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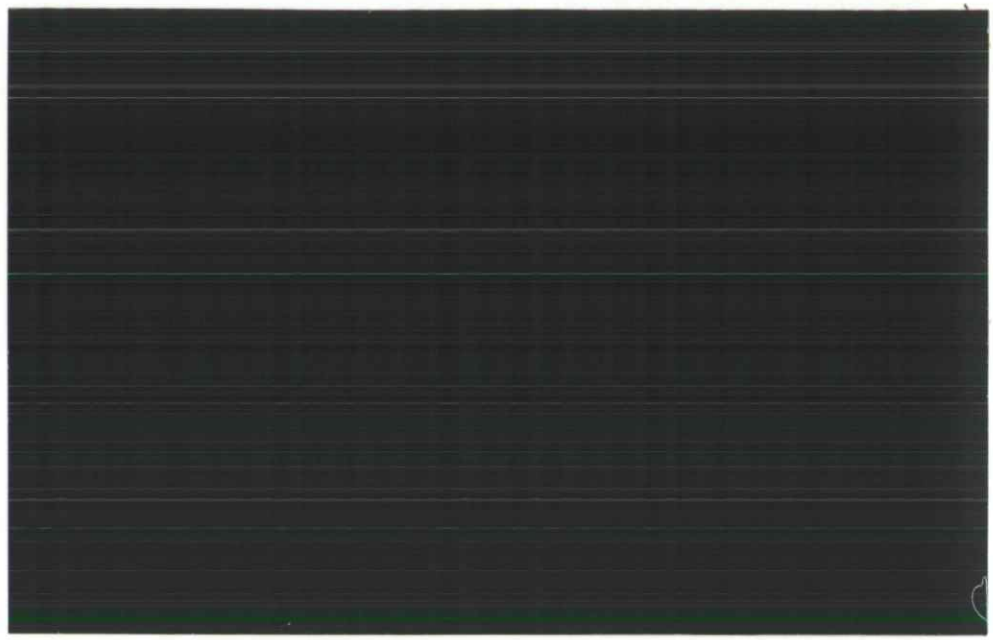

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Microbial Diversity and Ecosystem Function

CEH Integrating Fund Project T10062R6 (1995-1999)

Final Report to the Director of the Centre for Ecology and Hydrology

September 1999

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SUMMARY

- This report describes work carried out within the CEH Integrating Fund project entitled *The Role of Microbial Diversity in Regulating Ecosystem Function.* The project involved staff working at IFE (Windermere) and IVEM (Oxford). There was also a minor involvement of staff from the ITE stable isotope analysis facility at ITE Merlewood.

- The ecosystem studied was a one hectare, 3.5m-deep hyper-eutrophic pond known as 'Priest Pot', located at the north end of Esthwaite Water in the English Lake District (Cumbria). The dominating biology of the pond is microbiological and a considerable diversity of microbes – 'species' and functional groups, are now known to be present. The task of understanding the role this diversity plays in the way the ecosystem functions, is the general objective of the work presented here.

- The most significant event in the calendar of Priest Pot is thermal stratification of the water column, from May until the end of September. This process initiates a complex series of physical-chemical changes that generate three superimposed water column compartments (aerobic epilimnion; metalimnion with steep opposing gradients of dissolved oxygen and reductants; anoxic hypolimnion). Each compartment sustains a functionally coherent assemblage of species. Direct interactions (e.g. predation) between compartments are relatively rare, but the compartments are linked by the fluxes of essential nutrients (e.g. carbon dioxide and inorganic nitrogen, both in solution). The community in each compartment is further divided by vertical separation (in the order of 10 cm) of functionally similar species-populations. This further reduces competitive interactions and allows a greater number of species to co-exist within a compartment.

- Ecosystem functions such as carbon-fixation and nutrient cycling appear to be sustained by a plethora of *reciprocal* interactions involving physical, chemical and microbiological factors. For example, the rate of microbial activity depends on such factors as temperature and the concentrations of essential nutrients, but microbial activity itself also influences nutrient concentrations, redox chemistry, and the sub-surface light climate (which in part determines water temperature). Microbial activity also operates in conjunction with physical and chemical factors to create new microbial niches (e.g. for anaerobic photosynthetic, and nitrogen-fixing bacteria). The net result of all these reciprocal interactions, and the resulting microbial activities that are enabled, is the way the ecosystem functions.

- Microbial activity and microbial biodiversity are both a part of, and inseparable from, ecosystem function. As most free-living microbial species appear to be ubiquitous, newly-created microbial niches are quickly filled, and microbial diversity is probably never so impoverished that ecosystem function is impaired.

- The emergence of this holistic view of the nature of the interaction between microbial diversity and ecosystem function is the principal scientific product of this work.

- There are several further important implications of this work: (a) CONSERVATION - concerns for the extinction of animal and plant species cannot be extended to biodiversity at the microbial level; (b) 'BIOREMEDIATION' - If the habitat changes, new species will soon appear to fill vacant niches; (c) EXTRAPOLATION - contrary to many recent scientific publications, it is not possible to make realistic extrapolations from microbial diversity, to biodiversity in general.

- Much additional work has been carried out which supports or confirms the fundamental point that the absolute abundance of micro-organisms drives their dispersal and makes them ubiquitous. Future work will focus on investigating the mechanism and scale of microbial dispersal; and in verifying the cosmopolitan distributions of microbial groups with which we are very familiar. It may be possible for example, to find in Priest Pot the entire global inventory of certain microbial groups.

The remainder of this report is an extended version of the verbal presentation made to the SMA Group during their visit to IFE(Windermere) in March 1999. It does not include most of the illustrative material already presented in the First and Second Progress Reports (Aug 95 – Oct 96, and Nov 96 – Sep 97 respectively).

INTRODUCTION

Much effort is currently directed at understanding the role of biodiversity in the natural environment. This area of science is, however, not new. Darwin believed that species-rich plant communities were more productive than impoverished ones, and Elton (1958) advanced the hypothesis that "diversity begets stability". What is new, is that the questions have become more specific. Ecosystem functions such as carbon-fixation and nutrient cycling are identified and quantified, and related to the species richness of natural and manipulated communities. At the heart of all such studies is the desire to discover if it makes any difference how many species are present – i.e. is biodiversity important?

