Carbon and Nitrogen Mineralization and Microbial Succession in Oil Sands Reclamation Soils Amended with Pyrogenic Carbon

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REPORT SUMMARY

Land reclamation of oil sands disturbed boreal forests in Alberta is a challenging task facing companies with surface mine leases. The government requires reclamation to equivalent land capability, which is a vague statement at best. Agronomic theories and methodologies have been applied in the past with mixed success. We believe that ecological theory and new methods, designed to tease apart ecosystem function, should be applied to reclaiming ecosystems similar to native forests. This new reclamation ecology should start with disturbance theory and in boreal Alberta that means recovery from wildfire. All organisms in boreal forests, from the biggest trees to the smallest bacterium, are adapted to regular pulses of fire. Fire causes plant mortality and therefore changes in competition and resource availability. It also results in the partial combustion of organic matter, which creates pyrogenic carbon (PyC) and changes the soil chemical environment.

Pyrogenic C is the substrate legacy of fire. It is resistant to decomposition and remains in the soil for hundreds of years. It has high surface area and adsorbs organic and inorganic compounds readily, which affects the availability of nutrients. It can be manufactured by an industrial process called pyrolysis, where is referred to as biochar. We believe that rebuilding native forest soils in the reclamation environment will require the use of biochar to stimulate functional similarity to native ecosystems, in terms of nutrient availability and microbial community succession. This is because peat is being used as a reclamation surface soil, but does not follow the typical first-order decomposition kinetics of native forest soils, due to distinct differences in organic matter quality. We believe that PyC will help to align peat decomposition kinetics and retain nutrients in surface soils. We also believe that it will create microbial community diversity and structure to be similar to the NFS.

A 90 day laboratory incubation was conducted to examine the effect of PyC additions on carbon (C) and nitrogen (N) mineralization in two common oil sands reclamation surface soils, peat mineral mix (PMM) and forest floor mineral mix (FFM), and one native forest soil (NFS) recovering from wildfire. Three different kinds of PyC were used in the incubation, including charcoal collected from a local wildfire event, biochar pyrolyzed from willow chips, and petroleum coke, a by-product of oil sands upgrading. Micro-lysimeter chambers were used to build small soil columns of each soil type, to which PyC was added in replicate. These micro-lysimeters allowed for gas sampling from a soil head space for analysis of microbial respiration and therefore activity, and soil solution sampling for analysis of inorganic N. Samples were collected and analyzed on days 0, 1, 3, 7, 10, 14 and then every week after that for the duration of the incubation. After incubation, soil samples were extracted for microbial sequencing by paired end Illumina sequencing of the 16S rRNA gene for bacteria and ITS 1-2 gene for fungi to examine microbial community diversity and structure.

Results indicated that the different PyC types increased C mineralization compared to the control, which suggests that it stimulates microbial activity and therefore respiration similar to the NFS. Literature also suggests that it undergoes some surface modifications at the molecular level upon addition to soils, which we also feel is reflected in the increased respiration. In contrast, PyC caused a decrease in N mineralization, which we believe is the result of N retention

on PyC. This stems mostly from the fact that it is counter-intuitive that microbial activity would be increased (respiration), but the product of that activity (inorganic N) decreased. In reality, it is incredibly difficult to measure nutrient retention on PyC, but some literature provides evidence for this theory. PyC did not align decomposition kinetics for PMM as we believed it would, except in the case of N mineralization with biochar perhaps making it a soil amendment worth more attention. Pet-coke consistently performed the same or worse than the control in terms of C and N mineralization.

Molecular sequencing of bacterial DNA showed that PyC, except for pet-coke, increased diversity in both the FFM and NFS, but not PMM which was higher to begin with. In ordination space, there is clear microbial succession from the control to the biochar, which indicates that biochar has a strong effect on the community structure. Fungal sequencing indicated that FFM had the highest diversity which was lowered by biochar to the level of NFS. However, no clear effect of PyC could be established for fungal community structure.

These results clearly indicate that the addition of PyC has an effect on C and N mineralization, and microbial community diversity and structure in oil sands reclamation surface soils. The direction and magnitude of the effects has some similarity to the effect of PyC in NFS, and therefore should be considered as a soil amendment. However, the full interpretation of these results requires more work in terms of prescribing surface soil mixtures that will lead to a high degree of similarity between reclaimed ecosystems and native ecosystems. This work provides some preliminary evidence to support a paradigm shift towards reclamation ecology.

ACKNOWLEDGEMENTS

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1 INTRODUCTION

Surface mining in the Athabasca oil sands region (AOSR) is the second largest disturbance in the boreal region of Alberta, Canada, after naturally occurring fires. Northern Alberta is home to one of the world's largest deposits of petroleum in the form of bitumen (Allen 2008). To date, about 750 km^2 of land have been disturbed by oil sands extraction (Government of Alberta 2014). Land reclamation after surface mining necessitates the reconstruction of soil-like profiles using salvaged surface mineral materials, mining by-products, and peat amendments. Peat is used as the primary organic amendment in the reclaimed soils of the AOSR, and influences resident microflora and nutrient cycling. When possible, salvage operations strip the forest floor layer from pre-mined upland areas, as it is a rich source of native plant propagules (Mackenzie and Naeth 2009). In turn, this forest floor mixture (FFM), whether used alone or in addition to the peat material, stimulates microbial activity as indicated by increased microbial biomass and nitrification rates when compared to the use of peat alone (McMillan et al. 2007). However, FFM is less abundant than peat and current reclamation practices using peat are giving rise to novel ecosystems (Rowland et al. 2009). Bacterial communities at reclaimed sites using peat differ markedly from those of natural boreal settings, in both composition and activity (Dimitriu and Grayston 2010). Typically, lower microbial diversity, quantity, and overall activity is observed at reclaimed sites containing peat (Dimitriu and Grayston 2010, McMillan et al. 2007), likely due to altered soil abiotic properties (Dimitriu et al. 2010).

