Laboratory-based nitrogen mineralization and biogeochemistry of two soils used in oil sands reclamation

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MacKenzie, M. D. and Quideau, S. A. 2012. **Laboratory-based nitrogen mineralization and biogeochemistry of two soils used in oil sands reclamation**. Can. J. Soil Sci. **92**: 131–142. In the Athabasca oil sands region of Alberta, Canada, peat mineral and upland forest floor mineral soils are salvaged and stockpiled for reclamation. Previous work showed that sites reclaimed with forest floor mineral soil had better understory regeneration and nitrogen dynamics more similar to naturally disturbed ecosystems. Both soils and a mixture of the two were compared in laboratory incubations by examining nitrogen mineralization (over 45 wk) and factorial fertility additions (4 wk trial with NPK) on microbial community structure and nutrient availability. Nitrogen mineralization indicated forest floor mineral soil had lower release rates and a higher estimated labile nitrogen pool than peat mineral soil. Nitrogen mineralization in mixed soil started like peat mineral soil and finished like forest floor mineral soil. Fertility additions influenced microbial community structure less than soil type. Multi-response permutation procedure indicated the forest floor mineral soil microbial community was significantly different from peat mineral and mixed soil communities. Control nutrient profiles differed from those with added NPK. Forest floor mineral soil retained nitrogen as ammonium, while peat mineral and mixed soils were nitrate dominated. Reclamation will require all soil types to be used and these data will help determine soil placement prescriptions.

Key words: Rate constant (k), first order equation, ionic resin analysis, phospholipid fatty acid, microlysimeter, metabolic quotient

MacKenzie, M. D. et Quideau, S. A. 2012. **Minéralisation de l'azote en laboratoire et biogéochimie de deux sols employés pour la restauration des sables bitumineux**. Can. J. Soil Sci. **92**: 131–142. Dans la région de l'Athabasca, en Alberta (Canada), où l'on exploite les sables bitumineux, on met de côté et empile les sols minéraux des tourbières et des forêts des hauts plateaux en vue de les réutiliser pour restaurer les sols. Des recherches antérieures ont montré que les sites restaurés avec le sol minéral forestier suscitent un meilleur rétablissement du sous-étage et que la dynamique de l'azote ressemble davantage à celle observée dans les écosystèmes perturbés par un phénomène naturel. Les auteurs ont comparé les deux types de sol et un mélange de ceux-ci lors d'une incubation en laboratoire, en examinant la minéralisation de l'azote (sur 45 semaines) et des hausses factorielles de la fertilité (essai de 4 semaines avec des engrais NPK) ainsi que leur incidence sur la structure de la microflore et les éléments nutritifs disponibles. La minéralisation de l'azote indique que le sol minéral forestier libère l'azote plus lentement que le sol minéral tourbeux et possède une plus grande réserve d'azote labile. Dans les mélanges, la minéralisation de l'azote débute comme dans le sol minéral des tourbières et se termine comme dans le sol minéral des forêts. L'addition d'engrais affecte moins la population de microorganismes que la nature du sol. La technique de permutation des réactions multiples révèle que la microflore du sol minéral forestier diffère sensiblement de celle du sol minéral tourbeux et du sol mixte. Le profil des éléments nutritifs dans les sols témoins diffère de celui des sols amendés avec de l'engrais NPK. Le sol minéral forestier conserve l'azote sous forme d'ammonium, alors que les nitrates dominent dans le sol minéral tourbeux et les sols mixtes. La restauration exigera qu'on recoure à tous ces sols et les données présentées ici aideront à déterminer où et comment les employer.

Mots clés: Constante de vitesse (k), équation du premier degré, analyse ionique sur résine, acides gras phospholipidiques, microlysimètre, quotient métabolique

Resource extraction in the Athabasca oil sands region of north eastern Alberta, Canada, involves removal of complete ecosystems by surface mining, including geological materials to 100 m depths (Johnson and Miyanishi 2008). Currently, 650 km^2 of the boreal plains ecozone have been disturbed by this industry and the disturbed area is projected to increase to over 1700 km^2 in the next 10–15 yr (Government of Alberta 2010). Large scale land reclamation projects have already begun, and at least one reclaimed site, Gateway Hills, has met the legal requirements for a reclamation certificate. However, questions remain concerning the biogeochemical knowledge base for reclaiming large areas of multiple interconnected ecosystems (Johnson and Miyanishi 2008).

Land reclamation is limited by the types of materials available within the geographic area of the industrial activity, and re-establishing healthy ecosystems on reclaimed land is largely dictated by the type of surface soil material available (Bradshaw 2000). In the Athabasca oil sands region there are two broad categories of

Abbreviations: **MRPP**, multiple response permutations procedure; **NMS**, non-metric multidimensional scaling

selectively salvaged materials, a peat mineral soil and an upland forest floor mineral soil (McMillan et al. 2007). The nitrogen mineralization rate from these soil types has never been tested with laboratory microlysimeter techniques and questions remain as to whether these soils can provide sufficient nitrogen for plant uptake. Simulations with FORECAST, a process-based model with empirical constraints (Seely et al. 1999, 2002), showed that modelled plant productivity on peat mineral soil would be different based on assumptions of slow versus fast decomposition rates (Welham 2005), among other factors. To date, the actual rates have not been measured, and we hypothesize that they are higher than originally speculated in these model runs based on other work (McMillan et al. 2007; Hemstock et al. 2009; MacKenzie and Quideau 2010).

To overcome perceived deficiencies, newly reclaimed sites are often fertilized with nitrogen (N), phosphorus (P), and potassium (K) at rates similar to agricultural settings (250–350 Kg ha^{-1}, 10-30-15 N-P-K), but no data are available on how this may affect microbial community structure and the profile of other macro- and micronutrients. Researchers have shown that soil microbial communities are affected by environmental factors, such as plant community assemblages (Bever 1994; Hannam et al. 2006), carbon substrate (Bardgett et al. 1999b; Marschner et al. 2003) and season (Bardgett et al. 1999a; MacKenzie and Quideau 2010). Some researchers also suggest that inorganic nutrient additions can change microbial community composition from that of undisturbed environments (Bardgett et al. 1999b; Marschner et al. 2003), including lowering total microbial biomass and shifting prevalence of bacteria and fungi (Strickland and Rousk 2010).

