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RESEARCH ARTICLE

Biodiversity and Ecosystem Functioning in Transitional Waters; the point of view of a microbial ecologist

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Abstract

1 - The study of biodiversity of prokaryotes in transitional waters is hampered by the fact that we do not posses any comprehensive inventories of prokaryotic species. Microbial biodiversity is thus often estimated indirectly by extraction of nucleic acids from the natural environment and by using 16S ribosomal RNA gene sequences as phylogenetic and taxonomic markers. Experimental microbial ecology has contributed over the years to developing niche-assembly theories of community assemblage and I review some of their major findings. Finally, the aim of establishing a link between biodiversity and ecosystem functioning is quite recent and has prompted a novel experimental approach that can be applied in microbial ecology. The following observations have been forwarded.

2 - The species richness for prokaryotes is difficult to assess in transitional waters, because of an unclearly defined species concept and because the Bacteriological Code requires living cultures as type strains for taxonomic descriptions; therefore, the number of validly described prokaryote species is still very limited (i.e. currently less than 10,000).

3 - The major emphasis on niche assembly theories in microbial ecology is a consequence of the paradigm "everything is everywhere, but the environment selects" quoted by Baas Becking in 1934. Hence, insights on how competition mechanisms can contribute to sustaining microbial biodiversity in relatively homogeneous model systems have tremendously increased during the last decade. Nevertheless, I argue that coastal lagoons and other transitional waters are environments characterised by strong exchanges with adjacent local microbial communities, where community assemblage may be strongly impacted by dispersal processes.

4 - The study of biodiversity and ecosystem functioning is based on quantifying a process rate or a yield (the ecosystem function) as a function of a manipulated biodiversity in an artificial community. A positive relation between biodiversity and ecosystem function has been explained by 1) sampling effect (i.e. a higher biodiversity correlates with a higher chance of providing a home for the most productive species), 2) more efficient resource exploitation for species showing complementary niche differentiation. I argue that synergistic phenomena due to positive interactions among species may also play an important role.

Keywords: coastal lagoon, microbial biodiversity, resource ratio theory, dispersal, positive interaction

Introduction

Providing a home for biodiversity is an important ecosystem service of coastal lagoons. A long tradition of natural history studies has provided data bases of the species richness of macro-organisms in many coastal ecosystems including lagoons. Many of such records have been provided by dedicated professional and amateur field biologists. As a result, the accessibility and quality of such data bases are extremely variable between sites and between historical periods, as they depend on the taxonomic interest and knowledge of these field observers and on the extent and frequency of their field work as well as on the quality of the archive. Web-based publications of such data bases are now being prepared for different ecosystems and the data base held by the Hellenic Network on Research in Transitional Ecosystems is a good example for lagoon ecosystems (http://www.medobis.org/dbase-elnet/). In some cases detailed accounts do exist for microscopic organisms such as microalgae and cyanobacteria and to a lesser extent for the microscopic protozoa, as these have been described by their morphological features using microscopy (e.g. Krammer and Lange-Bertalot, 1986-1988-1991; Geitler, 1932). In many countries, systematic surveys and monitoring of phytoplankton species in coastal waters based on light microscopy have been implemented, because they can be used as indicators for water quality and because of the need for providing alarm systems for harmful algal blooms (Gailhard *et al.* 2002). In contrast, we do not posses any comprehensive, detailed and reliable species lists and inventories of prokaryotes in coastal lagoons. Even the estimations of the actual number of species in a given marine environment are vague and subject to controversies (Hong *et al.*, 2006).

Bacteria and Archaea are prokaryotes as opposed to eukaryotes, which also include microbes such as microalgae and protozoa. In addition, there are the viruses, which are very important in natural environments. However, the viruses are not self-replicating as they depend on a host organism for replication of genetic material and reproduction. Therefore, I have not included the viruses in my analyses of microbial diversity. While most of the prokaryotic forms of life are small and morphologically rather simple, they comprise an extremely wide spectrum of metabolic options. These include aerobic and anaerobic metabolisms and encompass capabilities to synthesise and degrade the largest spectrum of organic compounds, to perform redox conversions of a large number of inorganic minerals, and to harvest light for photosynthesis from a wide light spectrum, i.e. from ultraviolet UV A (370 nm) into the near infrared (up to 1030 nm). Therefore, prokaryotes play pivotal roles in all biogeochemical cycles and their activities are paramount for the functioning of coastal lagoon ecosystems. An example of a collaborative study of coastal lagoons where microbial processes received strong attention was the EU project entitled "ROle of BUffering capacities in STabilising coastal lagoon ecosystems", in which it was found that prokaryotes are major actors in providing buffering capacities in coastal lagoons (De Wit *et al.*, 2001). This research project paid some attention to microbial biodiversity (e.g. Cifuentes *et al.*, 2000, 2003), but more frequently the microbial compartment has been studied as a black box, as is common in many biogeochemical studies in coastal environments.

