Utilising microarray technology to investigate soil microbial ecology

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Abstract
Microarray technology provides a tool for assessing the transcriptional response or abundance of thousands of microbial functional or structural genes concurrently. Two new microarrays have been designed to detect a range of microbial functional gene targets for enzymes associated with the cycling of soil C, N, P and in antibiotic production, biodegradation and methane oxidation. A Phylochip structural array based upon 16S ribosomal RNA sequences of bacteria and archaea was also used to examine soil microbial communities. The three microarrays have been used in two experimental designs, one based on contrasting soil types and land-uses, the second in a climate change scenario of elevated CO_2 and warming. The functional microarrays provided valuable tools for the quantitative description of key soil microbial process associated with soil health, that are essential for the productive and sustainable management of natural and agricultural ecosystems. The Phylochip structural microarray elucidated major differences in bacterial community structure based upon the effects of climate change. Collectively, microarray technology represents a significant improvement in rapid and precise measurements of soil functions and responses to management. It therefore has enormous potential for application in land management decision support processes.

Key Words
Microarray, microbial ecology, functional genes, 16S, climate change

Introduction
Australian soils are under increasing pressure to provide a range of agroecological goods and services to meet the growing global demand for food, fibre and bioenergy. Current land-use practices are likely to influence soil ecosystem health and the underlying microbially-mediated functions related to plant nutrient supply (Kibblewhite *et al.* 2008). Furthermore, the influence of climate change on soil ecosystem health related to plant-available soil moisture and uptake efficiencies of soil-immobile nutrients will require detailed analyses of below ground process. A combination of high resolution molecular tools as well as lower resolution methods such as microbial biomass, gross N mineralization, microbial immobilization and net N mineralization may assist in predicting how microbial activity and rates of soil carbon and nitrogen cycling will change with increases in atmospheric CO_2.

Microarrays offer specific, sensitive, quantitative, and high-throughput tools for microbial detection, identification, and characterisation in soil ecosystems (Blalock 2004). While there are issues involved with their adoption such as the cost of the technology, sample preparation for hybridisation and the data analysis of information captured from thousands of probes simultaneously, progress is being made in the use of microarray technology for studying microbial ecology. Phylogenetic microarrays based upon the conserved marker 16S ribosomal RNA (rRNA) gene have been used to compare the relatedness of communities in different environments (Brodie *et al.* 2006). Functional gene arrays based upon functional genes that code for proteins catalysing processes such as the carbon, nitrogen, and sulfur cycles have been utilised to ascertain the potential of microbial populations to conduct these processes in soil (Wu *et al.* 2001).

Here we report on the development and demonstration as a proof of concept of two microarrays designed using CombiMatrix CustomArray technology to assess microbial functions associated with the health of Australian soils and climate change. The Soil Health Array was designed to quantify the microbial activity associated with environmental functions including nitrogen and phosphorus cycling, organic compound degradation, and antibiotic and plant growth promoting substances in a range of contrasting soils, in order to tentatively identify the environmental factors driving the change in soil function. The functional nitrogen and carbon (FuNC) array was designed to focus on nitrogen and carbon cycling by microbes in an environment

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under the simulated effects of climate change with elevated CO_2 and warming at TasFACE (a Free Air Carbon dioxide Enrichment facility) in Tasmania. The Phylochip structural array, developed at the Lawrence Berkeley National Laboratory, was also used in parallel with the FuNC array to investigate the effects of climate change on microbial community structure at TasFACE. The use of microarrays will provide valuable tools for the quantitative description of key soil microbial processes and microbial community structure associated with climate change and soil health. Knowledge of soil microbial communities and their function gained from the use of arrays can be used to inform decision making for the productive and sustainable management of natural and agricultural ecosystems.

Methods

Soil Ecosystems

As a proof of concept for the Soil Health Array, soil samples were collected from paired sites of two different land-uses (managed and remnant) within two different geomorphic zones and Soil Orders in Victoria. Three locations were sampled in the 'Eastern Uplands' of North-East Victoria where the Dermosol soil is characterised by a strongly acidic surface soil and three locations in the 'North Western Dunefields and Plains' zone of North-West Victoria where the Calcarosol soil has a neutral to slightly alkaline surface soil. The 'managed' sites were agricultural sites that were cropped or under pasture, with varying levels of agricultural inputs. The 'remnant' sites comprised small parcels of land that were either State Parks or shelter belts where native plant ecosystems remained. At each location, soil cores were collected from the paired land-use sites, and DNA extracted using a Bio101 Fast DNA Spin Kit. The sites studied were chosen because of the additional metadata (chemical and microbial) available for comparison to the microarray gene data.

