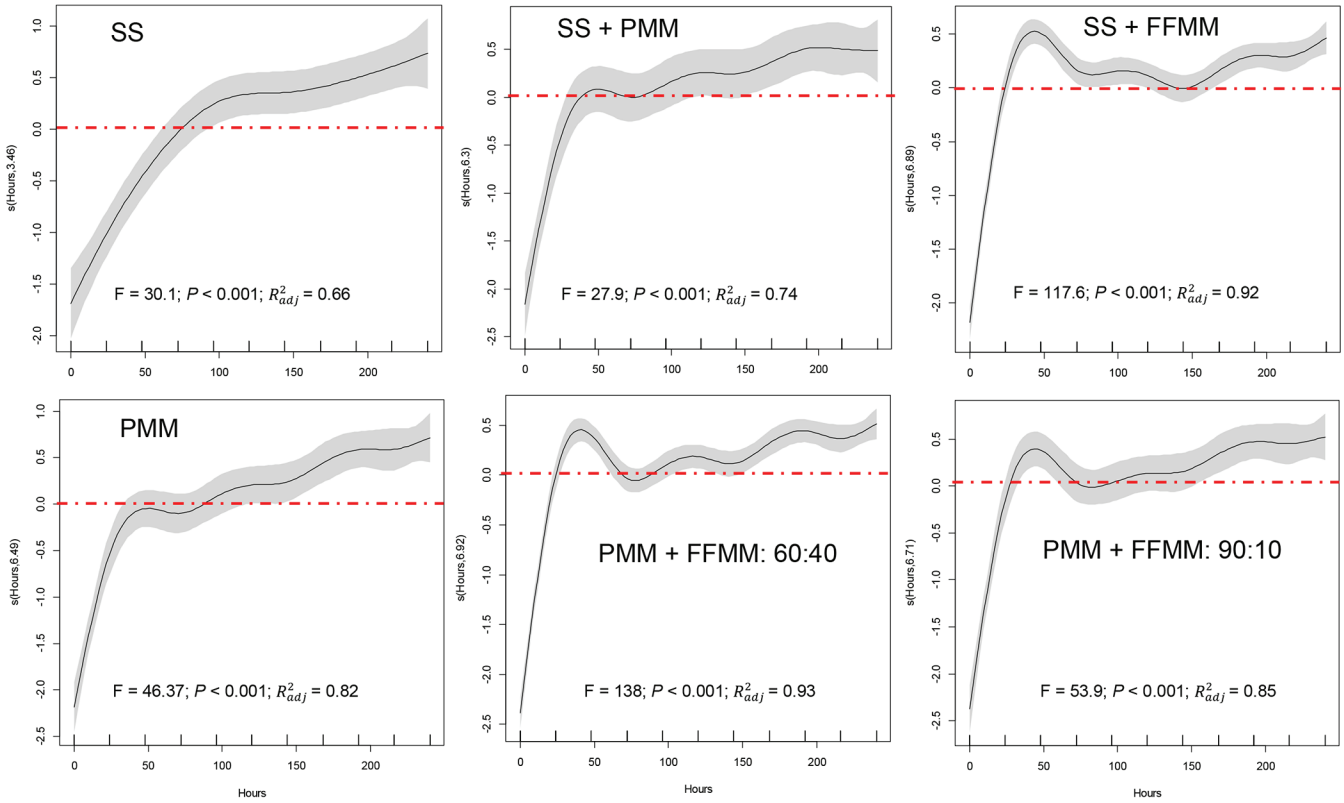

Fig. 4. Estimated flexible changes in the microbial functional diversity (FD) with incubation time as calculated using generalized additive modeling (GAM). Sub-soil (SS), peat mineral soil mix (PMM), and forest floor mineral soil mix (FFMM) were used and mixed at different ratios. The gray band represents 95% confidence interval and the red dotted line indicates hypothetical constant changes in FD set at zero for inter-comparisons. The model fit statistics and significances from GAM analysis are also shown.