The study of microbial diversity in natural environments is still in its infancy and in this paper I will discuss some of the major problems and challenges in this developing field. Because microbial ecology has typically been linked to general microbiology and to biogeochemistry, this discipline has developed into a different branch with too little interaction with mainstream ecology. Nevertheless, traditionally, experimental microbial ecology has contributed to developing niche concepts and increased our understanding of ecological interactions between species. By using model organisms, this discipline has drawn advantages from the small size and short generation times of micro-organisms. Thus, it has been possible to test the outcome of ecological interactions in communities comprising billions of individuals for the long term (tens to hundreds of generations) and, for example, to test the principle of competitive exclusion (Hardin, 1960). I briefly review the major findings of these studies and discuss their relevance for understanding biodiversity. In a world that is increasingly impacted by humans and faces important biodiversity losses, the question of how biodiversity impacts ecosystem functioning is urgent. This calls for developing a novel research approach using experimental systems with a manipulated biodiversity. Some good studies are now available for microbes, which will be discussed. Finally I will conclude and focus on the importance of understanding biodiversity and ecosystem functioning in coastal lagoons and other transitional waters.

Describing microbial biodiversity

The taxonomy of prokaryotes is governed by the Bacteriological Code (i.e. International Code of Nomenclature of Bacteria, 1990 revision; Lapage *et al.*, 1992), which refers to living type strains and uses many physiological features as diacritic criteria in the determination of bacterial species. This is entirely different from botanic systematics, which is based on dried and dead specimens stored as type taxa in a herbarium. Hence, identification of bacteria is mainly dependent on cultivation techniques. There are, however, some conspicuous forms of bacteria that allow them to be assigned to a genus, as is the case for *Beggiatoa*, *Chromatium*, *Achromatium*, *Bacillus* and *Clostridium*. But most bacteria observed under the light microscope have a simple morphology like coccoid, bacil shaped or vibriod single cells with little detail, and their taxonomic identity remains elusive even for a trained microscopist. Therefore, the study of the natural history of bacteria is poorly developed.

Because the reproduction of prokaryotes is mostly asexual, and therefore we cannot apply the criterion of interbreeding in the natural environment, the application of the biological species concept according to Mayr and Ashlock (1991) is not appropriate. Hence, a phylogenetic concept of species seems more appropriate for prokaryotes (Ogunseitan, 2004), although in practice prokaryotic taxonomy currently uses a combination of genomic and phenotypic data (phylophenetic concept). Accordingly, the prokaryotic species should ideally be described as “a category that circumscribes a genomically coherent group of individual isolates/strains sharing a high degree of similarity in many independent features comparatively tested under highly standardised conditions” (Oren, 2004). Relevant genomic properties include the nucleotide base ratio in DNA (expressed as the molar % of guanine plus cytosine, i.e., G+C), DNA-DNA hybridisation and the sequence of selected phylogenetic markers, which particularly includes the sequence of the 16S ribosomal RNA genes.

Another particularity is that for a long time the taxonomic diversity of prokaryotes has been grossly underestimated. Thus, in 1969, Whitaker (1969) claimed that the so-called Monera kingdom comprised about 5000 bacterial species, which he compared to more than a million animal species (insects alone represent about a million species) and hundreds of thousands of plant species. The taxonomy he used was based on five major kingdoms, i.e. Monera, Protists, Fungi, Plants and Animals (see Fig. 1).

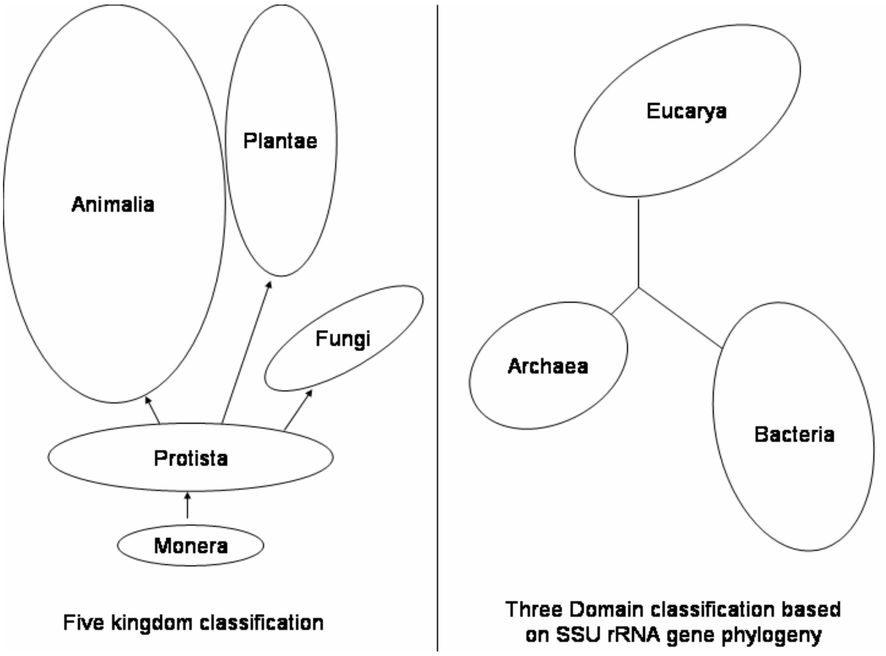

Fig. 1 Left: The Five Kingdom classification proposed by Whittaker. This taxonomic system is currently used by the European Register of Marine Species (ERMS). Note that all the prokaryotes belong to the Monera Kingdom. For a long time it was thought that the Monera represented only a marginal proportion (i.e. less than 0.5 %) of species numbers (Whittaker, 1969). This idea and this scheme are nowadays rejected by most microbiologists. Right: Classification of all self-replicating life forms into Three Domains based on phylogenetic clustering inferred from sequence analyses of SSU rRNA genes. Note that all eukaryotes are grouped into the single Eucarya Domain, while the prokaryotes are split into two domains.

