

Biodiversity: So much more than legs and leaves

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Microorganisms inhabit virtually every possible niche on Earth, including those at the outer envelope of survival. However, the focus of most conservation authorities and ecologists is the 'legs and leaves' side of biology – the 'macrobiology' that can be seen with the naked eye. There is little apparent concern for the preservation of microbial diversity, or of unique microbial habitats. Here we show examples of the astounding microbial diversity supported by South Africa's ecosystems and argue that because microbes constitute the vast majority of our planet's species they should be considered seriously in the future protection of our genetic resources.

Introduction: The microbial world

Despite suggestions from popular science programmes, planet Earth is not owned by insects (or humans): it is a microbial world. (For the purposes of this article, we classify 'microbial' as including the bacteria and archaea (prokarya), fungi and yeasts (eukarya) and viruses.) Microorganisms inhabit virtually every possible niche on Earth, including those at the outer envelope of survival.¹ Deep sea hydrothermal vent waters at 120 °C, the ancient sub-glacial lakes of the permanently frozen Antarctic continent, fractures in geological strata kilometres below our feet, sediments far below the ocean floors and even the clouds far above us are habitats for microbial life. In fact, the only sterile environments on Earth are those where conditions exceed the stability limits of molecular structures, such as in molten volcanic lava.

The total number of microbes on the planet is certainly not known with any accuracy, but estimates suggest >1x10³¹ microbial cells.² Various commonly quoted statistics give some sense of the vastness of the microbial biosphere: there are more microbial cells in a single human body than human cells³; the deep subterranean biosphere contains more microbial biomass than the entire photosphere⁴; and if all the 2x10³¹ virus particles on the planet were laid end to end, they would extend from Earth, past the Andromeda galaxy, to the next galactic super-cluster over 100 million light years away (own calculation). Indeed, viral genes probably represent the largest single grouping of genetic material on this planet.⁵

Cellular microbial 'species' diversity is also staggeringly high. Current estimates for fungi and bacteria are around 1x10⁶ and 1x10⁷, respectively,^{6,7} but these are made more insecure by the definition of what a microbial species actually is. (The 'species' concept is not particularly well designed for the microbial world, where lateral gene transfer allows the regular interchange of genetic material across taxonomic boundaries. However, in the absence of a better system of classification, it is still used ubiquitously.) Nevertheless, the cellular microbial pan-genome, representing the total genetic complement of all these 'species', constitutes the next largest genetic resource on the planet after viruses. Indeed, the bacteria living in us⁸ contribute far more genes – 100-fold more – to our metagenome than we do!

Accessing this resource is another matter. Their tiny size and lack of obviousness is only one barrier to discovery: with the development of modern molecular phylogenetic methods in the 1970s came the realisation that most of the world's microbial, and therefore total genetic resource, had yet to be accessed.⁹ This inaccessibility is now widely known as the problem of the 'unculturables' as far as cellular organisms are concerned,¹⁰ probably more accurately termed the 'uncultured', with reference to the fact that the culturing of these species is not impossible but science has yet to devise and apply the appropriate methods. For viruses, which are absolutely dependent on a cellular host for their life cycle, the problem is compounded by our very limited knowledge of what the hosts are for the viruses we find in soil, seawater and even in the human body.

Thus, our general understanding of biodiversity is due a major overhaul. Particularly because, until recently, conservation authorities were largely concerned with the 'legs and leaves' side of biology, or the 'macrobiology' that can be seen with the naked eye.¹¹ Unlike their larger and more charismatic cousins, no one was particularly concerned with preserving microbial diversity or unique microbial habitats – yet these organisms constitute the largest part of our biosphere, both in terms of mass and in diversity, and we know next to nothing of how they may be linked in interactive and co-dependent webs. A case in point here is the Karoo fracking proposal: companies intend to inject vast quantities of water and other substances deep underground in order to fracture ancient rocks and release gas – a substance that has almost certainly been created, over millions of years, by biological processes by organisms that we are largely ignorant of. What damage might we be doing to an environment that is largely unknown to us, yet seems ripe for exploitation? What processes might we be disturbing that could take millions of years to re-establish?

