Forgotten Kingdoms
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Proceedings of a seminar on the conservation of small and hidden species at the occasion of the retirement of Wim Bergmans as scientific director of IUCN NL

Editors

Wim Bergmans
Hans de Iongh
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Farewell to Wim Bergmans

Doeke Eisma & Willem Ferwerda

The symposium ‘Forgotten Kingdoms’ was organized in honour of the retiring scientific director of IUCN National Committee of the Netherlands (IUCN NL), Dr Wim Bergmans.

Wim Bergmans has dedicated himself to national and international nature conservation for many decades. With a background in zoology and taxonomy (University of Amsterdam), he has been an active and professional advocate of international nature conservation since the 1960s, always stressing the intrinsic value of nature.

Over the years, IUCN NL has evolved into a professional organization, offering a platform to Dutch IUCN members and Dutch IUCN Commission members and developing and executing many projects, the largest of which is the managing of a large ecosystem based grants programme funded by the Dutch Ministry of Foreign Affairs, and occupying a specific niche within the Dutch international conservation movement. Wim Bergmans has greatly contributed to the development and outreach of IUCN NL. Apart from his focus on nature conservation, in which he has always emphasized, among other things, the importance of the unseen and often unknown forms of life that
play a crucial role in the very existence of ecosystems, Wim Bergmans has always stressed the importance of linking biodiversity conservation with the broader development agenda and to use the Biodiversity Convention as a basis for scientific and applied collaboration with developing countries. Nowadays, part of the Dutch development aid is spent on the protection of important ecosystems, which is a step towards the realization of this concept.

His first project for IUCN NL, in 1983, was the establishment of the magazine ‘Environment and Development’, which was the precursor of ‘Ecology and Development’. From 1984 to 2000, Wim Bergmans was the successor of Irene Dankelman as secretary of the Netherlands Committee for IUCN and he became its scientific director in 2000. For many years, he was the organisation’s biodiversity expert and conscience.

In addition to his work for IUCN NL, Wim was an active member of the Bat Specialist Group of IUCN’s Species Survival Commission and he was a board member and later president of IUCN member the Van Tienhoven Foundation. As a member of the Dutch Scientific Authority he was actively involved in the implementation of CITES in the Netherlands. He maintained an active relation with science through his position at the University of Amsterdam. In 1998, he obtained his PhD on the subject of “Taxonomy and biogeography of African fruit bats”.

IUCN NL is very grateful for Wim’s many years of dedication and commitment to the organisation. Because of his special contributions to nature protection in the Netherlands and abroad, Wim Bergmans was awarded a knighthood, which was presented to him at the symposium.

Doeke Eisma, Chairman of IUCN NL
Willem Ferwerda, Director of IUCN NL
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Introduction

Hans de Iongh and Henk Simons

On the occasion of the retirement and farewell of its scientific director Wim Bergmans, the IUCN National Committee of the Netherlands jointly with the Institute of Environmental Sciences Leiden (CML) organised a seminar on 13 December 2006 on ‘Forgotten Kingdoms’: tiny organisms or cryptobiota, mostly invisible to the human eye and often neglected by nature conservationists, even more so by policy makers. This seminar was sponsored by the Van Tienhoeven Foundation. The objective of the seminar was to highlight the crucial role of the Forgotten Kingdoms for maintaining life on earth, and to identify strategies for conservation, including approaches for improving the status of knowledge.

The change in status of threatened plant and animal species is an important indicator for biodiversity. It is one of the core indicators selected by the Convention on Biological Diversity (CBD) to measure progress towards the 2010 target (= to significantly reduce the loss of biodiversity by 2010). At the species level, international nature conservation before 1970 has particularly been devoted to ‘higher’ animal species or vertebrates. The first IUCN Red Data Books, published in the sixties and the authoritative reference for species information since then, listed threatened species of mammals, birds, reptiles and amphibians. The available information on fish species was still too scattered and poor to allow inclusion in this first Red Data Book series. Conspicuously absent were the invertebrates, often referred to as ‘lower’ animal species, with a diversity many times higher than the diversity of vertebrates. Surprisingly, plants were lacking as well in the first Red Data Books. The first Red Data Book on invertebrates was not published until the eighties. The status of information was still poor, and a comprehensive overview of distribution and conservation status was available for just a few species of invertebrates. For instance, it was known that certain species of butterflies and shells were under increasing pressure from collectors, both amateurs and professionals. Other invertebrate species suffered from pollution, habitat conversion or introduction of invasive species. The Red Data Book presented a few of such examples to illustrate that not only
large, well-known and appealing animals (such as tiger and rhino) are endangered, but also less conspicuous species. It also emphasized that nature conservation is not only about protecting individual species, but needs to encompass all biodiversity.

By 2007, much progress has been made: specialist groups of IUCN’s Species Survival Commissions have been established, focusing on invertebrates and on plants and providing specific advice and recommendations on the conservation of those species. No earlier than 1998 were threatened plants and trees added to what is now known as the IUCN Red List of threatened species. In principle all threatened species can be included, and the Red List is now also available in digital format which facilitates the ongoing update of information.

With this increasing knowledge, particularly on higher plants and animals, the question is whether our knowledge on the whole spectrum of ‘Life on Earth’ is becoming complete. The answer is negative. In addition to the visible invertebrates, including the butterflies and shells mentioned earlier, and larger plant species (trees, flowering plants, ferns), a largely undiscovered universe of tiny organisms exists, which still remains beyond the scope of international nature conservation. The reasons for this neglect are not of a scientific nature. The evolution of nature conservation is a stepwise process, with an expanding interest for and knowledge of ‘new’ species groups: starting from mammals to fish, higher plants and invertebrates. Recent additions include groups of lower plants, i.e. fungi, mosses and lichens. Some of these species groups, e.g. lichens, are excellent indicators of air and water quality.

In terms of our current knowledge and interest, the hidden and forgotten Kingdoms of lower plant and animal species and micro-organisms – also referred to as cryptobiota – occupy the lowest end of the hierarchy. They are dominated by the Kingdoms of Prokaryotae (or Monera), Protoctista and Fungi, with the addition of tiny plant and animal species. These species are largely absent from Red Lists, with the exception of the larger Fungi. The latter are characterized by visible, aboveground parts i.e. mushrooms, which draw attention. However, the term Forgotten Kingdoms captures well the status of these
organisms in international conservation. At the same time, crypto­
biota strongly dominate the planet's biodiversity, both in numbers
and biomass.

Forgotten, unknown, and often invisible to the human eye, the cryp­
tobiota play a crucial role in the functioning of ecosystems, for in­
stance in controlling geochemical cycles, decomposition of organic
material, and pollination. And although some species are considered
as harmful from an anthropogenic perspective, as they cause diseas­
es to crops, cattle or humans, their ecological and economic benefits
outweigh these 'negative' aspects by far.

Concluding, the Forgotten Kingdoms occupy a central position in main­
taining the existence of life on earth, which justifies more explicit and
systematic attention for these organisms in both taxonomy and na­
ture conservation.

These proceedings are the outcome of the seminar on the conserva­
tion of ‘Forgotten Kingdoms’. They contain the papers that were pre­
sented during the seminar and a summary of the final discussion. The IUCN National Committee of the Netherlands will share these proceedings with a broad range of institutions, including the Dutch Government, nature conservation organisations in the IUCN com­
mi­nity, and knowledge institutions.
Forgotten, neglected, or unknown, maybe forever: variations on the theme ‘Forgotten Kingdoms’

Edi Gittenberger

The subdivision of the living world into the Kingdoms of plants, animals, fungi, and small creatures, is still widespread in daily life. In modern biosystematics, however, three domains are considered the highest taxa in the hierarchy of life, viz. Bacteria, Archaea and Eukarya. Only among the Eukarya there are truly multicellular creatures and not just micro-organisms. Among the many twigs of the tree of life, animals and plants are Kingdoms next to dozens of other taxa of a comparable rank, all of which represent groups of unicellular micro-organisms. Biodiversity is by far the largest among the smallest organisms, the inconspicuous ones, most of which are unknown and will remain so for a long time, in particular if they are not in any way related to human well-being.

The distinguishing, describing and naming of species, is among the earliest scientific activities on earth. It is told that Noah already had to bother himself with it. A unifying nomenclature was only introduced in the 18th century by the well-known Swedish scientist Linnaeus (1707-1778). For animals the tenth edition of his Systema Naturae, published 1758, is fixed as the starting point of binary nomenclature which is still generally used without any serious alternative. Initially, the classification of the various species used to be rather artificial. The expertise of the specialist, his/her subjective preferences and authority used to be of major importance in systematic practice. An important step forward was achieved by Willi Hennig (1913-1976), who brought the real data and their objective processing back to the frontline of taxonomic research. The system that should be aimed at, in not exclusively his view, should mirror the course of evolution as well as possible. Hennig provided the innovative, basic methodology, to approach that goal more successfully than before. Phylogeny reconstruction became falsifiable and the Tree of Life could be unraveled in more detail than previously and in a scientifically sound way. Later on, when molecular methods became available, in particular DNA sequencing, the amount of factual data that can be used increased immensely. Shortly afterwards, when ancient DNA turned out to be hidden in old museum collections, this opened an
additional, broad array of potentially new lines of research to unravel the history of life on earth in an even better way. In biogeography, phylogeography evolved as a new subdiscipline to reconstruct the history of populations in much detail. The idea of bar-coding species came up: molecular markers should enable their easy, objective identification. Finally, the internet became increasingly important as a source for data mining, and the start of web-based taxonomy was proclaimed. Despite this impressive increase in potential, biosystematics cannot be considered a flourishing, biological subdiscipline. Nearly globally this once dominating part of biology has been removed from academic training programmes. Funding became difficult since mainly innovative methods and not their application are funded. All this made the museums of natural history more important than ever, not only because of their illustrative exhibitions for the general public, and as archives, but especially also for biodiversity research and centres of anti-reductionism in evolutionary biology. At the universities, systematics and phylogenetics have been neglected long enough to be forgotten or nearly so.

There are hardly groups of organisms that were known well once and are completely forgotten now, but many taxa are known to only a very small and decreasing number of specialists. The majority of invertebrate species is still unknown instead of forgotten and there are not enough competent systematists available or in training to change this situation in the foreseeable future. Only in 1995 the phylum Cycliophora was discovered, not in a remote part of the world, but nearby in Scandinavia. Mainly because of their small size, these animals were overlooked for a long time. Dozens of new species of micromolluscs were discovered when litter from some limestone hills in Borneo was analysed. Obviously, there will be many more to discover in similar habitats elsewhere in tropical countries. Not only the smaller, relatively inconspicuous, invertebrates are poorly known in many parts of the world. Recently, DNA sequencing brought to light that there are many more species of relatively large parasitic snails, hosted by stone corals, than could be identified as separate entities morphologically. Similar cryptic radiations may be found in other groups of parasites. Sibling species resulting from convergent or parallel evolution are probably more common than hitherto thought, which implies that much is still unknown here.