To better equip land reclamation practitioners a quantification of nitrogen release rates from peat mineral and forest floor mineral soils that can be used in simulation models is needed. An evaluation of the effect of agricultural levels of fertilizer application on microorganisms and other plant nutrients will help determine possible shifts in functional diversity. Therefore, the objectives of this study were to determine and compare nitrogen mineralization rate constants (k), examine the effect of NPK additions on microbial community structure, and examine the effect of these additions on nutrient profiles in these surface soil types.

MATERIALS AND METHODS

Reclamation Soil Types

In the Athabasca oil sands, land reclamation involves backfilling up to 40 m by 1–2 km wide surface mine excavations with overburden and waste materials from the upgrading process (Fung and Macyk 2000). Overburden geologic material includes Pleistocene clay deposits, with relatively high alkalinity and saline-sodic Cretaceous deposits. By-products include tailings sand and lean oil sands, which have a bitumen content too low for extraction to be economically feasible. Once landforms built from these materials have been engineered for subsurface hydrology, they are currently capped with 1–1.5 m of reconstructed soil, which usually includes a 20–50 cm horizon of surface soil high in organic matter. The main soil types applied as capping material in the study area are salvaged peat mixed with mineral soil and salvaged upland forest floor mixed with mineral soil. Organic material is over stripped at a ratio of 1:1 (vol/vol) with underlying mineral soil (Fung and Macyk 2000), although this ratio can shift by as much as 20% in either direction. Peat and forest cover types from which these materials are collected, vary considerably; however, soils used in this study are representative of the types of material used for reclamation.

Soils were collected from a sphagnum (*Sphagnum angustifolium* [Warnstorf] C.E.O. Jensen, Bih) dominated peatland and an aspen (*Populus tremuloides* Michx.) dominated upland forest (Wayne Tedder, Suncor Energy Inc., personal communication), and both had an organic matter to mineral soil ratio of 30:70 (vol/vol). Peat mineral and forest floor mineral soils were collected in late winter of 2007, shipped cold, and air dried before being used in experiments. A third soil type (mix) was created by mixing equal amounts (wt/wt) of peat mineral and forest floor mineral soils. These three soil types were mixed with quartz sand (Fisher Scientific product number S80156-1) at a ratio of 1:1 (wt/wt) to reduce compaction upon rewetting during incubation experiments.

Soil Properties

Soil pH was measured on air-dried samples in a 2:1 slurry of 0.01 M calcium chloride ($CaCl_2$) using a glass electrode (Kalra and Maynard 1991). Water-holding capacity was measured by placing three replicates of each soil type into rubber rings and saturating them on a 15 kPa pressure plate (Kalra and Maynard 1991). Subsamples of each soil type were ground to <150 μm and approximately 30 mg was encapsulated for determination of total carbon and total nitrogen by dry combustion analysis (Nelson and Sommers 1996) on an elemental analyzer (Costech Model 4010, Valencia, CA).

Nitrogen Mineralization

To measure nitrogen mineralization over time, 50 g of each soil type was placed in a microlysimeter (Nalgene, product number N3004050), replicated three times, for a total of nine samples. Microlysimeters were incubated in the dark at 60% water-holding capacity (vol/vol) and 25°C for 315 d. Samples were periodically extracted with 100 ml of 0.01 M $CaCl_2$ and 25 mL of a nitrogen minus nutrient solution (Campbell et al. 1993). The extracting solution was added to the microlysimeters through the top chamber and removed through the bottom chamber under pressure (80 kPa), thus returning samples to approximately 60% water-holding capacity. Ammonium

(NH_4^+) and nitrate (NO_3) nitrogen concentrations were analyzed colorimetrically by segmented flow (Smartchem 200 Discrete Analyzer, Westco Scientific Instruments, Inc., Brookfield, CT), using the nitroprusside/salicylate method and the cadmium reduction method, respectively (Bundy and Meisinger 1994).

Fertilizer Application

We performed an additional incubation to determine if fertilizer additions shift microbial community structure and the overall nutrient availability profile. Two hundred grams of each soil type were weighed in sealable plastic bags, at three replicates per treatment; factorial combinations of N and PK were prepared, for a total of 36 bags. Sealable plastic bags were used as they are impervious to water, but allow some gas exchange. The bags were opened once a week to maintain aerobic conditions. Treatments were created by adding N, as ammonium chloride, and PK, as potassium phosphate, at a rate of 250 kg ha^{-1} of 10-30-15 (N-P-K), as per industrial reclamation practices (Marty Yarmuch, Syncrude Canada Ltd., personal communication) and by assuming a bulk density of 0.8 g cm^{-3}. Nutrients were added in solution to bring samples up to 80% water-holding capacity. Ionic resin analysis (probes – see below) was used to determine nutrient availability over the incubation period of 30 d at 30°C. At the end of the incubation period the probes were removed and analyzed, and subsamples were taken for phospholipid fatty acid analysis.

Phospholipid Fatty Acid Analysis

Subsamples from the control, N, PK and NPK treatments were collected from each replicate under sterile conditions, frozen at −86°C, and freeze dried prior to phospholipid fatty acid analysis. Polar lipids were extracted from 5 g of mineral soil using a modified Bligh and Dyer extraction (Bligh and Dyer 1959; Frostegaård et al. 1991; White and Ringelberg 1998). Polar lipid extracts were purified on packed silicic acid columns (Agilent Technologies, Wilmington, DE) and subjected to mild alkaline methanolysis to form fatty acid methyl esters. Fatty acid methyl esters were separated and quantified using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column and hydrogen carrier gas. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI Inc., Newark, DE). Standardized nomenclature for fatty acids (Hannam et al. 2007) was used to identify peaks and only fatty acids with ≤20 carbons were used in statistical analyses.

