Impact of Biochar Application to Soil on the Root-Associated Bacterial Community Structure of Fully Developed Greenhouse Pepper Plants

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Adding biochar to soil has environmental and agricultural potential due to its long-term carbon sequestration capacity and its ability to improve crop productivity. Recent studies have demonstrated that soil-applied biochar promotes the systemic resistance of plants to several prominent foliar pathogens. One potential mechanism for this phenomenon is root-associated microbial elicitors whose presence is somehow augmented in the biochar-amended soils. The objective of this study was to assess the effect of biochar amendment on the root-associated bacterial community composition of mature sweet pepper (Capsicum annuum L.) plants. Molecular fingerprinting (denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism) of 16S rRNA gene fragments showed a clear differentiation between the root-associated bacterial community structures of biochar-amended and control plants. The pyrosequencing of 16S rRNA amplicons from the rhizoplone of both treatments generated a total of 20,142 sequences, 92 to 95% of which were affiliated with the Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes phyla. The relative abundance of members of the Bacteroidetes phylum increased from 12 to 30% as a result of biochar amendment, while that of the Proteobacteria decreased from 71 to 47%. The Bacteroidetes-affiliated Flavobacterium was the strongest biochar-induced genus. The relative abundance of this group increased from 4.2% of total root-associated operational taxonomic units (OTUs) in control samples to 19.6% in biochar-amended samples. Additional biochar-induced genera included chitin and cellulose degraders (Chitinophaga and Cellvibrio, respectively) and aromatic compound degraders (Hydrogenophaga and Dechloromonas). We hypothesize that these biochar-augmented genera may be at least partially responsible for the beneficial effect of biochar amendment on plant growth and viability.

Fears of global climate change, attributed mainly to the accumulation of anthropogenic greenhouse gases emitted by fossil fuel use, are driving the development of alternative energy sources based on renewable resources, including biomass. Pyrolysis, the direct thermal decomposition of biomass in the absence of oxygen to solid (biochar or charcoal), liquid (bio-oil), and gas (syngas) bioenergy coproducts, is one of the tools suggested to help drive this paradigm shift (41). Because the half-life of biochar in soil is estimated to range from hundreds to several thousand years, the amendment of soil with biochar is thought to have long-term carbon sequestration potential (58). Importantly, various types of biochar used along with organic and inorganic fertilizers have been reported to significantly improve soil fertility (22), crop productivity (24, 51), and the availability of nutrients to plants (37, 50). Improved crop response as a result of biochar amendment can be attributed to its nutrient content and to several indirect effects, including the neutralization of phytotoxic compounds in the soil (56), the promotion of mycorrhizal fungi (57), and the alteration of soil microbial populations and functions (51).

Elad and colleagues (18) recently demonstrated that soil-applied biochar induces systemic resistance to the foliar fungal pathogens Botrytis cinerea (gray mold) and Leveillula taurica (powdery mildew) on sweet pepper and tomato and Podosphaera aphanis (powdery mildew) on strawberry plants (43), showing that biochar also has a positive impact on plant resistance to disease (18). Interaction between certain bacteria and plant roots can result in a phenomenon termed induced systemic resistance (ISR), where plants become resistant to selected pathogenic bacteria, fungi, viruses, insects, and nematodes. Both biological (virulent, avirulent, and nonpathogenic microorganisms) and chemical (methyl jasmonate, chitin, chitosan, laminarin, and alginate) elicitors can trigger ISR (26, 54). Species of soil microorganisms such as Bacillus, Pseudomonas, and Trichoderma are well-known to mediate ISR in numerous plant systems, including tomato, pepper, and bean plants (26, 28, 36). Phylogenetic characterization based on 16S rRNA gene analysis revealed that a large fraction of these isolates from