Accordingly, the Monera comprised all the prokaryotes. It was realised by Stanier and his colleagues that the formerly-called blue-green algae are in fact prokaryotes and are, therefore, nowadays called cyanobacteria (Rippka *et al.*, 1979). The taxonomic system based on five kingdoms is still promoted today by Margulis and Schwartz (1998) and is used as a reference for the European Register of Marine Species (ERMS) drawn up by the European Union network of excellence of marine biodiversity (MarBEF Data System - ERMS Taxon search). Nevertheless, most microbiologists today do not accept this five-kingdom taxonomic scheme (see Fig. 1). As a matter of fact, our view of prokaryotic biodiversity and phylogeny has been revolutionised by the accumulated wealth of genetic sequence information on selected genes. Thus, Woese (1987) and his followers selected ribosomal RNA (rRNA) genes, which are present in all self-replicating organisms, as a basis for phylogenetic analyses of all of them in a coherent scheme. The basic idea was that this gene can be used as a so-called molecular chronometer, implying that sequence dissimilarity is a measure of evolutionary divergence among species, which makes it possible to infer phylogenetic trees. The phylogenetic tree of life based on sequence analyses of the small subunit (SSU) rRNA genes does not support the five kingdom concept; rather, three major clusters emerge (Woese *et al.*, 1990), which are depicted in Fig. 1 (see also The UC Museum of Palaeontology at Berkeley: http://www.ucmp.berkeley.edu/alllife/threedomains.html). Two of these major clusters or so-called domains are prokaryotic, i.e. the Bacteria and the Archaea. All the eukaryotes are grouped together in the Eukarya domain. This scheme, rather than the five kingdom taxonomic system, is more likely to reflect the evolution of life since its beginnings about 3.9 10^9 years BP. The primitive life forms were prokaryotes and the first eukaryotes probably appeared 1.9 10^9 years BP; it was only in the Phanerozoic (0.55 10^9 years BP – present) that the eukaryotes showed their strong evolutionary diversification. Hence, the prokaryote domains have had a much longer time span to evolve and it is not surprising that they have evolved into two of the three widely branching domains. An illustrated guide to life following the SSU RNA universal tree of life has been presented by Lecointre and Le Guyader (2001). Such illustrated guides are important to disseminate this new view of phylogeny and evolution among those interested in natural history and make it possible to bridge the gap between microbial ecologists and field biologists mainly interested in macro-organisms.

It is surprising that the numbers of described species are about 9,000 both for birds and for prokaryotes each, i.e. 9,762 bird species and 9,280 prokaryotes (the latter comprising 9,021 Bacteria and 259 Archaea species (Harrison *et al.*, http://cnx.rice.edu/content/m12174/latest/#landg based on data provided by Lecointre and Le Guyader, 2001). Nevertheless, this comparison is a bit ridiculous, because to make it comparable one should restrict the number of bird species to those that are successfully raised and reproduced in captivity. The number of 9,280 described prokaryotic species contrasts with the 197,833 SSU rRNA gene sequences of prokaryotes (i.e. the SSU of prokaryotes has a size of 16 S, hence 16S rRNA genes for prokaryotes) deposited by 4 January 2006 in the Ribosomal Data Base Project (http://rdp.cme.msu.edu/index.jsp). This data base is rapidly expanding and has increased fourfold the number of gene sequences since its first release in September 2002 (see Fig. 2).

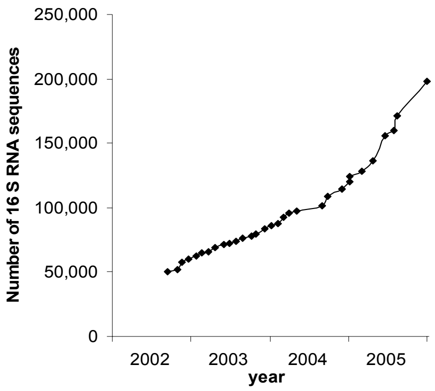

Fig. 2 Increase in the number of 16 S rRNA gene sequences of prokaryotes deposited in the Ribosomal Data Base Project (http://rdp.cme.msu.edu/index.jsp) maintained as a public service at the University of Michigan. Each researcher who has retrieved a 16 S rRNA gene sequence, either from a culture of a prokaryote or directly from the environment, can compare his sequence with the sequences in this data base by internet access. This way, he will find the nearest neighbour with which it shares the maximum of sequence homology and he will be able to project the source organism of his sequence in the phylogenetic tree.

However, it remains to be decided whether two different 16S rRNA gene sequences indicate that the source organisms belong to different species. As a rule of thumb it has been proposed that when two sequences differ by more than 3 % (i.e. less than 97 % sequence homology) the source organisms may represent different species (Stackebrandt and Göbel, 1994), although this criterion remains arbitrary and controversial. Using a proximal approach, an SSU rRNA gene sequence retrieved from the environment makes it possible to project the source organism in the phylogenetic tree of life. Nevertheless, for a validated description of a new prokaryotic species, one still needs to conform to the Bacteriological Code and therefore to deposit a living culture of an isolated strain as the type strain for that taxon. A major problem is, however, that we are currently unable to culture the vast majority of microbes and to isolate them as pure strains. This phenomenon has been described as non-culturable micro-organisms or rather not-yet cultured micro-organisms, thus expressing the hope that we will perhaps be able to culture them in the future.

