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Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus

Craig R. Anderson[a,*], Leo M. Condron[a,b], Tim J. Clough[b], Mark Fiers[c], Alison Stewart[a], Robert A. Hill[a], Robert R. Sherlock[b]

[a] Bio-Protection Research Centre, Lincoln University, PO Box 84, Lincoln, 7647, New Zealand
[b] Soil and Physical Sciences Department, Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 84, Lincoln, 7647, New Zealand
[c] The New Zealand Institute for Plant & Food Research Limited, Lincoln Campus, Private Bag 4704, Christchurch, 8140, New Zealand

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ABSTRACT

It is generally accepted that biochar-C is largely unavailable to soil microbes but changes in soil physico-chemical properties and the introduction of metabolically available labile-C compounds associated with the biochar may shift the soil microbial community structure. In the event that biochar becomes widely applied to agricultural soils as a soil conditioner, this research focuses on understanding how biochar application to soil influences bacterial community structure and biogeochemical function. A controlled pot experiment was designed to investigate temporal changes in soil microbial ecology and physico-chemical parameters in the presence and absence of biochar. Bacterial communities were investigated in both the rhizosphere and bulk soil using TRFLP coupled with a 454 new generation sequencing database to make tentative taxonomic assignments to TRFLP peaks that showed statistically significant change over time. When comparing biochar amended soils with controls, temporal changes in bacterial family abundances that were > 5% included: *Bradyrhizobiaceae* (∼8%), *Hyphomicrobiaceae* (∼14%), *Streptosporangineae* (∼6%) and *Thermomonosporaceae* (∼8%), where the biochar had a positive influence – either promoting an increase in abundance or reducing the magnitude of loss, and; *Streptomycetaceae* (∼−11%) and *Micromonosporaceae* (∼−7%), where biochar was perceived to have a negative effect on bacterial family abundance. The *Bradyrhizobiaceae* and *Hyphomicrobiaceae* have significant involvement nitrogen cycling, with genera/species identified by 454 involved in nitrate (NO_3^-) denitrification through to N_2. The data also suggests that organisms involved in nitrification of ammonium (NH_4^+) to nitrite (NO_2^-) are less abundant while mycobacterial nitrate reduction to NH_4^+ increases along with N_2 fixation. Coupled to the observation that biochar can adsorb NH_4^+, these results provide an explanation for the reductions in N_2O emissions observed from soils when biochar is added. Results from this study also indicate that biochar promotes phosphate solubilising bacteria, alters C-fluxes through increasing the abundance of bacterial families that can degrade more recalcitrant C compounds, and potentially decreases bacterial plant pathogens.

Introduction

Incorporation of biochar into soil is currently being promoted as one of the tools that could be deployed to combat anthropogenically induced increases in atmospheric CO_2 concentrations (Laird 2008; Novak et al. 2009). Biochar is a by-product of biomass pyrolysis, which is undertaken to generate energy (Lee et al. 2010). To date, the beneficial effects of adding biochar to soil include: increased pH and soil moisture retention, improved soil structure, increased nutrient retention, decreases in N_2O and CH_4 emissions, reductions in leaching of inorganic N, adsorption of anthropogenic chemicals such as steroid hormones, and adsorption of heavy metals (Cao et al. 2009; Spokas et al. 2009; Atkinson et al. 2010; Sohi et al. 2010).

These beneficial effects are extremely promising and could have a profound influence on how we manage our agro-ecosystems. However, to date, very little work has focused on the potential effects that biochar may have on soil microbial community structure and the biogeochemical processes that underpin many key ecosystem functions essential for soil and plant health. It is generally accepted that the majority of the biochar-C is largely unavailable to microbes (Theis and Rillig 2009), yet there is also evidence that biomass derived charcoal enhances soil microbial biomass, growth and activity (Steiner et al. 2008; Liang et al. 2010; Smith et al. 2010). With this in mind, biochar addition to soil will also undoubtedly shift the microbial community structure and function by simply changing the physicochemical properties of the

* Corresponding author. Tel.: +64 3 325 3693; fax: +64 3 325 3864.
E-mail address: craig.anderson@lincoln.ac.nz (C.R. Anderson).

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soil and introducing metabolically available labile-C compounds (Smith et al. 2010; Zimmerman 2010).

Biochar addition has mixed effects on ecto- and abuscular mycorrhizal fungal biomass and root colonisation of plants. Some studies have reported improvements in plant health through improved nutrient availability and pathogen resistance (Ishii and Kadoya 1994; Matsubara et al. 2002; Herrmann et al. 2004) while other studies report significant declines in both root colonisation, hyphal lengths and P availability (Warnock et al. 2010). Warnock et al. (2007) proposed four mechanisms to explain how biochar could affect mycorrhizal fungi in soils: (1) changes in nutrient availability; (2) changes in other soil microbial communities such as phosphate solubilising bacteria; (3) alterations in plant-fungi signalling, and; (4) habitat formation and refuge from hyphal grazers. All these mechanisms are inherently interrelated. Nutrient availability is affected by physicochemical properties such as changes in cation exchange capacity (CEC), water holding capacity (WHC) and pH, and these changes influence the microbial community best able to thrive, which in turn influences how that community interacts with the plants, soil and other soil biota.

O'Neill et al. (2009) provide the most detailed description so far of bacterial community change in anthrosols (Amazonian dark earth soils containing high levels of black carbon) versus adjacent soils. These authors used a culturing approach, followed by screening of cultured isolates to demonstrate that the presence of black carbon substantially influenced the microbial community structure. Such results are obviously biased toward culturable organisms, but a culture-independent PLFA study by Pietikäinen et al. (2000) also supports substantial microbial community change in forest soils containing wildfire derived charcoal layers.

In the event that biochar becomes widely applied to agricultural soils as a soil conditioner, this research focused on understanding how biochar application influences bacterial community structure and biogeochemical function. A controlled pot experiment was designed to investigate temporal changes in soil microbial ecology and physicochemical parameters in the presence and absence of biochar. Bacterial communities were investigated in both the bulk soil and the rhizosphere to gauge spatial differences between these two soil compartments. Inert sintered glass was used as a control to normalise soil volumes and associated soil derived nutrient concentrations. A biochar conditioning treatment was also used to evaluate the potential negative effects of adsorptive biochar nutrient immobilisation as suggested by Deenik et al. (2010).

Materials and methods

All aqueous solutions were prepared using ultrapure water from a MilliQ water system (18 MΩ-cm resistivity) and all chemicals used were analytical grade, unless otherwise stated.

Experimental design

A runout perennial ryegrass (*Lolium perenne* L.) pasture situated at Lincoln University (43°38′58″S, 172°27′53″E) on a Templeton silt-loam soil (Hewitt 1998), was renovated in May (autumn) 2009 for a field trial. The pasture was cultivated to a depth of 0.30 m using a rotocultivator, and then unweathered biochar manufactured from *Pinus radiata* was added at 3 amendment rates (0, 15 and 30 t ha^{-1}) on 5 replicate plot sets by spreading the biochar on the plots and then mixing it into the first 0.1 m by making a shallow pass with the rotocultivator (for more information about the field trial, along with biochar and soil properties refer to Taghizadeh-Toosi et al. 2011). Prior to resowing for the field trial, soil (18–20% moisture content) was collected for the pot trial described in this study from each of the 0 t ha^{-1} plots and sieved to 4 mm.

The soil collected was mixed and then divided into five separate volumes. Two soil volumes were amended at a rate of 10% by volume with 1–5 mm sized chips of the same unweathered biochar added to the plots in the field trial. The biochar in one of these soil volumes was left untreated while the biochar added to the other volume was pre-conditioned for 7 days in full-strength Hoagland's nutrient solution (Gamborg and Wetter 1975). The remaining two soil volumes were prepared as controls and contained a 10% by volume addition of Biomax® porous sintered glass (Fluval, Hagen Biomax, Montreal, Canada) crushed and sieved to the same dimensions as the biochar and acid washed in 10% HCl. Like the biochar treatments, one soil volume contained untreated Biomax®, while the other contained Biomax® pre-conditioned in full-strength Hoagland's nutrient solution. The remaining soil volume was unamended.

The rhizosphere sampling pots consisted of two 40 mm sections of PVC pipe with a 38 mm internal diameter. The base of the upper section was covered with a 20 μm mesh (Sefar Nitex, Heiden, Switzerland) to inhibit root penetration and was filled with 45 g of unamended soil. Each of the upper sections was planted with a forage perennial rygrass (*L. perenne*, cultivar 'Samson'). Twenty ryegrass seeds were distributed on the surface and covered with a further 5 g of unamended soil. No fertilisers were added. After germination (7 days), the seedlings were thinned to seven per pot and allowed to develop a thick root mat at the surface of the 20 μm mesh for a further 21 days. The lower section of the rhizosphere sampling pots had a 100 μm mesh at the base and was filled with 45 g of amended soil that was allowed to settle with watering before a further 5 g of amended soil was added. Lower sections were taped to upper sections using duct tape and the first sampling occurred 14 days later to allow fungal hyphal development from the upper section of the pot, through the 20 μm mesh to the lower section of the pot with the soil amendments.

Overall, there were 2 main treatments (biochar versus Biomax® sintered glass), each divided into three sub-treatments with three replicates of each sub-treatment taken at each sampling (including no plant controls). Sub-treatments were as follows: biochar with ryegrass (BC), pre-conditioned biochar with ryegrass (BC_{PC}), unplanted biochar (BC_{NP}), sintered glass with ryegrass (SG), pre-conditioned sintered glass with ryegrass (SG_{PC}) and unplanted sintered glass (SG_{NP}).