To illustrate just how long biological processes can be when microbes are involved, consider the fact that bacteria which are found 30 m below the Pacific Ocean floor in sediments,¹² laid down over 86 million years ago, are living so slowly that individual cells may qualify as the oldest organisms on this planet. We have only just discovered them; we have no idea what else is there or how it lives.

The fact that the small size of microbes potentially makes long-distance dispersal by water¹³ and wind¹⁴ a major mechanism, and given their fast growth rates and their enormous population sizes, it has been assumed that microbial populations do not exhibit any biogeographical pattern, that is, that the concept of 'everything is everywhere, but the environment selects'¹⁵ may hold for microorganisms. Thus, if all microbes are geographically ubiquitous: why be concerned with any individual environment? They are unlikely to be at risk of extinction. However, this assumption does not hold anymore, as the existence of microbial endemism is now widely recognised.¹⁶ Indeed, many pathogens and plant-associated microbes have (or had until humans transported them) well-defined biogeographical distributions.

Protecting genetic resources

The issue of endemism is of great academic and practical interest and has now become a critical issue in the global focus on microbial (and other) genetic catalogues as an economically valuable resource. Following the adoption in 1992 of a global convention relating to the sovereignty of genetic resources (The Convention on Biological Diversity: www.cbd.int), a number of countries have established national legislation relating to the protection of their 'endemic' genetic resources. South Africa is one of these countries; in 2004, the government promulgated the South African *National Environmental Management: Biodiversity Act 10*, establishing a set of guidelines for the protection, management, use and exploitation of South African genetic resources, along with a legal requirement for income relating to the use of such resources to be shared equitably with the 'owners' of that resource (South Africa's Bioprospecting, Access and Benefit-Sharing Regulatory Framework; www.environment.gov.za).

The legislation was also designed to protect the country against biopiracy; the loss of the intellectual property relating to the development of widely commercialised appetite-suppressive preparations from the near-indigenous *Hoodia gordonii* undoubtedly influenced the development of this legislation.¹⁷

While the new bioprotection legislation is entirely within the spirit of the Convention on Biological Diversity, its practical implementation has raised certain anomalies and complications – exemplifying 'the law of unintended consequences'. For example, a microbiologist wishing to publish the characterisation of a new bacterial or fungal isolate is required by all international journals to first deposit a culture in one or more international repositories. However, doing so in this country would be a breach of the legislation if an export permit has not been obtained, and is theoretically subject to a substantial fine and/or jail sentence – whether or not the new isolate is in fact ubiquitous in a global sense.

Irrespective of the framing of South Africa's biosecurity legislation, the issue of the nation's genetic resources remains a critical element of future conservation and commercial exploitation considerations. This is particularly important for endangered soil habitats (e.g. those threatened

by agriculture and urban development) and soil types, where the most diverse microbial communities exist.¹⁸

Why understanding microorganisms is important

We live in a world dominated by microbes. However, microbes are important not only because of their astonishing numbers, but because of the services they provide to ecosystems/society. Here are three very different examples:

(1) In hydrothermal vent communities (Figure 1) and other reducing habitats, chemoautotrophic symbionts provide organic nutrients for animal hosts in at least seven different phyla. The activities of these individual symbioses contribute to nested communities that include non-symbiotic animal and microbial species that are able to exist through the symbiotic primary production that is not driven by solar energy but rather by sulphide, hydrogen, methane, and other reduced energy sources.^{19,20}

(2) At both ends of the biotic temperature scale, primary production and nitrogen fixation, carried out by bacterial communities that thrive beneath quartz and translucent rocks (Figure 2) are thought to be important food sources for grazing nematodes and protozoans, and therefore the basis for the survival of whole ecosystems.²¹

(3) Bacterial communities in floral nectar can influence the way animals such as pollinators interact with plants. The common bacterium *Gluconobacter* sp. changes the chemical properties of the nectar, reducing nectar consumption by pollinators, and weakening the plant-pollinator mutualism.²²

Many of the global-change drivers that affect plant and animal populations, such as rising CO₂ concentrations, global warming, and altered precipitation regimes, simultaneously influence the abundance and composition of microbial communities.²³ Equally, global climate change²⁴ and the movement of plants²⁵ and animals²⁶, including humans, could act synergistically to promote microbial invasions; which, in turn, could pose a challenge to endemic microbial communities.