Total phospholipid fatty acids were calculated as ng g^{-1} soil material. Phospholipid fatty acid biomarkers (Frostegard and Baath 1996; Hassett and Zak 2005; Hannam et al. 2007) were used to identify organisms in specific functional groups and were calculated as mole % of total microbial biomass basis to standardize for differences in total amount of phospholipid fatty acids produced as a result of different reclamation soil types and fertility treatments.

Ion Exchange Resin Analysis

Plant root simulator (PRS™) probes (Western Ag Innovations Inc., Saskatoon, SK) use an ion exchange resin membrane to capture nutrient anions and cations from soil solution, and integrate the effect of temperature and water content on nutrient fluxes when left to incubate in situ (Qian and Schoenau 2002). Prior to installation, cation and anion probes were saturated with sodium (Na^+) and bicarbonate (HCO_3^-) ions, respectively, to recharge the probes. Anion and cation probes were installed in the N and PK fertilizer experiment as described above. Following the incubation period, probes were removed, washed with deionized water, and returned to Western Ag Innovations Inc. for elution with 0.5 M hydrochloric acid (HCl) and nutrient analysis. Ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) were quantified colorimetrically on a segmented flow Autoanalyzer III (Bran and Lubbe, Inc., Buffalo, NY). Potassium (K^+), sulphate (SO_4^{2-}), calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}) and boron (B^+) were quantified by inductively coupled plasma spectroscopy (PerkinElmer Optima 3000-DV, PerkinElmer Inc., Shelton, CT).

Table 1. Soil properties of reclamation soils from the Athabasca oil sands region

Reclamation soil type	pH	Water-holding capacity (%)	Total carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Carbon to nitrogen ratio
Peat mineral	6.3	32.2[z] (3.7)*a*	30.6 (7.8)*a*	1.4 (0.3)*a*	21.9
Forest floor mineral	5.3	7.9 (0.3)*b*	11.4 (1.3)*b*	0.4 (0.1)*b*	28.5
Peat mineral and forest floor mineral mix	5.85	32.9 (5.2)*a*	24.1 (6.5)*a*	1.1 (0.3)*ab*	21.9
P value		<0.000	0.027	0.100	

[z]Mean (standard error), $n = 3$.
a, *b* Significant differences at $\alpha \leq 0.100$.

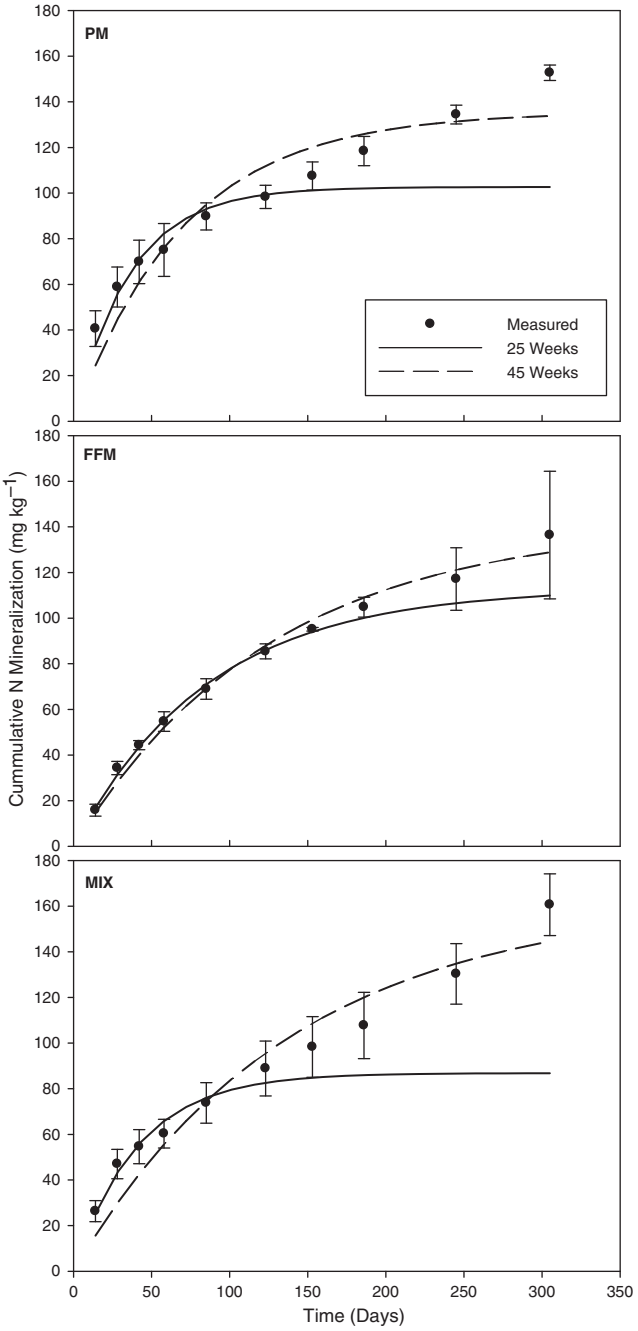

Fig. 1. Cumulative nitrogen mineralization (mg kg^{-1}) in (a) peat mineral, (b) forest floor mineral and (c) peat/forest floor mineral mix reclamation soils from the Athabasca oil sands region.

Microbial Respiration

Basal respiration after incubation was measured with the MicroResp™ system, which uses a 96 well detection microplate containing Cresol red indicator (12.5 μg mL^{-1}), potassium chloride (150 nM) and sodium bicarbonate (2.5 mM) suspended in a 1% agar solution (Campbell et al. 2003). Duplicate soil samples (~0.5 g each) and distilled water (0.3 mL) were placed in a 1.2 mL deep well plate and connected to the inverted reading plate by a MicroResp™ silicon gasket. Small clamps were used to prevent gas leakage between the deep well and detection plates. The assembled plates were incubated at 30°C over a period of 6 h to allow evolved carbon dioxide (CO_2) to equilibrate with sodium bicarbonate and indicator dye of the detection plate. Microbial respiration was determined colorimetrically on a microplate reader (Synergy HT, Multi-detection Microplate Reader, Bio-Tek Instruments, Inc., Winooski, VT) and calibrated with the standard curve provided by MicroResp™ (Macaulay Institute, Aberdeen, Scotland, UK). The metabolic quotient was calculated as basal respiration divided by total microbial biomass as derived from phospholipid fatty acid analysis (MacKenzie and Quideau 2010).

