IMPROVING MINE REHABILITATION SUCCESS THROUGH MICROBIAL MANAGEMENT

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INTRODUCTION

At present there are 441 operating mines in Australia mining commodities such as coal, mineral sands, iron ore, base, light and precious metals, uranium, and diamonds (Geoscience Australia, 2013). A significant number of these have an open cut or surface component to them. Open cut mines are usually easier, cheaper, and quicker to bring into production than underground mines, but often have a relatively short life-span, after which it may become necessary to move to underground mining to access deeper commodities.

Government authorization must be obtained to mine Crown or private land in Australia and land disturbed by mining activities must be returned to a sustainable post-mining land use. In more recent times, miners usually undertake progressive rehabilitation programs where mine closure planning is developed within the initial stages of mine operations rather than only being considered after mining ceases; however, Australia also has a legacy of unplanned mine closures, unsafe workings, hazardous mine sites, and unreclaimed lands, resulting from previously inadequate or non-existent mine closure practices and legislation (Smith, 2007). For example, in New South Wales alone there were more than 550 derelict mine sites requiring rehabilitation in 2008 (Nolan, 2008) where no individual or company is held responsible for their management or rehabilitation and no particular government agency has statutory responsibility for their rehabilitation (New South Wales Government, 2013). To minimize the risk of future derelict mines occurring, current mines are strictly regulated and must lodge a security deposit to cover rehabilitation costs if the mine becomes insolvent.

Land preparation prior to mining depends upon the mining technique to be adopted, the vegetation, and terrain. Surface or open cut mining involves a general process of removal of the topsoil layer and any other soil layers necessary to get to the substance that is being mined. The first stage of the mining process involves the removal of the existing trees and vegetation. Cleared timber and vegetation may be mulched and stockpiled to be re-used in rehabilitation. The topsoil is then removed and stockpiled and the soil layer below, usually referred to as overburden, is also removed and stockpiled separately. Once established, topsoil stockpiles may be revegetated until

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reused. When mining operations are complete, the overburden material is reapplied and contoured and then topsoil is reapplied, spread, and incorporated with the overburden to provide a planting medium. As post-mined soils often have a less developed structure, reduced organic matter, and lower nutrient contents than original soils, amendments are often made to change their physical and chemical properties. In particular, fertilizer addition is common practice and the addition of sewage sludge/biosolids and compost can also occur.

A major challenge in rehabilitating land that has been subjected to mining is re-establishment of a self-sustaining vegetative cover. This can be difficult because of the post-mining soils aforementioned reduced structure, organic matter, and nutrient content. As decomposers, soil microbial communities mediate critical ecosystem processes, and microorganisms are an important element for successful reclamation because of their role in nutrient cycling, plant establishment, geochemical transformations and soil formation. In addition, symbiotic nitrogen-fixing bacteria and mycorrhizal fungi are important microbial groups intimately involved in plant establishment on soils. Yet microorganisms generally remain an undervalued asset on mine sites. For example, prior to mine closure, companies need to demonstrate that they have rehabilitated the site to meet a number of predetermined criteria that usually fall into some general categories such as landform stability, topsoil, vegetation, fauna, water and safety, with the aim being to return disturbed land to a stable, productive, and self-sustaining condition and taking future land use into account. Microorganisms such as bacteria, actinomycetes, and fungi have an effect on most of these criteria, yet in Australia for example, they are not specifically included in any of them (Australian Government, 2006a). Furthermore, the mining industry's governance in Australia is administered by the State and Territories and all State and Territories have their own mine closure policies which require mining companies to develop site-specific post-mining rehabilitation plans for approval by the relevant State authority as part of the development assessment process. Under Australia's federal system, Commonwealth legislation via the Environment Protection and Biodiversity Conservation Act 1999, which came into effect in 2000, is only applicable in areas under national jurisdiction, on projects where matters of national environmental significance are concerned, such as mining operations in national parks, or which may have impacts on threatened or migratory species.

For the agencies with responsibility for sign-off, assessing the attainment of a self-sustaining restoration which emulates the structure, diversity, function, and dynamics of a specific ecosystem is more complicated than simply having an area which appears stabilized, contains growing plants, and a number of types of animals. Over time, for example, some of the fast-growing but short-lived plants may die, trees may cease to grow as the effects of initial fertilizer treatments decline, and the soil may not develop the desired texture or structure of the original substrate. Furthermore, a significant number of mines have unexpected or unplanned closures and a more integrated approach to mine closure planning can help achieve effective and earlier mine closure

and completion (Australian Government, 2006a). Success criteria for rehabilitation need to be based on ecological principles and those based on a narrow set of vegetation indices or single chemical parameters have generally been found to be inadequate. A combination of attributes at both the landscape level and addressing more specific ecosystem properties are thought to be necessary (Australian Government, 2006b).

This paper will outline the importance of microorganisms to soil health and the rehabilitation of disturbed soils and how microbial community metrics can be used to monitor and quantify the soil microbial community status to demonstrate their recovery in rehabilitated soils. The focus is on decomposer soil microorganisms rather than symbiotic microorganisms, as the role of the latter in the rehabilitation of degraded and mined soils is better recognized in the industry and has been covered by other authors (e.g., Barea, Requena & Jiménez, 1996; Corbett, 1999).

KEYWORDS

microorganisms, bacteria, actinomycetes, fungi, water, organic matter, soil health, mine rehabilitation

SOIL MICROORGANISMS ARE VITAL TO ECOSYSTEM HEALTH

Microorganisms are increasingly recognized as being important for successful reclamation. They play a vital role in promoting organic matter turnover and nutrient cycling, soil formation and its aggregation, plant establishment and growth through microbial symbioses (fungal mycorrhizae and bacterial nodulation), and through suppression of diseases and pests. In addition, macrofungi are an important food source for Australian native animals, with over two-thirds of terrestrial ground-dwelling mammals eating some fungi, and with fungi making up 60–70% of the diet of some small mammals (Vernes, 2007). Microorganisms are also a food source for microfauna such as insects.

