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## Phytoplankton diversity and ecosystem functioning in freshwater ecosystems

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FACULTÉ DES SCIENCES

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UNIVERSIDAD DE INGENIERIA Y TECNOLOGIA

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# **Phytoplankton Diversity and Ecosystem Functioning in Freshwater Ecosystems**

THÈSE

présentée aux Facultés de médecine et des sciences de l'Université de Genève  
pour obtenir le grade de Docteur ès sciences en sciences de la vie,  
mention Ecologie et évolution

par

**Ziyu GUAN**

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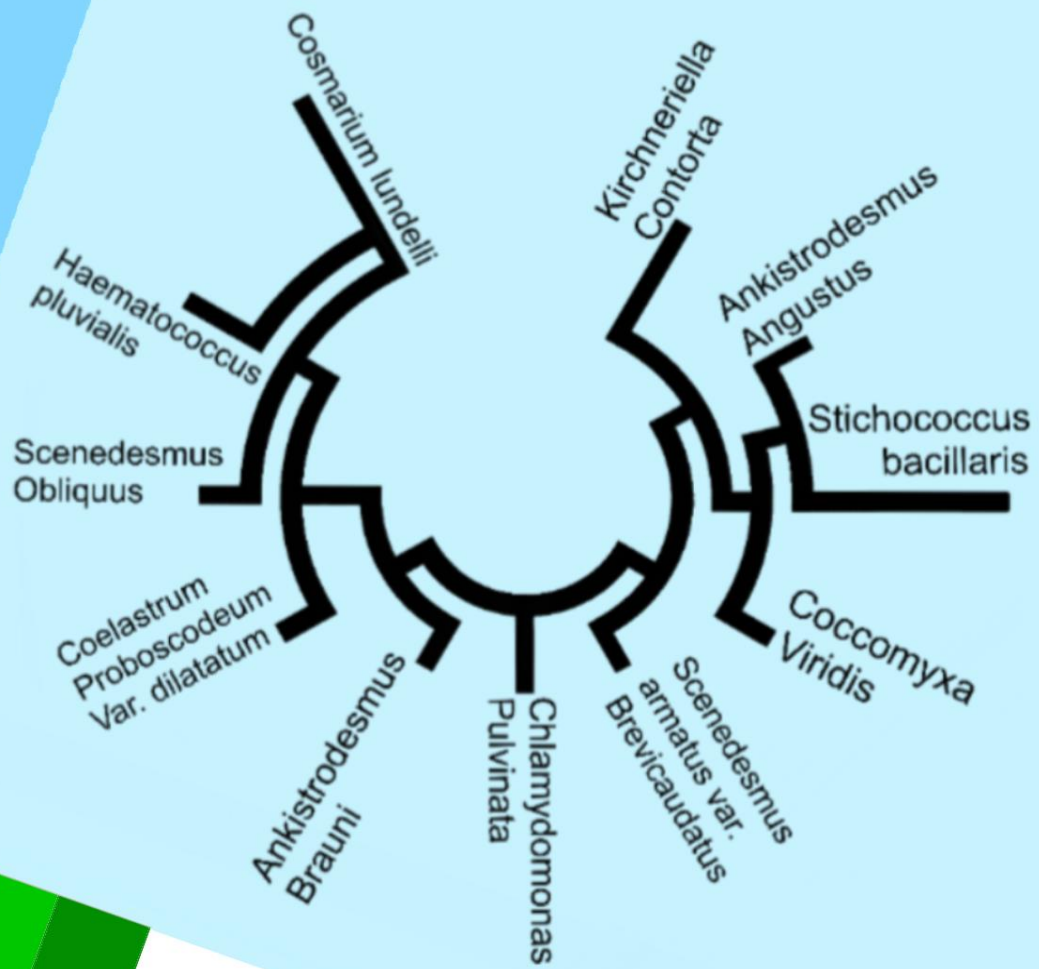
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# Phytoplankton diversity and ecosystems functioning in freshwater ecosystems

*Ziyu. Guan*

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ÉCOLOGIE ET ÉVOLUTION

**Thèse de Madame Ziyu GUAN**

intitulée :

**«Phytoplankton Diversity and Ecosystem Functioning in  
Freshwater Ecosystems»**

Les Facultés de médecine et des sciences, sur le préavis de Monsieur B. W. IBELINGS, professeur ordinaire et directeur de thèse (Département F.-A. Forel des sciences de l'environnement et de l'eau), Monsieur P. VENAIL, professeur et codirecteur de thèse (Department of Environmental Engineering/ Water Research and Technology Center, Universidad de Ingeniería y Tecnología - UTEC, Lima, Peru), Madame L. WEI, docteure (Département F.-A. Forel des sciences de l'environnement et de l'eau), Madame A. J.T. NARWANI, docteure (Department of Aquatic Ecology, EAWAG - The Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland), autorisent l'impression de la présente thèse, sans exprimer d'opinion sur les propositions qui y sont énoncées.

