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Measurements of the soil microbial community for estimating the success of restoration

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Summary

Land degradation is of concern in many countries. In order for timely and effective interventions to be made to reverse this degradation it is necessary to have objective measurements of ecosystem status. By measuring characteristics of the soil microbial community we can assess the status of the microbial ecosystem and in that sense the quality of the soil, and the potential for, and progress of, restoration after degradation.

Recent research has shown quantitatively how by measuring the soil microbial community we can assess degradation and the effects of management designed to reverse it. The size, composition and activity of the soil microbial community convincingly distinguish between systems, and between the impact of management strategies upon them. Measurements of these characteristics of the microbial community provide invaluable information for restoring degraded land and are ready for routine use. Specifically, profiles of phospholipid fatty acid contents, and substrate induced respiratory responses to different carbon substrates, will yield significant data upon which management decisions may be based.

Introduction

The problem of land degradation is one of concern in most nations of the world. In England alone 0.4% of the land surface is classified as derelict and requires treatment before it can be put to beneficial reuse. In addition there is a considerable area of land where the soil–plant system becomes degraded by civil engineering practices (such as opencast coal-mining) or intensive agriculture. The need for large-scale programmes aimed at restoring ecosystem structure, composition and function has never been more pressing (Cairns, 1999; Hobbs & Harris, 2001). In the UK there is increasing recognition of the potential for ecological restoration to deal with several problems simultaneously, such as rise in sea level, protection of catchments, and agricultural reform (Sutherland, 2002).

The Society for Ecological Restoration's (2002) definition of ecological restoration is as follows: 'Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed'. This leads to the central question facing the land manager attempting to remediate or restore degraded land of how to measure success or failure on a particular site or landscape. An important component of this is the need to have an early indication that the processes set in motion are putting the system of interest on the correct trajectory of recovery, which may be interpreted as either recovery from a disturbance to an established system, or, in extreme cases, primary succession on nascent soil-forming materials. It is essential that some objective measures of the status of an ecosystem are available if we are to assess such programmes. Central to this is the need to provide an objective measurement of the soil subsystem.

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Current methods for assessing soil quality give only an incomplete picture of the status of the soil system. Two indicators of that quality have been suggested by MAFF (2000): organic matter content and accumulation of heavy metals, both in agricultural topsoils. These are limited in use at best, and the measurement of heavy metals is irrelevant for many semi-natural situations, and give no indication of the ecological status of the soils under investigation. Arshad & Martin (2002) have suggested several indicators for assessing soil quality in agro-ecosystems, namely organic matter, topsoil depth, infiltration, aggregation, pH, electrical conductivity, suspected pollutants, and soil respiration. Although knowing these aids management decisions, they lack any indication of the dynamic ways in which soils need to respond to stresses and disturbances. A comprehensive determination of soil microbial community characteristics is one way in which this question may be addressed.

Measurements of the soil microbial community may certainly be used to determine biodiversity, ecological processes and structures (Society for Ecological Restoration, 2002), a suggestion of some long standing (Harris & Birch, 1992). In this context there are two main approaches for determining the success of a restoration scheme, as follows.

1 Return to conditions that approximate a target or reference ecosystem.

2 Maximization of efficiency of the ecosystem with respect to its function.

These two operational aims are not mutually exclusive, but the first approach may be easier to achieve than the latter. In choosing a target, it is necessary to determine how appropriate that target is. The advantage of microbially based measures over those involving larger organisms is that it is not easy to fake the signals from the recovering ecosystem microbial community. For example, one might plant trees to produce a vegetational structure that approximates to National Vegetation Classification (NVC), but it is not possible to do this with a microbial community because of its sensitivity to all biotic and abiotic characteristics (e.g. oxygen tension, carbon quality and quantity).

Wolters (2001) has indicated that although there may be multiple functional redundancy in the soil biota, the link between diversity and functional capabilities is still one worthy of investigation. This is particularly true in land restoration – the systems involved have often been pared back to a minimum, and crucial break-points in functional collapse have been passed, such as the transformation and supply of nutrients to plants, and the absence of mycobiont propagules essential for the establishment of certain species (Requena *et al.*, 2001). Measurement of the microbial community has utility as an indicator of the re-establishment of connections between the biota and restoration of function in degraded systems.