Although microorganisms may account for only 1–5% of the organic matter content in soils they can make up over 90% of the biomass and the microbial biomass can be ten times that of the soil microfauna (Satchell, 1971). The top 10cm of soil is the most valuable rehabilitation asset on a mine site with 50% of the microbial biomass residing in this layer. This can equate to ¼ ton dry weight of microbial biomass per hectare of forest soil, or something like 25 sheep's worth of fresh biomass. So not knowing the microbial status of a soil means not having the full picture of it as an asset.

Despite this, the majority of soil and environmental monitoring programs include only measurements of soil nutrients, chemical properties, texture, and heavy metal content. There has been much less emphasis placed on biological properties in monitoring programs, even though microorganisms are central to ecosystem function. In addition, changes in soil organism populations or soil biotic activity may precede detectable changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation.

HOW IS THE MICROBIAL STATUS IN SOILS DETERMINED?

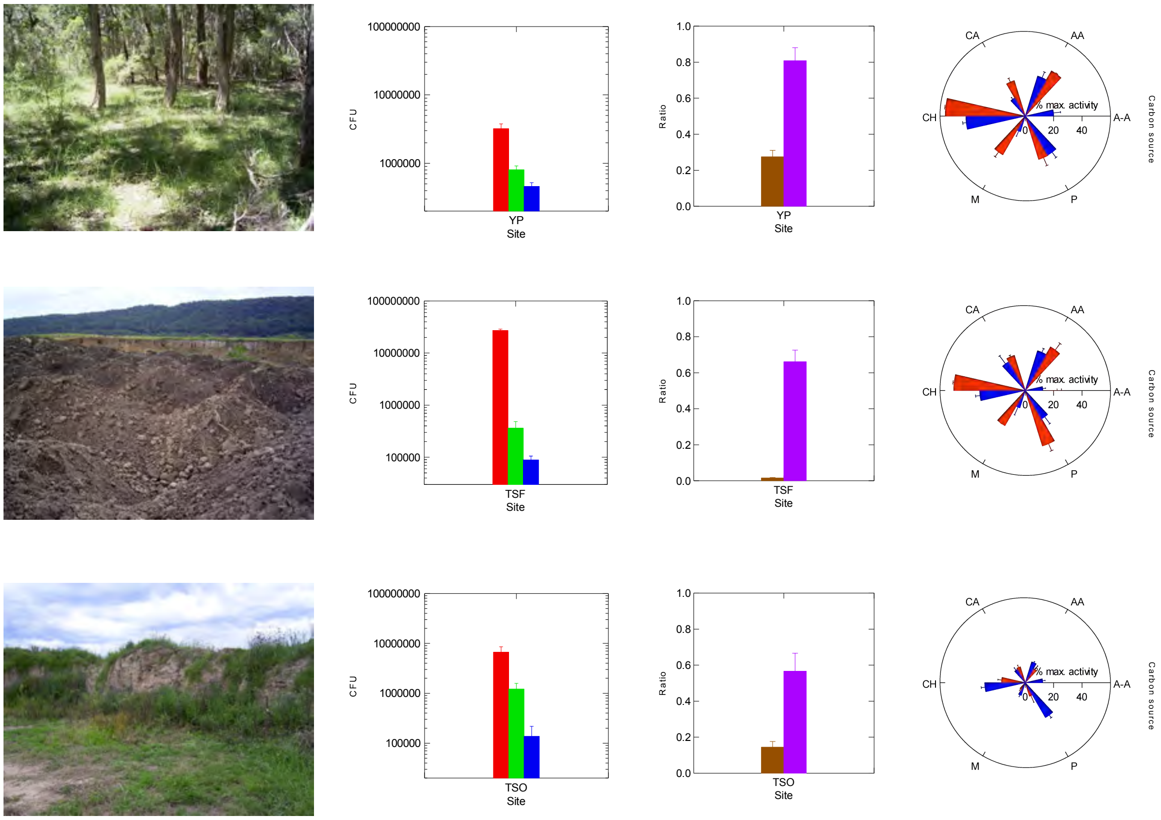
It is a challenge to identify soil microbial properties that are sufficiently simple and robust enough for routine measurement, yet provide enough information to give meaningful insights into the state of a soil and whether or not any soil functions are deteriorating. A significant functional redundancy amongst microorganisms means that many of the major functions of the soil microbial biomass, for example, are unaffected by its exact species composition. This limits the value of using only taxonomic and molecular approaches to microbial monitoring. Other indicators of microbial status include measuring levels of microbial activity, amounts of microbial biomass carbon and nitrogen, and soil enzyme activities.

We have successfully monitored microorganisms through a combination of direct isolation and metabolic fingerprinting methods. These involve both:

- the isolation and counting of bacteria, actinomycetes (filamentous bacteria), and fungi—including the presence of key indicator species, and
- determining the functional properties of the bacterial and fungal communities by examining carbon-source utilization by the communities.

Together these methods have proved to be sensitive indicators of changes in soil microbial community structure (Figure 1).

FIGURE 1. Soil disturbance and topsoil storage result in changes in bacterial and fungal numbers and community functionality. Data are for (top to bottom) native vegetation; a fresh topsoil stockpile; and an old topsoil stockpile. Graphs are of (left to right) numbers of bacteria, actinomycetes and fungi; ratios of actinomycetes: bacteria and the proportion of copiotrophic bacteria (rapidly growing bacteria usually associated with nutrient rich conditions); functional properties of bacterial and fungal communities.