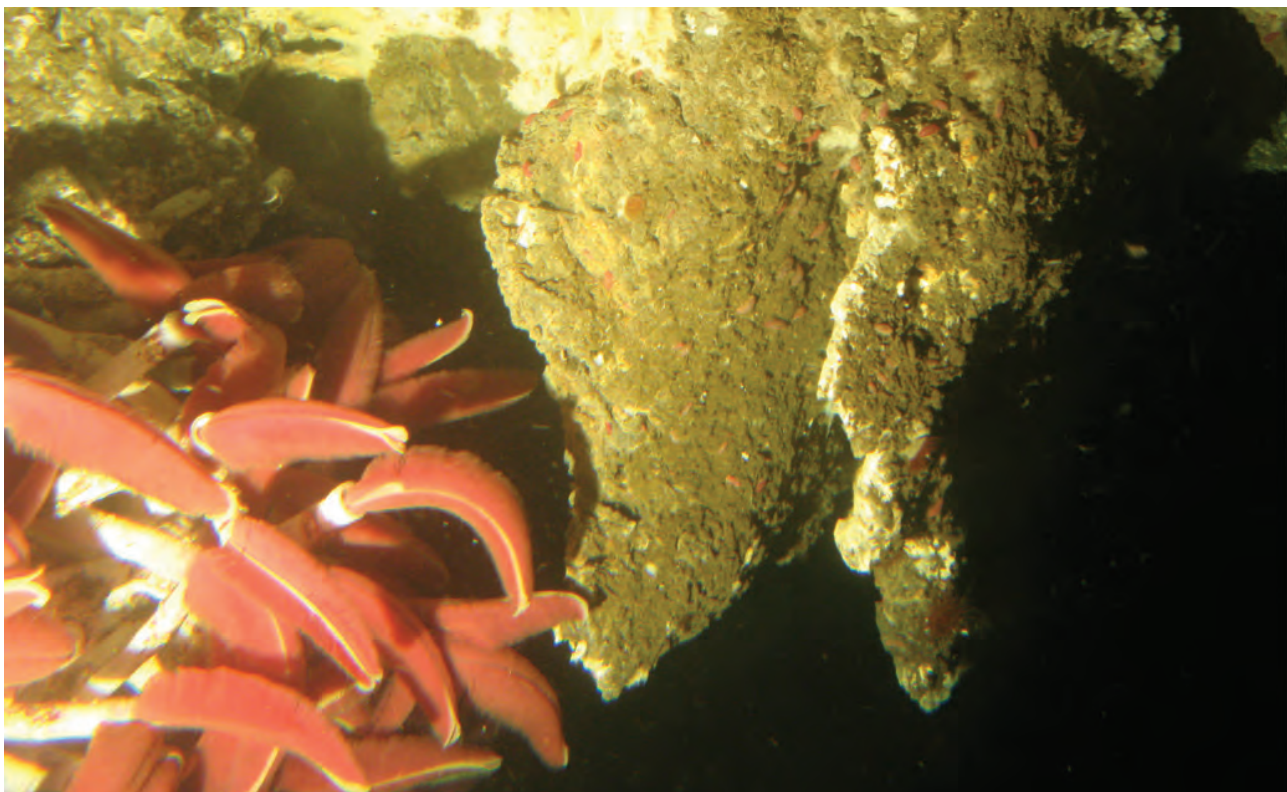


Photo: Don Cowan

Figure 1: *Riftia* tubeworms on a hydrothermal vent chimney (2010 m deep at East Pacific Rise 9°50'N), which contains populations of symbiotic chemoautotrophic microorganisms.



Photos: Don Cowan and Ed Rybicki

Figure 2: South Africa's cryptic biodiversity. Deserts, which cover a substantial proportion of South Africa's land area, are extreme environments where microorganisms inhabit specialised, often cryptic (hidden) niches. Hypolithic communities, found on the undersides of translucent rocks, represent 'hotspots' of microbial biodiversity.