In situ and inherent properties of soil

When employing measurements of microbial community to determine the status of an ecosystem with respect to its target or reference system, we should keep in mind that both inherent and *in situ* characteristics have to be measured. Inherent characteristics are those independent of location (in the short term) and can be measured by taking samples in the field, fixing or preserving them, and returning them to the laboratory for analysis. *In situ* characteristics, however, are those engendered by a combination of topology and hydrology, which may be lost upon destructive sampling. Soil microorganisms have also been much used in bioassays of contaminated land, and to follow the effects of a particular pollutant, particularly when engineered to contain the *lux* gene (e.g. Strachan *et al.*, 2002).

The focus of this review is principally the inherent characteristics of the soil microbial community, and in most studies of degraded systems these tend to be those chosen for investigation.

Methods

There are numerous reviews of the methods for the assay of soil microbial characteristics (e.g. Alef & Nannipieri, 1995), and it would be of little utility here to repeat the details. The techniques are manifold, and one type of measurement may often supply information relating to more than one characteristic. Essentially, there are three main conceptual classes to be determined, as follows.

- Size – the total mass of the *viable* microbial community, usually expressed in units of carbon, but also as other essential elements or component parts.
- Composition – the *abundances* of particular species or functional groups.
- Activity – the *metabolic turnover* of the biomass, from internal metabolism to conversion of nutrient pools.

Additionally, some methods focus on the importance of the physical arrangement of the community (Nunan *et al.*, 2001), but this takes us beyond simple consideration of the biomass as a conceptual unit, and is difficult to apply routinely to the large number of samples required in a real survey.

These single-factor measurements are often combined with one another, or with other components, to produce ratios. For example, the microbial metabolic quotient (respiration-to-biomass ratio), or $q\mathrm{CO}_2$, has often been used in interpreting trends in the development and disturbance of an ecosystem (Ohtonen *et al.*, 1999; Saviozzi *et al.*, 2001), which is of particular interest in this present text.

Trends in naturally disturbed systems

For studies of trends in the microbial community during the re-establishment and restoration of ecosystems on severely degraded sites, such as opencast coal mines, data from naturally disturbed and nascent substrates offer a valid and useful comparison.

Insam & Haselwandter (1989) investigated the changes in microbial carbon along a transect taken from a retreating glacier. They found steady increases in microbial biomass with time since retreat of the ice front, and they showed that these were related to the development of vegetation cover (Figure 1). Ohtonen *et al.* (1999) found a similar trend at the forefront of the Lyman Glacier in Washington State, and they showed that there was a shift from communities dominated by bacteria to ones dominated by fungi.

Similar trends have been reported in other disturbed and regenerating sites, such as landslides in forests in Nepal (Singh *et al.*, 2001) and primary succession transects on the uplifted coast in Western Finland (Merila *et al.*, 2002).

Trends in disturbed and reclaimed sites

Biomass measurements

Insam & Domsch (1988) sampled the soil on two chronosequence transects from opencast mining, one to agriculture

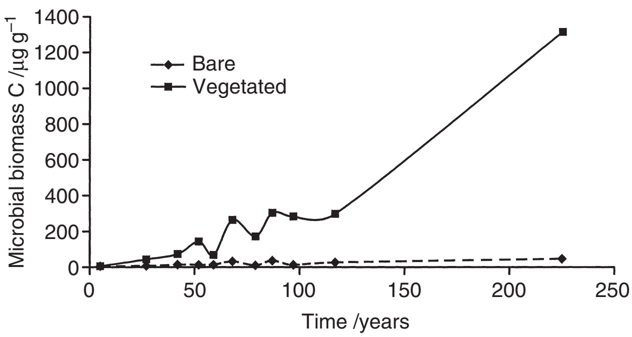

Figure 1 Changes in microbial biomass C with time on a retreating glacier forefront (redrawn from Insam & Haselwandter, 1989).