In most groups of arthropods and in many other groups of invertebrates, the percentage of the unknown is certainly much higher still than among more ‘popular’ invertebrate groups, like molluscs with at-
tractive shells or marine slugs with beautiful colours. On the one hand, so-called amateurs have been, and still are, extremely important in providing specimens for study and by their substantial contributions to the literature. On the other hand, their preferences and those of the few remaining professionals, are largely responsible for our actual, biased, incomplete picture of biodiversity on earth. The places of residence of the host of volunteers became increasingly less important since travelling became easier and less expensive, but a large and growing number of local regulations hamper collecting for scientific purposes in many countries. Systematists doing field work without causing any environmental damage, are now in danger of acting illegally. As a consequence, sampling is reduced, which will have irreversible consequences. What is not collected now, may become extinct by ongoing deterioration of natural habitats and is thus inaccessible for study in the future, remaining unknown forever. Therefore, selective collecting, which sounds reasonable in view of the number of available systematists, should be considered a minimum strategy of some institutes aiming at biodiversity research and housing collections. Not only a lack of specialists but especially lack of space is the most frequently heard excuse for this short-sightedness. The species groups that have been traditionally or periodically neglected will be most seriously affected by this approach. Species of the category ‘endemic, small and inconspicuous’ are going extinct continuously. With every single, isolated limestone hill that is dug away for cement, all its endemic species become extinct. Therefore we may assume that many of those will never be known anymore, since representative specimens, or bottom samples in which they might be found, are kept waiting nowhere in a ‘selective’ research collection. As a consequence, every reconstruction of the Tree of Life will be and will inevitably remain incomplete. The most complex ecosystems will never be known completely, despite the availability of modern molecular methods offering various opportunities to do more than only a morphological analysis (e.g., analysing DNA of stomach contents). Interactions among unknown species cannot be studied. This is not a matter of being forgotten. It is a matter of neglect, resulting in ‘unknown forever’.

In comparison with the systematists, so-called modern biologists, usually without any training in systematics, may have a more distorted, simplified picture of the results of the evolutionary process. For them Drosophila spec., C. elegans, the zebra fish and a few more model organisms may represent life and evolution. To students specializing in what is cynically called Life Sciences in The Netherlands, sponges, sea squirts, etc., may be unknown creatures. The students should not
be blamed for that, however. The fact that biodiversity is to some extent irreducibly diverse is more generally neglected, forgotten or unknown.
Microbial diversity underground: do we need to protect the Earth’s greatest source of biodiversity?

George A. Kowalchuk

The wealth of soil-borne microbial diversity has generally been unappreciated and left largely untapped. The recent application of molecular and genomics approaches in microbial ecology is providing access to this reservoir of diversity, providing the opportunity to better understand how Earth’s ecosystems work and how microbial activities might better be exploited. Most significantly, microbial ecology is moving forward from just describing microbial diversity to trying to elucidate what it actually does. In plant-soil systems, methods that attempt to link microbial identity with function are providing insight into the many roles that microbes play, including the recycling of nutrients, buffering against perturbations such as climate and land use change, and controlling plant pathogens. The commentary provided here seeks to highlight the advances being made in our understanding of, and attempts to, unlock the riches of soil-borne microbial Kingdoms.

The Earth’s biodiversity is mostly microbial

Soil-borne microorganisms are the greatest source of biological diversity on the planet. Indeed, as we may pursue far-reaching plans to delve into space for the discovery of new life forms, we have yet to uncover the vast majority of the microbial diversity under our own feet. Not only are microbes the oldest and most diverse life forms on this planet (Whitman et al. 1998), they also control the majority of the Earth’s geochemical cycles, thereby occupying a central position in maintaining the existence of life on this planet.

The number and diversity of microorganisms on this planet are truly enormous. The number of living microbes on Earth is estimated to be in the neighborhood of $10^{31}$ cells, a number beyond comprehension. Microorganisms are obviously small as individuals, but as a whole, they weigh as much as all people and livestock on Earth combined, and are tremendous storehouses of carbon, nitrogen, phosphorus and other basic elements of Earth’s biology. Although only less than 10,000 bacterial species are properly described, as compared
for instance to approximately one million characterized insect species, estimates of the total number of bacterial species on Earth vary from several million to hundreds of billions (Curtis et al. 2002; Gans et al. 2005). Of course, this also has to do with the definition of a bacterial species, which represents a topic of too much debate to be addressed here.

This tremendous microbial diversity does not simply consist of a multitude of slight variations to a common theme, but covers the full range of lifestyles possible on Earth, making this truly the planet of the microorganisms. Microbial life not only takes advantage of Earth as an attractive place to live and evolve, but is responsible for shaping the planet. Life as we know it does not exist because the Earth is a habitable place. Rather, the Earth, as a suitable habitat, exists because it has been shaped by life to be this way. The environmental range within which eukaryotic organisms, especially higher eukaryotes, can function is rather narrow. Indeed, the limits of life for prokaryotes extend far beyond conditions that are generally considered conducive to life with respect to temperature, pH, redox potential, pressure, etc. But certainly even more impressive is the diversity of chemistry that can be utilized by prokaryotes. As opposed to eukaryotes, which can only breath oxygen to produce carbon dioxide, prokaryotes have evolved to utilize every conceivable electron donor/acceptor combination that is capable of yielding sufficient energy. The versatility of prokaryotes is equally as impressive when it comes to what they can eat. Whereas we may be limited to eating a narrow range of organic compounds, prokaryotic life has evolved to use a wide range of organic and inorganic substrates for growth.

The dominance of microbial diversity in the Earth’s total genetic pool has become clear only relatively recently. Only a quarter of a century ago, the tree of life was organized subjectively into five or six Kingdoms, in which the lowly microbes resided at the bottom. One of the most revolutionary concepts introduced to biology in the past half century is the use of DNA sequences, especially those that encode ribosomal RNA, to infer the evolutionary relationships between organisms. Recognizing the power of such molecular markers, Carl Woese revolutionized our perspective of life, proposing a far more objective tree based upon small subunit ribosomal RNA gene sequences (Figure 2.1; Woese et al. 1990). This tree recognizes three so-called domains of life, and is clearly dominated by microbial diversity. The prokaryotes constitute two of these domains, now recognized as the Bacteria and the Archaea. All macroscopic life forms are found within the third domain,
the Eucarya, to which plants and animals belong. However, the eukaryotic domain is also mostly comprised of microorganisms. Thus, also from the perspective of molecular diversity, this planet is clearly dominated by microbial life forms.

Figure 2.1 Phylogenetic tree of life based upon 16S rRNA gene sequences from selected organisms. The red shape highlights microbial organisms (Woese et al. 1990).

**Gaining access to the microbial majority**

So, if microorganisms are so very abundant and important, why is it that we know relatively little about the diversity and ecology of microorganisms compared to plants and animals? This knowledge gap with respect to microbial ecology stems mostly from the inherent difficulties in studying these organisms. In addition to their small size, most microorganisms lack distinguishing characters, making them impossible to identify based upon morphology alone. Furthermore, the majority of microorganisms are recalcitrant to known culture methods, resulting in what has been coined ‘the great plate count anomaly’ (Table 2.1; Amann et al. 1995; Staley & Konopka 1985). This refers to the fact that many more viable cells can be counted in a typical environmental sample than can be cultured using defined media.
in the laboratory. Despite the great successes of novel culturing methods in recent years (Zengler et al. 2002; Stevenson et al. 2004), the fact remains that in some environments, such as soils, only a very small fraction of the total microorganisms can be cultured by conventional methods.

Table 2.1  The percentage of cells that can be cultivated from various habitats using standard solid culture media (Amann et al. 1995; Staley and Konopka 1985).

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Culturability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>0.001 - 0.1</td>
</tr>
<tr>
<td>Freshwater</td>
<td>0.25</td>
</tr>
<tr>
<td>Mesotrophic lake</td>
<td>0.1 - 1</td>
</tr>
<tr>
<td>Unpolluted estuarine waters</td>
<td>0.1 - 3</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>1 - 15</td>
</tr>
<tr>
<td>Sediments</td>
<td>0.25</td>
</tr>
<tr>
<td>Soil</td>
<td>0.3</td>
</tr>
</tbody>
</table>

However, the use of molecular methods in the past two decades has opened up a tremendous window into the understanding of microbial diversity in the environment. For instance, the number of recognized bacterial phyla has quadrupled to over fifty in the last twenty years. Among these newly discovered phyla, many are only known from DNA sequence information and have never been successfully cultured in the laboratory. Even with the advance of molecular methods, we are still not able to describe the incredible diversity contained in soil environments adequately. Indeed, it has been estimated that a single gram of soil may contain tens of thousands of bacterial species with perhaps millions of species represented in a single field (Torsvik et al. 1990; Gans et al. 2005).

Is there a need to preserve microbial diversity?

From a conservationist’s point of view, it is perhaps difficult to conserve the unknown, and the vast majority of microbial diversity is clearly still not known. But before considering the point of conservation, one must first step back and decide whether microbial diversity really matters, and whether loss of microbial diversity may be of importance. There are essentially three arguments to the importance of microbial diversity: 1) microbial diversity is responsible for a vast
range of local and global ecosystem services, 2) microbial genomes encode a great wealth of useful products and activities, and 3) studies of microbial diversity may provide valuable insight into general ecological and evolutionary principles.

To the first of these points, although it has been demonstrated that microbial activities drive a wide range of essential ecosystem processes, it is far from clear that a relationship exists between the diversity of the microbial communities involved and the ecosystem processes performed (Finlay et al. 1997). Similar to the debate surrounding macro-ecological diversity/function relationships (Cardinale et al. 2006; Schwartz et al. 2000; Hooper et al. 2005), it is generally agreed that although there may be some positive effects of microbial diversity on ecosystem function, microbial functioning is primarily a function of microbial community composition as opposed to diversity (Finlay et al. 1997; Cook et al. 2006). It has been hypothesized that, even for relatively specialized soil-borne microbial functions, the functional redundancy present in the vast diversity of the soil-borne microorganisms makes soil ecosystems functioning largely insensitive to biodiversity loss (Wertz et al. 2006).

With respect to useful products and activities, soil-borne microbes represent a seemingly boundless potential resource. Soil-borne microbes are already the single largest source of biological products. Given this recognized wealth of natural products, and the fact that the vast majority of microbial genomes have yet to be explored, it follows that microbial communities in soil contain a great untapped economic potential. Indeed, microbial collective genomes, or meta-genomes (see below) are already being studied for commercially valuable molecules with agricultural, chemical, industrial, and pharmaceutical applications, and soil environments are the habitats of choice for such meta-genome exploration (Daniel 2005).

To the issue of the scientific value of microbial diversity, it is clear that progress in understanding microbial functioning is not only providing invaluable knowledge relevant to human cellular functioning, but also yielding unique insights into the evolution and origin of life on this planet. Indeed, the majority of the Earth's living history has only known microbial life forms, and understanding of the Earth’s evolution is inextricably linked to microbial ecology and evolution.

There is great concern that the Earth is losing biodiversity, mainly due to man's increasing demand on the planet's ecosystems. This alarming
trend is obvious for certain well-known and appreciated animals and plants. With regard to microbial diversity, two fundamental questions arise: 1) Is microbial diversity being compromised by human activities? and 2) Does it matter if microbial diversity is lost? Unfortunately, to a large extent, we do not yet know the answers to these questions.

That microbial diversity is lost with the simplification of ecosystems is well established, but whether this actually has an impact on the total diversity of the world’s microorganisms is not known. Also, similar to the case for plants and animals, it is difficult to prove that loss of biodiversity per se, and not specific species, has an impact on ecosystem functioning. Whatever the answers to these questions, it remains the case that any habitat that supports viable chemistry for life, will be exploited by microbes. Destruction of habitats, whether they be internal environments of extinguished animal and plant species or exceptional and/or isolated natural settings, may lead to a loss of unique microbial traits. Thus, at present, it appears to make little sense to speak of preservation of individual microbial species. Rather, conservation of microbial diversity requires a habitat level approach, to sustain the genetic variation and wealth of functions that has evolved over billions of years. The flipside of human impact on microbial communities is the potential for adaptation to numerous novel anthropogenic pressures. As stated above, microorganisms have been remarkably successful in exploiting Earth’s diverse and sometimes seemingly impossible niches, and microbial communities continue to evolve and adapt in response to modern human-imposed challenges. Thus, while man may be reducing microbial diversity due to ‘habitat’ degradation, he may also be creating it by facilitating evolution to rapidly changing environments.