There are some excellent and well-documented examples of microbial endemism.¹⁶ Cho and Tiedje²⁷ isolated fluorescent pseudomonads from 38 undisturbed pristine soil samples from 10 sites on four continents and revealed no overlap in genotypes between sites or between continents. Geographical isolation has also been demonstrated for archaea thriving in hot springs²⁸, and bacteria found in seawater²⁹ and sediments³⁰. Furthermore, recent global surveys indicate that most bacteria are restricted to broad habitat types, and there is little overlap among bacterial taxa found in soils, sediments, fresh water and seawater.^{31,32} Hence, despite the 'everything is everywhere' hypothesis, dispersal limitation and differences in environmental factors deeply affect the establishment of endemic microbes. It is, therefore, a valid assumption that South Africa, given its enormous geographical and habitat variation, harbours a high level of microbial endemism.

South Africa's (microbial) genetic resources

Marine ecosystems

Microorganisms constitute more than 90% of the living biomass in the sea, are responsible for 98% of primary production and are a major force behind the nutrient and energy cycles in the world's oceans.³³ The various oceanic zones present profoundly different physical, chemical and biological properties.³⁴ Therefore, understanding the patterns of microbial distribution is crucial in order to anticipate the responses of marine ecosystems to environmental changes.³⁴ Global ocean studies have identified remarkable large-scale patterns across different ecosystems,³³⁻³⁵ and the general consensus is that the ocean is an 'underexplored and rare biosphere'³⁵ with respect to the microbial diversity contained within it. The inescapable inference is that microbes account for the majority of the genetic and metabolic variation in the oceans, which remains under-sampled and essentially uncharted. For example, viruses are seldom taken into account in estimating rates of carbon cycling in the world's oceans – yet they are the most abundant marine organisms; it is estimated that they cause the death of 50% of oceanic bacteria every day, and they undoubtedly significantly influence the availability of soluble organic carbon in ways that are only beginning to be understood.³⁶

It is no surprise that despite an approximately 3000-km long southern African coastline, almost nothing is known regarding the microbial biodiversity in this biome (Figure 3). Previous efforts in assessing marine biodiversity have focused only on the identification and study of the flora and fauna, a significant proportion of which are endemic.³⁷ Yet it is a little recognised fact that even greater microbial endemism is associated with these macroorganisms. Southern African marine invertebrates (particularly sponges, tunicates and tube worms) have attracted much attention because of their production of potent compounds with biomedical potential.³⁸ Interestingly, it is becoming apparent that these compounds do not actually originate from the invertebrates, but are rather produced as secondary metabolites by the symbiotic microorganisms that inhabit them. However, very little is known about the diversity of sponge-associated microbes and the viruses that may regulate these communities. Only one study has reported on the bacterial community associated with a South African sponge: *Tsitsikamma favus*, from which a unique pyrroloiminoquinone was isolated.³⁹

A variety of South African derived marine sediments and samples have been recently screened for bacteria able to produce a range of activities or products for biotechnological application. These organisms include, for example, those displaying hydrocarbon utilisation⁴⁰ and bioflocculant production⁴¹⁻⁴³. Culturable fungi from marine sediments were characterised in St. Helena Bay⁴⁴: extracellular cellulases were produced by filamentous fungal isolates, indicating their probable role in detrital decay processes and therefore in the carbon cycle on the ocean bed. But, to the best of our knowledge, only one study has focused on microbial distribution patterns in South African open water, with the aim of understanding their role in ecosystem functioning.⁴⁵ The Micro B3 Ocean Sampling Day initiative (<http://www.microb3.eu/>), a sampling campaign of the world's oceans in the year 2014, could, however, be an important starting point for a better understanding of the diversity and function harboured in our oceans.

Non-pathogenic plant-associated microbes

Plant-associated microbes may provide the plant with physiologically accessible nutrients and phytohormones that improve plant growth, may suppress phytopathogens or may help plants to cope with stressors such as heat, drought and salt.⁴⁶ Symbiotic associations between rhizobia, a group of nitrogen-fixing bacteria, and legumes are one of the best



Photos: Don Cowan

Figure 3: South Africa's marine coastal zones, especially those impacted by the western Benguela Upwelling System, are particularly rich in biomass and biodiversity. Much of the eukaryotic (and most of the prokaryotic) diversity is completely unexplored.