Fire is the primary natural disturbance regime of boreal forest and peatland ecosystems in western North America, and is a major driver of ecosystem processes and carbon (C) cycling (Hicke et al. 2003, Larsen and MacDonald 1998a,b). Fire affects biotic ecosystem processes through increased plant mortality, volatilization of organic matter, and the production of pyrogenic carbon (PyC) (see Preston and Schmidt (2006) for a review). The range of charred plant biomass that constitutes PyC extends from chars that retain physical structures of the original plant material, to recalcitrant graphite (Seiler and Crutzen 1980). The environmental persistence and ubiquity of PyC, coupled to regular fire disturbance, has made PyC an integral part of natural boreal soils. Evidence of PyC in this regard extends as far back as 350 My to the late Devonian (Schmidt and Noack 2000). PyC has been shown to influence soil biogeochemistry by modifying microbial community structure and function (Ball et al. 2010), and can significantly influence specific groups of microorganisms (Pietikainen et al. 2000, Wardle et al. 1998, Zackrisson et al. 1996). For example, PyC can stimulate the establishment of ectomycorrhizae (Harvey et al. 1976), and can increase microbial biomass when present in humus (Zackrisson et al. 1996). PyC also enhances nutrient retention in soils (Lehmann et al. 2003), and mitigates N-limiting conditions (MacKenzie and DeLuca 2006).

Current reclamation practices do not actively incorporate PyC as a soil amendment, and little knowledge exists with respect to PyC and its influence in a reclamation setting. The propensity of PyC to release adsorbed nutrients slowly over time may be of particular interest to reclamation efforts. In a recent study in the Alberta oil sands region, a controlled release fertilizer elicited greater tree growth performance (based on diameter) than immediate release fertilizers (Sloan and Jacobs 2013). Resonating with this observation is a greenhouse study in which *Populus* *tremuloides* (trembling aspen) performed better in reclamation soils amended with organic-mineral materials (slow release of nutrients) versus direct fertilizer amendments (Pinno et al. 2012). A slow nutrient release scheme may be more favorable to boreal reclamation – a process that PyC could facilitate – and may be a natural condition of healthy boreal soils. Using a parent material closer to that found in the boreal setting, such as forest floor amendments, can place the microbial community on a faster trajectory to ecosystem recovery (Hahn and Quideau 2013), with the added benefit of indigenous plant propagules (Brown and Naeth 2014).

In this study we examine the effects of three types of PyC on microbial activity and diversity in three different soil types found in the AOSR. Carbon (C) and nitrogen (N) mineralization were monitored over 90 days in a lab to assess microbial activity. Genetic analyses were done at the end of incubation to compare bacterial and fungal diversity. We hypothesized that:

1. PyC amendments would increase C mineralization and decrease N mineralization in all soils
2. Both C and N mineralization in the peat-amended soils will not follow first-order kinetics, and that PyC will align it to this model
3. Both bacterial and fungal diversity would be highest in the NFS soils, with PyC increasing diversity across all soil types.

2 MATERIALS AND METHODS

2.1 Soil Sample Collection and PyC Amendment

Bulk soil samples were collected from the Athabasca Oil Sands Region (AOSR) and mixed with PyC for use in microlysimeter trials. Reclamation soils were collected from Syncrude's Aurora Capping Study and included a peat-mineral mix (PMM) and a forest floor mineral mix (FFM). A third soil type was collected from a native forest (post-fire b Ecosite – NFS) on the Canadian Natural Resources Limited lease. All three soils were individually mixed with quartz sand (Fisher Scientific, product number S80156-1) at a ratio of 1:1 (w/w) to reduce compaction upon rewetting during incubation experiments.

Three sources of PyC were mixed at 5% (w/w) in triplicate with each soil mixture; a negative control (no PyC) for each soil type was also maintained. The PyC sources included: charcoal collected from the Richardson fire of 2011, petroleum coke produced as a by-product of oil sands upgrading and collected from Syncrude Canada Ltd., and biochar made by pyrolysis of willow feedstock at 500°C with a 15 minute retention time (see Alessi et al. 2014 for more details). The mixing process was replicated in triplicate for a total of 36 samples. Samples were stored at 4°C until needed.

2.2 Microlysimeter Setup

Soil mixtures were placed in microlysimeter chambers to assess C and N mineralization over time. Each soil sample (150 to 200 g) was placed in a microlysimeter (Nalgene, product number N3004050), and incubated in the dark at approximately 60% water-holding capacity (v/v; WHC) and constant temperature (25°C) for the duration of the experiment. Sampling was conducted on days 1, 3, 7, 10, 14, and then weekly over the course of 90 days.

For sampling of C, each chamber was initially closed with a rubber septum for one hour then, using a Gastight™ syringe, 1 mL of the headspace was removed and injected into a Hewlett Packard 5890 Series II thermal conductivity detector (TCD) gas chromatograph (GC) to determine the concentration of CO_2, which was then used to calculate the C respired in mg g^{-1} of soil.

Mineralization of N was measured by mixing each soil sample with 40 mL 0.01 M $CaCl_2$ solution and 10 mL of non-nitrogen-containing nutrient solution (0.002 M $CaSO_4$, 0.005 M $CaHPO_4$, 0.002 M $MgSO_4$, and 0.0025 M K_2SO_4) (Campbell et al. 1993), followed by immediate extraction at -80 kPa. 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% WHC. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were analyzed colorimetrically by segmented flow (Smartchem 200 Discrete Analyzer, Westco Scientific Instruments, Inc., Brookfield, Connecticut), using the nitro-prusside / salicylate method and the cadmium reduction method, respectively (Bundy and Meisinger 1994). Total mineralized N was reported as μg g^{-1} of soil (Smith et al. 1980).

Non-linear curve fitting for mineralized C and N was done in Microsoft Excel using a first-order kinetics equation shown to work well for the decomposition of organic compounds (C and N mineralization) from natural ecosystems (Stanford and Smith 1972):

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

Where

N_m = nitrogen mineralized (μg g^{-1});

N_o = theoretical pool of labile nitrogen (μg g^{-1});

k = rate of mineralization (μg g^{-1} d^{-1});

t = time (d).

2.3 DNA Isolation, Amplification, and Sequencing

For each of the samples, DNA was extracted in duplicates from 0.5 g of homogenized soil by bead beating using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, Ohio, USA) as instructed by the manufacturer. DNA was quantified fluorometrically (NanoDrop 3300 fluorometer, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and duplicate extractions were pulled in equal concentrations prior to amplification and sequencing.