The pots were randomly placed in four irrigation trays lined with felt and filled with fine sand, with each pot randomly cycled between the trays every 4 days. Reverse osmosis (RO) treated water was gravity fed to a level 1 mm above the sand surface with plants relying on capillary action to wet the soil within the pots. Every 48 h, 5 mL of RO water was pipetted into the upper section of the rhizosphere sampling pots. Growth conditions in the climate chamber were set to a 12 h light/dark cycle with respective temperatures of 20 °C and 15 °C and a constant humidity of 70%.

The rhizosphere sampling pots were sampled destructively at weeks 2, 3, 4, 5, 6, 8, 10 and 12. Samples for molecular microbial ecology were taken at weeks 2, 4, 6, 8, 10 and 12. Destructive soil sampling involved separating the two sections of the pot and scraping off the first 3 mm of soil that was in direct contact with the 20 μm mesh, at the surface of the bottom section, using a sterile spatula. This soil was designated 'rhizosphere.' After sterilizing the spatula again with 70% ethanol, a further 10 mm of soil was removed and discarded. The next 5 mm was then sampled and designated 'bulk soil.' The ryegrass was trimmed every 2 weeks to 50 mm above the soil surface. Ryegrass dry matter yields were measured after the final sampling by harvesting the grass 5 mm above the soil surface, drying (65 °C for 48 h) and weighing.

Due to the constraints related to the soil volume available in the rhizosphere sampling pots, the analysis regime was ranked by importance: Molecular microbial ecology > soil moisture > pH > dehydrogenase activity > inorganic-N.

Soil physicochemical analysis

Two 0.5 g subsamples of soil were taken and frozen immediately for molecular microbial ecology analysis as described below. One gram aliquots were taken for soil moisture/pH, dehydrogenase and inorganic-N determinations. Soil was dried at 105 °C for 48 h to determine gravimetric moisture content (θ_g), and pH was measured following overnight equilibration of the 1 g subsamples in 2.5 mL of deionised water (Blakemore et al. 1987).

Measurement of dehydrogenase activity (estimate of microbial activity) followed an adaptation of von Mersi and Schinner's (1991) INT colorimetric method where 0.5 g aliquots of soil were mixed with 1 mL of INT solution (9.88 mM), 5 mL of extractant solution (50:50 mix of *N*,*N*-dimethlformamide and ethanol) and 1.5 mL Tris Buffer (1 M, pH 7.0). The samples were shaken every 20 min for 2 h, followed by centrifugation at 210 × *g* for 10 min and measurement against a blank at 464 nm using a Varian Cary 50 UV–visible spectrophotometer equipped with a dip probe. For inorganic N analysis, 1 g subsamples of moist soil were shaken with 20 mL of 2 M KCl for 1 h and then filtered (0.2 μm syringe filters) with the extracts analysed, using flow injection analysis (Blakemore et al. 1987), for ammonium–N (NH_4^+–N), nitrate–N (NO_3^-–N) and nitrite–N (NO_2^-–N).

Statistical analyses were performed using Genstat – Release 12.2. ANOVA statistics compared treatment effects and temporal sampling effects.

DNA/RNA extraction and cDNA preparation

Total nucleic acids from each triplicate sample were extracted from soil using a CTAB extraction protocol (Griffiths et al. 2000). Briefly, 0.5 g of soil (wet weight) was put into a BIO-101 matrix B tube (Mo Bio Laboratories, California) along with 0.5 mL CTAB extraction buffer and 0.5 mL of phenol–chloroform–isoamyl alcohol (25:24:1). Cells were lysed using two 2-min 50 Hz beating sessions in a Fritzh bead beater, with chilling on ice between sessions. Samples were then centrifuged for 5 min at 16,000 × *g* and the aqueous layer was transferred to a fresh tube and an equal volume of chloroform–isoamyl alcohol (24:1) was added. The tubes were mixed and chilled on ice before centrifugation for 5 min at 16,000 × *g*. The supernatant was transferred to a new tube and two volumes of 30% PEG 6000/1.6 M NaCl was added followed by precipitation for 2 h at room temperature. Samples were then centrifuged at 18,000 × *g* at 4 °C for 10 min, the PEG solution was drawn off and the nucleic acid pellet was washed 3 times with 200 μL 70% ice-cold ethanol. Clean pellets were air-dried and then resuspended in 60 μL ultrapure H_2O.

The resuspended nucleic acids were separated into two aliquots, with one aliquot DNAse treated using the TURBO DNA-free kit as per the manufacturer's instructions (Ambion, Scoresby, Australia). PCR (conditions outlined below but with 35 cycles) with general 16S DNA primers (27F and 1492R) was used to ensure that DNA was completely removed from the RNA. The RNA was then converted to cDNA using First Strand cDNA Synthesis (Invitrogen, Superscript III Supermix) as per the manufacturer's instructions (Invitrogen, Auckland, New Zealand).

The concentration of DNA and cDNA in the separate aliquots was measured using a Nanodrop ND-1000 spectrophotometer and each sample was then diluted to obtain a final concentration of ~10 ng μL^{-1} with ultrapure H_2O.

DNA/cDNA amplification and TRFLP preparation

Samples of extracted DNA were prepared for analysis according to the terminal restriction fragment length polymorphism (TRFLP) protocol outlined in Davis and Moyer (2008) except that only 25 cycles were used for the PCR amplification. Duplicate PCRs were performed using bacterial domain specific primers, 50 ng of total DNA/cDNA and Phusion DNA polymerase (Finnzymes, New England Biolabs). The specific primers used for bacteria were 68F (5′-TNA NAC ATG CAA GTC GRR CG-3′) and 1492R (5′-YGR TAC CTT GTT ACG ACT T-3′). The forward primer was labelled with the fluorochrome 6-FAM (6-carboxyfluorescein) on the 5′ end. The resulting PCR products were visually assayed for size using a 1% agarose gel with a 1-kb DNA size ladder. The fluorescently labelled PCR products from the duplicate reactions were pooled together, desalted using a 100 KD Montage PCR centrifugal filter device (Millipore) and eluted in 150 μL of ultrapure H_2O.

Fifteen μL aliquots of the labeled PCR products were digested separately with 5 units of the enzymes *Alu*I, *Hha*I, *Mbo*I and *Msp*I (final volume 30 μL) (New England Biolabs). The resulting restriction fragments were desalted using Sephadex G-75 columns and dehydrated. The restriction fragments were resuspended in 15 μL of Hi-Di deionized formamide with Genescan LIZ-600 internal size standard (Applied Biosystems, Foster City, CA). The digests were then denatured by heating at 95 °C for 2 min, and were separated with capillary electrophoresis on an ABI 3100 Genetic Analyzer fitted with a 50 cm capillary array and POP7 polymer and chemistry (Applied Biosystems).

TRFLP profiles were first checked for stable baselines, voltage and calibration and peaks in the range of 50–500 bp were selected. Absolute peak areas were initially determined with GeneMapper software v 4.0 (Applied Biosystems) using a minimum peak height of 5 fluorescence units. Final minimum peak height threshold was determined with the T-REX TRFLP online analysis tool (http://trex.biohpc.org/) (Culman et al. 2009), which removed peaks whose height was less than twice the standard deviation computed over all peaks. Data comprising the area of these "true" peaks was exported for conversion to samples-by-fragments tables (where peak heights in each sample are computed as a percentage of total height) and subsequently to samples-by-binned-OTUs tables by the custom R script "interactive binner" (Ramette 2009) using a relative fluorescence intensity (RFI) cut-off of 0.09%, a window size of 1 and a shift size of 0.1.

Statistical analysis between TRFLP profiles was performed using PRIMER – Release 6.1 with the PERMANOVA add-on (using Bray Curtis similarity, and default parameters throughout). Multi-dimensional scaling (MDS) analysis, permutational ANOVA (PERMANOVA) analysis and similarity percentage (SIMPER) analysis were performed. The General ANOVA function in GENSTAT version 12.2 was used to compare individual TRFLP relative peak height changes for different treatments. ANOVA analysis was only performed on peaks where the average abundance in either the bulk soil or the rhizosphere soil was >0.95% of the sum of the total peak heights after SIMPER analysis. ANOVA analysis was performed using only the main treatments biochar versus Biomax® sintered glass i.e. it was assumed that there was no discernable difference between the microbial community structure between the sub-treatments of either biochar or the sintered glass.

454-Pyrosequencing library generation

In order to identify peaks in individual TRFLP profiles and to partially identify species responsible for the distribution patterns in MDS plots a 454-pyrosequencing library was generated. Ten core samples (20 mm internal diameter × 100 mm depth) were taken from the field-site where the soil was obtained for the

pot experiments. DNA was extracted from duplicate subsamples as per the protocol outlined above, and amplified using 25 PCR cycles with Bacterial domain specific primers, 50 ng of total DNA and Phusion DNA polymerase (Finzymes, New England Biolabs). The specific primers used for bacteria were 68F_454A (5′-CGTATCGCCTCCCTCGCGCCATCAG–TNA NAC ATG CAA GTC GRR CG) and 536R_454B (5′-CTATGCGCCTTGCCAGCCCGCTCAG–GTA TTA CCG CGG CTG CTG G). The resulting PCR products were visually assayed for size using a 1% agarose gel with a 1-kb ladder DNA size standard, pooled together, and desalted using a 100 KD Montage PCR centrifugal filter device (Millipore). The 454 sequencing was performed at The University of Otago (Dunedin, New Zealand), Department of Anatomy and Structural Biology High Throughput Sequencing Service using the Roche/454 GS FLX platform and titanium chemistry.

The 454 run resulted in 12,792 raw reads that were assembled using Roche's Newbler software. Within these 12,792 raw reads, 90% >200 bp, 83% >300 bp, 78% >400 bp, 62% >450 bp and 13% >500 bp. These raw reads represented 4 subsets which were extracted using unique tags: BF (TNANACATGCAAGTCGRRCG) for bacterial forward primer sequences; BR (GTATTACCGCGGCTGCTGG) for bacterial reverse primer sequences; FF (GGAAGTAAAAGTCGTAACAAGG) for fungal forward primer sequences and FR (TCCTCCGCTTATTGATATGC) for fungal reverse primer sequences. The fungal sequences are not relevant to this study. The number of reads in the BF set was 2531, while the BR set contained 7351. Sequencing from the reverse primer was far more successful, but these sequences could not be used in the current study because the reverse primer in the TRFLP was not labelled and corresponding fragment lengths are therefore unknown.