Tilman's (1996) work with terrestrial plant communities indicates that the biggest gains in community stability and productivity come with the first ten species; and additional species do not appear to bring additional 'benefits' to community performance. This could indicate that natural ecosystems, often containing many hundreds of plant species, have much greater species richness than is ever likely to be needed to reach peak productivity. But does this apparent surfeit of plant species in natural communities have any role to play with respect to ecosystem function? The question is difficult to answer for terrestrial communities of higher plants and animals, and the problem is essentially one of spatial and temporal scale. Niche diversification may be more intricate than can easily be observed because of micro-scale environmental heterogeneity, and so-called "redundant" species occupying cryptic niches. And the niche will be especially difficult to detect if the species has a relatively long life-span, or if it produces long-lived propagules, so that its maintenance in the community may be supported by environmental conditions that appear with low frequency and short duration; such as rainfall in the desert.

Microbial Biodiversity in Aquatic Ecosystems

These problems are less important when dealing with free-living microbes in aquatic systems. The short generation times of microbes – days or even hours – permit rapid changes in population abundance. Moreover, changes in aquatic microbial community structure are often the result of environmental conditions (e.g. dissolved oxygen tension) that vary with high amplitude and frequency. In a productive freshwater pond, the spectacular variation in microbial activity and diversity observed during a single day probably exceeds that of the most dramatic successions within terrestrial plant communities spanning many years. In the course of a few days, the habitat experienced by a microbial community may shift from oxygen supersaturation to anoxia; from an excess of dissolved nutrients to complete nitrogen depletion. By recording the accompanying rapid succession of microbial species, we may quickly gather evidence of the relationship between species-richness and ecosystem function, and the typically high rate of turnover of microbial species will increase the probability that we will occasionally glimpse (and understand) bursts of activity from these 'redundant' species.

Microbial Biodiversity – the fundamental characteristics

The debate about microbial diversity is currently driven by two divergent views (Table 1). On one hand, free-living microbial species may behave like most animals and plants. They may be restricted to specific geographical areas. And if many of these species have locally restricted distributions, the global number of microbial species will be very large.

Table 1 Two divergent views – the fundamental characteristics of plant and animal biodiversity differ from those of microbial biodiversity

	Macroscopic plants and animals	Free-living microbes
Absolute abundance	Low	HIGH
Rate of migration	Low	HIGH
Rate of speciation	HIGH	Low
Rate of extinction	HIGH	Low
Number of endemics	HIGH	Low
Global number of species	HIGH	Low
Local:global number of species	Low	HIGH
Cryptic persistence	variable	Yes

The opposite view emerges from recognition of the astronomical abundance of organisms within each microbial species. So for purely statistical reasons, migration rates will be high, rates of speciation and extinction will be low, the global number of microbial species will be low, and local microbial diversity will account for a large proportion of global microbial diversity. According to this second view, a microbial species may be capable of thriving in the biosphere wherever a suitable habitat exists. It has been difficult to demonstrate the ubiquity of microbial species because of the rapid turnover of active species, and because at any moment in time, most species in a habitat are relatively rare, or in some cryptic state. However, we have recently been able to show, with the example of microbes that leave traces of their recent population growth in the form of siliceous scale structures, that all species in the chrysomonad flagellate genus *Paraphysomonas* are probably ubiquitous.

Paraphysomonas consists of 50 species, all of which are distinguished using the morphology of their surface scales. The scales remain recognisable for several months after cell death, so by looking at their remains in the sediment of a pond, we should find evidence of the preceding species succession. We used transmission electron microscopy to examine the superficial ~2 mm of sediment collected from a one-hectare freshwater pond (Priest Pot, Cumbria, UK). ^{210}Pb–dating indicated deposition of this sediment layer within the previous 3 months. We identified and quantified all scales and cell remains of all *Paraphysomonas* species present, then used this information to reconstruct whole cells. Our examination of 25.2 µl of sediment yielded data for the relative abundance of 32 species.

We then compared these data with the information in 73 published surveys of *Paraphysomonas* species carried out across all biogeographic regions of the world. The total number of species registered in these surveys is 41, so 78% of species in the global inventory were also detected in a tiny volume of pond sediment. The pattern of relative abundance of species in Priest Pot is remarkably similar to that for the relative commonness of species in the global data (Fig. 1).

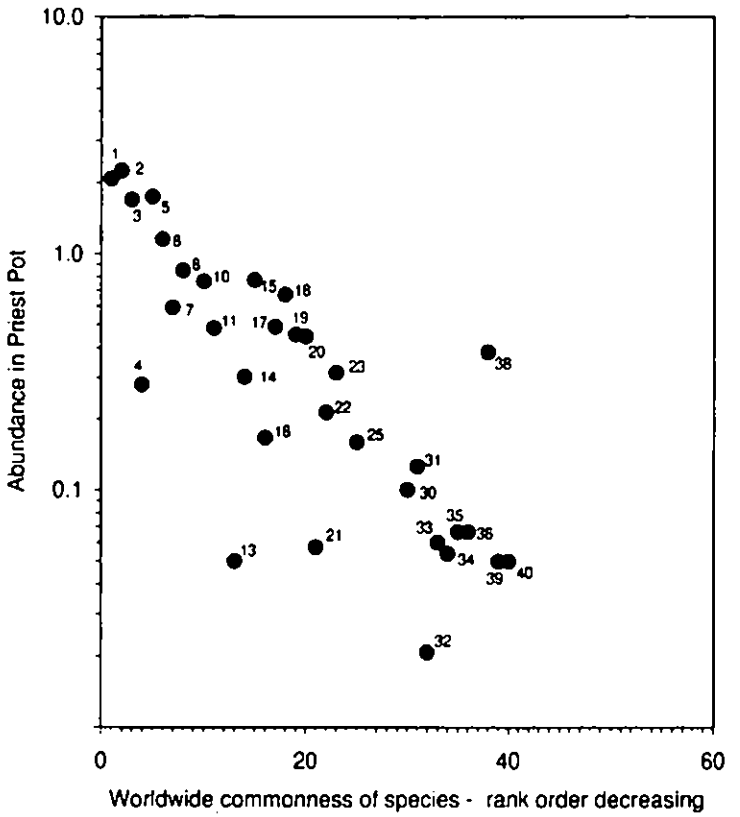


Fig. 1. The abundance of each Paraphysomonas species in 25.2 µl (equivalent to ~0.1 cm^2) of Priest Pot sediment, plotted against its worldwide commonness. The commonness data are ranked in order, decreasing from left to right. Species 1 and 2 are P. vestita and P. imperforata respectively.