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# Abstract

To date, much of biodiversity and ecosystem functioning (BEF) research has been focused on terrestrial plant ecosystems, and we know less well how diversity influences functioning in freshwater ecosystems. The aim of this thesis is to provide a better understanding on how and why biodiversity influences ecosystem functioning in freshwater phytoplankton communities. I focused on phytoplankton because it is the primary producer in aquatic ecosystems, thus representing the base of most aquatic food webs. Phytoplankton is responsible for several major functions such as nutrient uptake, oxygen production, CO<sub>2</sub> fixation and biomass production. The final purpose of my thesis work is to generate a better mechanistic understanding of the influence of phytoplankton biodiversity on biomass production and nutrient uptake. It includes seven chapters. **Chapter 1** introduces some of the key concepts in the thesis, giving the reader a basic idea of its content. Additionally, this chapter explains the importance and current status of this field of research. **Chapter 2** includes an exhaustive review of the literature on experimental and field studies linking phytoplankton diversity to ecosystem functioning. It provides central information on how much attention different aspects of BEF research in freshwater lentic ecosystems have received. We did so by performing a quantitative synthesis of previous BEF studies in freshwater ecosystems. We also discuss the proposed mechanisms by which diversity influences functioning and identify major research gaps and limitations. Finally, we propose some ways to move forward in BEF research. **Chapter 3** reports a first controlled laboratory experiment. It deals with the impact of cell size composition on functioning using phytoplankton microcosms. We found that resource uptake benefited from the presence of relatively smaller phytoplankton species with larger surface area to volume ratios. This was mainly determined by a compositional effect over which species interactions had a limited impact. **Chapter 4** reports a second controlled experiment. This time, we explore facilitative interactions in experimental phytoplankton communities. By using an additive design in controlled laboratory conditions and using nine species of green algae, we found that phosphate concentration influenced the prevalence facilitation, but not its strength. Reciprocal facilitation was a rare outcome, with most of the time only one species in a pair showing evidence of facilitation. Overall, some species acted as providers of facilitative interactions (facilitators), and other benefited from the presence of a second species (facilitated). **Chapter 5** reports a field study on BEF in tropical high-altitude lakes from Southern Ecuador. We explored the links between several environmental variables and productivity, measured as

chlorophyll-a concentration and total phytoplankton biovolume. We found that a combination of four abiotic factors explained over three quarters of the variation in chlorophyll-a concentration amongst lakes. Contrary to what studies from temperate regions suggest, taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, Shannon's diversity index was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume, presumable due to a strong compositional effect. To go further, **Chapter 6** discusses the applicability and perspective components of BEF studies. A series of environmental and human related topics could benefit from BEF research, including biodiversity conservation, habitat restoration, sustainable agriculture and biomass production. We first introduce biodiversity conservation and habitat restoration in aquatic ecosystems. Then, we summarize the potential applicability of incorporating facilitative interactions into aquatic restoration and conservation. We also give two examples on how our findings can be used for wastewater treatment and industrial production purposes. Finally, **Chapter 7** offers a general discussion of the thesis. It shows how all chapters fit together and contribute to the field and put the different parts of my thesis into a wider perspective. First, I discuss the underlying mechanisms of BEF relations. Then, I provide some personal opinions about the ecological way of thinking and the difficulty of transmitting scientific research results to a non-specialized public. I end-up by summarizing all the main results of my research.

# Résumé

À ce jour, la plupart de la recherche sur la biodiversité et le fonctionnement des écosystèmes (BEF) porte sur les écosystèmes végétaux terrestres et nous connaissons moins bien comment la diversité influe sur le fonctionnement des écosystèmes d'eau douce. Le but de cette thèse est de mieux comprendre comment et pourquoi la biodiversité influence le fonctionnement des communautés de phytoplancton d'eau douce. Dans cette thèse, nous nous concentrons sur le phytoplancton, car il est le principal producteur des écosystèmes aquatiques, représentant la base de nombreux réseaux trophiques aquatiques. Le phytoplancton est aussi responsable de plusieurs fonctions majeures telles que l'absorption de nutriments, la production d'oxygène, la fixation de CO<sub>2</sub> et la production de biomasse. Notre objectif final est de générer une meilleure compréhension mécanistique de l'influence de la biodiversité du phytoplancton sur la production de biomasse et l'absorption de nutriments. Cette thèse inclut sept chapitres. **Le chapitre 1** présente et explique de manière générale les sujets importants de la thèse. Donnant ainsi au lecteur une idée de base du contenu de cette thèse. **Le chapitre 2** comprend une revue exhaustive de la littérature sur les études expérimentales et sur le terrain associant la diversité du phytoplancton au fonctionnement des écosystèmes. Dans ce chapitre, nous fournissons un résumé quantitatif exhaustif de l'état actuel des études BEF sur les écosystèmes d'eau douce, afin d'adapter les deux limites de l'état actuel des études BEF. **Le chapitre 3** présente un rapport sur les études d'une expérience de laboratoire que nous avons réalisée. Dans ce chapitre, nous montrons, en manipulant directement une série de variables liées à la taille des cellules et en mesurant pour la première fois leur impact sur de multiples fonctions, que la multifonctionnalité a tiré parti de la présence d'espèces relativement plus petites présentant des ratios surface / volume plus importants. Ceci était principalement déterminé par un effet de composition des communautés sur lequel les interactions entre espèces avaient un impact limité. **Le chapitre 4** décrit une deuxième expérience de laboratoire qui explore les interactions facilitantes dans les communautés de phytoplancton. En utilisant un design additif, dans des conditions de laboratoire contrôlées et avec sept espèces d'algues vertes, nous avons constaté que la concentration en phosphate influençait sur la fréquence de la facilitation, mais pas sur sa magnitude. La facilitation réciproque était un résultat rare, avec la plupart du temps une seule espèce dans une paire montrant des signes de facilitation. Dans l'ensemble, certaines espèces ont joué le rôle de fournisseurs d'interactions facilitantes (facilitateurs) et d'autres ont bénéficié de la présence d'une deuxième espèce (facilitée). **Le chapitre 5** présente une étude de terrain sur BEF dans des lacs tropicaux de haute altitude du sud de l'Équateur. Nous avons exploré les liens entre plusieurs variables environnementales et

