MICROBIAL COMMUNITY STRUCTURAL AND FUNCTIONAL DIVERSITY IN THE RHIZOSPHERE OF CO-OCCURRING FOREST TREES

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INTRODUCTION

The rhizosphere, the narrow zone of soil immediately surrounding plant roots, is a zone of high biological activity in soils and is important in nutrient uptake and biogeochemical cycling in forest ecosystems. Tree roots exude organic compounds that have the potential to directly influence metal mobilisation through ligand exchange, acidification of the rhizosphere, oxidation-reduction reactions and dissolution through ion exchange (Curl & Truelove 1986, Jones 1998). Further, the addition of root exudates and organic matter such as sloughed cells into the rhizosphere provides quality nutrition and substrates for a myriad of soil micro-organisms (Paul & Clark 1996, Dakora & Phillips 2002). Interactions between plant roots, soil microbial communities and soil minerals may be particularly important in the deeply weathered, nutrient depleted landscapes and soils seen across the Australian continent. However, until recently there have been few attempts to understand these interactions in Australian forest soils (Marschner *et al.* 2005). Here we investigate one aspect, being shifts in structural and functional diversity of microbial communities in the rhizosphere of a dry sclerophyll forest soil.

Specifically this research examined soil microbial community structural and functional diversity in the rhizosphere of co-occurring *E. mannifera* and *A. falciformis* growing on Yellow Chromosols and Kandosols in a near-natural dry sclerophyll forest. Focus is given to the potential implications for biogeochemical cycling and mineral weathering in these soils.

METHODS

Denaturing Gradient Gel Electrophoresis of Polymerase Chain Reaction (PCR) amplified 16S and 18S *r*DNA was used to examine the structural diversity of the soil bacterial and fungal communities. Rhizosphere and non-rhizosphere soil DNA was extracted from approximately 0.75 g (dry weight equivalent) samples using a MoBio soil DNA extraction kit. Serial 1:100 dilutions were made of the neat DNA extract using sterile milli-Q water. The bacterial 16S and fungal 18S *r*DNA of triplicate samples were amplified using separate PCRs containing bacteria- and fungi-specific primers and confirmed on 2% agarose gels using UV-transluminescence. Products from the bacterial 16S and fungal 18S *r*DNA PCR reactions were separated using Denaturing Gradient Gel Electrophoresis (40-60%, and 15-50% formamide-urea gradient, respectively) at 60 V for 19 hours and stained using a Silver staining procedure (BioRad). A number of cluster analyses of the bacterial and fungal community structures were undertaken using duplicate samples run on the same gels. BIOLOG® sole C source utilisation profiles (SCSUP) were examined as an indicator of potential microbial functionality in rhizosphere and non-rhizosphere soils beneath co-occurring *E. mannifera* and *A. falciformis*. BIOLOG MT2™ microtitre plates were prepared using sterile techniques to contain 4 replicate sets of 23 C-sources and one blank well. The C-sources used were representative of low molecular weight organic compounds, commonly observed in root exudates and the rhizosphere (Dakora & Phillips 2002, Macdonald *et al.* 2004). Additional MT plates were supplemented with sterile grains of bytownite feldspar (ca. 75% Anorthite / 25% Albite). Bacterial and fungal patterns of potential C utilisation were determined through creating soil suspensions containing streptomycin (1 mg/mL) and chlortetracycline (0.5 mg/mL) respectively. Plates were inoculated (100 µL of rhizosphere or non-rhizospshere dilution), incubated at 22ºC, and colour development (590 nm for bacterial, and 650 nm for fungal plates) measured at regular intervals over 15 days on a Biolog Emax™ Microstation microplate reader.

The 16S and 18S *r*DNA community structures were examined using cluster analyses in the Diversity Database (Biorad). A combination of techniques was used to examine BIOLOG substrate utilisation patterns. First the data was normalised by the Average Well Colour Development of each replicate set of C-sources.

Principal components analysis (PCA) was used to reduce the data to contain a more manageable number of variables, and then finally the data was analysed by canonical variate analysis (CVA). In order to relate the potential C-source utilisation patterns to 16s and 18s DGGE community structures and geochemical data it is appropriate to use Canonical correspondence analysis (CCA). Experimental data pertaining to the biogeochemistry in these soils (Little *et al.* 2004a, Little *et al.* 2004b) were used to gain an insight into potential pedogenic implications for any shifts in the microbial community structural and functional diversity.

