Soil Teeming with Life: New Frontiers for Soil Science

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Introduction

Soil science in its sub-disciplines of physics, chemistry, biology and taxonomy/genesis is a century old. Many of the basic principles have been established and many practical questions answered. Some would argue that the significant discoveries have been made, the work done and it is time to move resources to emerging fields. In some respects, there is truth in this argument, but at others it is short-sighted and lacking in vision. If we restrict our questions and approaches to those of the past, the criticism applies, but if we consider the challenges of understanding, managing and harvesting the most complex biological community, then we have in our hands one of the greatest opportunities in science.

Many see biology at the heart of the scientific enterprise of the next century. We would agree with this projection, but we also see soil science as an integral part of the biological research enterprise. This may mean that some of our goals and the context of our research changes, but it does not mean that knowledge from any of the soil science sub-disciplines is lacking in importance. We are suggesting that soil biology can become at least one, if not the major driver, for soil science research in the next century.

Important practical issues require soil biology knowledge. These include understanding the role of soil processes in global warming and strategies to ameliorate it; enhanced and safe recycling of waste from manures, urban and industrial activity; pollutant destruction at waste disposal sites as well as on landscapes contaminated from natural processes; biological control of rhizosphere pests; enhanced groundwater

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quality, including safety from the emerging water-borne pathogens; discovery of new biotechnological products, including new pharmaceuticals, pesticides and enzymes from the undiscovered microbial diversity of soil; and optimizing recycling of soil nutrients, soil texture and water content for sustainable agricultural and forestry. The terrestrial (soil) environment hosts almost all of the world's human population and provides much of its basic resources. The biology of soil and its control by the soil's chemical and physical features play a daily role in sustaining those resources. Hence, there should be no question about the importance of soil science in the 21st century.

Biology at the current level of understanding is recognized as complex, i.e. the interactions at the molecular, organismal and environmental level are multiple and often non-linear, making predictability difficult. Understanding this complexity will require expertise from most scientific disciplines including the geosciences, chemistry, physics, computational sciences and even the social sciences. The soil environment is arguably the most complex biological community because of the extremely high diversity at small scales and a chemical environment of complex and changing gradients housed in a heterogeneous physical environment. These features are influenced further by larger scale effects such as climate, geological history and human activity. Several basic facts are important in appreciating the complexity of this community, including:

1. *Soil harbours high population density*. Fertile surface soils typically contain a few billion prokaryotes (bacteria and archaea) per gram and often an equivalent amount of fungal biomass. While soil particle surfaces are not crowded with life at this density, it nonetheless means that the potential for diverse biological activity resides at virtually every microsite.

2. *Soil harbours enormous microbial diversity*. This diversity is exhibited as metabolic, genetic, kinetic, morphological and life history variation. Furthermore, and most significant, it appears that only 0.1% or so of the soil microorganisms have been cultured and hence their metabolic role understood. One of the greatest frontiers in biology remains the discovery and characterization of the particularly novel organisms that reside in soil. Understanding complexity requires knowledge about its component parts; hence novel approaches are needed to understand better the undiscovered diversity.

3. *Soil harbours a tremendous range of physical and chemical conditions*. Life in soil experiences a complexity of gradients of nutrients, oxygen, carbon and other salts which are rarely held constant. Furthermore, the types of carbon compounds are numerous, an important point in understanding a heterotroph-dominated community such as soil. Different mineral surfaces, organic coatings of different ages and composition and the extent and depth of organic surfaces add further to the microbe's complex environment. Also, the physical environment, especially as it influences moisture and the

rate of supply of nutrients and electron-accepting resources, is also critical to the microbial community.

4. *The soil microbial community is a product of more than 3.5 billion years of evolution*. The fossil record indicates that prokaryotes have been on Earth for an extremely long period of time; 85% of their history occurred before Pangea separated. This long period of evolution and natural selection under a wide range of conditions is probably responsible for the enormous microbial diversification. It has also probably selected for organisms that survive stress conditions including starvation, desiccation and freezing. In some sense, a gram of soil may contain a reasonable historical record of the early evolutionary history of life.

The basic premise behind an attempt to understand the complex soil community is that further knowledge will pay off in improved agriculture, environmental decision making and management, and many of the major practical issues listed above. In the past, soil biological processes have been studied at the level of the 'Grand Mean', i.e. lumping all of the diversity and complexity as an average value per gram, kilogram or hectare, for example. This approach has been what was feasible and no doubt useful. The basic question now is can, or in what cases will, a more detailed level of understanding or a mechanistic level of insight be useful? Schimel (1995) has suggested that in some cases it will be and in some cases it will not be. An example of the former is when particular communities selected by one environment has kinetic features or tolerance properties somewhat different from those of communities selected under a different condition. In this case, models of nutrient flux, for example based on Grand Mean coefficients, will not be accurate for both cases. Other examples where knowledge about particular organisms matters would be a PGPR (plant growth-promoting rhizobacterium) that works in one soil type but not in another, or that atmospheric methane is consumed by soils of one ecosystem type but not by another. In other cases, the populations may not differ in ways that affect function, but instead a new level of understanding can be obtained which provides more insight into how or how fast a process is controlled, e.g. the triggering of the molecular regulation of denitrification or the response of quorum sensors that initiate root pathogenesis.

Operational Model for Understanding Soil Biocomplexity

A more in-depth understanding of the soil community and its activity implies exploring biological processes at the organism and molecular levels *and* understanding how those levels are controlled by soil physical, chemical and climatic factors and by the overlying vegetation. Figure 6.1 shows the continuum in biological organization in the soil community and the adaptive features important at each scale of organization. The adaptive features

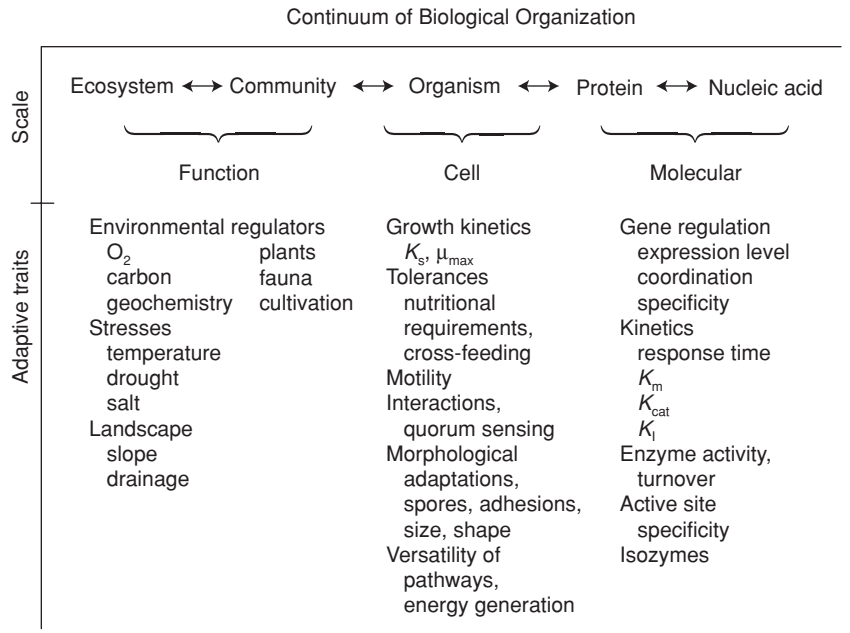

Fig. 6.1. The traits that control microbiological activity and can exhibit variation at different levels of biological organization.

reflect biodiversity that can be important to function. In the past, soil science research typically has stopped at the level of function, but in the future we argue that we should take the lead in extending the continuum, not stopping artificially at the level of function. This model provides a wealth of opportunity for research in the future, a true frontier.