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biochar-amended soils were closely related to previously described plant growth-promoting and/or biocontrol agents (24, 43). Recent studies have applied both traditional isolation techniques and culture-independent molecular techniques to examine the impact of both pre-Columbian (terra preta) and modern pyrolysis-generated biochar application on soil microbial community structure. These studies showed that biochar amendment was generally characterized by higher soil pH levels and an increase in the relative abundance of members of the Actinobacteria and Bacteroidetes phyla (14, 33, 45). Although these studies shed light on the influence of charcoal on bulk soil microbiota, they did not explore the impact of biochar amendment on root-associated bacteria that potentially are involved in both plant growth promotion and priming that is responsible for induced plant resistance. The objective of this study was to assess the effect of soil biochar amendment (3%, wt/wt) on the root-associated bacterial community composition of mature sweet pepper (Capsicum annuum L.) plants, specifically focusing on biochar-induced phyla. Given that only a negligible fraction of the soil microbial community can be cultured (40), a molecular approach combining microbial fingerprinting techniques (denaturing gradient gel electrophoresis [DGGE] and terminal restriction fragment length polymorphism [T-RFLP]) with high-throughput 16S rRNA gene pyrosequencing was employed.

**MATERIALS AND METHODS**

Overview of biochar characteristics and greenhouse experimental setup. The greenhouse experimental setup and physicochemical properties of the biochar were described in detail previously (18, 24). Briefly, biochar was prepared from citrus wood in a traditional charcoal kiln (lump charcoal). Accounting for ash content (10.9%), the elemental composition of the biochar was 70.6% C, 0.6% N, 2.3% H, and 15.5% O, giving an O/C atomic ratio of 0.16, H/C ratio of 0.40, C/N ratio of 131, and H/O ratio of 2.4 (24). Prepared biochar was ground into a ratio of 131, and H/O ratio of 2.4 (24). Prepared biochar was ground into a powder of 0.1- to 1.0-mm particles and mixed with an organic matter (OM)-poor sandy soil (Besor region, western Negev, Israel; OM, 0.4%; 92% sand, 1.5% silt, 6.5% clay) (18). Sweet pepper cv. Maccabi plants (Hazera Genetics, Berorim, Israel) were obtained from a commercial nursery (Hishtil, Ashkelon, Israel) 40 days after the initial seeding stage and transplanted into 1-liter pots containing sandy soil without or with biochar (3%, wt/wt). Plants were fertilized with nitrogen, phosphorus, and potassium before the initial seeding stage and transplanted into 1-liter pots. The plants were maintained in a greenhouse at 20 to 30°C in a pest- and disease-free greenhouse for 3 months prior to harvest for 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and elongation at 72°C for 45 s. PCR was performed with 25 U Taq DNA polymerase (DreamTaq, Fermentas Life Science, Vilnius, Lithuania), Taq buffer containing a final magnesium concentration of 2.5 mM, deoxynucleoside triphosphates (dNTPs) (20 nmol each), 12.5 mM glycine serum albumin, and 25 pmol of each primer. The PCR program consisted of an initial denaturation step of 95°C for 180 s followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. Cycling was completed with a final elongation step of 72°C for 5 min. PCR amplicons were checked by agarose gel electrophoresis (1%) and staining with ethidium bromide. DGGE was performed in 6% (wt/vol) acrylamide gels containing a linear urea-formamide gradient ranging from 20 to 70% denaturant (with 100% defined as 7 M urea and 40% [vol/vol] formamide). Gels were run for 17 h at 100 V in the Dcode Universal Mutation System (Bio-Rad Laboratories, Hercules, CA). DNA was visualized after staining with Gelstar (Invitrogen Corporation, Carlsbad, CA) by UV transillumination (302 nm) and photographed with a Kodak DCS digital camera (Kodak Co., New Haven, CT).

Cluster analysis of DGGE community fingerprints. DNA fingerprints obtained from the 16S rRNA gene banding patterns on the DGGE gels were analyzed using Fingerprinting II Informatix software (Bio-Rad Laboratories). The lanes were normalized (T-RFs) by dividing the total signal of normal counts of total signal after background subtraction. Clustering was determined by the unweighted pair group with mathematical averages (UPGMA) method.

