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Microalgae in Ecology: Ecosystem Functioning Experiments

Bastian Steudel[1,2*]

[1]*Experimental Phycology and Culture Collection of Algae, Georg-August-University Göttingen, Albrecht-von-Haller Institute, Nikolausbergerweg 18, 37073 Göttingen, Germany*

[2]*Biodiversity, Macroecology and Conservation Ecology, Georg-August-University Göttingen, Büsgenweg 1, 37077 Göttingen, Germany*

Corresponding author: Bastian Steudel, Georg-August-University Göttingen, Albrecht-von-Haller Institute, Experimental Phycology and Culture Collection of Algae, Nikolausbergerweg 18, 37073 Göttingen, Germany, Tel: 49-0551-39-137; E mail: bastiansteudel@aol.com

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Ecosystem functioning is one of the most striking topics in ecological research. All human beings depend on the functioning of ecosystems and the services provided by ecosystems [1]. Ecosystem services provide food from e.g. agriculture and fish as well as clean water and air. Thus, the functioning of ecosystems is a crucial topic of experimental studies in ecology. The first known ecological experiment was on increases of biomass in mixed cultures of grasses compared to monocultures of the species [2,3]. Although, this experiment gave an empirical evidence of positive effects of species richness on biomass production [2] similar experiments were repeated in the 20th century and finally a series of meta-analyses was necessary to reach a consensus about positive biodiversity-ecosystem functioning effects, i.e., biodiversity effects [4-7]. Most of these manipulation experiments observing biodiversity effects on ecosystem functioning were conducted in grassland ecosystems or with grassland species [6,7]. This surprises not because terrestrial ecosystems are of high commercial interest [8,9]. However, aquatic ecosystems provide about half of global net primary production [10]. The understanding of the processes underlying the ecosystem functioning of aquatic systems and the impact of global change is far beyond our understanding of the terrestrial systems [11,12]. As results from terrestrial systems cannot be transferred directly to aquatic systems [13], studies on the functioning of aquatic ecosystems are required.

The general biodiversity-ecosystem functioning effects of species richness, i.e., increase in ecosystem functioning and increase of the stability of ecosystem functioning under ambient environmental conditions, of aquatic systems show similar patterns like terrestrial systems [4]. However, compared to their importance of primary production, aquatic ecosystems are seldom studied regarding their functioning. In a similar way, in most cases species richness was manipulated and other aspects, such as functional diversity, genetic diversity, or epigenetic diversity was less studied [4,6,7,14]. This is particularly interesting, since most theoretical approaches like the "insurance hypothesis" do not differentiate between the different "levels" of biodiversity [15]. Thus, any increase of biological diversity, i.e. biodiversity, is considered to contribute to an increase of ecosystem functioning. And indeed, genetic [16-18] and even epigenetic diversity of one single species increases ecosystem functioning [14].

Although ecosystems are faced to natural fluctuations most ecosystem-functioning experiments are conducted under ambient conditions. Ecosystems can be stressed to some degree without direct destruction of the functioning of the system, but a decrease of functioning was found in several systems [19-22].

In observational studies it is hard to distinguish the influence of environmental factors like nutrient availability on species richness from the effects of biodiversity on the functioning [23]. As an example imagine a species-rich grassland with low biomass production due to low nutrient availability. If nutrients are applied productivity, i.e., total biomass production, may increase but species richness will decrease. In this context a clear connection is known. But if the differences in nutrient concentrations between two study sites are unknown this may be different. One could compare a species rich, but nutrient poor, grassland with another plot with low species number and good nutrient availability. It will be found that low species richness increases productivity. However, the cause of species loss influenced directly the productivity so that nutrient availability overrides the effects of species richness on productivity. Because in many cases the determinant of Species richness in unknown I do not discuss such studies in detail even if species distribution patterns were shown for microorganisms recently [24-28]. In open oceanic systems a differentiation between species richness influencing environmental factors and the consequences of species richness per se is even more difficult since clear borders between the sites are lacking or difficult do observe. However, even in highly dispersed marine environments limits of genetic connectivity were reported in several studies [29,30]. Recently, progress on the patterns of functional traits of planktonic species revealed biogeographic distribution patterns as well [31]. Nonetheless, the patterns of traits do not represent species richness patterns and are further based on only a few aspects of ecosystem functioning [31]. Thus, it reveals unknown how species composition is distributed globally and how it is affected by environmental factors.

Instead of focusing on observational studies I here will refer to ecosystem functioning studies manipulating biodiversity to observe effects on ecosystem functioning and pinpoint to possible applications of algae models in ecosystem functioning experiments.