la productivité, mesurés en tant que concentration de chlorophylle-a et biovolume de phytoplancton total. Nous avons constaté qu'une combinaison de quatre facteurs abiotiques expliquait plus des trois quarts de la variation de la concentration de chlorophylle-a entre les lacs. Contrairement à ce que suggèrent des études menées dans les régions tempérées, la richesse spécifique n'était pas liée aux concentrations de chlorophylle-a ni au biovolume du phytoplancton total. De plus, l'indice de diversité de Shannon était corrélé négativement aux concentrations de chlorophylle-a et au biovolume total du phytoplancton, vraisemblablement en raison d'un fort effet de composition. **Le chapitre 6** est l'application et la prospective des études BEF. Dans ce chapitre, nous avons d'abord introduit d'une manière générale la conservation de la biodiversité et la restauration de l'habitat dans les écosystèmes aquatiques. Deuxièmement, nous avons résumé l'application et donné quelques perspectives d'interaction positive dans la restauration et la conservation aquatiques. Enfin, nous avons donné deux exemples, qui utilisent nos résultats expérimentaux dans le traitement des eaux usées et la production industrielle. **Le chapitre 7** propose une discussion générale de la thèse. Il montre comment tous les chapitres s'emboîtent, contribuant à ce domaine de recherche et nous plaçons les différentes parties de ma thèse dans une perspective plus large. Premièrement, je discute des mécanismes sous-jacents des relations BEF. Ensuite, je donne quelques opinions personnelles sur la manière de penser écologique et sur la difficulté de transmettre les résultats de la recherche scientifique à un public non spécialisé. Je termine en résumant tous les principaux résultats de mes recherches.

# CHAPTER 1

## General Introduction

### Biodiversity and ecosystem functioning (BEF)

Biological diversity or *Biodiversity* is the variety of life forms across several scales of organization, including variation among ecosystems, communities, species, individuals, traits, and genes (Cardinale *et al.* 2012). Biodiversity is generally classified and quantified according to three approaches: taxonomy-based, functional trait-based and gene-based variation. Each of these approaches covers different aspects of biodiversity and has its benefits and drawbacks (Steudel *et al.* 2016). The term *Ecosystem* is an abbreviation for “ecological system”. An ecosystem consists of different organisms living together and relying on each other for survival. Ecosystem functioning refers to all the ecological processes that control the fluxes of energy and matter through a biological compartment, such as primary productivity and nutrient cycling (Cardinale *et al.* 2012). All living things rely on ecosystems to get food and habitat. For example, humans require oxygen to breath, water to drink, food to eat and a place to rest, all of which are provided by ecosystems. In other words, the survival of each species, including humans, relies upon the proper functioning of ecosystems.

Biodiversity is in crisis, with incredibly high extinction rates reported over the last few decades (Ceballos *et al.* 2015). Therefore, scientists are increasingly concerned about the possible ecological consequences of this reduction in biodiversity (Chapin *et al.* 1998). The Convention on Biological Diversity, opened for signature at the Earth Summit in Rio de Janeiro on June 1992, illustrates well the increasing the general awareness on this issue. The increasing interest on the consequences of biodiversity erosion led to the development of a research field named as biodiversity-ecosystem functioning (hereafter *BEF*). It focuses on the consequences of variations in biodiversity on the functioning of ecological systems. Thus, studying the relationship between biodiversity and ecosystem functioning is key to understand and predict the ecological consequences of diversity loss (Hooper *et al.* 2005, Srivastava & Vellend 2005, Cardinale *et al.* 2012).

After over two decades of intense BEF research, it has been established that biodiversity influences multiple ecosystem functions, with consistent evidence supporting that a reduction in biodiversity negatively affects ecosystem functioning and the services it provides (Balvanera *et al.* 2006). However, the majority of BEF studies followed a fixed procedure and barely innovate. These studies are mostly empirical examinations of terrestrial plant ecosystems in which some aspect of biodiversity, usually the number of species, is manipulated as an explanatory variable of ecosystem functions. Approaches primarily consist in generating units of study with increasing diversity and measuring the performance of each unit. Then, the direction and shape of the relationship between these two variables is established and interpreted. Despite the important achievements made by previous BEF studies, two major limitations are: 1) the low explanatory power of species richness as measure of biodiversity, and 2) the poor mechanistic understanding of the relationship between diversity and ecosystem functioning.

Two main mechanisms have been proposed to explain biodiversity's influence on community functioning (Balvanera *et al.* 2006). On one hand, the *sampling effect*, suggesting that some species make a disproportionate contribution to functioning. Dominance of the community by a species with low functioning levels results in low community functioning (negative selection effect) whereas dominance by a species with a high functioning level turns into a high functioning community (positive selection effect). Increasing the number of species in a community increases the chances of including a dominant productive species. Thus, regarding the sampling effect, community functioning is not dependent on diversity per se but on the probability of the presence of a dominant species with a disproportionate contribution to community functioning. When no dominant species contributes disproportionately to community functioning, or when positive and negative selection effects cancel each other, the overall selection effect is null. On the other hand, the *complementarity effect* is the result of interactions among species and of their influence on functioning. Species can either have negative interactions (e.g., competition); or positive interactions (e.g., facilitation), resulting in communities performing better than the constitutive monocultures (positive complementarity effect). In the absence of species interactions influencing functioning (functionally neutral interactions), community's functioning is simply the accumulation of individual species performances (null complementarity effect). In the end, it is the balance between the relative contributions of sampling and complementarity effects that determines the shape of the relationship between species richness and community functioning. However, the number of

species in a community (i.e., species richness) is not meant to determine or to predict the relative contribution of a species to functioning (sampling effect) or the nature and/or strength of species interactions that influence functioning (complementarity effect). This leads very often to a limited capacity of species richness to predict ecosystem functioning and an incapacity to provide a clear mechanistic understanding of BEF relationships (Borics *et al.* 2012). As an alternative, an increasing number of BEF studies have incorporated trait-based information (Petchey, Hector & Gaston 2004; Cadotte, Carscadden & Mirotchnick 2011; Flynn *et al.* 2011). Traits are the foundation of biodiversity effects on ecosystem functioning (Tilman, Lehman & Thomson 1997; Loreau 1998). Some traits are expected to be directly linked to the nature and strength of species interactions or have disproportionate effects on functioning. Thus, trait-based information may provide a better understanding of biodiversity's impact on ecosystem functioning than species richness. In general, trait-based information explains greater variation in ecosystem functioning than species richness (Petchey, Hector & Gaston 2004; de Bello *et al.* 2010) and provides a clearer mechanistic understanding (de Bello *et al.* 2010), but determining the traits that are relevant for ecosystem functioning remains a major challenge in BEF studies.