and the other to forest, representing a 50-year period. They found that there were increases in microbial biomass and decreases in qCO_2 with increasing time on the agricultural sequence, but not under the forest. The forest soils also had a much smaller contribution of microbial carbon to total carbon than the soil of the agricultural sequence. The authors concluded that combined measurements were better than those of single variables in tracking the changes in ecosystem characteristics during restoration. This approach has been used by several other authors; for example, Johnson & Williamson (1994), also working on opencast sites, found very small ratios of biomass C:organic C on sites 3 years after re-instatement. Harris *et al.* (1989) also used measurements of microbial biomass to determine the impacts of storing topsoil during opencast mining, the results of which indicated the serious additional degradation caused by the stockpiling. Harris *et al.* (1993) took this work further by suggesting a theoretical approach based on microbial lifestyle strategies to the interpretation of such data.

Hart *et al.* (1999) investigated the combined effect of ripping, fertilizer N application and grazing management on subsoils at sites from which the topsoil had been stripped (i.e. removal to 30 cm depth). They reported that microbial biomass C had recovered to 62% on undisturbed topsoil, and mineralizable N to 65% after 3 years. The productivity of the pasture was 70% of reference system, and the authors concluded that the cost of continued application of N to achieve even this depressed yield made the remediation uneconomic. A particularly interesting feature of their paper is the link made between soil measurements and setting policy guidelines, in particular the proposed incentive scheme resulting from this work, designed to encourage land owners and topsoil miners to use successful restoration techniques on these sites.

Ruzek *et al.* (2001) demonstrated that although there were clear relationships between time since restoration and increases in soil microbial biomass, distinctly different algorithms had to be developed for reclaimed sites in the Czech Republic and Germany. Ruzek and co-workers indicated that this was related to both organic matter content as a starting point in new reclamations and the textural characteristics of the soils reclaimed. This underlines the need for caution when fitting microbial parameters into general models, and the importance of having defined target areas to allow direct comparison.

Zeller *et al.* (2001) demonstrated a shift from bacterial to fungal biomass in subalpine meadows abandoned by farmers in the European Alps. Assessing this type of shift in community composition is more likely to yield useful information than gross biomass estimates where changes in management practice, rather than restoration of significantly perturbed sites, is being followed. Emmerling *et al.* (2001) followed the changes in a site where intensive agriculture had given way to a low-input system over a 10-year period, and they found a 10–15% increase in all of the following: microbial biomass, the C_{mic}:C_{org} ratio (where C_{mic} is the microbial carbon and C_{org} the total organic carbon in the soil), and soil organic matter. This indicates that gross microbial measurement may be superfluous or insufficient in such cases.

In contrast to many studies showing increases in total microbial biomass with time, Lukesova (2001) found decreases in numbers of unicellular algae in mining spoils in artificial chronosequences in the Czech Republic and Germany. This was linked to the development of tree cover on these sites, and indicated that the development of ecosystems on minespoil is analogous to that on raw substrates exposed by natural processes, as when glaciers retreat (Insam & Haselwandter, 1989).

Activity measurements

Degens & Harris (1997) developed a method for determining the soil microbial community's catabolic capabilities based on the respiratory response of the soil microbial community to a variety of carbon substrates. They measured substrate induced respiration (SIR) during 4 hours, and found that the results discriminated between different management regimes. From these primary data they calculated a 'catabolic evenness' index using the Simpson–Yule index. Schipper *et al.* (2001) applied the method to several successional sequences of plants on soil substrates newly exposed after natural disturbances, namely landslips, volcanic eruptions, glacial retreat, and a variety of islands supporting different successional stages. The results suggest that heterotrophic evenness of response re-established quickly after disturbance once significant organic matter inputs occur, but then declined. This is consistent with the humpback model of species diversity with respect to the availability of resources (Grime, 1979).