Molecular Research LP (Shallowater, Texas, USA) performed paired end Illumina sequencing (2 X 300) of the V1 to V3 regions of the bacterial 16S rRNA gene for bacteria and ITS 1-2 for fungi, as described by Dowd et al. (2008). Amplification was performed using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, California) under the following conditions: 94ºC for 3 minutes, followed by 28 cycles of 94ºC for 30 seconds; 53ºC for 40 seconds and 72ºC for

1 minute with a final elongation step at 72ºC for 5 minutes. Gene-specific forward PCR primer sequences were tagged with the sequencing adapters for paired end Illumina MiSeq chemistry, an 8 base barcode, and a linker sequence. All PCR reactions were performed in triplicate, mixed in equal concentrations, and purified using Agencourt AMPure beads (Agencourt Bioscience Corporation, Massachusetts, USA). Paired end amplicon sequencing was performed using the Illumina MiSeq platform (see Appendix 1 for more details).

2.4 DNA Assemblage Analysis

Mothur v. 1.33.3 was used for all assemblage analysis and statistics (Schloss et al. 2009). Alpha diversity was estimated using the Chao1 and ACE nonparametric richness estimators, reciprocal Simpson's diversity index (invsimpson), and Shannon diversity index (Chao 1984, Chao and Shen 2003, Shannon 1947, Simpson 1949). Yue and Clayton's theta measure of dissimilarity (θyc) and Jaccard's similarity coefficient (Jclass) were used to compare the composition and membership of the different assemblages respectively in an OTU-based, phylogeny-independent manner (Yue and Clayton 2005). Non metric multi-dimensional scaling (NMDS) and principal co-ordinate analysis (PCoA) were used to ordinate the different samples in multidimensional space, based on the distance matrixes generated by θyc and Jclass. The nonparametric analysis of molecular variance (AMOVA) was used to test the significance of the grouping based on the NMDS ordination (Lozupone et al. 2006). Microsoft Excel 2007 was used to generate all graphs.

3 RESULTS

3.1 Carbon and Nitrogen Mineralization

Gas samples and liquid extracts from microlysimeters were analyzed to determine C and N mineralization, respectively. Mineralization of C follows first-order kinetics for all samples (Table 1). All soil mixture types exhibited increased C respiration in the presence of PyC (Figure 1), except for petroleum coke in the FFM. Petroleum coke exhibited the poorest performance with respect to C mineralization. Of all the soil types used, the NFS soil mixtures exhibited an approximately 3-fold higher level of final C mineralization relative to respective treatments in PMM and FFM (Figure 1). Addition of charcoal in the NFS treatment had the greatest effect on respiration, followed by the biochar.

N mineralization was lower in all samples amended with a form of PyC (Figure 2). The amount of N mineralized was highest on the undisturbed forest soil recovering from fire, and lowest in the PMM. The PMM did not fit first-order kinetics, as hypothesized (Table 1), although this was improved marginally by the addition of PyC.

Table 1. Goodness of fit statistics (R^2) and modeled parameters (C_o, N_o, k-value) for first order decomposition kinetics of C and N mineralization in the presence and absence of pyrogenic C on two oil sands reclamation soils (PMM and FFM) and one native forest soil (NFS).

Treatment	C_m(Average)	C_m(Model)	C_o	k	R^2	N_m(Average)	N_m(Model)	N_o	k	R^2
PMM-Control	0.196	0.186	0.204	0.027	0.970	9.254	7.124	7.124	0.145	0.603
PMM-Biochar	0.235	0.225	0.250	0.025	0.975	3.440	3.022	1.179	0.000	0.730
PMM-Coke	0.217	0.207	0.232	0.025	0.974	6.316	5.228	5.228	0.442	0.551
PMM-Charcoal	0.239	0.227	0.242	0.030	0.965	7.589	6.066	6.198	0.042	0.494
FFM-Control	0.255	0.240	0.247	0.039	0.953	56.485	55.094	58.999	0.030	0.959
FFM-Biochar	0.293	0.278	0.286	0.038	0.964	27.961	23.972	23.985	0.083	0.868
FFM-Coke	0.202	0.189	0.194	0.041	0.952	42.204	40.280	42.093	0.035	0.958
FFM-Charcoal	0.298	0.279	0.284	0.044	0.933	42.851	40.391	41.919	0.036	0.949
NFS-Control	0.640	0.607	0.640	0.033	0.956	77.191	76.242	98.298	0.016	0.996
NFS-Biochar	1.156	1.099	1.151	0.034	0.962	27.896	26.876	45.581	0.010	0.968
NFS-Coke	0.707	0.671	0.705	0.033	0.959	43.974	41.979	45.680	0.028	0.980
NFS-Charcoal	1.423	1.348	1.385	0.040	0.955	50.770	47.198	60.725	0.017	0.959

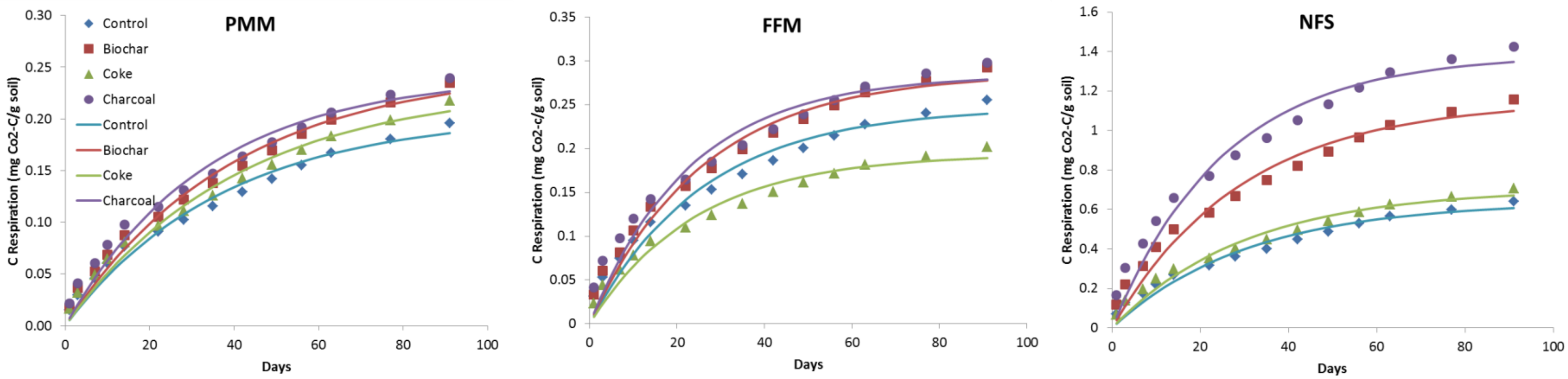

Figure 1. Carbon mineralization over 90 days, with and without pyrogenic C additions, in two different oil sands reclamation soils (PMM and FFM) and one native forest soil (NFS).
The points represent measured data and the line represents modeled first order decomposition kinetics
The scale is different on each graph to highlight how the measured data fits the modeled data.