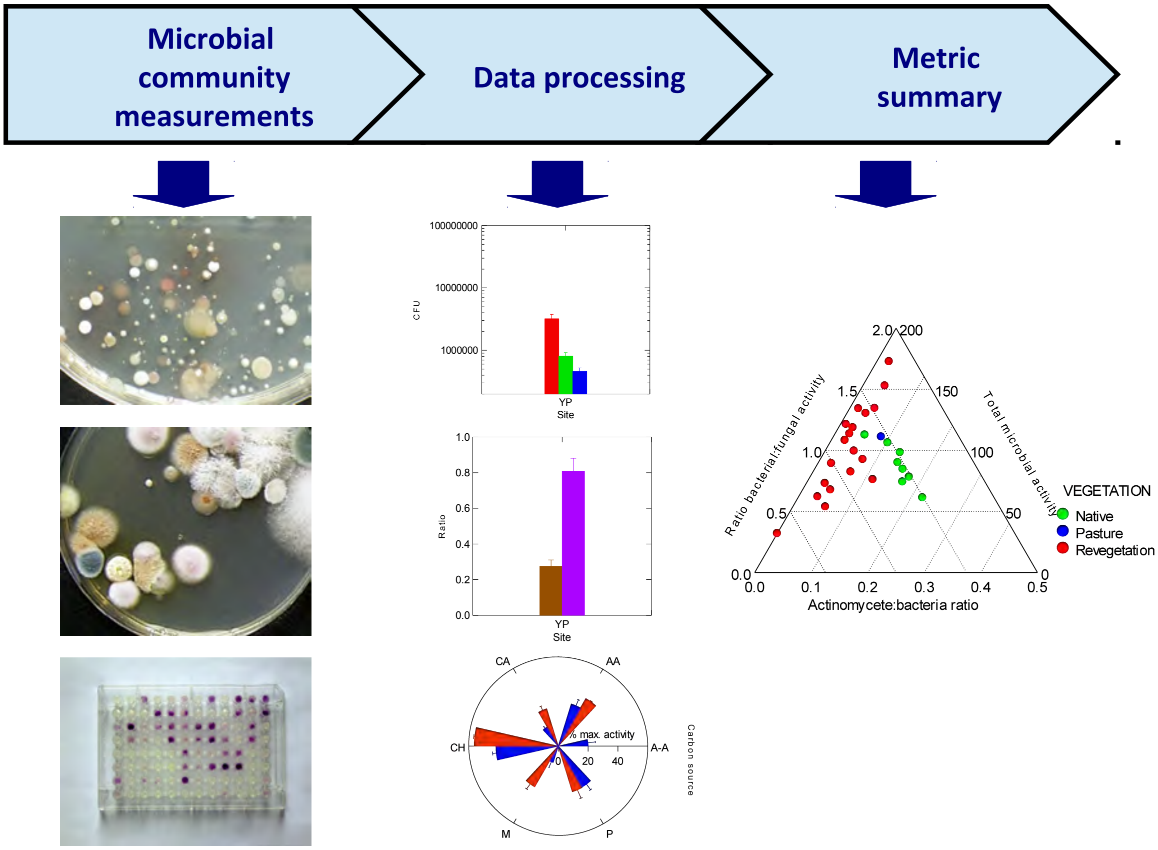
Moreover, we have devised metrics to provide a summary of the data obtained from these microbial measurements and which allow easy comparisons, tracking, and summary documentation of soil microbial community status for the annual environmental management reports of mining clients who may not have microbiological expertise and/or who may just want to know if the microbiology of their soils is “okay” (Figure 2). We are not aware of the previous use of metrics to summarize microbial numbers and community functionality data in soils.

The metric used here is of the:

1. The actinomycete:bacteria ratio. This is a key indicator of native versus disturbed soils, with lower values (< 0.2 and often < 0.1) being indicative of disturbed soils.
2. The sum of the bacterial and fungal community activities as measured by the utilization of carbon sources. Consistently higher and stable values over time indicate greater community functionality and are generally associated with native vegetation.
3. The ratio of the bacterial community:fungal community activities. The size of this ratio is an indication of the relative activities of the bacterial and fungal communities and is influenced by soil water and organic matter. A stable value over time is generally indicative of native vegetation.

It should be noted that a wealth of data on numbers and types of soil microorganisms and microbial community activities underlies the metric and this can be used to provide information on the causes of change in the metric.

FIGURE 2. Metrics can be devised to summarize the status of soil microbial communities.



THERE ARE CAVEATS WHEN UNDERTAKING MICROBIAL MONITORING

A number of factors need to be considered when undertaking microbial monitoring of rehabilitating soils and these are discussed using examples from studies we have undertaken. These include:

- The use of reference sites
- Organic matter is not microbially inert
- The early stage disconnect between vegetation status and microbiota
- The use of key microbial species as rehabilitation indicators
- How long does rehabilitation take?
- What are rehabilitation trajectories?
- How often should monitoring be done?
- When should monitoring begin?