The harvesting and PCR amplification of SSU rRNA and of SSU rRNA genes directly from the environment and their sequence analyses is nowadays a common technique in molecular ecology. In this way, the study of microbial diversity has become independent of cultivation techniques (Amann, 1995). The SSU rRNA gene from prokaryotes has a lower weight than that of eukaryotes, which are accordingly referred to as 16S rRNA and 18S rRNA, respectively. Typically, 16S rRNA gene sequences that share more than 97 % of sequence homology are lumped together in a so-called phylotype, which is sometimes also called an operational taxonomic unit (OTU) that can be considered as a proxy for a prokaryotic species. From these studies it has been inferred that prokaryotic biodiversity is much higher than expected and that the total number of species is more likely to be in the millions (Curtis *et al.*, 2002; Oren, 2004). Nonetheless, this view is controversial and extrapolations suffer from many uncertainties (Hong *et al.*, 2006). For example, the theoretical calculations of Curtis *et al.* (2002) suggested that a ton of soil may contain 4 million different bacterial taxa, while the global biodiversity of the pelagic compartment in the ocean comprises significantly less – although it still might reach 2 million. For the ocean, this number was questioned by Hägstrom *et al.* (2002) who analysed the 16S rRNA gene sequences in the global data base for documented marine representatives. They concluded that the number of new marine phylotypes added to the data base peaked in 1999, since then there has been a deceleration and by 2002 they reported 1,117 phylotypes. Hence, it was concluded that the apparent marine bacterioplancton species diversity is relatively low (Hägstrom *et al.*, 2002).

Molecular ecology techniques thus make it possible to study the biodiversity of specific environments in accordance with these phylogenetic approaches. An attractive approach is to construct a clone library based on sequence analyses of 16 S rRNA genes retrieved from a specific environment. Examples of such studies applied in coastal lagoon environments are provided by Benlloch *et al.* (1995) and Cifuentes *et al.* (2000, 2003). By counting the number of phylotypes (using the 97 % sequence homology as a criterion for their delineation) and considering their frequency of occurrence, one can calculate the classical different biodiversity indices. For an evaluation of the different indices in the context of highly diverse microbial communities see e.g. Hill *et al.* (2003). The total number of species in a sample can be inferred from the coverage, by studying the rarefaction curves, or from statistical inference based on models of species rank abundance distributions (Hong *et al.*, 2006).

How to explain biodiversity?

For a long time biodiversity research has been dominated by the question of what causes biodiversity? Therefore, many studies have aimed to plot a measure of biodiversity (ordinate) as a function of environmental parameters (abscissa) – see Fig. 3. In most cases a difference is made between α and β biodiversity. β biodiversity relates to spatial differences in environmental factors and therefore supports different habitats providing opportunities for different species. In the context of coastal zone management and nature conservation, habitat conservation (maintenance of gradients, mosaics and ecotones) is a most important issue. Microorganisms will experience environmental variation at small spatial and temporal scales. Particularly where microorganisms can adhere on surfaces e.g. as epiphyton or epilithon and in soils or sediments, many microhabitats are provided allowing a high degree of β biodiversity.

α biodiversity relates to the coexistence of species in the same habitat and ecological theory is required to explain coexistence patterns. Classically, the coexistence of species in the same habitat is explained by different and competing theories, i.e. niche assembly rules, or alternatively assuming dispersal limitation. The first theory is fully deterministic and assumes that the final community assemblage is determined by the traits of the different species and that the final community is in equilibrium with the environmental conditions. The latter theory reserves a paramount role for stochasticity and predicts that the species composition of the community will dynamically fluctuate in time as inhabitants die off and are replaced by newcomers from adjacent communities.

