Characterisation and consequences of soil microbial biodiversity

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What is meant by biodiversity? The single most important factor generating reduction in biodiversity is human land use, and agriculture is the most important factor affecting Europe's landscape and biodiversity. Despite this, it is still unclear how biodiversity affects ecosystem functioning, and how ecosystems supporting different levels of biodiversity respond to extreme environmental perturbation. The main conclusion of a recent European Working Group on Research and Biodiversity Report was the urgent need for research to investigate the role of biodiversity in soil processes, or, in other words, to go beyond merely *describing* biodiversity in all its forms and to understand its *consequences*.

Classic concepts of biodiversity tend to revolve around the fundamental taxonomic unit of species. However, operational definitions of biodiversity are not as straightforward as the mass-media would wish. In essence, biodiversity is a concept that aims to rationalise a complex set of factors that encompass the basic genetic, taxonomic, trophic and functional components of communities and their spatio-temporal dynamics, at a variety of scales. The concept also needs to include the number of different biological forms, entities or units from each of these perspectives, their relative abundance and the degree of interconnectedness between them. Food-web diagrams (Fig. 1) illustrate many of these points, and show that life in soils is exceedingly diverse, especially considering that each box encompasses entire organismal groups. If we consider within-group diversity, then things get really interesting. A handful (100g) of forest soil can harbour up to 4,000 bacterial species (genetically distinct units); a sandy agricultural soil can contain over 350 distinct bacterial species, which is of the same order as the total number of plant species in the UK.

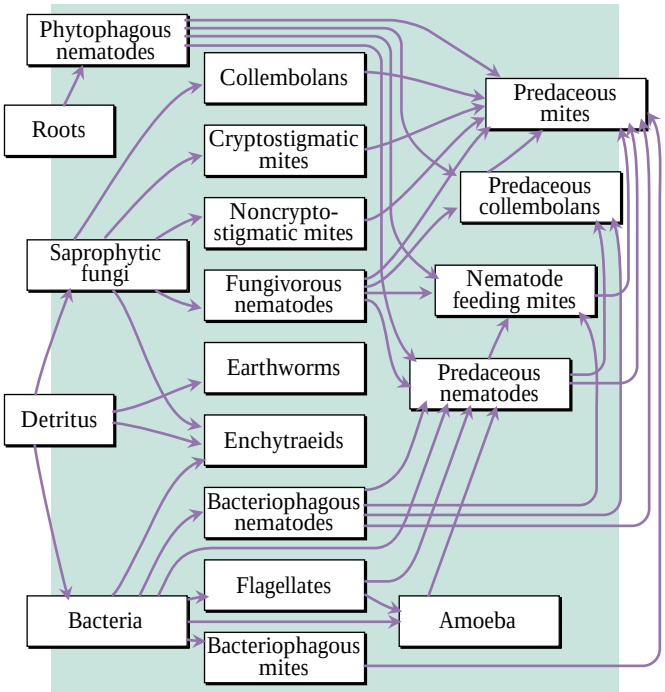

Figure 1 A typical food web from an agricultural soil showing the relationship between the major classes of soil organisms. Organisms in the same trophic group appear in the same column, progressing from the primary substrate supply on the left to the top predator on the right. Figure taken from de Ruiter *et al.* [2].

We need to be aware that biodiversity is entirely a human concept invented to describe complex biological systems. Ecosystems function through the interaction between individual species and communities, and there is no intrinsic mechanistic relationship between biodiversity and ecosystem function.