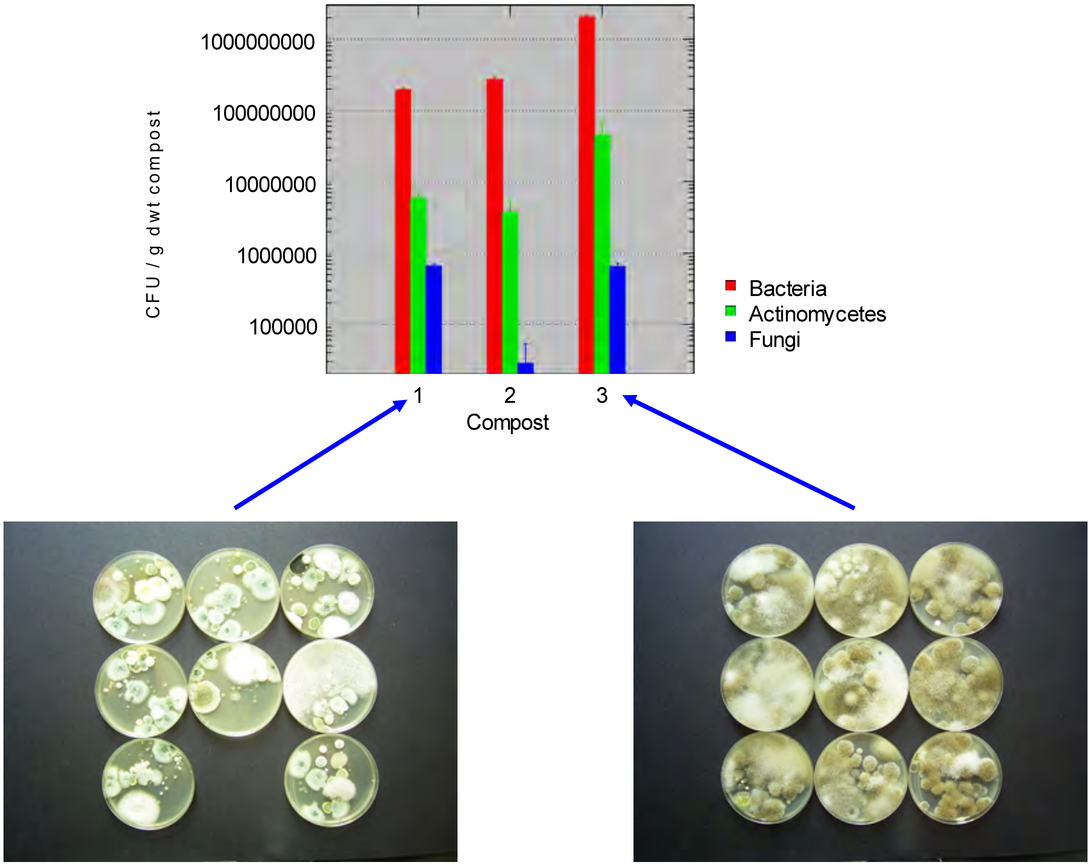
Appropriate reference sites should be used for assessing a soil microbial community since microbial numbers vary between soils and there are no absolute target numbers for a soil to be considered healthy. For example, we have observed some golf greens to have 10-fold greater bacterial and actinomycete numbers than forest soils, but these numbers are associated with a very low biodiversity and a requirement for artificial energy inputs in the form of fertilizers to maintain these numbers.

Organic matter inputs to rehabilitating soils are not microbially inert—some have very high microbial numbers and activities (e.g., biosolids and compost) and others do not. For example, there were significant differences between the numbers and biodiversity of microorganisms in composts that we tested from three different suppliers (Figure 3). The compost containing the greatest numbers of microorganisms had the lowest microbial diversity. Furthermore, microorganisms not normally seen in soils but which are introduced with certain types of organic applications, such as biosolids, can still be detected in rehabilitating soils after 5 years.

Despite the high numbers of microorganisms associated with some types of organic matter, unless soils are particularly devoid of microorganisms the percentage of new microbes added to a soil through the addition of organic matter can be low unless the organic matter has high numbers of microorganisms associated with it (e.g, biosolids) and the application rate is high (e.g., > 50 t dwt ha^{-1}). Furthermore, these microorganisms will have to compete with a significant number of indigenous microorganisms already present in the soil. Therefore, it is more likely that organic matter additions generally act as a source of nutrients that are able to be used by the existing soil microorganisms rather than as a source of new microorganisms.

In the early stages of revegetation there is often a disconnect between the status of the vegetation of a rehabilitating area and that of the soil microbiota as organic matter inputs do not occur naturally as they do in native vegetation areas. For example, Chen *et al.* (2012) examined the relationship of microbial biomass carbon and tree root distribution in an urban environment four years after different soil rehabilitation treatments and found that there was no relationship between microbial biomass carbon and plant root biomass; however, the soil total organic carbon was positively related to microbial biomass carbon. Several studies we have undertaken of early stage rehabilitating mine soils have indicated that soil microbial communities are influenced by the quality and quantity of the organic matter already in the soils until natural organic matter inputs begin to occur. This demonstrates the strong interrelationship between soil organic matter and the soil microbiota.

FIGURE 3. Significantly different numbers and diversities of bacteria, actinomycetes, and fungi are associated with different composts. Photographs are of the fungi isolated from the composts. The highest biodiversity was observed in compost 1. Compost 3 had high numbers but of only a single fungal genus.



There are some microorganisms that can act as surrogate markers of the microbial community and organic matter status of soils. For example, *Trichoderma* is an important cellulose-decomposing fungus commonly observed in native soils with natural inputs of organic matter. Its increase in abundance over time on rehabilitating mine sites is associated with organic matter inputs to the soils in the form of leaf litter and/or increasing grass cover (Figure 4).

Declines in numbers of *Trichoderma* have been noted in the soils of some early stage rehabilitating sites in the initial few years after topsoil additions. This indicates a decline in cellulosic substrates in these soils as organic matter inputs have not had a chance to occur naturally as they do on native and well-vegetated sites.