RESULTS

Cluster analyses of the rhizosphere and non-rhizosphere community structures of co-occurring trees showed distinct variation between rhizosphere and non-rhizosphere 16S and 18S *r*DNA bacterial and fungal community structures (Figure 1). Generally:

- 16S and 18S soil bacterial and fungal community diversity, indicated by numbers of bands, show most differentiation down-profile:
    - Averages of 12 to 15 bands were observed for the A_1 horizon 18S fungal communities, with 8 to 12 bands observed for the B_2 horizon fungal communities:
        - The highest average number of bands (14 to 15) was observed in the A_1 horizon beneath the eucalypt and the lowest number in the B_2 horizon beneath the acacia;
        - The absolute maximum number of bands (25) was observed in the non-rhizosphere A_1 horizon soils beneath the acacia.
    - Averages of 9 to 11 bands were observed for the A_1 horizon 16S bacterial communities, with 8 to 14 bands observed for the B_2 horizon bacterial communities:
        - The highest average number of bands (11 to 14) was observed in the B_2 horizon beneath the eucalypt and the lowest number (8-9) in the B_2 horizon beneath the acacia;
        - The absolute maximum number of bands (19) was observed in the rhizosphere soils in the eucalypt B_2 horizon soils.
- Similarities in community structure, indicated by band position, were observed between 16S microbial in the B_2 horizon beneath *E. mannifera* and the A_1 horizon beneath *A. falciformis*:
    - 16S microbial community structure in the B_2 horizon beneath *A. falciformis* were least similar to any of the other soil microbial communities in the study;
    - Some clustering of non-rhizosphere is also observed regardless of tree species and horizon.

The SCSUP observed during bacterial rhizosphere C-source utilisation experiments reflected the observed 16S *r*DNA community structures of rhizosphere and non-rhizosphere soils of the co-occurring trees. Potential C-source utilisation by the microbial suspensions was most rapid (72 to 78 hrs) on microplates inoculated with bacterial suspensions from rhizosphere and non-rhizosphere soils of the A_1 horizon beneath *A. falciformis* and the B_2 horizon beneath the *E. mannifera* (Figure 2). In contrast, AWCD was slower in the respective plates inoculated with microbial suspensions from the B_2 and A_1 horizons of these species respectively.

The addition of the bytownite feldspar to the wells generally caused higher background absorbance at the beginning, and hindering readings of absorbance throughout the experiment. This highlights problems with adding the mineral. A greater diversity of C-source utilisation by the fungal communities was also observed in the bytownite containing wells, though this could not be adequately resolved due to interference by the added mineral.

DISCUSSION

The results of the BIOLOG and DGGE examinations of the soil microbial communities are consistent with results of geochemical investigation of the rhizosphere and non-rhizosphere soils at the site (see Little *et al.* 2004a, Little *et al.* 2004b, Little *et al.* 2005). Interestingly the soil microbial communities were more culturable, or may have had a higher inoculation density in the A_1 horizon beneath the *A. falciformis* and in the B_2 horizon beneath the *E. mannifera* compared to the other soils used in the experiment.