General Questions Applied on Microalgae Models

Ecosystem functioning of primary producers

In aquatic systems positive biodiversity effects were observed in manipulation experiments, i.e., experiments in which biodiversity was artificially manipulated to simulate species loss or artificial communities, with higher plants [20,32,33], macroalgae [34-38], and microalgae [22,39-42]. Manipulation experiments with species from oceanic systems show a positive biodiversity- ecosystem functioning effect for primary producers, i.e., plants and algae [33]. Additionally to species richness a positive effect of genetic diversity was reported for sea grass clones [33]. Manipulation experiments on plankton microalgae are scarce [42].

Microcosm experiments with microalgae are particularly appealing because they grow fast and microcosms are small. Thus, a high number of replicates can be conducted. However, natural species richness and abundance of microalgae were not used in the mentioned experiments yet. Microalgae may be combined in abundances as found in nature to enhance the transferability of the results to natural conditions. Using "natural" communities combined from monocultures in the lab can avoid the difficult discrimination between the influence of biodiversity and those factors determining biodiversity on the ecosystem functioning of observational studies. Further, a long-lasting criticism of ecosystem-functioning experiments, i.e., that random "species lost" is too artificial to be transferred to natural conditions [43,44], can be faced.

High number of species

Most communities comprise a lot of different species. This is a major problem in manipulation experiments of large species since the instalment of mesocosms is logistically difficult and time consuming. Combining microalgae from monocultures is very fast done, especially if they are grown in liquid culture media. Thus, species richness levels found under natural conditions can be used for the experiments.

Different levels of biodiversity

In a similar way, other measurements of biodiversity such as genetic diversity or functional diversity are easier to study in small systems. A major aspect for using microorganisms in this particular point is the possibility of isolation of single autogenic strains (clones) or the use of a few individuals as initial population to obtain populations of reduced genetic or functional diversity. Once isolated, such strains or populations can be combined to artificial communities and experiments on their functioning can be conducted. Further, epigenetic influences on ecosystem functioning may be studied in these systems as well as changes in epigenetic characteristics may be induced by different treatments of the strains previously. Even if this does not lead to a more realistic community of organisms it might be of interest to combine physiological research with ecology. As an example a study comparing niche differences and phylogenetic diversity in paired species assemblages of 37 freshwater green algae revealed no influence of evolutionary relatedness on ecological mechanisms [45]. However, the influence of niche differences to relative fitness differences was shown [45].

Different ecosystem functions

Most ecosystem functioning experiments of primary producers focused on biomass production [4].Other functions like nutrient concentrations or primary production are less studied [4]. Here, microalgae provide the opportunity to measure photosynthesis directly by using pulse-amplitude-modulated fluorescence and the pigment content can be determined for several target pigments. The determination of different functions in an ecosystem functioning experiment will lead to a more detailed understanding of the functioning of the whole communities.

Temporal performance of communities functioning

The temporal performance of biodiversity dependent ecosystem functioning was only tested in a few studies [46-48]. In microcosm experiments with microalgae the temporal performance can be studied with dozens of generation turnovers in some weeks. Such an experiment with grassland species would last several years.

Environmental fluctuation and stress

Including environmental fluctuations in the range of natural incidence can lead to a more detailed understanding of ecosystem functioning under realistic conditions. A stress application, i.e., a change in environmental conditions which may not be observed in nature, on the other hand can help to understand the general impact on the functioning of ecosystems. Here two main strategies of studies on stress applications in biodiversity-ecosystem functioning experiments can be seen. First, studying the influence of different stress types on ecosystem functioning. This was done in only a few experiments in aquatic systems [22]. Similarly to the observation of different ecosystem functions, the comparison between the influence of different stress types will help to understand ecosystem functioning. Different stress types may lead to different plant community response and thus the underlying mechanisms of biodiversity effects. Here, two main groups of mechanisms can be differentiated, selection effects (sometimes called portfolio or sampling effects) and complementarity effects. Selection effects refer to an over-proportional growth of one or only a few species on the cost of others, while complementarity effects refer to a better performance of most species in mixture compared to the monocultures. To differentiate these mechanism groups monoculture data are crucial [49]. Future experiments may compare a "limiting", i.e., a stress leading to lower levels of nutrient or water availability e.g. salinity or drought, with a "destructing", i.e., a stress physically damaging for example the photorespiratory apparatus e.g. UV radiation. Here, different stress types may or may not lead to different underlying mechanism groups. A second aspect of stress is its intensity. Although stress intensity is was included in theoretical approaches since about 20 years [50] there are only a few studies on ecosystem functioning effects manipulating simultaneously stress intensity and biodiversity [51]. Three of those studies [52-54] were conducted in terrestrial systems, while one [22] used microalgae. The latter experiment required a total of 3456 microcosms because two different stress types in six stress intensities were applied. Such an experiment would have been almost impossible with larger plant species. In general, the relationship between biodiversity and stress intensity regarding ecosystem functioning was identified as poorly studied [51]. A focus on the influence of biodiversity on ecosystem functioning at different stress intensity levels will contribute to maintain effective ecosystem functioning in future ecosystems. Another aspect is to combine the important insights of physiological research and ecology. Since physiology focuses on processes of a single species it is artificial in the stress application itself and in the point that almost all species co-occur with other species. However, as we need to understand how ecosystems work our aim should be to understand the influence of both, biodiversity and external factors on the functioning of ecosystems. As there isa great variety of physiological studies on microalgae an approach to combine both disciplines would be possible.