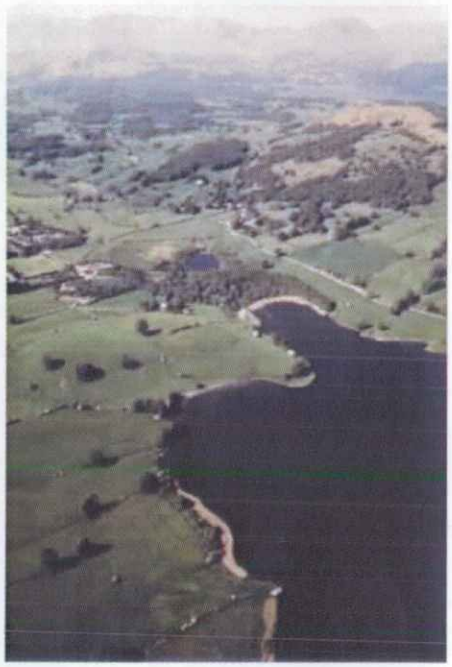
Species that are frequently recorded globally, are also abundant in Priest Pot sediment. And species that are rarely found globally, are not abundant in Priest Pot. Our explanation is as follows. Species that are globally abundant will, through neutral migration, 'seed' the pond more frequently than rare species. The reason these species are abundant is probably that they are capable of population growth in a broad range of conditions, so these species will more frequently find opportunities for population growth. Finally, termination of population growth is accompanied by the production of resting cysts. As the 'cyst-bank' size for each species is likely to be proportional to its global abundance, repeated cyst production will effectively strengthen the pattern of relative abundance of species that results from neutral migration. This is the clearest evidence yet for the global ubiquity of eukaryotic microbial species. The fundamental reason for species ubiquity is probably the sheer weight of numbers of organisms that drives large-scale dispersal across the physical and geographical barriers that normally halt migrations of larger animals and plants (the water column of Priest Pot supports ~4 x 10^{14} living *Paraphysomonas*). And as free-living bacteria are about three orders of magnitude more numerous than heterotrophic flagellates, it is even more likely that they too are ubiquitous, and that the global richness of free-living microbial species in general is modest.

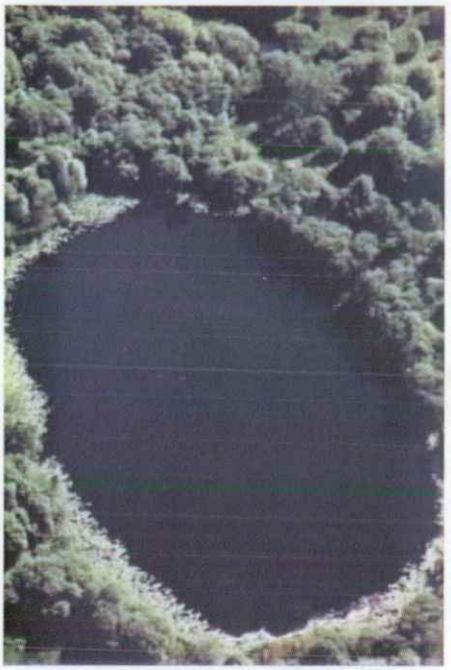
These ideas are also supported by empirical evidence – e.g. from soil isolates of the bacteria *Bacillus subtilis* and *Bacillus mojavensis* obtained from three continents. Recombination was shown to occur within each species at about the same rate as neutral mutation, whatever the geographical scale over which strains were sampled. The rate of migration was shown to be

so high that during the time span of each generation, more than one viable cell is transported between populations on different continents (Roberts & Cohan 1995).

Reciprocal Interactions

But microbes have some other special characteristics, as illustrated below from our investigation of a small pond. The one-hectare pond known as Priest Pot (Cumbria, UK) has been studied scientifically for more than 40 years (e.g. Gorham 1960, Belcher et al. 1966, Goulder 1971, Robinson et al. 1984, Finlay 1985, Berninger et al. 1986, 1991, 1993, Davison & Finlay 1986, Stewart & George 1987, Finlay et al 1988, 1996b, Guhl et al. 1994).





Figs 2 and 3. Aerial views of the pond known as Priest Pot, which lies at the northern end of Esthwaite Water in the English Lake District, Cumbria.

It is clear from the results of these studies, and from the current study, that many biological and non-biological factors interact to make the pond function in the way it does. These are invariably *reciprocal* interactions, and this implies that microbial biodiversity, far from being something that acts upon or responds to ecosystem function, may actually be *a part of* ecosystem function. Preliminary evidence for this is apparent in Fig. 4, which synthesises information abstracted from the large body of published research on Priest Pot, and interlaces it with the spatial and temporal framework of events and processes observed in 1996:-