mineralization rate was maintained in SSF (FFMM-admixed SS) which again suggests that the microbial functions are favored in systems that experience similar biogeochemical processes. Both FFMM and SS in nature are presumably the continuum of the same soil profile and may, therefore, receive similar products of decomposition, leaching, percolation, and other hydrological inputs, and the inhabiting microbiota in the two soil zones are more familiar with the resulting chemical environment than that created by PMM admixing. A drastic drop in the C mineralization rate in SSP compared to SSF (yet significantly greater than that in SS) indicates a negative impact of PMM admixing on the biochemical processes. Moreover, FFMM admixing can also results in a faster establishment of microbial functional diversity as found for both SS and PMM soils in the current study. This is probably due to the greater microbial substrate use efficiency and diverse community structure in FFMM soils as demonstrated by several other studies (MacKenzie and Quideau 2010; Quideau et al., 2013b). Accordingly, Stefani et al. (2018) showed that FFMM and PMM harbor completely different microbiota even if the aboveground vegetation is of same genetic make-up.

Nutrient Drivers

As hypothesized, drivers of major macronutrients were different between fresh and stockpiled soils, mostly in the PMM soils. A greater importance of polymers in stockpiled PMM indicates dependency of microbial communities on rather complex C substrates for biomass and energy gains. Variation in pH, temperature and oxygen availability in stockpiles can preferentially favor degradation of specific substrates. Studies on peat soils (Kaal et al., 2007; Barkovskii et al., 2009; Perez-Rodriguez and Cortizas, 2014) showed that polysaccharides decrease exponentially with depth and the labile compounds are more degraded on surface, whereas more recalcitrant substrates are found at greater depth. Carbohydrates and carboxylic acids were the dominant drivers of nutrient bioavailability in fPMM, which also corroborates the chemical characterization of PMM done by Béasse et al. (2015) and Quideau et al. (2017), indicating the presence of labile short chain C substrates. Major drivers of nutrients in FFMM also shifted from mostly carbohydrates in the fresh to mostly amino acids in the stockpiled soils. This could be due to the breakdown of aggregates during soil handling which may have released the occluded enzymatic and microbial decomposition products in the system. This was, however, beyond the scope of this current study and could not be confirmed. Destabilization of soil aggregates is one of the major effects of soil handling during stockpiling which significantly impacts nutrient mineralization as reported in previous studies (Wick et al., 2008; Larney and Angers, 2012).

Although OM admixing significantly increased MBC and BR in SS, this did not cause a major shift in the guild specific nutrient drivers, especially in FFMM-admix. However, the observed shift in the nutrient availability in FFMM-admixed SS indicates an importance of other mechanisms such as the change in chemical environment (e.g., pH and EC) or mediation effects of newly added