Another major limitation of most BEF studies, contributing to the poor descriptive power of diversity on ecosystem functioning, is that they have primarily focused on the effects of diversity on single ecosystem functions. Frequently, effects of diversity on single ecosystem functions saturate at low diversity levels, underestimating its full potential as a driver of ecosystem functioning. Therefore, it has been suggested that the explanatory power of biodiversity on ecosystem functioning might increase when multiple functions are considered simultaneously. However, different functions might also be oppositely influenced by biodiversity (trade-offs among functions), resulting in weak or null impacts on multifunctionality (Gamfeldt, Hillebrand & Jonsson 2008; Zavaleta *et al.* 2010; Gamfeldt & Roger 2017). The approach that considers the influence of biodiversity on multiple functions referred as multifunctionality is not new (Hector *et al.* 2002; Hector & Bagchi 2007; Gamfeldt, Hillebrand & Jonsson 2008) but remains relatively underexplored (Maestre *et al.* 2012, Lefcheck *et al.* 2015). Overall, combining trait-based information together with multiple functions may lead to a more comprehensive description of biodiversity's impact on ecosystem functioning and shed more light into the underlying biological mechanisms. Such studies are very rare (Mouillot *et al.* 2011; Gross *et al.* 2014; Valencia *et al.* 2015; Gross *et al.* 2017) and we are unaware of any in which the trait structure of communities was directly manipulated to determine its impact on multiple different functions at a time.



Finally, despite all the evidence collected from controlled laboratory BEF experiments, the importance of diversity as a main driver of ecosystem functioning in real-world conditions is still debated (Duffy *et al.* 2017). For some authors, a myriad of abiotic factors (such as climate or nutrients) may dominate over biodiversity effects on ecosystem functioning in natural conditions. In a recent quantitative meta-analysis of BEF studies both terrestrial and aquatic systems, it has been shown that increases in biomass with species richness are stronger in nature than in the laboratory after controlling for environmental variables (Duffy, Godwin & Cardinale 2017). Moreover, species richness ranked more frequently as the first predictor of biomass production compared to climate and nutrient predictors. In nature, the role of community trait structure - as opposed to species richness - in BEF relations, however, is almost completely unknown.

## General characteristics of freshwater ecosystems

Over 71% of the surface of Earth is covered by water, but 99% of this water is in the ocean. Plants, animals and humans all need freshwater to live, illustrating the vital role of freshwater ecosystems in our planet. Firstly, they provide habitat for myriads of organisms. Vegetated wetlands play a major role in water purification, flood control and carbon sequestration. In addition, the fish production of freshwater ecosystems has large commercial value. Moreover, freshwater ecosystems provide water for irrigation, power generation and industrial purposes. Freshwater ecosystems are very sensitive to climate change, especially to temperature increase that affects their thermal structure. It is also well known that ice cover is decreasing, leading to major changes in the freshwater cycle. Water pollution is another major environmental issue. Thus, freshwater ecosystems are one of the most diverse but are also one of the most threatened by pollution and climate change. The loss of biodiversity in freshwater ecosystems is higher compared to other ecosystems (Dudgeon *et al.* 2006). Freshwater fish represent one-fourth of all living vertebrate species, with over 30% being threatened (Abellán *et al.* 2005). Most amphibian, aquatic reptile and aquatic mammal species are also endangered.

Lakes are important natural freshwater reservoir units. Understanding their physical, chemical structure and dynamics can help assessing how the environmental factors influence the metabolism, growth and reproduction of phytoplankton. Lakes also play an important role on the freshwater cycle. Most lakes were formed by catastrophic events, such as the displacement of the terrestrial crust, volcanic activity, landslides into valleys or the erosional and depositional

activity of glaciers. As such, the physical, chemical and biological properties of lakes are influenced by the morphology of the lake basin. As one example, the productivity of small shallow lakes is usually negatively correlated with their mean depth (Beyter *et al.* 2016). Solar radiation is the major energy source in freshwater ecosystems and is used for photosynthesis. Light is not evenly distributed in water. Of the total light entering the water, a certain portion is scattered, and the rest is absorbed by the suspended particulate matter and dissolved compounds. Light attenuation results in a diminution of radiant energy with depth. Oxygen is another important element in lakes. The metabolism of aerobic organisms relies on the oxygen dissolved in water which comes from the contact of the surface water with the air and comes from photosynthetic activities of plants and phytoplankton.

Dissolved carbon dioxide (CO<sub>2</sub>) is the main source of carbon for photosynthesis. The dissolving of carbon dioxide in water is very complex. Carbon dioxide is about 200 times more soluble in water than oxygen. After carbon dioxide dissolves in water, it hydrates and transforms into carbonic acid. However carbonic acid dissociates immediately, leading to a change in the pH of water. The relative proportions of carbon dioxide, carbonate and bicarbonate results in different values of pH in water.