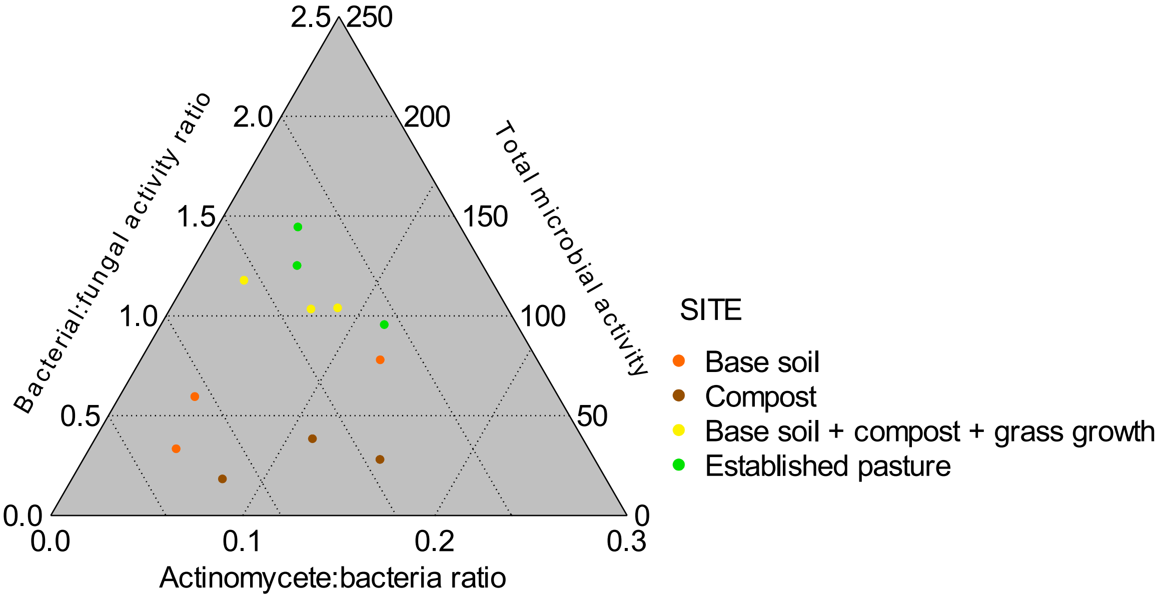
A study of the recovery of microbial biomass in rehabilitating bauxite mine soils in Western Australia found that biomass levels recovered to those of native jarrah forest soils after six years when using direct-returned topsoil and nine years when using stockpiled topsoil (Sawada, 1996). Our own work has shown that 5 years after topsoil and seed application the soil microbial communities of some rehabilitating coal mine soils were similar in status to those of native vegetation sites. Thus, it can take a number of years for the status of soil microbial communities of rehabilitating sites to approach those of analogue sites and, in addition, not all rehabilitating sites will follow the same recovery trajectory.

FIGURE 4. Key microbial species can help identify the functional status of soil microbial communities. The increased presence of *Trichoderma* (large green colonies) in rehabilitating soils is often associated with organic matter inputs such as leaf litter.

There are a number of different paths that a soil microbial community can take in its development and several factors influence the path. These include the initial microbial status of the overburden used, the microorganism and nutrient additions associated with organic matter or topsoil applications, the site slope and it's effect on soil erosion, and changes in the water status of the soil.

However, constant monitoring of a soil's microbial status may not be necessary. Given that it might take some years for stable soil microbial communities to develop in rehabilitating soils, it is not necessary to monitor sites annually unless a problem with soils is suspected or has obviously occurred. Strategic monitoring is of greater value and soil microbial monitoring should commence at the most appropriate time to maximize the value of the data. For example, determining the microbial status of topsoil stockpiles as they are established and again prior to their re-use can provide information on the effectiveness of stockpiling conditions. It can also show whether treatments might be required to rejuvenate or augment the microbial community of the stockpiled soils. This is important as the origins of the topsoil and its management prior to reuse are important factors in determining the microbial populations that are applied to rehabilitating sites and the likelihood of them establishing in their new environment without further assistance.

FIGURE 5. Compost addition to a microbially-poor soil assisted microbial community development towards that of an established pasture and also improved establishing grass growth.



The initial monitoring of newly-established rehabilitating soils also provides a benchmark for subsequent status checks. Figure 5 shows that adding compost to a microbially-poor soil had assisted the microbial community development towards that of an established pasture community within 7 months as well as improving new grass growth.

SOIL DISTURBANCE, MOISTURE, AND ORGANIC MATTER ARE PRIMARY REGULATORS OF SOIL MICROBIAL COMMUNITIES

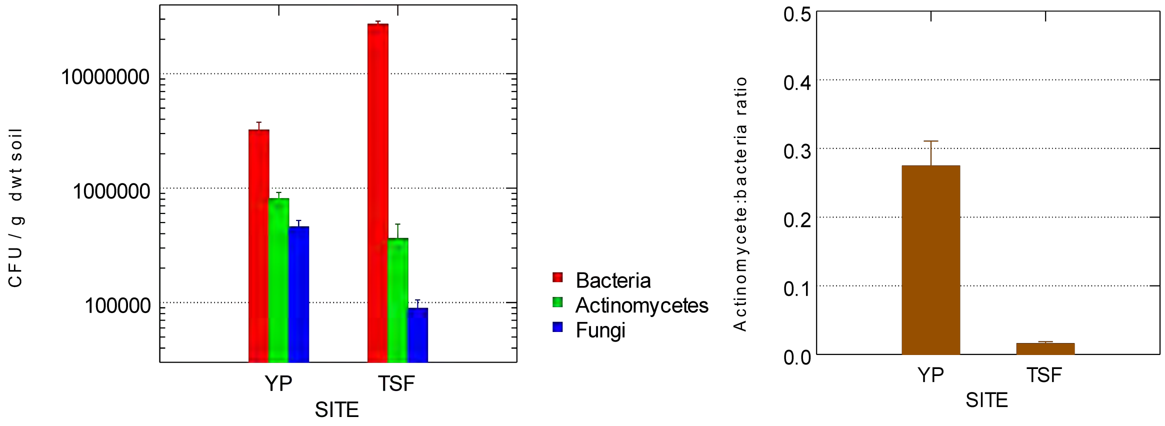
Soil Disturbance

As earthquakes can disrupt complex rail or road networks, disturbance ‘earthquakes’ comprising the physical, chemical, and biological actions that occur during topsoil stripping and stockpiling more readily disturb the filamentous microbial networks in soils than the unicellular bacteria; however, the filamentous actinomycetes and fungi are important for decomposition of complex carbon substrates such as chitin, celluloses, hemicelluloses, and lignin to create humus in soils. Bacterial numbers often exhibit an initial rise with soil disturbance due to a flush of readily-utilisable nutrients associated with the disturbance; however, actinomycete and fungal numbers usually decline which makes them sensitive indicators of soil disturbance. The changes in bacterial and actinomycete numbers means that the actinomycete:bacteria ratio is a sensitive indicator of disturbed soils and is usually much less than 0.1 in these soils (Figure 6).