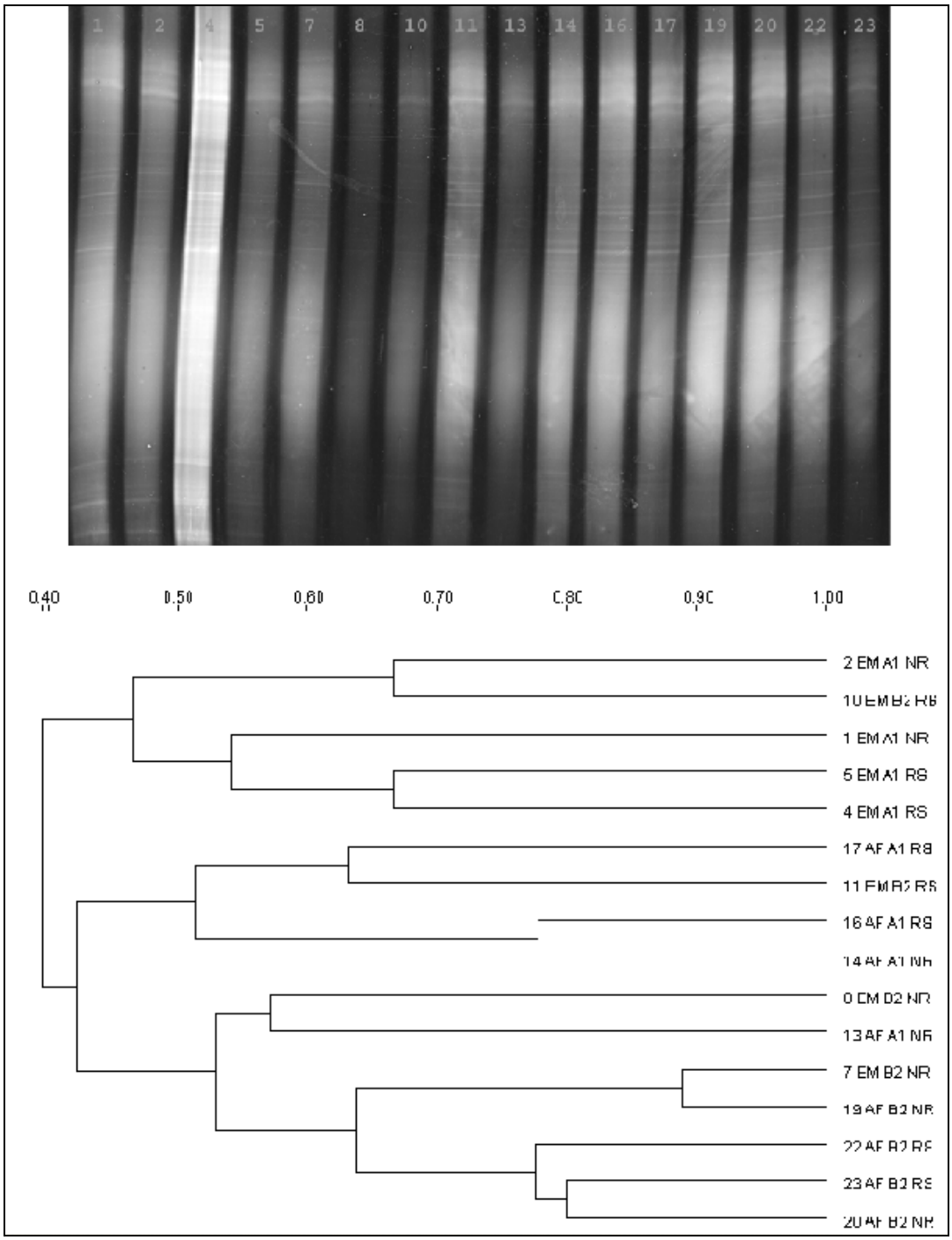

Figure 1: Cluster tree (UPGAMA) and acrylamide gel (40 to 60% chemical denaturants) showing 16S bacterial community structure in forest soils beneath co-occurring *Eucalyptus mannifera* and *Acacia falciformis* (EM – *E. mannifera*; AF – *A. falciformis* A – A_1 horizon, B – B_2 horizon; NR – non-rhizosphere; RS – rhizosphere).

The rhizospheric influence on structural and potential functional diversity of the soil microbial community seemed greatest in the A_1 horizon under the acacia, where a greater proportion of metal nutrients are likely to occur in association with soil organic matter rather than inorganic minerals (Little *et al.* 2004a, Little *et al.* 2004b, Little *et al.* 2005). The eucalypt B_2 horizon soil microbial communities may scavenge for vital metal nutrients by collecting nutrients leaked from the surface soils beneath the acacia, as well as by interacting with clays and sesquioxide materials. This is also supported by the greater abundance fine roots (< 3 mm diameter) in the acacia soils, compared with under the eucalypt, where there is predominance of very large roots (≥ 10 cm diameter) (data not shown). In addition the apparent greater functionality of the soil microbial community in the eucalypt B_2 horizon compared with the A_1 horizon suggests an association with Al and Fe

oxy-hydroxides, which have strong associations with many insoluble and trace elements. Thus it is the eucalypt-associated microbial communities that are likely to have a greater influence on mineral weathering, compared to the acacia-associated microbial communities, which seems more likely associated with soil organic matter accumulation and biogeochemical cycling.

Further research is needed to elucidate the nature of low molecular weight carboxylic acids beneath these co-occurring *E. mannifera* and *A. falciformis* in order to more fully understand the potential functionality of the soil microbial communities in rhizosphere and non-rhizosphere soils at this site.