Characterisation of soil microbial biodiversity Traditional measures of biodiversity rely on species identification, the counting of individuals and knowledge of the ecological role of each of the species. When considering microbial communities, these parameters simply cannot be determined. There are no methods currently available, or likely to be available in the foreseeable future, that can determine the identity, frequency and evenness of all microbial species present. Rather, microbial ecologists are limited by the data that can be obtained and have to devise experimental approaches to overcome the technical shortcomings. The non-cultivability of the majority of microbial species present in soil is now well established, and the analysis of environmental, or whole-community, DNA is being used to overcome this. Environmental DNA can be characterised in a number of ways. For example, its complexity can be measured using reasso-

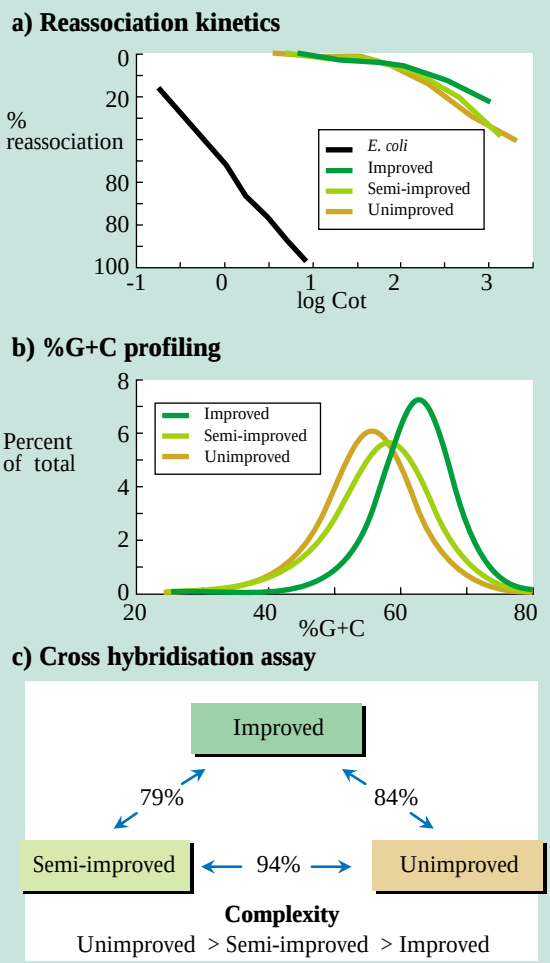

Figure 2 Broad-scale analysis of soil community DNA extracted from upland grasslands receiving different degrees of management. (a) reassociation curves indicating relative complexity of soil communities – the black line shows the rate associated with one bacterial species; note the x-axis is a log scale. (b) %G+C profiles indicating the community composition on the basis of guanine and cytosine bases in the whole soil DNA. (c) cross-hybridisation assay, allowing quantification of degree of similarity between the community DNA, plus another measure of relative complexity. Data obtained within SERAD MICRONET Programme.

ciation kinetics assays, while cross-hybridisation and % guanine+cytosine profiles allow the comparison of DNA from samples to determine how much of the DNA is in common. Some examples of the application of these broad-scale assays to characterise the impact of management systems upon microbial communities in upland grasslands are given in Figure 2. Communities comprised of completely different species could be equally diverse but, as ecological function is more related to species composition than it is to diversity, such characterisation is important.

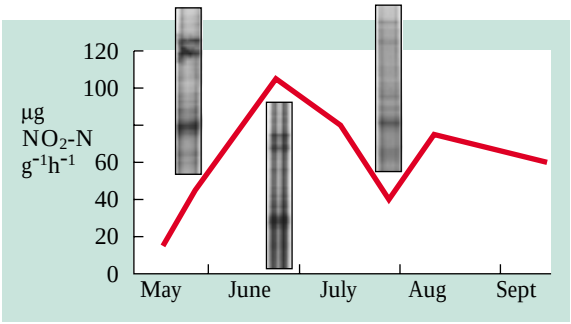

Figure 3 The time-course of potential nitrification through the spring and summer in soil from a crop of field beans at SCRI. The insets show profiles of the microbial community, analysed by DGGE, taken at times of high and low activity.