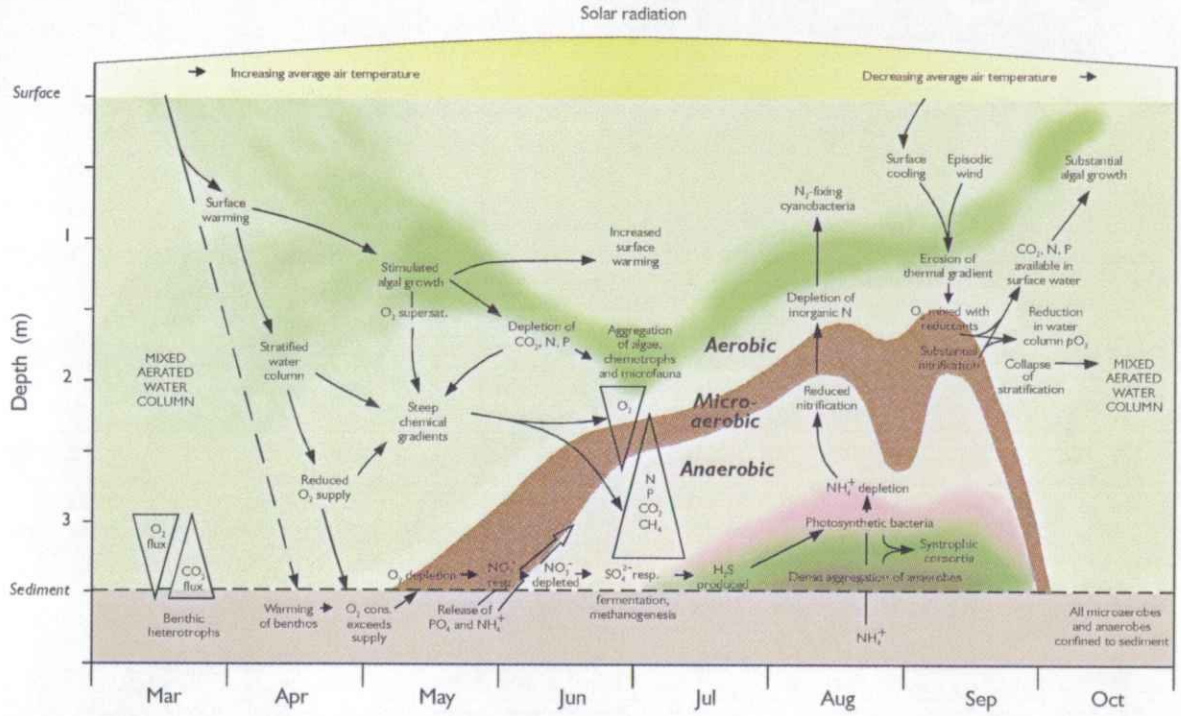


Fig. 4 An illustration of some reciprocal interactions in Priest Pot in 1996. During the course of each summer, the water column evolves into three superimposed redox compartments (aerobic, micro-aerobic and anaerobic). This figure does accurately reflect the spatial-temporal distribution of these compartments. Some processes, and many microbial functional groups, have been excluded for simplicity, e.g. the diversity of chemoautotrophic prokaryotes.

Some of the more important events in the seasonal cycle are as follows:-

1. Increasing solar radiation in the spring, and the establishment of physical stratification, reduces the rate of oxygen supply to the sediment and eventually produces an anoxic hypolimnion.
2. Aerobic microbes migrate out of the sediment and into the overlying water.
3. Degradation of the oxidised sediment-water interface solubilises bound phosphate; and nutrients such as ammonia and CO_2 diffuse into the water column.
4. The oxic-anoxic boundary eventually stabilises at an intermediate depth, where a dense food web based on phototrophic and chemotrophic micro-aerophiles (prokaryotes and eukaryotes) develops, sustained by the opposing fluxes of light and dissolved oxygen from above, and dissolved CO_2, nitrogen and phosphorus from below.
5. Meanwhile, in the anoxic hypolimnion, a transient niche opens for nitrate respirers. These soon exhaust the nitrate, and sulphate-reducing bacteria grow to replace them.
6. At the same time, fermenting bacteria in the sediment continue to degrade organic matter to CO_2 and acetate. Syntrophic consortia are established, and anaerobic protozoa (which have now excysted) appear, carrying symbiotic bacteria, especially methanogens.
7. At this point – around the beginning of July – the metalimnetic community reaches its maximum biomass, and intercepts most of the transmitted light in the 400-700nm band.
8. In the anoxic hypolimnion, the sulphide produced by sulphate reducers increases in concentration. Thus a niche is created for anaerobic photosynthetic bacteria that use long wavelength (mainly >700nm) light, and sulphide as electron donor.

9. By mid-August, these bacteria have reached maximum abundance about 90 cm above the sediment, where they produce a thick layer of slow-growing biomass that fixes nutrients, including ammonia, that would otherwise pass to the overlying metalimnetic community.
10. Thus nitrification is depressed, and in the absence of rainfall (as in 1996), the entire aerobic water column becomes depleted in inorganic nitrogen.
11. Nitrogen-fixing cyanobacteria suddenly appear, and symbiotic associations between ciliates and chlorellae (the ciliate providing the chlorellae with nitrogen) now dominate the protozoan community.
12. At this stage of seasonal development, the water consists of three superimpsed 'compartments' (anoxic hypolimnion, microaerobic metalimnion, and oxygenated epilimnion), each supporting a characteristic community. Direct interactions between species in different compartments are relatively rare, although the compartments are connected by diffusion and transport of soluble reactants of the nutrient cycles (e.g. CO_2 produced by anaerobic bacteria may be fixed by microaerobic algae in the overlying compartment).
13. By the beginning of September, decreasing solar radiation leads to cooling of surface water and the deep penetration of dissolved oxygen. The renewed co-occurrence of O_2, CO_2, and ammonia, promotes an intense burst of chemotrophic activity whose activity causes a rapid increase in nitrate, and a reduction in dissolved oxygen.
14. The re-supply of inorganic nitrogen to the upper water causes a temporary bloom of algae.
15. Stratification then breaks down, and water temperatures fall slowly to winter values.
16. All of the microbial species that appeared during the period of stratification return to the sediment, where they presumably remain viable, as they appear in subsequent years whenever suitable niches are created.

Thus Priest Pot provides us with a comprehensible sequence of reciprocal interactions involving physical factors, microbial activity, and water chemistry. These interactions produce a large number of potential niches in space and time which, in conjunction with short microbial generation times, can support high biodiversity. This is apparent from the rapidly growing inventory of microbial species recorded for Priest Pot.

