# Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments

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## ARTICLE INFO

## ABSTRACT

The effects of pyrogenic carbon on the microbial diversity of forest soils were examined by comparing two soil types, fire-impacted and non-impacted, that were incubated with laboratory-generated biochars. Molecular and culture-dependent analyses of the biochar-treated forest soils revealed shifts in the relative abundance and diversity of key taxa upon the addition of biochars, which were dependent on biochar and soil type. Specifically, there was an overall loss of microbial diversity in all soils treated with oak and grass-derived biochar as detected by automated ribosomal intergenic spacer analysis. Although the overall diversity decreased upon biochar amendments, there were increases in specific taxa during biochar-amended incubation. DNA sequencing of these taxa revealed an increase in the relative abundance of bacteria within the phyla Actinobacteria and Gemmatimonadetes in biochar-treated soils. Together, these results reveal a pronounced impact of pyrogenic carbon on soil microbial community composition and an enrichment of key taxa within the parent soil microbial community.

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## 1. Introduction

Long-term carbon sequestration strategies are urgently being sought to counter the effects of rising levels of atmospheric carbon dioxide ($CO_2$). The production and application of pyrogenic, biomass-derived black carbon, or biochar, to soils have emerged as a viable tool for the stable, long-term storage of carbon in terrestrial ecosystems (Glaser et al., 2001; Lehmann et al., 2009; Marris, 2006). Whereas 80–90% of the carbon in uncharred biomass remineralizes within 5–10 years, the carbon in biochar undergoes slower degradation with carbon half-lives in the order of $10^3$–$10^7$ years (Zimmerman, 2010). In addition, biochar amendments can enhance soil fertility while reducing water and fertilizer needs (Glaser et al., 2001; Lehmann et al., 2009; Chan et al., 2007; Glaser, 2007). The cause of this fertility enhancement is likely related to the ability of the biochar to absorb and exchange nutrients and natural organic matter. Prior research has suggested that changes in soil microbial community composition may also play an important role. First, ancient pyrogenic carbon-enriched soils, such as Amazonian Dark Earths (Terra Preta), have been shown to contain greater microbial biomass and, in some cases, greater diversity than surrounding non-enriched soils (Kim et al., 2007; Jesus et al., 2009; O'Neill et al., 2009). Second, filamentous microbes, including fungi, have been observed to infiltrate biochar particles over time, thus facilitating their degradation (Hockaday et al., 2007). Lastly, in soils amended with yeast and glucose derived pyrogenic biochar, increases in fungal and Gram-negative bacterial biomass have been observed using phospholipid fatty acid analyses (Steinbeiss et al., 2009).

In this current study, we expanded upon these previous biochemical studies by using molecular methods to examine the overall taxonomic changes in bacterial community structure due to experimental pyrogenic carbon amendments. We hypothesized that the soil type and source of the pyrogenic carbon would influence the microbial composition and target the growth of specific taxa within the soil community. To address this hypothesis we compared changes in biomass and microbial diversity within the soil communities and assessed whether these changes varied with pyrogenic carbon type and soil pre-conditioning to pyrogenic carbon sources.

## 2. Materials and methods

### 2.1. Soil sampling and biochar synthesis

Soils were collected in November 2008 from the surface layers (upper 10 cm) of two forests in north-central Florida. The first site

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was an upland hardwood forest located within a residential area near Gainesville, FL and had no historical record of burning (latitude: 29.6011; longitude: −82.3623). This unburned forest soil had an organic carbon content of 3.8 wt%, was primarily sandy and had no visible evidence of charcoal. The second sampling site was primarily a longleaf-slash pine-palmetto (i.e. pine flatwood) forest located in the Austin Cary Memorial Forest (ACMF), near Gainesville, FL (latitude: 29.7340; longitude −82.2171) that has been managed through controlled burns carried out periodically since the 1950s and annually since 1982. The ACMF burned forest soil was also sandy but had visible flecks of charcoal and an organic carbon content of 2.5 wt%. The unburned and burned forest soils were classified as Entisols and had sand contents of 76.1 and 98.4%, clay contents of 18.2 and 1.6%, and pH of 5.4 and 5.9, respectively.

The soils were augmented with two synthesized biochars derived from Laurel oak heartwood (*Quercus laurifolia* Michx.) and Eastern gama grass (*Tripsacum dactyloides* (L.) L.). The oak and grass biochars were generated by combusting 10−20 g of the material in a 0.04 $m^3$ standard oven under full atmosphere for 3 h at 250 °C (hereafter denoted Oak250) or by pyrolyzing 5−10 g of the biomass under $N_2$ in a custom-built pyrolyzer (5.5 cm diameter × 50 cm length pipe) for 3 h at 650 °C (hereafter denoted Oak650 or Grass650). The chars were lightly crushed with a mortar and pestle and sieved into a uniform size fraction of between 250 μm and 2 mm and sterilized in an autoclave. All biochars were then rinsed briefly with sterile water to remove fine ash.

The chemical characteristics of these biochars have been previously described (Zimmerman, 2010; Kasozi et al., 2010). Briefly, the Oak650 and Grass650 biochars were nanoporous (225 and 77 m2 $g^{-1}$; BET surface area, respectively) but all the biochars were microporous (331−528 $m^2 \ g^{-1}$ $CO_2$ adsorption surface area). The Oak250, Oak650, and Grass650 biochars had organic C weight contents of 55, 79, and 64%, volatile contents of 66, 21, and 33%, respectively, and a pH of 3.5, 9.1 and 10 respectively. The pH of all soil and biochar mixtures ranged between 6.1 and 6.3.