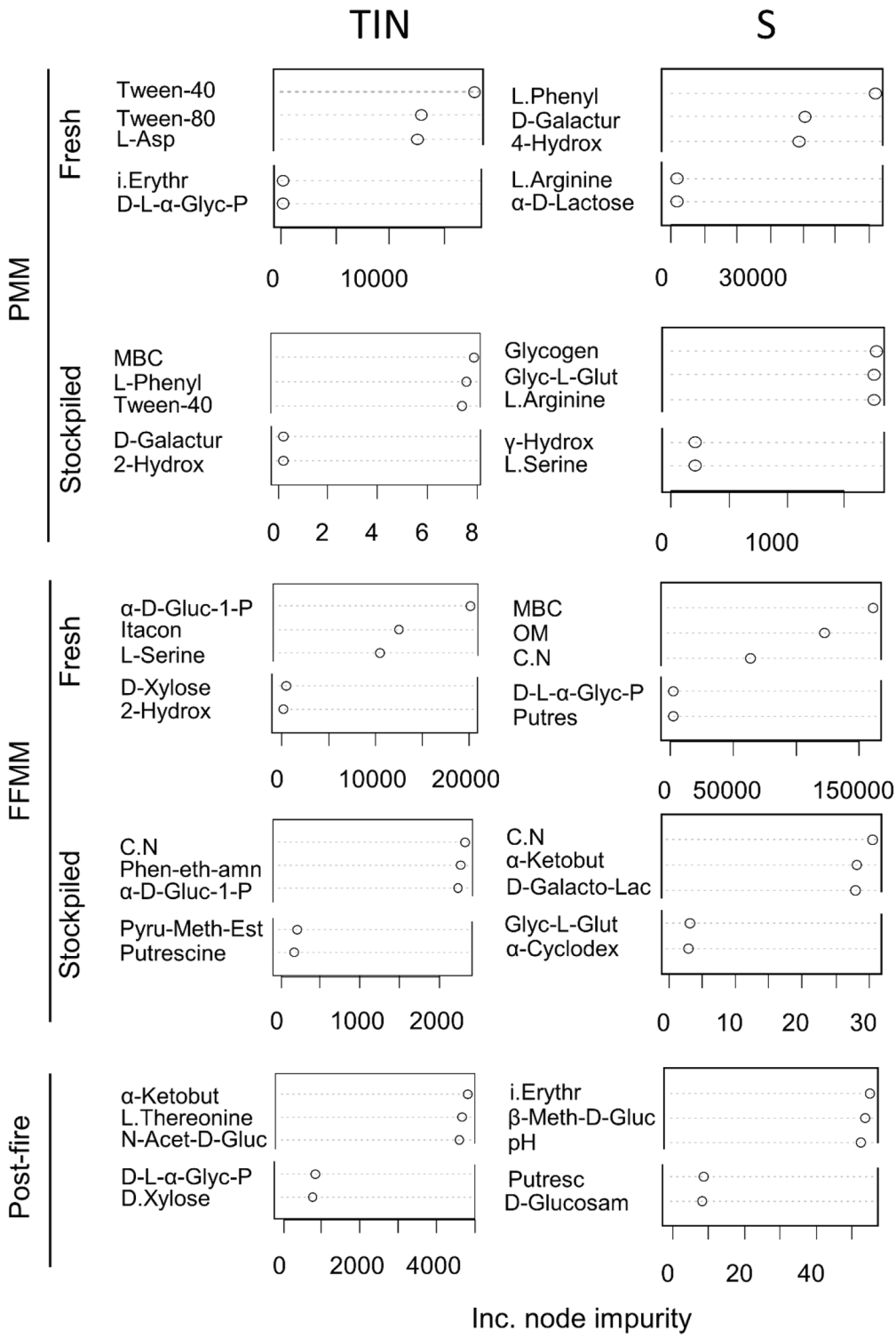

Fig. 5. The most significant drivers of total inorganic N (TIN) and S availability in the fresh, stockpiled and benchmark soils as determined by the Random Forest models. Only top three and lowest two drivers are shown. The *y* axis in the figures indicates mean decrease in node impurity and a higher value indicates higher significance. PMM, peat mineral soil mix; FFMM, forest floor mineral soil mix.

microbial pool on the existing community (Fontaine et al., 2003, 2004). The FFMM-admixed PMM, however, showed shifts in C-guilds with a noticeable dominance of similar guilds as seen in the FFMM soils. The FFMM soils are selectively salvaged from nearby forest stands which include a mixture of organic layer (LFH), coarse woody debris, and top mineral horizon. These soils are reported to have diverse and more active microbial community than the PMM soils (MacKenzie and Quideau, 2010; Béasse et al., 2015). While this is a possible explanation for the shift in the nutrient drivers, this was confounded by the little to no increase in microbial activity in the admixed soils (Table 2). Another possibility for such decoupled phenomenon is the microbial preference of substrate selection. FFMM had much lower C to N ratio and possibly higher lability than the PMM soils which contributed to the overall shift in nutrient