T-RFLP analyses. The heterogeneity of 16S rRNA gene sequences from duplicate control and biochar-amended bulk soil and root-associated DNA samples was analyzed by terminal restriction fragment length polymorphism (T-RFLP) (17). An approximately 600-bp fragment of the 16S rRNA gene was amplified by PCR using the bacterial primers 341F, labeled with 6-carboxyfluorescein (FAM) at the 5' end and 907R. PCR amplification consisted of an initial denaturation step of 95°C for 180 s followed by 25 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s, and elongation at 72°C for 180 s. Cycling was completed with a final elongation step of 72°C for 10 min. PCR products from triplicate reactions were pooled and digested with 25 U mung bean nuclease (New England BioLabs, Inc.) in reaction buffer at 30°C for 1.5 h to remove single-stranded fragments that may bias the analyses (17). Nucleotide- and PCR products were purified using the Intron PCR purification kit (nBRN Biotechnology, Gyeonggi-do, South Korea) and quantified spectrophotometrically by NanoDrop. Approximately 2-mg aliquots of each pooled PCR product were digested for 8 h at 37°C with MseI orMspI (New England BioLabs, Ipswich, MA) according to instructions provided by the manufacturer and purified by phenol-chloroform (1:1, vol/vol) extraction followed by ethanol precipitation. Finally, the purified DNA pellet was resuspended in nuclease-free double-distilled water at a concentration of 100 ng/ml. The size (in base pairs) of each peak termed a T-RF was used to indicate an operational taxonomic unit (OTU), whereas the height of the peak was used to determine its relative abundance in the profile. True peaks (as opposed to background noise) were determined according to Abdo et al. (1) using T-rex (13) (http://tlex.biocnp.org), and T-RF sizes were aligned to the nearest integer. In each sample, T-RF heights were normalized by the total height of T-RFs in that sample. The UPGMA clustering analysis was performed using the WPGMA cluster analysis based on Jaccard similarity then was confirmed by performing the PAIe-co-ecology STatistics (PAST) freeware package, version 2.03 (27) (http://folk.uio.no/ohammer/past). Multiresponse permutation procedures (MRPP) in PCORD 5.10 (MJM Software) were used for testing significant differences in T-RF population structure between the different experimental groups.

Pyrosequencing of bar-coded 16S rRNA amplicons. To assess the diversity and phylogenetic affiliation of the root-associated bacteria in the biochar-treated plants relative to the controls, triplicates of extracted DNA were pooled together, and bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed on root-associated control and biochar-amended DNA samples in duplicate plants. The bTEFAP analyses were performed using universal eubacterial 16S rRNA gene primers 530F and 1003R as previously described (125, 16). The bTEFAP procedures were performed at the Research and Testing Laboratory (RTL, Lubbock, TX) using previously described protocols (51a).