While a biologist may identify more easily with the model in Fig. 6.1 than a soil chemist physicist, mineralogist or taxonomist, it is extremely important that the latter provide their expertise in understanding the environmental conditions so that environmental control of these processes can be understood at the organismal and molecular levels. Understanding this complexity at a mechanistic level demands a multidisciplinary effort. Some of the basic questions to be addressed include:

1. Biological diversity is much greater in soil than elsewhere; why? This observation suggests that basic features of the soil matrix promote and sustain diversification. What are the soil features which are most important, does soil management alter these features, and hence diversification?
2. Are there microbial patterns that can be explained by soil taxonomy or by vegetative history? Are current soil taxonomic traits appropriate for mapping microbial biogeography? Microbial communities are selected by growth of the successful competitors; the outcome reflects the primary

chemistry (types of organic carbon, available), hence is it the vegetation in soil that determines biogeographic patterns?
3. Can microbial activity be mapped at the microaggregate scale? Are microbes in the centre of aggregates inactive relics? Are microbial processes primarily patchy? At what size scales? When? In response to what conditions? The primary regulation of cell activity is thought to be at the level of gene expression. Can mRNA synthesis be measured at aggregate scales? How fast is that expression under realistic soil conditions? For example, what is the time scale for molecular events controlling denitrification following a rainfall?
4. What is the degree of coupling between redox active elements and microbial processes? Are these couplings tight, in effect a symbiosis? How does such coupling influence soil geochemistry over time?
5. What poorly studied processes might be triggered by the microbes' *in situ* environment? Does the starvation state induce synthesis of a protective coat, e.g. produce hydrophobic organic matter, or a physiological state resistant to stresses such as desiccation? Such responses could change the nature of soil carbon and result in a physiology that we do not yet recognize. For example, obligate non-spore-forming anaerobes survive in well-drained aerobic sandy soils; why?
6. How can we introduce or manage desired microbial populations to be more effective? How do we improve their dissemination, by earthworms or similar animal vehicles? By a combination of chemotaxis and water management, or by mechanical devices? Once the organisms are dispersed, how do we ensure gene expression?

The three following sections illustrate some of the points made above and hopefully show opportunities for better understanding of the soil community in the future. The first shows how spatial isolation provided by the soil matrix apparently sustains soil diversity, the second suggests that soil populations are geographically distinct and the third provides an introduction to the use of genomic and DNA microarray technologies in microbial ecology studies. The latter is projected to have great value for understanding microbes in nature and should provide a natural synergy for collaborative research between basic biologists and environmental scientists.

What is the Role of the Soil Matrix in Structuring Microbial Communities?

While there is likely to be general acceptance among microbiologists that soil microbial populations are highly complex, recent advances in the molecular analysis of soil communities have revealed a level of diversity previously unimagined. For example, small subunit ribosomal DNA (rDNA)-based studies have shown that clone libraries constructed from

soils can be composed of almost entirely unique members (Borneman *et al.*, 1996, 1997; B. Xia and J.M. Tiedje, unpublished). These studies agree with earlier work where DNA reassociation rates were used to estimate that 4000 non-homologous genomes were present in a forest soil sample (Torsvik *et al.*, 1990). One explanation for these high levels of microbial diversity is that some quality of the soil matrix must promote the development and maintenance of complex microbial communities. The aim of this section is to identify the soil qualities that are most active in shaping microbial community structure and to detail methods by which these qualities can be better defined.

An understanding of the mechanisms that control the structure of microbial communities would clearly benefit any strategy designed to enhance the growth and dominance of a microbial community member, whether that member is indigenous or introduced. Measures of species diversity in plant and animal communities often show that the majority of species are rare and a few species are abundant, suggesting that competitive interaction is a key determinant of community structure (Fig. 6.2a). To determine if competitive interactions also play a key role in structuring soil microbial communities, a small subunit rRNA gene-based approach (Zhou *et al.*, 1997) was carried out on surface, vadose and saturated zone soils. In surface samples, this analysis yielded a nearly uniform distribution of rDNA restriction patterns (operational taxonomic units) indicating that

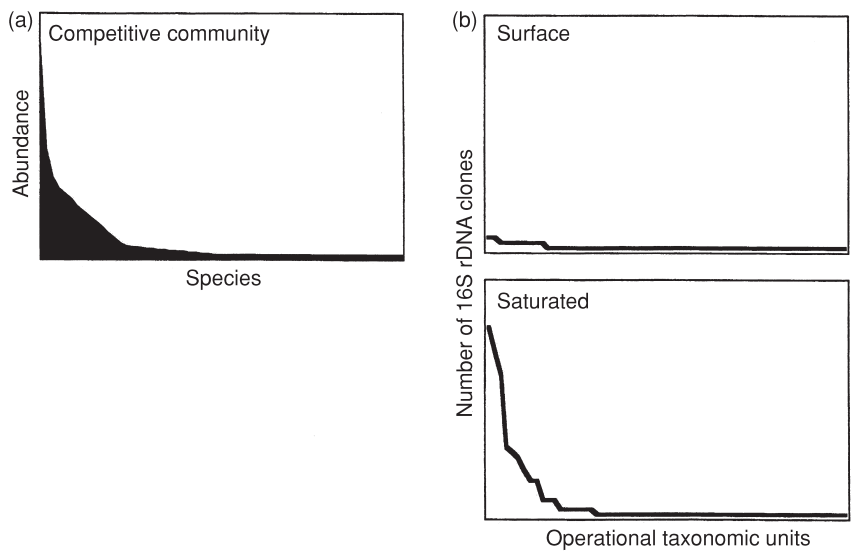

Fig. 6.2. Community diversity profiles. (a) A common diversity pattern seen by ecologists for macroorganisms where competitive interactions define community structure, compared with (b) microbial community diversity patterns for bacteria (determined by ARDRA) in surface and saturated zone soils.

a high level of microbial diversity was maintained (Fig. 6.2b). This type of community distribution where no one member is dominant suggests that competition must be nearly absent, leading us to term this a non-competitive diversity pattern. In contrast, the saturated samples exhibited much less diversity of restriction types, and dominance of one or a few community members leading to a competitive diversity pattern (Fig. 6.2b). In these samples, it appears that a few community members were able to out-compete the rest of the community for nutrients. The vadose zone soils showed community patterns intermediate between the surface and saturated samples; not as diverse as surface communities, but lacking the strong appearance of dominance observed in the saturated zone.

Can spatial isolation and resource heterogeneity explain these community patterns?

Two hypotheses could explain how the non-competitive and competitive diversity patterns are formed. First, spatial isolation (because of low moisture) in the surface samples could allow for the maintenance of diverse types of microbes and lead to a high level of diversity. At the surface, water films are transient, existing only after a rainfall. As gravity removes this moisture, there will be a low level of connectiveness (high spatial isolation) of soil particles, and microbial species that would normally be lost by competitive exclusion are able to persist. In saturated soils, excess water allows for a high level of connectiveness (low spatial isolation) which offers ample opportunity for the transfer of nutrients and microbes. Under these conditions, the organism best able to scavenge nutrients or migrate to a nutrient source will outgrow less fit types and become dominant.

While the spatial isolation hypothesis fits well with the varied moisture content of our soil samples, an alternative hypothesis is that greater resource heterogeneity at the surface allows for the maintenance of high microbial diversity. The merit of this proposal is that indeed total organic carbon, and probably the variety of carbon types, decreases with increasing soil depth. Thus, multiple resources at the surface could create a variety of microhabitats that support a diverse collection of species. In the saturated zone, the lack of diverse carbon sources means that fewer species will dominate the community.

Do the non-competitive and competitive diversity patterns appear as a general theme in soils?

If spatial isolation is an important determinant of the diversity pattern, then one would predict that smaller particles, e.g. clays, would contribute more

strongly to a spatially isolated environment. Hence, we would predict that moisture and clay content would shape community structure in a manner such as that hypothesized in Fig. 6.3. We currently are testing whether this hypothesis is supported by examining the existing microbial communities in soils that vary in these two features.

Test of the hypotheses that spatial isolation and resource heterogeneity act to structure soil communities

In addition to examining existing soil microbial communities, we are conducting controlled laboratory studies with simple two-strain microcosms to evaluate the spatial isolation and resource heterogeneity hypotheses. While our examination of existing communities will reveal community diversity patterns, it is with these simple microcosms that we can test what forces impact on most microbial community structure. The advantage of using this simple system is that many of the abiotic soil components can be held constant while the impact of a single variable, such as moisture, undergoes evaluation. The low complexity of the two-strain community ensures that the dynamics of each population can be measured precisely.