We can add a further layer of detail to this model, with information from two recent years. 1995 was an unusual summer - there was virtually no rainfall. So by the beginning of August, the entire water column of Priest Pot was devoid of detectable inorganic nitrogen, and most Protozoa had algae living symbiotically inside them, consuming their waste nitrogen:-

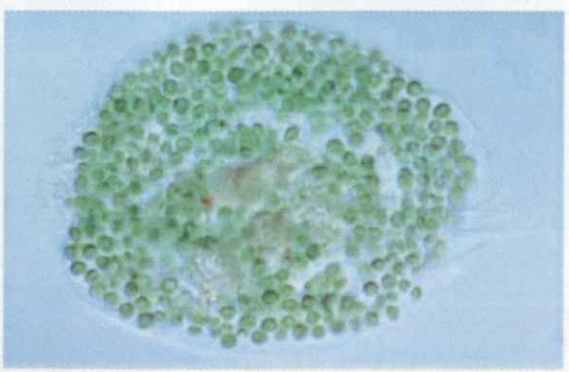


Fig. 5. The ciliate Euplotes daidaleos *packed with endosymbiotic zoochlorellae (green algae)*

And just below the oxic-anoxic boundary, where most ciliates were concentrated, 96% of them had symbiotic algae – providing sufficient oxygen to enable the ciliates to live in anoxic water.

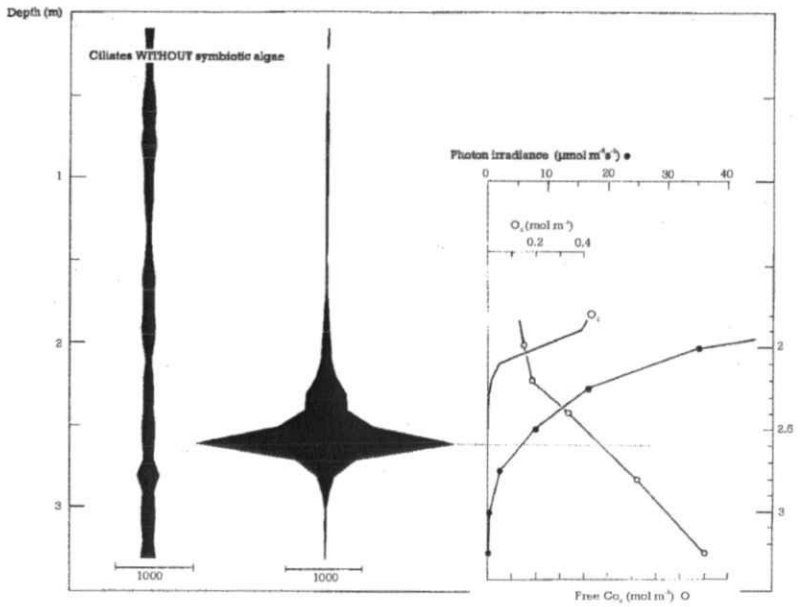


Fig. 6. Vertical distribution of ciliates (with and without symbiotic algae) in the water column of Priest Pot. 96% of the ciliates had zoochlorellae. The inset shows profiles of O_2, CO_2 and light with depth.

But in 1997, conditions in Priest Pot were quite different. There was no shortage of dissolved nutrients in the water column, peak ciliate abundance lay precisely at the oxic-anoxic boundary, and the community composition was quite different.

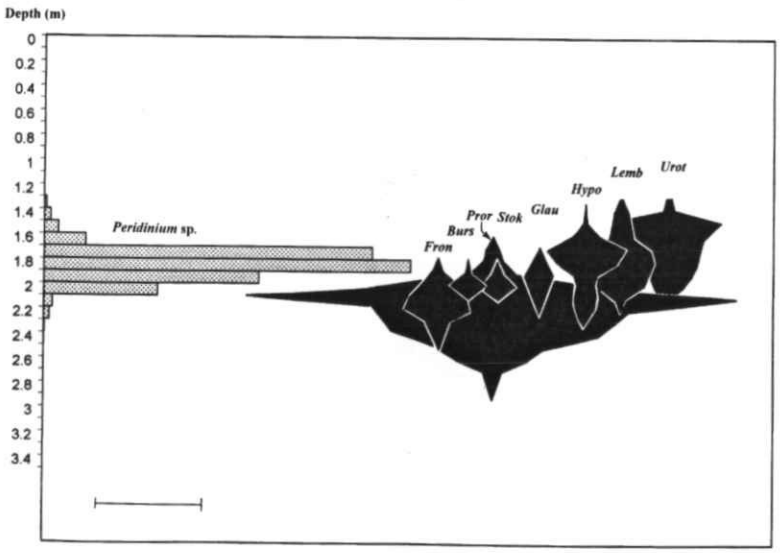


Fig. 7. June '97 – vertical distribution of ciliate species associated with a Peridinium *bloom*

The key reason for the totally different ciliate community was probably the presence, in bloom proportions, of the dinoflagellate *Peridinium*:-

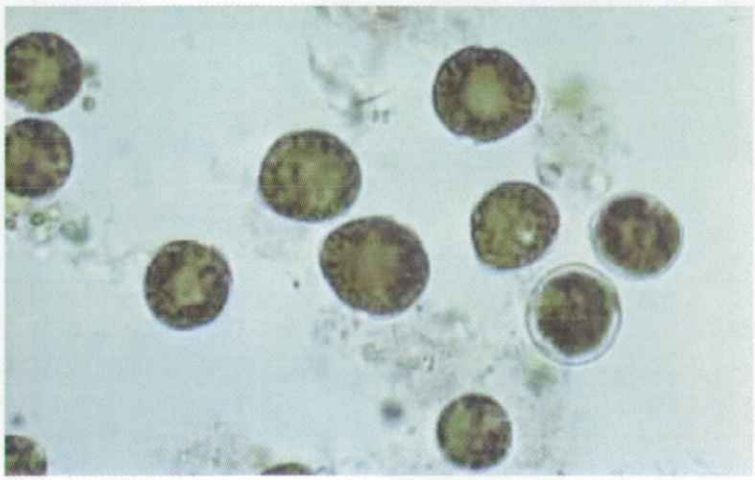


Fig. 8. The dinoflagellate Peridinium *sp. in Priest Pot, June 1997. Organisms are approximately 25 µm in diameter.*

These produced a sharp peak of abundance at the metalimnion. Because of its size and shape *Peridinium* is easily ingested by a variety of raptorial ciliates, and a diverse range of new ciliates of this type – not previously seen in Priest Pot in the recent past - rapidly appeared in the water column.