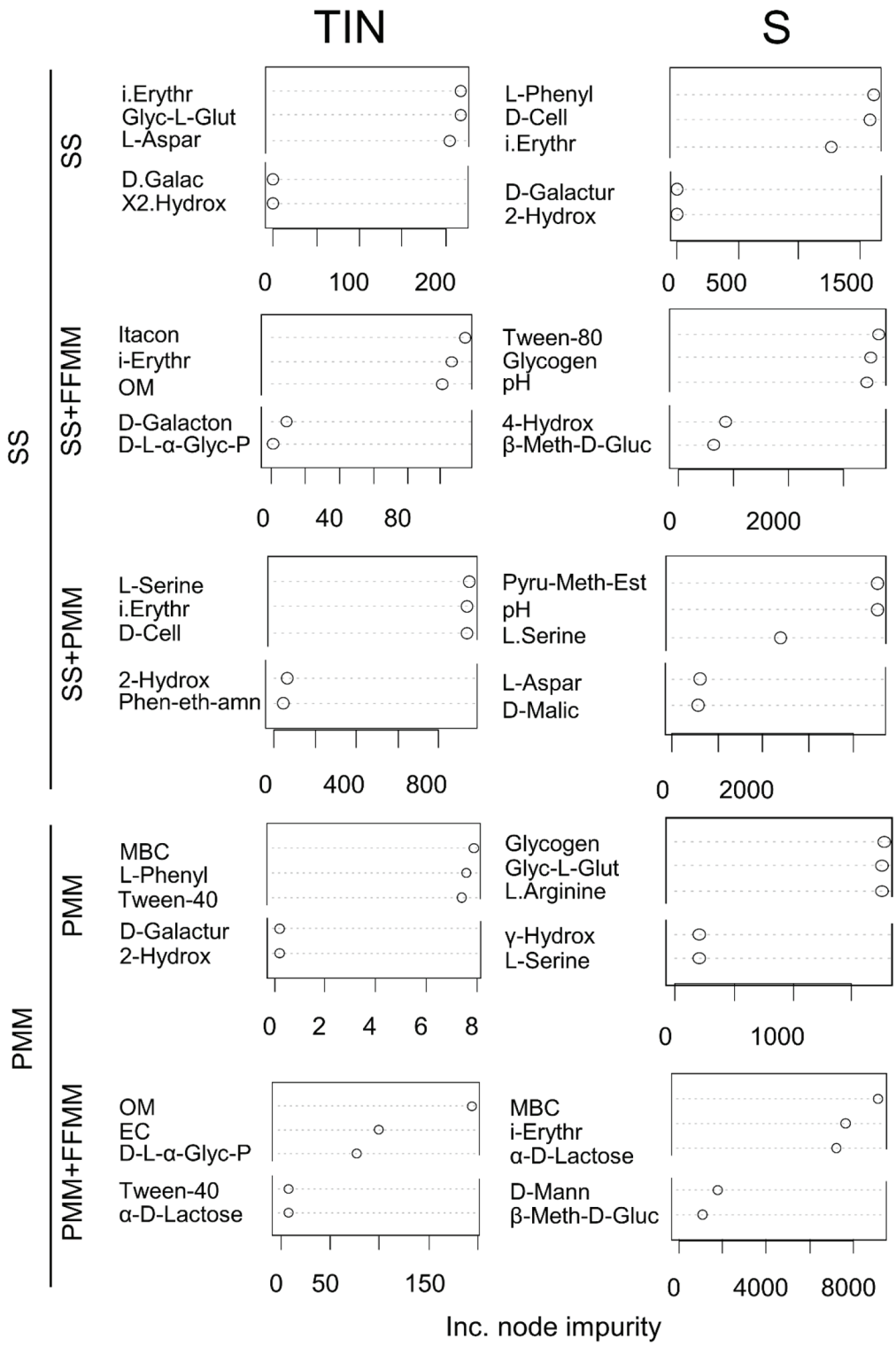

Fig. 6. The most significant drivers of total inorganic N (TIN) and S availability in admixed soils as determined by the Random Forest models. Only top three and lowest two drivers are shown. The *y* axis in the figures indicates mean decrease in node impurity and a higher value indicates higher significance. Mixtures at ratios of 60:40 (PF64) and 90:10 (PF91) were grouped together as the drivers were similar. PMM, peat mineral soil mix; FFMM, forest floor mineral soil mix; SS, sub-soil.

drivers without necessarily changing the nutrient availability due to the lower proportion of FFMM substrates in the mixture. Such priming effect of OM addition has been shown to alter microbial activity, substrate mineralization, and nutrient availability by giving a competitive advantage to specific microbial communities (r- or k-selective species) (Nottingham et al., 2009; Chen et al., 2014).