Harris & Birch (1989) followed changes in the soil at sites reclaimed after opencast mining by enzyme assay and measuring nitrifying potential, and found increases in both with time. Restoration can evidently enhance the rate of mineralization of N. Kaye & Hart (1998a) investigated several restoration treatments of ponderosa pine–bunchgrass communities near Flagstaff, Arizona. Their treatments comprised: partial thinning to presettlement conditions, or complete removal of trees, plus addition of native plant litter and a prescribed burn. They found that the restoration treatments had 2–3 times greater annual net N mineralization and 3–5 times greater annual net nitrification than the control. They also found (Kaye & Hart, 1998b) significant effects on soil respiration. The amount of nitrogen in an ecosystem will also control the size of other populations, notably that of herbivores. Hendrix *et al.* (1998) showed that earthworms increased the rate of microbially mediated turnover of N in soils, whilst reducing the total microbial biomass. Coyne *et al.* (1998) examined the addition of various organic wastes to stimulate N recycling on a former surface coal mine in Muhlenberg County, Kentucky, with the intention of reclaiming it for prime farmland. Specifically they set out to test the hypothesis that adding organic waste stimulates microbial activity on such mines. The organic amendments consisted of a mixture of poultry waste and sawdust at 25 and 40 t ha^{-1}, respectively. The net effect of this was to increase gross N mineralization, nitrification, and immobilization by up to 4.5 times that of the controls, and to magnitudes similar to those in the reference ecosystems.

Vance & Entry (2000) sought appropriate soil measurements to track the success of restoration on barren land and adjacent Shasta red fir forest in the Siskyou Mountains, Oregon. They found that enzyme activity was a better indicator than microbial biomass in this respect, and that it reflected the accumulation of organic matter well. However, this may be due to the methods employed. The authors used direct biomass staining, which is both time-consuming and sensitive to the operator. There might have been some value in making comparison of these approaches with non-direct methods such as ATP or fumigation–extraction methods.

An interesting technique for combining activity with diversity measurement has been devised and employed by Yin *et al.* (2000). The authors took soil samples along a transect on the Jamari tin mine site in the Jamari National Forest, Brazil, from bare minespoil through restored and recovering land to undisturbed forest. They then amended subsamples with individual carbon substrates (L-serine, L-threonine, sodium citrate, and α-lactose hydrate) in the presence of bromodeoxyuridine (BrdU), which would become incorporated into bacterial DNA as a result of metabolizing the added carbon. This enabled them to identify what proportion of the bacterial biomass had been actively involved in the metabolism of the added substrates, and therefore to obtain an index of functional redundancy. They demonstrated clearly that bacterial functional redundancy increased as they went from disturbed to undisturbed land, and that this increase could be related to the re-establishment of plant species. Significantly, the increase in total diversity (Shannon's index) increased as a step change from that in bare spoil to that under a pioneer tree, whereas the immuno-captured, i.e. active, richness increased in proportion to the increase in plant community cover (Figure 2). A total richness score of zero in the minespoil samples is a puzzle when compared with a value of 5 for the active portion; this is, nevertheless, highly suggestive of the scenario that the *arrival* of bacterial species depends on time since exposure or end of disturbance of the substrate (and this occurs fairly quickly), but the *activation* of the bacterial species depends on input from primary producers. This is both fundamentally important and of great significance when planning restoration because the delayed arrival of species essential for successful vegetational establishment may represent a bottleneck to the establishment of late successional species, and points to the importance of immigration rates of microorganisms.

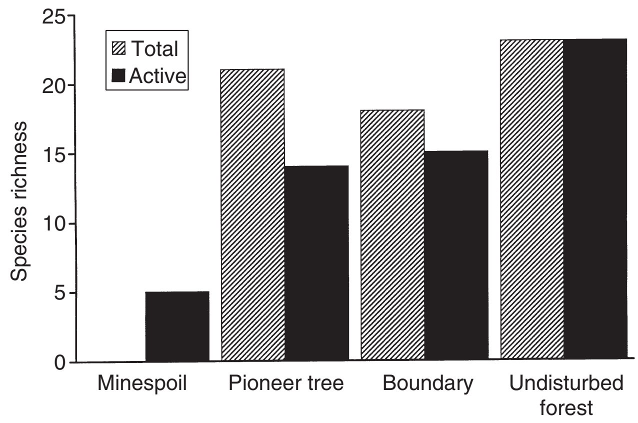

Figure 2 Averages of total and active bacterial species richness along a restoration gradient (redrawn from Yin *et al.*, 2000).

