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Analysis of soil microbial community level physiological profiles in native and post-mining rehabilitation forest: Which substrates discriminate?

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ABSTRACT

The interpretation of community level physiological profiles (CLPP) may be made ecologically relevant by including carbon (C) substrates that reflect organic molecules likely to be present in a soil. In this study, whole-soil CLPP assays were conducted using 86 C substrates selected firstly on the basis of relevance to soil ecosystems and secondly to provide a range in structural complexity. The impact of mining and rehabilitation on soil CLPP (rehabilitated 3, 13 and 26 years previously) was tested by comparison with adjacent non-mined native jarrah (*Eucalyptus marginata*) forest soil CLPP. The effect of prescription burning (2 years prior) on rehabilitation and non-mined forest CLPP was also investigated. Our first hypothesis that by 26 years the CLPP of rehabilitation soils would be indistinguishable from non-mined soil CLPP was not supported. Significant differences in CLPP were found between all rehabilitation ages and non-mined forest soil; the extent of differences depending on which substrates were included in the analysis. Conversely, prescription burning was not found to result in any significant differences ($P > 0.1$) in CLPP of rehabilitation soil (13 year old) or non-mined forest soil. The hypothesis that any differences found in CLPP would be due to greater utilization of more structurally complex C substrates in non-mined soils compared to rehabilitation soils was supported. As a proportion of the total substrate response, non-mined forest soils had a significantly higher response to the group of complex substrates tested. However, a large proportion (up to 37%) of the variation between rehabilitation and non-mined forest soils was also attributable to differential responses to simple organic compounds, in particular the carboxylic acids. The study demonstrated that the discriminatory power and ecological relevance of CLPP was improved through the selection of specific substrates to include in the assay.

1. Introduction

There is evidence to suggest that soil microbial communities may be conditioned or adapted to decompose carbon (C) substrates specific to their soil environment (Grayston et al., 2004; Hamer and Marschner, 2005; Orwin et al., 2006). Mature ecosystems may have a greater diversity of C substrates and also greater quantities of complex C substrates than younger ecosystems (Nierop et al., 2001; Rumpel et al., 1999). This may result from C stabilization processes that occur during ecological succession, producing structurally complex C substrates in soils (Schipper et al., 2001). In addition, ecosystem disturbances such as fire result in thermal oxidation of organic matter to form a myriad of complex macromolecular and heterocyclic C compounds (Gonzalez-Perez et al., 2004). Hence, an increase in the diversity of C substrates in soil as a result of successional processes and ecological disturbance may result in more diverse soil microbial communities capable of degrading these substrates or microbial adaptation to these substrates (Grayston et al., 1998).

It has been argued that returning the diversity of soil ecosystem functions to degraded lands may be more important than returning soil microbial diversity *per se* (Setälä et al., 2005). A common approach taken to assess the functional diversity of soils is to measure microbial heterotrophic capacity. This involves measurement of short-term soil respiration responses to a range of C substrates that vary in structural complexity, to produce community level physiological profiles (CLPP). This type of profile does not represent a measure of microbial community composition, such as that provided by genetic-based profiling (Ros et al., 2008), but the technique has been applied successfully to detect a change in soil functional ability post-disturbance (Campbell et al., 2008; Cookson et al., 2008; Degens et al., 2001; Insam, 1997; Lewis et al., 2010) or in response to soil management practices (Campbell et al., 2003; Degens, 2001).

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Large-scale (approx. 550 ha per year) bauxite mining and rehabilitation practices within the jarrah (*Eucalyptus marginata*) forest in the south-west of Western Australia has created a mosaic landscape consisting of newly cleared and mined areas, rehabilitation and non-mined forest in various states of succession. The objective of this mine rehabilitation is to restore the biodiversity and long-term sustainability of a native Jarrah forest ecosystem (Koch and Hobbs, 2007), including characteristics such as resilience to prescription burning practices (Grant et al., 2007). Most studies to date assessing the success of forest rehabilitation have focussed on above-ground diversity, biomass and vegetation structure (Grant and Loneragan, 2003; Koch, 2007b; Norman et al., 2006) and soil nutrient status (Morley et al., 2004; Todd et al., 2000b; Ward, 2000) while comparatively little attention has been given to the recovery of soil microbial biomass, diversity or specific functions (Banning et al., 2008; Lalor et al., 2007; Todd et al., 2000a).

The only published comparison between rehabilitation and non-mined jarrah forest soil CLPPs reported a time-frame of between three and 16 years for re-establishment of CLPP in top-soil, dependent on the location within the surface micro-topography created during initial site preparation (Lalor et al., 2007). In the Lalor et al. (2007) study, CLPP were produced using a range of substrates, originally described by Degens and Harris (1997), that were selected based on giving the greatest discriminatory power across seven U.K. soils under different management. Campbell et al. (1997) suggested that more ecologically relevant conclusions could be drawn from CLPP created using C substrates likely to be present in the soil ecosystem of interest. They demonstrated that the use of root exudate C sources provided greater discriminatory power than the C sources provided by the commonly-used commercially available Biolog plates. This raised the question whether the particular substrates selected for optimal CLPP discrimination of U.K. arable soils would also provide the best discriminatory power and ecological relevance in the highly weathered nutrient-poor lateritic soils (McArthur, 2004) of an Australian eucalypt forest.