Statistical Analyses

One-way analysis of variance (ANOVA) was used to determine differences in baseline soil properties of the three soil types. Two-way ANOVA was used to examine the effect of soil type and fertility treatment (N and PK additions). In all cases, the data fit the assumptions of normality, independence and heteroscedasticity of residuals (Underwood 1997). We used an α of 0.10 to determine significant differences among ANOVA results, as the number of replicates in most cases was low ($n = 9$ or 36), resulting in low power to detect differences (Di Stefano 2001). The statistical package used for these analyses was Systat 11.0 (Wilkinson 2004).

Non-linear regression variables were fit to the following equation using the solver function in Excel 2003 (Microsoft Office Professional Edition 2003, Microsoft Corporation):

$$N_m = N_o(1 - e^{-kt}) \quad (1)$$

where N_m is mineralizable nitrogen at time t, N_o is the theoretical labile pool of nitrogen and k (wk^{-1}) is the nitrogen mineralization rate constant (Campbell et al. 1993). Variables N_o and k were calculated by the least squares error method (Wraith and Or 1998), and by trying to maximize goodness of fit (r^2).

Patterns in the phospholipid fatty acid and ion exchange resin data were examined with a non-metric multidimensional scaling (NMS) ordination technique (Kruskal 1964; Mather 1976) using PCORD software (Version 5, MjM Software Design, Gleneden Beach, OR). The main matrix contained either phospholipid fatty acid data or ionic exchange resin data, and was log square root transformed and relativized before analysis. For phospholipid fatty acid data, samples with less than 7 non-zero numbers were removed to reduce effect of rare species, which left 32 phospholipid fatty acid biomarkers for analysis. The secondary matrix contained categorical variables including soil type,

Table 2. Nitrogen mineralization at 25 and 45 wk in reclamation soils from the Athabasca oil sands region

Reclamation soil type	25 wk				45 wk			
	N^{z}_{min}	N_o	k	r^2	N_{min}	N_o	k	r^2
	(mg kg^{-1})		(mg kg^{-1} wk^{-1})		(mg kg^{-1})		(mg kg^{-1} wk^{-1})	
Peat mineral	107.49[y] (3.57)	102.92 (3.81)	0.203 (0.045)*a*	0.938 (0.009)*c*	152.73 (1.93)	137.40 (7.34)	0.102 (0.021)*a*	0.817 (0.073)
Forest floor mineral	95.14 (0.45)	114.47 (5.59)	0.081 (0.008)*b*	0.993 (0.003)*a*	136.40 (16.14)	151.00 (22.49)	0.057 (0.019)*ab*	0.974 (0.011)
Peat and forest floor mineral mix	98.29 (7.67)	105.13 (17.02)	0.144 (0.046)*ab*	0.961 (0.011)*b*	160.63 (7.80)	162.57 (12.79)	0.051 (0.003)*b*	0.866 (0.076)
P value	0.256	0.727	0.001	0.012	0.315	0.552	0.100	0.258

[z]Measured (N_{min}) and modeled (N_o) data, the rate constant (k) and goodness of fit (r^2).
[y]Mean (standard error), $n = 3$.
a, *b* Significant differences at $\alpha \leq 0.100$.

fertilizer treatment and all ionic exchange resin data for the phospholipid fatty acid ordination. The Sorensen (Bray-Curtis) distance measure was used in the analyses with the autopilot function set to slow and thorough, which included 250 runs of real data and 250 runs of random data. The multiple response permutations procedure (MRPP) was used to compare distances between points corresponding to different soil types and fertilizer treatments to determine whether these groups were statistically different from each other. Bonferroni corrections were used to determine a family error rate for the MRPP analyses (Legendre and Legendre 1998).

RESULTS

There were significant differences in all soil properties tested for the three soil types (Table 1). However, in most cases the mix soil type was not different from peat mineral soil and both had higher water-holding capacity and total carbon than forest floor mineral soil. If a bulk density of 0.8 g cm^{-3} (McMillan et al. 2007; Hemstock et al. 2009) and a sampling depth of 5 cm are assumed, then these soils contain 4000 to 14 000 kg carbon ha^{-1} and 160 to 600 kg nitrogen ha^{-1}. Although not tested statistically, pH of forest floor mineral soil was slightly higher (5.3) than the other soil types (4.8), but all three were in the acidic range of plant nutrient availability (4–6).

Nitrogen Mineralization

Nitrogen mineralization modeled by non-linear regression for two incubation periods, 25 wk and 45 wk, produced different results for the three soil types (Fig. 1, Table 2). For 25 wk, the k-value and r^2 were significantly different among soil types (Table 2), where peat mineral soil had the highest k-value and was different from forest floor mineral soil which had the highest r^2. However, 25 wk results indicated that N_o for the peat mineral soil was lower than measured N_{min}, implying a poor model fit (Table 2). At 45 wk, only the k-value was significantly different; however, peat mineral soil was significantly greater than the mix soil and forest floor mineral soil was in the middle (Table 2). The model for cumulative N_{min} after 25 wk did not fit the measured data well for either peat mineral or mix soil types (Fig. 1a and c), but model output for both 25 and 45 wk corresponded well to measured N_{min} data from forest floor mineral soil (Fig. 1b). A comparison of measured N_{min} by day showed peat mineral soil decreased exponentially for the first four measurements, then remained relatively constant and low (Fig. 2a). The measured N_{min} for forest floor mineral soil gradually increased for the first four measurements, up to a maximum of 2 mg kg^{-1} d^{-1} (Fig. 2b), and then decreased slowly for the rest of the experiment. The mix soil type had measured N_{min} by day trends similar to both peat mineral and forest floor mineral soils with exponential losses at first and then high mineralization rates later on (Fig. 2c).