Measurements of composition and diversity

These types of measurements fall into two principal classes:

- measurements of species numbers by taxonomically based counts, and
- broad diversity estimates based on molecular or biochemical measurements or both.

Measurements of species numbers by taxonomically based counts

As in many early studies of soil microbial communities, isolation and culturing by means of solid and liquid media have been carried out in restoration programmes. However, these suffer severe limitations, all of which lead to underestimations in terms of biomass and species composition. Although some general observations have been made on the type of effect that soil handling techniques have had on the community, e.g. compaction decreases species number, these have been largely revisited and greatly improved by indirect techniques. Similarly, microscopy-based counting has proved to be time-consuming and subjective.

Broad diversity estimates based on molecular and/or biochemical measurements

Phospholipid fatty acid analysis. Phospholipid fatty acid (PLFA) analysis provides a broad diversity measurement of microbial community composition. The types and amounts of different PLFAs extracted from samples reflect both taxonomic and functional diversity. There has been great interest in and a significant amount of research carried out with the procedures for extracting, derivitizing and estimating the quantities and identities of PLFAs in order to derive community profiles of the soil microbial biomass. These have tended to focus on the polar lipid fractions, but the results from such studies must be interpreted with caution, and should ideally be extended to cover the whole lipid fraction, polar, neutral and glycol-lipids (Zelles, 1999).

In a study of the effects of disturbance caused by military vehicles at Fort Benning, Georgia, Peacock *et al.* (2001) used PLFA analysis on five sites: light disturbance (infantry training); moderate disturbance (areas adjacent to tracked vehicle training); heavy disturbance (tracked vehicle training); remediated (previously heavily used, now planted with trees, and unused); and an unused reference area. The quantities of microbial biomass in these sites estimated by total PLFA are shown in Figure 3. Key conclusions here are: the significantly smaller value for the heavily disturbed area; the recovery of the remediated area; and the great variation of the disturbed and remediated areas compared with the reference area values. Peacock and co-workers further show that increased disturbance caused decreases in those PLFAs associated with Gram-negative bacteria and microeukaryotes, but increases in relative proportions of Gram-positive bacterial and actinomycete biomarkers. They also went on to apply a predictive linear discriminant model and non-linear artificial neural network (ANN) discrimination to the data, both of which successfully distinguished the amount of traffic a soil had received, and clearly showed the differences between the communities of the lightly and undisturbed areas and the moderately and heavily trafficked areas (a result not clear from the biomass determinations alone). Both techniques had a predictive effectiveness of 66% (i.e. in correctly ascribing PLFA profiles to site class), but the ANN included all PLFA and biomass data, without having to make assumptions of normal distributions or linear relationships. This approach holds great promise for predicting the effects of stress and disturbance in systems, and the efforts made to restore and remediate systems.

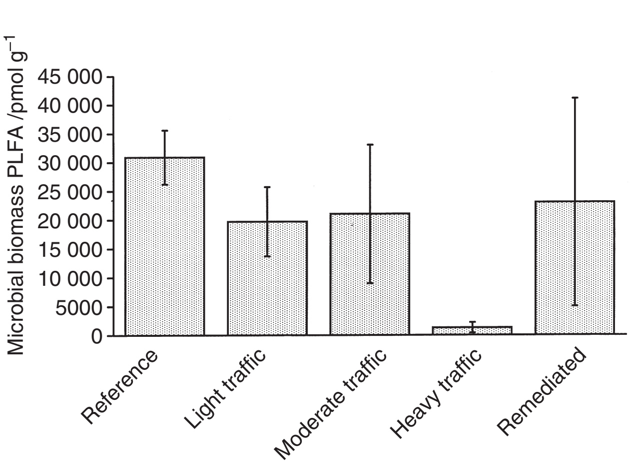

Figure 3 Effect of trafficking pressure by military vehicles on microbial biomass phospholipid fatty acid (PLFA) content. Bars represent standard errors (redrawn from Peacock *et al.*, 2001).