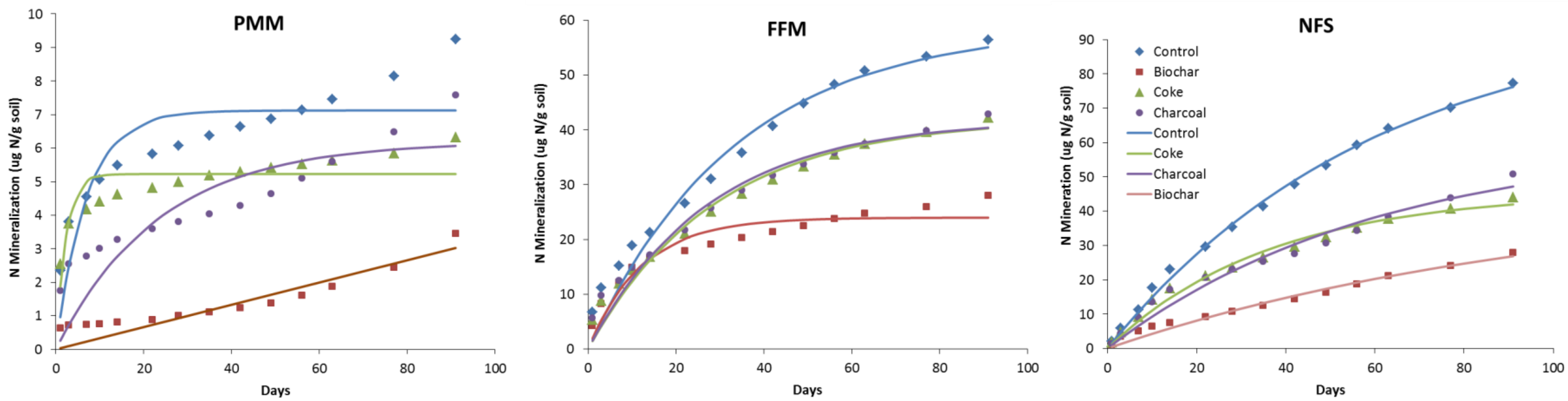

Figure 2. Nitrogen mineralization over 90 days, with and without pyrogenc C additions, in two different oil sands reclamation soils (PMM and FFM) and one native forest soil (NFS).
The points represent measured data and the line represents modeled first order decomposition kinetics
The scale is different on each graph to highlight how the measured data fits the modeled data.

3.2 Bacterial Population Dynamics

Genetic analyses were done on all soil samples at the end of the 90 d period to assess the final bacterial and fungal populations. Observed number of bacterial operational taxonomic units (OTUs) shows an increase in FFM and NFS with the addition of biochar and charcoal (Figure 3a). The increase in observed richness as compared to the non-treated control appears to be similar between the two treatments for the two soil types however NFS show a higher increase in estimated richness between the two treatments and the non-treated control.

Both diversity indices indicate an increase in the overall diversity of the bacterial communities within the treated groups when compared to the non-treated control (Figure 3b). While coke did show a slight increase in the diversity of the FFM bacterial community this response was much lower than other treatments. In all other cases either no change or a slight decrease in richness and diversity were registered (Figure 3a and b). Peat showed higher richness and diversity than other soil types, and perhaps decreased with the addition of PyC (Figure 3a and b). Coke and charcoal treatments appear to increase observed number of OTUs in FFM and NFS respectively. However this effect does not carry over to diversity (Figure 3a and b).

Ordination of distances between community structures based on θyc showed both charcoal and biochar treatments shift the bacterial community structure of FFM and NFS with a trajectory up and to the right when compared to the no treatment control (Figure 4a and b). For both soil types, biochar drove the community structure further away from the non-treated control then charcoal did (Figure 4a and b). Biochar drove the community of FFM soil further from the non-treated control than it did in NFS (Figure 4d). The bacterial community of peat did not respond to any of the treatments (Figure 4c).