**Microbial communities as ‘meta’ organisms**

Advances in DNA sequencing technologies (Shendure et al. 2004), as stimulated by the human genome project, have opened the door to elucidation of hundreds of microbial genomes in the past decade, and this number is increasing exponentially (see www.genomesonline.org/references.htm; Liolios et al. 2006). This genomic revolution is providing unprecedented insight into microbial processes, signaling and evolution. However, such efforts have necessarily focused on cultivated organisms, especially those relevant to medical, agricultural and biotechnology industries. Thus, since the majority of microbial populations in nature still elude cultivation, genome sequencing ef-
forts provide only limited insight into the natural genetic diversity contained within microbial communities.

Circumventing this limitation, microbial community sequencing efforts, or so-called metagenomics approaches, have recently emerged (Rondon et al. 2000; Beja et al. 2000; Handelsman 2004). In such approaches, the entire community is taken as a complex meta-organism with a complex composite genome. With enough sequencing and computational effort, it should theoretically be possible to reconstruct all the genomes within a community from the collective DNA sequence information. This strategy has been elegantly demonstrated by Banfield and coworkers in the elucidation of the organisms composing a biofilm community from an acid mine drainage site (Tyson et al. 2004). Now, just as with isolated organisms, this community can be interrogated using a number of post-genomics methods to describe how the various parts of the system interact and how they collectively respond to change (Allen & Banfield 2005). However, as the complexity of the community increases, the prospect of community sequencing becomes more daunting, even when vast amounts of DNA sequencing power are brought to bear. In a landmark metagenomics study of the Sargasso Sea, Venter and colleagues (Venter et al. 2004) used a massive sequencing effort, which is now expanding around the globe (www.sorcerer2expedition.org/version1/HTML/main.htm) to gain insight into the diversity and metabolism of microbial communities of the open ocean. In doing so, many partial genomes were recovered, as well as countless novel genes, but it was obvious that such an approach did not come close to describing the full meta-genome of the samples examined. Extending this approach to soil, Tringe et al. (2005) found no sequenced fragments of DNA that could be assembled together, because of the extreme diversity of the sample. Many novel genes were found, but it was obvious that even a Herculean sequencing effort was not going to come anywhere close to providing a complete soil community genome.

Although it is theoretically possible to infer the activities and interactions of a microbial community when the genomes of all the players are known, this becomes increasingly difficult as the proportion of the community that is known diminishes. Although such blind sequencing approaches are expanding our knowledge of the globe’s genetic diversity, it is clear that more directed approaches are necessary if one wishes to focus on those populations that are particularly relevant to ecosystem functioning, such as the dominant organisms interacting with plants in the rhizosphere. With this goal in mind, meta-genome approaches with large inserts (Rondon et al. 2000; Beja
et al. 2000) and high throughput screening methods are offering a means to access genomic information more efficiently (Figure 2.2). With efficient interrogation of meta-genomes in mind, microbial ecologists must be clever in their pursuit of ecologically relevant, valuable and niche-defining genomic information, within the vast wilderness of microbial diversity. Keys to the success of such strategies will be the ability to extract and recover large genomic fragments from environmental samples and the efficiency of high throughput screening of these fragments to fish out the targets of interest (Kowalchuk et al. 2007).

![Figure 2.2 Schematic depiction of the steps in the application of large-insert metagenomic strategies.](image)

**Microbial diversity: need to study at a microbial scale**

Much research effort within the study of biodiversity has been devoted to discerning mechanisms that facilitate the development and maintenance of biodiversity. Given that soil-borne microbes are the greatest source of biodiversity on the planet, it seems fitting that we ask, ‘Why are there so many microbial species in soil?’ A number of arguments have been put forth to help explain the incredible diversity of Earth’s microorganisms, but they still seem to fall short of a complete explanation. These include the fact that soil habitats are highly heterogeneous, thereby offering many niches for different microbes to exploit. Further hypotheses have centered around the large popula-
tion sizes and broad distributions of microbes and their rapid rates of mutation, whereby species are extinguished at relatively low rates, yet created at relatively high rates. Microbial species richness may also be a product of selective and variable predation pressures from other bacteria, viruses, protists and soil animals, or complex patterns of substrate affinities and utilization efficiencies (Huisman & Weissing, 1999; Weinbauer & Rassoulzadegan, 2004).

The above hypotheses are principally based upon established ecological theory, derived from study of macroscopic communities. Although they certainly will often hold true for microbial communities, and predict high levels of microbial diversity, they generally fail to account for the most marked property of microorganisms, namely their very small size. Environmental landscapes and selective pressures at scales relevant to microbial populations are very different from those generally perceived. Soil-borne microbes typically reside in micro-colonies of var-
ious sizes, separated by a considerable distance from nearby neighbour micro-colonies (Figure 2.3). So on a scale relevant to microbes, life in soil may be highly analogous to one enormous archipelago, making principles of island biogeography highly relevant. Analogous to the role of island biogeography in explaining high levels of plant and animal diversity, microbial diversity may be shaped by similar forces, when examined at the appropriate spatial scale (Figure 2.3).

Fortunately, we are now in a position to test this hypothesis, as precision sampling technologies and molecular biological methods are now becoming available to examine microbial communities at microbiologically-relevant scales. Reverting back the discussion of optimizing the efficiency of metagenomic studies, the precise targeting of specific microbial aggregations (i.e. a single island in Figure 2.3), coupled with DNA amplification methods, will now allow us to study mixtures of microbial genomes that actually interact with each other, thereby providing direct data on microbial activities and interactions. Taking this a step further, whole genome amplification of individual cells, specifically plucked from micro-environments of interest, should allow for the recovery of genome sequences without cultivation, essentially describing the meta-genome of one species at a time (Raghunathan et al. 2005; Zhang et al. 2006).

**Microbial diversity and the environment**

As stated above, microbial activities have global feedback consequences, and the profound climatic changes being induced by human activities are superimposed upon existing biogeochemical cycles. Indeed, microbial communities can facilitate carbon sequestration in soils and oceans, thereby buffering against rapid increases in atmospheric CO$_2$ concentrations. On the other hand, temperature rises, especially in polar climates, may induce the release of soil-borne carbon stores, thereby exacerbating the greenhouse effect. Thus, it is of global importance to know how microbial communities and their activities will respond to changing climatic scenarios. To this end, we have directed a number of studies towards describing environmental change-induced impacts on microbial communities. Via the implementation of the growing molecular and genomics toolbox, we are beginning to gain understanding on how soil-borne microbial communities respond to changes in atmospheric CO$_2$ and temperature. Although microbial communities generally show little direct response to changes in these factors, large indirect effects are found via
interactions with vegetation and other environmental variables such as moisture and nutrient availability (Yergeau et al. 2007a & b; Drigo et al. 2007).

Similarly, a number of projects within the Dutch Ecogenomics (www.ecogenomics.nl) and Ecology of Genetically Modified Crops programs (www.nwo.nl/nwohome.nsf/pages/NWOA_6JNP94) are attempting to examine the normal operating range of soil-borne community structure and function, with the goal of using this knowledge to help devise strategies for sustainable land use. These and other efforts are taking the approach of determining which microbial populations and functions are affected by specific environmental changes. Such studies show the promise not only of deepening our understanding of microbial diversity and microbial processes, but also of providing potential mitigation routes for environmental problems and human pressure on terrestrial ecosystems.

References


Conservation of the cryptobiota

Willem N. Ellis

Introduction

We are used to divide the living world, the biota, into fauna and flora. This bisection, that probably dates back to the time of Aristotle, is totally oblivious of modern insights that recognize not two, but four or five, in some views even more, Kingdoms. Nevertheless, this bisection still is both commonplace and practical, and in this paper I will do like any layman or politician, and treat all Kingdoms other than animals as plants, i.e., flora.

A little more detail, also both antiquated and popular, divides the fauna into vertebrates and invertebrates, and the flora into flowering plants (or, more precisely, vascular plants) and cryptogams (including, as just noted, Fungi, Bacteria etc.). I will use these crude distinctions to make a different, and rather less traditional grouping. I take the vertebrates and vascular plants together into what may be called the macrobiota (the larger-sized biota), and invertebrates and cryptogams into the cryptobiota (the hidden biota). Needless to say, these ‘groups’ are ecological, certainly not phylogenetical units.¹

By far the most attention of conservationists has always been devoted to the macrobiota (e.g. Collins & Thomas 1991; Jiménez-Peydró & Marcos-García 1995). There is a growing awareness that also the cryptobiota is in need of active conservation. The aim of this paper is to argue that the cryptobiota requires a conservational strategy of its own, and that the methodologies applied to the macrobiota cannot simply be expanded to the cryptobiota, some even being counterproductive.

Body size

In order to be a useful concept, the cryptobiota, as defined above, must be more than a loose aggregate of neglected species. In other

¹ Both the words macrobiota and cryptobiota have received a different meaning in some circles. Neither has, in my opinion, a scientific value and I feel free to use them as was done originally in Ellis & Krikken (1992).
words, is there something else that cryptobionts (the members of the cryptobiota) have in common?

Broadly speaking, we can say that cryptobionts have a small body size. Of course, some vertebrates may be quite small (like the tiny fish *Paedocypris spiniceps* that was discovered recently, just 8 mm long) as are some diminutive flowering plants (especially annual ones). On the other hand, some cryptobionts (like the bat-capturing centipede *Scolopendra gigantea*) may be in the decimetre range. Yet, taking the cryptobiota as a whole, the size difference is what most clearly distinguishes it from the macrobiota. Only in the sea the distinction seems blurred to such an extent that I explicitly will limit this paper to the terrestrial biota. The implications of this single trait – size – are quite ramified.

**No emotional appeal**

Small, tiny, and even more microscopical organisms are little known to the general public, and do not trigger emotional affection. For many persons the sense of ‘strangeness’ of invertebrate animals makes them aliens, often even threatening or repulsive. Their beauty, moreover, is little visible at best. Cryptobiota conservation for this reason cannot rely on the emotional or aesthetic appeal that forms the carriage of macrobiota conservation. Some of the largest and most colourful forms, butterflies and dragonflies foremost, are an exception (only the adults!), but, because of their large size they are also exceptional cryptobionts.

Of course, many cryptobionts are ‘useful’. Some of them are indispensable pollinators (Buchmann & Nabhan 1996), others serve as human food, or act as biocontrol agents. In more general terms, the cryptobiota delivers crucial services to the functioning of ecosystems (to reduce kitchen refuse and garden litter to compost, to give a domestic example). But it is not so easy to translate gratitude for ecosystem services into arguments for conservation. Often it has been postulated that these ecosystem services, to be performed well and reliably, require a high biodiversity. Yet, despite a number of studies this supposition has little satisfactory experimental underpinning so far (Srivastava & Vellend 2005).

Since the arguments in favour of cryptobiota conservation cannot fall back on aesthetic, emotional or utilitarian arguments, they must rely solely on ethical reasoning, and on the intellectual appreciation of the multifarious outcomes of organismal evolution. The ethical argument essentially is a very simple one: no one should destroy without a compelling reason something that he or she is not able to reconstruct. The loss of a species, however humble and inconspicuous is an irrevo-
cable loss of complexity that no amount of money of effort can undo. The intellectual aspect is reflected in the role cryptobionts play in various disciplines of comparative biology, genetics, pharmacology, ecology, etc. Each organism and species is the bearer of a unique body of information, that may be exploited practically or scientifically. Practically, in for instance the monitoring of the quality of the environment. Scientifically, in that almost everything that is conceivable in biology does exist in the cryptobiota: organisms with a tremendous genetic variation, with just one, or hundreds, of chromosomes, with all sorts of sexuality and reproductive strategies, all sorts of life cycles, all quaint biochemistries, signalling systems, symbiotic or antagonistic relationships. Many future discoveries in biology are to be expected in connection with the cryptobiota – or what has remained of it by then.