Microbial Community Structure

Microbial community structure was significantly different among soil types (Fig. 3a), but was not related to fertility treatment (Fig. 3b). The NMS ordination of phospholipid fatty acid data resulted in a two-dimensional solution with a stress of 8.598 and an instability of <0.0000 after 69 iterations. The MRPP analysis indicated that soil types were significantly different ($T = -6.577$, $A = 0.144$, $P = 0.001$); however, pairwise comparisons revealed forest floor mineral soil was only significantly different from peat mineral soil ($T = -2.817$, $A = 0.076$, $P = 0.022$). Vector analysis ($r^2 \geq 0.500$) indicated differences in the microbial community fingerprint of the soil types (Fig. 3a). Forest floor mineral soil fell into quadrants 2 and 3, which were strongly correlated with actinomycetes, total microbial biomass, fungi and the fungi to bacteria ratio. Peat mineral soil, located in quadrant 1 and 4, was correlated with an increasing metabolic quotient, gram negative bacteria and general bacteria markers.

It appeared that fertilizer treatment had no consistent effect on microbial community structure in ordination space (Fig. 3b). MRPP analysis indicated no

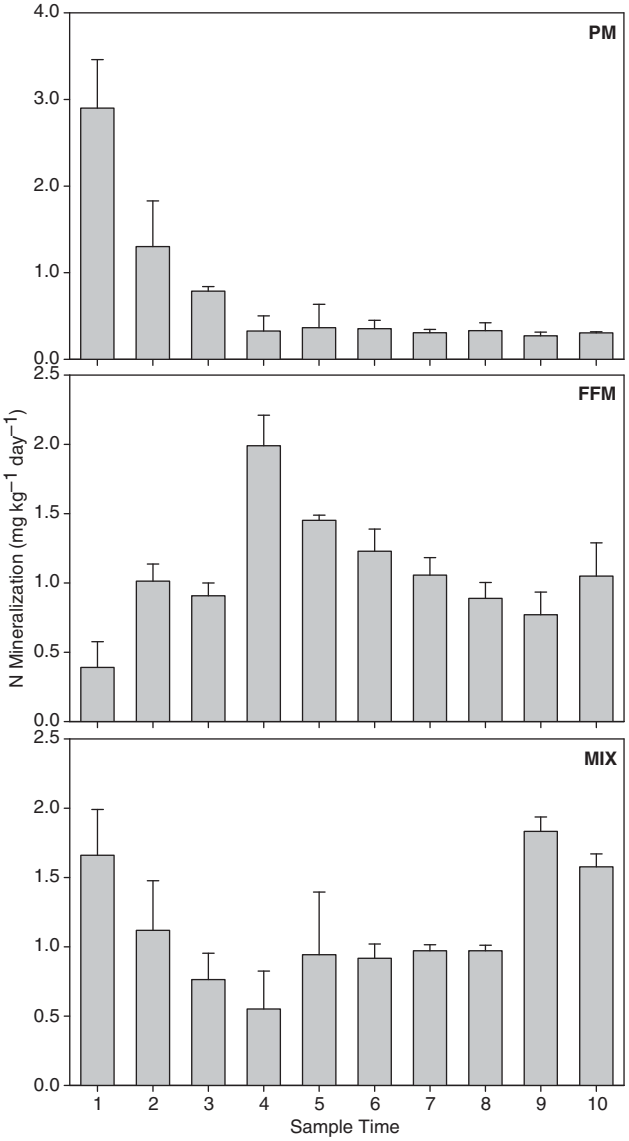

Fig. 2. Nitrogen mineralization (mg kg^{-1} d^{-1}) over a 45 wk incubation of (a) peat mineral, (b) forest floor mineral and (c) peat/forest floor mineral mix reclamation soils from the Athabasca oil sands region. Samples were collected at 2 wk intervals for the first four sample periods, at 4.5 wk intervals for the next four sample periods, and at 8 wk intervals for the last two sample periods.

significant patterns within the individual soil types or between them. There was some clustering of some treatments, but all nine replicates of one treatment never clustered together. No nutrient vectors were significantly correlated to either axis of the phospholipid fatty acid data beyond an $r^2 \geq 0.100$.

Soil type had an effect on total microbial biomass and the proportion of some of the biomarkers (Table 3), including fungi, bacteria, the ratio of fungi to bacteria and actinomycetes. However, fertility treatment and the interaction between soil and fertility were not significant by ANOVA, similar to ordination results.

Plant Nutrient Availability

There were significant differences among soil types and fertility treatments for all plant nutrients tested (Table 4 and Fig. 4). Resin analysis revealed higher availability of nitrate (NO_3^-) in peat mineral and mix controls, and additions of ammonium (NH_4^+) nitrogen appeared to be converted to nitrate in these soils. There was higher ammonium availability in the forest floor mineral soil control, in which added ammonium nitrogen was adsorbed as ammonium (Table 3). Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) were higher in peat mineral and mix soils, but addition of ions in fertility treatments reduced adsorption of both cations. Potassium and phosphorus were higher in forest floor mineral soil, and additions of these ions in fertility treatments were better retained by mix than peat mineral soils. Mix soil had highest boron in the control, but lowest in the PK treatment. Sulphur was highest in peat mineral soil, which was reduced by fertilizer additions; it was intermediate in forest floor mineral soil, where it increased with fertilizer additions, and was lowest in mix fertility treatments.

Ordination analysis showed significant clustering of plant available nutrient profiles both by soil type and fertility treatment (Fig. 4). NMS ordination of ionic exchange resin data resulted in a three-dimensional solution with a stress of 7.696 and an instability of <0.000 after 113 iterations. Axes one and three explained the most variation in ordination space (28.7 and 45.6%, respectively), and showed significant clustering by soil type (Fig. 3a). MRPP analysis confirmed that these groups were statistically different ($T = -8.738$, $A = 0.188$, $P < 0.000$) and pairwise comparisons showed forest floor mineral soil was statistically separate from peat mineral and mix soils ($T = -9.410$, $A = 0.214$, $P = < 0.000$), but these were not significantly different from each other ($T = -0.560$, $A = 0.017$, $P = 0.207$). Vector analysis indicated that ammonium was strongly correlated with axis 1 ($r^2 = 0.890$), while calcium and magnesium were negatively correlated with axis 3 ($r^2 = -0.692$ and -0.780, respectively), and respiration was negatively correlated with axes 1 and 3 ($r^2 = -0.291$ and -0.274). Soil type influenced distribution of fertility treatments in ordination space (Fig. 3b), and MRPP analysis indicated fertility treatments were significantly different from each other ($T = -10.898$, $A = 0.292$, $P < 0.000$). Pairwise comparisons demonstrated control and PK treatments were significantly different from the N and NPK treatments (Fig. 3c), which showed distribution of nutrient profiles for axes 1 and 2, and vector analysis revealed that axis 2 was strongly correlated to nitrate ($r^2 = 0.410$).