Competition experiments performed by Gause (1934) with two species of *Paramecium* demonstrated that the more competitive species predominated in a uniform, single-nutrient environment. This pioneering work led to the concept of competitive exclusion, the idea that competitors cannot coexist on a single limiting resource (Hardin, 1960). In many ways, a species pair that exhibits strong competitive exclusion, where one species is rapidly out-competed, would be ideal for evaluating our spatial isolation and resource heterogeneity hypotheses. Thus we chose pairs of species that differed in their growth kinetics in liquid culture, when spatial isolation is low. Under these conditions, the species with superior growth kinetics was demonstrated to predominate in a predictable manner. Once these competitive interactions are defined under highly connected conditions, the impact of varied levels of isolation or resources can be tested.

With two-species competition experiments, it must be ensured that positive or negative interactions between the species do not interfere with the hypothesis being tested. For example, if strain A cross-feeds on secondary metabolites produced by strain B, then coexistence will occur even under conditions of low spatial isolation. One solution to this problem is to use two variants of the same species that differ in their growth kinetics. In this case, it may be necessary to distinguish the strains by introduction of a marker, such as B-galactosidase (LacZ) or the green fluorescent protein (Tombolini *et al.*, 1997). Ideal for this second strain pair would be a collection of strains isolated from the same environment. For example, we have evaluated a collection of closely-related 2,4-dichlorophenoxyacetic

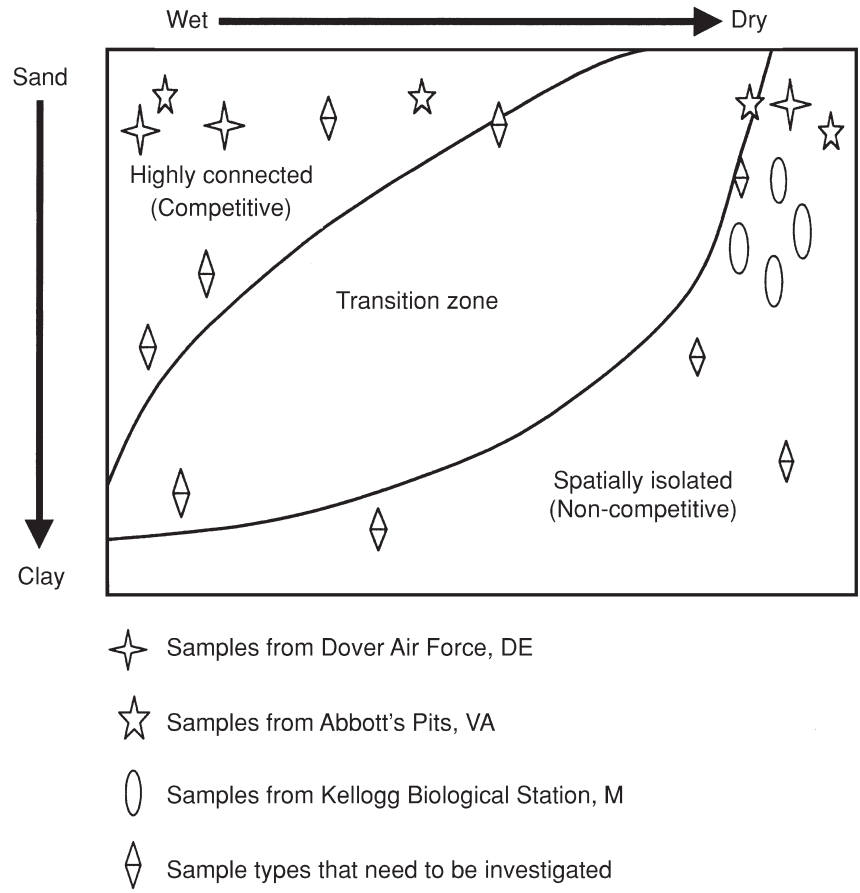

Fig. 6.3. Hypothesized relationships of microbial community structure to the texture and moisture content of different soils.

acid (2,4-D)-degrading *Sphingomonas* sp. isolated from an agroecosystem study site (Ka *et al.*, 1994) for use in our microcosm studies. Our initial experiments have focused on two microbial species in competition for a single nutrient in uniform clean sand. Each species is easily distinguished by colony morphology, and in liquid culture and saturated sand (low spatial isolation) one of the strains dominates because of a shorter lag time and superior growth rate. However, as the level of spatial isolation increases, because of decreasing moisture, we have observed that the population sizes of the two species become nearly equal. These results mimic the

non-competitive diversity pattern observed in surface soils (Fig. 6.2), and suggest that even in a highly uniform large-particle matrix, such as sand, the impact of spatial isolation appears dramatic. One would predict that in smaller particle matricies such as soil these spatial isolation effects would be experienced even more readily.

Although simple, the two-species microcosm design is remarkably flexible. They should allow us to tease apart the contributions that spatial isolation and resource heterogeneity make to the maintenance of microbial diversity. The results hopefully will shed some light on how factors such as soil particle size, total organic carbon and clay content of soils impact on microbial diversity.

Are Soil Heterotrophic Communities Geographically Unique?

The soil matrix that maintains diversity can also promote an ongoing diversification if the rate of local genetic change exceeds the rate of microbe dispersal. The dogma in the past has been that microbes, being small, are readily transported globally by wind, birds and human activity to name the most likely. This implies that the microbes inhabiting the Edinburgh valley soils are the same as those inhabiting Michigan and New Zealand valley soils. Is this true? The question has not been seriously addressed. Until the development of molecular tools, we did not have a means for realistically addressing microbial biogeography. Most countries have quarantine systems directed against spread of plant pathogens, but these organisms are usually host-associated organisms in their growth habitat, not free-living heterotrophic soil bacteria. Hence, the experience from quarantine is not particularly helpful for resolving the question of soil microbial biogeography.

The question of whether soil microbes are basically cosmopolitan (everywhere) or endemic (geographically unique) has important implications. If endemic, the estimate of global microbial diversity expands tremendously. The answer has important implications for strategies for discovery of new biotechnology products and for national intellectual property rights. Furthermore, if endemism predominates, it means that soil microbial process information is not transported so reliably between different geographic locations. We have addressed the question of whether soil heterotrophic bacteria are endemic with two types of bacterial populations, one set selected on the member's ability to degrade 3-chlorobenzoic acid (3-CBA), a rare property in nature, and the other a coherent taxonomic group, the fluorescent *Pseudomonas*.

Our strategy was that the major ecological features influencing bacterial selection should be the same at least within ecosystem type, i.e. climate, soil

group and the same or similar vegetation, and hence population differences would be more likely to be due to distance. Soil samples were collected from two ecosystem types (Mediterranean and Boreal Forest) that exist in widely separated global regions (southwest Australia, southwest South Africa, central Chile and central California for the former, and northern Saskatchewan and northwest Russia for the latter) (Fulthorpe *et al.*, 1996). We used a hierarchical geographic sampling strategy scaling from samples 5 m apart in 200-m transects, to multiple sites in the same continental region (100–850 km apart), to sites on different continents. The sites either were in parks or nature preserves, or otherwise unimpacted by human activity. Importantly, all soil samples were collected from below the soil surface (5–10 cm) using a soil core sterilized between each sampling. By sampling below the soil surface, we hoped to improve the probability of sampling long-term resident soil bacteria not influenced by human activity. The 3-CBA-degrading isolates obtained from this global sampling showed an endemic pattern; no genotype determined by rep-polymerase chain reaction (PCR) (a rapid measure of chromosome structure) was found on more than one of the six continental regions and most genotypes were not found at multiple sites within the region, although they were found repeatedly in samples from the same 200-m transect (Fulthorpe *et al.*, 1998). The chlorobenzoate-degrading trait, however, is often borne on transmissible plasmids which could mean that the isolate collection contains organisms from multiple phylogenies. Hence, we also examined a readily isolated soil colonizer, the fluorescent *Pseudomonas*, from the same soil collection. In this case, we explored three levels of genetic difference ranging from coarse to fine resolution: (i) amplified ribosomal DNA restriction analysis (ARDRA); (ii) 16S–23S rDNA intergenic transcribed spacer fragment length polymorphism (ITS-RFLP) and rep-PCR genomic fingerprinting (Rademaker *et al.*, 1998). As expected, no endemicity was seen at the coarse level of resolution (ARDRA method) since the rRNA operon is highly conserved. The ITS-RFLP analysis, however, showed a weak level of endemicity. This species to sub-species level of resolution also analyses a more conserved part of the genome. At the finest level of resolution (rep-PCR), however, we observed strong endemicity. No genotypes were found in more than one continental region, nor in more than one site of the same continental region; however, seven genotypes were found repeatedly along particular 200-m transects. Hence, this second biological example supports the hypothesis that soil heterotrophic populations are endemic, but only at a rather fine scale of resolution. This scale, however, is significant to many ecologically important properties such as pathogenesis, rates of reaction and biotechnological value.