**Strongly specialized**

Small size comes with a short life. Again with exceptions (think of many lichens), the lifespan of most cryptobionts generally does not exceed one year, and often is much shorter. Having many generations per unit of time implies a relatively fast evolution, which is part of the explanation of the extremely high number of species composing the cryptobiota (Hutchinson 1959; Janzen 1977; Dykhuizen 1998; Kawecki 1998).

Many cryptobionts are strongly specialized. This can be understood partly as the result of the fast evolution just mentioned. Another facet is the fact that in many cases a cryptobiont is small relatively to its food. This means that an organism can, and often must, spend most of its life span on one single food item (figure 3.1). This is possible only when the organism is sufficiently specialized. This is interesting in itself. From the viewpoint of a conservationist, however, this strong specialisation implies that management measures cannot possibly be favourable to all cryptobiont species at the same time.

**Erratic population sizes**

Their small body size makes cryptobionts particularly vulnerable to abiotic factors, weather in particular. They have little volume, and a big surface relative to their body mass. They are easily washed away by a heavy rain, swept off by the wind, have little water reserve to resist desiccation during a spell of drought, almost no possibility of defence against cold. Strong abiotic effects, in combination with a short generation time, will lead therefore to strong fluctuations in population size, even in the absence of man-made disturbances. Therefore
one of the main tools of macrobiota conservation, population counts, can be applied only with difficulty to the conservation of cryptobiota. Measurement of success in cryptobiota conservation is no easy matter.

Monitoring is especially difficult in the many instances that an organism is virtually invisible during a large part of its life cycle. Many insect species for instance can only be witnessed and identified in the adult stage: their eggs are next to invisible, larvae and pupae are often hidden, and moreover often not so well described in the literature that identification at the species level is possible.

**Badly known**

This introduces another typical cryptobiota problem. The sheer number of species makes that scientific, taxonomic effort is spread over so many species that the knowledge about individual species mostly is quite restricted. Globally, the majority of cryptobiont species even still awaits formal description (Samway 1993). This poses not only problems with identification; our knowledge of the biology, ethology, and ecological preferences of many species is limited at best.
Big numbers

Small size also means many individuals. The total biomass of the cryptobiota of an area usually exceeds that of the vertebrate fauna (Hölldobler & Wilson 1990). Its ecological impact is enormous; for instance, think of the feat that the soil cryptobiota manages to mineralize within a year the package of dead leaves that is deposited in autumn. Many individuals per area implies that a viable cryptobiont population can exist on a surface area that is far too small to maintain a viable vertebrate or flowering plant population. It also has a very practical implication: collecting, if not done at a commercial scale, is very improbable of damaging the population to any degree.

The unknown biota

It can be stated with little exaggeration that it is impossible to draw a complete species list of the cryptobiota of a plot of land, even as small as a garden. There are too many species groups involved, each requiring a specialist of its own. Too many species can be found and/or identified only during a few weeks in the year. Too many different collecting devices and techniques have to be deployed, many of them detrimental to non-target organisms. Identification, moreover, in many cases is possible only after killing the object. In other words, it is possible by experience to make an estimate of the conservation value of the cryptobiota of a terrain, but in large part our efforts must be directed to the safekeeping of an unknown biota (Samways 1993; Ellis 1998). It is perfectly all right to create a reserve because it harbours a population of, for example, a rare butterfly species. But the management of the reserve should be aimed at the complete biota, not just a single species.

Traditional approaches to cryptobiota conservation

Nature conservation nowadays has discovered the cryptobiota, but most of the time a few, large and colourful groups are picked out: butterflies, dragonflies and grasshoppers. Precisely these groups are least typical for the cryptobiota: they are large, lovable, easy to identify and not rich in species. Conservalional interest in, to mention just one example, springtails, still is close to zero.
A traditional approach is to reckon that a reserve that is well managed as to its macrobiota offers the best chances for the cryptobiota as well. I am inclined to agree, provided that we are speaking about really large reserves, like the Kruger or Yellowstone Parks, where maintenance is minimal. Most reserves however, particularly those in Western Europe, require more or less intensive maintenance: mowing, cutting (to artificially maintain a pre-climax successional stage), sometimes in combination with some way of harvesting. In these situations there is little evidence to support the statement that (all) cryptobionts require the same measurements as do the macrobionts. An example is the strongly negative impact mowing can have on the insect fauna, when done at a high speed, large scale, and at the wrong time. Another is the disappearance of field crickets because of trampling of their burrows by large grazers. Many cryptobionts moreover live in situations that simply are devoid of almost any macrobiont: rock faces, beaches, drift sands, vegetationless river banks, etc. (Hewitt et al. 2005) (figure 3.2). Hotspots of macrofungi diversity in the Netherlands are situated in landscapes with little or no apparent floristical interest (Keizer 1994); arable land is a key habitat for a

Figure 3.2  An almost vegetationless patch like this harbours a number of mosses, lichens and algae. Light spots indicate nests of digger wasps.
number of rare bryophytes (British Bryological Society 2006). Also small, often ephemeral, elements in the landscape without much value for the macrobiota can be vital for the cryptobiota, most clearly in the case of dead wood (Speight 1989; Kirby & Drake 1993; Samuelson et al. 1994; Reemer 2003; Jagers op Akkerhuis et al. 2005) but this applies to cadavers and dung as well. The elements may be quite small indeed, as the role that is played by shells and pebbles in paths for lichens (Aptroot et al. 1998).

**Threats to the cryptobiota and macrobiota alike**

In a number of aspects there is indeed little difference in the response of the cryptobiota, taken broadly, and that of the macrobiota. I will not dwell on habitat loss, no doubt the most biota-wide threat, because that is too obvious.

The dispersal power of cryptobionts varies strongly. Fungi, tardigrades, migrating insects are among the most powerful dispersers in existence. On the other hand, many saproxylic insects, molluscs, annelids, etc. hardly ever venture beyond the tree on which they were born. A similarly wide spectrum of dispersability exists in the macrobiota. In the avoidance of habitat fragmentation the management of cryptobionts and macrobionts therefore must follow the same guidelines. Dispersal power and width of ecological niche also are equally decisive in both for a species’ ability to survive the effects of global warming.

Also the deleterious effect of a landscape-wide lowering of the ground water table has a strongly negative impact on the cryptobiota and macrobiota alike (Bos et al. 2006). Air pollution has impacted on the macrobiota in many ways, that often proved difficult to unravel (‘Waldsterben’); its effects on the cryptobiota are certainly present, but are even more elusive.

**Three pitfalls**

Cryptobiota and macrobiota are thus in step at several points. Yet, the thoughtless extrapolation of some conservation methods, originally designed for the macrobiota, towards the cryptobiota leads to failure at three points.
**Red Lists**

The first Red Lists concerned threatened vertebrates; later flowering plants were added. The role these lists have played, and still play, in macrobiota conservation can hardly be overstated (Hoogeveen 1998). But Red Lists are a tool that should be used with discretion. What we see now is a proliferation of Red Lists of various more or less well known cryptobiont groups. At best the end result is a collection of documents that list endangered species, based on standardized criterions. But while the cumulative list gets longer, its effect diminishes, and each new addition dilutes the impact of its predecessors. More and more it becomes just a long list of names that have little meaning for the general reader or policy maker. The result is a devaluation of the importance that is attached to the occurrence of a redlisted species, and irritation among the public when another project is obstructed (mostly temporarily only) in case this happens.

Even the persons responsible for the management of a reserve cannot have the expertise to recognize all redlisted species under their care. Our knowledge of the ecological needs of most rare and declining cryptobionts also is so sketchy that it is impossible to define precise measurements to be taken for their conservation (Barendregt et al. 1998, Dekoninck et al. 2003). Moreover, as said above, measurements intended for the benefit of one species may well be deleterious to another species that is perhaps equally redlisted, but will inevitably be differently specialized. It also is unthinkable that the Red List collection will ever be complete, introducing the risk that redlisted species groups may ‘outcompete’ ones that are not.

Finally, monitoring of many cryptobiont species is such a difficult task that including them in Red Lists, to be used in conservation strategy, will lead to erratic effects.

Taking everything together, the problem with cryptobiont Red Lists is not so much their existence, as their status. Knowledge about the vulnerability of species is always valuable, but this should be seen in a broad perspective, that encompasses our limited knowledge of the cryptobiota at large, and its threats.

**Bans on collecting**

A ban on the collecting of relatively slow growing and striking macrobiots mostly is an obvious conservation tool. Cryptobionts, however, generally are so difficult to spot and catch, so ephemeral in time, and so large in number that non-commercial collecting does not damage the cryptobiota, unless this is done in very fragile or restricted sites.
What a general ban does, however, is to demotivate and stigmatise amateur collectors, precisely those people able to gauge the value of a particular plot. There are ample examples of highly qualified amateurs, in particular butterfly specialists, whose work is made almost impossible or illegal. A general ban on collecting also makes it difficult for young people to obtain both the skill and fascination that are necessary for a career, professional or otherwise, in cryptobiota research.

There is an obvious connection between Red Lists and collecting bans. It is understandable that one wishes to restrict the collecting of endangered species. But for a non-specialist site manager the distinction between a common and an endangered cryptobiont species usually is impossible. In practice then one takes the safe side, which means that a ban on, for instance, some rare butterfly species is expanded to a ban on Lepidoptera collecting, or even insect collecting in general.

Of course as stated before, this is not to mean that collecting of specific taxa in specific sites should not be restricted, like the collection of lichens in a small quarry or butterflies in the remainder of a chalk grassland or of a peat bog.

**Target species**

There are many, and very different, interpretations of this term. Here it is meant to include species that are very rare, strongly declining, yet relatively well represented nationally (Bal et al. 1995; Van der Zande & Hoogeveen 1995; Hoogeveen 1998). In other words, target species are Red List species for which a national government should feel particularly responsible. Even for macrobiota the latter criterion is a dangerous one, because it does not take into account geographical variation and area border effects: individuals of a species are far from identical over their geographical range (Barendregt et al. 1998). By their very nature, target species are to be found in utterly restricted situations. To protect them there naturally is all right; but to use them as focal points for the protection of a biota at large is a strategy that misses the countless species with a different ecology.

**What rather should be done**

Conservation measures of the cryptobiota can be envisioned at several scales: the level of the single reserve, with its practical problems
and local solutions; an intermediate level of conservation organisations and scientific societies, and finally the governmental level.

At the practical level, and in the context of our small European reserves, the best general approach is the ‘90 percent rule’: carry out each management effort only for 90%. When mowing, leave some unmowed, never cut it all, never clean all ditches equally thoroughly. Simple as this rule is to formulate, it is surprisingly difficult to teach technical staff such a seemingly sloppy management practice (figure 3.3). Another facet, almost as difficult to teach, is to learn and understand the importance of small landscape elements like a rock face, a dead or bleeding tree, a cadaver, a trickle, big stones etc. This also means that management should best be done on a small scale (e.g. Verberk & Esselink 2002), and with due respect for structural variation.

Figure 3.3 This meadow was mown at the right time (late October), but with a relentless thoroughness. Not even a transition zone to the forest edge was allowed (inset).

At the middle level, rather than drawing up more Red Lists, I advocate drawing up a list of valuable sites, rather similar to the British list of Sites of Special Scientific Interest. The general idea is that specialists
are invited to report sites where they have found a particularly inter-
esting flora or fauna (either exceptionally rich in species or harbour-
ing a number of rarities). Sites like these may be expected to represent
an unusually favourable ecological situation; by the protection of such
a site also a number of species of different taxonomic groups will
benefit (Koomen & Van Tol 1993). As explained above, for cryptobi-
onts even relatively small sites may be quite valuable. A comparable
attempt at a European scale is the compilation of prime butterfly are-
as by van Swaay & Warren (2003).