examples of these plant–microbe interactions.⁴⁷ Nitrogen-fixing bacteria are important regulators of plant productivity because plants cannot fix atmospheric nitrogen and because nitrogen is, together with phosphorus and potassium, the main element that limits plant productivity.⁴⁸ To date very little is known about the types of rhizobia nodulating South African legumes. Although the majority of legumes form symbioses with members of the genus *Rhizobium* and its relatives (alpha-proteobacteria), some legumes, such as those of the genus *Aspalathus*, are nodulated by beta-proteobacteria of the genus *Burkholderia*.^{49–51} Although beta-rhizobia are particularly associated with mimosas and acacias, they also nodulate several agriculturally important legumes, including honeybush tea (*Cyclopia* spp.), thus raising the possibility that they could be used as biofertilisers. Their particular characteristics (e.g. tolerance to pH extremes and high salt concentrations) make them suited to specific environments, such as those in the Cape region⁴⁹ (Figure 4). The negative aspect of this mutually beneficial relationship is that it can also impact plant diversity and community composition⁵² by enhancing a species' invasive capacity.^{33,54} Many thousand hectares of unique fynbos vegetation has been colonised by imported *Acacia* sp.⁵⁵ While the success of acacia in colonising these habitats was thought to be connected with their ability to associate with indigenous nitrogen-fixing bacteria,⁵⁵ recent studies have shown that invasive acacias in South African ecosystems tend to nodulate with bacteria that do not overlap with native legumes, and sometimes even with bacteria co-introduced from Australia.⁵⁶

Another important group of plant symbionts that enhance plant productivity by supplying limiting nutrients are mycorrhizal fungi (Figure 5). Mycorrhizal fungi are widespread in nature, where they form symbiotic associations with the roots of 70–80% of all terrestrial plant species.⁵⁷ Mycorrhizal fungi can provide resistance to disease and drought, and supply nutrients to the plant in exchange for carbon. The most abundant and important groups of mycorrhiza are the arbuscular mycorrhiza, the ectomycorrhiza and the ericoid mycorrhiza. A number of studies have reported mycorrhizal fungi in South Africa.^{58,59} However,

very little is known about the community composition, although the high beta diversity of aboveground plant species is expected to influence fungal diversity. Notably, mycorrhizal fungi associate extensively with the Ericaceae and Orchidaceae families in South Africa, but not with the Proteaceae,⁵⁹ implying that other microorganisms may fill this niche. In the Cape Floristic Region co-occurring orchid species seem to use different fungal partners, consistent with the expected role of these fungi in reducing competition for nutrients.⁶⁰



Photos: Don Cowan

Figure 4: South Africa harbours a wealth of floristic diversity. The Cape Floristic Biome alone has over 6000 endemic plant species. There is growing evidence that many of these species may harbour unique rhizospheric and endophytic microorganisms.



Photos: Wilhelm de Beer, University of Pretoria (reproduced with permission).

Figure 5: South African fungal diversity is vast and largely unexplored. Mostly invisible, except when the fruiting bodies appear, fungi play critical roles in environmental processes such as lignocellulosic degradation by, for example (a) *Gleophyllum* spp.; as mycorrhizal symbionts of trees, including (b) *Amanita* spp.; (c,d) as *Termitomyces* symbionts of termites; and as saprophytes, for example (e) *Coprinus* spp., that break down dead plant tissues; and in many other processes including as causal agents in plant and animal diseases.

Non-mycorrhizal fungi also associate with plants (Figure 5). An intensive study by Marincowitz et al.⁶¹ on the saprophytic fungi of plants from the Cape Floristic Region has revealed a large number of undescribed species. Likewise, ophiostomatoid fungi were found to be highly diverse in the infructescences of *Protea* species.⁶² Next-generation sequencing applied to the study of fungal endophytes in a single *Eucalyptus* tree has shown that there are about ten times more species present than those that can be isolated.⁶³ This finding is not surprising and is similar to those of sampling of many other environments.