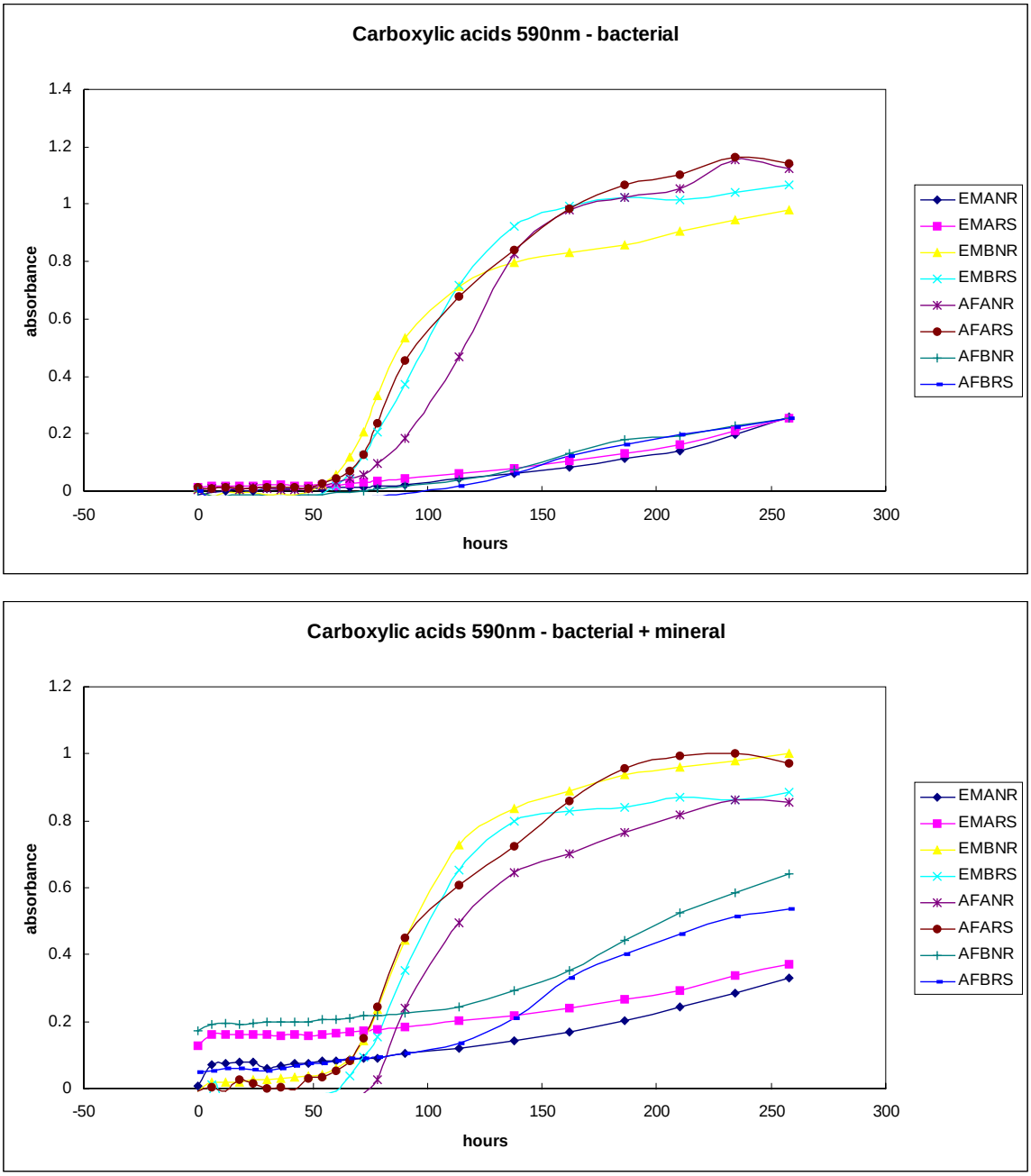

Figure 2: Averaged utilisation of (12) carboxylic acids by 8 rhizosphere and non-rhizosphere soil bacterial communities on Biolog MT2 microplates, as indicated by absorbance at 590 nm (EM – *Eucalyptus mannifera*; AF – *Acacia falciformis*; A – A_1 horizon, B – B_2 horizon; NR – non-rhizosphere; RS – rhizosphere).

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