A related approach is the inventorization and classification of hab-
itats, such as has been attempted in Great Britain (Fry & Lonsdale
1991; Kirby 2001; Buglife 2006) and Germany (Röser 1988; Jedicke
1994; Riecken, Ries & Ssymank 1994; Pott 1996); see also Koomen &

To stimulate and assist field workers there are a few prerequisites:
a stable nomenclature, a body of vernacular names, and good, acces-
sible identification keys. Scientific societies with an eye for cryptobi-
ta conservation should consider these tools a top priority.

At the governmental level it is imperative that the vital role played by
amateur biologists is better appreciated. It is a sad fact that systemat-
ics is a field of research that is held in low esteem. The number of pro-
fessional systematists is at its lowest level in over a century, and those
that are left mostly deal with tropical regions, where their work is
even more urgently needed. For an ongoing study of the cryptobiota
of a region or country one has to rely therefore largely on amateur bi-
ologists. Their activities and organisations should be intensively stim-
ulated, and their results taken seriously.

The Third World has hardly been addressed in this paper. This is be-
because there most of the threats are similar for macrobionts and cryp-
tobionts alike: loss of habitat surface, structure, and quality in its
broadest sense. But there is one difference. Our rich Western World
has a large – although not large enough – body of capable amateur
specialists devoted to cryptobionts, that have a wide range of identifi-
cation literature at their disposal. In the Third World the situation is
the opposite. Providing the Third World with accessible identification
keys to their cryptobiota is the first step that may lead to a reversal of
this deleterious situation. With these tools a body of local amateurs
may be able to develop, and take a stand in the defence of their local
cryptobiota.
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References


Ants are social: their workers can communicate and divide their tasks quite well. The generations overlap, which implies that the younger workers can learn from the older ones. Consequently the colony is able to adapt to some extent to changes in its environment. Ants occur in nearly all terrestrial biotopes. Most species are living in tropical and subtropical areas.

At the moment, about 12,000 ant species have been described. This number may increase, despite the continuing destruction of those ecosystems which are most rich in ant species, like tropical forests. In 1976, c. 6,000 species were known, in 1990 8,000, in 2006 12,000 species, while the total number is estimated at 22,000 species (Agosti & Johnson 2003).

Ants play an important role in the biotopes (ecosystems) in which they occur: as predator, scavenger, aphid keeper, seed disperser, plant eater, honey collector, guest, slaveholder or as parasite.

**Monitoring species**

Generally, ant species can be protected by protecting and managing the ecosystems in which they occur. However, some control by means of monitoring target species will be necessary (Agosti et al. 2000). A few species, belonging to the following categories, can be selected as a target species and can be monitored:

- ‘Umbrella – species’ (e.g. red wood ants as guest of parasites)
- ‘Keystone – species’ (e.g. red wood ants as polyphagous predator)
- Vulnerable and threatened species (Red List species, among others)
  - Species which are dependent on other species (parasites and slaveholders) and other habitat specialists (stenotopic species)
  - Species with a very limited distribution area (some endemic species)
Species which are sensitive to changes in the environment, like desiccation (e.g. species of peat bogs), eutrophication (e.g. species of nutrient poor grasslands), habitat fragmentation (species with a poor colonization capacity), pollution (species which accumulate harmful substances).

From the group of vulnerable and threatened species a few species can be selected for the IUCN Red List with the help of the following criteria:

- Species is vulnerable
- Distribution area is known (more or less)
- Indications for shrinking of the distribution area and/or strong decrease of local populations
- Causes of decline are known.

Of the selected species data should be presented on which basis decisions can be taken to add them to the Red List of Threatened species.

**Red List species**

Nowadays 149 species are mentioned in the recent IUCN Red List, while only 11 species were mentioned in the IUCN Red List of 1986 (IUCN 1986, 2006). In the former Red List information was given about the status of the species, but such information is lacking in the recent Red List. Most species mentioned in this List are parasites. These species can be protected by protecting their host species, or at least the habitat of their host species. However, as long as these data are lacking, the Red List will not be an effective tool for the protection of the species mentioned.

The following data are relevant to collect and to be mentioned in the Red List:

- Possible dependency of a species on other species (host or slaves)
- Distribution area of the species (in case of parasites also of the host species)
- Stenotopy of the species (in case of parasites stenotopy of the host species)
- Distribution of the habitat of the species (in case of parasites the nests of the host species)
Generally host species can be found more easily than their parasites. Therefore social parasites can be protected more effectively by protecting their host species.

Two examples: The parasite *Formicoxenus nitidulus* is mentioned in the IUCN Red List of Threatened Species. It lives in nests of at least six *Formica* species (*Formica polyctena*, *F. rufa*, *F. pratensis*, *F. truncorum*, *F. exsecta* and *F. pressilabris*). The nest mounds of these species can be found easily, in contrast to the parasite. The parasite will disperse in late summer, during which young (fertilized) queens will leave the *Formica* nest flying (or walking) in search for other nests. In a polydomous red wood ant colony the queens and workers of the parasite can follow red wood ant trails to neighbouring nests. In such a situation the local population of the parasite will have a high survival probability. Figure 4.1 shows the distribution area of the host species in The Netherlands, as well as the distribution area of the para-

![Figure 4.1](image-url)
site. It appears that the distribution area of the parasite overlaps the distribution area of its host species nearly completely, also of the host species which have a protected status, i.e. the red wood ants. Formicoxenus nitidulus can be protected most easily by managing the biotopes of red wood ants in a way that they can survive. That is why Formicoxenus nitidulus is removed from the List of protected species in The Netherlands, while its host species F. truncorum is added. There may be also good arguments to add the mound building ants of the F. exsecta group to the Red List. They are living in nutrient poor grasslands, which area is shrinking quickly.

Figure 4.2 Distribution of nests of Tetramorium caespitum and Strongylonathus testaceus
The parasite Strongylognathus testaceus lives in nests of Tetramorium species. The nests of this host species can be found easily, while the parasite can be found only by opening Tetramorium nests or by observing sexuals which leave the nest for a wedding flight in summer. Figure 4.2 shows the distribution area of both species in The Netherlands. It appears that the distribution area of the parasite is much smaller than that of its host species. It may be that the parasite has a poor dispersal capacity. If so, then the parasite will be sensitive to habitat fragmentation, i.e. a decrease in the density of Tetramorium nests. For an effective protection we should take the dispersal capacity of young (fertilized) queens into account. This parasite is considered as threatened in several European countries, but not mentioned in the IUCN Red List (figure 4.3).

Figure 4.3 Tetramorium caespitum and parasite.

Ant specialist group

Relevant data for the protection of threatened ant species were collected by the Social Insect Specialist Group (SISG), which is the successor of the Ant Specialist Group. Agosti et al. made a start with presenting data about the distribution of Red List species on internet in order to make them available for everybody (see: antbase.org). However, this process develops slowly, due to lack of financial support. Recently, I tried to find SISG on internet to check some data of Red List species, but only an Invertebrate Specialist Group (ISG) was mentioned. At the moment this group can only deliver data about Odonata and Mollusca. Do ants belong to the
‘Forgotten Kingdoms’? I asked this question to the chairman of the SSC and the IUCN Red List Officer. They explained that many Invertebrate Specialist Groups, among which the SISG, are temporarily dissolved as part of the reconstruction of the Species Survival Commission (SSC). According to the chairman of the SSC ant specialists can deliver data of Red List species to a so called ‘Ant Focal Point’. However, who will give advice about what species should be mentioned in the Red List and who will process data about their status?

Protection of red wood ants

Eight species of red wood ants are on the IUCN Red List of Threatened species. Three of them occur in The Netherlands: Formica polyctena, F. rufa and F. pratensis. The fourth indigenous species, the Trunk ant (Formica truncorum), is not mentioned in the List, although this species seems to be more vulnerable than the other three: local colonies have a higher extinction probability and it is doubtful if this can be compensated by a higher probability to colonize empty habitat patches (Mabelis & Korczyńska 2001, Mabelis & Chardon 2006).

Most Formica species on the Red List are not threatened, but they are vulnerable for disturbance, e.g. by pupae robbers, wild boars and woodpeckers. The Formica species are not only protected

Figure 4.4 Nest of Formica truncorum.
for their intrinsic value, but mainly to maintain their role in the forest ecosystem:

- Red wood ants are polyphagous predators and as such they can stabilize prey populations
- The nest of red wood ants is the habitat of many ant guests (in The Netherlands: 32 species of beetles, among others)
- Red wood ants disperse seeds of myrmecochorous forest plants (Viola species, among others)
- Red wood ants are a reliable food source for several other species (woodpeckers, ant lions)

In a lot of forests throughout Europe red wood ants are (re-)introduced as a method of biological control of harmful insects. This is mainly done in production forests, but sometimes also in National Parks. For the re-introduction of a species in a National Park (or another protected area) a few conditions should be fulfilled:

- The species occurred in the past, but became extinct
- Habitat quality is good
- Distance to nearest source is too great for re-colonization of the species
- Re-introduction may not be at the cost of other characteristic species

Nests of red wood ants are often protected by foresters with fences against wild boars. However, it may be better to manage the forest and its wild boar population in a way that red wood ants can survive, despite some local losses due to disturbances by these animals. Sometimes nests are protected with nets against woodpeckers. This should be dissuaded. Red wood ants are a reliable food source for these birds in winter and the protection of red wood ants should not be at the cost of woodpeckers, which can also be qualified as keystone species, because their tree holes are important for hollow breeding birds and bats.

**Habitat fragmentation**

Red wood ants prefer to build their nests in open forests and on the southern edge of dense forests. In the trees they can find aphids which provide the ants of aphid-milk, which is an important source of energy, while prey, as an important source of protein, can be found mainly in open areas. Red wood ant species have a different survival strategy, which is related to the tolerance
of workers to accept more queens in their nest, either fertilized daughters or young queens from other nests. A colony of a species which accepts many queens, like Formica polyctena, has a lower probability to become extinct than the colony of a species which accepts generally just one or only a few queens, like F. rufa (Mabelis 1986). Moreover, a species which has many queens in the nest can disperse by means of budding, during which process workers will transport several queens from the mother nest to newly build daughter nests. This is a much safer means of dispersal than trying to colonize an area by means of flying queens (Rosengren & Pamilo 1983; Rosengren et al. 1993). However, the advantage of budding is lost if the habitat area is small. In that case it may be a better option for a young queen to leave the nest flying, which occurs now and then. To establish a new colony, a queen has to penetrate a nest of a Serviformica species (generally F. fusca), because she is not able to care for her own brood. Only very rarely workers of this host species will adopt a red wood ant queen as her own queen (Gösswald 1952). So, colonizing new areas by means of flying queens is a risky venture. Nevertheless it may be a good survival strategy in a situation in which the habitat is fragmented, like small patches of woodland in an open area. In this case it seems that the higher extinction probability of a colony with one or a few queens can be compensated by a higher probability to colonize a patch which is still unoccupied. Consequently, a monogynous/oligogynous species, like F. rufa, is better adapted to a situation in which the habitat is fragmented, while a polygynous species, like F. polyctena, will thrive better in an area where habitat patches are connected. The distribution pattern of their nests reflects their survival strategy: F. polyctena colonies occur more often in woody areas without barriers, while F. rufa is more common in small and isolated habitat patches (Mabelis 1992, 1994). This difference appears to be more clear in an area where the edge of the patches has a good habitat quality than in a situation where the edge of habitat patches is contaminated with animal manure and pesticides, sprayed from adjacent agricultural fields (which is the case in The Netherlands).

Forest management

In a dense forest the southern exposed edge of open areas provide habitat for red wood ants. If the open areas are small, a species which disperses mainly by flying will have a higher probability to
survive than a species which disperses mainly by walking, that is to say if the distances between the open areas are not too great for flying queens. In natural conditions small open areas can arise by windfall, while big herbivores sometimes can keep the areas open long enough for providing a Serviformica species and consequently a red wood ant species a good chance to establish themselves. However, the greatest part of the forest area in Europe is planted and managed for wood production and consequently rather dense. In those forests open areas can be created by felling trees or by pulling down trees with a winch. It will not only create habitat for red wood ants, but for many other forest species as well. Biodiversity of a forest can be maintained (or enlarged) by maintaining a varied forest structure with small open areas.