The BF and BR sequences were annotated using a BLASTn (Altschul et al. 1990) search against the NCBI non-redundant nucleotide (nt) database and the RDP database (Cole et al. 2009). Custom written software and EMBOSS (Rice et al. 2000) tools were used to digest the sequences *in silico* using the same restriction enzymes that were used in the TRFLP. The first 5 BLASTn annotations for each sequence were extracted and coupled with their corresponding predicted digested fragment sizes and individual sequence identifiers in a Microsoft Excel worksheet for subsequent TRFLP peak identification.

To taxonomically identify TRFLP peaks, the Microsoft Excel filter function was used to select the appropriate fragment length representing the peak plus or minus 2 bp either side to account for analytical error in TRFLP fragment migration patterns. Taxonomic information relating each 5 bp TRFLP peak range was then exported to a separate spreadsheet and was organised as taxonomically deep as possible. The phylogenetic software MEGAN (http://ab.inf.uni-tuebingen.de/software/megan/) was used to build diversity trees for sequences from both the BF and BR sets resulting in very similar pictures despite the difference in the number of reads per set (data not shown).

Results

Soil physicochemical characteristics

Biochar increased the average soil pH in the bulk soil by approximately 0.1–0.2 pH units with the rhizosphere containing biochar demonstrating the greatest variation (Fig. 1). Because of this variation, a statistically significant biochar treatment effect could not be discerned between the rhizosphere and bulk soil. However, rhizosphere soil had up to 5.7% less moisture by weight than the bulk soil. When compared to the Biomax® sintered glass (SG) controls, soils with biochar (BC) retained 0.9–3.5% more moisture by weight (Fig. 2).

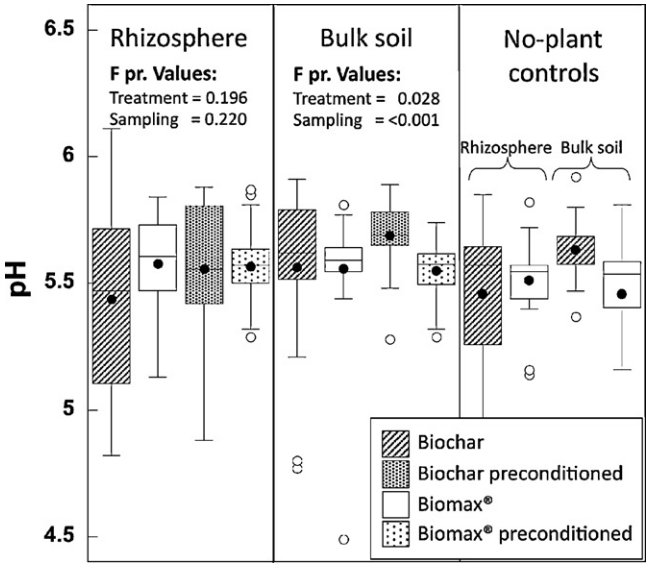

Fig. 1. Representation of pH data spread over 12 weeks for Templeton silt-loam soil with and without biochar. Soils with biochar are up to 0.2 pH units higher than control soils with treatment effects having statistical significance (*F* pr. value = 0.028). The box represents upper quartile, median and lower quartile while the whiskers end at 1.5 times the interquartile range. Open circles represent outlying values beyond the 1.5 interquartile range. Closed circles represent overall mean values. The no-plant controls did not include the preconditioning in nutrient solution treatment.

Dehydrogenase activity was used as a proxy for microbial activity in these experiments. The results were highly variable with statistically significant differences only found in the rhizosphere between pre-conditioned treatments (BC_{PC} and SG_{PC}) and biochar (BC) or Biomax® controls (SG) alone (*P* value <0.05). When comparing the no-plant controls (BC_{NP} with SG_{NP}), the average activity in the presence of biochar was 15% greater ($P < 0.05$) than the Biomax® control (Fig. 3).

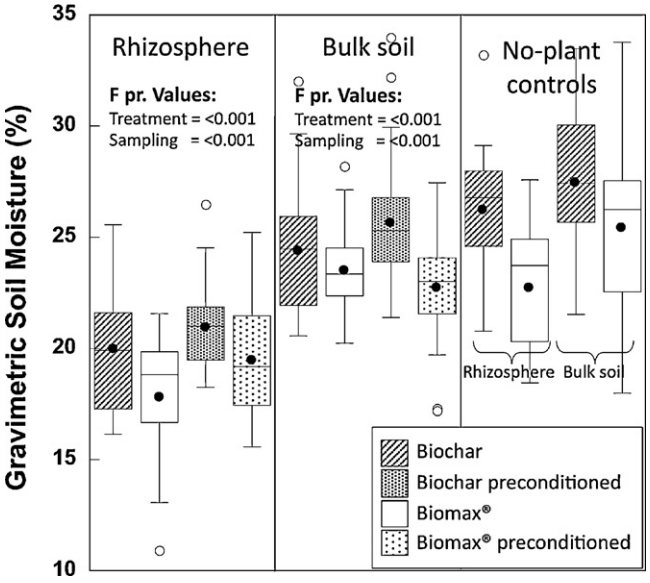

Fig. 2. Soil moisture data over 12 weeks for Templeton silt-loam soil with and without biochar. Soil with biochar is significantly wetter (up to 3.5%), while the rhizosphere is significantly drier (>5%). The box represents upper quartile, median and lower quartile while the whiskers end at 1.5 times the interquartile range. Open circles represent outlying values beyond the 1.5 interquartile range. Closed circles represent overall mean values. The no-plant controls did not include the preconditioning in nutrient solution treatment.

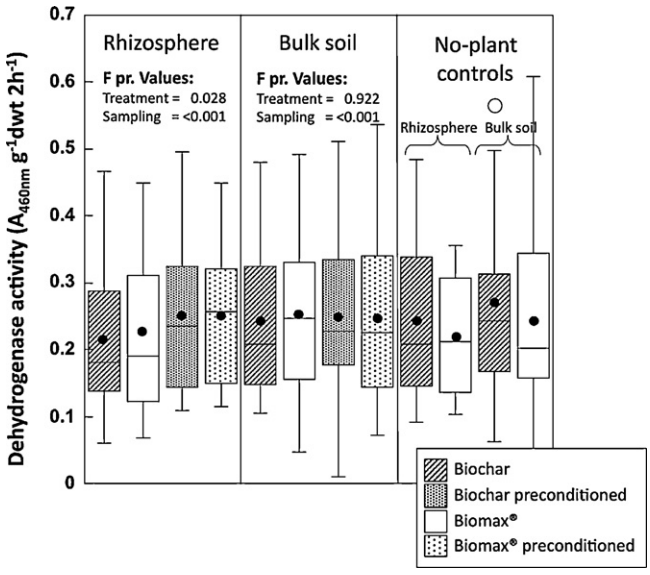

Fig. 3. Dehydrogenase activity in the rhizosphere and bulk soils. Dehydrogenase activity represents a measure of microbial activity. Microbial activity in the rhizosphere is lower in the presence of bio-char but the controls are also lower. Presoaking biochar and the Biomax® control in Hoagland's nutrient solution restores the activity to the same levels as the bulk soil. The box represents upper quartile, median and lower quartile while the whiskers end at 1.5 times the interquartile range. Open circles represent outlying values beyond the 1.5 interquartile range. Closed circles represent overall mean values. The no-plant controls did not include the preconditioning in nutrient solution treatment.

Nitrate decreased rapidly in the first 2 weeks from 20 $\mu g\,g^{-1}$ soil at the start of the experiment to approximately 1 $\mu g\,g^{-1}$ by the end of the experiment with no statistically significant treatment differences (data not shown). After the initial loss of NO_3^-–N, the levels of NO_3^-–N in the BC_{NP} and SG_{NP} treatments started to increase by the end of the experiment (data not shown). The majority of NH_4^+–N and NO_2^-–N concentrations were below detection. Differences in dry matter yields between biochar (BC) treatments and Biomax® controls (SG) were not statistically significant except between the SG and the SG_{PC} treatments ($P < 0.05$) (126 and 153 $g\,m^{-2}$ respectively).

Microbial community characteristics

A total of 96 TRFLP profiles were analysed and compared using the multidimensional scaling (MDS) algorithm within the Primer 6 software. The TRFLP data separated into two distinct data sets, these being DNA versus cDNA (RNA). After analysing these two data sets separately, the DNA group formed distinct clusters with the strongest predictors in the being the soil compartment (C) and time (T). The permutational ANOVA (PERMANOVA) P-value ($C \times T$) was 0.006. Communities were broadly clustered according to treatment and sub-treatment with rhizosphere communities in the presence of biochar diverging the most after 12 weeks (Fig. 4A). The cDNA data demonstrated less separation (Fig. 4B) with the ($C \times T$) P-value being 0.07. Clustering was similar whether profiles from all restriction enzymes were used in the MDS analysis or if they were analysed separately (data not shown).

