

The composition and diversity of prokaryotic and eukaryotic communities from an Australian Vertisol: An experimental study

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Abstract

Biological diversity in soils has been linked to many functional processes. A conjoint measurement of biodiversity of all three Domains (Archaea, Bacteria and Eukarya) has seldom been attempted in soils. We measured biodiversity of bacteria and eukarya (fungi, micro- and mesofauna) under controlled laboratory conditions, studying the dynamics of a detrital food web in a self-mulching Vertisol from New South Wales, Australia. Previous site use history (including vetch in the rotation) had a greater impact on biotic diversity than short-term additions of stubble.

Key Words

Soil food web, biodiversity, 16S rRNA, prokaryotes, eukaryotes, microfauna

Introduction

Soil disturbances caused by natural or human activities have direct impacts on ecosystem properties and function, such as nutrient cycling and physical and chemical complexity. Soil, one of the largest reservoirs for bacteria on earth, and its processes are greatly influenced by bacterial, fungal and faunal community structure, activity, and stability (Whitman *et al.* 1998; Coleman & Whitman 2005). The use of molecular, culture-independent based techniques has led to a new understanding of microbial diversity (Hugenholtz *et al.* 1998; Janssen 2006). Most approaches exploit sequence variation in the small subunit of the ribosomal RNA gene (ssu rRNA). Using DNA extracted from whole soil communities, ssu rRNA sequence variation can be used to rapidly profile the community structure and diversity within each of the three domains (DGGE, TRFLP, SSCP, ARISA etc). Detailed analysis of near full-length ssu rRNA gene clone library sequences can then be used to determine the composition of species within soil microbial environments, an essential step towards understanding the role of these communities and their effects on ecosystem processes.

Detrital food webs have been analyzed in a wide range of soils and ecosystem types. Many of these have been described and analyzed in agricultural and forested lands, usually in heavily-textured soils, such as sandy loams or loamy sands in Alfisols, Mollisols, and occasionally in Ultisols (Wardle 2002; Coleman *et al.* 2004). To our knowledge, no detrital food-webs have been investigated in heavy clay soils, such as self-mulching Vertisols. To identify differences in the composition and diversity of the bacterial and eukaryotic communities in an agricultural soil, we carried out a study using a self-mulching Vertisol from eastern New South Wales, Australia.

The objective of this study was to measure the prokaryotic and eukaryotic diversity in an Australian Vertisol that had varying previous histories of land-use. In pursuing this study, we hypothesized that treatments that had vetch grown in the crop rotation would have more available organic and inorganic N, and this would be reflected in a more speciose array of bacteria, fungi and soil micro- and mesofauna as well.

Methods

Soil and site description

Vertisol soils were taken from two field sites at the Australian Cotton Research Centre, Narrabri, NSW. The previous history of the sites was as follows: one site had cotton-wheat-cotton (designated cwc) in summer-winter rotation. The other field had cotton-wheat-vetch-cotton rotation (designated cwvc). Samples (10kg apiece, of 0-10 cm soils) were shipped to Adelaide, sieved and air dried before use. The pH was near neutral, soil organic carbon ranged from 0.95 to 1.03 % in the two treatments. Total nitrate-N for the cwvc treatment was 50 mg/kg, while the cwc rotation was nearly 142 mg/kg. All other inorganic characteristics were very similar.

Handling of soil, setup of the experiment

Triplicate samples of each soil type were set up in 5 cm. dia x 5 cm depth PVC cores to a bulk density of 1.2 in four permutations: cwc soil alone; cwc soil with 1 g. wheat stubble sprinkled on top of it (cwcs); cwvc soil alone; cwvc soil with 1 g. wheat stubble on top (cwvcs). Replicate cores were moistened to field moisture capacity and maintained there, and others were subjected to a wetting and drying regime.

