



Soil amendments improve microbial ecology parameters of “topsoil inoculum” used in post-mining restoration

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Mine operations generate substantial volumes of waste substrates, which are crushed and/or chemically treated waste rock from which ores are extracted.

Establishment of rehabilitated landforms using post-mining substrates (i.e. waste rocks, tailings) that typically exhibit extreme conditions (such as high pH, nutrient deficiency, excessive salinity and metal toxicity) can be a major environmental problem and a critical issue during mine closure operations. More importantly, mine sites are located predominantly in arid or semi-arid lands where our understanding of basic ecosystem processes and microbial interactions with native plants (e.g. *Eucalyptus* spp., *Acacia* spp., *Grevillea* spp. in Western Australia) are limited. Despite the wide acknowledgement on the impact of microbial functional diversity on overall soil and plant health, no detailed attention has been paid to understand the role of belowground microbial functional diversity in the context of mine rehabilitation strategies. In this research, we investigated the role of nitrogen-based and microbial consortia amendments on improving the microbial ecology parameters of “topsoil inoculum” and subsequently its cascading effect on seedling establishment and plant morphology of *Acacia ancistrocarpa*, a legume native to the Pilbara and other regions of Western Australia and commonly used in arid zone restoration. The study was conducted under controlled environmental conditions in potted plants using topsoil retrieved from previously stockpiled material as growth media. A morphological assessment was undertaken to measure shoot length, shoot weight, root length, root area and root weight. Soil chemical properties, e.g. carbon, nitrogen and trace metals concentrations were determined. Microbial activity was measured with the 1-day CO₂ test, which determines soil microbial respiration rate based on the measurement of the CO₂ burst produced after moistening dry soil (Muñoz-Rojas et al., 2016). Bacterial and archaeal community composition was assessed using high-throughput sequencing of the 16S rRNA marker gene analysis (Whiteley et al., 2012). Our results showed significant ($P < 0.05$) larger rates of microbial activity and shoot:root ratio in those pots amended with both N and microbial consortia. Both amendments significantly influenced the chemical and bacterial-archaeal community composition in the topsoil inoculum. Topsoil amended with inorganic nitrogen revealed higher relative abundance of 16S rRNA gene sequences related to phylum Nitrospira, Planctomycetes, Gemmatimonadetes and Cyanobacteria/Chloroplast in comparison to “water-only” (control) and “microbial consortia” treatment. Addition of microbial consortia to topsoil significantly increased the concentrations of soil potassium, iron, copper and manganese. Interestingly, a large proportion of sequences that can only be assigned at the domain level (either Bacteria or Archaea) were detected in the control samples suggesting the presence of novel taxa/lineages in the native soil and their relative abundances are significantly influenced by the addition of amendments. Our data indicates that whilst amendments results significant improvement of plant growth and establishment at early stages it also results in considerable phylogenetic divergence within soil bacterial/archaeal communities, in particular loss of indigenous taxa/lineages that could have above-below ground feedback with native plants.

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