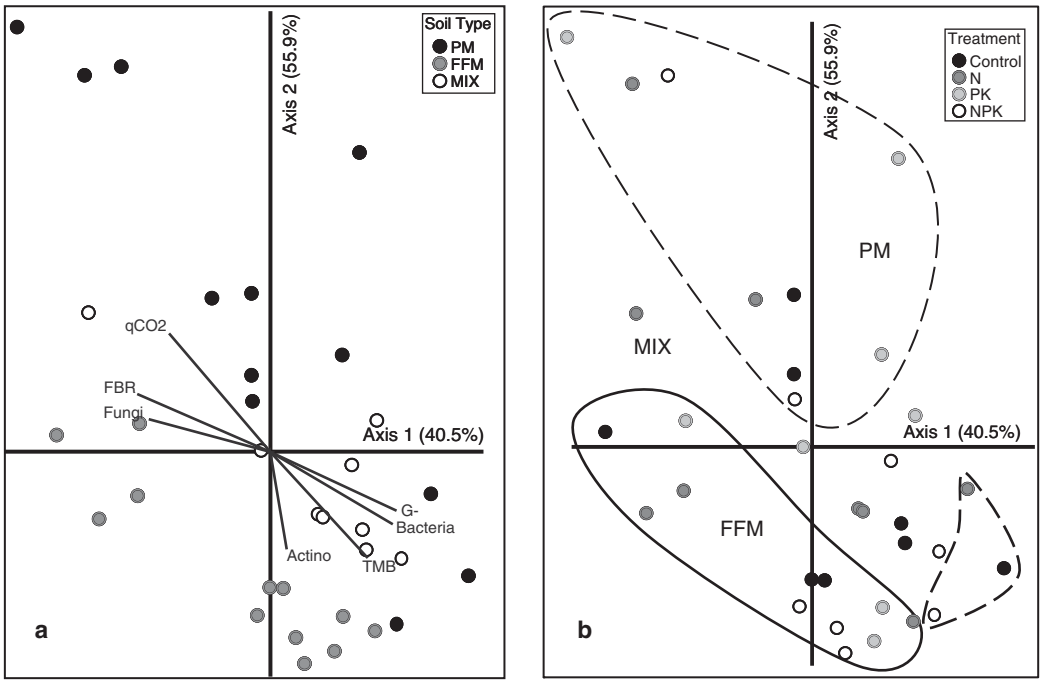

Fig. 3. Non-metric multi-dimensional scaling of microbial community structure by phospholipid fatty acid analysis after a 4 wk incubation of reclamation soils from the Athabasca oil sands region. Graphs are differentiated by (a) soil type (b) and fertility treatment.

DISCUSSION

Natural vs. Reclaimed Soil Nitrogen Mineralization

Microlysimeter results for common reclamation soil types were different from what has been reported in the literature for natural forest stands (Fyles and McGill 1987). Peat mineral soil *k*-values were an order of magnitude higher than those in jack pine forest soils from the boreal plains (Fyles and McGill 1987), while forest floor mineral soil had the same magnitude as LFH incubations, but was 1–2 times higher than A and B horizon incubations. This is not surprising given that forest soils release nitrogen following disturbance (Likens and Bormann 1970; Vitousek and Melillo 1979; MacKenzie et al. 2006). However, the fact that the nitrogen mineralization rate (*k*) in peat mineral soil is similar to that in agricultural systems (Stanford and Smith 1972) may be cause for re-evaluation of its exclusive use in land reclamation. The goal of land reclamation in the oil sands is to re-create forest ecosystems with similar land capability (Oil Sands Vegetation and Reclamation Committee 1998) and in the boreal plains of northern Alberta this means ecosystems with tight nitrogen cycling in the absence of fire (Fyles and McGill 1987; McMillan et al. 2007).

It is clear that the first order equation used to model nitrogen mineralization over time represents a poor model fit for peat mineral soil depending on incubation period and a good fit for forest floor mineral soil regardless of time (Table 2). The 25 wk incubation period represents the average of what is commonly reported in the literature (Stanford and Smith 1972; Smith et al. 1980; Fyles and McGill 1987; Campbell et al. 1993) and a different story would have emerged if analysis had stopped there. Instead we ran the experiment an additional 20 wk to measure long-term nutrient release, after which time it appeared that mix soil had nitrogen mineralization patterns more similar to forest floor mineral soil (Table 2, 45 wk; Figs. 1 and 2). This is significant because it provides evidence that mixing these reclamation substrates may be a viable option for future land reclamation projects.

FORECAST model simulations applied to land reclamation prescriptions in the Athabasca oil sands region show deficiencies in plant productivity may result from using <50 cm of peat mineral soil, partially based on slow and fast decomposition rates of 8 and 84 mg N kg^{-1} yr^{-1}, respectively (Welham 2005). This study found that the actual mineralization rates of all soils tested were approximately double the fast decomposition rate estimate (Table 2, Model 2). Laboratory incubations do not include all parameters from which a process based simulation model is made, including empirical constraints due to planting density, understory composition and plant nutrient uptake (Seely et al. 1999, 2002). However, these results do show the need to run further simulations. Field incubation results from the literature show that nitrogen mineralization rates in the boreal are highly variable, ranging from approximately 150 to 1300 mg N kg^{-1} yr^{-1} (Hemstock et al. 2009).