To simplify the identification of bacterial groups contributing to differences between biochar amended soil and Biomax® sintered glass controls, only data from the *Alu*I TRFLP profiles was chosen for downstream analysis. Similarity percentage (SIMPER) analysis of *Alu*I TRFLP profiles representing the DNA group from bulk soil and rhizosphere soil returned 79 TRFLP peaks in the profiles that contributed to 90% of the dissimilarity between sub-treatments from the different soil compartments. From these 79 DNA peaks, 38 had

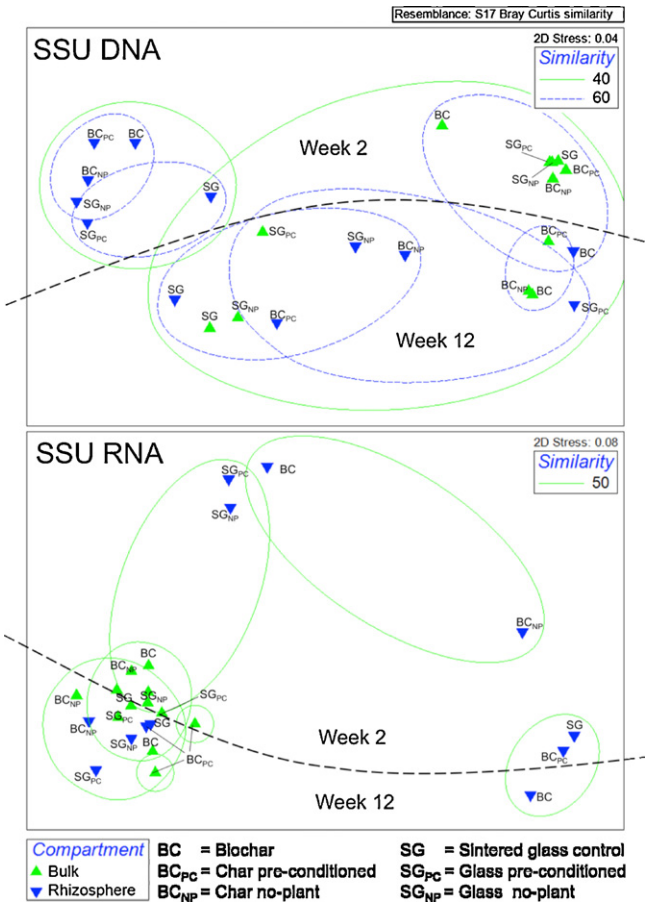

Fig. 4. Multi-dimensional scaling (MDS) plot from weeks 2 and 12 depicting differences in bacterial TRFLPs. The strongest predictors in the data set are the soil compartment (C) and time (T). The P-value for ($C \times T$) was 0.006 for DNA and 0.07 for RNA. Communities are broadly clustered according to overall treatment (BC versus SG) and sub-treatments at the 60% similarity level (blue dashed lines). Rhizosphere communities in the presence of both BC and SG diverge the most after 12 weeks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

an average abundance >1% of the sum of the total peak heights recorded within each treatment. For the cDNA data there were 74 TRFLP peaks in the profiles that contributed to 90% of the dissimilarity between sub-treatments from the different soil compartments.

When collectively investigating the effect of biochar in bulk soil and rhizosphere soil for the DNA data set, only 22 of the 79 peaks had statistically significant change in relative peak height between weeks 2 and 12. These 22 peaks contributed to ~55% of the SIMPER dissimilarity. In the bulk soil, the average change in relative peak intensities between weeks 2 and 12 was deemed significant by T-test ($P < 0.05$) for 19 of these peaks when considering Biomax® controls separately and 6 peaks within the biochar treatments when considered separately (Table 1). ANOVA analysis between biochar and Biomax® controls returned 13 peaks where there were statistically significant differences between the treatments ($P < 0.05$). The corresponding data for DNA from the rhizosphere soil returned only 4 peaks in the biochar treatments with statistically significant differences between weeks 2 and 12, no significant peaks indicating temporal change in the Biomax® controls and only 1 significant difference in the ANOVA analysis between biochar versus the Biomax® controls.

For the cDNA data set, only 4 peaks out of 74 had statistically significant changes in relative peak height, these peaks contributed to only ~13% of the SIMPER dissimilarity (Table S1). Three peaks in the bulk soil had statistically significant average relative peak intensity

Table 1
Similarity percentage (SIMPER) analysis of 16S SSU DNA *Alu*I TRFLP peaks from soils amended with biochar or sintered glass between weeks 2 and 12. The average dissimilarity between treatments was 61.36%. Peaks represented are those where the change (Δ) between week 2 and week 12 for either biochar or sintered glass in either the bulk soil or the rhizosphere was statistically significant ($P < 0.05$). Subtrt. = subtreatments.

*Alu*I peak	Average abundance (%), weeks 2–12			Average Δ, weeks 2–12 – bulk soil (%)				Average Δ, weeks 2–12 – rhizosphere (%)			
	Bulk soil	Rhizosphere	Cumulative Contribution %	Biochar (BC)	Sintered glass (SG)	ANOVA (*F* values)		Biochar (BC)	Sintered glass (SG)	ANOVA (*F* values)	
						BC versus SG	Subtrt.			BC versus SG	Subtrt.
164	16.84	6.29	10.50	−8.20*	−21.20*	0.01*	0.52	13.00	8.30	0.81	0.97
156	4.21	11.30	18.56	−1.60	12.30*	0.05*	0.69	−21.00	−11.60	0.61	0.87
126	0.89	6.20	23.26	−0.13	2.96*	0.01*	0.37	−9.40	−5.60	0.65	0.62
131	5.51	1.89	27.07	2.12	−7.77*	0.06	0.56	4.40	3.00	0.88	0.89
189	1.86	4.73	30.36	−0.94	6.22*	0.03*	0.67	−4.50	−4.80	0.96	0.70
196	2.81	1.17	32.66	−4.69*	−6.54*	0.07	0.19	−1.98*	−2.11	0.57	0.64
176	4.18	2.55	34.80	0.75	−2.29*	0.01*	0.13	3.50	4.20	0.90	0.64
158	2.74	1.34	36.84	−1.53*	−3.95*	0.05*	0.96	1.94	−4.70	0.08	0.07
113	0.95	2.46	38.71	0	3.79*	0.002*	0.50	−1.85	−3.73	0.40	0.88
103	2.56	1.08	40.57	2.32	−2.16*	0.83	0.26	2.20	2.50	0.96	0.91
195	1.74	1.73	42.30	2.47*	4.48*	0.03*	0.31	2.88	3.27	0.87	0.37
169	1.39	3.12	43.95	0.23	2.23*	0.03*	0.41	−2.62*	−1.62	0.65	0.52
98	3.85	2.50	45.52	−0.70	−2.09	0.153	0.14	2.38*	0.60	0.04*	0.32
161	2.25	1.07	46.94	1.97	−2.23*	0.72	0.49	1.99	1.57	0.86	0.82
128	0.73	1.15	48.16	0	2.90	0.18	0.50	−1.98*	3.26	0.39	0.52
185	1.06	1.06	49.33	2.99*	1.24*	0.03*	0.25	3.10	2.70	0.89	0.98
104	0.94	1.95	50.34	0.287	1.20*	0.02*	0.12	−1.24	−1.83	0.64	0.87
190	1.79	1.08	51.27	−0.97	−1.11*	0.80	0.78	2.04	1.03	0.48	0.98
145	0.10	1.12	52.15	0.03	0.36*	0.02*	0.35	−2.11	0.26	0.23	0.45
186	1.10	0.37	52.98	−1.29	−1.80*	0.33	0.43	−0.28	0.95	0.51	0.50
153	0.19	0.96	53.80	0.15*	0.60	0.25	0.43	−0.48	0.99	0.76	0.86
111	1.04	0.34	54.51	−0.43	−1.44*	0.02*	0.38	0.52	0.85	0.80	0.79

* Statistically significant at 95% level.

change between weeks 2 and 12, and only 2 peaks in the rhizosphere (Peak 104 demonstrated significant change in both bulk and rhizosphere soils). ANOVA analysis between biochar and Biomax® sintered glass controls was significant in only two cases for peaks 104 and 181.

Each of the TRFLP peaks that had statistically significant temporal change was queried against the 454-pyrosequencing database to identify them as taxonomically deep as possible. To account for some of the differential migration and sizing discrepancies induced by flourophores, purine content and run-to-run variability (Schütte et al. 2008), taxonomic assignments were taken from the database that were plus or minus 2 bp from the location of the fragment length identified in the TRFLP profiles. All the peaks could be identified at Phylum level, 17 at Order level and 16 at Family level. The 16 Family level peaks could be taxonomically defined at the Genus level and 10 could be taxonomically assigned at the species level. Genus and species level identifications were only used as a guide to make speculative statements about function. The majority of peaks represented multiple bacterial phyla and families. Fourteen families were represented among the peaks from the cDNA data (Table S2).

Overall, 16 bacterial phyla, 16 bacterial orders and 30 bacterial families were represented in the TRFLP profiles from both DNA and cDNA data. Although it was impossible to determine the individual bacterial Family contribution to individual relative peak heights (most peaks represent multiple families), when summing the average temporal change in abundance for each Family associated with each TRFLP peak there were 27 bacterial families whose potential relative abundance changed with statistical significance either when assessed temporally in the same treatment, and/or when assessed temporally between treatments (Table 2 and Table S2).

When comparing biochar amended soils with sintered glass controls, temporal changes in bacterial family abundances that were >5% (cumulative average peak height differences for biochar minus sintered glass control) included: *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, *Streptosporangineae* and *Thermomonosporaceae*, where the biochar had a positive influence – either promoting an increase in the abundance over time or reducing the magnitude of loss, and; *Streptomycetaceae* and *Micromonosporaceae* where biochar was perceived to have a negative effect on bacterial family abundance over time (Table 2). Of these, *Bradyrhizobiaceae*, *Hyphomicrobiaceae* and *Streptomycetaceae* had the largest differences between their average temporal change of 8, 14 and 11% respectively. The implications of these results in terms of ecosystem function related to the genera/species tentatively identified (in parentheses throughout the discussion) are discussed below. Within the cDNA data the largest differences recorded are for the *Bacilliaceae*/*Intrasporangiaceae* families with 6% increase in the bulk soil while the combined *Bradyrhizobiaceae*/*Hyphomicrobiaceae* show an 11% decrease in the rhizosphere (Table S2).