Soil Moisture

Soil microbial numbers and community activities are primarily regulated by moisture availability in the form of the timing and magnitude of rainfall. As such, microorganisms are often water limited in soils and rely on windows of opportunity provided by rainfall inputs in order

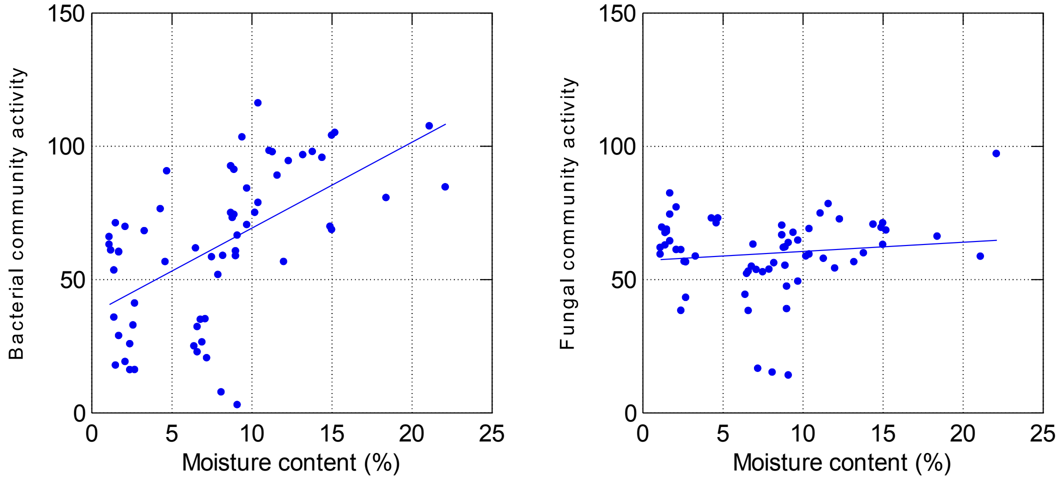
FIGURE 6. Actinomycetes and fungi are sensitive indicators of soil disturbance. The actinomycete:bacteria ratio is a sensitive indicator of disturbed soils. The pre-disturbance values are the bars on the left in both graphs.



to function. In general, bacteria require greater soil moisture for growth than fungi, so fungi compete more successfully at lower moisture values. Figure 7 illustrates the greater decline in bacterial than fungal community activities as soil moisture levels decrease in mine site soils.

Most soil practitioners are familiar with the concept of soil water potential where water potential is the measure of water's energy state and is the sum of matric, osmotic, and gravitational potentials. Water potential is related to water activity, which is much easier to measure, especially in the field. Water activity is a thermodynamic parameter (on a scale of 0–1) defined in relation to the chemical potential of water and represents the unbound or available water in a sample. Small changes in the water activity have large impacts on plants and microorganisms, especially in the wet range. Plant water availability occurs between 1 and 0.99. The majority of bacteria require water activities above 0.98 for growth and few grow at values below 0.90. However, a number of fungi can grow at water activities between 0.90 and 0.80 and a few can grow at very low water activities.

FIGURE 7. Bacterial community activity declines more rapidly than fungal community activity as soil moisture levels decline.



The relationship between soil moisture content and water activity is unique to a soil due to the relationship between the physical and chemical nature of the soil and its organic matter content. This can be important in soil rehabilitation on mine sites. Figure 8 shows a number of native and rehabilitating soils where bacterial growth-limiting water activities can be seen in some but not all the soils at low soil moisture contents. Bacterial growth-limiting water activities at low soil moisture levels were more common in rehabilitating soils and topsoil stockpiles. In addition, most of these soils would be water limited for plants.

The unique relationship between soil moisture content and water activity in a soil can lead to the paradox of soil bacterial communities, in particular, being water-limited and non-functional at higher soil moisture levels in some soils than others. In Figure 8 some native soils can be seen to have lower moisture content than an old topsoil stockpile, yet they have higher soil water activities which enable their microbial communities to perform their ecological functions.

Soil drying can also have a long-lasting effect on microbial communities. The effect of soil drying on microbial communities is not a simple event with the rewetting returning microbial community status to how it was. Microbial community activity can decline after soil drying and rewetting and changes in microbial community structure can also occur (Fierer, Schimel & Holden, 2003). The extent of stress associated with soil drying and the subsequent recovery of the soil microbial communities after rewetting is dependent on the soil type (Chowdhury, 2011). Therefore, the long-lasting effects of soil drying on microbial communities are an important consideration when soil is removed, stockpiled, and subsequently reused for rehabilitation.

Soil organic matter

Soil organic matter is a keystone component of ecosystems. Organic matter cover prevents soil erosion and water loss, buffers soil temperature, and can be a source of microorganisms. Organic matter incorporation into soils will store water for plants and microorganisms, is an energy store driving many soil-based processes, adds to the available nutrient pool, and can be a source of microorganisms.