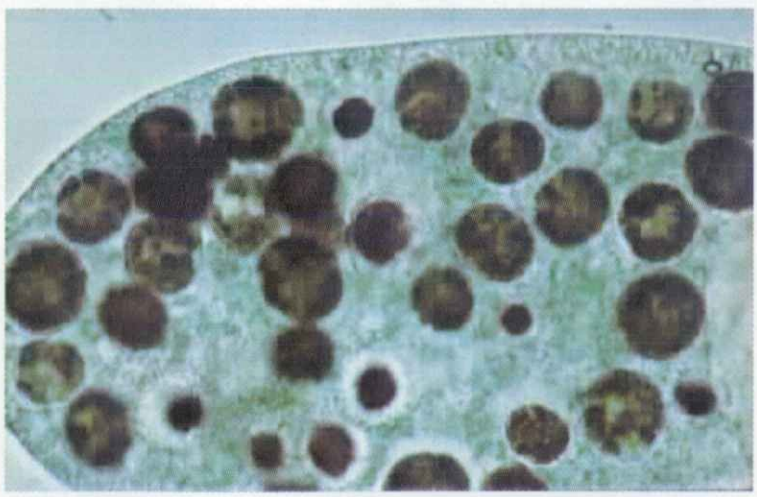


Fig. 9 The ciliate Frontonia leucas *with many ingested cells of* Peridinium, *in the water column of Priest Pot in 1997*

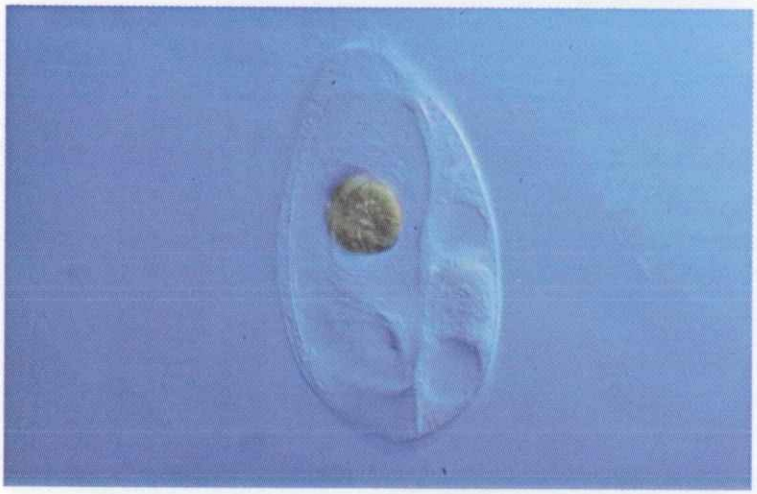


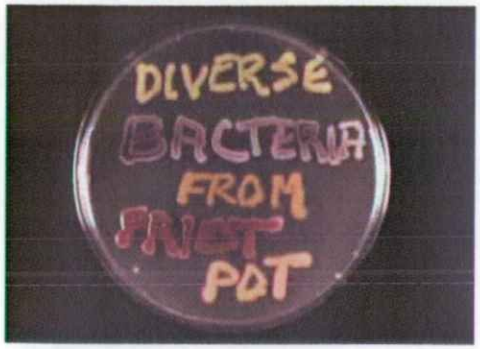
Fig.10 The ciliate Lembadion magnum, *at the point of ingesting a* Peridinium

So the presence of *Peridinium* in 1997 dictated the species composition of the 'active' ciliate community. And as these ciliates did not support oxygen-producing endosymbionts, the abundance peak lay precisely at the oxic-anoxic boundary. So the bloom of *Peridinium* determined not only which species would be present, but also the depth in the water column where the ciliate peak was found.

In this little pond, we have found roughly half of all species ever described worldwide, in several large protozoan groups. Local microbial diversity does indeed seem to account for a large proportion of global diversity. And as protozoa are amongst the rarest of microbes, we might be confident that most, if not all types of free-living bacteria that exist, also exist in this pond. This of course would mean that the pond can produce a microbial community in response to any conditions that are ever likely to arise.

Bacteria and viruses

There is indeed information that the local diversity of bacteria is a substantial proportion of global diversity. 903 bacterial isolates of free-living heterotrophic bacteria were obtained from the water column of Priest Pot. These were identified from their FAME profiles (fatty acid methyl esters) as 101 different species. 100 of these species were positively identified from existing FAME databases; so virtually all of these species had already been isolated and identified from places in the world other than Priest Pot. It is also clear that these 101 species represent only a small fraction of the species in Priest Pot.



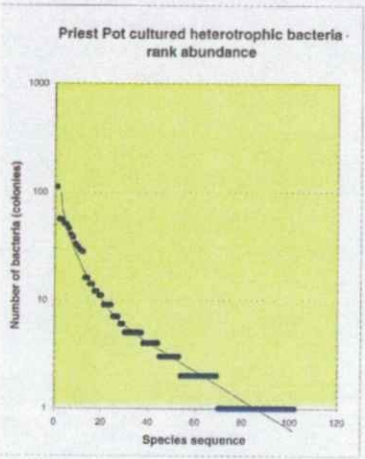


Fig. 11 'Diverse' Priest Pot heterotrophic bacteria
Fig. 12 Rank abundance plot of cultivated heterotrophic bacteria from Priest Pot

Figure 12 is simply a rank abundance plot for the 101 species. The key point is that no fewer than 33 of these species were represented by a single colony, indicating that very many more, even rarer species, would have been detected with additional sampling.