We calculated the relationship between the genetic distance based on the rep-PCR fingerprinting and the corresponding geographic distance (Fig. 6.4). We used one genotype from our reference site in Australia as the

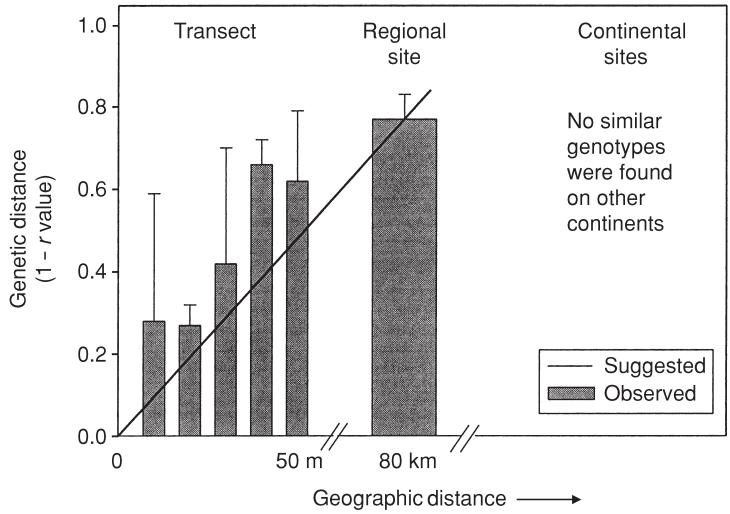

Fig. 6.4. The relationship between genetic distance based on genomic fingerprinting (rep-PCR) and geographic distance between isolate sources. The method does not resolve large genetic distances well but does confirm that similar genotypes were not isolated at other sites in the region or on other continents.

base and calculated similarity coefficients to every other genotype in the transect, to all transect isolates of a second Australian site and to all transect isolates in different regions. Those values plotted against geographic distance revealed a relationship of increasing diversity with distance. The methodology we have used so far provides its best resolution at transect scale genetic differences. We currently are working to obtain additional measures that will allow this relationship to be evaluated at larger geographic scales. While these findings support the endemism hypothesis, they also suggest that the resulting corollary is true, i.e. that bacterial diversification is actively ongoing.

Applications of DNA Microarray Technology to Environmental Microbiology

Introduction to microarray technology

Since the advent of microbial genome sequencing programmes less than 8 years ago, a massive amount of microbial genome sequence information has been collected. The complete sequences for > 20 microbial genomes have been determined, and > 100 microbial genome sequencing projects are now in progress (www.tigr.org). The next step in the era of microbial

genomics is extracting functional and evolutionary information from these large data sets and, from an ecological point of view, applying genomics technology to relevant questions in microbial ecology. This technology can have a tremendous impact on soil microbiology. Hence, in this section, we introduce DNA microarray technology, describe the basic method and detail some of the potential applications of this technology to microbial ecology as well as some of the current limitations in this field. We hope that this introduction will facilitate entry of this technology into soil science.

DNA microarrays are microscopic arrays of large sets of DNA sequences immobilized on solid substrates. Microarrays are used in hybridization experiments designed to detect gene expression under defined experimental conditions, or to detect the presence of the arrayed sequences in a given sample. There are two general types of arrays: (i) cDNA microarrays, which are constructed either with partial (expressed sequence tag; EST) or full-length complementary DNA (cDNA) sequences typically generated with PCR; and (ii) oligonucleotide microarrays, which are constructed with short (15–40 mer) or longer (i.e. 75 mer) oligonucleotide sequences, designed to be complementary to specific coding regions of interest. In cases when short oligonucleotides are used, often 10–20 probes per gene and mismatch probes are put on the array. There are numerous advantages of microarrays over other hybridization strategies: (i) the high capacity of printing the array on solid substrate (either microscope slides, or 1×1 cm^2 wafers) allows tens of thousands of samples to be arrayed; (ii) the overall reduction in size of the experiment reduces amounts of probe and hybridization volume, and increases sample concentration and reaction kinetics (Eisen and Brown, 1999); (iii) global information can be accessed in studies with completely sequenced genomes, or with large numbers of ESTs, such that coverage is broad, and a collective picture of whole organism gene expression can be developed; (iv) speed and high throughput design using robotic printing of DNA samples allows the mass production of cDNA arrays, increasing quality control; (v) parallel design facilitates substantial data acquisition; and (vi) when used with two-colour fluorescence detection, direct comparison of independent experimental samples is readily obtained.

Microarray hybridization approaches promise to revolutionize biology, much in the same way that DNA sequencing and PCR have in recent years. DNA microarrays allow thousands of genes to be surveyed under copious experimental conditions in parallel. Initial studies used cDNA microarrays to determine gene function (i.e. Schena *et al.*, 1995, 1996). For organisms in which the complete genome sequence information is available, it is possible to study the expression of all genes in a single experiment (Eisen and Brown, 1999). Studies have been completed in this regard utilizing the full sequence of *Saccharomyces cerevisiae* (i.e. DeRisi *et al.*, 1997; Wodicka *et al.*, 1997). Additional applications of microarray technology have included

screening for mutations in specific genes (Hacia *et al.*, 1996), identifying genes involved in genetic diseases (Heller *et al.*, 1997), evolutionary sequence comparisons of closely related species (Hacia *et al.*, 1998), studying mutation incurred during adaptive evolution (Ferea *et al.*, 1999) and detecting genetic variants, or genetic expression studies in temporally expressed viral genes (Chambers *et al.*, 1999). Oligonucleotide arrays are also used for DNA sequencing and genotyping (Gingeras *et al.*, 1998), which is a promising application of the high-density oligonucleotide hybridization platform. Affymetrix (Santa Clara, California) is developing sequencing by hybridization technology, and currently is marketing oligonucleotide-based arrays (GeneChips) in which the probes are synthesized *in situ* utilizing photolithographic technology, in which all oligonucleotides are synthesized in parallel. Currently, GeneChips for rat, human and yeast open reading frames are available, with applications directed towards expression analysis, polymorphism analysis and genotyping, and disease management.

Development of microarray technology for studies in microbial ecology is just being launched (Guschin *et al.*, 1997; Kelly *et al.*, 1999). The use of microarrays in prokaryote applications is also in its infancy, though the number of funded projects in the field of functional genomics, and the numbers of biotechnology and pharmaceutical companies involved in microarray research, is growing rapidly, suggesting that the future in this field is very promising. There are several microbiology/microbial ecology-oriented research areas that will benefit from microarray technology such as: determining the metabolic effects of novel antibiotics or mutations; identifying the presence of specific messages, DNA sequences or genomes in natural samples; understanding gene regulation coincident with pathogenicity; identifying pathways and regulatory networks involved in bioremediation and biogeochemical processes; and screening natural populations for evolutionary divergence. Commercial chips for soil bacteria are not likely to be available; the market is too small. Hence, we will need to make our own.

Microarray basics

Due to the variety of schemes for which DNA microarrays can be used, we will discuss, in general, the types of equipment and gene information that will be necessary for development of DNA microarrays that appear to be useful for microbial ecological studies. For specific methods, we refer the reader to a recent publication by Eisen and Brown (1999), which covers cDNA microarray technology as applied to gene expression studies.