Polymerase chain reaction (PCR) based techniques for analysing soil DNA allow for a higher-resolution analysis of community structure. PCR product analysis by degrading gel gradient electrophoresis (DGGE) can be used to assess commonality in microbial community structure between samples. Currently, the usefulness of this approach is limited by the number of DNA bands and complexity of the banding patterns obtained. However, the banding patterns obtained from an arable soil throughout the growing season suggest that this is an effective approach for studying soil microbial biodiversity. For example, changes in microbial community structure related to temporal variations in soil processes such as nitrification can be studied (Fig. 3). Here, primers were utilised such that the gel profiles represent the entire bacterial community, but primer design is progressing at a rapid pace and it is now possible to generate profiles representative of specific components of the community (such as actinomycetes, archaebacteria, pseudomonads and fungi). Through the use of RNA technology, it is also

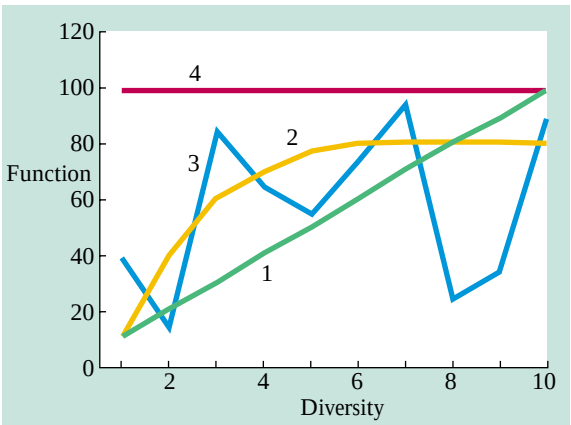

Figure 4 Graphs depicting the theoretical relationships between biodiversity and ecosystem function.

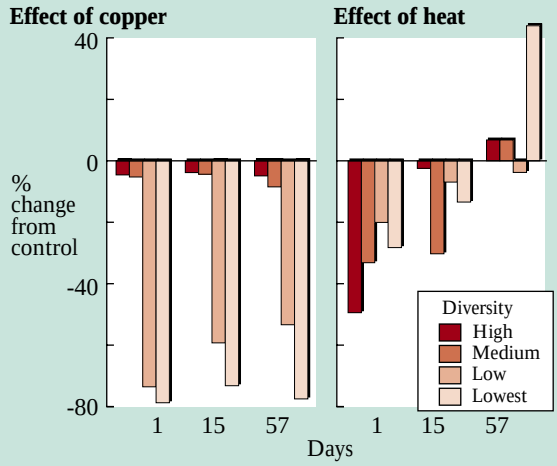

Figure 5 The resilience of fumigated soil differing in biodiversity to the applied stresses of copper or heat. Stress was applied at day 0, and the reduction in decomposition (relative to an unstressed control) determined subsequently. The least diverse soils were very susceptible to copper and decomposition did not recover, while after heat, decomposition recovered fastest in the most diverse soils.

possible to determine which of these components are active in any particular sample.

Theoretical consequences of changes in biodiversity for the functioning of ecosystems Various theories exist of how the functioning of ecosystems could vary as biodiversity changes (Fig. 4). Each species may fulfil a certain role in the environment and so as species are lost, function diminishes (i.e. Curve 1 below). Given the enormous biodiversity of soil microbes, there is undoubtedly some redundancy in function, so that more than one species can fulfil each task and that species can be lost, possibly until some critical threshold value of biodiversity is reached (Curve 2). This is more realistic. However, given the complexity of interactions in soil, the removal of species is likely to affect how other species behave in an entirely unpredictable fashion (e.g. Curve 3). This is the most likely scenario. It is also theoretically possible that loss of species will have no effect on the functioning of the system (Curve 4).

Given that the most likely outcome is Curve 3, it is unlikely that any theoretical framework could be constructed to directly link biodiversity with specific ecosystem functions. It is, however, much more likely that the resilience of the soil microbial community (resilience being its ability to withstand or recover from perturbation) is directly linked to biodiversity.