The presence of soil organic matter encourages microbial community development in soils as microorganisms are often energy limited in soils and rely on organic matter inputs for

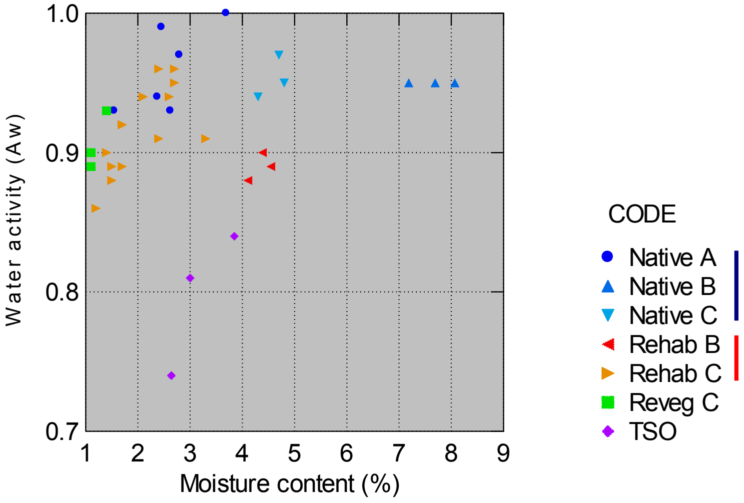
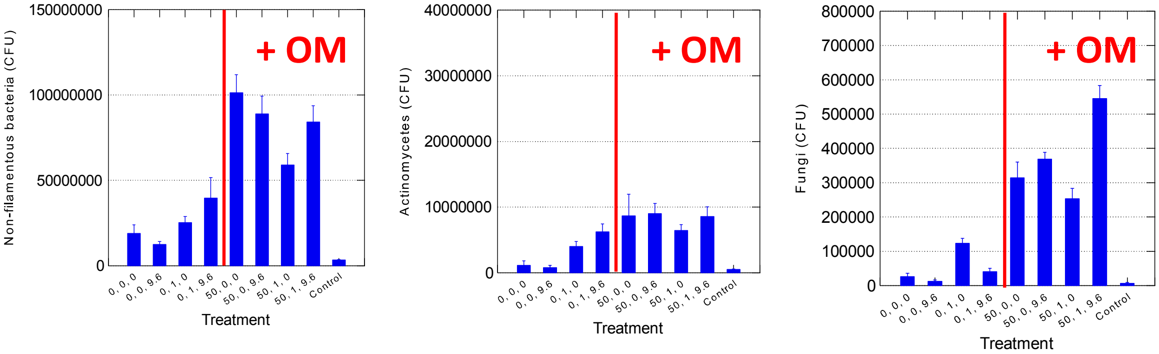
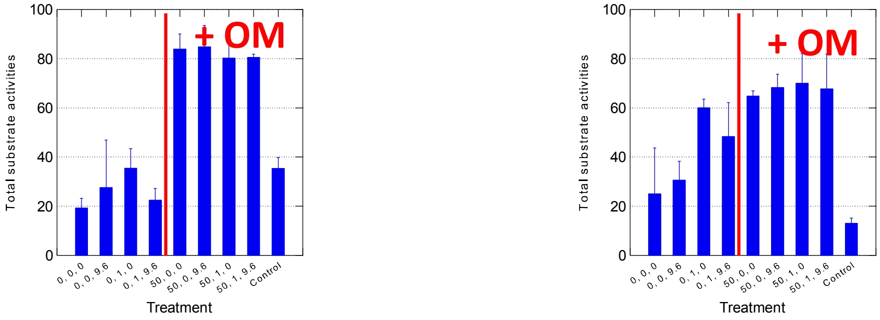


FIGURE 8. The relationship between soil moisture content and soil water activity is unique to a soil. Bacterial growth-limiting water activities at low soil moisture levels are more common in rehabilitating soils (Rehab B, Rehab C) and topsoil stockpiles (TSO).

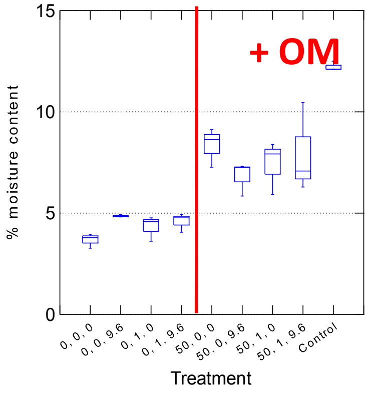
FIGURE 9. Organic matter additions increased microbial numbers and community activities as well as retaining soil moisture in rehabilitating soils. Treatments that included an organic matter component (+OM) had the greatest effect.



Bacterial (left), actinomycete (middle) & fungal (right) numbers



Bacterial (left) & fungal (right) community activities



Soil moisture

this energy. The stimulating effects of organic matter additions on both microbial numbers and community activities in a newly rehabilitating sandy soil can be seen in Figure 9. A number of soil treatments were tested, but those that included an organic matter addition had the greatest effect. Although soil water was not observed to be limiting microbial growth, the organic matter additions also helped maintain higher moisture levels in the soil.

TOPSOIL IS A VALUABLE RESOURCE WHICH NEEDS TO BE KEPT ALIVE AND IN PLACE

As noted previously, topsoil is the most valuable rehabilitation resource on a mine site and needs to be kept alive and in place (Figure 10). However, it can be lost as dust through wind erosion and by mass erosion and soil de-differentiation through water flows. The greatest soil erosion risk is before vegetation establishes and the window-of-erosion risk is greatest before the vegetation cover is above 50%. Water erosion can cause the greatest soil losses from bare ground or sites with low vegetation cover but soil losses from rehabilitated sites are usually significantly less. For example, soil losses of several mm per year can occur on sites with low surface cover compared with < 0.5 mm per year on rehabilitated sites (1 mm depth $\mathrm{yr}^{-1} \approx 12$ t ha^{-1} yr^{-1}). Soil de-differentiation can also result from water flows redistributing soil particles along a slope.