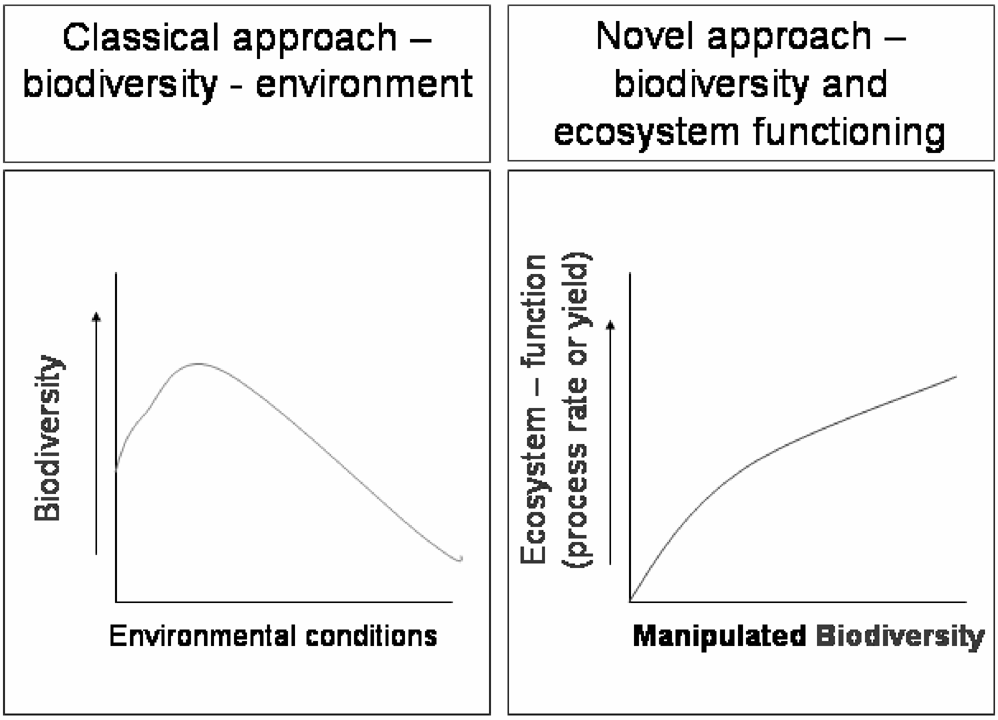

Fig. 3 Comparing the classical approach in biodiversity research with a novel approach seeking to describe the relationship between biodiversity and ecosystem functioning. The classical approach seeks to establish a relationship between environmental parameters and biodiversity measures. The depicted curve represents the frequently reported case where biodiversity is optimal at intermediate values of the environmental parameter. The novel approach seeks to establish a relationship between biodiversity and ecosystem functioning. For a rigorous experimental approach it is required that the biodiversity is manipulated experimentally. Therefore, different artificial communities have been established at different species richness levels based on a random selection of the species added to the different artificial communities. For each of the artificial communities a specific process or yield will be quantified to study this variable as a function of biodiversity.

For theory development in some cases, it has even been assumed that the interaction between the species within the habitat is neutral (e.g. Hubbell, 2001). Most microbiologists adhered to the statement that "Everything is everywhere, but, the environment selects" (Baas Becking, 1934). This statement implicitly rejects the neutral dispersal limitation theory, which is based on biogeographical patterns and stochasticity. Therefore, Baas Becking took a strongly deterministic approach and can be considered as a predecessor of a deterministic niche-assembly theory (De Wit and Bouvier, 2006). Nonetheless, while Baas Becking claimed that proliferation of bacterial species is fully determined by environmental conditions, he was not really aware of the importance of competition. An experimental study of microorganisms (two *Paramecium* species) was conducted by Gause in the same period (Gause, 1934). Most theories and experimental work developed by microbial ecologists have been based on the statement by Baas Becking, which has been used as a real paradigm. Currently, this paradigm is questioned in the microbiological community and the study of the biogeography of micro-organisms is a developing field of research.

Transitional waters are typical environments where dispersal of microorganisms is obviously an important phenomenon, particularly for the pelagic compartments. Thus, coastal lagoons for example receive high inputs of prokaryotes and micro-algae through the tributaries from their watershed and from the coastal ocean (see Fig. 4). While such