Extracted nucleic acid based techniques. Although techniques based on extracted nucleic acids have seen wide application in studies on soil ecosystems, their use specifically in tracking land restoration has been rather limited. There has been significant work in metal-polluted systems (McGrath *et al.*, 1995; Torsvik *et al.*, 1998; Sandaa *et al.*, 1999), but this has tended to focus on the consequences of metal contamination as a means of elucidating biodiversity of communities, rather than as a tool for following reclamation treatments. Nucleic acid tools have also been used for assessing relationships between symbionts in disturbed and reclaimed lands (Schwenke & Caru, 2001). Here the importance of microbial symbionts for the establishment of particular plant groups, such as legumes, has been confirmed.

Combined measurements

Bentham *et al.* (1992) illustrated clearly the efficacy of measurements of soil microbial communities in comparing the status of disturbed ecosystems with that of undisturbed reference sites. Size (ATP biomass), composition (fungal ergosterol) and activity (dehydrogenase enzyme) were determined, and samples were taken from a variety of sites. The resultant cross-ordination of these variables proved better at discriminating between sites than physico-chemical measurements alone, or when used in combination with them. A similar approach was taken by Tscherko & Kandeler (1999), who combined microbial biomass assay (determined by SIR) with measurements of urease, denitrification, arylsulfatase and xylanase activities, at experimental sites subject to a variety of perturbations. This approach is not only powerful in discriminating between different systems, but its presentation is easily comprehensible.

Odum (1997) suggested that qCO_2 should decline during succession and increase during disturbance. This is predicated on a small active biomass being present during the developing (immature) stages of an ecosystem where growth is essential for development, eventually leading to a larger less active biomass in the mature ecosystem. Harris & Hill (1995) took this approach to successfully discriminate between ancient and developing woodland, and ancient and restored floodmeadow. Wardle & Ghani (1995) have demonstrated, however, that

qCO_2 measurements do not always reflect the simple model developed by Odum. On re-analysing data from a number of publications using the qCO_2 approach they concluded that whilst it provides a useful index of microbial efficiency, it has limitations due to the confounding effects of stress and disturbance in degraded and recovering systems.

Effects of specific treatments

Measurements of microbial communities have been used to assay the effects of specific soil amendment strategies. Borken *et al.* (2002) added mature composted household waste to six degraded (acidified) forest sites in Saxony. They found that there were significant increases in microbial respiration, microbial C and N of between 14 and 21% at 0–5 cm, suggesting this may be a useful strategy for restoring the O horizons of forest soils suffering acidification, provided that the wastes contained little salt.

Relationships to other characteristics of the systems

The link between soil microbial measurements and other characteristics of a system is an important one to demonstrate if they are to be convincingly advocated for wider use as ecological indicators.

The genesis of water-stable aggregates is of crucial importance to the successful restoration of site structure and function; it has implications for erodibility, pore space availability and nutrient supply considerations. However, measuring aggregate stability *per se* is time-consuming and expensive. Edgerton *et al.* (1995) demonstrated a close relationship between microbial biomass in restored and reference systems with the water stability of soil aggregates. Jastrow *et al.* (1998), working on a chronosequence of sites restored to prairie, demonstrated strong relationships between microbial characteristics and the stability of soil aggregates, at several scales. All but the smallest aggregate size fraction were positively correlated, on a log-linear scale, with microbial biomass C (Figure 4), a finding similar to that of Edgerton *et al.* (1995), although by far the biggest influence on aggregate stability was the length of fine roots. This indicates, however, that microbial biomass C correlates with aggregate stabilization, and it is more easily and rapidly measured than fine root length.