**Summary**

- Ant species can be protected most easily by protecting their biotopes and managing these well.
- Besides biotope protection, attention should be paid to the most vulnerable and threatened species (Red List species).
- For these species data should be available on their status and threatening factors for an effective protection.
- Besides these species attention should be paid to ‘umbrella-species’, which can function as host for parasites, and to ‘keystone-species’, which play an important role in the ecosystem concerned.

**References**


All life on earth belongs to one of the five currently recognized Kingdoms: Bacteria, Fungi, Protoctista, Plantae or Animalia. The level at which we know these Kingdoms is far from balanced. On a global scale we are quite aware of the diversity of the Plantae, and the number of species described is estimated to cover 85% of the total number of living plant species. To date only the global richness of vertebrates, with 95% of all species having been described, is better known (Table 5.1).

Table 5.1  Estimates of global biodiversity (Groombridge and Jenkins 2002)

<table>
<thead>
<tr>
<th>Taxonomic level</th>
<th>Organism group</th>
<th>Global number of described species*</th>
<th>% described of estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom</td>
<td>Plantae</td>
<td>270 000</td>
<td>84</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Animalia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phylum</td>
<td>Craniata</td>
<td>52 500</td>
<td>95</td>
</tr>
<tr>
<td>Phylum</td>
<td>Mollusca</td>
<td>70 000</td>
<td>35</td>
</tr>
<tr>
<td>Phylum</td>
<td>Crustacea</td>
<td>40 000</td>
<td>27</td>
</tr>
<tr>
<td>Phylum</td>
<td>Arthropoda</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mandibulata</td>
<td>963 000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Chelicerata</td>
<td>75 000</td>
<td>10</td>
</tr>
<tr>
<td>Phylum</td>
<td>Nematoda</td>
<td>25 000</td>
<td>6</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Protoctista</td>
<td>80 000</td>
<td>13</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Fungi</td>
<td>72 000</td>
<td>5</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Bacteria</td>
<td>10 000</td>
<td>1</td>
</tr>
</tbody>
</table>

* terrestrial, fresh water & marine

The smaller the individual organisms, the less of their diversity we seem to have captured. It is thus not surprising – it is hard to protect what you do not know – that the Kingdoms of Bacteria, Fungi, Protoctista and invertebrate Animalia have globally been largely forgot-
ten in efforts aimed at biodiversity conservation and restoration. Nature conservation and restoration traditionally focus on conserving populations of endangered species. The success of conservation and restoration management practices can be evaluated by studying the occurrence of the focal species. However, the less mobile and the more specialised the species, the more attention the conservation of the whole habitat of the species attains.

In terrestrial ecosystems the forgotten, but abundant and diverse Kingdoms of Bacteria, Fungi, Protoctista and invertebrate Animalia are predominantly to be found in the soil (Wardle 2002). Despite their high abundance they are easily overlooked given their body diameters of less than one tenth of a millimetre for the micro-flora and -fauna, or up to two millimetres for the mesofauna. Their larger soil companions, the macro- and mega-invertebrate fauna, get noticed more easily. Unfortunately these multi-legged crawlers like millipedes and spiders, or non-legged earthworms and pot worms do not seem to be very charismatic to the general public. Small and perhaps not very appealing, but without them we would not be able to sustain human life on earth!

To date we do not know what each individual soil inhabiting species does, how unique it is in its appearance and function. But, we do know that a healthy ecosystem is one with a healthy soil, rich in microscopic life (Wall et al. 2001). Soil biota plays a key role in decomposition, nutrient mineralisation, formation of soil aggregates, biodegradation of pollutants, suppression of soil borne pests and diseases and carbon sequestration. It would thus not be surprising if biodiversity aboveground and belowground are, at least to some extend, interdependent.

During the last century terrestrial biodiversity declined tremendously, due to the reduction and fragmentation of suitable habitats. In grassland ecosystems agricultural intensification has played a major role in the reduction of plant species.
richness (Bakker 2005). The use of mineral fertilisers, increased mechanisation and ongoing seed selection yielded high productive but species poor grasslands (Poschlod et al. 2005). Over the last decades, however, intensively used grasslands are ‘given back to nature’ in order to serve nature conservation purposes (Bakker 2005). Restoration of the formerly species rich, traditionally managed hay meadows is, however, not as straightforward as it may seem. In the initial, high productive and species-poor stage fast growing grass species dominate the canopy due to superior competitive ability for light. Restoration management targets to reduce productivity by cessation of fertilizer application, haymaking and livestock grazing at low density. As a result the plant community becomes limited by soil nutrients rather than by light availability, which enhances the competitive status of slower growing sub-ordinate forb and grass species (Marss 1993). A major constraint to achieve the target species-rich plant community can be the lack of viable seeds of target species in the seed bank, poor dispersal and limited sites for establishments. Introduction of seeds and creation of establishment sites can help to overcome this hurdle (Bakker & Berendse 1999). Successful restoration may take decades and achieved richness is not readily predictable from potential plant species richness...

Plants interact with a high abundance and diversity of soil biota which may affect plant establishment and persistence in the plant community (Crawley 1997; Bardgett 2005). Mutualist symbionts, like mycorrhizal fungi and nitrogen fixing bacteria, can stimulate plant performance, while root pathogens and root herbivores can have a suppressive effect. In grasslands the most important invertebrate root-feeders are nematodes and insects (Mortimer et al. 1999). Nematodes, however, are also very abundant in other trophic levels in the soil food-web as bacterial-feeders, fungal-feeders, predators and omnivores (Yeates et al. 1993). Per square meter of grassland soil one can find ten million individual nematodes of sixty different species. Moreover, the structure of the nematode community proves to be very indicative for decomposition pathways and soil functioning (Bongers 1990). The second most abundant evertebrate group in grasslands are the microarthropods (predominantly Collembolans and mites). They appear in densities as high as 300 000 per square meter (Bardgett & Griffiths 1997), may comprise hundreds of species (Siepel & Van de Bund 1988) and are mostly active as detrivores, fungivores and predators (Petersen & Luxton 1982).
In order to investigate the potential role of these abundant soil invertebrates in grassland restoration, we assembled plant communities with grass and forb species characteristic for early and later stages of restoration (secondary succession) and grew them without or with soil invertebrates present. The plants were growing in mid successional soil from which soil biota were removed by irradiation. For the with-soil invertebrate-treatment we added nematodes, micro-arthropods and insect larvae collected from grasslands in different stages of restoration (De Deyn et al. 2003). Consequently we monitored the plant species dominance in soil with and without soil invertebrates from different locations. Over time the biomass of the early and mid successional plant species decreased, while that of later successional plant species increased in the plant communities with soil invertebrates present. In addition the presence of the soil invertebrates increased the overall diversity of the plant communities by reducing the dominance of the grasses and promoting the sub-ordinate forb species. Since the invertebrates were introduced in abundances and composition reflecting those in the field, we can not state to which extent the observed results were due to the diversity of the soil invertebrates. However, it appeared that the reduction of the dominant grasses was strongly related to the activity of root-feeders with selective feeding behaviour.

In a following outdoor experiment we investigated how plant diversity may be modified by the separate and combined effect of nematodes, wireworms (click beetle larvae, Elateridae) and grasshoppers (Van Ruijven et al. 2005). The plant communities consisted of sixteen plant species from species rich grasslands on sandy soil and also in this case field collected invertebrates were introduced. To our surprise grasshoppers reduced overall community productivity without affecting plant community diversity, while the soil invertebrate nematodes and wireworms altered plant community diversity but not their productivity. Overall, nematodes reduced plant diversity and wireworms promoted diversity. However, when wireworms as well as grasshoppers were present plant diversity was reduced. Both experiments demonstrated that soil invertebrates can direct plant community diversity through selective root-feeding and differential plant species tolerance to herbivory. The outcome is yet hard to predict because the degree of specificity and selectivity of root-feeders ranges from specific on few plant species to generalist on many plant species. Moreover the tolerance of plants to support herbivory and to stimulate herbivore populations is also dependent on soil fertility (Verschoor et al. 2002; De Deyn et al. 2004). The presence or absence of particular species or species combinations of soil invertebrates may thus affect aboveground
diversity more than the total number of species present. However, biodi-
versity can play a major role in the predictability of the response.
Having a diverse pool of soil biota present ensures the maintenance
of many interactions, which is expected to make the outcome of the
response less variable over time (Neutel et al. 2002).

The forgotten Kingdoms of Bacteria, Fungi and Protocista are all
well represented in the soil, albeit largely unknown in which diversity
(Wall et al. 2001). For the current status and advances concerning the
Bacteria and Fungi I like to refer to the contribution of Kowalchuk
(this volume). One group of soil fungi, however, I can not leave unad-
dressed in relation to aboveground biodiversity: the mycorrhizal fun-
ghi. These fungi form mutualistic associations with plant roots from
which they obtain carbohydrates in return for supply of mineral nu-
trients to their host. Mycorrhiza of trees and shrubs (ectomycorrhiza),
of Ericales (ericoid mycorrhiza) and of orchids (orchid mycorrhiza)
are classified in the fungal phyla of Ascomycota and Basidiomycota.
Most plant families, however, form mycorrhizal associations with ar-
buscular mycorrhizal fungi which are classified in the phylum Glomeromycota. The phylum comprises ten genera with a total of 150
described species. Given that most of the 250 000 species of plants
form mycorrhiza, host specificity appears to be low (Smith & Read
1997). Nevertheless aboveground plant diversity and productivity does

Figure 5.2 Diverse soil biota of click beetle larva, collembola, mites and nematodes ineract with plant roots and thereby affect plant competitiveness and diversity.
seem to be dependent on the diversity, or at least the species combination, of the arbuscular mycorrhizal fungi present in the soil (Van der Heijden et al. 1998).

Does soil biodiversity matter for aboveground biodiversity? How much belowground diversity do we need to sustain aboveground diversity? Who is driving who? The answer to these questions is not straightforward. Not only are many species still undiscovered, but also organisms operate at different spatial and temporal scales (De Deyn & Van der Putten 2005). Moreover interactions across the soil surface continuously feedback and span several trophic levels above- and belowground (Wardle et al. 2004). Yet we do know that the biota beneath our feet are key to understand large scale ecosystem processes such as plant succession (Van der Putten et al. 1993; Van der Putten 2003), maintenance of grassland ecosystems (Bardgett & Wardle 2003) and success of exotic invaders (Reinhart et al. 2003; Wolfe & Klironomos 2005). As most of us know, plant species differ in their optimal performance under different abiotic conditions like pH, availability of water, nutrients and light. Similarly, soil biota also differ in their optimal performance with respect to abiotic conditions. However, the range of conditions in which both plant species and soil biota can persist in a sustainable way appears to result from their intimate interaction. Which species will persist, disappear or will become a successful invader will depend on the relative specificity and the costs and benefits associated with the interactions.