Discussion

The biochar-amended treatments in this study show statistically significant changes in both pH and water holding capacity (WHC). These physicochemical changes reiterate observations noted by other researchers suggesting that biochar has a liming effect with some authors discussing the agronomic benefits of liming, specifically improved nitrogen cycling (Chan et al. 2007, 2008; Major et al. 2009). Changes in soil aeration/WHC associated with biochar addition can also reduce N_2O emissions potentially through decreasing the prevalence of anaerobic pockets where bacterial mediated denitrification processes are more likely to occur (Rondon et al. 2005; Yanai et al. 2007).

Table 2
Bacterial Families represented by 16S SSU DNA *AluI* TRFLP peaks identified in Table 1 with significant change between weeks 2 and 12 Figures represent the sum of the average relative peak heights between weeks 2 and 12, or the sum of the average Δ between weeks 2 and 12 of peaks that represent the bacterial Families identified. 'Biochar effect': +/− = 0–2.5% difference between biochar and sintered glass amended soil; ++/−− = 2.5–5% difference; +++/−−− = >5% difference. Positive 'Biochar effects' represent relative abundance increases or reduction in the magnitude of decrease over time.

Families	Sum average relative peak heights		Sum of average Δ (%), weeks 2–12, bulk soil		Sum of average Δ (%), weeks 2–12, rhizosphere Biochar effect			
	Sum %, Bulk	Sum %, Rhizosphere	Biochar (BC)	Sintered glass (SG)	Biochar (BC)	Sintered glass (SG)	Bulk	Rhizosphere
Acidothermaceae	4.18	2.55	0.75	−2.29	3.50	4.20	++	−
Bradyrhizobiaceae	14.10	11.84	7.60	−0.21	7.23	8.94	+++	−
Burkholderiales/Micrococcaceae/ Xanthobacteraceae	1.74	1.73	2.47	4.48	2.88	3.27	−	−
Cellulomonadaceae	4.60	2.25	−5.66	−7.65	0.06	−1.08	+	+
Conexibacteraceae/Cystobacterineae	0.89	6.20	−0.13	2.96	−9.40	−5.60	−−	−−
Rhizobiaceae/ Geodermatophilaceae	1.79	1.08	−0.97	−1.11	2.04	1.03	+	+
Frankiaceae	4.35	2.16	1.35	−3.27	4.24	3.53	++	+
Hyphomicrobiaceae	21.42	12.43	−5.71	−20.00	11.13	6.42	+++	++
Leptospiraceae/Sphingomonadaceae	2.56	1.08	2.32	−2.16	2.20	2.50	++	−
Microbacteriaceae	3.87	2.23	−1.70	−5.30	1.12	0.59	++	+
Micromonosporaceae	3.65	5.81	−1.91	5.11	−2.46	−3.77	−−−	+
Mycobacteriaceae	23.79	18.53	−11.33	−12.85	−6.06	−8.00	+	+
Nitrosomonadaceae	0.94	1.95	0.29	1.20	−1.24	−1.83	−	+
Nocardioidaceae	3.76	3.63	−4.69	−2.75	−3.83	−5.84	−−	+
Rhodospirillaceae	3.85	2.50	−0.70	−2.09	2.38	0.60	+	+
Streptomycetaceae	8.74	16.22	−4.10	7.24	−17.02	−15.27	−−−	−
Streptosporangiaceae	2.74	1.34	−1.53	−3.95	1.94	−4.70	+	+++
Thermomonosporaceae	4.53	2.42	−2.50	−5.06	3.98	−3.67	++	+++
Xanthomonadaceae	5.49	4.85	−1.93	−0.86	−0.34	−0.67	−	+

Broadly speaking, there are fewer statistically significant changes in relative microbial abundance associated with biochar treatments compared to sintered glass controls, suggesting that temporal microbial community structure and function may be more stable when biochar is added. The results indicate very little change in microbial activity, as assessed by dehydrogenase assays, but this data can be questioned since it was highly variable over time in the presence of both biochar and sintered glass.

It should be noted that the following discussion surrounding potential microbial community functional change is based on the genera and species identified for each temporally significant TRFLP peak. The reader should regard functional impact presented as speculative in light of the limitations surrounding PCR and the TRFLP fingerprinting methodology used. The changes in abundance used are only relative measures and are biased through PCR. Despite the methodological limitations, these results help narrow the field of potential bacterial groups to target in future research into the effect of biochar on bacterial communities.

The taxonomic assignments to TRFLP fragment lengths are purposefully broad, taking in database entries for *in silico* fragments that correspond to the TRFLP fragment length assigned by the Genemapper software (ABI Life technologies) plus any database entries within a plus or minus 2 bp range from the TRFLP fragment length assigned by the Genemapper software. This is to account for three main limitations in the fingerprinting methodology. Firstly, run-to-run variability results in approximately plus or minus 1 bp discrepancies, although this is accounted for by the binning process (Ramette 2009). Secondly, there are significant differences in the way fragments migrate during electrophoresis depending on the fluorophores used. In this study the DNA fragments were labelled with the fluorescein based dye 6FAM, while the internal standard was labelled with the ABI proprietary dye LIZ. The magnitude of migration discrepancies between different fluorescent dyes is not constant across fragment sizes, being up to 2–3 bp for fragments between approximately 100 and 500 bp, and more above and below these values (Schütte et al. 2008). Because of these inaccuracies below 100 bp, we have only attempted to taxonomically label TRFLP peaks that fall within the 100–500 bp range. Thirdly, the purine content of each fragment causes small migration discrepancies (approximately 1 bp) but the T-REX programme (Culman et al. 2009) and the interactive binner R-script (Ramette 2009) account for these discrepancies.

The largest relative abundance change occurred within the *Bradyrhizobiaceae* (*Rhodoblastus*, *Rhodopseudomonas*, *Bradyrhizobium*, and *Nitrobacter*) and the *Hyphomicrobiaceae* (*Rhodoplanes*, Starkeya). These families increased by up to 11% (depending on soil compartment) and are intimately involved in C and N cycling. Under anoxic conditions members of the *Bradyrhizobiaceae* and the *Hyphomicrobiaceae* can utilize N_2, NO_3^-, or NH_3 (Larimer et al. 2004; Kulichevskaya et al. 2006; Hiraishi and Imhoff 2007). *Bradyrhizobium* are both N_2-fixing bacteria and denitrifiers since they also possess the denitrification enzyme NosZ responsible for reduction of N_2O to N_2. *Nitrobacter* gains energy from oxidising NO_2^- to NO_3^- (Prescott et al. 2005). The increase in the abundance of these organisms provides both support and a mechanism for the notion that biochar addition to soils improves N cycling. This support is additional to liming and changes in aeration and WHC since denitrification could be promoted to the point where NO_3^- is reduced through to N_2 as opposed to just N_2O, thus reducing N_2O flux.

Rhodoblastus, *Rhodopseudomonas* and *Rhodoplanes* are all photo/chemotrophs using simple C compounds as electron donors and/or C sources such as formate, acetate, pyruvate, lactate and propionate (Hougardy et al. 2000; Larimer et al. 2004). They are also known for the metabolic trait of methylotrophy, using methanol or methane as C sources (Larimer et al. 2004). *Rhodopseudomonas* can also degrade lignin aromatics and *Stakeya* can utilize a variety of single-carbon compounds, sugar alcohols, amino acids, carboxylic acids, and fatty acids as primary sources of cell carbon as well as inorganic and organic sulfur compounds such as thiosulfate, tetrathionate, dimethylsulfide (DMS), and dimethylsulfoxide (Kelly et al. 2000; Im et al. 2006). Promotion of these organisms could lead to decreases in methane flux, especially when coupled to NH_4^+ adsorption to biochar and biochar induced increases in soil pH (Clough and Condron 2010, and references therein); as less NH_4^+ and alkaline pH are conditions conducive to increases in biological methane oxidation (Saari et al. 2004).

Mycobacteriaceae (specifically *Mycobacterium*) is a Family that received a modest benefit from biochar incorporation and had the highest average relative abundance, especially in the bulk soil (~16%). Mycobacteria are common soil organisms with several *Mycobacterium* species known to be NO_3^- reducers. Interestingly, some *Mycobacterium* species can degrade polyaromatic hydrocarbons and can metabolise ethylene, which can be produced in soils amended with biochar made from specific feedstocks (Hennessee et al. 2009; Spokas et al. 2010). In a recent study by Depkat-Jakob et al. (2010), *nar*G (indicative of dissimilatory nitrate reducers and denitrifiers) transcripts from organic rich uppermost soil were predominantly derived from *Mycobacterium* while *nos*Z (indicative of N_2O reduction to N_2 by denitrifiers) was from *Bradyrhizobium*. Similarly, in a study by Stres et al. (2004) a high diversity of *nos*Z sequences were found, specifically from the *Alphaproteobacteria* (incl. *Bradyrhizobium*). Therefore, in the presence of biochar, *Mycobacterium* could be a player in NO_3^- reduction coupled with *Bradyrhizobium*, reducing N_2O to N_2. This could be a mechanism to explain the reductions in N_2O emissions observed in the presence of biochar (Rondon et al. 2005; Taghizadeh-Toosi et al. 2011), simply through biochar promoting complete denitrification to N_2.