The FuNC array and Phylochip were utilised to examine the effects of climate change on the soil microbial community. TasFACE in Tasmania, Australia, is an experimental field site for examining the effects of increased temperature and atmospheric carbon dioxide concentrations on a native pasture. TasFACE has been running for more than 8 years with changes in the plant community documented over time (Hovenden *et al.* 2008). Soil samples were collected in October 2007 (the 6th year of treatment). Sixteen different treatments were tested to examine interactions between elevated CO_2 (FACE or control), elevated temperature (warmed or unwarmed), plants (the dominant grass *Themeda* spp. or non-*Themeda*), and soil depth (0-5cm or 5-10cm). DNA was isolated from soil using a MoBio Powersoil kit.

Soil Health Array

Several functional families were targeted for sequence selection including nitrogen cycling, phosphate cycling, 1-carbon oxidation, biodegradation and global activators. Gene sequences from the bacterial and archaeal domains were obtained for 68 functional groups in the nine functional families. Probes were designed from selected sequences using Oligoarray 2.1, with 2933 probes selected. The microarray probes were synthesised on a CombiMatrix 12K CustomArray and replicated four times across the array. Twelve samples of soil DNA (3 µg) were labelled and hybridised to the array and the resultant data analysed to select probes that showed substantial difference across the samples.

FuNC array

Functional families representing the processes of nitrogen and carbon cycling in bacteria, archaea and fungi were targeted for sequence selection for probe design. Carbon cycling functions such as photosynthesis, methanogenesis, C decomposition and, carbohydrate, pectin, cellulose, hemicellulose and lipid catabolism, were represented by 6678 sequences from Genbank. A suite of genes representing N cycle functions such as ammonia and nitrite oxidation, nitrogen fixation, and the reduction of nitrite, nitrate, nitric oxide and nitrous oxide were represented by 20738 sequences. The array included probes that were designed to provide either high levels of coverage within functional groups ("4-hitters") or high levels of specificity to target sequences ("specific"). The microarray probes were designed for a 40K block and synthesised onto a CombiMatrix 90K CustomArray as two replicated blocks. Forty-two samples of soil DNA (1 µg) from TasFACE were hybridised to the array and the resultant data analysed to select probes that showed a substantial difference across treatments.

Phylochip

The Phylochip is a structural Affymetrix array for identifying bacterial and archaeal populations to subfamily level and was designed at LBNL (Brodie *et al.* 2006). The array contains ~300,000 probes that detect 8741 different bacteria and archaea representing two domains, 63 phyla, 136 classes, 262 orders and 455 families.

Forty-two samples of soil DNA from TasFACE were amplified with primers specific to the 16S rRNA subunit of bacteria and the 16S rRNA subunit of archaea, then the resultant PCR products were hybridised to the Phylochip. Fluorescence readings for each array were normalised and data analysed to select probes that showed a substantial difference across treatments.

Results

Soil Health Array

Probes designed for the array mapped to 5456 targets from an input sequence set of 6420 sequences, representing a probe design success rate of 85% for all sequences submitted. The results of the two-way hierarchical clustering of probe signal intensity from the 12 samples for a filtered set of 2770 probes are presented in Figure 1. Hierarchical clustering revealed six distinct probe signal intensity profiles (probe clusters) from across the samples analysed (horizontal clusters on the heat map), while the 12 soil DNA samples demonstrated three major patterns of probe signal intensity (sample clusters) shown as vertical clusters on the heat map. Samples can be seen to cluster on the basis of geomorphic region (eg North-West [NW] or north-East [NE]) or land-use (managed [M] or remnant [R]). Different N cycle genes were present in the probe clusters: ammonia monooxygenase (*amoA*) in clusters 1, 2 and 4; nitrogenase (*nifH*) was dominant in probe clusters 3, 5 and 6; methanol dehydrogenase (*mxaF)* was dominant in clusters 5 and 6; and nitric-oxide reductase (*NOR)* was most abundant in cluster 3. Particulate methane monooxygenase (*pmoA*) was also dominant in probe clusters 1 and 2.