The flowchart in Fig. 6.5 depicts the basic strategy for a microarray project. Only general attributes of the scheme have been listed, as this is

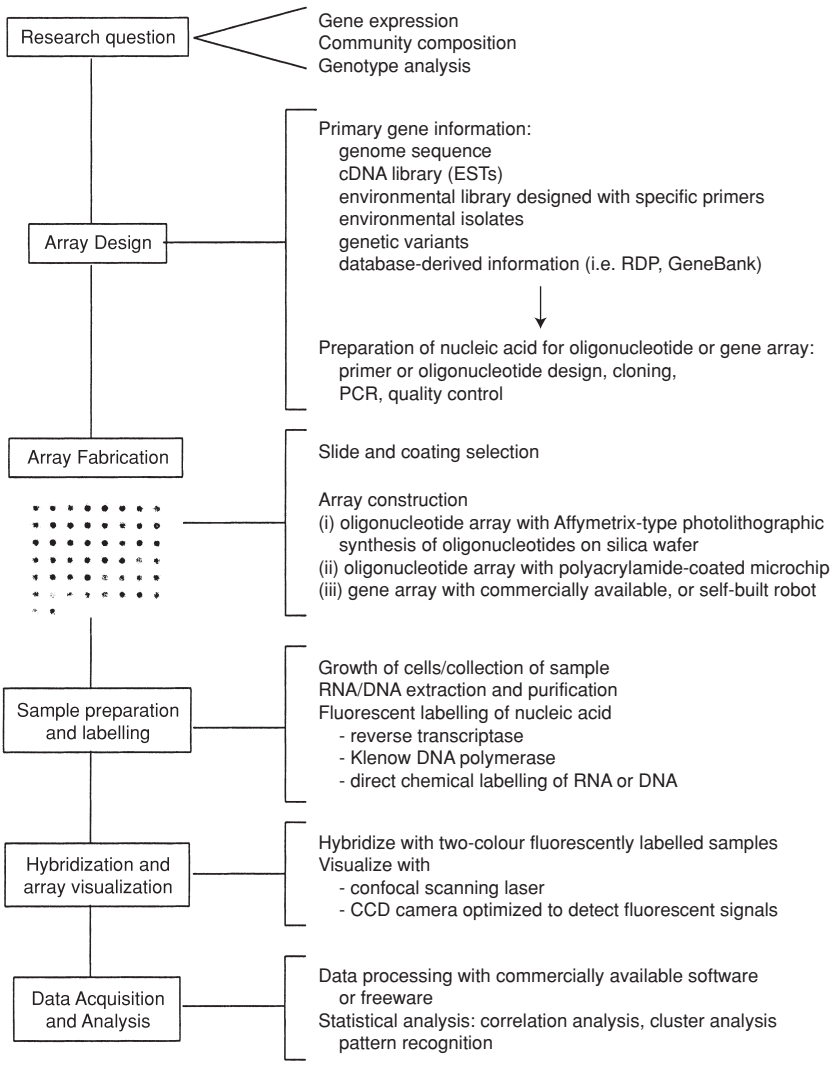

Fig. 6.5. Flowchart for DNA microarray experimental strategy. Results from a section of an actual array experiment are shown in which genes expressed under denitrifying conditions are compared with their expression under aerobic conditions. The array image has been converted from colour (red versus green), which is much more easily quantified, to black and white. Nonetheless, differences in expression can be seen. The microarray was constructed with PCR products designed from the *Shewanella oneidensis* strain MR-1 genome sequence. This organism is of interest because it can use a variety of electron acceptors for growth including Fe_3^+, Mn_4^+, NO_3^- and O_2.

intended to give perspective to the reader for what is needed to conduct a microarray experiment. To start, careful attention needs to be paid to developing a research question, and determining the appropriate array format. Arrays which can address a variety of questions will be most valuable, since the bulk of time and cost involves array design and preparing the nucleic acid samples (oligonucleotide synthesis, PCR) for placement on the array. Array fabrication is largely automated and, other than the initial cost of the arraying device (see Bowtell, 1999 for recent listing of products available), this step is quite affordable. The technical details of sample preparation are outlined briefly, though these are important details that need to be worked out, particularly for low biomass environmental samples. The hybridization itself is straightforward; specificity, normalization and sensitivity of the hybridization reaction can be assessed with internal controls on the array. Experiments are conducted with dual fluorochrome-labelled templates, with either gene expression compared under two experimental conditions, or a reference sample compared with the experimental sample. Microarrays are visualized with either a confocal scanning laser or a CCD camera specifically designed for microarrays. The image file representing the microarray is processed using commercially available software (see Bassett *et al.*, 1999) or shareware available on the web (http://rana.stanford.edu/software/).

Arrays work in much the same way that traditional hybridization approaches have operated. In a simple case, where the relative amount of gene expression is to be assessed, the target (labelled nucleic acid in solution) samples are varied experimentally. For example, DeRisi *et al.* (1997) compared mRNA isolated from starved cells with mRNA isolated from cells grown under nutrient-rich conditions. The two different mRNA populations were labelled with different fluorochromes (Cy3 and Cy5), and hybridized together on the same microarray. The scanner delivers two images (one for each fluorochrome) which are overlaid using the processing software. Signal intensities of each spot are determined and a ratio of signal intensities is derived. Using the relative representation of RNA to compare different samples is the most optimal way to use these data, due to differences between sample processing, variations in labelling and other experimental conditions (Eisen and Brown, 1999). The ratio values can be analysed by a variety of statistical methods to assess relationships between coexpressed genes.

Microarray uses in environmental microbiology

There are a number of ways in which environmental microbiology will benefit from microbial genomics. As mentioned earlier, microarrays are being used in microbial functional genomics research to determine patterns

of gene expression, and identify novel metabolic pathways and regulatory networks. These discoveries at the basic research level will provide invaluable information for environmental studies. Sequence information from completed genomes is being used to design arrays with full complements of all open reading frames for several microorganisms (*Bacillus subtilis*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori* (Matrubuthan *et al*., 1999), *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Shewanella putrefaciens*). Even for well-characterized microorganisms such as *E. coli*, < 60% of the genome is homologous to genes of known (or hypothesized) function. For organisms less well studied, an estimated 40–60% of the genome may have no homology to characterized genes.

Microarray expression profiles offer a quick way to access functional information for these genes of unknown function. This information can aid functional diversity studies by identifying highly expressed genes and genes critical in biogeochemical pathways, bioremediation or biocatalysis. Cell regulatory function will also become better understood, which could aid in understanding environmental regulation under varying conditions of carbon supply, energy source and electron acceptor availability. Genetic expression for other important environmentally controlled phenomena such as quorum sensing, chemotaxis and antibiotic production may also be monitored, once the regulation and genetic expression for these pathways are understood.

There are a number of direct environmental microbiology applications that we have envisaged for DNA microarrays (oligonucleotide and gene arrays). These include the following:

1. Community genome arrays (CGAs). Arrays constructed with genomes of hundreds to thousands of bacteria (environmental isolates) would be used to study community composition and community dynamics of reactors, soil, sediment, water, gut, etc. The utility of CGAs involving DNA–DNA hybridization depends on sample complexity, hybridization kinetics, relatively high biomass samples and the requirement for cultivation of the important organisms in the environment to be studied.
2. SSU rDNA arrays. Oligonucleotide arrays constructed for different taxa could be used in community analysis studies. These could be designed in a phylogenetic framework to survey different levels of sequence conservation, from highly conserved sequences giving broad taxonomic groupings, to hypervariable sequences giving genus (and potentially species) level groupings. These assays would not require high biomass sample if PCR or other signal amplification techniques were applied, and would be free from cultivability bias. SSU rDNA array design would be limited by the quality of database information available for SSU gene diversity, coverage of the SSU sequences (20 probes per SSU sequence designed from hypervariable regions) and the sensitivity of hybridization. With > 12,000 prokaryotic sequences in the ribosomal database project

(RDP, http://www.cme.msu.edu/RDP/html/index.html), a large resource of sequence information is available.

3. Environmental functional gene arrays. These arrays could come in a variety of styles. One concept would be to prepare oligonucleotide arrays for targeted gene expression, with genes of interest on the array. For example, oligonucleotide probes complementary to genes coding for key enzymes in all biogeochemical cycling processes could be arrayed. These would be used for specific detection of expression in the environment. Another style for an array could be designed to study functional diversity in nature. These gene arrays could include hundreds of PCR products representing the diversity found in nature (e.g. nitrate reductase, ammonia monooxygenase and dechlorinase). The limitations for these two concepts are similar. They rely on available sequence information for designing the array. Functional gene sequencing lags far behind the information available in SSU databases though, with the diversity of genome projects underway, this situation is changing rapidly. Additionally, samples of varying biomass concentration may present technical challenges, since large amounts of RNA are required for the hybridization experiments (5–10 μg total RNA per experiment). Developments in signal detection, and in signal amplification may aid in these problems.