Other bacteria identified that are involved in N cycling include: *Nitrosomonadaceae* (*Nitrosovibrio*), *Rhizobiaceae* (*Rhizobium*) and *Frankiaceae* (*Frankia*) (Prescott et al. 2005). In the current study the relative abundance of *Nitrosovibrio* in the presence of biochar was less than that of the controls. This Family of bacteria oxidize ammonia to NO_2^-, which suggests that the nitrification step from NH_4^+ to NO_2^- could be retarded. Whether this is the result of a decrease in the NH_4^+ pool, through adsorption to biochar (Taghizadeh-Toosi et al. 2011) or the presence of inhibitory substances (Clough et al. 2010) is unknown, but the implications are that this could be one mechanism to decrease the NO_3^-–N pool. A decrease in the NO_3^-–N pool could also affect the abundance of NO_3^- reducing bacteria (in the case of this study, *Conexibacteraceae* and *Nocardioidaceae*) and hence decrease N_2O flux from denitrification. Taking this one step further, a recent study by Deenik et al. (2010) suggests that biochar with high volatile content negatively affects plant growth due to a significant decline in NH_4^+, while at the same time promoting soil respiration. The increase in soil respiration was not attributed to any particular group of bacteria thus we could postulate that the presence of biochar shifts N-cycling toward organisms involved with dissimilatory NO_3^- reduction (DNR) and NO_3^- ammonification (DNRA) to balance the loss of NH_4^+.

The relative abundance of a TRFLP peak that includes N_2-fixing *Rhizobium* dropped in bulk soil while increasing slightly in the rhizosphere whereas the N_2 fixing *Frankiaceae* increased in both bulk and rhizosphere soil amended with biochar. *Frankiaceae* are symbionts of non-leguminous plants and also produce a variety of proteinaeses, pectinases and cellulases. Along with being N_2 fixers, *Rhizobiaceae* are also efficient P solubilising bacteria. Members of the *Microbacteriaceae* (*Microbacterium*) and the Order *Burkholderiales* are also efficient phosphate solubilising bacteria (Rodríguez and Fraga 1999; Malboobi et al. 2009). In this study the relative abundance of *Microbacteriaceae* increased in the presence of biochar while *Burkholderiales* decreased in both bulk soil and rhi-

zosphere soil when amended with biochar. Again, with regard to phosphate mobilisation, the relative abundance of the *Intrasporangiaceae* Family (*Humibacillus*, *Tetrasphaera* and *Terracocus*) and *Bacillaceae* (*Bacillus*) decreased slightly in biochar-amended soils when examining DNA data, but increases substantially (6%) when examining the cDNA data. Some of these organisms possess efficient acid phosphatases and accumulate phosphate in cell wall polymers that may improve phosphate availability to plants upon cell death (Prauser et al. 1997; Maszenan et al. 2000; Oberson and Joner 2005). To date there are no studies dealing explicitly with biochar effects on P availability but biochar could aid P mobilisation by promoting the growth of bacteria involved with this.

Within the rhizosphere the relative abundance of *Streptosporangiaceae* (*Streptosporangium*) and the *Thermomonosporaceae* (*Actinomadura* and *Actinoallomurus*) were promoted by the addition of biochar. Species within these bacterial families are chemo-organotrophs involved with turnover of organic matter, extensively degrading cellulose and lignocellulose residues. They can reduce NO_3^- and are known to produce bioactive compounds (Shearer et al. 1983; Goodfellow and Quintana 2006; Kroppenstedt and Goodfellow 2006). If the carbon in biochar is considered largely unavailable (Cheng et al. 2006; Lehmann et al. 2006; Liang et al. 2006), then increases in organisms that can degrade more recalcitrant organic matter in the presence of biochar could lead to an increase soil organic matter turnover with accompanying soil-C stock decline (as suggested by Wardle et al. 2008). Alternatively it could be that these bacteria, and those mentioned in the paragraphs below, receive only short-term simulation through metabolism of labile-C compounds associated with the biochar (Smith et al. 2010) and will consequentially have no effect on long-term soil-C dynamics and storage.

Acidothermaceae, *Cellulomonadaceae* (*Cellulomonas*), *Geodermatophilaceae* (*Geodermatophilis*), and *Microbacteriaceae* (*Microbacterium*, *Agroccoccus*) also increased in the presence of biochar. *Acidothermaceae* and *Geodermatophilaceae* (*Geodermatophilis*) are genetically related to *Frankiaceae* although they are not known to fix nitrogen (Normand 2006). Very little is known about these fastidious Actinobacterial families, but *Acidothermaceae cellulolyticus* is well known to grow on cellulose as the sole carbon source (Normand 2006). *Cellulomonadaceae* (*Cellulomonas*) are generally associated with the hydrolytic degradation of cellulose, starch and xylan (Rivas et al. 2004). Members of the *Microbacteriaceae* (*Agrococcus*) can also hydrolyse starch along with utlising other carbon sources such as malate and succinate that are commonly found in the rhizosphere (Groth et al. 1996).

Conexibacteraceae (*Conexibacter*), *Micrococcaceae* (*Arthrobacter*), *Micromonosporaceae, Nocardioidaceae* (*Marmoricola*) and *Steptomycetaceae* (*Streptomyces*) decreased in the presence of biochar, especially in the bulk soil. *Conexibacter* and *Marmoricola* can reduce NO_3^- and can utilize C sources like acetic acid and propionic acid, along with harbouring phosphatases (Monciardini et al. 2003; Dastager et al. 2008). *Micromonosporaceae* are associated with secondary metabolite production, and some species are efficient solubilizers of rock phosphate (Hamdali et al. 2008). There are also recent reports of the Micromonospora genus being involved in N_2 fixation (Trujillo et al. 2010). *Arthrobacter* species are interesting, with some able to reduce metals (e.g. hexavalent Cr), pesticides and herbicides (Megharaj et al. 2003). Arthrobacter species were originally thought to be aerobic but some species can use NO_3^- as an electron acceptor in anaerobic metabolism (reducing it to ammonia) and survive in very high levels of NO_3^- (Piñar and Ramos 1998; Eschbach et al. 2003). To date there are few studies dealing with rates of decay of pesticides and herbicides in the presence of biochar so a decline in the number of bacterial species that can aid agrochemical breakdown could be detrimental despite biochar being known to adsorb agrochemicals (Spokas et al. 2009).

A large number of *Streptomycetaceae* genetic sequences related to 12 different *Streptomyces* species were identified. Although generally thought to be beneficial, a decrease in the relative abundance of *Streptomycetaceae* in the presence of biochar could actually be seen as an agronomic benefit, specifically because sequences related to the plant pathogen *Streptomyces puniciscabiei* (potato scab) were identified in this study. Sequences related to another plant (e.g. on Brassica and citrus causing black rot and lesions, respectively) pathogen group, the *Xanthomonadaceae* (*Xanthomonas*), were also identified with their relative abundance decreasing in the presence of biochar. Although no specific species of *Xanthomonas* were identified in this study, the results imply that biochar additions to soil could reduce the prevalence of these plant pathogens could have benefits for arable, forage and forestry systems where *Xanthomonas* diseases are known to occur.

Other Proteobacterial families identified, aside from the *Xanthomonadaceae*, include: *Cystobacteriaceae* (*Cystobacter*) and *Xanthobacteriaceae*, whose relative abundance marginally decreased in the presence of biochar, and; *Rhodospirillaceae* (*Magnetospirillium*) and *Sphingomonadaceae*, whose relative abundance increased in the presence of biochar. These organisms have an interesting blend of metabolic attributes with *Magnetosprillium* involved in iron reduction, siderophore production and respiratory NO_3^- reduction (Fukumori et al. 1997); the *Sphingomonadaceae* family are known for their ability to utilize a wide variety of C sources (along with recalcitrant xenobiotic molecules) while *Cystobacter* is a chitin degrader (Adkins 1999; Pinyakong et al. 2003; Reichenbach 2007). Broadly, increases in the abundance of these organisms could point to biochar addition improving trace metal availability and xenobiotic breakdown.

In summary, the addition of biochar to soil potentially enhances the growth of organisms involved in N cycling in the soil, specifically those that may decrease the flux of N_2O, either because they promote the denitrification of N_2O through to N_2 or they potentially produce NH_4^+ that can then be adsorbed to biochar thus altering soil N dynamics (Fig. 5). Biochar could play an integral role in supporting the proliferation and interactions between these bacteria possibly because it brings with it C compounds they can easily utilize e.g. acetate (Fig. 5). Hence, the results of this study raise the hypothesis that biochar is acting as a transient 'nitrogen island' in the soil, moderating N cycling dynamics, thereby reducing N losses to leaching and gas fluxes. Obviously, these theories need to be tested in the field through isotope studies of N flux, but at this early stage the data suggest that biochar positively influences bacterial N cycling.

Generally the changes observed in this study are of agronomic benefit or of benefit to soil productivity. The discussion presented here is speculative concerning how much impact these bacteria actually have, but now that biochar has been shown to promote changes in individual bacterial families, and genera have been partially elucidated, we can now direct further research to more accurately quantify temporal changes and metabolism of specific bacterial genera and species. If similar bacterial community shifts can be observed in the field, then biochar can be considered beneficial, especially when considering reductions in greenhouse gas flux. Increases in N_2 fixation and general N cycling capabilities along with potential improvements in soil C cycling, P availability and decreases in potential plant pathogens are also beneficial in terms of reducing agricultural inputs. It must be stressed, however, that in-depth quantitative assessments of soil C dynamics must be made since it is possible that biochar promotes microorganisms that can degrade more recalcitrant C compounds. Despite the need for further research, these results support the notion that biochar can act as a soil conditioner to improve greenhouse gas emission profiles from agricultural systems, along with promoting more sustainable agricultural practice.