The bacteria colonising the rhizosphere (the area immediately surrounding the root) and the endophytic compartment (within the root) of South African plants are even less well understood. Local studies have demonstrated that microbial rhizospheric community structures differ between plant species. For example, Stafford et al.⁶⁴ investigated two endemic species of the Proteaceae family (*Leucospermum truncatum* and *Leucadendron xanthoconus*) and showed that the rhizospheric soil communities were different between plant species but more similar to each other than to non-rhizospheric soil. A study analysing the rhizospheric and endophytic microbial communities associated with South African sorghum reported that specific bacterial taxa are consistently detected in sorghum-created rhizospheric and endophytic environments, irrespective of any biogeographical factors.⁶⁵ The implication of these findings is that these organisms have significant potential in agricultural biotechnology as sorghum (and possibly other crops) improvement tools.

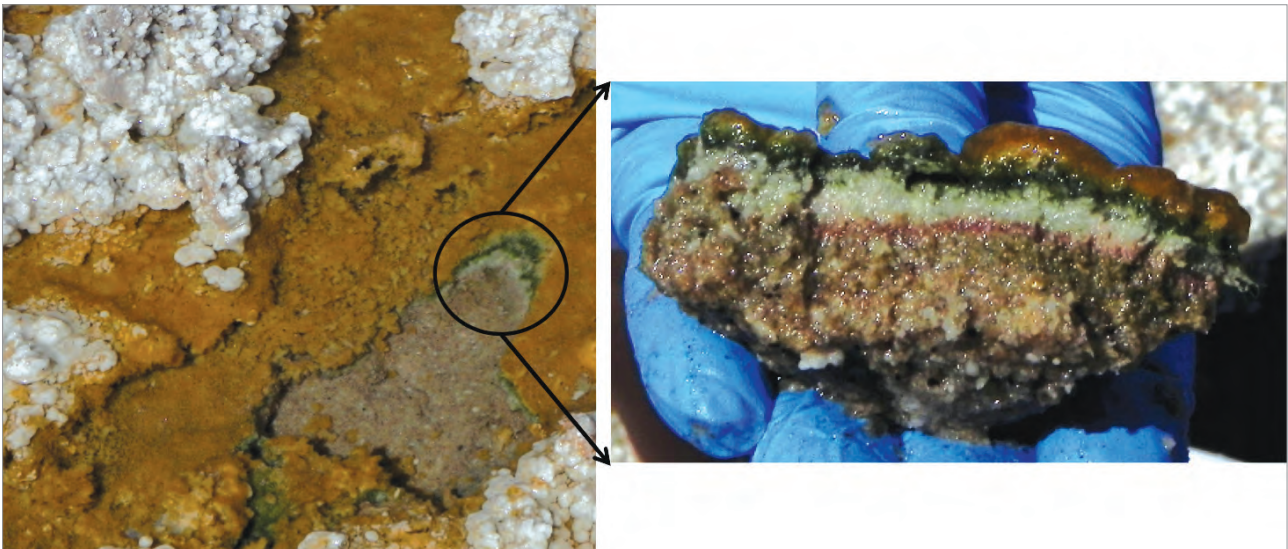
Plant virus diversity in South Africa has never been systematically investigated, although many have been found and characterised, and much damage is known to be done to crop plants in the country.⁶⁶ It is even possible that undiscovered plant viruses may be subtly influencing the distribution and success of native plant species in South Africa: recent findings in virus ecology elsewhere suggest that many viruses in natural ecosystems do no harm at all despite causing persistent infections, and may in fact be mutualistic in terms of providing clear benefit to their hosts.⁶⁷ However, no such investigations have ever been done in South Africa, despite the richness and uniqueness of its plant life.

Hypersaline habitats

Hypersaline environments are those with salt concentrations above that of seawater (3.3% total dissolved salts), and are widespread in both coastal and inland regions of South Africa (Figure 6). Microbial communities able to withstand these extreme saline conditions have the capacity to regulate the osmotic pressure to resist the denaturing effects of salt in the environment. In addition, a common phenomenon in these environments is the occurrence of gradients in salinity as a result of evaporation, and halophiles are able to rapidly adjust their osmotic equilibrium as required when the outside salinity is changed. These gradients also result in the establishment of very diverse microbial compositions, in which halophilic and halotolerant microorganisms from all four domains of life (eukarya, bacteria, archaea and viruses) can be found. The eukarya are a minority in high salt environments, mostly represented by the halotolerant green algae *Dunaliella* and only a few fungal species.⁶⁸ Salt-adapted bacteria are spread over a large number of phylogenetic groups⁶⁹ and the majority are moderate rather than extreme halophiles. The extreme hypersaline habitats are dominated by the archaea, and often display a bright red colour as a result of the large number of pigmented haloarchaea.⁷⁰ Nevertheless, very little is known about the diversity of viruses in these environments (Figure 7), although projects are underway in South Africa to start filling this gap (MI Tuffin, EP Rybicki, M Pfaff, unpublished results).