4. Population biology arrays. Genetic diversity or genetic polymorphisms within specific populations can be assessed with oligonucleotide arrays. This has already been done with *M. tuberculosis* (Gingeras *et al.*, 1998), *S. cervesiae* (Ferea *et al.*, 1999), and with the human cytomegalovirus (Chambers *et al.*, 1999) in which the potential for this application was demonstrated. Oligonucleotides representing all open reading frames of a reference organism genome can be arrayed, then assayed against strain-level variants. Similarly, cDNAs for a genome of interest could be arrayed then mRNA from isolated strains could be compared with the reference organism to study speciation and functional relationships between the isolates.

There are clearly a large number of different applications of microarray technology that can be applied to relevant problems in environmental microbiology. The field of soil microbiology will benefit invaluably from the contribution to our understanding of microbial content and function in the natural environment.

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Sustaining Soil Organic Matter 6.1

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Introduction

Concern about the loss of organic matter from soils and the implications of this for the sustainable functioning of soils is not new, but the increasing demands that are being placed on our environment are leading to an urgent need to reassess the role played by soils in the development of sustainable patterns of land use. This book has helped to achieve this by providing a wide-ranging selection of relevant research papers and reviews. In this chapter we review some highlights from the material presented and outline what we see as the main conclusions.

The concept of soil quality is used to define those attributes of soils that are essential to soil functions such as nutrient storage, the provision of a suitable physical environment for plant growth and the attenuation of pollutants. In the opening chapter of this book, this concept was reviewed by Carter, where he emphasized the importance of defining precisely those attributes that are pivotal in controlling organic matter quality as well as the need to develop standardized measurement and sampling procedures. Carter identified specific fractions of organic matter that describe soil quality. These include macroorganic matter, microbial biomass and carbohydrate contents. Silt- and sand-sized macroorganic matter is important in maintaining the protection effect of soil organic matter. This acts mainly in promoting and stabilizing soil aggregation. Loveland *et al.* proposed that the important component of soil organic matter is the 'active fraction', made from recent additions of crop residues and organic manures.

Cultivation and compaction can be associated with physical degradation. Cultivation generally depletes organic matter and reduces soil

structural stability. Chenu *et al*. showed that soil structural stability is largely dependent on complexes of clay and organic matter. In protecting soil from physical degradation, the quality of soil organic matter is probably more important than the overall content. Loveland *et al*. reviewed many research papers, and found that there was little consistent evidence for 'critical thresholds' of soil organic carbon above or below which soil physical properties change significantly. This does not imply that the role of soil organic matter is any less important. However, it creates more difficulties for those responsible for devising policies of soil protection, and suggests that evaluation of soil quality on a case by case basis may be required in order to ensure that soil functions are maintained adequately.

Soil organic matter modelling provides a valuable opportunity to explore ways of managing the terrestrial carbon cycle. Modelling at the regional scale is important for climate change issues. At this scale, Paustian advocated a whole ecosystem approach where the interactions between soil organic matter, crop yields, economic returns and subsequent changes in management feed back to determine organic matter and crop responses. This modelling has, amongst other things, highlighted the close linkage between C and N cycling processes. For example, Franko found that the organic matter content in some German soils has reached an optimum level. Above this level, nitrogen losses can exceed inputs resulting in a net loss to the environment. At a similar scale, organic matter modelling was applied by Gaunt *et al*. to predict the dynamics of soil nitrogen supply required for making more precise fertilizer recommendations. They suggested that measurable fractions of soil organic matter can be used to define the potentially available nutrient pools, making the models of greater practical value. Tillage has a large effect on carbon and nitrogen dynamics, with C and N lost after ploughing out grassland. Richter *et al*. used a modelling approach to show that these losses resulted in a decrease of net mineralization and a widening of the C : N ratio. They also showed the importance of microbial carbon and nitrogen associated with litter and crop debris in specifying the soil microbial biomass. Paustian identified a need for an increased collaboration between modellers. However, he stressed the continued need for long-term experimentation and for closer correspondence between theoretical and measured organic matter fractions. The availability of a richer set of field experimental data, and the use of isotopic tracers should in future allow more robust and constrained testing of conceptually based models.

Soil Organic Matter and Land Management

The sequence and type of crop rotations have been widely shown to influence plant productivity by affecting physical, chemical and biological

aspects of soils, as well as the prevalence of weeds, pests and diseases (Sumner, 1982). Rotations comprising only annual crops may cause a decline in soil organic matter by leaving relatively few plant residues (above and below ground) compared with perennial crops. In contrast, perennial forages have been shown to build up soil organic nitrogen (Clement and Williams, 1967), improve soil physical properties (Tisdall and Oades, 1982) and reduce the risk of soil erosion. Management of crop rotations by manipulating application rates of crop residues and manures, tillage and treatment of crop residues (e.g. mulching or incorporation) can have a significant effect on soil organic matter dynamics.

Organic farming systems represent an increasingly important land use in Europe and beyond. The land area farmed organically in Europe has grown from 100,000 ha in 1985 to 2.8 Mha in 1998 (N. Lampkin, personal communication). The principles of organic agriculture as defined by the International Federation of Organic Agricultural Movements (IFOAM, 1998) specifically include the maintenance of long-term soil fertility as a prerequisite. Increases in soil organic matter in soils under organic management are widely reported (e.g. Reganold *et al*., 1993, Clark *et al*., 1998). Wander and Traina (1994) have also measured higher levels of carbon in the 'light fraction' of soils under organic management which is thought to be an indication of a more biologically active pool. As the number of organic farmers grows, there are increasing numbers of conventional, specialist, arable farmers wanting to convert to organic production. This poses particular challenges for soil organic matter management. Grass–clover leys are traditionally the 'engine' of organic systems in Western Europe, but grass–clover leys are not an economic option without livestock to utilize their productivity. Philipps has shown that with less than 25% of nitrogen-fixing crops in the rotation (i.e. a legume grown 1 year in every 4), organic matter declines over a 10-year period. However, working with a rotation with a similar proportion of legumes, but comparing the use of mineral fertilizers and farmyard manure with or without biodynamic preparations, Raupp found that soil organic matter was higher in the manured + biodynamic treatment, and lowest in the treatment which received only mineral fertilizer.

The sustainable management of crop residues in agroecosystems is a global challenge. Lal (1995) calculated that on an area of 1×10^9 ha of agricultural land, ~3.5×10^9 Mg of crop residues are produced. Approximately 74% of this originated from cereals while the next biggest contributors were sugar crops at 11%. Crop residues have an important role not only in building soil organic matter and in conservation of soil and water, but also in supplying nutrients to subsequent crops in rotations and to simultaneous crops in, for example, agroforestry systems. The magnitude of benefits from crop residues depends on the quantity and quality of the residues, as well as the following crop, climatic, edaphic and management factors. Until

recently, it was widely accepted that equilibrium levels of carbon and nitrogen in soil were controlled largely by net input, and that qualitative aspects were relatively unimportant. Drinkwater *et al.* (1998), however, found that quantitative differences in inputs alone could not explain observed changes in soil carbon and nitrogen, and that plant species composition and litter quality influenced soil organic matter turnover. They suggested that managing the quality of inputs could help to increase carbon sequestration and reduce CO_2 emissions, in accordance with the Kyoto protocol. Cadisch and Giller move this idea forward by suggesting that soil organic matter management should begin with the decision as to whether we are managing organic matter for carbon sequestration or for crop nitrogen supply. The key residue characteristics that govern the outcome are carbon to nitrogen ratio, lignin and polyphenol content. They also highlight the importance of understanding differences in decomposition of root material compared with above-ground material. Recent research has suggested that root turnover is relatively short for many temperate species, for example ~30% of grass and clover roots survive for < 3 weeks under UK field conditions (Watson *et al.*, 2000). Although there are now reliable estimates of root turnover for many tree and agricultural species (Black *et al.*, 1998; Watson *et al.*, 2000), there is still a lack of quantitative data on soil organic matter inputs from this source.

In addition to the influence of quantity and quality of residues on potential nutrient release and soil organic matter accumulation, physical management of residues is also a key issue. Baggs *et al.* and Vinten *et al.* both address the question of particle size of residues. The use of crop residues and off-farm organic wastes within cropping systems may require alterations to normal fertilizer and cultivation practices in order to maximize crop uptake and minimize nutrient losses (Shepherd *et al.*, Robertson and Thorburn, and Vinten *et al.*).