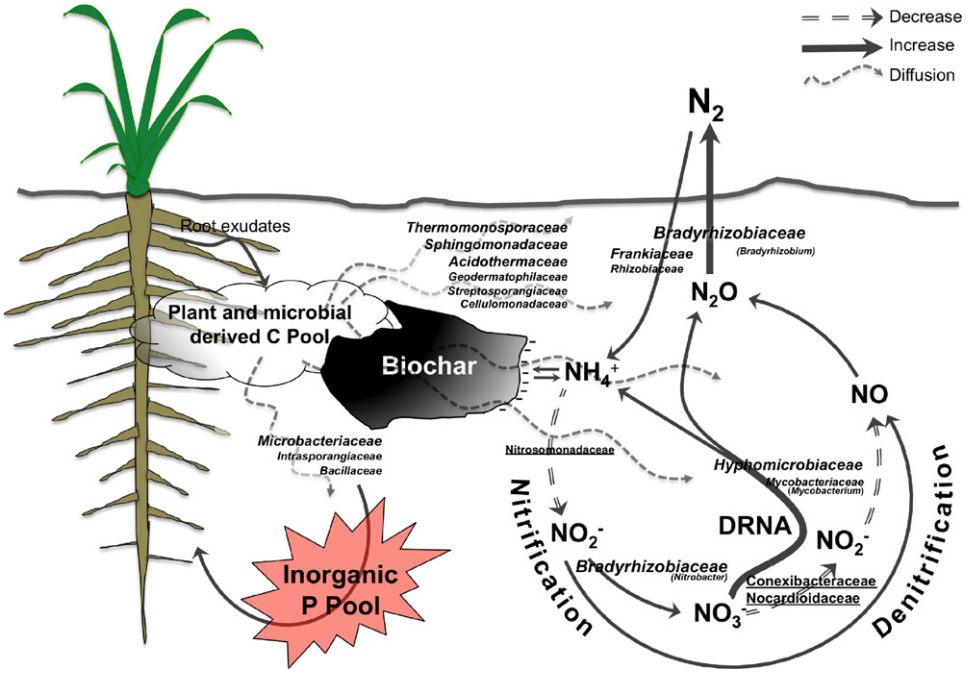

Fig. 5. Schematic depicting how biochar affects the different bacterial families identified in this study and the associated biogeochemical cycles that the genera and species identified from those families influence. Font size for bacterial families represents the relative magnitude of change with non-italicized underlined families decreasing in abundance. The plant and microbial derived C pool includes compounds such as cellulose, propionate, lignocellulose, malate, fumarate, succinate from plants and low molecular weight C compounds from biochar such as acetate and ethylene. Other labile-C compounds introduced with biochar could include a variety of acids, alcohols, aldehydes, esters, ethers, hydrocarbons, ketones and phenols. Organisms in the families *Thermomonosporaceae*, *Sphingomonadaceae*, *Acidothermaceae*, *Geodermatophilaceae*, *Streptosporangiaceae* and *Cellulomonadaceae* are likely candidates to metabolize a wide range of carbon compounds liberating useful carbon sources for other microorganisms. The growth promotion of *Microbacteriaceae*, *Intrasporangiaceae* and *Bacilliaceae* in the presence of biochar could influence inorganic P bioavailability. The possibility also exists that biochar improves dissimilatory reduction of NO_3^- to ammonium (DRNA) through promoting the growth of the *Hyphomicrobiaceae* and *Mycobacteriaceae* coupled to complete denitrification of N_2O to N_2 through the *Bradyrhizobiaceae*. More complete denitrification and less N leaching could also be aided through NH_4^+–N adsorption to biochar and a decrease in the relative abundance (or inhibition) of microorganisms such as *Nitrosomonadaceae*, decreasing the NO_3^-–N pool.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pedobi.2011.07.005.

References

Adkins, A., 1999. Degradation of the phenoxy acid herbicide diclofop-methyl by *Sphingomonas paucimobilis* isolated from a Canadian prairie soil. Journal of Industrial and Microbial Biotechnology 23, 332–335.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology 215, 403–410.

Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temerate soils: a review. Plant and Soil 337, 1–18.

Blakemore, L.C., Searle, P.L., Daly, B.K., 1987. Methods for chemical analysis for soils. NZ Soil Bureau, Scientific Report, pp. 9–12.

Cao, X., Ma, L., Gao, B., Harris, W., 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. Environmental Science and Technology 43, 3285–3291.

Chan, K.Y., van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of greenwaste biochar as a soil amendment. Australian Journal of Soil Research 45, 629–634.

Chan, K.Y., van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry little biochars as soil amendments. Australian Journal of Soil Research 46, 437–444.

Cheng, C.H., Lehmann, J., Theis, J.E., Burton, S.D., Englehard, M.H., 2006. Oxidation of black carbon by biotic and abiotic processes. Organic Geochemistry 37, 1477–1488.

Clough, T.J., Bertram, J.E., Ray, J.L., Condron, L.M., O'Callaghan, M., Sherlock, R.R., Wells, N.S., 2010. Unweathered wood biochar impact on nitrous oxide emissions from a bovine-urine-amended pasture soil. Soil Science Society of America Journal 74, 852–861.

Clough, T.J., Condron, L.M., 2010. Biochar and the nitrogen cycle: introduction. Journal of Environmental Quality 39, 1–6.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Teidje, J.M., 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Research 37, D141–D145.

Culman, S.W., Bukowski, R., Gauch, H.G., Cadillo-Quiroz, H., Buckley, D.H., 2009. T-REX: Software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10, 171.

Dastager, S.G., Lee, J.-C., Ju, Y.-J., Park, D.-J., Kim, C.-J., 2008. *Marmoricola bigeumensis* sp. nov., a member of the family *Nocardioidaceae*. International Journal of Systematic and Evolutionary Microbiology 58, 1060–1063.

Davis, R.E., Moyer, C.L., 2008. Extreme spatial and temporal variability of hydrothermal mat communities along the Mariana Island Arc and the southern Mariana back-arc system. Journal of Geophysical Research 113, art. no. B08S15.

Deenik, J.L., McClellan, T., Uehara, G., Antal, M.J., Campbell, S., 2010. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. Soil Science Society of America Journal 74, 1259–1269.

Depkat-Jakob, P.S., Hilgarth, M., Horn, M.A., Drake, H.L., 2010. Effect of earthworm feeding guilds on ingested dissimilatory nitrate reducers and denitrifiers in the alimentary canal of the earthworm. Applied and Environmental Microbiology 76, 6205–6214.

Eschbach, M., Möbitz, H., Romph, A., Jahn, D., 2003. Members of the genus *Arthrobacter* grow anaerobically using nitrate ammonification and fermentative processes: anaerobic adaptation of aerobic bacteria abundant in soil. FEMS Microbiology Letters 223, 227–230.

Fukumori, Y., Yoshimatsu, K., Noguchi, Y., Fujiwara, T., 1997. Enzymatic iron oxidation and reduction in magnetite synthesising *Magnetospirillum magnetotacticum*. Journal of Physiology IV 7, 659–662.

Gamborg, O.L., Wetter, L.R., 1975. Plant Tissue Culture Methods. National Research of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, p. 109.

Goodfellow, M., Quintana, E.T., 2006. The Family *Streptosporangiaceae*. In: Dworkin, M., et al. (Eds.), The Prokaryotes. Springer, New York, pp. 725–753.

Griffiths, R.I., Whitely, A.S., O'Donnell, A.G., Bailey, M.J., 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA and rRNA based microbial community composition. Applied and Environmental Microbiology 66, 5488–5491.

Groth, I., Schumann, P., Weiss, N., Martin, K., Rainey, F.A., 1996. *Agrococcus jenensis* gen. nov., sp. nov., a new genus of *Actinomycetes* with diaminobutyric acid in the cell wall. International Journal of Systematic Bacteriology 46, 234–239.

Hamdali, H., Hafidi, M., Virolle, M.J., Ouhdouch, Y., 2008. Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing *Actinomycetes* in a P-deficient soil under greenhouse conditions. Applied Soil Ecology 40, 510–517.

Hennessee, C.T., Seo, J.-S., Alvarez, A.M., Li, Q.X., 2009. Polycyclic aromatic hydrocarbon-degrading species isolated from Hawaiian soils: *Mycobacterium rutilum* sp. nov., *Mycobacterium rufum* sp. nov. and *Mycobacterium aromaticivorans* sp. nov. International Journal of Systematic and Evolutionary Microbiology 59, 378–387.

Herrmann, S., Oelmuller, R., Buscot, F., 2004. Manipulation of the onset of ectomycorrhiza formation by indole-3-acetic acid, activated charcoal or relative humidity in the association between oak micro-cuttings and *Piloderma croceum*: influence on plant development and photosynthesis. Journal of Plant Physiology 161, 509–517.

Hewitt, A.E., 1998. New Zealand Soil Classification. Landcare Research science series no. 1, 2nd ed. L. Manaaki Whenua Press, NZ.

Hiraishi, A., Imhoff, J.F., 2007. Rhodoplanes - Hiraishi and Ueda 1994b, 671^{VP}. In: Brenner, D.J., et al. (Eds.), Bergey's Manual of Systematic Bacteriology, vol. 2: The Proteobacteria, Part C, The Alpha-, Beta-, Delta-, and Epsilonproteobacteria. Springer, New York, pp. 545–549.

Hougardy, A., Tindall, B.J., Klemme, J.-H., 2000. *Rhodopseudomonas rhenobacensis* sp. nov., a new nitrate-reducing purple non-sulphur bacterium. International Journal of Systematic and Evolutionary Microbiology 50, 985–992.

Im, W.-T., Aslam, Z., Lee, M., Ten, L.N., Yang, D.-C., Lee, S.-T., 2006. *Starkeya koreensis* sp. nov., isolated from rice straw. International Journal of Systematic and Evolutionary Microbiology 56, 2409–2414.

Ishii, T., Kadoya, K., 1994. Effects of charcoal as a soil conditioner on citrus growth and vesicular–arbuscular mycorrhizal development. Journal of the Japanese Society for Horticultural Science 63, 529–535.

Kelly, D.P., McDonald, I.R., Wood, A.P., 2000. Proposal for the reclassification of *Thiobacillus novellus* as *Starkeya novella* gen. nov., comb. nov., in the Alpha-subclass of the *Proteobacteria*. International Journal of Systematic and Evolutionary Microbiology 50, 1797–1802.