Given the largely undiscovered diversity of soil biota there is an urgent need for taxonomists and for the maintenance of collections of the forgotten Kingdoms. Taxonomy should span the molecular, morphological and functional level. Clearly not an easy, quick and cheap task; long-term investment and close collaboration between taxonomists, ecologists and evolutionary biologist is warranted. Meanwhile soils – and thereby the diversity of genes, species and functions they harbor – are being degraded worldwide. This soil degradation has devastating effects on aboveground diversity, productivity, soil carbon pools and water quality (Sugden et al. 2004; Lal 2004). Therefore we need to protect the sustainability of soils and their biodiversity and increase our understanding of the role of soil biodiversity in soil quality and sustainability now (Harris et al. 2005; Usher 2005). Policy-makers became increasingly aware of this global need and at the European level strategies for the protection of soil biodiversity and sustainable use of soils are being proposed (www1). In view of this, tools to monitor and evaluate soil quality are crucial and soil inverte-
brates appear to be very suitable for this purpose (Van Straalen 2004). Amongst the ‘forgotten’ organisms especially the Nematoda are widely recognized and used for their biomonitoring potential (Bongers 1990; www2). Monitoring alone will not increase our understanding of the interdependency of life on earth. Continued exploration and elucidation of the amazing world we live in will only come from interdisciplinary research. For sure, further discovery of the forgotten Kingdoms and their functional importance promises to be at least as exciting and valuable as the discovery of water on Mars! But of course we all want to know which Kingdoms extraterrestrial water may harbour.

As background information a brief overview on the ecology, classification and diversity status of nematodes, mites and collembolans is provided below (for more detailed classification see www3 and Wall et al. 2001 for soil biota diversity estimates).

**Nematodes**

Nematodes are aquatic, triploblastic, bilaterally symmetric, unsegmented roundworms. They occur globally in marine, freshwater and terrestrial habitats, even on Antarctica! The majority of nematodes is free-living and feed on dead organic matter, bacteria and fungi or predate on other small animals. However, also parasitic nematodes, living in plants and animals, are common throughout and can be very abundant. Soil nematodes are on average shorter than 2mm and as thin as 20 micrometer. The community structure of nematodes is widely used as a bioindicator (Bongers 1990). Their indicative value was already noticed a century ago by Nathan A. Cobb in ‘Nematodes and Their Relationships’: In short, if all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes. The location of towns would be decipherable, since for every massing of human beings there would be a corresponding massing of certain nematodes. Trees would still stand in ghostly rows representing our streets and highways. The location of the various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases even their species could be determined by an examination of their erstwhile nematode parasites (Cobb 1915).

**Classification**

Phylum: Nematoda, comprising two classes: Secernentea (mainly terrestrial) and Adenophorea (mostly marien) under the traditional view.
Based on molecular analysis the phylum is viewed as consisting of 5 clades (Blaxter et al. 1998). The perspectives in nematode phylogeny and classification are still in full change because of new insights gained through molecular phylogenetics and bioinformatics (De Ley & Blaxter 2002).

Diversity
Globally 25 000 species are recorded, but it is estimated that this covers only 6-10% of the actual number of nematode species present. Of the soil inhabiting species 5000 are described but this would present only a tenth of all soil nematode species (Brussaard et al. 1997). Nematode diversity can be assessed with molecular tools and is then often evaluated at the level of sequence identity as operational taxonomic units (MOTU) rather than species (Floyd et al. 2002).

Mites
Mites are small (mostly smaller than 1 mm) chelicerate arthropods, bearing four pair of legs as an adult but only three pair of legs in the larval stage. They have a discrete gnathosoma but no primary segmentation. Like nematodes they have a global distribution and inhabit terrestrial, marine and fresh water habitats and occur free living as well as parasitic. Soil mites feed on detritus and microbes or predate on other microfauna, thereby regulating microbial processes.

Classification
Phylum: Arthropoda, class: Arachnida, orde: Acari, with three superorders: Opilioacariformes, Acariformes (containing the groups Oribatida, Astigmata and Prostigmata) and Parasitiformes (comprising ticks, parasitic mites and predatory Mesostigmata) (Evans 1992).

Diversity
Globally about 45 000 species of mites have been described, which is estimated to be about 5% of the number of species to be alive today. Acariformes are the most species rich with 30 000 known species, followed by the Mesostigmata with 10 000 known species. The estimate of described species of soil mites ranges from 20 000 to 30 000 species (Brussaard et al. 1997, Walter & Procter 1999).
Collembola

Collembola or springtails are small (mostly smaller than 2 mm) arthropods, bearing three pair of legs and antennae, but no wings or cerci. They have three body segments and their mouthparts are located within a ‘gnathal pouch’ (entognathous). Many springtails have a furca (a posterior ventral forked abdominal appendage) enabling them to jump. They can be found globally in terrestrial ecosystems, mainly inhabiting soil and leaf litter, feeding on fungi, detritus and algae or predate. They are also found in moss, on bark, in caves and ant and termite nests, but also in the intertidal zone at the coast, on the surfaces of lakes and ponds and snow fields of glaciers.

Classification

Diversity
Globally there are about 7 500 described species of collembola, which is estimated to be about 15% of the species actually alive worldwide (Hopkin 1998, www4).

References


www1. ec.europa.eu/environment/soil/index.htm

www2. www.nem.wur.nl/UK/MI

www3. The Tree of Life Web Project. tolweb.org

www4. www.collembola.org

Invasions by alien macroinvertebrates and ecological rehabilitation: lessons from the Rhine

Gerard van der Velde & Abraham bij de Vaate

Abstract
The large river Rhine has gone through periods of severe ecological deterioration due to human impact, alternating with periods of some ecological improvement. Measures taken since 1986 in the context of ecological rehabilitation programs have led to a dominance of alien macroinvertebrates, particularly crustaceans and molluscs, in the river’s main channel. However, ongoing river engineering in the river channel and nature development in the remnants of its floodplain will cause the system to enter a phase of permanent disturbance. More invasions of macroinvertebrates are therefore likely, with a trend towards increasing similarity to the river Danube.

Introduction

From an economical point of view the Rhine is the most important river in Europe. The river’s natural function is the discharge of melt water from the glaciers in the Alps and rain water from the river catchment. The meandering character of the natural lower river reaches creates many different habitats as sediments become differentiated by differences in flow rates, meanders become cut off by natural processes (oxbow lakes), and floods and seepage areas affect areas along the river. This has led to a high biodiversity in the river itself and its floodplain areas (Van der Velde et al. 2004). Habitat requirements to support the species-rich rheophilic flora and fauna are: a) relatively low water temperatures, b) a high oxygen supply, c) a regular food supply, c) various substrates differentiated into islands, sand and gravel banks, d) shallow sand and gravel banks for growth of benthic algae, the main food for grazers, e) water and bank vegetation, large woody debris (LWD), also for benthic algae production, providing attachment opportunities, spawning sites, shelter and food, f) floodplain forest for the reproduction of aquatic insects, shelter against sunlight and the production of LWD and other allochthonous litter.
The river is fed by streams in which the food source is mostly of terrestrial origin. Wood and fallen leaves (coarse particulate organic matter (CPOM)) is consumed by shredders such as insect larvae and by gammarids that digest the microbial fungi and bacteria that colonized the leaves in the water. These leaves also leach out dissolved organic matter (DOM), which can flocculate and be taken up by microbial organisms. Faeces, fragments and flocs form fine particulate organic matter (FPOM) which fuels the higher order head streams and is the main food source for collectors (filter-feeders). As the stream widens in downstream direction, more light is able to reach the water surface, creating growth conditions for macrophytes, benthic algae and periphyton. This leads to more grazers in the system. In the lower reaches of the river collectors (filter-feeders and deposit feeders) dominate the system because of phytoplankton development, detritus supply and low flow rate. The various processes in the course of a river from small stream to large lowland river have been summarized in the River Continuum Concept (RCC).

In historical times humans started to use the river for various other purposes leading to more and more anthropogenic influences by human activities. The river’s economic functions now include navigation, fisheries, recreation, discharge of substances, provision of cooling water, drinking water production, electricity production, sediment extraction, and the use of river water for irrigation and to stop salt intrusion. Not only was the river used for economic functions, but the river floodplain was narrowed and the land thus obtained was protected by dikes to improve safety. The function of the river as a habitat for flora and fauna has always been neglected, until the ‘Sandoz disaster’, a chemical spill in 1986. After this disaster, plans have been developed and realized to achieve ecological rehabilitation of the river. The Rhine Action Program was the umbrella for these plans in the decade after the disaster. Macroinvertebrate studies started to examine the effect of anthropogenic influences, in order to provide a basis for management measures and to formulate political decisions. Much effort was put into monitoring activities to make results of ecological measures visible.

**Deterioration**

The various anthropogenic influences on river biotopes were several, all leading to a severe degradation of the ecological state of the river (Cioc 2002). Deforestation and agriculture led to increased erosion and sediment load as well as increases in nutrient and pesticide lev-
els. Waste products of cooling water use (chlorination), industrialization and mining led to chemical, often toxic water pollution such as heavy metals, organic substances and salt through discharge and atmospheric deposition. The sewer discharge in towns led to high levels of organic matter, oxygen depletion and high nutrient levels. High nutrient levels led to phytoplankton blooms diminishing transparency of the water. River engineering influenced water velocity and channel depth by normalization and canalization and by the construction of summer and winter dikes influencing flooding and sedimentation patterns.

In a natural river, the main channel meanders and is characterized by natural discharge, an open estuary, riffles, pools and sand banks. It has no sewer or navigation functions and no dikes or levees. Today, the river is characterized by fixed river beds, artificial meander cut-offs, (partly) partial dams, an estuary with many arms closed off by dams, a dredged river bed, sewage, a major navigation function and dikes and/or levees. This process has led to a reduced number of lotic and lentic biotopes, unnatural discharge levels, migration barriers, reduced species richness and a smaller floodplain (river forelands). These river forelands are also used as meadows for cattle. Canalization and meander cut-offs, normalization, bank protection, sand and gravel extraction are irreversible effects, whereas the effects of waste discharge can be reversed. Chemical pollution increased after the start of the industrial revolution, and after a pause during World War II increased again making the Rhine a severely polluted and degraded river, which was regarded the sewer pipe of Europe. Effects on macroinvertebrates were severe, greatly reducing the numbers in insect species, particularly rheophilous mayflies (Ephemeroptera), caddis fly larvae (Trichoptera) and midges (Chironomidae). The greatest reductions occurred in the numbers of species living on hard substrates (e. g. wood), in sand and in vegetation (Klink 1989; Van den Brink et al. 1990) (Figures 6.1 and 6.2). A species which became completely extinct was the largest mayfly in Europe, Palingenia longicauda, the last specimens of which were observed around 1915. It died out not only in the river Rhine but in nearly all large rivers in Europe, with the exception of the river Tisza, a tributary of the river Danube. Their larvae burrow into silty-clayey sediments making U-shaped burrows up to 15 cm length and 6-8 mm wide. Densities can reach 4000 specimens per m². They feed on organic matter in the sediment. The burrowed sediment passes their intestines. This poor food may be one of the reasons of their slow larval development (Wesenberg-Lund 1943). The larvae live in the water for three years before they metamorphose into adults. Recolonisation of the Rhine by this species is not expected to


take place, because of the changes in water quality, hydrology and morphology, and because the Tisza is too far away.

**Rehabilitation**

Since 1970, however, the states along the Rhine have implemented measures to improve the water quality of the river. Between 1970 and 1990, this quality did indeed improve (Admiraal et al. 1993; Bij de Vaate et al. 2006). Concentrations of heavy metals like Cd and Hg, organic micropollutants and mineral oil were reduced to very low levels. Reduced concentrations of cholinesterase inhibitors led to the return of midges (Chironomidae) and caddis larvae (Trichoptera) in the river after 1976.
Minimum, average and maximum oxygen saturation values of the water increased to natural levels. On the other hand, average water temperatures in the Rhine have increased gradually by several degrees since 1911. Pesticides and other diffuse forms of pollution have remained, just like salt discharge, and the lack of habitat diversity has also persisted. In 1995, 59–63 macroinvertebrate species were found per biotope (rip-rap, channel bottom, artificial substrate); 21 species were only found in a specific biotope. Nevertheless, a clear indication of the improvement of the circumstances for the sand inhabiting fauna is the recolonization in 1991 by the mayfly Ephoron virgo after almost 50 years of absence (Bij de Vaate et al. 1992).

