EFFECTS OF SOIL AMENDMENTS ON SOIL BACTERIAL DIVERSITY

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Introduction

Soil is a complex biological system; approximately five percent of soil space is occupied by organisms, especially bacteria. A single gram of soil is estimated to contain to one hundred million to a billion individuals and from between three to ten thousand species. These organisms are responsible for many of the functions of soil such as cycling of nutrients, including carbon, nitrogen, sulphur and phosphorus, disease suppression, and the degradation of complex and often potentially toxic compounds such as pesticides. The makeup of soil bacterial is affected by many factors. The most important of these are organic carbon content, moisture, aeration, pH, temperature and inorganic nutrients. Amending the soil with compost, fertiliser and other additives can alter these factors. Diversity is a complex concept: not only does it include the range and abundance of species, but it also incorporates richness at the gene level. This study highlights the changes seen in bacterial diversity following the addition of compost and biochar to orchard soil. The changes at phyla level and in genes involved in the nitrogen cycle (Figure 1) were used to study this diversity.

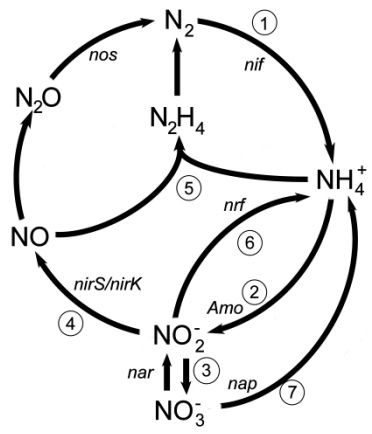

Figure 1. Reactions of the microbial nitrogen cycle.
(1) Nitrogen fixation;
(2) aerobic ammonium oxidation;
(3) aerobic nitrite oxidation;
(4)denitrification;
(5) anaerobic ammonium oxidation;
(6) dissimilatory nitrite reduction and
(7) dissimilatory nitrate reduction.
Genes examined in this study: *Amo*: ammonia monooxygenase, *nap*: periplasmic nitrate reductase, *nar*: nitrate reductase, *nif*: nitrogenase, *nirS*/*nirK*: nitrite reductase (cytochrome cd1 and copper containing forms), *nos*: nitrous oxide reductase and *nrf*: periplasmic cytochrome c nitrite reductase.

Methods

Soil was sourced from the Stevenson's Orchard Mountain River, Tasmania, Australia, where separate plots were amended with biochar, compost, both or nothing (control). For each of four treatments (control, compost, biochar and both compost and biochar), ten samples of soil (from the top 10 cm) were collected and mixed. DNA was extracted from 0.5 g soil samples using RNA PowerSoil™ Total Isolation kit with the DNA Elution Accessory kit (Mo Bio, Carlsbad, CA, USA). Relative levels of genes were determined by qPCR in a LightCycler 480 using LightCycler 480 SYBR Green I Master (Roche Applied Science) as described in the manufacturer's instructions. Each reaction contained 2.5 ng of. Primers used and their annealing temperatures and extension times for qPCR are given in Table 1. qPCR consisted of a pre-incubation period of 5 minutes at 95 ºC, forty amplification cycles of 95 ºC for 10 seconds, annealing temperature for 10 seconds and 72 ºC for extension time; finally melting curves were obtained by increasing temperature from 65 ºC to 97 ºC over 5 minutes. Negative controls consisted of water. Any reactions where amplification product in the sample was detected less than six cycles before that of the negative control were ignored in analysis. The raw relative abundance data were log transformed, as the large differences in abundance (up to one billion fold) would lead to some data being virtually ignored in the statistical analysis. These transformed data were used to generate box plots and a step-wise discriminant analysis to compare the effects of the different treatments on the bacterial diversity.

Target	Forward Primer	Reverse Primer	Source	Annealing Temperature	Extension Time
Eubacteria 16S rRNA	Eub338	Eub518	Fierer	55	12
Fungal 16S rRNA	ITS1f	5.8s	Fierer	55	12
Acidobacterium 16S rRNA	Acid31	Eub518	Lesaulnier	55	12

Table 1. Sources and conditions for primers used in this paper.