**PCR and DGGE.** PCR amplification for DGGE was performed on control and biochar-amended root-associated DNA samples using 20 ng of extracted DNA as a template with the general bacterial primer pair 907R and 341F with a 40-bp GC clamp attached to its 5' end (44). PCR mixtures (final volume of 50 ml) contained the following components: 1.5 U Taq DNA polymerase (DreamTaq, Fermentas Life Science, Vilnius, Lithuania), Taq buffer containing a final magnesium concentration of 2.5 mM, deoxynucleoside triphosphates (dNTPs) (20 nmol each), 12.5 mM glycine serum albumin, and 25 pmol of each primer. The PCR program consisted of an initial denaturation step of 95°C for 180 s followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. Cycling was completed with a final elongation step of 72°C for 5 min. PCR amplicons were checked by agarose gel electrophoresis (1%) and staining with ethidium bromide.
Pyrosequencing data analyses. Following sequencing, all failed sequence reads, sequences of less than 300 bp, low-quality sequence ends, and tags were removed, and sequences were depleted of any nonbacterial ribosome sequences and chimeras using Black Box Chimera Check custom software B2C2 (23) (Research and Testing Laboratory). The resulting high-throughput data sets were analyzed using Mothur (www.mothur.org) (49) as outlined at http://www.mothur.org/wiki/Costello_stool_analysis. The analysis involved the following steps, in brief. (i) A 50-bp-long moving window was slid across the reads, and its average Phred quality score was calculated; whenever mean quality dropped below 35, the read was trimmed to the end of the last window with a mean quality score higher than 35. In mock community experiments, P. D. Schloss (personal communication) found this strategy to decrease the error rate 10-fold compared to the rate after not trimming the sequences. In addition, we removed any sequence where the longest homopolymer (e.g., TTTT) was longer than 8 bases and if it contained an ambiguous base call (e.g., N'). (ii) Reads were aligned to the SILVA-compatible database of Mothur (www.mothur.org/wiki/images/9/98/Silva.bacteria.zip), containing nearly 15,000 sequences. (iii) Chimeras were removed using the native Mothur algorithm. (iv) Sequencing noise was reduced using a method based on that of Huse et al. (31). Since abundant sequences are likely to generate sequences that are less abundant and differ from the dominant sequence type by about 1 base every 100 bases, these noise sequences are merged with the abundant sequence type (31). The high-quality sequences were phylogenetically classified using the RDP-II Classifier (55) with a bootstrap confidence estimate threshold of 80%. The classifier, available at http://sourceforge.net/projects/rdp-classifier, assigns 16S rRNA gene sequences to the bacterial taxonomy of Garrity et al. (20); its classification is accurate, stable, independent of sequence alignments, and suitable for very large data sets (11, 39). Due to a lack of the normal distribution in the data, we used a nonparametric Mann-Whitney test to evaluate significant differences in abundances of phyla and genera as a result of biochar amendment at a confidence level of 95%.

RESULTS AND DISCUSSION

The objective of this study was to assess the effect of biochar soil application on the structure and composition of bacterial communities in bulk soil and on the roots of 3-month-old sweet pepper plants. We focused on root-associated communities which most likely are involved in plant-microbial interactions. We hypothesize that the previously observed increase in plant growth and the induction of systemic resistance to plant disease (18, 24, 43) may be at least partially attributed to the proliferation of microbial elicitors that occurs as a result of biochar-stimulated shifts in the rhizosphere microbial community.

Molecular fingerprinting of bulk soil and root-associated bacterial communities. Culture-independent molecular fingerprinting analyses (T-RFLP and PCR-DGGE) of 16S rRNA gene fragments were performed to compare the community composition of root-associated and bulk soil bacteria in biochar-amended and nonamended (control) soils (Fig. 1). Hierarchical clustering of T-RFs digested with both MseI andMspI showed two distinct statistically significant (MRPP tests; A > 0.09; P < 0.01) root-associated and bulk soil clusters, demonstrating a strong rhizosphere effect on the bacterial community composition. Because the hierarchical clustering of the MseI- andMspI-digested T-RFLP patterns were very similar to each other, Fig. 1 shows the cluster analysis usingMspI. Differentiation between microbial community composition in bulk soil and the rhizosphere is well documented in the literature and is attributed to carbon-rich root exudates, which can
account for as much as 30% of a plant’s fixed carbon (38). Bulk soil and root-associated T-RF clusters each were characterized by the presence of separate, statistically significant (MRPP tests; $A > 0.06; P < 0.01$) subclusters representing biochar-amended and control (nonamended) samples (Fig. 1). This was supported by UPGMA clustering analyses of 16S rRNA gene fragments using PCR-DGGE (Fig. 2), where root-associated bacterial communities also formed two distinct clusters depending on the biochar application. Collectively these techniques demonstrated that biochar treatment alters the bacterial community structures of both the rhizoplane and the bulk soil.