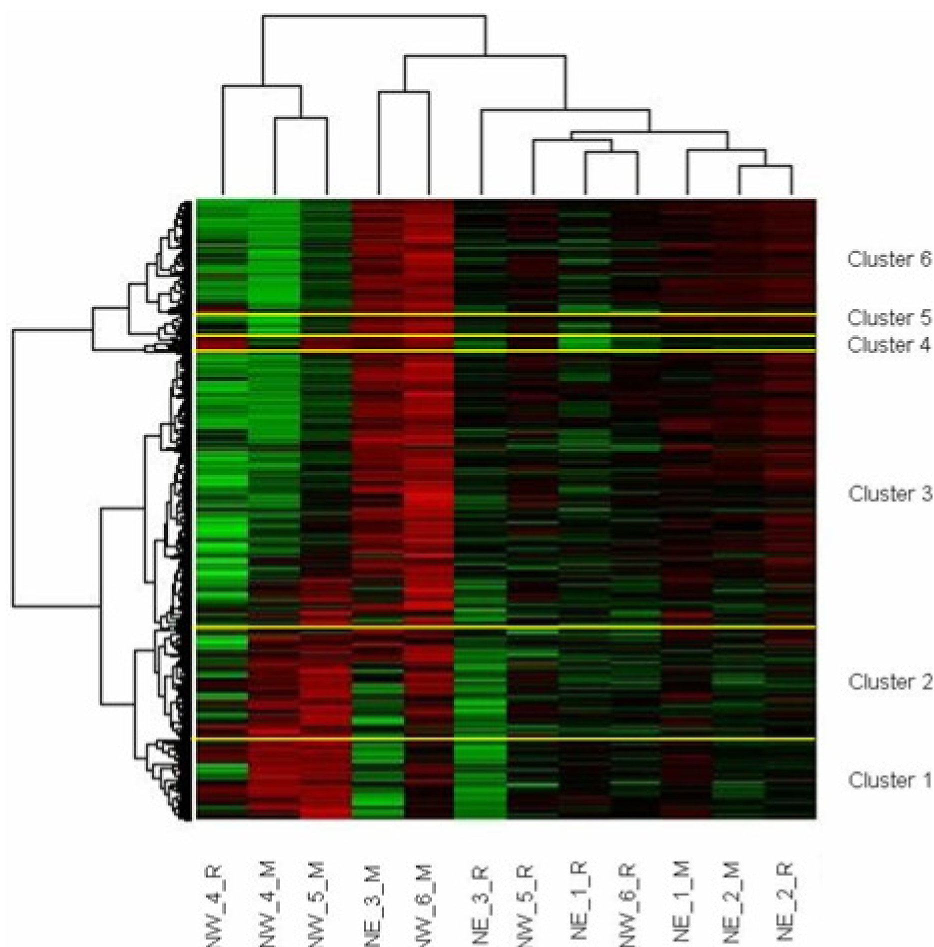

Figure 1. A heatmap generated using hierarchical clustering showing simultaneous relationships between probe signal intensities across and within soil samples. Red coloration indicates an increase in probe signal intensity while green indicates a decrease. Yellow horizontal lines indicate the boundaries between clusters of probes that show similar profiles across the samples.

FUNC Array

Data was collected based on analysis of variance from 7795 probes from functional genes showing strong signals across samples (Table 1). Carbon and nitrogen cycling genes were detected for all factor and interaction terms in the TasFACE experiment, with probe responses being similar between the “4-hitter” and

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“specific” probes. Probe signal intensity and the number of probes (proportional representation) from each functional group were summarised for the carbon and nitrogen cycling genes found to have a significant ($P <$ 0.05) response. Strong effects on functional gene response were observed for warming (and soil moisture) and FACE (elevated CO_2) treatments, and their interaction with plant communities (C4 species *Themeda* vs non-*Themeda* C3 species).

Table 1. Summary of the number of FuNC array probes significant ($P < 0.05$) within each factor and interaction in a 4 way ANOVA of TasFACE data.

Factor or interaction term	Number of significant probes
FACE (Elevated CO_2)	305
Warming	620
Themeda	23
Depth	28
FACE x Warming	124
FACE x Themeda	241
FACE x Depth	3
Warming x Themeda	168
Warming x Depth	814
Themeda x Depth	2
FACE x Warming x Themeda	611
Warming x Themeda x Depth	4
Warming x Themeda x Depth	31
FACE x Depth x Themeda	9

Phylochip

The Phylochip microarray identified that different phyla and classes were responding to the treatments (factors) and interactions tested at TasFACE. Dominant groups that responded to the different factors were the classes Acidobacteria and Alphaproteobacteria for temperature; Firmicutes and Bacteroidetes for FACE; and Actinobacteria and Bacteriodetes for the grass species *Themeda*. Taxa across all Phyla responded to depth with a decrease in probe fluorescence intensity. Of the interactions tested FACE x Temperature affected the most probes (n = 103) with four different phyla, including the classes Actinobacteria, Sphingobacteria, Clostridia, Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria responding. Four clusters based on similar probe responses were identified for the FACE x Temperature interaction.

Conclusion

The use of three different microarrays (Soil Health Array, FuNC array and Phylochip) on soil microbial communities has been described. The application of microarray technology to differing soil ecosystems has provided insight to microbial functional processes and community structure for many hundreds of genes simultaneously, and how these may be impacted by Soil Order, land-use or climate change.

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