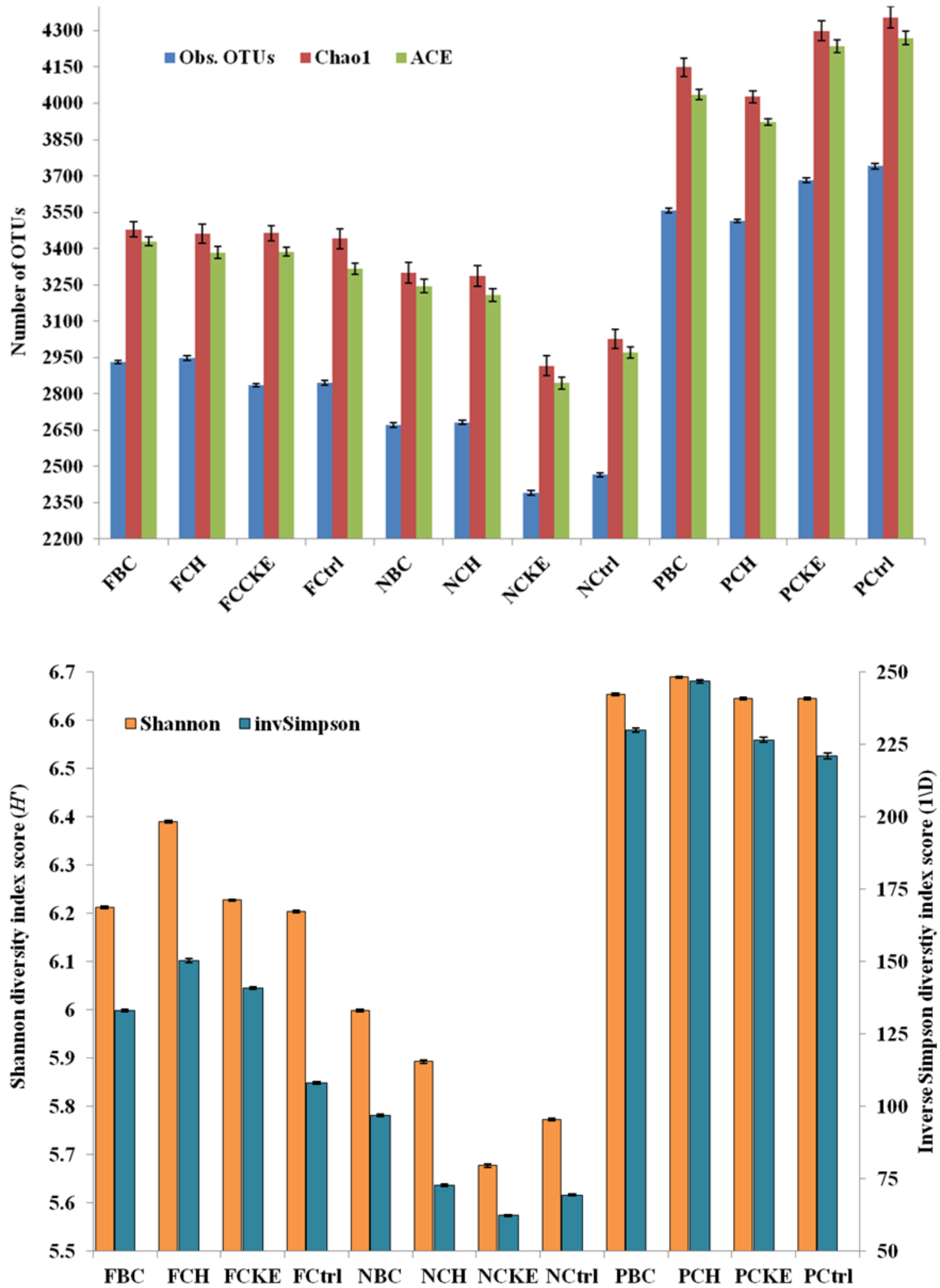

Figure 3. (a) Bacterial OTU richness measured by observed number of OTUs and estimated by Chao1 and ACE nonparametric richness estimators, and (b) estimated diversity as measured by the Shannon and inverse Simpson's diversity indices.
F = FFM, N = NFS, P = PMM, BC = biochar, CH = charcoal, CKE = coke, Ctrl = control.
Error bars represent Standard Deviation.

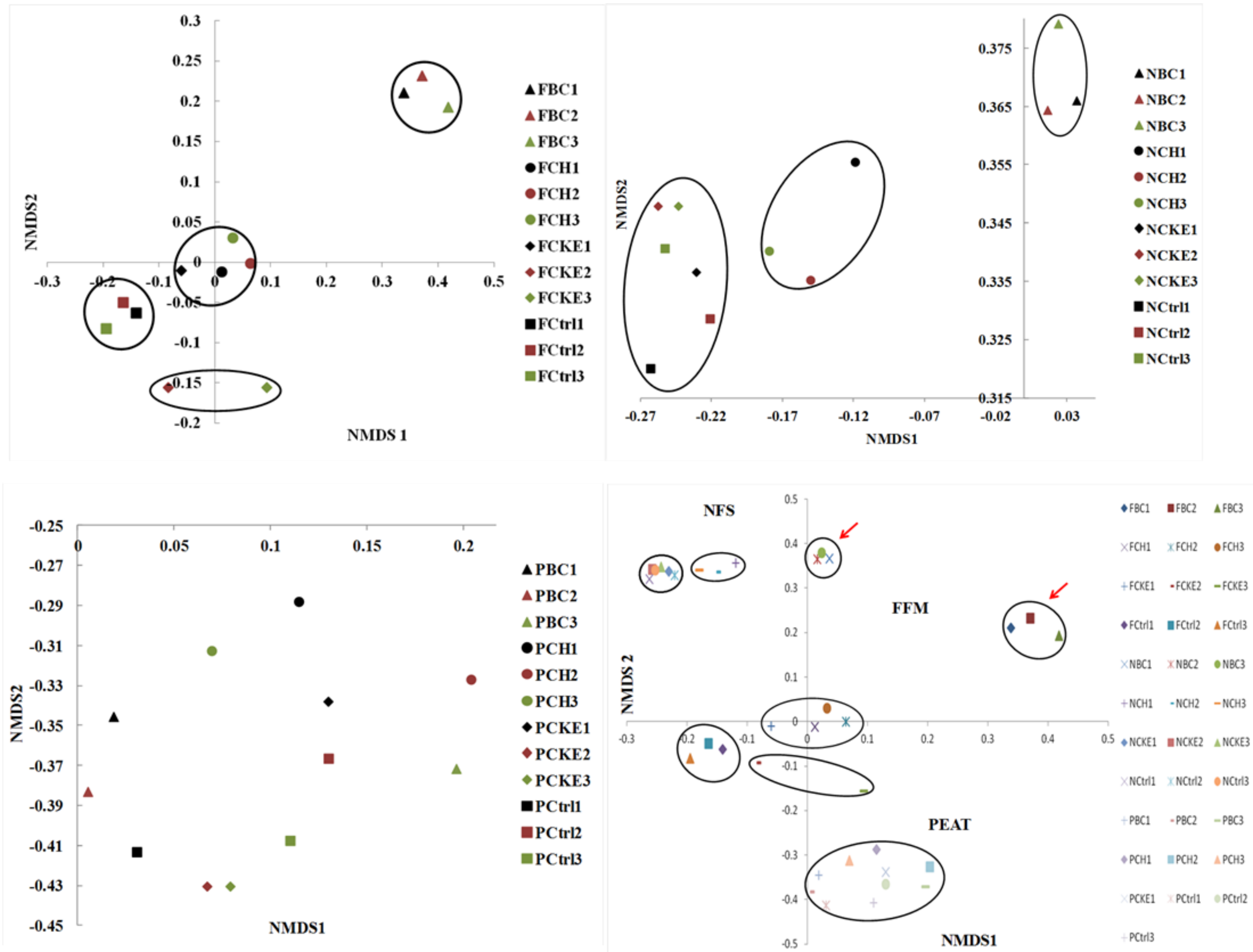

Figure 4. NMDS ordination of distances between bacterial communities based on θyc dissimilarity index, for (a) FFM, (b) NFS, (c) PMM, and (d) all samples together.