**Pyrosequencing of bar-coded 16S rRNA amplicons.** The pyrosequencing of bar-coded 16S rRNA amplicons is increasingly used for the comprehensive analysis of bacterial community composition in heterogeneous environments, including soil and the rhizosphere (2, 40, 47). We applied bTEFAP (bacterial tag-encoded FLX amplicon pyrosequencing) to evaluate root-associated bacterial community composition in biochar-amended and control plants, with the objective of identifying specific biochar-induced phyla potentially involved in induced plant resistance and plant growth promotion. Bacterial tag-encoded FLX amplicon pyrosequencing analysis was performed following the PCR amplification of root-associated DNA from duplicate 0 and 3% biochar-amended pots as described in Materials and Methods. A total of 20,142 OTUs were generated, with an average of 5,035 sequences per sample. A total of 1,134 and 2,611 OTUs for the control duplicates and 1,899 and 996 OTUs for the biochar-amended duplicates remained following quality control and filtering using the criteria detailed in Materials and Methods. These high-quality sequences were classified to the genus level and used for further phylogenetic analyses. The overwhelming majority of control (95.7%) and biochar-amended (92.5%) root-associated bacteria were associated with four primary phyla, *Proteobacteria, Bacteroidetes, Actinobacteria,* and *Firmicutes*, which represented 72% ± 7.4% and 47% ± 2.5%, 12% ± 1.0% and 30% ± 5.7%, 6.6% ± 3.8% and 10.4% ± 2.1%, and 5.9% ± 2.9% and 5.1% ± 0.2% of the total classified phyla in the control and biochar-amended samples, respectively (Fig. 3). Approximately 75% of the sequences from both the control and the biochar-amended samples belonged to the *Proteobacteria* and *Bacteroidetes* phyla, similarly to community trends documented in other previously described soil environments (4, 40, 47). The relative abundance of the *Bacteroidetes* phylum was substantially higher in the biochar-amended root-associated community, whereas the relative abundance of the *Proteobacteria* phylum was much greater in the control samples (12 versus 30% and 72 versus 48% for the control and biochar samples, respectively) (Fig. 3). Traditional isolation techniques conducted in this study using the selective medium HA-Agar (29) (data not shown) and previously conducted studies using both molecular and culture-dependent techniques (33, 43, 45) have indicated that the relative abundance of *Actinobacteria* is induced in biochar-amended soils. Nonetheless, pyrosequencing analysis did not show significantly higher abundance of this phylum in the biochar samples (Fig. 3).

Although no significant differences were observed in the levels of genus richness between the biochar-amended and control samples (averages of 195 and 172 unique genera for duplicate samples, respectively), the composition of selected genera significantly differed between the two treatments. Comparative analysis of pyrosequencing data identified several genera with significantly different levels of abundance in the control and biochar-amended samples (Fig. 4).

*Flavobacterium* appeared to be the genus most significantly affected by biochar. The relative abundance of this group was 4.2% ± 2.9% of the total genus-defined root-associated OTUs in control samples and it was 19.6% ± 4.5% in the 3% biochar-amended samples (Fig. 4), explaining to a great extent the substantially higher levels of *Bacteroidetes* detailed in Fig. 3. Multiple bacterial community characterization techniques, including classical plate isolation (21), 16S rRNA gene fragment clone library screening (34), and pyrosequencing (3, 40), have demonstrated the substantial abundance of *Flavobacterium*...
strains in plant root environments, which in some cases may exceed 5% of the total bacterial population.