As stated in the Introduction, nutrient budgets are used increasingly as international indicators of sustainability. Nutrient budgets can be used for a number of purposes; they can identify the long-term sustainability of a system and may be able to suggest management options that can improve nutrient retention. They can also be used to identify gaps in our knowledge of nutrient fluxes by using simple models to calculate fluxes that would otherwise be difficult to measure experimentally. Finally, they can be useful as a tool for policy makers in order to allow the synthesis of data at the scale of a catchment or region. Fortune *et al.* point out some of the difficulties in interpreting budgets, due in part to the different methodologies used in their compilation, and also the need to understand the reliability and limitations of the data available. Pilbeam *et al.*, working in Nepal, use nutrient budgets to illustrate how sustainability at one level, in this case the household, may jeopardize the sustainability of the system at a higher level, when the origin of imports to the household is taken into account. This

highlights the need to define the boundaries for compiling budgets in relation to use of the resulting information.

As it is no longer acceptable to judge land management practices simply on the basis of their current productivity, we must understand the long-term implications of current practices. The combination of archaeological knowledge with modern analytical techniques and modelling is potentially a very powerful tool. McCann *et al.* provide a fascinating insight into the origins of the Terra Preter soils in Amazonia, and the paper by Glaser *et al.* explores the scientific evidence underlying farmer observations that these soils are the most productive in the region. In a contrasting setting, Adderley *et al.* draw conclusions on the sustainability of traditional manuring practices on a remote Scottish island.

Major changes in land use, such as the ploughing out of long-term pasture, are known to result in major disturbances to the carbon and nitrogen cycles (Johnston, 1991; O'Sullivan *et al.*). The question of how best to maintain soil organic matter levels following a more major land use change is difficult. Hatley *et al.* and Mazzoncini *et al.* both assess the impact of different management treatments following land use change in the contrasting environments of East Anglia and the Mediterranean.

Nutrients

One of the key roles played by soil organic matter as discussed by Goulding *et al.* is that involving the supply of nutrients to plants and soil organisms. Soil organic matter contains substantial pools of organic N, P, S and a number of trace elements; however, the availability and mobility of these elements in organic compounds is generally very much less that that in the inorganic state. An understanding of the process of transfer between the organic and inorganic states (mineralization) and the reverse process of immobilization is therefore critical to our approach in this area. It is often assumed that high inputs of inorganic fertilizers can substitute for the nutrient supply from organic matter pools. Work with isotopically labelled N and P, however, has shown this not to be the case. Even where high inputs are supplied, organic matter plays an important controlling role. Results from field trials in the UK and USA have shown that even when N fertilizers are added in amounts that are sufficient to satisfy the crop's demand, the crop recovery of fertilizer-derived N is no more than 60% of that which was added, with the remainder being made up from N released from organic matter pools and small amounts from atmospheric inputs. In other words, if we wish to manage fertilizers efficiently, we must be in a position to understand the interactions between added fertilizer materials and the organic matter pools. Short-term immobilization by the microbial biomass potentially can result in crop nutrient shortages. However, work by Vinten *et al.* has shown that crop N recovery can be optimized whilst

minimizing losses of N through leaching by careful soil management that takes account of soil microbial processes.

Measuring the process of mineralization has for many years proved difficult, but is essential if we wish to be able to quantify and/or manage nutrient transformations within a given system. Using the recently developed pool dilution techniques, we now realize that flows of nitrogen in soils that result from gross mineralization processes are very much larger than previously thought. Goulding *et al.* have shown that gross transformations of N may exceed 18 mg kg day^{-1}, indicating that such fluxes may be many times the uptake of N by crop plants, although in this example the high mineralization rates were accompanied by high immobilization. Methodological problems remain in the use of such techniques, though they do provide a real indication of microbial activity and allow improved insight into the competition between plants and microbial populations for nitrogen.

The concept that mineralization of organic nitrogen is a necessary prerequisite for plant nitrogen acquisition has been questioned in a number of recent studies. Goulding *et al.* have found that extractable soil N in a range of arable soils in the south of England contains 55–65% of total soluble N in organic form, which clearly indicates the importance of possible N loss in this form and may indicate a possible source of N for plants. Nasholm *et al.* (1998) found rates of ^{15}N-labelled glycine uptake by coniferous trees, dwarf shrubs and grasses that were comparable with that of NH_4^+. Uptake of organic N is known to be of importance in upland and boreal vegetation, and has often been assumed to be mediated by ectomycorrhizas (Turnbull *et al.*, 1996). It has been suggested by Jonasson and Shaver (1999) that this may explain the reason why some plants adapted to these habitats show relatively little response to additions of inorganic N.

An understanding of the factors contributing to the turnover and decomposition of dissolved organic matter (DOM) fractions is important in allowing us to predict losses and potential mineralization from this source. Several papers in this book have identified the importance of DOM in contributing to losses of organic carbon with associated nutrients from soil profiles by leaching (Kaiser *et al.*, McTeirnan *et al.*, and Marschner and Bredow). It is likely, however, that site properties, such as hydrology, play an important role in mediating such losses (McTiernan *et al.*). Chapman *et al.* found that concentrations of dissolved organic nitrogen (DON) remained constant despite changes in net mineralization of N and suggested that this could be explained by the equilibrium between the DON produced and a larger reserve pool. The central role of organic matter as an intermediate in the process of mineralization (Appel and Mengel, 1990) underlines the importance of developing a better understanding of dissolved organic fractions in nutrient cycling processes.

The storage of nutrients in soils is closely linked to the availability and throughput of organic matter derived from plants. Current increases

in atmospheric CO_2 have led to an alteration in the equilibrium between soil organic matter and the pre-industrial CO_2 concentrations. This is complicated further by the enrichment of our environment with fixed N, which interacts with the added organic C in soils to produce effects on soil properties and soil organisms that are difficult to anticipate (Swift *et al.*, 1998). Most of the carbon held in terrestrial ecosystems is in the soil ($\sim 1500 \times 10^{15}$ g) and is derived from plant and animal material (Batjes, 1996). Changes in the soil carbon stores may result from the effects of elevated CO_2 on plant growth and from the climate changes resulting from the change in global atmospheric composition. Elevated levels of CO_2 can affect the quality of leaf and fine root litter, their decomposition rates and the relationship between litter quality and decomposition (Cotrufo *et al.*, 1998). Elevated levels of atmospheric CO_2 were shown by Torbert *et al.* to increase both soil organic carbon and total nitrogen content under soybean and sorghum. Martin-Olmdèo *et al.* found no direct plant-mediated effect of elevated CO_2 on nitrous oxide production or emission from soil. However, they considered that the positive effect on plant growth and microbial biomass by the CO_2 might affect potential feedback effects between soils and atmosphere.

The build-up of greenhouse gases can be limited quite considerably through improved soil management; according to Smith *et al.* agricultural soils can be particularly important. The sequestration of carbon in organic matter in agricultural soils is an important mitigation option. This can be achieved using organic amendments, improved residue management and tillage techniques, alternative cropping regimes and changes in land use cover. Afforestation and bioenergy production are the changes with the greatest mitigation potential.

Soils of the boreal and sub-arctic vegetation zones are important for carbon storage, particularly in the sub-soil. Guggenberger *et al.* stated that, in Siberia, where global warming is relatively rapid, belts of vegetation may shift northward enabling more soil organic matter to be stored, though emissions of greenhouse gases may increase.

A future problem in many areas of the world will be an increasing incidence of forest fires which leave soil exposed and vulnerable to degradation. Haslam *et al.* showed how solid-state ^{13}C-nuclear magnetic resonance spectroscopy can be used to estimate the changes in soil organic matter quality as organic material reaccumulates after fires.

Biodiversity

Organic matter sustains the life of soil and this is inherently important to the concept of soil health. Microorganisms in particular play an essential role in the transformations of organic matter and nutrients that underpin

many soil processes. It has long been recognized that soil is the most complex of all environments and yet there is a great need to find effective and sensitive ways of monitoring its health. Being able to measure the response of soil microorganisms to environmental change may prove to be a valuable and rapid way of measuring the health of our soil. A number of soil microbiological parameters, notably microbial biomass carbon and basal respiration, have been suggested as possible indicators of soil quality and are employed in national and international monitoring programmes. More recently, we have moved into the 'age of communities' (Tiedje, 2000) and microbial diversity has also been recommended as a biological indicator of soil quality (Kennedy and Smith, 1995). But how do we quantify this intractable diversity? New methods to characterize, isolate and identify soil biota suggest that we have only just scratched the surface of a large and undiscovered gene bank, the reasons for which are intriguing. Organic matter also has a major effect on the soil physical environment. Indeed, the heterogeneity of the soil physical environment may in fact partly explain why soil has such a large diversity (Tiedje *et al.*). The pore structure of soil and its interaction with soil-water relationships can create microhabitats that lead to spatial isolation (islands), which may explain the diverse biogeography that we are only now starting to discover (Tiedje *et al.*).