Nitrogen and phosphorus are the two main limiting nutrients for phytoplankton. However, due to human activities such as fertilization, more nutrients can enter freshwater systems and generate eutrophication issues. Nitrogen in water can be found as organic compounds such as amino acids and inorganic reactive nitrogen such as ammonia, nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>). Phosphate is mostly present in water as organic phosphates in living cells or dead organic material. The most vital inorganic form of phosphate is orthophosphate. The requirements for nutrients are variable among species and can lead to selective advantages of certain taxa as nutrient supplies fluctuate seasonally.

## Phytoplankton in lakes

Phytoplankton in lakes consists of a diverse assemblage of taxonomic groups. The presence of photosynthetic pigments is the primary characteristic of phytoplankton. These pigments include the chlorophylls, carotenoids, and phycobilins. Chlorophyll *a* is the most common chlorophyll and is present in all algae and cyanobacteria. Besides chlorophyll *a*, there are chlorophyll *b*, *c* and *d* than are present in some special groups of algae. The cyanobacteria, also called blue-green algae, are bacteria with photosynthetic pigments. As prokaryotic organisms, they lack

cell membranous structures and the cytoplasm contains the proteins and nucleic acids. Cyanobacteria can be unicellular or colonial. Green algae are another group of phytoplankton. They are eukaryote and extremely morphologically diverse in lakes. Besides the green algae, other algae abundant in freshwater lakes are the Xanthophyceae (yellow-green algae), Chrysophyceae (golden-brown algae). Diatoms are another group of phytoplankton with silicified cell walls. Diatoms are unicellular or colonial as well. The unique silicified cell wall gives diatoms beautiful and complex structures. Additionally, their relatively dense cell wall causes them to sink easier compared to green algae and cyanobacteria. They are mostly nonmotile.

The fact that there are so many kinds of phytoplankton in different shapes and sizes, with different or similar characters living in lakes attracted scientists' attention. An outstanding feature of phytoplankton communities is the coexistence of algal species. Early in the 60's, Hutchison (Hutchinson 1961) reported the paradox of phytoplankton, wondering how can many species to coexist in a relatively isotropic or unstructured environment with all competing for the same limited array of resources. This contradicts the competitive exclusion principle, suggesting that there can be no more species coexisting than limiting resources. This special feature of phytoplankton communities makes them a popular tool for community ecology studies.

## **Biodiversity and ecosystem functioning research in freshwater phytoplankton**

Despite being the largest contributor to global primary production and its crucial role in global energy fluxes and element cycles, phytoplankton BEF studies remain relatively scarce compared to studies in terrestrial plants (Balvanera *et al.* 2006; Cardinale *et al.* 2011; Cadotte, Dinnage & Tilman 2012). Some general BEF patterns obtained for terrestrial plants, such as the positive effect of species richness on biomass production, have also been reported in experiments using freshwater microalgae (Fox 2004, Cardinale *et al.* 2006, Weis *et al.* 2007, Cardinale *et al.* 2011, Gross *et al.* 2014), but are far from universal. Some evidence suggests that biodiversity-ecosystem functioning patterns observed for terrestrial plants may not be generalizable to phytoplankton and require specific studies (Schmidtke, Gaedke & Weithoff 2010).

A widespread use of species richness as the sole measure of diversity in BEF studies with freshwater phytoplankton has led to two major limitations: 1) a low explanatory power of diversity, with a large proportion of unexplained functioning, even under highly controlled lab-conditions and 2) a poor mechanistic understanding of the relationship between diversity and ecosystem functioning. To overcome both limitations, BEF studies have recently started to incorporate the functional differentiation among species as a measure of diversity (Shurin *et al.* 2014, Steudel *et al.* 2016). While some progress in depicting the functional traits controlling species interactions (e.g., competition) and community structure in phytoplankton has been achieved (Litchman *et al.* 2007, 2010; Edwards *et al.* 2011; Schwaderer *et al.* 2011), the functional differentiation among species is often difficult to quantify directly because the functions performed by organisms are controlled by a wide-variety of biological traits, many of which are hard to identify and to measure. The capacity of functional diversity metrics to explain ecosystem functioning better than species richness depends on the capacity to incorporate the relevant set of traits associated with the ecosystem function of interest.

As stated earlier in this introduction, most of the current mechanistic understanding of the effect of phytoplankton diversity on ecosystem functioning relies on frequently unsupported claims about effects colloquially known as complementarity and selection, generally studied using a variety of *ad hoc* statistical tests. While informative, these effects do not necessarily correspond to real biological mechanisms. Real improvements into the mechanistic understanding of the impact of diversity on ecosystem functioning will rely on the capacity to better depict the nature and strength of species interactions (Cardinale *et al.* 2002), either negative (i.e., competition), positive (i.e., facilitation) or neutral (i.e., resource partitioning). For instance, recent efforts focused on establishing how the nature and strength of species interactions depend on species identity (Venail & Vives 2013; Lyu *et al.* 2017) or the presence or absence of functional traits (Litchman *et al.* 2010; Edwards *et al.* 2011). It has been argued that when considering a large range of algal taxonomic groups, competitive abilities for nitrate and phosphate are negatively correlated, suggesting that species performing well under nitrate limited conditions perform badly under phosphate limited conditions and vice-versa (Edwards *et al.* 2011). It has also been suggested that in addition to competition, positive species interactions such as facilitation (e.g., positive allelopathy) might be very important for understanding the link between phytoplankton diversity and ecosystem functioning (Venail *et al.* 2014, Fritschie *et al.* 2014, Wright *et al.* 2017). Until today, little is known about the nature of such interactions and the conditions under which these positive interactions emerge. BEF research in general has been too focused on

competition and that a better characterization of the facilitation process is urgently required (Cardinale *et al.* 2002, Wright *et al.* 2017).