Members of the Flavobacterium genus are widely distributed in nature, where they play a role in mineralizing various types of organic matter (carbohydrates, amino acids, proteins, and polysaccharides). They often possess an arsenal of extracellular enzymes, such as proteinases and chitinases, which enable them to degrade bacteria, fungi, insects, and nematode constituents (9), and often they produce secondary metabolites, including a wide range of antibiotics (12, 42). Most Flavobacterium species are characterized by gliding motility that is facilitated by a novel unique secretion system (48) that enables rapid movement of up to 5 mm s\(^{-1}\) on solid surfaces (32). Interestingly, this secretion system also is responsible for the transport of extracellular chitinase. Certain Flavobacterium isolates have been shown to have biocontrol capabilities. For example, selected Flavobacterium isolates from sunflowers, apples, and bananas were highly antagonistic toward the soil-borne fungal pathogens Sclerotium rolfsii, Lasiodiplodia theobromae, Cellulosirhodans musae, and Phytophthora cactorum, which can infect a wide range of agricultural and horticultural crops (5, 25, 30). In addition, some flavobacterial strains possess ISR-inducing capacities, potentially attributed to oligomeric elicitors generated from macromolecule degradation (6, 7). We hypothesize that the gliding motility, wide spectrum of extracellular enzymes, and antibiotic production capacity enhances the rhizosphere competence of these flavobacteria, potentially explaining their ubiquitous presence on plant roots and their potential role in induced plant growth and disease resistance. Nonetheless, it still is not clear why the relative abundance of this genus increased so significantly on the biochar-amended roots.

Besides Flavobacterium strains, which are well-known for their ability to rapidly digest insoluble chitin (42), we also observed the significant induction of other hydrolytic enzyme-producing genera, such as Chitinophaga (Bacteroidetes) and Cellvibrio (Betaproteobacteria). The relative abundance of these genera was 0.05% ± 0.06% of total root-associated OTUs in control samples versus 0.5% ± 0.3% in the biochar-amended samples and 0.06% ± 0.03% in control samples versus 1.6 ± 0.2% in biochar-amended samples, respectively (Fig. 4). We suggest that these biopolymer-degrading bacteria mineralize chitin in the outer shells and cell walls of rhizosphere-associated arthropods and fungi (35). Oligomeric products of chitin degradation are well-known elicitors of ISR (46), and this may partially explain the induced resistance observed in the biochar-amended experiments (18). However, it currently is not clear why these chitin-degrading genera were induced in the biochar-amended samples.

Additional biochar-stimulated genera not affiliated with plant growth stimulation or induced plant resistance in the literature included Hydrogenophaga and Dechloromonas, whose relative abundance was 0.2% ± 0.02% in control samples versus 0.7% ± 0.16% in the biochar-amended samples and 0.06% ± 0.08% in control samples versus 2.2% ± 1.6% in the biochar-amended sample, respectively (Fig. 4). Hydrogenophaga spp. were shown to dominate biphenyl catabolism in a horseradish (Armoracia rusticana) rhizosphere contaminated with polychlorinated biphenyls (PCBs), which are naturally present in coal tar, crude oil, and natural gas (53). In addition, Hydrogenophaga spp. can utilize the aromatic contaminant 4-aminobenzenesulfonate (4-ABS) as the sole carbon, nitrogen, and sulfur source under aerobic conditions (19). Dechloromonas spp. are found in soil environments, where they can oxidize toluene, benzene, and chlorobenzene, generally with no detrimental effects on adjacent plants (10). Previously, the chemical analyses of the biochar used in this study revealed that it is enriched with an array of aromatic compounds, such as phenol, methyl-phenol, and dihydroxybenzenes (24). This may explain the enrichment of these aromatic compound degraders in the biochar-amended samples.

Biochar amendment appeared to be antagonistic toward the Pseudoxanthomonas genus (Gammaproteobacteria), where the average number of characterized OTUs was 1.6% ± 0.5% versus 7.3% ± 7.0% of the total characterized genera in the control samples (Fig. 4). Several Pseudoxanthomonas species are known opportunistic plant pathogens that are able to attack a diverse array of economically important crops (52).

Conclusions. This study shows a clear shift in the root-associated microbial community structure of sweet pepper plants grown in biochar-amended soil, characterized by a substantial induction of several chitin- and aromatic compound-degrading genera. We suggest that physical and chemical factors (biochar-associated organic compounds) collectively are responsible for the observed community shift and that induced bacterial communities are at least partially responsible for the induced growth and plant resistance phenomena observed in previously described experiments (18, 24). Current research is focusing on assessing the influence of specific biochar components (the biochar carbon skeleton, particle size, specific biochar-associated organic fractions, etc.) on the bacterial community composition in correlation with the observed plant physiology (in-
duced disease resistance and growth promotion) and on assessing the potential role of biochar-amended soil-associated flavobacterial isolates in plant growth and disease resistance.