Soil DNA extraction

Samples of soil from individual cores were taken at the end of the experiment (40 d) and mixed community DNA was extracted from duplicate 0.4 g soil samples using the MoBio UltraClean soil DNA extraction kit (MoBio Laboratories, CA). Mechanical disruption via bead-beating (FastPrep FP100; Bio101) was used to increase recovery of DNA from the heavy-textured soil. Following DNA extraction, the duplicate aliquots of DNA were combined giving a final volume of 100uL in Tris-buffer. The bacterial cultures were re-grown at the UGA sequencing facility for isolation of plasmid DNA and the 16S rRNA gene inserts were partially sequenced using the primer 27F. Quality checks of the sequences, editing, chimera analyses, and alignments were carried out as described in Jangid *et al.* (2008).

Fungal PCR-DGGE

DGGE was used separate the mixture of fungal sequences within the PCR based on sequence variability (binding strength) and used the Ingeny phorU system (Ingeny International, The Netherlands).

Extraction and enumeration of the soil mesofauna

Protists were identified in dilution-well plates with inverted microscopy, using bacterial (*Pseudomonas* sp.) broths to feed the protozoans. Nematodes were extracted in modified Baermann funnels over 48 h and preserved in 4% formalin (Coleman *et al.* 1999). They were counted in trophic groups and analyzed accordingly.

Results

Bacterial diversity, 16S rRNA gene libraries

We sampled four soil treatments, with three replicates taken per treatment for a total of twelve., Between 70-80 clones from the 16S rRNA gene library for each soil sample resulted in good quality sequences (Table 1). Our low PCR cycle amplification resulted in a very low frequency of chimeric sequences, a total of 14, amongst the 937 sequences analyzed, resulting in a total of 923 sequences (Table 1) that were analyzed further. LIBSHUFF comparisons of the replicate libraries indicated no significant difference between them. Thus, the methods for extracting DNA and cloning were reproducible, and the samples generally appeared to be representative of each site. However, all three replicates of cwc were different, $p = 0.002$. Sequences from the replica cores were then combined for further analyses. Because all libraries contained sequences from bacterial groups that were difficult to lyse (e.g., *Actinobacteria*), cell lysis during the extraction was considered complete.

Phylogenic groups represented in clone libraries

Close to one-half of the sequences within each library were only distantly related to cultured organisms. These clones were placed by RDPquery into the "unclassified" group (Table 1), indicating that they possessed less than 80 % sequence similarity to a sequence from a type strain in the RDP database.

In spite of their low similarity to genes from cultured organisms, many of these clones were closely related to other environmental clones obtained from soil. Thus, the composition of these libraries was similar to that found in other soil communities, and these communities are well represented in the data bases. The large number of unclassified clones was due to poor representation of soil bacteria among the type strains in culture collections. Many of these clones represented deep branches of phyla with only a few cultured representatives, such as *Acidobacteria*, *Nitrospira* and *Chloroflexi*.

For clones that were classified by RDPquery, the most abundant phylum was *Proteobacteria*, which consisted of 37% of the total number of clones (Table 1). Amongst them, the β -*Proteobacteria* was the largest proteobacterial group within all the libraries (12%) and included clones similar to many common soil bacteria, such as nitrifying bacteria and *Rhizobiaceae*. The second most abundant phylogenetic group was *Firmicutes*, with about 19% of clones. The third most abundant phylum was *Acidobacteria*. The fourth largest phylum, 8.6% of clones, represented another well known soil group, *Bacteroidetes*. The remaining phyla present within the libraries included *Planctomycetes*, *Verrucomicrobia*, and *Gemmatimonadetes*. Each consisted of less than 3% of the clones.

Table 1. Australian Vertisol microbial analyses, showing Taxa and experimental treatments: 1-3 = soil alone; 4-6 = soil plus straw; 10-12 = vetch soil alone; 13-15 = vetch soil plus straw.