To date, only three studies have tested the effect of phytoplankton functional diversity on ecosystem functioning in natural freshwater systems (reviewed in Venail 2017), with positive, negative and null relationships being observed (Vogt, Beisner & Prairie 2010; Borics *et al.* 2012; Pálffy, Présing & Vörös 2013). Such discrepancies may stem from the variety of traits being studied. In the current environmental context, marked by an intense degradation of freshwater systems (Dudgeon *et al.* 2006), controversies about the actual role of diversity as a driver of ecosystem functions in natural conditions can be misleading. More studies focused on the importance of phytoplankton trait structure for ecosystem functioning are urgently required.

## Achievements in BEF research from members of my research group

Before I started my project, Dr. Patrick Venail co-supervisor and member of my research group had important achievements in BEF studies. His research is mostly focused on testing basic ecological hypotheses on community assembly and the relationship between biodiversity and ecosystem functioning (BEF). He's been working for the last eight years with microscopic algae as model system to address BEF related issues (Venail & Vives 2013; Venail *et al.* 2014; Alexandrou *et al.* 2015; Venail 2017). He has also analyzed data from grassland systems (Venail *et al.* 2015, Cardinale *et al.* 2015, Lyu *et al.* 2017), which is the model system more frequently used in BEF studies. Currently, one of his main research interests is to better understand the mechanisms influencing diversity effects on ecosystem functioning, with special focus on the role of traits and their connection to species interactions. He has participated over the last five years in 10 peer-reviewed publications. His most recent and current research in the field of biodiversity and ecosystem functioning using freshwater phytoplankton as model system can be divided into four topics that are briefly described below.

*Phylogenetic diversity does not predict community biomass stability nor the nature and strength of species interactions in experimental phytoplankton communities.*

Dr. Venail and his colleagues experimentally explored the influence of the evolutionary relatedness of freshwater green algae on the temporal stability of community biomass production (Venail *et al.* 2013), and the nature and strength of species interactions (Venail *et al.* 2014). In both cases, they performed laboratory experiments in which we manipulated the

phylogenetic distance between freshwater green algae species. In Venail et al. 2013, they explored how the different components of community temporal stability in the face of environmental fluctuations changed as species got less related. They found that species interactions were more important for community stability than the differences among individual species in their responses to environmental fluctuations. In Venail *et al.* 2014, they first reviewed the empirical evidence linking evolutionary relatedness to the nature and strength of species interactions and found that most of studies performed up to date offered no evidence that relatedness influences species interactions. Then, they tested the hypothesis suggesting that closely related species compete stronger than distantly related species through an experimental approach. They found that neither the nature nor the strength of species interactions among freshwater algae was determined by their evolutionary relatedness.

*Evolutionary relatedness does not predict competition or species co-occurrence in natural and experimental communities of green algae.*

To explore more natural systems, Dr. Veail and colleagues included transcriptomic analysis intended to identify the genes responsible for species coexistence and the production of biomass in both experimental and natural freshwater green algae (Alexandrou *et al.* 2015). For this, they developed molecular phylogenetics to answer specific questions about the evolution of green algae, to characterize the process of algal diversification and to evaluate the impact of their diversity on ecosystem functioning. They found that evolutionary relatedness did not predict competition or species co-occurrence in natural or experimental communities of green algae.

*Phylogenetic relationships of species, independent of species richness, do not relate to the temporal stability of grassland primary production nor to their specific interactions.*

While not on freshwater phytoplankton, this study provided interesting insights related to BEF research. A similar approach may be applied to phytoplankton, when enough data will become available. Dr. Venail and colleagues first performed a data-synthesis on the effect of phylogenetic diversity on the temporal stability of community biomass production in grassland plants (Venail *et al.* 2015, Cardinale *et al.* 2015). For this, they compiled data from sixteen different studies performed in eleven different locations around the world and including a total of over 800 plots. They showed that the phylogenetic relationships of species, independent of species richness, do not relate to the temporal stability of primary production in grasslands. In a later study, they used data from grassland plant communities in the Tibetan plateau to illustrate

the limited capacity of phylogenetic relatedness as predictor of species interactions (Lyu *et al.* 2017).

*Niche differences trump fitness differences in predicting phytoplankton coexistence in size differences-based invasion experiments.*

The modern framework of coexistence, suggesting a balance between stabilizing and equalizing forces for understanding the maintenance of diversity, may benefit from the incorporation of trait information. In this study, Dr. Venail and colleagues (Gallego *et al.* 2019) focused on size, a key trait known for capturing several of the physiological and ecological functions of phytoplankton (Litchman *et al.* 2010) but whose influence at the community level remains largely unexplored. They tested if size differences among cyanobacteria species could determine their coexistence by analyzing the influence of size variability on interspecific niche and relative fitness differences and their relative contribution to the outcome of competition. Coexistence of pairwise combinations of freshwater cyanobacterial species was experimentally tested using an invasion-from-rare approach under controlled laboratory microcosms. Their study included thirty unique pairs of residents vs. invading cyanobacteria, whose average diameter ranged over two orders of magnitude. They found that differences in size among competing species directly influenced both niche and fitness inequalities, but that species coexistence was mainly driven by the niche differences based on such size differences.

## Outline of this thesis

This thesis focuses on three key concepts to address major knowledge gaps in BEF: traits, facilitation and multifunctionality.