Samples in black circles are statistically significant groupings based on AMOVA at $p<0.05$.

F = FFM, N = NFS, P = peat mineral, BC = biochar, CH = charcoal, CKE = coke, Ctrl = control.

3.3 Fungal Population Dynamics

Unlike with bacteria, the fungal community seems the richest and most diverse in FFM (Figure 5a and b)[1]. The exception to that is the fungal community diversity from FFM which drops in response to biochar (Figure 5b). This is due to two OTUs related to the genera Coprinus and Coprinullus which increased 2 to 3 orders of magnitude in representation when compared to the control and represent 30% of the total sequences.

Ordination of distances between community structures based on Jclass showed that only the NFS community responded to the biochar treatment (Figure 6b). Two of the charcoal treatment replicates in PMM were grouping differently than all treatments and controls however the third one did not (Figure 6 c). No other treatment in any of the soils elicited statistically significant response for the fungal community.

4 DISCUSSION

4.1 C and N Mineralization in the Presence of PyC

The increased C respiration exhibited by all soil types in the presence of PyC is not unexpected. The addition of PyC to soil represents the addition of an organic substrate, and the initial pulse of C emitted during incubation is synonymous with increased microbial activity during the breakdown of easily consumed organic components (Lehmann and Joseph 2009). As the pool of organic compounds shrinks and becomes enriched with recalcitrant forms of PyC, mineralization tapers off. Thus, the observed mineralization of C follows first-order kinetics for all samples.

The NFS soil mixtures all exhibited an approximately 3-fold higher level of final C mineralization relative to their respective treatments in PMM and FFM (Figure 1). Due to the relatively undisturbed nature of this material, we suggest that the resident microflora remains functional to a greater extent than in the PMM and FFM. The PMM and FFM soils have undergone a relatively greater degree of anthropogenic disturbance, most notably their transport to sites alien to their origin.

Charcoal in the NFS treatment had the greatest effect on respiration, followed by the biochar. Charcoal used in this study was obtained from the Richardson Fire of 2011, and is derived from plant matter commonly found in the boreal. The NFS mixtures contain a resident microflora derived from the boreal and which may be readily capable of breaking down organic compounds typically found within plant-derived charcoal compared to the biochar and coke used. The PMM and FFM, in contrast, may simply not have the established microflora needed in abundance to break down these compounds at similar rates. However, the addition of plant-derived PyC stimulates respiration, even within PMM and FFM.

[1] For more on boreal soil fungal communities see Richardson, E., G. Walker, G. MacIntyre, S. Quideau, J.B. Dacks and S. Adl, 2014. Next-Generation Sequencing of Protists as a Measure of the Microbial Community in Oil Sand-Associated Soils. OSRIN Report No. TR-69. 26 pp. http://hdl.handle.net/10402/era.40343