Bacilli 16S rRNA	BL3342F	Eub518	Lesaulnier	60	10
Firmicutes 16S rRNA	Lgc353	Eub518	Fierer	60	10
□-Proteobacteria 16S rRNA	ADF618F	Alf685	Lesaulnier	60	14
□-Proteobacteria 16S rRNA	Eub338	Bet680	Lesaulnier	60	14
Planctomycetes 16S rRNA	Plancto352f	Plancto920r	Mühling	60	24
□-Proteobacteria 16S rRNA	Gamma395F	Gamma871R	Mühling	55	20
Cyanobacteria 16S rRNA	CYA361f	CYA785r	Mühling	60	17
Basidiomycota 16S rRNA	ITS4b	5.8sr	Fierer	60	20
AmoA	A189f	A682r	Holmes	55	24
nirS	NirS832F	NirS3R	Smith	60	28
nosZ	NosZ2F	NosZ2R	Henry	60	20
nifH	nifH-F	nifH-R	Rösch	60	18
nirK	nirK876F	nirK5R	Henry	60	7
narG	narG1960m2f	narG2050m2r	Smith	60	5
napA	napA67F	napA67R	Smith	60	16
nrfA	nrfAF1	nrfA7R1	Smith	60	20

Results

There was considerable variation in the relative abundances measured within the ten samples of a single treatment. Figure 2 shows the distribution of abundances for the bacterial phyla under the four treatments, and the variation between the highest and lowest is seldom less than tenfold. The nitrogen metabolism genes displayed a similar result and have been omitted for clarity. The variation within a treatment was often similar to that between the different treatments, and the difference between treatments was not statistically significant for any of the phyla tested. Those phyla of bacteria that were most common, Bacteroidetes and

Firmicutes, are those most common in nearly all soils, and together make up more than 99% of the bacteria measured. Alphaproteobacteria are a subphylum containing many phototrophic bacteria, but do include the genus *Paracoccus*, able to oxidise nitrate.

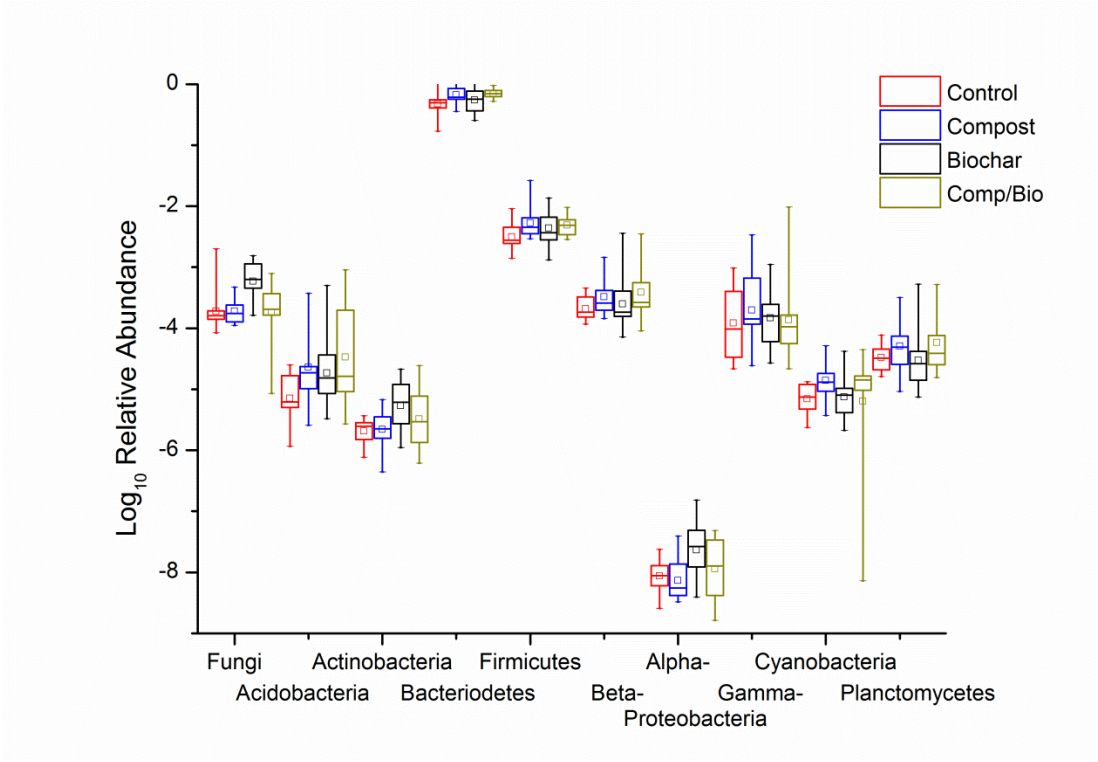

Figure 2. The spread of relative abundances for each treatment for all the bacterial phyla measured. The whiskers give the spread from highest to lowest value for each, with the large box enclosing the first to third quartiles. The median is the bar within the box, while the average is the small square.