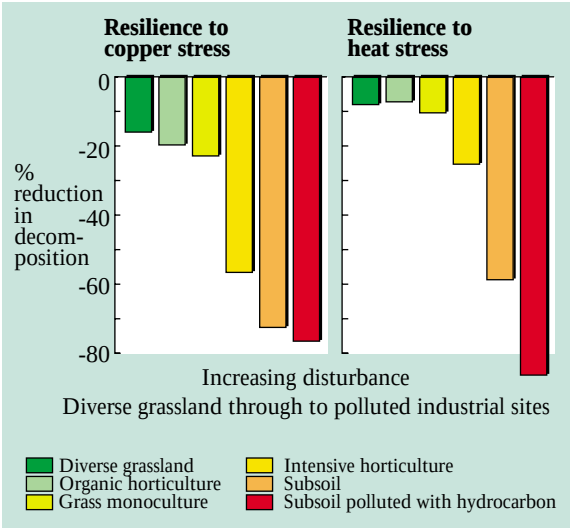

Figure 6 The resilience of soils exposed to increasing degrees of environmental stress to applied copper or heat.

Recent experimental diversity:function studies at SCRI

1. Destructive reduction in biodiversity We were one of the first research groups to use progressive fumigation with chloroform to reduce soil biodiversity experimentally, and to determine the consequences of this for soil processes[1]. Although fumigation reduced soil biodiversity by up to 40%, there were no consistent effects on soil functions. Activities like decomposition, respiration, growth and denitrification were increased in the least diverse soils; while others such as nitrification and methane oxidation were decreased. The most significant finding was that the most diverse soils were more resilient to environmental stresses, in this case experimentally applied copper or heat treatments. This experimental evidence supports the theoretical conclusions given above (Fig. 5).

2. Constructive increases in biodiversity There was some evidence from the chloroform experiment described above that the communities resulting from fumigation were physiologically different from those in unfumigated soil. They might have been selected by the treatment. To overcome this, in further experiments sterile soil was inoculated with microbial communities, differing in biodiversity, that had been extracted from soil. Again there were no consistent effects of biodiversity on soil functions, but also no consistent effects on resilience. It seems likely now that the measure of resilience is an indicator of microbial community stress, such that communities which have been stressed are less able to withstand subsequent stresses. Thus, the communities inoculated into

sterile soil had all been stressed to the same extent and so had equal resilience. In the fumigated soils, although the communities were reduced in biodiversity, they had also been stressed by the fumigation and so differed in resilience.

3. Response of soils naturally differing in biodiversity The functioning and resilience of microbial communities exposed to different degrees of environmental stress, and therefore expected to differ in biodiversity, were also tested. Thus, soils with different aboveground biodiversity (i.e. monoculture *vs* a diverse sward), soils with and without hydrocarbon pollution, and with intensive or organic management, were examined. Protozoan populations, which have been proposed as environmental indicators because of their sensitivity to environmental conditions, were effective at differentiating soils from the same site, but showed little relation to biodiversity between the different soils. The functioning of the soils, measured as the ability to decompose substrates, was not related to biodiversity. In particular, the polluted soil, with a particularly low biodiversity, was more able to decompose a range of substrates than the unpolluted soil. Resilience, on the other hand, was a good measure of previous environmental stress and, therefore, correlated with biodiversity. Given the results of the constructive experiment described above, it is likely that resilience is affected by the previous stresses that the microbial community has been exposed to rather than the biodiversity of that community (Fig. 6).

Conclusions Changes in soil microbial biodiversity *per se* do not impact on the provision of ecosystem services by soil systems. Biodiversity can be altered by many factors, and it is the effects of those factors which are important in determining the outcome for soil processes. The measurement of resilience has been seen to provide useful information about the soil microbial community, and will be studied in more detail in future work. The characterisation of microbial communities from their DNA, and more particularly in terms of RNA to access the active components, is important in understanding what the key components are, how they behave and how this will be affected by the effects of future land use and management changes.

References

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