Everything microbial is everywhere – the environment selects (*attrib.* Beijerinck *ca* 1895). But that environment may have microbial dimensions – and the environment may even exist inside another cell. A symbiotic *Chlorella* - a green alga - was isolated from a ciliated protozoon in Germany, and the *Chlorella* was cultured at IVEM. We then took water from different depths in Priest Pot.

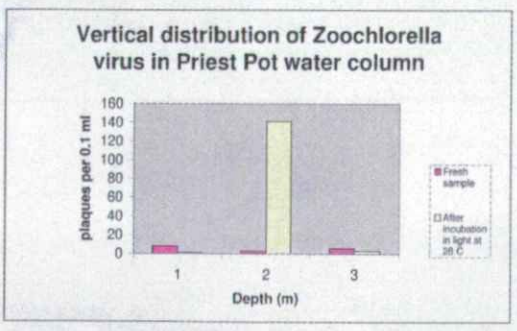
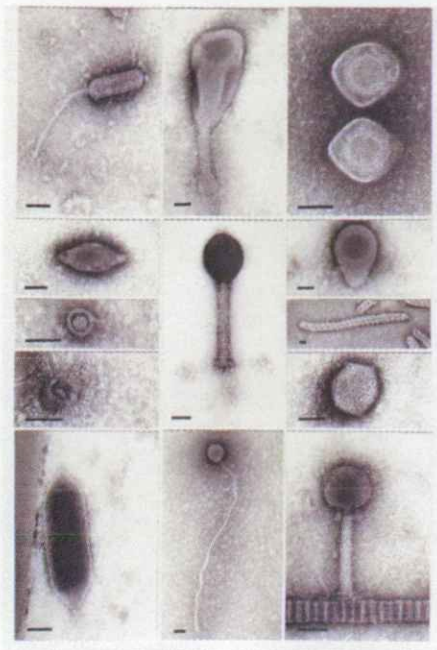


Fig. 13 Plaques forming on a 'lawn' of Chlorella *after addition of raw water from Priest Pot*
Fig. 14 Vertical distribution of zoochlorella virus

A water sample from the middle layer (where lived most of the ciliates with endosymbiotic *Chlorella*) - produced a very large quantity of virus that killed the *Chlorella* taken from the ciliate from Germany. The environment – in this case a lawn of *Chlorella* in a Petri dish – selects. And the strong indication here is that the ciliate, its symbiotic *Chlorella*, and the virus that infects the symbiont, can be found in freshwater ponds in England and Germany (and probably also further afield).

Viruses are extraordinarily abundant in the water column of Priest Pot – roughly 10^8 per ml, or 100 times the abundance of bacteria.



Examples from the range of >100 free-virus morphotypes found in the water column of Priest Pot. Bar = 50 nm

Fig. 15 – Representative selection of Priest Pot viruses (TEM)

Viruses are probably also implicated in the rather complex battles between bacterial species. Using bacteria isolated from Priest Pot, it is apparent that different species are quite capable of killing each other – either with viruses or with bacteriocins (antibiotics produced by one bacterium that kill closely related bacterial species). But our progress on this to date amounts to simply documenting the phenomenon.

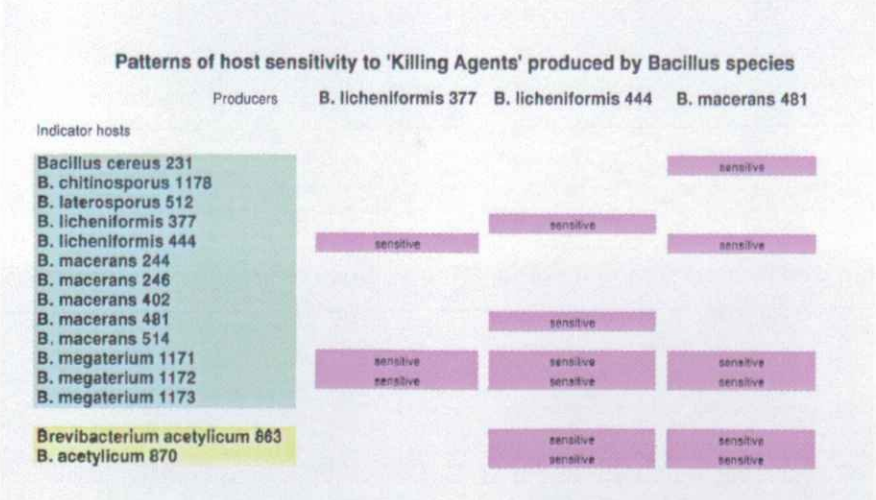


Fig.16 Bacterial 'killing agents' detected in the Priest Pot water column

If we are seriously interested in trying to obtain deep understanding of the relationship between microbial diversity and ecosystem function, we will need to devote much more effort to the natural history of viruses.

The FUTURE

Like all large projects, this one has raised at least as many questions as it has answered. It has also highlighted some work that definitely has to be done. One problem is that different types of methods are needed to characterise different microbial groups. Phenotypic methods (even cell morphology) are as useful as anything for most of the eukaryotes, whereas DNA-based methods, while often not quantitative, are believed to be the only realistic means of characterising prokaryote communities.

There is a pressing need for a method that can be used to monitor the natural dynamics of whole microbial communities in the water column. The method should be quantitative and non-selective, and it should not require cultivation of micro-organisms. Unlike most DNA-based methods, fatty acid methyl ester (FAME) analysis seems to meet these requirements. Tentative evidence from FAME analyses of the three principal water layers in Priest Pot, does confirm gross phenotypic differences in the microbial communities. Certain fatty acids are uniquely associated with specific depth zones, or completely absent from them; and there are striking similarities within each pair of profiles from each of the depth zones.