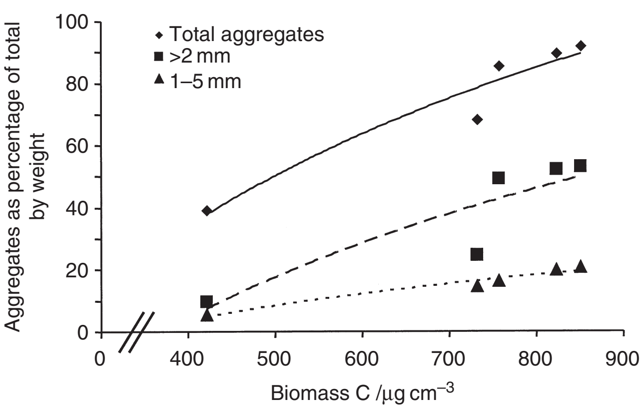

Figure 4 Relation between microbial biomass C and aggregate stability classes (redrawn from Jastrow *et al.*, 1998).

Malik & Scullion (1998) showed that increases in microbial biomass during restoration are not related simply to the passage of time. They found that although soil organic matter did increase over time in soils re-instated after opencast mining, there were not proportional increases in soil microbial biomass, carbohydrates or aggregate stability. This indicates that there were restrictions to successional processes on these sites, possibly related to management. The suppression of earthworm populations by cultivations may have been a crucial factor. Indeed, in further work Scullion & Malik (2000) showed that this relation may well be mediated through the activity of the earthworms in soils restored after opencast mining. Where earthworms were present there were larger microbial biomasses, but less active ones, and this again was linked to greater water-stable aggregation.

A synthesis and recommendations – the future

The measurement of a comprehensive suite of soil microbial biomarkers promises to be powerful in assessing the state of restoration sites (White *et al.*, 1998). By producing a comprehensive profile of the community's size, functional composition, activity, and physiological status, we might be able to identify the effects of particular management for reclamation and restoration. This biomarker approach could be extended beyond the soil microbial community to include other key groups, such as nematodes (Ruess *et al.*, 2002).

Most studies of soil microbial communities in restoration sites have been descriptive along artificial chronosequences, or have reported the effect of management interventions in an experimental setting (although the latter have been rather few). The utility of such approaches has been well demonstrated in a research context, but what is required now is for such measurements to be routinely applied and used iteratively in management programmes. How might this be achieved?

One obstacle to the use of such measurements of microbial communities is the complexity of the data, which are not easy for a site manager to use. Ways to facilitate their use could be:

- multidimensional ordination,
- neural network analysis,
- expert system development,
- development of simple indices, and
- presentation of a minimum number of incisive measurements.

All these must be set within the context of well-characterized reference target systems.

Could measurement of the soil microbial community be used in wider applications, particularly in contributing to determinations of ecosystem integrity? Andreasen *et al.* (2001) provide a checklist of five criteria against which the potential of a particular ecosystem metric could be judged, as follows.

1 Are they relevant to the ecosystem(s) under study and to the objectives of the assessment programmes?

2 Are they sensitive to anthropogenic changes?

3 Can they provide a response that can be differentiated from natural variation?

4 Are they environmentally benign?

5 Are they cost effective to measure?

Most current methodologies meet criteria 4 and 5; analysis of the soil microbial community meets all five. Dale & Beyeler (2001) produce a similar list of criteria, adding that measurements should be easy to make, of small variance and integrative; again, measurements of the microbial community address these points, and have been suggested to do so in agriculture (Stenberg, 1999).

What is also clear is the potential for using such disturbed and degraded sites to test the very fabric of ecological theory. Opportunities for this type of project abound, with huge forces of stress and disturbance being imparted, and the potential for using the data gained to test theory (e.g. Harris *et al.*, 1993; Schipper *et al.*, 2001).

Conclusion

Recent research has demonstrated unambiguously that we can assess the quality of soil by measuring characteristics of the microbial community in it. The measurements enable us to characterize the state of degradation and the effects of management practices aimed at restoring ecosystem structure and function. In particular phenotypic profiling (phospholipid fatty acid contents) coupled with functional profiles (substrate induced respiratory responses to different carbon substrates) yield sufficient data upon which to base management decisions, and to test underlying ecological theory. The techniques, supported by discriminant analysis and neural networks, are now ready for use in a routine way.

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