Increasing invasions

Monitoring the Rhine during the last decades has revealed that alien species, mostly crustaceans (Van der Velde et al. 2000) and molluscs have become dominant over native species, mostly insect species, numerically as well as in terms of biomass. Large rivers are normally insect-dominated. At the start of the water quality improvement campaign in 1970, the alien invaders were pollution-tolerant species. After the Sandoz accident in 1986, the invasion rate accelerated, as the river was invaded by Asiatic clams (Corbicula fluminalis, C. fluminea) in 1988, which moved in an upstream direction from the estuary (Bij de Vaate 1991), and by Ponto-Caspian macroinvertebrates, mostly crustaceans, invading in a downstream direction. The Ponto-Caspian invaders came from the Mittelland canal. This canal is connected with the Rhine and a series of canals connecting all south-north flowing rivers discharging into the Baltic and North Sea. After the opening of the Main-Danube canal in 1992, the invasion rate increased again, due to invasions by more and more Ponto-Caspian species. The increasing number of invasions is suggested to be the result of incomplete communities with vacant niches, due to major disturbances, viz. pollution followed by water quality improvement and then by a chemical spill, which cleared the river free of macroinvertebrates and eels over a stretch of hundreds of kilometers followed by further water quality improvements (Den Hartog et al. 1992). These invaders also cause disturbance leading to rapid turn-overs in the dominating species. Interactions between species of similar origin can quickly establish new communities, as new invaders are facilitated by the earlier invaders, a phenomenon which is known as invasional meltdown (Van der Velde et al. 2006 and literature therein). A series of Ponto-Caspian species invaded the Rhine through the Main-Danube canal, whereas only two alien species invaded the Danube through this canal.

Only a few invaders became highly dominant in the Rhine, and these are considered here in more detail. The North-American freshwater shrimp Gammarus tigrinus became very numerous after its appearance in the Rhine in 1982 together with the Ponto-Caspian Zebra mussel (Dreissena polymorpha), which returned to the river when cadmium concentrations in the water dropped in the 1970s and 80s (Van der Velde et al. 1991 and literature therein). After 1986, other Ponto-Caspian species invaded the Rhine, viz. the mudshrimp Chelicorophium curvispinum from the Mittelland canal followed by the freshwater shrimp Echinogammarus ischnus in 1989. After the opening of the Main-Danube canal new Ponto-Caspian species invaded the Rhine and became dominant, viz. the freshwater shrimp Dikerogammarus villosus in
1994/5, the isopod Jaera istri in 1995/7 and two mysid species in 1997 (Van der Velde et al. 2000). These dominant invaders had a large impact on the communities in the Rhine and can be regarded as ecosystem engineers.

Only two biotopes are present in the main channel, viz. sediment (sand and gravel) and stones (rip-rap, groynes). Densities of macroinvertebrates on the sand are low, the community being dominated by Asiatic clams. Densities on the stones, by contrast, are very high and there is severe competition for space. The impact of an invader on the communities can work in a bottom-up or top-down direction. Mutual interactions are responsible for species replacements and subsequent dominance.

The mudshrimp Chelicorophium curvispinum (Figure 6.3) changed the surface of the stones into muddy substrates by building the tubes in which they live (Figure 6.4). C. curvispinum is a filter-feeder taking advantage of algal blooms in the river due to eutrophication (Van den Brink et al. 1991, 1993). As densities ran into hundreds of thousands of individuals per square metre this mud layer became up to 4 cm thick, smothering Zebra mussels and other sessile organisms under the mud (Van der Velde et al. 1994, 1998). This led to a distinct reduction
in biodiversity on the stones (Van der Velde et al. 2002). Predation is another way to dominate the communities. Gammarus tigrinus replaced the native G. pulex and later became replaced itself by Dikerogammarus villosus. Such replacement processes occur mainly by intraguild predation (IGP), which is a method used by generalists to exclude competitors through size-dependent predation, and which can be interspecific or intraspecific (cannibalism). This leads also to avoidance of size classes of these species in space and/or time. Although freshwater shrimps are mainly omnivorous, D. villosus appeared to be the largest, most predatory and competitively strongest of the freshwater shrimps occurring in the Rhine. It not only replaced G. tigrinus (Van Riel et al. 2004) but was also responsible for a severe reduction in the densities of C. curvispinum, thus favouring the Zebra mussels and the freshwater limpets (Ancylus fluviatilis) (Van Riel et al. 2006). The appearance of this freshwater shrimp species prevents insect dominance in the Rhine because insect larvae form an easy prey. However, more and more Ponto-Caspian species can invade the Rhine in which predators and parasites of the invaders are already present, thus weakening their control over the communities till other invaders can take over this role. The decline of C. curvispinum coincided not only

Figure 6.4 Groyne completely covered by mud tubes of Chelicorophium curvispinum becomes visible at low discharge of the Rhine.
with the invasion of *D. villosus* but also with that of endo- and ectoparasites, other predatory macroinvertebrates and fish (Kelleher *et al.* 1998, 2000; Van Riel *et al.* 2003).

**Discussion**

The ecological rehabilitation scheme for the River Rhine has shown that recovery may be only partly successful. The greatest successes were obtained by improving the water quality. However, the river is still far from natural, due to irreversible anthropogenic changes relating to the large number of functions the river is expected to fulfil. Measures like habitat and the digging of new side channels are only possible in the river forelands. The river’s navigation and discharge functions prevent continuing natural succession of the riparian forest, which leads to persistent human interference. It must be realized that the Rhine ecosystem has been destroyed over a period of more than a century, and it is not likely that it will return to its original state as long as human impact remains (Lenders *et al.* 1998). The canals connecting various river catchments which were in the past isolated from each other will remain and continue to be used as routes for more and more invading species, which are already underway. This will continue to change macroinvertebrate communities in the Rhine making them increasingly resemble those of the Danube (Bij de Vaate *et al.* 2002). Attempts have to be made to achieve a further reduction of pollutants from diffuse sources, while a further lowering of the salt concentrations can perhaps provide more chances for rheophilous insect species than for crustaceans. It is also to be expected that more and more thermophilous species will enter the Rhine. Native species will only return if more diverse habitats are created, increasing heterogeneity and providing more shelter options and a variety of food sources. Water quality improvement following pollution has also led to the invasion of alien species in other waters, as the barrier of polluted stretches was removed. Strayer *et al.* (2005) studied the Hudson River and state that restoration projects in large rivers are often accompanied by alien species in one way or another. The plans currently being implemented in The Netherlands, whose intention is to provide the river with more space to move in, will also lead to greater dynamics in the macroinvertebrate communities (increased turn-overs), with unpredictable outcomes. Larger numbers of these species will also invade our canals and lakes and can cause economical and ecological damage (Van der Velde 2001).
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Discussion and concluding remarks

This final section summarizes the concluding plenary discussion and also captures some of the main issues brought forward by the various speakers. Discussions and recommendations focused on the following themes:

1. The forgotten or unknown Kingdoms: status and trends in knowledge (challenges and constraints)
2. Strategies for protection
3. Red List and cryptobiota

The forgotten or unknown Kingdoms: status and trends in knowledge; challenges and constraints

From the various presentations and the discussions it appears that several terms can be used to describe the status or appeal of small organisms – or cryptobiota: forgotten, unknown, neglected, overlooked. Despite the advancement of modern biosystematics, for instance through the application of DNA-sequencing, we know relatively little about the diversity and ecology of micro-organisms compared to higher plants and animals. We know even less about distribution, status and trends of species. Partly as a result of the above factors, cryptobiota are still largely neglected by nature conservation and policy makers. The crucial role of taxonomists in changing this unfavorable situation was stressed and supported by the seminar. Gaining more taxonomic knowledge will also benefit other fields, including zoology, botany, microbiology and nature conservation. There is a continuing need for well-trained taxonomists, recognition of their niche, and financial support for taxonomic research and data collection.

No agreement was reached on the proposition that stronger emphasis on the economic value of cryptobiota would help to draw more attention (and funds) to taxonomy. Some argued that there is a risk when only economic values are taken into account, which could lead to further neglect and decline of ‘useless’ species. A stronger ‘economiza-
tion’ could also threaten the independent position of taxonomists. Others supported the proposition, stating that factual evidence and visibility of the economic value, for instance for agriculture and the health sector, would certainly help to conserve forgotten Kingdoms. A growing number of examples confirm this view, including the conservation of Catskills catchment area which is vital for water supply for New York City.

A third issue that was discussed in relation to the status of knowledge, was the role of amateurs. The seminar concluded that amateurs play an important role in data collection, particularly for attractive groups such as butterflies and shells. Their contribution, however, is often undervalued by the government and policy makers. Amateurs complement scientific data collection, and both activities are interlinked. The difference is that only scientists receive a salary for their efforts.

**Strategies for protection of cryptobiota**

Effective habitat protection is the key for conservation of both forgotten and known Kingdoms – or cryptobiota and macrobiota. Focusing on conservation of single species is a temporary solution, although it may help to raise awareness for the habitats they depend upon. However, this only applies to the larger and popular species which have a strong link to a particular habitat, such as for instance polar bears (arctic), panda bear (bamboo forests) and lion (savanna).

Participants stressed again that too little is known about cryptobiota, particularly of their conservation status. A number of factors complicate progress, including the lack of appeal to the larger public, small size and short life, strong fluctuations in population size, and difficulties in monitoring. As a result, measurement of success in cryptobiota conservation is no easy matter.

Given the above constraints, the seminar discussed possible ways to effectively conserve cryptobiota. The following suggestions were made:

- The focus of nature conservation should be on the protection of healthy habitats and whole ecosystems, both inside and outside protected areas. Larger, appealing macrobiota species – flagship species – could help to draw attention to a particular ecosystem
under threat, also benefiting the conservation of less conspicuous species such as cryptobiota.

- Small creatures need small scale management, and reserve managers need to learn and understand the importance of small landscape elements like rocks, dead trees, etc. Even small sites may be quite valuable for cryptobiota.
- Dutch conservation policy is focusing on hotspots and target species. However, such a rather static approach has its shortcomings. More attention should be paid to spatial and temporal dynamics of species and ecosystems in designing conservation policies, and a critical analysis is needed of the various assumptions that form the basis of such policies.
- There is a tendency to apply rigorous management measures, for instance in river restoration, without knowledge of their impacts on the various ecosystem components. Particularly cryptobiota could suffer from such improperly planned approaches.

**IUCN Red List and cryptobiota**

This final theme gave rise to heated discussions and different views were aired on the usefulness of the Red List for cryptobiota (conservation). Some participants expressed concern regarding developments in the IUCN Species Survival Commission and Species Specialist Groups. The attention and focus on cryptobiota, for instance ants, has declined. Others argued that this should not prevent organizations and individuals from continuing their data collection and survey work, and that it could also offer new opportunities and initiatives.

The Red List of threatened species has two main functions: 1) informing and influencing national and international government policies; and 2) raising public awareness. Currently, few representatives of the cryptobiota – e.g. ants, beetles, dragonflies – have been assessed and listed. Factors for this poor representation have been mentioned earlier: low public appeal, poor status of information, hard to monitor, etc. On the one hand, some participants recommended gradually expanding the Red List to include cryptobiota. On the other hand, other participants expressed strong doubts about the usefulness of the Red List. The usefulness is doubtful because of the current poor status of information on the cryptobionts, while it was also argued that the addition of more Kingdoms and taxa may even weaken the effectiveness of the Red List itself (particularly when the information is sketchy and less reliable).
Finally, the seminar recommended to list taxonomists as a vulnerable or even threatened species. Taxonomy is being marginalized as a scientific discipline, and financial support for taxonomic institutions is declining, while taxonomy is at the basis of all biology. There is a growing discrepancy between the ambitious targets and work programs agreed upon by international agreements, especially the Convention on Biological Diversity (CBD), and the actual efforts on the ground.
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