Table 3. Biomarkers from phospholipid fatty acid analysis after factorial additions of nitrogen and phosphorus in reclamation soils from the Athabasca oil sands region

Reclamation soil type	Treatment	Total microbial biomass (ng g^{-1})	Fungi	Bacteria	Gram −	Actinomycetes	Fungi/bacteria	qCO_2
				(% total microbial biomass)				
Peat mineral	Control	19.15[z] (3.06)	0.017 (0.001)	0.354 (0.021)	0.268 (0.022)	0.022 (0.017)	0.051 (0.007)	0.014 (0.004)
	N[y]	15.13 (4.26)	0.026 (0.006)	0.322 (0.043)	0.255 (0.029)	0.017 (0.010)	0.096 (0.034)	0.015 (0.004)
	PK	14.62 (3.84)	0.022 (0.007)	0.312 (0.048)	0.257 (0.034)	0.005 (0.010)	0.089 (0.046)	0.018 (0.006)
	NPK	17.90 (6.60)	0.020 (0.005)	0.313 (0.044)	0.242 (0.028)	0.033 (0.017)	0.071 (0.033)	0.015 (0.007)
Forest floor mineral	Control	21.89 (6.40)	0.019 (0.004)	0.316 (0.043)	0.259 (0.027)	0.043 (0.010)	0.066 (0.027)	0.012 (0.005)
	N	18.90 (6.31)	0.019 (0.003)	0.318 (0.033)	0.256 (0.017)	0.047 (0.004)	0.064 (0.015)	0.012 (0.003)
	PK	23.87 (5.94)	0.018 (0.003)	0.324 (0.029)	0.265 (0.014)	0.044 (0.003)	0.058 (0.015)	0.009 (0.003)
	NPK	30.76 (0.72)	0.016 (0.001)	0.406 (0.007)	0.264 (0.005)	0.066 (0.001)	0.040 (0.003)	0.005 (0.001)
Peat mineral and forest floor mineral mix	Control	23.97 (0.03)	0.016 (0.001)	0.365 (0.003)	0.298 (0.007)	0.047 (0.003)	0.045 (0.001)	0.008 (0.001)
	N	19.21 (4.81)	0.021 (0.006)	0.339 (0.034)	0.256 (0.018)	0.031 (0.014)	0.067 (0.027)	0.012 (0.004)
	PK	18.51 (5.23)	0.019 (0.003)	0.351 (0.036)	0.256 (0.024)	0.046 (0.006)	0.054 (0.014)	0.011 (0.003)
	NPK	26.51 (2.97)	0.012 (0.002)	0.398 (0.001)	0.298 (0.013)	0.042 (0.002)	0.029 (0.004)	0.007 (0.001)
P values								
Soil type		0.114	0.091	0.016	0.443	0.001	0.845	0.037
Treatment		0.318	0.818	0.294	0.745	0.262	0.764	0.580
Soil type × Treatment		0.938	0.947	0.998	0.851	0.765	0.905	0.988

[z]Mean (standard error), $n = 3$.
[y]Fertilizer treatments including nitrogen (N), phosphorus/potassium (PK), and a combination (NPK).

Hemstock et al. (2009) also reported field nitrogen mineralization rates for peat mineral soil, averaging 18 mg N kg^{-1} yr^{-1}, well below the 45 wk incubation data reported here, so clearly more work is needed in this area.

Fertilizer and Soil Microorganisms

After 4 wk of incubation in aerobic conditions there were still distinct differences in microbial community structure among soils. Rowland et al. (2009) suggested it takes 15–20 yr for the plant community and associated biogeochemical processes in peat mineral soil to converge towards those of natural stands. A similar time frame is required for microbial communities to change under influence of natural forest floor accumulation on reclaimed sites (Sorenson 2010). In this study, the metabolic quotient was highest in peat mineral soil indicating that aerobic microorganisms must work harder to derive energy from this substrate (Wardle 1993) and that differences in microbial composition (decreased fungi and fungi/bacteria) are perhaps some of the reasons why it takes longer for peat mineral soils to resemble natural stands. Microbial community structure of forest floor mineral soil is assumed to be more similar to natural stands, but evidence to confirm this is necessary. Changes in fungi/bacteria are related to site recovery from disturbance (Mummey et al. 2002a, b), but here it was already higher on forest floor mineral soil, which may be some preliminary evidence for more rapid development of similarity to natural sites. Ordination revealed that most forest floor mineral and mix soils, along with a few peat mineral soil laboratory replicates, were dominated by high total microbial biomass, which was not related to fertility treatments (Fig. 3a and b). In the absence of plant growth, applications of NPK are not the main drivers of microbial structure in ordination space, but rather soil is, as seen before (Dimitriu et al. 2010; MacKenzie and Quideau 2010). However, given the very short incubation time of the second experiment and the fact that it eliminates mycorrhizal associations from contributing to fungi/bacteria (Strickland and Rousk 2010), further work is needed to reach a conclusion.

Ionic exchange resin analysis showed peat mineral and mix soils were loaded with nitrogen (Table 4); three times more than forest floor mineral soils. These results differ from those of the microlysimeters in that pools of nitrogen that accumulated were trapped on resin membranes allowing examination of differences in speciation. Large amounts of nitrate in peat mineral and mix soils indicates higher nitrifier activity, as reflected by higher gram negative occurrence on these soils (Fig. 3a, Paul and Clark 1996). Ordination of nutrient profiles showed forest floor mineral soil retained N additions as ammonium, while added N was converted to nitrate on peat mineral and mix soils (Fig. 4c). High nitrate indicates severe disturbance (Likens et al. 1970) and represents a risk for nitrogen

Fig. 4. Non-metric multi-dimensional scaling of plant nutrient availability by ion exchange resin analysis after a 4 wk incubation of reclamation soils from the Athabasca oil sands region. The three-dimensional solution was differentiated by soil type (a), and fertility treatment, axes 1 and 3 (b) and axes 1 and 2 (c).

loss from the site, as nitrate is more mobile than ammonium (Stevenson and Cole 1999). Previous findings showed peat mineral and forest floor mineral reclaimed sites had higher nitrification rates than that of a natural site (McMillan et al. 2007), which we believed was possibly related to higher pH on the reclaimed sites. In this study, peat mineral soil had slightly higher pH than forest floor mineral soil and higher nitrification, but work suggests that certain strains of nitrifying bacteria do very well in acidic soils (Kowalchuk and Stephen 2001).