In a 45-wk long incubation experiment, MacKenzie and Quideau (2012) found no significant difference in N mineralization and microbial community structure between FFMM-admixed PMM and FFMM soils. Nitrogen mineralization rate of mixed soils in their experiment did behave like pure PMM until 20 wk after which it was more comparable to FFMM. The pattern observed

in the current study may, therefore, be a consequence of the short-term nature of the experiment, where the shift in drivers may not have had enough time to cause a noticeable shift in nutrient bioavailability. The positive effects of OM admixing, either in the form of nutrition shift or the change in the nutrient drivers, might be used as an evidence to adopt this as a viable reclamation practice which can rely on using locally available substrates. However, a field-level study is necessary to confirm the observations from the controlled laboratory experiments. Despite this caveat, the substrate-based microbial functional characterization technique used in the current study can be easily replicated in determining the effects of mine soil handling on soil microbial properties and nutrient cycling. Average well color development (AWCD; a proxy of substrate degradation) values are the simple most index of microbial activity in this profiling technique that is useful in detecting differences in C use efficiency between soils and can be ascribed to processes related to nutrient cycling.

CONCLUSIONS AND OPERATIONAL IMPLICATIONS

Although stockpiling can be detrimental for soil quality, it is a part of progressive mining and cannot be avoided for practical reasons. Maintaining fertility and biological integrity of reclamation soils, on the other hand, is crucial for meeting reclamation goals and regulatory standards. The evidence presented in this study confirms the strong stockpiling effects on soil properties reported in other mine conditions; however, stockpiling effects were soil specific and interaction effects were much stronger than simple stockpiling effects in the current study. This suggests that inherent soil properties at donor site and post-handling must be considered to tease apart the effects of storage condition. In general, stockpiling FFMM should be avoided, if possible, to preserve the fertility and microbial benefits. Organic matter admixing in poor quality soils such as mineral sub-soil may improve fertility conditions and microbial functional diversity. Selectively salvaged FFMM has many advantages as reclamation topsoil over PMM soils (Mackenzie and Naeth, 2010; Quideau et al., 2013b). However, a better tree growth has also been observed on PMM soils (Pinno and Errington, 2015). Therefore, admixing these two could be a potential option to get some of the desired nutritional and microbial benefits of both reclamation soils. Overall, a 20 to 40% admixing of FFMM to SS and PMM soils showed the best results and this can be tested in further field trials.

The current study also demonstrated the use of simple microbial functional profiles (CLPP) in assessing the effects of operational management practices on soil quality and the findings are comparable to other studies that used more sophisticated methods (e.g., Béasse et al., 2015; Quideau et al., 2017). Similar approach can be adopted for routine soil measurements and monitoring changes in the reclaimed and other disturbed sites.

SUPPLEMENTAL MATERIAL

Supplemental material is available with the online version of this article. The supplemental document contains Table S1. Grouping of ecoplate substrates in different C-guilds; Fig. S1. Estimated flexible changes in the microbial functional diversity with incubation time; Fig. S2. Relative changes in mineralization rate and area under curve of the ecoplate carbon guilds; Fig. S3. Average well color development of ecoplate C guilds at 120 hours of incubation in different reclamation soils; Fig. S4. Most significant drivers of K and P availability in the fresh, stockpiled and benchmark soils as determined by the Random Forest models; and Fig. S5. Most significant drivers of K and P availability in admixed soils as determined by the Random Forest models.

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AUTHOR CONTRIBUTIONS

SDG and BP conceived the idea. SDG designed the experiment. SDG and WK conducted the data analysis. SDG wrote the manuscript. All the authors reviewed and approved the final version of the manuscript.

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