To address this question, 86 C substrates were selected for conducting CLPP assays on the basis of providing a range in structural complexity and relevance to soil ecosystems. The MicroResp™ approach (Campbell et al., 2003) was used for whole-soil CLPP assays, as this had been previously found to provide greater discrimination than the Degens and Harris (1997) approach (Lalor et al., 2007). Whole-soil CLPP assays measure respiration and are not dependant on the extraction and cultivation of microorganisms, unlike the Biolog CLPP approach (Campbell et al., 2003). We hypothesized that microbial functional diversity, as assessed by CLPP, would be different in rehabilitation forest soil compared with non-mined forest soil but that they would become more similar to each other with increasing successional age. We also hypothesized that a prescribed burn of 13 year old rehabilitation and non-mined forest, would have affected the soil CLPP (two years' post-burn), particularly in the rehabilitation forest soil. Although a previous study found no difference in soil CLPP ('Degens and Harris' approach) from a 12 year old rehabilitation forest one year following prescribed burning (Cookson et al., 2008), increases in soil pH, organic C and total N following burning were found in this study and elsewhere (Morley et al., 2004). Prescribed burning may also change the plant community structure, particularly in rehabilitation sites in which the fire is used as a tool to decrease the midstorey layer density (predominantly *Acacia* species) and stimulate understorey plant growth (Grant, 2003).

A further aim of this study was to determine which substrates provided the highest discriminatory power for the CLPP assay; either individually or grouped into functional types (amino acids + amines; alcohols; carbohydrates; carboxylic acids; 'complex' substrates defined as those containing benzene or heterocyclic rings, long-chain fatty acids or polymers). Considering that successional age and burning are likely to influence the structural complexity of C substrates in soil (Gonzalez-Perez et al., 2004; Schipper et al., 2001), it was hypothesized that any differences in CLPP would be due to a greater relative response to complex C substrates in non-mined soils or burnt soils compared to rehabilitation soils or non-burnt soils.

2. Materials and methods

2.1. Study area and experimental design

The study area was located within Alcoa of Australia's (Alcoa) Huntly mine site situated in the jarrah forest, 110 km south of Perth, Western Australia (32.7103°S and 116.0594°E). The area has a Mediterranean-type climate, with hot dry summers and cool wet winters with mean annual rainfall of 1258 mm. Soils consist of an upper layer of coarse ferruginous gravels and yellow/brown sands overlying a layer of caprock (Todd et al., 2000b). A detailed description of Alcoa's procedures used to rehabilitate mined areas in south-west Western Australia is found in Koch (2007a). Briefly, mine-site rehabilitation practices include the return of topsoil, contour ripping, seeding and planting with native plant species and initial fertiliser inputs. Contour ripping (depth of >1.2 m and at 1.6 m spacing) creates a distinct micro-topographical pattern (i.e. mound and furrow). A mixed fertilizer of nitrogen (N), phosphorus (P), potassium (K), and micronutrients was applied following seeding at ~500 kg ha^{-1}.

Four replicate plots (20 m × 20 m) were established in post-mined rehabilitation sites (approximately 3–4 ha each) that were initially subject to rehabilitation in 1979 (26 year old), 1992 (13 year old) or 2002 (3 year old). Four replicate plots were also established in post-mined 13 year old rehabilitation sites that had been burnt in a moderate intensity prescription burn two years prior to sampling. In addition, four replicate plots (20 m × 20 m) were established in adjacent areas of both non-burnt and burnt non-mined forest. All non-burnt rehabilitation sites had not been burnt previously, whilst the non-burnt non-mined forest was last burnt 14–16 years earlier. Composite bags of 12 soil cores (0–5 cm) were randomly collected from each replicate plot in Spring, sieved <2 mm and stored at 4 °C. Soil was collected separately from both the mound and furrow of the contour rip lines in rehabilitation sites. Soil was pre-incubated at 40% water holding capacity (WHC) for 7 days prior to analyses.

2.2. General soil chemical properties

Air-dried (40 °C) samples of the <2 mm fraction were analysed for inorganic N, Colwell (available) P, organic C, electrical conductivity (EC) and pH in 0.01 M ($CaCl_2$) using standard analytical techniques (Nelson and Sommers, 1996; Rayment and Higginson, 1992) and are given in Table 1.

2.3. Community level physiological profiles

Upon review of the literature, 86 C substrates consisting of 23 carboxylic acids, 11 carbohydrates, 28 amino acids and amines, 3 alcohols, 2 vitamins, 1 polymer, 3 long chain fatty acids and 15 aromatic organic compounds were selected (Table 2). Substrates classed as structurally 'complex' were those with benzene or heterocyclic rings, the long-chain fatty acids, the vitamins and the polymer ($n = 21$). Substrates were included based on their known occurrence in Jarrah forest ecosystems (Abbott and Loneragan, 1986; Malajczuk and McComb, 1977), as substrates produced during the thermal oxidation of organic matter (Gonzalez-Perez et al., 2004) or known plant root exudates (Campbell et al., 1997; Degens and Harris, 1997).