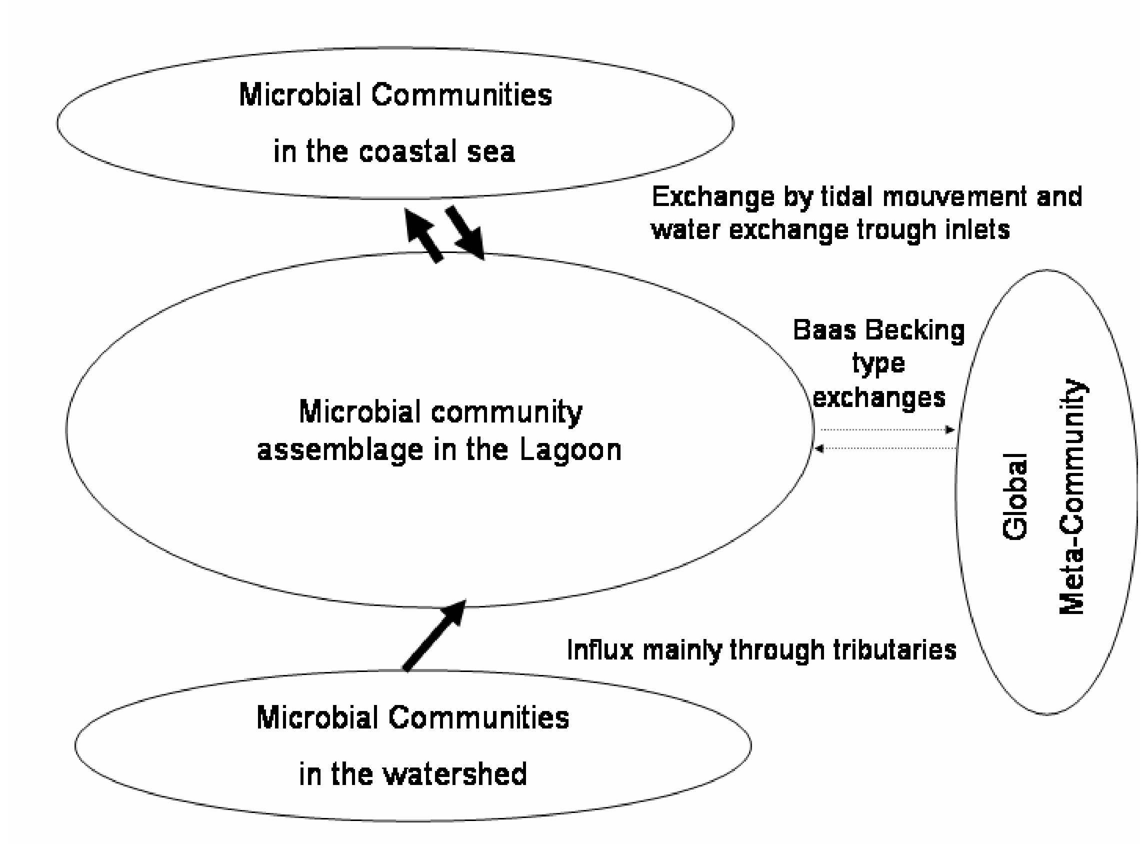

Fig. 4 Major exchanges and interactions among local microbial communities in a landscape with a coastal lagoon. Obviously, the study of microbial communities in coastal lagoons needs to take into account these exchanges and should therefore incorporate the pertinent information on dispersion and exchanges among these communities. Therefore, the water columns of coastal lagoons and other Transitional Water Bodies represent typical environments where dispersal-limitation community assembly theories may provide a pertinent framework. However, coastal lagoon waters have specific characteristic features (e.g. salinity), which may present a competitive advantage for certain species. Therefore, strict neutrality as an assumption seems inappropriate.

"allochthonous" organisms are probably not optimally adapted to proliferate in the transitional water body, they may survive and even grow slowly. In this way they contribute to biogeochemical processes in the transitional water body and when they immigrate with high densities they may even compete for resources with micro-organisms that are in principle better adapted to the local conditions.
Rather than the alternating straight line segments.

Thus, in some cases, it is perhaps possible that immigrants from adjacent communities completely prevail over the micro-organisms that are in principle better suited to the prevailing environmental conditions, which is in contradiction with "the environment selects" statement. Hence, community structure in transitional waters may be strongly influenced by the dispersal phenomena. While, personally, I can not accept the hypothesis of complete neutrality between species, I certainly think that transitional waters represent an attractive model for microbial ecologists to study the influence of dispersal mechanisms and of exchange between adjacent communities on microbial community structure. Recently, an elegant study by Lindström and Bergström (2004) described the extent to which the bacterioplancton communities of lakes can be determined by the bacterioplancton communities from the tributaries. Thus in a Swedish lake with a short hydraulic retention time (0.3 years), the bacterioplancton community was estimated to be 80 to above 90 % similar to the communities in the major inlets. The authors carefully considered that such a similarity can be explained by either the direct input of riverine bacteria that grow and survive in the lake or alternatively by the similarity of environmental conditions between the lake and its inlets, which would favour the development of similar communities. A statistical analysis showed best correlations with the amount of imported cells into the lake and it was therefore concluded that input of riverine bacteria was a major factor in shaping the assemblage of communities in lakes with short hydraulic retention times (Lindström and Bergström 2004).

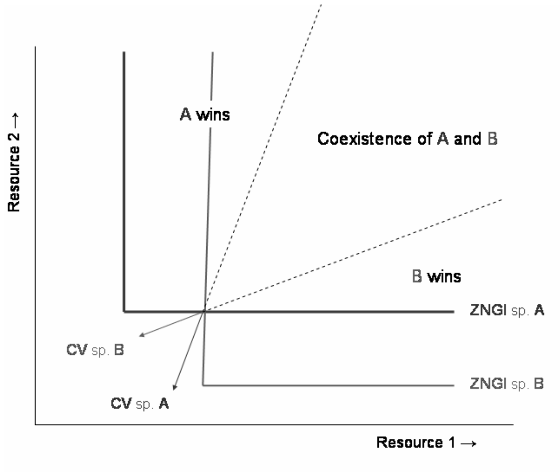

Fig. 5 Graphical representation by Tilman (1982) of Resource Ratio theory. For the given conditions, this theory predicts an area of resource supplies where species A and B can coexist stably. The grey and black line segments depict the so-called Zero Net Growth Isoclines (ZNGI) for species A and B, respectively. In general, each species will deplete the resources delivered to the habitat to a level described by its so-called ZNGI. A stable coexistence of A and B is only possible if the intersection point between the ZNGI's of both species becomes the stable attractor of that system. Therefore, one needs to consider the so-called consumption vectors (CV) for both species. The grey and black vectors define the CV for species A and B, respectively. Stable coexistence of both species is only possible if each species consumes most of the resource that is most limiting for its growth (as in this example). Otherwise, the coexistence is not stable and the intersection point represents a labile equilibrium, which is disturbed at any minor deviation. In the stable case, a sector can be defined where A and B are predicted to coexist stably as starting from the ZNGI intersection point and delimited on either side by the slopes of both CV's. Note that in this example, the ZNGI's show that only a single resource can limit the growth of a species. Nevertheless, this graphical model can accommodate multiple resource limitation by using hyperbolic ZNGI's