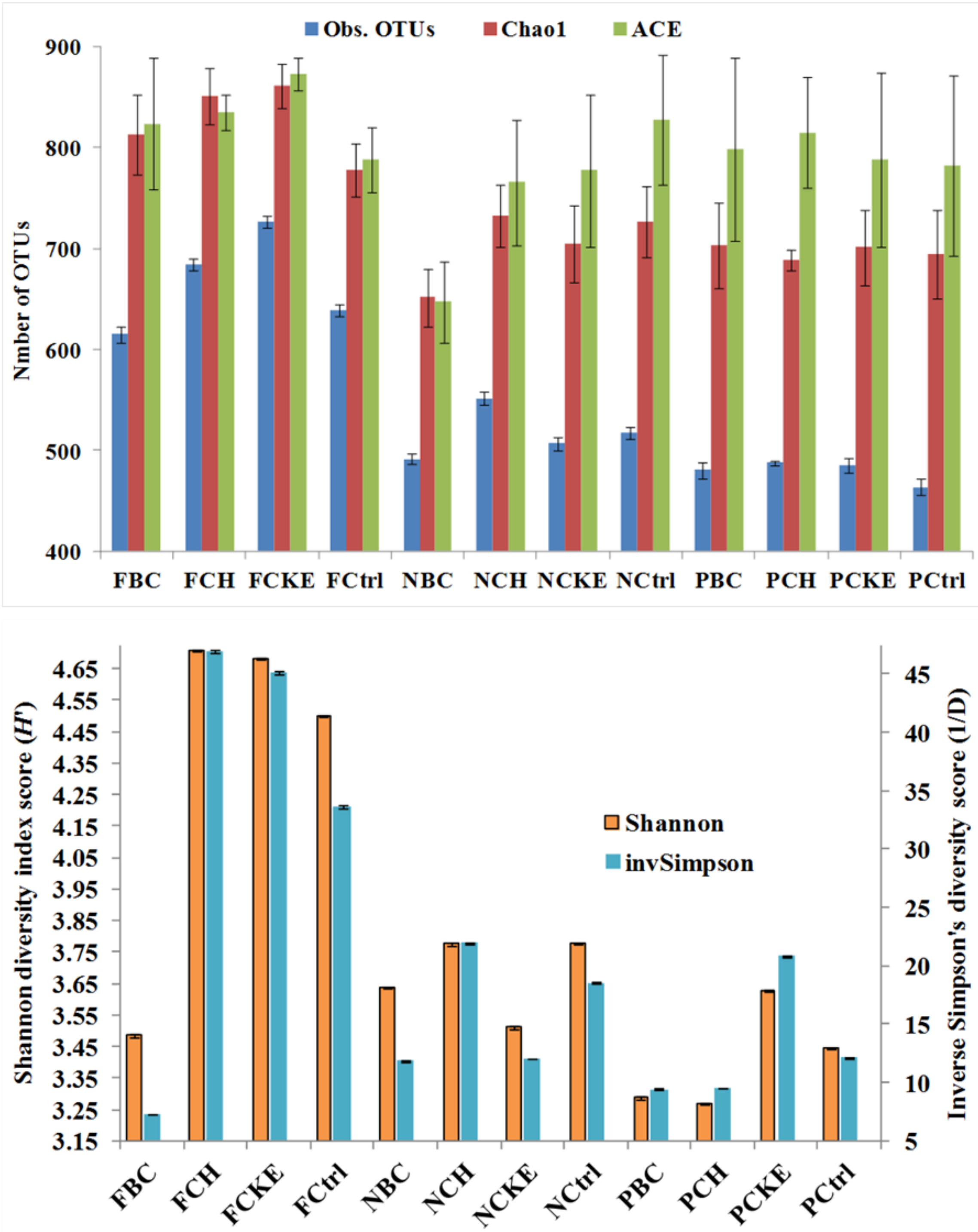

Figure 5. (a) Fungal OTU richness measured by observed number of OTUs and estimated by Chao1 and ACE nonparametric richness estimators, and (b) estimated diversity as measured by the Shannon and inverse Simpson's diversity indices.
F = FFM, N = NFS, P = PMM, BC = biochar, CH = charcoal, CKE = coke, Ctrl = control.
Error bars represent standard deviation.

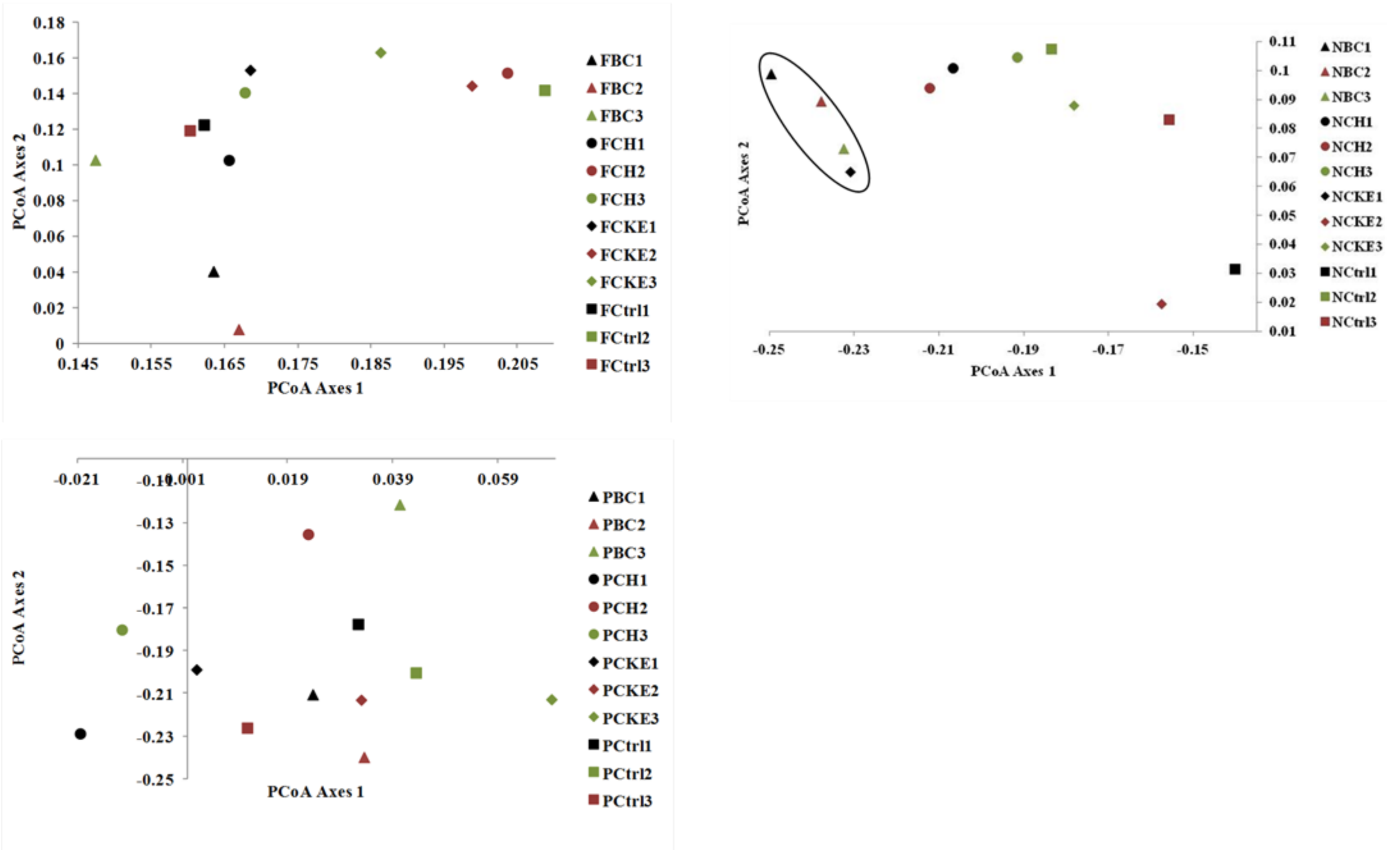

Figure 6. PCoA ordination of distances between fungal communities based on Jclass dissimilarity index, for (a) FFM, (b) NFS, and (c) PMM.
Samples in black circles are statistically significant groupings based on AMOVA $p<0.05$.
F = FFM, N = NFS, P = PMM, BC = biochar, CH = charcoal, CKE = coke, Ctrl = control.

In contrast to charcoal and biochar amendments, petroleum coke addition to soil lowers levels of C mineralization. These low levels of microbial activity in the presence of petroleum-coke are likely due, in part, to the recalcitrance of petroleum-coke. It may also be due to an overall unfavourable milieu of metals and organic compounds in the coke (Alessi et al. 2014). The resident microflora within boreal soils may have adapted to fire disturbance in an opportunistic manner, and are likely better adapted to the breakdown of plant-derived, thermally altered organic compounds than to the hydrocarbons present within petroleum coke.

In contrast to C mineralization, N mineralization was lower in all samples amended with a form of PyC. This suggests a sorption effect, and is consistent with observations of PyC adsorption of compounds observed elsewhere. The N mineralization was highest in the NFS, and may be due to the resident microflora being less disturbed than that of the PMM and FFM. The PMM did not exhibit first-order kinetics in the presence of PyC as hypothesized. We suggest that this is, in part, due to the presence of microflora inconsistent with that found in FFM and NFS. The physico-chemical structure of peat may also prevent N mineralization consistent with first-order kinetics.