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REFERENCES

1. Abdo, Z., et al. 2006. Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Environ. Microbiol. 8:292–938.

2. Acosta-Martinez, V., D. Acosta-Mercado, D. Sotomayor-Ramirez, and L. Cruz. 2010. Flavobacterium communities and enzymatic activities under different management in semiarid soils. Appl. Soil Ecol. 39:249–260.

3. Acosta-Martinez, V., S. Dowd, Y. Sun, and V. Allen. 2008. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Sci. Biochim. 40:2762–2770.

4. Acort, Maait M. V., S. E. Dowd, Y. Sun, D. Wester, and V. Allen. 2010. Pyrosequencing analysis for characterization of soil bacterial populations as affected by an integrated livestock-cotton production system. Appl. Soil Ecol. 45:27–35.

5. Alexander, B. J. R., and A. Stewart. 2001. Glasshouse screening for biological control agents of Phytophthora cactorum on apple (Malus domestica). N. Z. J. Crop Hortic. Sci. 29:159–169.

6. An, O. D., et al. 2008. Properties of an alginate-degrading Flavobacterium sp. strain LXA isolated from rotting algae from coastal China. Can. J. Microbiol. 54:314–320.

7. An, Q. D., et al. 2009. Alginase-degrading oligosaccharide production by alginate from newly isolated Flavobacterium sp. LXA and its potential application in protection against pathogens. J. Appl. Microbiol. 106:161–170.

8. Angel, R., M. I. M. Soares, E. D. Ungar, and O. Gillor. 2010. Biogeography of soil archaea and bacteria along a steep precipitation gradient. ISME J. 4:553–563.

9. Bernardel, J. F., and J. P. Bowman. 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing. BMC Bioinformatics. 2009. T-REX: software for the processing and analysis of T-RFLP data. Flavobacterium johnsoniae, a gliding bacterium community in potato roots. Microb. Ecol. 58:270–281.

10. Graber, E. R., et al. 2010. Biochar impact on development and productivity of pepper and tomato grown in fettigated soilless media. Plant Soil 337:481–496.

11. Gunasinghe, W. K. R. N., and A. M. Karunaratne. 2009. Interactions of Colletotrichum musae and Lactosiphon theobromae and their biocontrol by Panoea agglomerans and Flavobacterium sp. in expression of crown rot of "Embul" banana. Biocontrol 54:587–596.

12. Haas, D., and G. Defago. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. 3:307–319.

13. Hammer, O., D. Harper, and P. Ryan. 2001. PAST: palaeontological statistics package for education and data analysis. Paleont. Electron. 4:9.

14. Harman, G. E., C. R. Howell, A. Viterbo, I. Chet, and M. Lorito. 2004. Trichoderma species—opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2:43–50.

15. Hayakawa, M., and H. Nonomura. 1989. A new method for the isolation of Actinomyces from soil. Actinomycetologica 4:95–104.

16. Hebbar, P., O. Berge, T. Heulin, and S. P. Singh. 1991. Bacterial antagonists of sunflower (Helianthus Annuus) fungal pathogens. Plant Soil 138:131–140.

17. Huse, S. M., D. M. Welch, H. G. Morrison, and M. L. Sogin. 2010. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ. Microbiol. 12:1889–1898.

18. Jarrell, K. F., and M. J. Mcbride. 2008. The surprisingly diverse ways that prokaryotes move. Nat. Rev. Microbiol. 6:466–476.

19. Khodadad, C. L. M., A. R. Zimmerman, S. J. Green, S. Uthandi, and S. J. Foster. 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biol. Biochem. 43:356–392.

20. Kloepper, J. W., C. M. Ryu, and S. A. Zhang. 2004. Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94:1259–1266.

21. Lehmann, J., et al. 2003. Nutrient availability and leaching in an archeological anthrosol and a ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil 249:343–357.