Why limiting to primary producers?

As most biodiversity-ecosystem functioning research was concentrated on a single trophic level, namely primary production, in the past, we now need to include trophic interaction into our experimental approaches. This statement is not new [54,55]. However, studies on food webs are difficult to conduct due to a variety of reasons. One major reason is the logistically difficult instalment of such experiments with large species in terrestrial systems. An advantage of using species from aquatic ecosystems would be that the diversity of different trophic levels can be manipulated in microcosm experiments easily as primary producers, associated bacteria and grazers are small. As stated above, oceanic plankton communities are important to global primary production and further build the basis of food webs crucial to a variety of human ecosystem services like fisheries and pollutant fixation. The complexity of trophic flows between organisms reaching from primary producers to mesozooplankton is high and difficult to model [42,56-58]. On the other hand, exactly this complexity of organisms in oceanic plankton offers a great opportunity for manipulation experiments addressing specific questions.

General Short comings of Microalgae in Experiments

Identification of the included species

The combination of microalgae is quite easily done. However, as they are small and often lack a great variety of morphological characters they are difficult to identify. An example is the "genus" *Chlorella* (Trebouxiophyceae) in which some species described on morphological characters are now treated in another class of green algae (Chlorophyceae), e.g. the strains SAG 2334 and SAG 2337, "*Chlorella*" emersonii, Culture Collection of Algae Göttingen. The morphological differentiation is particularly difficult if close relatives are used in the experiments or the organisms are faced to stress leading to morphological deformations. However, this may be faced with modern molecular biological methods like real-time PCR or FISH labeling [59-61].

Contaminations

Using axenic, i.e., only one species containing cultures with no infection of bacteria, fungi or viruses, cultures would be ideally for the mentioned experiments. However, since most oceanic organisms are not cultivable in axenic cultures, influences of contaminants may influence the experimental results. Especially through their fast growth virus replication may be critical. However, as studies on such influences on biodiversity - ecosystem functioning effects were not conduced yet it is unknown how and to which degree such contaminants will affect the functioning of the communities compared to the monocultures containing the same contaminations. If the included strains stem from the same environment such influences may be negligible since all organisms were faced to the contaminants previously. Further, seen a strain as consortium, i.e. a combination of different organisms, may be one way to deal with this problem. Additionally, this can be studied by cross-experiments combining all species under study with each other and the observation of the development of bacterial or fungi growth or virus infections.

Transferability of the experiments to other biomes

As mentioned above, the results of ecosystem-functioning experiments under fluctuating environmental conditions conducted in aquatic systems cannot be transferred directly to terrestrial ecosystems. However, aquatic, and particularly oceanic, ecosystems are crucial for the primary production of the earth. Thus, the principles of the functioning of these ecosystems should be better understood. By using model microcosms manipulations of the environmental conditions for the communities can be applied, leading to a better understanding of the mechanisms and responses of such communities. Using aquatic model organisms thus does not per se have the limitation to a model for other biomes. However, experimental studies on aquatic ecosystems provide qualitatively comparable results to terrestrial ecosystem functioning.

Conclusion

Overall, algae and their associated species can provide a great variety of model systems. With these model systems specific questions about ecosystem functioning can be addressed. Even if the results of such model systems may not validly be transferred to other biomes the increased thread of oceanic ecosystems should be reason enough for research in this field. Further, results from microcosm experiments can be expected faster than from experiments with large species. My aim is to promote the use of microorganisms, especially primary producers, in ecological research. At least for pilot studies the advantages through their fast growth and the small scales of the microcosms are obvious.

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