Nonetheless, mainstream experimental microbial ecology has focussed on niche assembly rules and mainly studied competitive interactions and cross-feeding phenomena (cooperation and commensalism). Since the 1940's it has developed a superb experimental tool for such studies, namely the continuous cultivation technique (Monod, 1950; Veldkamp and Kuenen, 1973). In this system, all species that have specific growth rates below the dilution or washout rate imposed by the system progressively disappear, and the device can in theory be maintained under constant environmental conditions indefinitely. In practice, experimental microbial communities can be monitored in this system for tens or hundreds of generation times. Hence, it is a perfect tool to test the "competitive exclusion principle" (Hardin, 1960). If growth is limited by a single nutrient, it is predicted and has been generally observed that the outcome of the selection process is a monospecific culture. By studying a range of dilution rates, it has been observed that some species tend to be more competitive at high growth rates, while others perform better at low growth rates (Veldkamp and Kuenen, 1973), which correspond to r and K species, respectively. Since the 1970's, mathematical models have predicted that a stable coexistence of two competing species is possible when these are competing for two limiting resources (Taylor and Williams 1975, Gottschal and Thingstad, 1982, Tilman, 1982), and such coexistence has been confirmed experimentally (Tilman, 1982; Gottschal and Thingstad, 1982). A graphical presentation of such mathematical models is depicted in Fig. 5. These findings resulted in the formulation of a resource ratio theory, promoted by Tilman (1982) and Smith (1993), which provides a mechanistic explanation of how resource supply ratios competitively regulate microbial community structures. Interspecific variability of specific resource uptake ratios and of net-zero growth isoclines allow the stable coexistence of species, each limited to a different extent by the different substrates. Thus, under constant resource supply, the maximum number of stably coexisting species at equilibrium is expected to be equal to the number of limiting nutrients. It further predicts that a change in resource supply ratios will induce a shift in the competitive dominance of different microbial species. A good example predicted by this theory is the shift observed from diatom-dominated communities to dinoflagellate-dominated communities in coastal environments when the N to Si ratio goes above 1 mole/mole (Rabelais *et al.*, 1996).

A basic version of resource ratio theory expects communities to achieve equilibrium under constant environmental conditions. In the 1970's it was experimentally observed that fluctuating resource supply makes it possible to increase the number of coexisting species above the number of limiting nutrients (Van Gemerden, 1974; Turpin *et al.*, 1979). This is also predicted by the Intermediate Disturbance Hypothesis (Connell, 1978). Thus, long stable conditions allow competitive exclusion and favour K-strategists, while disturbance creates new conditions for r-strategists. At the intermediate level of disturbance, both r and K specialists will be able to coexist with fluctuating densities.

The resource ratio theory defined by Tilman (1982) is solidly based on experimental observations with two limiting nutrients (see Fig. 5) and has perhaps too heedlessly been extrapolated to much larger numbers of limiting nutrients. Hence, it was a spectacular surprise when Huisman and Weissing (1999) found that when three or more substrates are limiting then in many cases a stable equilibrium is not achieved. For example, model predictions showed that nine species can coexist when only three substrates are limiting, albeit at highly fluctuating densities. The variations in population densities are not imposed by externally changing conditions, but rather induced by the competition process. Such wax and wane phenomena have been described as deterministic chaos. In the long-term, this would explain the coexistence of a very high number of microbial species in aquatic environments and it has been invoked as a mechanism explaining "the paradox of the plankton" (Scheffer *et al.*, 2003). This paradox was phrased originally by G.E. Hutchinson (1961) and raised the question of how so many species can coexist in an apparently very homogenous environment such as the mixed water column.

Finally, I highlight the importance of novel ecological theories that describe the possibility of multiple stable states of the ecosystem, each of them characterised by a different community structure (Scheffer *et al.*, 1993). This theory was originally developed for shallow freshwater lakes, which may show the following alternate conditions i) clear water with an abundant macrophyte vegetation and ii) highly turbid water where productivity is dominated by phytoplankton. These alternate states represent alternative attractors each stabilised by positive feedback loops (Scheffer, 2001). A good study in the coastal environment has been provided by De Koppel *et al.* (2001) who showed two stable states for communities on intertidal flats, i.e. i) a silt-rich diatom-dominated surface and ii) bare sand. An important conclusion from this work is that at relatively small scales these multiple stable states can generate mosaic structures in a spatial setting and thus contribute to generating β biodiversity in the environment.

In conclusion, some theories have put a lot of emphasis on the role of the external variability of environmental factors in explaining biodiversity. These theories claim that equilibrium will not be achieved in the natural environment, which thus prevents competitive exclusion and allows for the coexistence of larger numbers of species. However, recent studies show that external variability is not necessary to prevent a stable equilibrium, because fluctuations in time and spatial heterogeneity can be induced by the biological interactions between the different species in the community.

A novel approach – biodiversity and ecosystem functioning

Interest in the link between biodiversity and ecosystem functioning is quite recent and has been promoted by Kinzig *et al.* (2001). Accordingly, a process rate or a yield (the selected ecosystem function) is measured as a function of a manipulated biodiversity using an artificial community. Hence, the selected ecosystem function is plotted on the ordinate and a measure of the manipulated biodiversity (often the number of species) is plotted on the abscissa – see Figs. 3 and 6. A rigorous application of this approach requires that species contributing to the artificial communities are sampled at random from a large species pool and that experiments are replicated many times. This is necessary, because inherent large variation (see Fig. 6) is caused by the community species composition, and this needs to be statistically separated from the effect of the number of species as such. Bell *et al.* (2005) present an early study of bacteria, while complying with these conditions. Therefore, they isolated 347 strains of heterotrophic bacteria from the semi-permanent rain-pools in bark-lined depressions at the base of beech trees (*Fagus sylvatica*). The strains were assigned to 103 species, and microcosms were incubated with a defined number of species sampled from this pool (ranging from 1 to 72). The CO_2 production rate (respiration) was measured as the selected ecosystem function. On average, a positive relation was observed between species richness of the inoculum and respiration rates. Unfortunately, they did not check whether all of the inoculated species survived and grew in the microcosms and, therefore, there is some doubt as to whether the biodiversity at the end of the incubation was the same as that originally added from the inoculum.