22. Lichtfouse, E., et al. 2009. Rhizodeposition of organic C by plant: mechanisms and controls, p. 97. Sustainable agriculture. Springer, Amsterdam, Netherlands.

23. Liu, Z., T. Z. DeSantis, G. L. Andersen, and R. Knight. 2008. Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. Nucleic Acids Res. 36:e120.

24. Manter, D. K., J. A. Delgado, D. G. Holm, and R. A. Stong. 2010. Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microb. Ecol. 60:157–166.

25. Mathews, J. A. 2008. Carbon-negative biofuels. Energy Policy 36:940–945.

26. Mcbride, M. J., et al. 2009. Novel features of the polysaccharide-digesting gliding bacterium Flavobacterium johnsoniae as revealed by genome sequence analysis. Appl. Environ. Microbiol. 75:6864–6875.

27. Meller Harel, Y., et al. 2010. Induced systemic resistance to disease in plants by biochar. ICBMC/WRPS Bull., pp. press.

28. Menger, G., E. C. Deswaal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695–700.

29. O'Neill, B., et al. 2009. Bacterial community composition in Brazilian anthrosols and adjacent soils characterized using culturing and molecular identification. Microb. Ecol. 58:23–35.

30. Rajkumar, M., K. J. Lee, and H. Freitas. 2008. Effects of chitin and salicylic acid on biological control activity of Lasiodiplodia theobromae and their biocontrol by Panoea agglomerans spp. in damping off of pepper. J. Phycol. 44:268–273.

31. Roesch, L. F., et al. 2007. Pyrosequencing enumerates and contrasts soil microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Environ. Microbiol. 8:292–938.

32. Ruvkun, G., and L. H. Wolkenstein. 2010. Black box chimera check (B2C2): a Windows-based software for batch depletion of chimeras from bacterial 16S rRNA gene datasets. Open Microbiol. J. 4:47–52.

33. Sato, K., et al. 2010. A protein secretion system linked to bacterioide growth motility and pathogenesis. Proc. Natl. Acad. Sci. U. S. A. 107:276–281.

34. Schloss, P. D., et al. 2009. Introducing mothur: open-source, platform-inde-
dependent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75:7537–7541.

50. Silber, A., I. Levkovich, and E. R. Graber. 2010. pH-dependent mineral release and surface properties of corn straw biochar: agronomic implications. Environ. Sci. Technol. 44:9318–9323.

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

51a. Sun, Y., R. D. Wolcott, and S. E. Dowd. 2011. Tag-encoded FLX amplicon pyrosequencing for the elucidation of microbial and functional gene diversity in any environment. Methods Mol. Biol. 733:129–141.

52. Thierry, S., et al. 2004. Pseudoxanthomonas mexicana sp. nov. and Pseudoxanthomonas japonensis sp. nov., isolated from diverse environments, and emended descriptions of the genus Pseudoxanthomonas Finkmann et al. 2000 and of its type species. Int. J. Syst. Evol. Microbiol. 54:2245–2255.

53. Uhlik, O., et al. 2009. Biphenyl-metabolizing bacteria in the rhizosphere of horseradish and bulk soil contaminated by polychlorinated biphenyls as revealed by stable isotope probing. Appl. Environ. Microbiol. 75:6471–6477.

54. Vallad, G. E., and R. M. Goodman. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Sci. 44:1920–1934.

55. Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73:5261–5267.

56. Wardle, D. A., O. Zackrisson, and M. C. Nilsson. 1998. The charcoal effect in boreal forests: mechanisms and ecological consequences. Oecologia 115:419–426.

57. Warnock, D. D., J. Lehmann, T. W. Kuyper, and M. C. Rillig. 2007. Mycorrhizal responses to biochar in soil—concepts and mechanisms. Plant Soil 300:9–20.

58. Zimmerman, A. R. 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environ. Sci. Technol. 44:1295–1301.