*Traits*, as theoretically they should represent the foundation of biodiversity effects on ecosystem functioning. The widespread use of species richness as the sole measure of diversity in most BEF studies has led to two major limitations: 1) a low explanatory power of diversity, with a large proportion of unexplained functioning, even under highly controlled lab-conditions and 2) a poor mechanistic understanding of the relationship between diversity and ecosystem functioning. To overcome both limitations, BEF studies started to incorporate the functional differentiation among species as a measure of diversity (Shurin *et al.* 2014, Steudel *et al.* 2016). Some progress in depicting the functional traits controlling species interactions (e.g., competition) and community structure in phytoplankton has been achieved (Litchman *et al.*

2007, 2010; Edwards *et al.* 2011; Schwaderer *et al.* 2011). However, the functional differentiation among species is often difficult to quantify directly because the functions performed by organisms are controlled by a wide-variety of biological traits, many of which are hard to identify and to measure. The capacity of functional diversity metrics to explain ecosystem functioning better than species richness depends on the capacity to incorporate the relevant set of traits associated with the ecosystem functions of interest.

*Facilitation*, as they are frequent and may provide a mechanistic understanding for positive BEF relationships. Real improvements into the mechanistic understanding of the impact of diversity on ecosystem functioning will rely on the capacity to better depict the nature and strength of species interactions (Cardinale *et al.* 2002), either negative (i.e., competition), positive (i.e., facilitation) or neutral (i.e., resource partitioning). It has been suggested that in addition to competition, positive species interactions such as facilitation (e.g., positive allelopathy) might be very important for understanding the link between phytoplankton diversity and ecosystem functioning (Venail *et al.* 2014, Fritschie *et al.* 2014, Wright *et al.* 2017). Until today, little is known about the nature of such positive interactions and the conditions under which they emerge. BEF research in general has been too focused on competition and a better characterization of the facilitation process is urgently required (Cardinale *et al.* 2002, Wright *et al.* 2017).

*Multifunctionality*, because synergies or trade-offs among functions are possible. Another major limitation of most BEF studies is that they have primarily focused on the effects of diversity on single ecosystem functions, underestimating its full potential as a driver of ecosystem functioning and contributing to the poor descriptive power of diversity on ecosystem functioning. Therefore, it has been suggested that the explanatory power of biodiversity on ecosystem functioning might increase when multiple functions are considered simultaneously. However, different functions might also be oppositely influenced by biodiversity (trade-offs among functions), resulting in weak or null impacts on multifunctionality (Zavaleta *et al.* 2010, Gamfeldt *et al.* 2017). This approach, that considers the influence of biodiversity on multiple functions referred as multifunctionality is not new (Hector & Bagchi 2007, Gamfeldt *et al.* 2008) but remains underexplored (Maestre *et al.* 2012, Lefcheck *et al.* 2015).

In this thesis, I wanted to merge these three concepts by putting emphasis into understanding the influence of traits on the nature and strength of species interactions and on how that scales into multifunctioning terms. Overall, combining trait-based information with species



interactions and their effects on multiple functions may lead to a more comprehensive description of biodiversity's impact on ecosystem functioning and shed more light into the underlying biological mechanisms, promising to make big steps forward in BEF research. This research may hold the key to better comprehend the role of diversity as a driver of ecosystem functioning.

As previously mentioned, much of BEF research has focused on terrestrial ecosystems, and we have little knowledge about how diversity loss in freshwater ecosystems influences their functioning. The aim of my thesis is to understand how biodiversity influences the ecosystem functioning in freshwater phytoplankton communities. In this thesis, we focused on phytoplankton because it is the primary producer in freshwater ecosystems, representing the base of the entire trophic web. Phytoplankton is responsible for major functions such as nutrient uptake, oxygen production, CO<sub>2</sub> fixation and biomass production. Our major goal is to improve the mechanistic understanding of the influence of phytoplankton biodiversity to several kinds of ecosystem functions such as biomass production and nutrient uptake. This thesis proposes a data synthesis, two original laboratory experiments and a fieldwork study that would provide elements to fill current gaps in our BEF knowledge.

**Chapter 2** emerges as a first natural step to evaluate the state of the art in the field of BEF using phytoplankton as a model system, both in laboratory and natural conditions. This chapter includes an exhaustive review of the literature on experimental and field studies linking phytoplankton diversity to ecosystem functioning. It provides some key information on how much attention different aspects of BEF research in freshwater lentic ecosystems have received. We present a quantitative overview of BEF studies in freshwater ecosystems. For this, we first summarized the information from previous BEF meta-analyses. Then, we reviewed the patterns derived from previous individual BEF studies in freshwater lakes and describe the possible mechanisms by which diversity influences functioning. We also identified research gaps and limitations. Finally, we discussed some ways to move forward in BEF research. A draft of this chapter has been written and is current under edition to be submitted for publication before the end of 2019.

**Chapter 3** reports a controlled laboratory experiment about the impact of phytoplankton cell size composition on functioning using microcosms. Previous studies suggest that cell size is a master trait in phytoplankton, affecting a wide array of physiological processes including nutrient uptake and growth. Similarly, several population and community processes such as

abundance, biomass and primary production correlate well with changes in the cell size composition of phytoplankton in both marine and freshwater ecosystems. However, the direct influence of cell size on functioning remains empirically underexplored and little is known about the ecosystem level consequences of changes in the cell size composition of phytoplankton assemblages. Here we show, by directly manipulating a series of variables related to cell size and by measuring their impact on multiple functions for the first time, that multifunctionality benefited from the presence of relatively smaller species with larger surface area to volume ratios. Overall, the impact of cell size on multifunctionality was mainly determined by a compositional effect over which species interactions had a limited impact. As an exception, biovolume production did not relate to cell size or to the production levels of the component species, suggesting that interspecific interactions influenced this function. In an environmental context with smaller taxa dominating phytoplankton communities, our results suggest that this might ensure higher levels of multifunctionality via the improvement of functions related to resource uptake, but without necessarily influencing biomass production. This chapter is currently under review in *Freshwater Biology*.