Microorganisms can play a role in stabilizing soils in the critical time before vegetation establishes through soil aggregation by both chemical and physical means. Polymer production is a common characteristic of soil microorganisms and especially the cyano- or “blue-green” bacteria and yeasts. Microbially-produced polymers can affect soil properties in a positive way and can act to mitigate soil loss. They can increase soil wettability and water-holding capacity, and increase soil aggregate formation via chemical means and through stimulating growth of fungal mycelium, which in turn can physically bind soil particles. Microbial polymers have also been shown to reduce the sediment load in water run-off in irrigated agriculture where they may not be as effective as the synthetic polymers that are commercially available but they are less hazardous to handle and are environmentally friendly (Sojka, Entry, Orts, Morishits, Ross & Horne, 2003).

Microbial polymers can be produced inexpensively using organic wastes as substrates. Furthermore, there is no necessity for purification of the polymer if the producing microorganism is environmentally benign. The polymer, leftover substrate, and producing microorganism can be applied as a separate application to soils or could be included as a part of some other stage of the rehabilitation process. We have scaled up production of a microbial-produced polymer to 5L including using non-sterile conditions similar to those employed in home beer brewing, thus allowing the prospect of its production being done close to where it is to be utilized. The polymer is produced by an environmentally safe, commonly-occurring

FIGURE 10. Topsoil stockpiles must be stabilized to avoid subsequent mass soil erosion and de-differentiation issues. A fresh topsoil stockpile (left) and an unstabilized stockpile (right) are shown.

soil fungus and preliminary testing in the laboratory has shown it improves soil wettability, water holding capacity and aggregation, together with reducing sediment load in water runoff. The US Army Corp of Engineers has also scaled-up the production of a different biopolymer to 1500L using an unsophisticated fermentation system (Griggs, 2010).

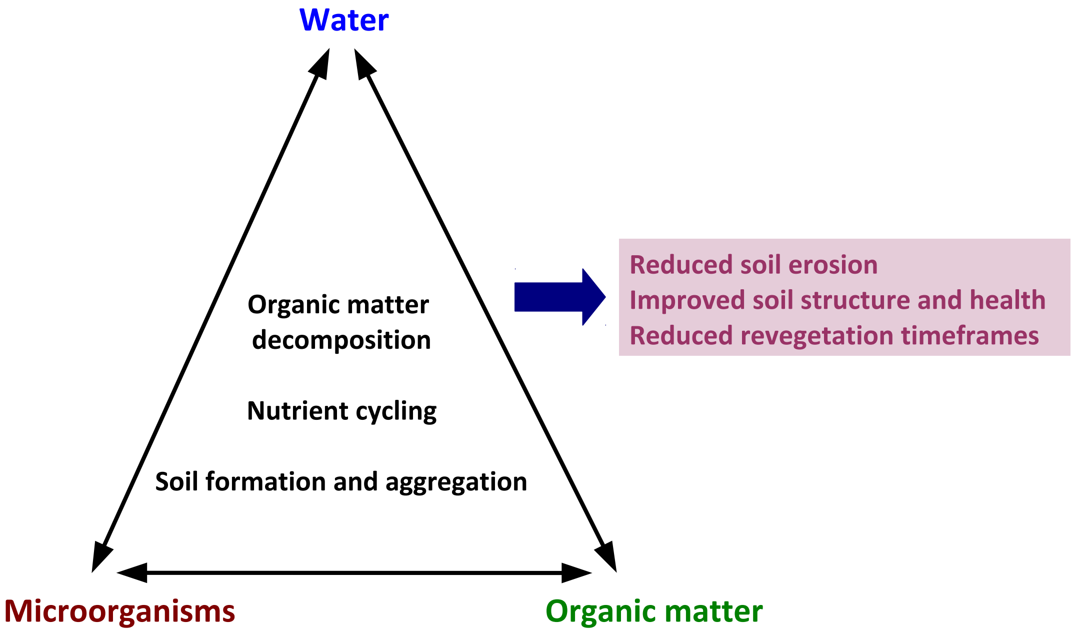
Physical stabilization of soils occurs by the binding of soil particles into aggregates by the networks of filamentous microorganisms such as cyanobacteria, actinomycetes and fungi (both decomposer and mycorrhizal). As noted previously, however, disturbances caused by the physical, chemical, and biological actions that occur during topsoil handling more readily disturb these filamentous microbial networks than the unicellular bacteria. Consequently, the minimization of soil disturbances will reduce the need to rebuild physical microbial networks which is a more complex task than chemically stabilizing soils.

CONCLUSIONS

Microorganisms generally remain an undervalued asset on mine sites and, in Australia to date, are not part of any predetermined regulatory criteria that are required to show that disturbed land has been restored to a stable, productive, and self-sustaining condition, and taking future land use into account. However, with recent papers suggesting that radically disturbed sites might be restored to hybrid or novel ecosystems comprising new combinations of physical and biological components (e.g., Doley & Audet, 2013) the use of multiple quantitative indicators of ecosystem condition—including the microbial status in soils—become more necessary.

Microorganisms should not be an afterthought during soil rehabilitation works on mines. They are the major living component of topsoil, which is the most important rehabilitation asset on a mine site, and their status should be known in order to ensure that they are working to their full potential. Figure 11 emphasizes the important interactions of water, organic matter, and microorganisms in soil creation and soil function and how any one of

FIGURE 11. A simple model of the interaction of water, organic matter and microorganisms in soil creation and soil function.



them can rate limit these processes. These three components should be at the forefront of managing any soils for rehabilitation.

Our work on a number of rehabilitating mine soils has provided evidence of increased microbial activity over time in these soils, but this has largely been as an indirect consequence of rehabilitation activities rather than through the positive manipulation or management of soil microorganisms. Further work needs to be undertaken to quantify the benefits of reduced soil erosion, improved soil structure and health, and reduced revegetation timeframes in mine rehabilitation through pro-actively managing microbial populations in soils during their stripping, stockpiling, and re-use.

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