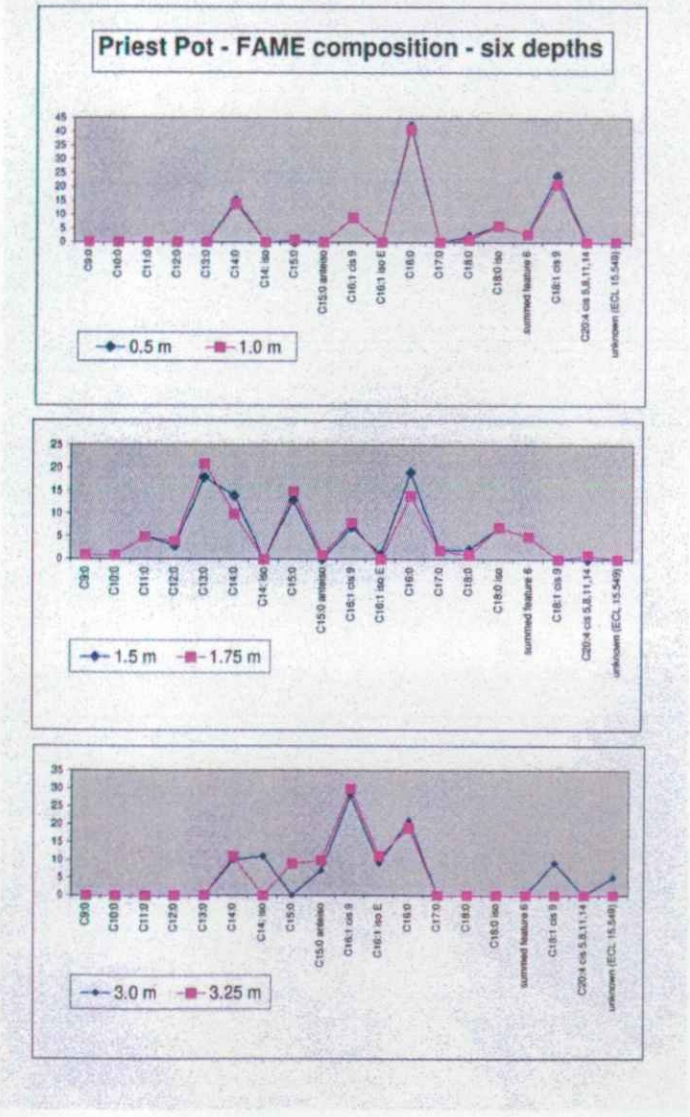


Fig. 17 FAME analyses of the whole microbial community in the water column of Priest Pot – three superimposed layers (epilimnion, metalimnion and hypolimnion; two depths from each layer)

The utility of this technique is currently being assessed (again, using samples from the stratified water column of Priest Pot) to determine if it should be developed further. At present it appears as if the technique may have value as a tool for characterising complete microbial communities in the water column – and certainly with much greater resolution than is achievable in the analysis of soil microbial communities.

In the course of this work we have come across many examples where considerably more is known about microbially-mediated processes and their quantification than about the identities and species richness of the micro-organisms responsible for these processes. For example, the flux of methane and carbon dioxide from the pond to the atmosphere, its seasonal variation, and the relative importance of diffusion and ebullition is now - from the work carried out within this project - well known (Fig. 18). We know what broad functional groups of microbes probably drive these processes, but that, at present, is the extent of our 'taxonomic' knowledge.

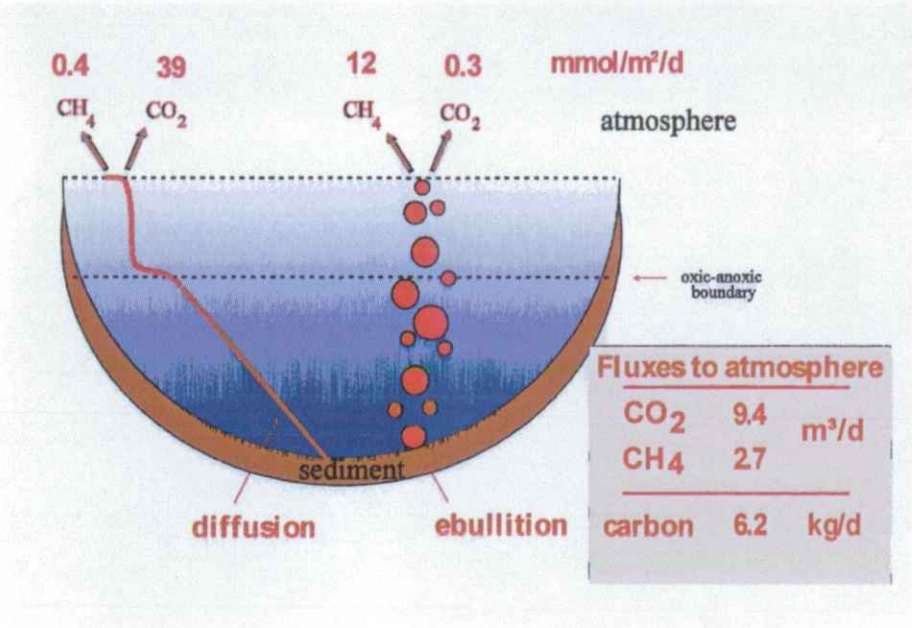


Fig. 18 Fluxes of CO_2 and CH_4 to the atmosphere, by ebullition and diffusion from Priest Pot, determined from intensive sampling and analysis over a period of one year.

Conclusions

Biodiversity at the microbial level does, apparently, have characteristics that are not shared by animals and plants:

- most species of free-living micro-organisms are probably ubiquitous
- empty microbial niches are quickly filled
- microbial diversity is probably never so impoverished that ecosystem function is impaired.

Implications

- CONSERVATION: concerns for the extinction of animal and plant species cannot be extended to biodiversity at the microbial level.
- BIOREMEDIATION: if the habitat changes, new species will soon appear to fill vacant niches.

- EXTRAPOLATION: it is not possible to make realistic extrapolations from microbial diversity, to biodiversity in general. To take a trivial example - a microbial population responds to starvation and the loss of habitat by shutting down most of its metabolic activity and waiting – perhaps for many years - for better conditions and the chance to return to population growth. But if the Bengal tiger is deprived of its habitat, immediate extinction of the species is virtually guaranteed.

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