### 2.2. Soil incubations

To assess the effects of biochar on the microbial soil community structure, each soil type was incubated in triplicate with and without biochar supplements. Soils were aliquoted (3 g) into individual glass vials with gas permeable covers. A subset of the soils was amended with 300 mg of oak (Oak250 and Oak650) and grass (Grass650) biochar. Sterilized distilled water (1.2 mL) was added to each vial at time zero to bring soils to their maximum holding capacity. The biochar-treated soils along with the unamended controls were incubated at 32 °C for 188 days in the dark. These conditions were chosen to optimize growth of heterotrophic microbes. Two additional replicate incubations were conducted in parallel for cell cultivation and $CO_2$ respiration analyses. These parallel incubations were carried out in sterilized 12 mL borosilicate vials with rubber septum and were scaled down to one-third size (1 g soil; 100 mg biochar; 0.4 mL water).

### 2.3. $CO_2$ evolution

To monitor microbial respiration within the soil treatments, $CO_2$ evolution was measured from the parallel incubations and compared on Day 62, 109 and 188 of the incubations. Headspace $CO_2$ was measured by purging, using $CO_2$-free air as a carrier gas, into an automated $CO_2$ coulometer (UIC Inc., Joliet, IL), leaving the vials refilled with $CO_2$-free air for continued incubation. The analytical detection limit for $CO_2$, determined using acidification of $CaCO_3$ standards, was found to be 0.1 μg C. 'Total' $CO_2$ evolution was the sum of three individual $CO_2$ measurements over the 188 days, whereas the 'mean' evolution rate was the mean of each of the

three individual respiration rates calculated as evolved $CO_2$ divided by the number of days incubated since the last measurement.

### 2.4. Quantitative real time PCR

The microbes of the treated and untreated soils were assessed using quantitative real time PCR (qPCR). Genomic DNA was extracted in quadruplicate from approximately 100 mg of each soil treatment using the MoBio DNA Powersoil kit (Carlsbad, CA), quantified using the Quant-iT PicoGreen kit (Invitrogen, Carlsbad, CA) and normalized. Total recovery of genomic DNA ranged from 300 to 800 ng of DNA per 100 mg of sample material. Amplification and detection of the small subunit (SSU) ribosomal RNA (i.e. 16S rRNA) gene by qPCR were performed using the Roche Light Cycler-480 platform (Roche Diagnostics, Indianapolis, IN) in quadruplicate and the mean values were calculated. PCR reactions were run in 20 μL total volume containing final concentrations of 1× SYBR Green Master Mix (Roche Diagnostics, Indianapolis, IN), 0.4 μg $mL^{-1}$ BSA, 0.5 μM each primer and 5 ng template DNA. The primers used in this study targeted the 16S rRNA genes of organisms from the domain Bacteria and have been previously employed for community composition analysis in soils (Fierer et al., 2005). The bacterial primer set included the forward primer EUB338 (5′-ACTCCTACGGGAGGCAGCAG; Lane, 1991) and the reverse primer EUB518 (5′-ATTACCGCGGCTGCTGG; Muyzer et al., 1993). The reaction conditions included incubation at 95 °C for 10 min followed by 30 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 20 s. A melting curve analysis was performed at 95 °C for 30 s; 65 °C, for 30 s with continuous monitoring at 95 °C to ensure that the derived fluorescent measurements were PCR products and not primer-dimers. DNA standards were generated from 16S rRNA amplicon library of unburned forest soil generating a 5-point curve. Copy numbers were based on the standard curve and an assumption that the average molecular mass of double stranded DNA is 660 g per mol (Fierer et al., 2005).

### 2.5. Cultivation of bacteria and Actinobacteria

Subsets of the soil treatments (0.1 g) were stirred with 10 mL of distilled water for 5 min. These suspensions were serially diluted and culturable cells were quantified using the pour plate method. Briefly, 1 mL of each dilution was added to 9 mL of agar-media maintained at 45 °C and then poured into sterile Petri dishes. To assess the non-fastidious culturable bacterial community a Nutrient agar containing (w/v) 0.3% beef extract, 0.5% peptone, 0.8% NaCl, and 1.5% agar adjusted to pH 6.5 was used. Soil treatments plated on the Nutrient agar were incubated at 37 °C for 2 d before quantification. Actinobacteria were cultivated using an arginine−glycerol−salt (AGS) media (El-Nakeeb and Lechavalier, 1963; Küster and Williams, 1964) that contained (w/v) 0.1% arginine, 1.25% glycerol, 0.1% $K_2HPO_4$, 0.1% NaCl, 0.05% $MgSO_4$, 0.001% $Fe_2(SO_4)_3$, 0.0001% $CuSO_4$, 0.0001% $ZnSO_4$; 0.0001% $MnSO_4$, and 1.5% agar at pH 6.9. The AGS plates were incubated at 37 °C for 7 d. The arginine−glycerol−salt (AGS) medium used in this study has been shown to be selective for the isolation of cultivable soil bacteria from the phylum Actinobacteria (i.e. actinomycetes) (El-Nakeeb and Lechavalier, 1963) as L-arginine as been shown to be a selective nitrogen source for actinomycetes. Although it is possible that non-target organisms may be capable of using the L-arginine as a nitrogen source the high carbon to nitrogen ratios in AGS media also helps reduce background bacteria levels (Labeda and Shearer, 1990), and Actinobacteria have a unique cell and colony morphology that facilitates their identification.

### 2.6. Automated ribosomal RNA intergenic spacer analysis (ARISA)

Microbial community fingerprinting of the biochar-treated soils was performed using ARISA, as previously described (Havemann