Phylum	Treatments												
	1	2	3	4	5	6	10	11	12	13	14	15	
Acidobacteria	11	4	9	5	9	8	13	7	13	12	9	7	
Actinobacteria	5	5	8	9	9	3	7	11	5	5	4	6	
Bacteroidetes	4	9	3	10	11	9	3	6	4	4	8	9	
Firmicutes	2	0	1	2	2	1	2	1	0	0	0	0	
Gemmatimonadetes	3	5	3	6	4	2	3	1	2	2	1	2	
Alphaproteobacteria	8	5	10	19	8	8	3	1	2	2	13	8	
Betaproteobacteria	8	6	5	9	8	7	16	3	15	15	7	12	
Deltaproteobacteria	4	3	3	3	1	6	3	0	4	3	1	4	
Gammaaproteobacteria	6	5	5	5	3	9	3	8	2	2	3	3	
Unclassified Proteobacteria	4	2	4	2	4	2	6	1	6	8	8	6	
Verrucomicrobia	2	4	5	2	4	1	1	5	0	0	2	0	
Others	25	23	28	12	19	13	16	27	26	23	14	22	
Total (all taxa)	82	71	84	84	82	69	76	71	79	76	70	79	923

Diversity indices

The soil bacterial community was very diverse. The Shannon indices for each library were 0.88-0.93 of their maximum values, and the Chao1 estimators were much higher than the number of sequences examined. Similarly, the rarefaction curves failed to plateau, even when similar libraries were combined. For these reasons, the libraries only sampled a small portion of the bacterial diversity present in the samples.

Fungal numbers and diversity

Crop and stubble treatments significantly ($p < 0.001$) affected fungal community structure (data not shown).

Mesofaunal numbers and diversity

Nematodes were more diverse in soils with a previous history of vetch in the field rotation history; only stubble treatments in wetting-drying regimes were significantly separated (Figure 1).

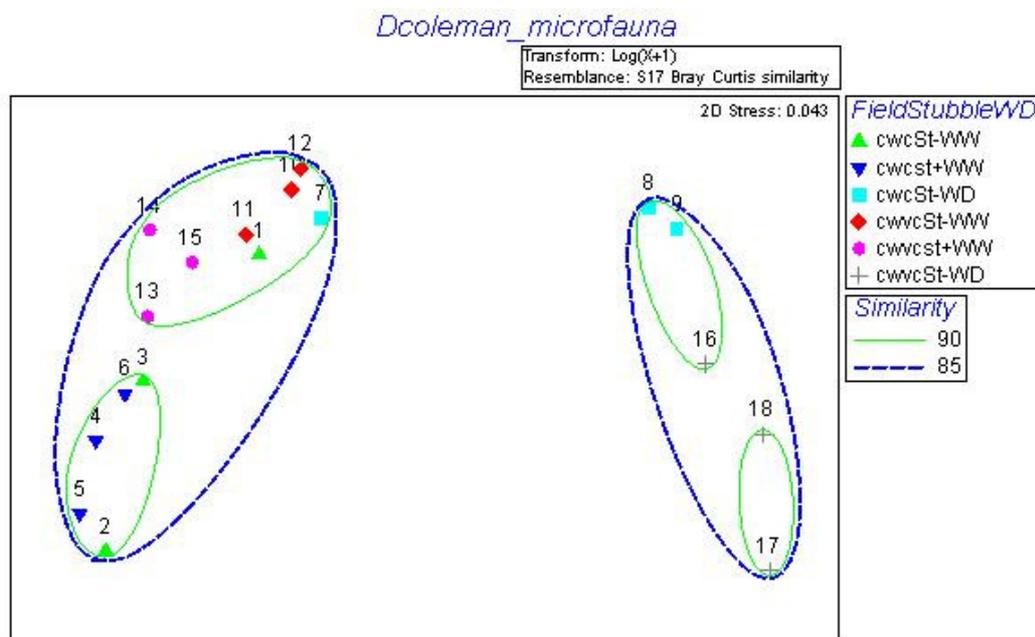


Figure 1. PCA-plots of nematodes in Narrabri Vertisol microcosms.

Conclusion

The Rep Clones that were significantly greater ($p < 0.01$) in the vetch rotation were: Proteobacteria-Betaproteobacteria and Proteobacteria-unclassified bacteria. Proteobacteria-Alphaproteobacteria, and Gemmatimonadetes. Only Bacteroidetes and Alphaproteobacteria were significantly lower in straw-amended soils. Site history seems to have greater influence than short-term carbon additions in these experimental conditions.

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