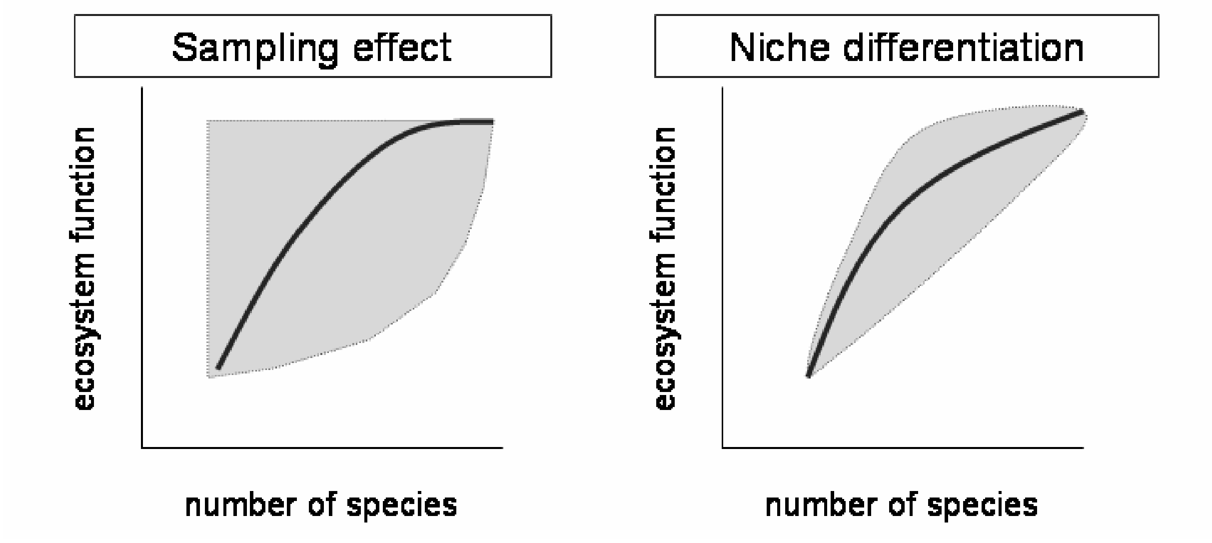

Fig. 6 Shapes of the possible outcomes depicted as domains (light gray) and of the averaged tendency of a selected ecosystem function as a function of species richness. Left panel: sampling effect case; right-panel: complementary niche exploitation case. See text for explanation.

In general, a positive relation between biodiversity and ecosystem function has been explained by 1) a sampling effect (i.e. a higher biodiversity correlates with a higher chance of providing a home for the most productive species), or by 2) more efficient resource exploitation for species showing complementary niche differentiation (Tilman and Lehman, 2001). Both patterns are characterised by different shapes of the outcome domains and the averaged tendencies, as depicted in Fig. 6. When there is a large difference between the performances of different species with respect to the selected function, we will observe a very large dispersion of possible outcomes for the sampling effect case. The upper limit of the domain will be constrained by a single value that corresponds to the value achieved by the best performing species. In contrast, complementary niche exploitation will result in less dispersion of outcomes and in a steeper slope for the initial part of the average curve. The upper limit of low-species-number communities is constrained by the incomplete use of resources and will increase with increasing species diversity. Notice that Bell *et al.* (2005) refer to "selection effect" instead of sampling effect and "complementary effect" for complementary niche differentiation.

Complementary niche differentiation may show on average a linear increase with species number at low species richness, which then decelerates at higher species richness. In contrast, the average curve for the sampling effect starts with a steep increase and progressively decelerates. Both curves tend to an asymptote that represents the carrying capacity for the selected ecosystem function in that environment. In my opinion, Tilman and Lehman (2001) neglect the possibility of synergistic interactions among species. Synergism is, however, mentioned by Bell *et al.* (2005), but their assertion that complementary niche differentiation can be assimilated to synergism, which is not true because synergism means working together to produce an effect that is greater than the sum of their individual effects. Obviously, complementary niche differentiation will merely result in the summing of the individual effects. A case of real synergism was observed by Massé *et al.* (2003), who grew monocultures and a mixed culture in laboratory microcosms of a green and a purple sulfur phototrophic bacterium, which were both originally isolated from coastal lagoon sediments in France. In this case, it was observed that the final pigment yield (a biomarker for biomass) greatly exceeded the sum of the values observed in the monocultures. Hence, synergism will introduce an acceleration of the curves shown in Fig. 6 and allow an even more pronounced positive impact of biodiversity on ecosystem functioning. This possibility deserves more attention in theory development.

Conclusion and perspective

The study of the relationship between biodiversity and ecosystem functioning is still in its infancy, and this is particularly so for micro-organisms. Coastal lagoons represent an interesting, albeit very complex site for microbial biodiversity studies (continent – ocean interface). In general, transitional waters represent attractive models for studying exchange between adjacent microbial communities and can therefore contribute to understanding the role of dispersal phenomena with respect to niche assembly mechanisms for microbial community assembly. So far, in experimental microbial ecology, the emphasis has been too narrowly focussed on studying the niche assembly rules. While these biodiversity studies are very important from a fundamental point of view, I argue that there is still a place for classical biogeochemical approaches (microbes as a black box) in the study of transitional waters. This is because biogeochemical processes and the microbial component are very important for understanding the biodiversity of macroscopic organisms.

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