The current momentum in soil biodiversity research is fuelled not only by the prospects of species and product discovery and the development of new molecular tools, but also by the opportunity to test new ecological theories and the pressing need to solve intractable problems associated with producing food and protecting fragile ecosystems. Linking biodiversity to ecosystem function is an exciting area scientifically and of broad interest in contemporary ecology. It is often assumed that soils with the greatest diversity of microorganisms may be the most resilient to pollutant stress. Ritz and Griffiths point out that there are many potential pitfalls when testing such hypotheses and also show that they may depend on how transient the stress is. Interestingly, they found that a physical, transient stress (heat shock) produced a different response from a persistent chemical stress (Cu contamination). The link between resilience and pollution effects is also made more difficult to understand because the same soil properties that affect diversity (e.g. organic matter, pH, texture; see Tiedje *et al.*) will also alter the bioavailability of many pollutants. It is important, therefore, when studying the effect of pollutants on diversity also to measure their availability to ensure that the selective pressures/or toxicity in different soils are unequivocal.

The effects of organic or biodynamic farming systems on biodiversity are areas where increased 'biodiversity' is often put forward as justification of the merits of different systems. Fliebßach *et al.* did find increased diversity in biodynamic systems compared with conventional fertilized

soils, and this was inversely related to the metabolic quotient of the soil microbial biomass. However, they did not find significant effects in the quality of the soil organic matter, measured by ^{13}C-NMR. O'Flaherty *et al.* showed that metal-rich sludges applied to land, which might otherwise have been assumed to cause stress and a reduction in diversity, actually increased diversity (measured using molecular methods). Clearly, simple generalizations may be hard to come by.

Several papers have quantified functional diversity (Fliebßach *et al.*, O'Flaherty *et al.* and Degens) and attempt to relate the quality of organic matter and/or changes in land use to diversity. Fleibßach *et al.* and O'Flaherty *et al.* tested soil extracts using Biolog plates to construct community-level physiological profiles (CLPPs), while Degens has pioneered the use of whole-soil substrate-induced respiration methods to produce catabolic response profiles (CRP). There is still debate on how well such methodologies measure functional diversity. Both approaches measure the potential utilization of different carbon sources at relatively high levels of C amendment, but clearly the functional approach is seen as a useful way to gain new insight.

Biological indicators of soil health should ideally be rapid and sensitive but there also needs to be a substantive amount of background information on natural variation and what constitutes 'normal' responses before value judgements can be made. Degen's use of catabolic diversity as a generic indicator of changes in soil functioning due to land use and the application of wastes to soil is a case in point. If rapid methods could be found for measuring such parameters, then this is an approach that might be attractive to agencies that have to monitor and regulate soil protection policies. The vision of the future presented by Tiedje *et al.* suggests that functional genomics will eventually allow us to measure important functional attributes, possibly at the mRNA level, so that the limitations of the potential measures and culturability will one day be overcome.

What then, after we have fully quantified this diversity? How do we then manage or manipulate it to create a more sustainable system? The ability to manage soils to enhance key species such as earthworms (Scullion and Malik) or rhizobium is clearly an advantage. Microbial communities might in the future be managed for environmental protection as well as to enhance nutrient supply. For example, organic matter (sawdust) added in trenches adjacent to streams has be used to stimulate denitrifying organisms and create a 'denitrification wall' to protect waters from excess nitrate (Schipper and Vojvodic-Vukovic, 2000). Thus management of key functional groups or species responsible for key processes is arguably quite realistic, but how to manage the more complex generalist communities? The importance of the rhizosphere as the interface between plant–soil–microbial interactions (de Neergaard and Magid) is also now realized and research is being directed at 'rhizosphere engineering' to achieve, for

example, remediation of pollutants or the biological control of pathogens. How can we then start to think of engineering the transformation of organic matter – to release nutrients in protected organic matter in low fertility soils or to enhance protection of organic matter in soil subject to physical degradation? These are some of the challenges that might be addressed by gaining a greater understanding of how microbial diversity interacts with organic matter.

Concluding Remarks

Land management must play a critical role in developing sustainable strategies of land use in the coming decades. Although cultivation too often in the past has been associated with organic matter loss and soil degradation, we are now in a position to apply our understanding of crop sequences and cultivations, many of which have been described in this volume, in a way that can actively restore organic matter storage, thereby restoring the functions that the soil supports. One of the characteristic features of organic matter, unlike many other important soil properties, is that it is significantly affected by management. Falloon *et al*. have shown that land management can have a significant impact on the sequestration of carbon by soils, thereby partially offsetting the imbalance between carbon release and uptake by terrestrial systems. We now have a better understanding of the relationships between organic matter quality and its function in soil (Cadisch and Giller). However, there is still much progress to be made in understanding how land management contributes to patterns of spatial and temporal heterogeneity in soils, particularly given the problems that this causes in relation to attempts to try to scale-up processes from the micro scale to the level of an ecosystem.

Modelling of soil organic matter dynamics is likely to continue to help in the understanding and management of the carbon cycle. This is important both at the global scale, enabling projections of carbon mitigation potentials in agriculture, and at the regional scale, enabling an 'optimum' organic matter level to be specified. Exceeding the optimum is likely to cause significant losses of nitrogen in addition to carbon, particularly during land use changes. Future developments are likely to include a wider range of scales of modelling. At small scales, we need further reconciliation of experimental and theoretical descriptions of soil organic matter. At larger scales, where technical development of models is well advanced, more acquisition of historical data on organic matter and land use practices is required.

The scope of the papers presented here highlights the breadth of approaches to soil organic matter research within the soil science community, and the inter-disciplinary nature of soil science *per se*. If we

are to address fully the issues associated with the sustainable management of soil organic matter, however, we need to continue to move beyond the traditional disciplines of soil biology, soil physics and soil chemistry and to work with other natural scientists and, increasingly, with social scientists. Real and perceived divisions between researchers and advisors, or between social and natural scientists, that exist within higher education and research institutes are undoubtedly barriers to the development of inter-disciplinary research. Current research funding methods may also hamper collaboration.

Interestingly, despite the importance of legumes on a global scale in contributing to N cycling and organic matter management, they received little attention at this conference. This perhaps reflects the current emphasis on the study of N fixation by plant physiologists not soil scientists, rather than a lack of research in the subject. Overcoming these barriers, together with our ability to harness the ever-increasing range of molecular and chemical techniques, not only will aid our understanding of soil organic management, but also our ability to influence policy that will protect and enhance soil organic matter across the world. In developing countries, indigenous knowledge and management systems have an important role to play in research (Pretty, 1995). Participative research approaches, which involve farmers, land managers and the extension service in research on soil fertility are being used extensively in developing countries (e.g. Corbeels *et al.*, 2000; White *et al.*, this volume). Such alternative approaches are beginning to gain more widespread acceptance in developed countries, and potentially could result in greater awareness of the importance of soil organic matter amongst farmers and other land managers.

Management of soil organic matter has to date involved primarily chemical and physical (mechanical) treatment to improve structure, incorporate residues and stimulate decomposition. While such field practices will continue, management in the future might also be based on greater biological understanding that seeks to manipulate or engineer the microbial population to enhance crop production and protect soils. Greater understanding of soil biodiversity may also lead to rapid biological tests that can be used to monitor and protect soil health.

The need to manage organic matter sustainably is clear. Much damage has already been caused to the world's ecosystem through neglect of the natural environment and the support systems that it maintains. This book illustrates that progress has been made in linking our understanding of soil processes with functions. It will be necessary to build upon this understanding through engagement with advisors, farmers and land managers to develop strategies that not only halt the degradation of soils but, in time, also reverse it. This will require international effort supported through national governments to value the use of natural resources and investment

in people and technologies that can achieve the ultimate goal of sustainable development.

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