There was strong association between respiration and nutrient availability in peat mineral and mix soils as seen with the microbial data. Although fertility additions did not affect microbial growth in this study, there may have been a slight reduction in respiration (Fig. 4a). Higher potassium and phosphorus in forest floor mineral soils might make it more suitable to upland plants (Ballard and Carter 1986) and we are currently conducting greenhouse research with aspen to examine this (Pinno et al. 2012). Peat mineral and mix soils had higher boron, an important nutrient for plant growth, but often found deficient (Ballard and Carter 1986; White and Krause 2001), which might also be good evidence for some mixing of these substrates in future land reclamation prescriptions.

Table 4. Ionic resin adsorption of plant nutrients after factorial additions of nitrogen and phosphorus in reclamation soils from the Athabasca oil sands region

Reclamation soil type	Treatment	Nitrate	Ammonium	Calcium	Magnesium	Potassium	Phosphorus	Boron	Sulphur
					(μg 10 cm^{-1} month^{-1})				
Peat mineral	Control	405.00[a] (27.94)	0.53 (0.26)	2825.33 (109.91)	285.67 (4.81)	15.07 (1.87)	0.47 (0.07)	4.60 (0.53)	562.27 (63.11)
	N[b]	890.47 (11.69)	26.47 (8.03)	2452.01 (101.53)	224.40 (7.67)	12.00 (1.14)	0.20 (0.01)	2.67 (1.07)	351.87 (0.96)
	PK	315.07 (22.42)	4.07 (0.26)	2190.67 (25.09)	248.73 (4.45)	507.07 (12.33)	148.47 (8.80)	3.20 (1.10)	210.93 (35.33)
	NPK	794.80 (49.78)	55.00 (8.97)	2374.00 (121.33)	244.93 (15.88)	312.53 (9.06)	110.07 (3.89)	2.60 (0.81)	51.33 (3.88)
Forest floor mineral	Control	121.00 (20.69)	11.27 (2.79)	855.40 (46.31)	120.27 (7.91)	291.33 (18.46)	3.73 (0.41)	2.33 (0.93)	148.47 (2.39)
	N	52.67 (9.08)	277.27 (13.82)	1174.53 (157.58)	183.00 (19.54)	261.80 (17.08)	3.07 (0.41)	2.53 (0.98)	282.93 (2.79)
	PK	87.40 (10.02)	26.80 (5.30)	710.80 (47.92)	91.87 (5.61)	826.40 (93.50)	158.67 (34.24)	2.20 (0.92)	415.13 (3.07)
	NPK	6.20 (1.62)	261.33 (4.85)	785.40 (30.05)	118.67 (4.64)	1239.60 (73.58)	236.40 (10.23)	1.47 (0.56)	317.73 (1.77)
Peat and forest floor mineral	Control	422.70 (9.10)	0.00 (0.00)	2805.00 (141.00)	295.40 (3.21)	54.30 (1.32)	0.90 (0.01)	7.00 (0.61)	181.27 (49.20)
Mix	N	1057.53 (41.13)	38.27 (5.03)	2522.00 (66.52)	214.53 (4.93)	32.87 (0.84)	1.67 (0.35)	2.73 (1.63)	51.80 (4.00)
	PK	299.80 (47.80)	0.00 (0.00)	2377.00 (97.00)	260.80 (1.65)	589.60 (78.21)	501.40 (90.10)	0.70 (0.10)	53.00 (48.90)
	NPK	786.60 (24.80)	55.80 (17.40)	2492.00 (4.00)	230.00 (7.60)	350.40 (27.63)	201.10 (25.30)	1.90 (1.71)	55.07 (4.40)
P values									
Soil type		<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	0.139	<0.000
Treatment		<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	0.008	<0.000
Soil type × treatment		<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	0.084	<0.000

[a]Mean (standard error), $n = 3$.
[b]Fertilizer treatments including nitrogen (N), phosphorus/potassium (PK), and a combination (NPK).

Management Applications

In this laboratory incubation study, it initially appeared as though peat mineral soil was a better choice for capping material as it had higher water-holding capacity and lower carbon:nitrogen ratios than forest floor mineral soil. However, MacKenzie and Naeth (2009) found that 10 to 20 cm of forest floor mineral soil was an excellent source of native seeds and propagules. They observed a reduction in upland species germination on peat mineral soil and suggested that either depth of forest floor mineral soil may work if mixing can be avoided. Given that mix soil was more similar to peat mineral soil in initial nitrogen mineralization rate, nitrogen forms and microbial community structure, layering the two soils might be a better strategy than mixing them. McMillan et al. (2007) reported forest floor mineral soil layered on top of peat mineral did not differ significantly from peat mineral alone, except when water was added, in which case forest floor mineral soil and the layered soil had properties more consistent with natural soil. The two soil types examined here differ in physical and chemical properties, nutrient dynamics and microbial community structure. Not all of these properties are projected to have a positive effect on ecosystem health; however, given the scale of industrial disturbance and the necessity of using local reclamation materials, both soils and a mixture of the two will most likely be used for future land reclamation. As such, more in-depth analysis of the in situ behaviour of these soils with different layering strategies is necessary.

CONCLUSION

The evidence presented here suggests that nitrogen mineralization in common land reclamation soil substrates of the Athabasca oil sands region is much higher than previously believed, and that the peat mineral soil does not follow first order decomposition kinetics under aerobic conditions. The 1 yr incubation also showed that a mixture of peat mineral and forest floor mineral soils may begin to resemble the forest floor mineral soil, which is already conditioned for aerobic decomposition. A short-term incubation revealed that the microbial community structure was different between soil types, but the mix soil was not different from either peat mineral or forest floor mineral soil, and no community was affected by fertility treatment other than to stimulate nitrate production, which may lead to nitrogen losses. Given the high nitrogen availability in these soils after disturbance, further work needs to be conducted in order to establish best management practices for the use of fertilizer when recreating ecosystems dominated by low nutrient availability.

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