The increased respiration observed in the NFS samples is paralleled by higher levels of N mineralization, relative to the PMM and FFM samples.

4.2 Microbial Population Dynamics with the Addition of PyC

Peat originates in an anoxic environment and is rich in labile organic carbon. The high levels of diversity and richness for peat along with the lack of response to any of the treatments may stem from the fact that the microbial community of peat is undergoing a successional process responding to the transition to an oxic environment. This may indicate that peat is not the right material to use in oil sands land reclamation.

The addition of biochar and charcoal alter the structure of FFM and NFS bacterial communities. FFM and NFS bacterial taxa richness and diversity increase with the addition of charcoal and biochar but not with coke. FFM and NFS appear to be primed to respond to the addition of charcoal and biochar but not coke. Though coke is also black carbon it differs in its chemical and physical properties. This indicates the source of black carbon is important to elicit a response from the bacterial community.

These results largely support previous points:

- Peat seems uninfluenced by treatments, possibly due to it undergoing a successional process from the transition to an oxic environment
- Coke does not impact community structure
- FFM and NFS appear to be primed to respond to charcoal and biochar
- Fungal community does not show a clear trend of response to treatments

All in all these results indicate that the fungal community may not respond to treatments as rapidly as the bacterial community has. This might mean that a longer incubation time is required for fungi to respond to treatments. Other researchers have found that the amount of NO_3^- leached in forests of Sweden is lower where a higher ratio of fungi to bacteria is observed (Högberg et al. 2013).

5 CONCLUSION

Plant-derived PyC contributed to the highest amount of C mineralization over the 90-day incubation. This suggests that these forms of PyC help to stimulate the decomposing community and allow them to access soil organic matter.

Further study should include temperature as a key variable when assessing C and N mineralization. The influence of temperature on both soil organic carbon pools and microbial activity / biomass are of growing interest (Allison and Treseder 2011, Li et al. 2013, Ziegler et al. 2013). For example, as temperatures increase, fungal communities appear to preferentially mineralize slow-turnover C pools in boreal organic soils (Ziegler et al. 2013). Shifts in microbial population activities based on temperature are evident, based on PLFA analyses, but the genetic identities of those communities remain unclear.

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7 GLOSSARY

7.1 Terms

Black Carbon

A continuum of organic matter thermally altered to have a specific molecular structure dominated by graphene sheets and with O/C and H/C ratios in the range of 0 to 2.0.

Biochar

An industrial product created through the pyrolysis of organic matter with indirect heat and in the absence of oxygen.

Charcoal

Organic matter partially combusted during fire.

First-Order Kinetics

A mathematical model that reflects a one pool equation driven by a rate constant and time, it has been used to examine decomposition of organic matter in native soils.

Microlysimeter

A small chamber that allows a soil column to be built and incubated under lab conditions, with a gas tight upper compartment for measuring carbon dioxide generated by microbial activity and a lower compartment for drawing out soil solution for measuring the by-product of microbial activity, inorganic nutrients.

Mineralization

Conversion of organic compounds to inorganic compounds.

Oxic

The presence of oxygen.

Petroleum Coke

A by-product of petroleum upgrading, it represents a soot-like material that condenses on the walls of the upgrader.

7.2 Acronyms

AMOVA	Analysis of Molecular Variance
AOSR	Athabasca Oil Sands Region
DNA	Deoxyribonucleic Acid
FFM	Forest Floor Mineral Mix
GC	Gas Chromatograph
NFS	Native Forest Soil
NMDS	Non Metric Multi-Dimensional Scaling
PCR	Polymerase Chain Reaction
PMM	Peat Mineral Mix
PyC	Pyrogenic Carbon
OSRIN	Oil Sands Research and Information Network
OTU	Operational Taxonomic Unit
rRNA	Ribosomal Ribonucleic Acid
SEE	School of Energy and the Environment

TCD	Thermal Conductivity Detector
v/v	Volume to Volume
WHC	Water Holding Capacity
w/w	Weight to Weight

7.3 Chemicals

$CaCl_2$	Calcium chloride
$CaHPO_4$	Calcium hydrogenphosphate
$CaSO_4$	Calcium sulphate
K_2SO_4	Potassium sulphate
$MgSO_4$	Magnesium sulphate
NH_4^+	Ammonium
NO_3^-	Nitrate

APPENDIX 1: DNA Isolation, Amplification and Sequencing

Molecular Pre-Processing and Quality Control of Raw Sequences

All pre-processing and sequence quality control steps were performed using USEARCH (V 7.0.1090 for Windows 32bit) according to the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_pipeline.html, date accessed 1 Oct 2014) and python V 2.74 for Windows (Edgar et al. 2011). In brief, fastq files of paired ends where combined using USEARCH's fastq_mergepairs command. Reads where than de-multiplexed and sequences containing primer/barcode mismatches discarded. Remaining sequences screened based on maximum expected error probability and those with a probability higher than 0.25 were discarded. The remaining sequences where than globally trimmed (450 bp for 16S rRNA sequences, 250 bp for fungal ITS sequences) sequences shorter than the specified lengths were discarded. Remaining sequences where than pre-clustered for further noise reduction as recommended (Huse et al. 2010).

OTUs were assigned using the UPARSE greedy algorithm for an OTU definition of 97% sequence similarity. Though this step also removes chimeras it is recommended by the USEARCH pipeline to supplement it with a dedicated chimera removal step. Therefore chimeras were additionally detected and removed using UCHIME (Edgar et al. 2011). Globular singleton OTUs (those that are represented by one single sequence in the entire dataset) where removed due to their unknown nature.

The assigned bacterial OTUs were classified to phylum, class, and/or genus level using the Ribosomal Database Project classifier (train set 10) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). The assigned fungal OTUs were classified to the phylum, class, or genus level using the UNIT fungal ITS database (Kozich et al. 2013). The sequences were subsampled to the lower than smallest library size (80,000 for merged bacterial samples, 20,000 for individual bacterial samples, 60,000 for merged fungal samples, 11,300 for individual fungal samples) to allow identical sequencing depth for each sample before further alpha and beta diversity analyses (Gihring et al. 2012). Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive.

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