The dense microbial communities occurring in these environments often exhibit high activities of photosynthesis, dissimilatory sulphate reduction and other microbial processes, thereby exerting a profound influence on biogeochemical cycles.⁷¹ During the highly evaporative stages, the microorganisms become entrapped within the halite crystals enabling them to retain their viability for long periods.⁷² Microbial survival within salt crystals has become a popular topic, relevant to a range of disciplines including geology, biogeography, evolution and even space exploration.^{68,73} In addition, halophilic and halotolerant microorganisms have found interesting biotechnological applications as a result of the large variety of stable and unique biomolecules they are able to produce.

Salt lakes (also referred to as salt pans or vleis) are numerous and geographically widespread in South Africa, but the majority are inland, shallow and contain water only ephemerally.⁷⁴ Vleis in the southwestern Cape Province are usually temporary or permanent



Photos: Ed Rybicki

Figure 6: A microbial mat from a hypersaline stream, showing stratified biomass of microorganisms embedded in extracellular polymeric and mineralised materials. Light penetrates only a few millimetres into the mats and oxygen produced by photosynthesis rapidly diminishes with depth. Turnover of carbon fixed in the upper portion of the mats is affected by fermentation processes and sulphate reduction deeper in the mat.

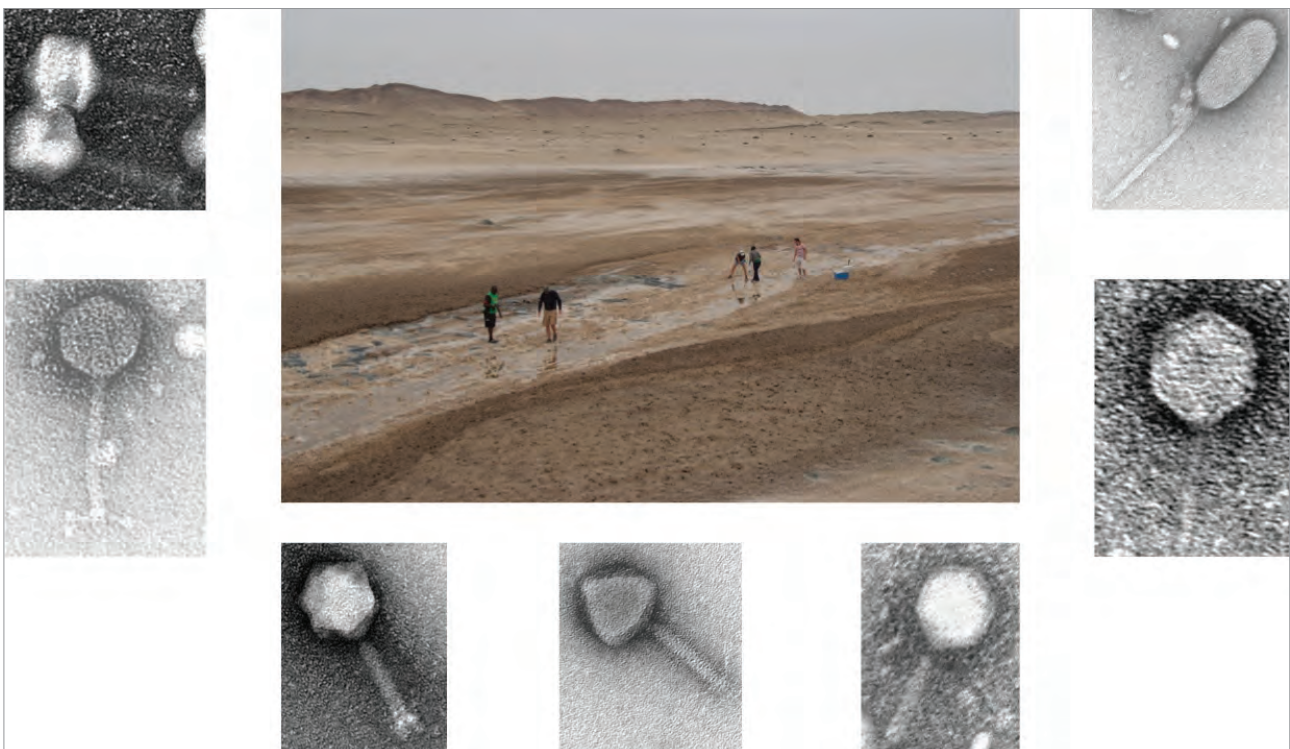


Photo: Don Cowan; micrographs: Lonnie van Zyl, University of the Western Cape (reproduced with permission).

Figure 7: South Africa's extensive desert areas, and their associated saline springs, harbour a wide variety of bacteriophages, about which almost nothing is known. Soil and saline water filtrates show large numbers of myoviruses and siphoviruses.

lakelets and/or marshes, or even estuarine lagoons. The best studied are the hypersaline pans in the Darling and Yzerfontein regions, which include coastal and inland brackish to saline and brine pans.⁷⁵ To our knowledge, only four reports have been published on the microbial diversity associated with South African salt pans.⁷⁶⁻⁷⁹ One of these studies⁷⁷ reported nanoarchaea, previously found in hyperthermophilic and mesophilic halophilic environments, demonstrating that this group

of very unusual and rare microorganisms may be much more widely distributed and more physiologically flexible than previously thought. Sulphate-reducing bacterial community structures have also been investigated.^{79,80} Spatial and temporal analyses demonstrated that similar microbial populations were generally found in individual pans but varied from one pan to another. Phylogenetic analyses showed that most of the sulphate reducers were members of the Desulfobacteraceae

and Desulfohalobiaceae families. Sulphate reducers are important for sulphur and carbon cycling, accounting for more than 50% of the organic carbon mineralisation in marine sediments. Therefore, an understanding of sulphate-reducing microbial communities is important to predict changes in ecosystem functioning.