Kroppenstedt, R.M., Goodfellow, M., 2006. The Family *Thermomonosporaceae*: *Actinocorallia*, *Actinomadura*, *Spirillospora* and *Thermomonospora*. In: Falkow, S., et al. (Eds.), The Prokaryotes. Springer, New York, pp. 682–724.

Kulichevskaya, I.S., Guzev, V.S., Gorlenko, V.M., Liesach, W., Dedysh, S.N., 2006. *Rhodoblastus sphagnicola* sp. nov., a novel acidophilic purple non-sulfur bacterium from Sphagnum peat bog. International Journal of Systematic and Evolutionary Microbiology 56, 1397–1402.

Laird, D.A., 2008. The charcoal vision: a win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. Agronomy Journal 100, 178–181.

Larimer, F.W., Chain, P., Hauser, L., Lamerdin, J., Malfatti, S., Do, L., Land, M.L., Pelletier, D.A., Beatty, J.T., Lang, A.S., Tabita, F.R., Gibson, J.L., Hanson, T.E., Bobst, C., Torres, J.L., Peres, C., Harrison, F.H., Gibson, J., Harwood, C.S., 2004. Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. Nature Biotechnology 22, 55–61.

Lee, J.W., Hawkins, B., Day, D.M., Reicosky, D.C., 2010. Sustainability: the capacity of smokeless biomass pyrolysis for energy production, global carbon capture and sequestration. Energy and Environmental Science 3, 1695–1705.

Lehmann, J., Gaunt, J., Rondon, M.A., 2006. Biochar sequestration in terrestrial ecosystems – a review. Mitigation and Adaptation Strategies for Global Change 11, 403–427.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Theis, J.E., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal 70, 1719–1730.

Liang, B., Lehmann, J., Sohi, S.P., Theis, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G., Luizão, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Organic Geochemistry 41, 206–213.

Major, J., Steiner, C., Downie, A., Lehmann, J., 2009. Biochar effects on nutrient leaching. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management, Science and Technology. Earthscan, London.

Malboobi, M.A., Owlia, P., Behbahani, M., Sarokhani, E., Moradi, S., Yakhchali, B., Deljou, A., Heravi, K.M., 2009. Solubilization of organic and inorganic phosphates by three highly efficient soil bacterial isolates. World Journal of Microbiology and Biotechnology 25, 1471–1477.

Maszenan, A.M., Seviour, R.J., Patel, B.K.C., Schumann, P., Burghardt, J., Tokiwa, Y., Stratton, H.M., 2000. Three isolates of a novel polyphosphate-accumulating Gram-positive cocci, obtained from activated sludge, belong to new genus, *Tetrasphaera* gen. nov., and description of two new species *Tetrasphaera japonica* sp. nov. and *Tetrasphaera australiensis* sp. nov. International Journal of Systematic and Evolutionary Microbiology 50, 593–603.

Matsubara, Y.-I., Hasegawa, N., Fukui, H., 2002. Incidence of Fusarium root rot in asparagus seedlings infected with arbuscular mycorrhizal fungus as affected by several soil amendments. Journal of the Japanese Society for Horticultural Science 71, 370–374.

Megharaj, M., Avudainayagam, S., Naidu, R., 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Current Microbiology 47, 51–54.

Monciardini, P., Cavaletti, L., Schumann, P., Rohde, M., Donadio, S., 2003. *Conexibacter woesei* gen. nov., sp. nov., a novel representative of a deep evolutionary line of decent within the class *Actinobacteria*. International Journal of Systematic and Evolutionary Microbiology 53, 569–576.

Normand, P., 2006. The Families *Frankiacece*, *Geodermatophilaceae*, *Acidothermaceae* and *Sporichthyaceae*. In: Falkow, S., et al. (Eds.), The Prokaryotes. Springer, New York, pp. 669–681.

Novak, J.M., Busscher, W.J., Laird, D.L., Ahmedna, M., Watts, D.W., Niandou, M.A.S., 2009. Impact of biochar amendment on fertility of a south-eastern coastal plain soil. Soil Science 174, 105–112.

O'Neill, B., Grossman, J., Tsai, M.T., Gomes, J.E., Lehmann, J., Peterson, J., Neves, E.G., Theis, J.E., 2009. Bacterial community composition in Brazilian Anthrosols and adjacent soils characterized using culturing and molecular identification. Microbial Ecology 58, 23–35.

Oberson, A., Joner, E.J., 2005. Microbial turnover of phosphorus in soil. In: Turner, B.L., et al. (Eds.), Organic Phosphorus in the Environment. CABI Publishing, CAB International, Wallingford, UK, p. 399.

Pietikäinen, J., Kiikkilä, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. OIKOS 89, 231–242.

Piñar, G., Ramos, J.L., 1998. A strain of *Arthrobacter* that tolerates high concentrations of nitrate. Biodegradation 8, 393–399.

Pinyakong, O., Habe, H., Omori, T., 2003. The unique aromatic catabolic genes in sphingomonads degrading polycyclic aromatic hydrocarbons (PAHs). Journal of General and Applied Microbiology 49, 1–19.

Prauser, H., Schumann, P., Rainey, F.A., Kroppenstedt, R.M., Stackebrandt, E., 1997. *Terracoccus luteus* gen. nov., sp. nov., an LL-Diaminopimelic acid-containing coccoid *Actinomycete* from soil. International Journal of Systematic Bacteriology 47, 1218–1224.

Prescott, L.M., Harley, J.P., Klein, D.A., 2005. Microbiology, 6th ed. McGraw-Hill, New York.

Ramette, A., 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. Applied and Environmental microbiology 75, 2495–2505.

Reichenbach, H., 2007. Cystobacter - Schroeter 1886, 170^{AL}. In: Brenner, D.J., et al. (Eds.), Bergey's Manual of Systematic Bacteriology, vol. 2: The Proteobacteria, Part C, The Alpha-, Beta-, Delta-, and Epsilonproteobacteria. Springer, New York, pp. 1086–1096.

Rice, P., Longden, I., Bleasby, A., 2000. EMBOSS: the European Molecular Biology Open Software Suite. Trends in Genetics 16, 276–277.

Rivas, R., Trujillo, M.E., Mateos, P.F., Martínez-Molina, E., Velázquez, E., 2004. *Cellulomonas xylanilytica* sp. nov., a cellulolytic and xylanolytic bacterium isolated from a decayed elm tree. International Journal of Systematic and Evolutionary Microbiology 54, 533–536.

Rodríguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17, 319–339.

Rondon, M., Ramirez, A., Lehmann, J., 2005. Charcoal additions reduce net emissions of greenhouse gases to the atmosphere. In: Proc. of the 3rd Symp. on Greenhouse Gases and Carbon Sequestration, USDA, Baltimore, MA, p. 208.

Saari, A., Rinnan, R., Martikainen, P.J., 2004. Methane oxidation in boreal forest soils: kinetics and sensitivity to pH and ammonium. Soil Biology and Biochemistry 36, 1037–1046.

Schütte, U.M.E., Abdo, Z., Bent, S.J., Shyu, C., Williams, C.J., Pierson, J.D., Forney, L.J., 2008. Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterise microbial communities. Applied Microbiology and Biotechnology 80, 365–380.

Shearer, M.C., Colman, P.M., Nash, C.H., 1983. *Streptosporangium fragile* sp. nov. International Journal of Systematic Bacteriology 33, 364–368.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biology and Biochemistry 42, 2345–2347.

Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil. Advances in Agronomy 105, 47–82.

Spokas, K.A., Baker, J.M., Reicosky, D.C., 2010. Ethylene: potential key for biochar amendment impacts. Plant and Soil 333, 443–452.

Spokas, K.A., Koskinen, W.C., Baker, J.M., Reicosky, D.C., 2009. Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere 77, 574–581.

Steiner, C., Das, K.C., Garcia, M., Förster, B., Zech, W., 2008. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic ferralsol. Pedobiologia 51, 359–366.

Stres, B., Mahne, I., Avguštin, G., Tiedje, J., 2004. Nitrous oxide reductase (*nosZ*) gene fragments differ between native and cultivated Michigan soils. Applied and Environmental microbiology 70, 301–309.

Taghizadeh-Toosi, A., Clough, T.J., Condron, L.M., Sherlock, R.R., Anderson, C.R., Craigie, R.A., 2011. Biochar incorporation into pasture soil suppresses *in situ* N_2O emissions from ruminant urine patches. Journal of Environmental Quality, doi:10.2134/jeq2010.0419.

Theis, J.E., Rillig, M.C., 2009. Characteristics of biochar: biological properties. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management. Earthscan, Dunstan House, London, UK.

Trujillo, M.E., Alonso-Vega, P., Rodriguez, R., Carro, L., Cerda, E., Alsonso, P., Martinez-Molina, E., 2010. The genus *Micromonospora* is widespread in legume root nodules: the example of *Lupinus angustifolius*. ISME Journal 4, 1265–1281.

von Mersi, W., Schinner, F., 1991. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biology and Fertility of Soils, 216–220.

Wardle, D.A., Nilsson, M.-C., Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. Science 320, 629.

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil – concepts and mechanisms. Plant and Soil 2007, 9–20.
Warnock, D.D., Mummey, D.L., McBride, B., Major, J., Lehmann, J., Rillig, M.C., 2010. Influences of non-herbaceous biochar on arbuscular mycchorhizal fungal abundances in roots and soils: results from growth-chamber and field experiments. Applied Soil Ecology 46, 450–456.
Yanai, Y., Toyota, K., Okazaki, M., 2007. Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. Soil Science and Plant Nutrition 53, 181–188.
Zimmerman, A.R., 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environmental Science & Technology 44, 1295–1301.