A step-wise discriminant analysis adds one factor at a time (in this case the qPCR results for a gene) looking for those that separate the treatments as effectively as possible, until the treatments are best separated. The result is a diagram of the separations and the underlying vectors used to create the diagram; the longer the vector, the more important the factor is in resolving the treatments.

The analysis of these results required fourteen factors to give the best separation, and even then the Biochar and Biochar/Compost treatments are not fully resolved (Figure 3). However, there are clear transformations associated with additions. The control soil is in the bottom left corner, and adding biochar shifts the treatment to the left, while adding compost shifts it up. Looking at the underlying vectors shows that addition of biochar leads to increases of Alphaproteobacteria and nitrate and nitrous oxide reductase. These last two are involved in denitrification, the oxidation of nitrate to nitrogen and subsequent loss as atmospheric nitrogen; this process tends to happen in conditions of low oxygen and the enzymes involved are mainly found within Proteobacteria. Here, since Beta- and Gammaproteobacteria decrease, this suggests that the increase is directly linked to Alphaproteobacteria.

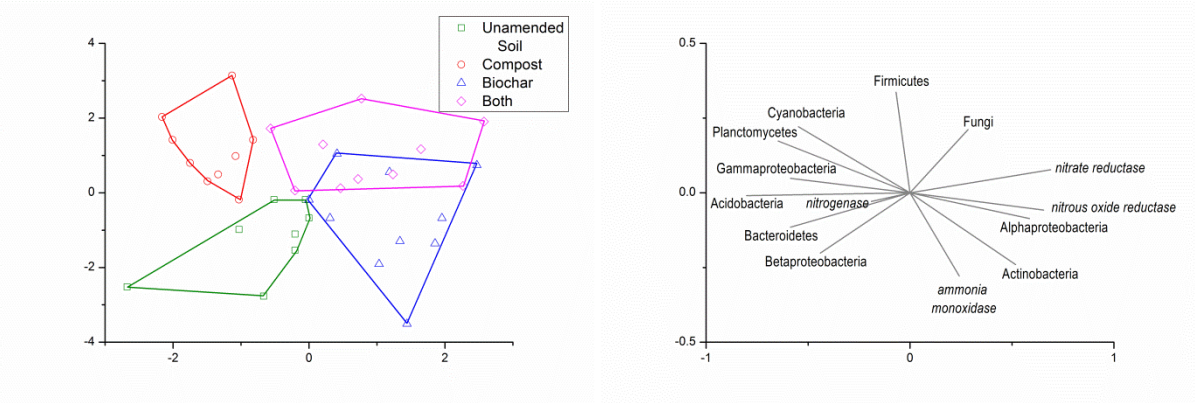

Figure 3. Stepwise Discriminant Analysis. Both the visualisation of the analysis and the underlying vectors used to create the visualisation are given.

Conclusions

This study shows that the amendment of soil with compost and biochar does lead to changes in bacterial diversity. However, these changes are modest, and require sophisticated analysis over a number of factors over many samples to be distinguished. It is possible that measuring a narrower group of bacteria, such as the genus *Paracoccus*, to hone in on specific factors may give a better idea of changes in diversity associated with soil emendation.

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References

Fierer N, Jackson JA, Vilgalas R, Jackson RB. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71:4117-4120, 2005.

Henry S, Bru D. Stres B, Hallet S, Philippot L. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Applied and Environmental Microbiology 72:5181-5189, 2006.

Holmes AJ, Costello A, Lidstrom ME, Murrell JC. Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related FEMS Microbiology Letters 132:203–208, 1995.

Lesaulnier C, Papamichail D, McCorkle S, Ollivier B, Skiena S, Taghavi S, Zak D, van der Lelie D. Elevated CO2 affects soil microbial diversity associated with trembling aspen. Environmental Microbiology 10:926-941, 2008.

Mühling M, Woolven-Allen J, Murrell JC, Joint I. Improved group-specific PCR primers for denaturing gradient gel analysis of the genetic diversity of complex microbial communities. The ISME Journal 2:379-392, 2008.

Rösch C, Mergel A, Bothe H. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Applied and Environmental Microbiology 68:3818-3829, 2002.

Smith CJ, Nedwell DB, Dong LF, Osborn AM. Diversity and abundance of nitrate reductase genes (narG and napA), nitrite reductase genes (nirS and nrfA), and their transcripts in estuarine sediments. Applied and Environmental Microbiology 73:3612-3622, 2007.