Factors negatively affecting discovery of microbial diversity

Until recently, microbial diversity and ecology were certainly not priority research areas, in comparison with plant ecology, for example. However, we note with some gratification that the South African National Biodiversity Institute (www.sanbi.org.za) has now identified microbial diversity as a priority area for research.

The problem has been exacerbated by the relatively small size of the academic community involved in environmental microbiology research, compared with that dedicated to the study of (macro) flora and fauna – possibly because of the more charismatic nature of the latter. The relatively high cost of modern molecular technologies (deep sequencing of phylogenetic markers, metatranscriptomics, metaproteomics etc.) has also contributed to the slow growth of the microbial ecology research community.

We argue that it should not be this way: given our limited understanding of the immense contribution of microbes to everything from human digestion to soil and oceanic carbon cycling, our research priorities should surely swing to more properly understanding these contributions, how they affect the diversity of plants and animals, and, more selfishly, how they affect our species.

Opportunities: New technologies

The opportunities afforded to budding microbial ecologists by South Africa's amazing microbial diversity are literally boundless. Not only do our many terrestrial and marine microenvironments offer the promise of an almost unimaginable microbial diversity, but we now have ready access to the advanced technology needed to catalogue it. Next-generation and even third-generation nucleic acid sequencing techniques are available in the country, or via courier service to Asia, Europe or even the USA. The computer requirements for analysis are largely met within South Africa; the software is increasingly available, and often free. As with many other fields of research, the high profile and strong funding base of the biomedical community will drive much of the transition from culture-based and immunological identification of microbes to metagenomic identification – and will make it easier for everyone else to join in.

The rewards will be immediate, in the sense of information on medical risks, and short term, with regard to information on environmental hazards and unsuspected role-players in carbon trafficking and other energy flows, to long term in the sense of understanding how global oceanic and soil carbon flows actually work. We will, for the first time, understand how *all* the players in an ecosphere actually interrelate – from the nanoscale (viruses) to the macroscale (plants and animals).

It is time that we engaged across the entire spectrum of biodiversity and time to consider how everything meshes together to govern all the processes that we consider important. And it is time to acknowledge the presence of and to study organisms beyond those that generate tourist revenue.

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