**Chapter 4** explores facilitative interaction in controlled phytoplankton communities. Species interactions are considered as a key mechanism by which diversity influences ecosystem functioning. Studies on the influence of species interactions on ecosystem functioning have been largely focused on competition, a negative species interaction that leads to a reduction in ecosystem functioning as diversity increases (Wright *et al.* 2017). When positive interactions such as facilitation are present, combining multiple species may prove beneficial for ecosystem functioning. Recent evidence suggesting that positive interactions are quite common among freshwater algae (Venail *et al.* 2014, Fritschie *et al.* 2014). However, the conditions under which these positive interactions emerge are unknown. Even though in the past two decades BEF research has helped to deepen our understanding of the role of facilitation, its prevalence and magnitude in freshwater ecosystems remains poorly studied. Beyond this, there is a need to study how facilitative mechanisms can affect BEF relationships for other ecosystem functions than biomass production only. We investigate, in a controlled experimental setting, the importance of facilitation for the functioning of green algal communities. We used an additive design, combining two species with an equal initial biovolume and growing them under different nutrient conditions. This allowed determining how the contribution of each species to biovolume production differs in presence of another species, relative to when alone, and under different resource availability conditions. We focus on the prevalence of such positive

interactions (how often they occur) and their magnitude (how strong these effects are). This study aims to answer four questions. Firstly, do positive interactions between the tested species occur in our experiment and if yes, what kind of positive interaction is involved, niche differentiation or facilitation? Secondly, is the prevalence and/or magnitude of positive interactions linked to or dependent on the nutrient (phosphate) conditions? Third, is facilitation reciprocal between the interacting species? Lastly, and most importantly, if yes, how does phosphate condition influence the positive interaction? We found that phosphate concentration influenced facilitation by changing its prevalence, but not its strength. Reciprocal facilitation was a rare outcome, with most of the time only one species showing evidence of facilitation. Overall, some species acted as providers of facilitative interactions (facilitators), and other benefited from the presence of a second species (facilitated). The data of this chapter are still being analyzed and organized for publication.

**Chapter 5** is about BEF in tropical high-altitude lakes. Thanks to the literature review from chapter 2, we observed a total absence of BEF studies from high altitude tropical regions. Tropical high-altitude lakes are vital freshwater reservoirs in the Andes, heavily threatened by human activities that may alter their functioning and hamper the provisioning of key ecosystem services such as water supply. Despite their ecological and social relevance, we know little about these waterbodies, especially regarding the factors influencing their functioning. Here, we explored the links between several environmental variables and productivity, measured as chlorophyll-a concentration and total phytoplankton biovolume, across twenty-four tropical high-altitude lakes located over three-thousand meter above sea level in Southern Ecuador. We found that a combination of four abiotic factors explained over three quarters of the variation in chlorophyll-a concentration amongst lakes. Contrary to what studies from temperate regions suggest, taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, Shannon's diversity index was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume, presumably due to a strong compositional effect. Our results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activities can indirectly impact their functioning and their capacity to provide vital ecosystem services. This chapter is been published in the journal *Sustainability*.

**Chapter 6** deals with the applicability and perspective components of BEF studies with phytoplankton. A series of environmental and human related topics could benefit from BEF

research, including biodiversity conservation, habitat restoration, sustainable agriculture and biomass production. We first introduced biodiversity conservation and habitat restoration in aquatic ecosystems. Second, we summarized the potential applicability of incorporating facilitative interactions into aquatic restoration and conservation, Finally, we give two examples on how our experimental results can be used for wastewater treatment and industrial production purposes.

Finally, **Chapter 7** offers a general discussion of the thesis. It shows how all chapters fit together and contribute to the field and put the different parts of my thesis into a wider perspective. First, I discuss the underlying mechanisms of BEF relations. Then, I provide some personal opinions about the ecological way of thinking and the difficulty of transmitting scientific research results to a non-specialized public. I finish by summarizing all the main results of my research.



## CHAPTER 2

# A synthesis of biodiversity and ecosystem functioning research in freshwater lentic systems

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*Paper in preparation (first draft written by Ziyu, waiting for revisions by co-authors)*

## Abstract

Biodiversity and Ecosystem Functioning (BEF) research deals with understanding the ecosystem level consequences of losing biological diversity. Extensive evidence collected for over two decades, suggests that in general higher biodiversity results in higher ecosystem functioning. However, these conclusions were based on datasets that barely included freshwater information. Also, some studies that compared terrestrial to aquatic ecosystem opened a debate on whether the findings for terrestrial plants should be extrapolated to freshwater ecosystems or not. We collated all the published BEF studies in lentic systems (lakes, reservoirs and ponds), summarized the data and depicted the suggested underlying mechanisms. Our synthesis revealed that the majority of BEF studies in freshwater lentic ecosystems used species richness-based metrics as measures of biodiversity and productivity-based variables as measures of functioning. Besides, we found that the distribution of the types of BEF relationships differed between controlled laboratory studies and observational field studies. We found that the suggested claims about the underlying mechanisms are not well supported by proper evidence. To finish, we propose some directions for future BEF research, such as focusing more in trait-related diversity, exploring multiple functions simultaneously and facilitation in freshwater ecosystems. More importantly, we claim for efforts to build a stronger mechanistic understanding about the impact of biodiversity on ecosystem functioning.

## Introduction

The diversity of life, as the most unique feature on Earth, attracts much attention. All the variety of life forms, including variation among species, functional traits and genes are different components of biodiversity (Cardinale *et al.* 2012). Hot biodiversity related topics range from understanding the emergence and coexistence of so many life forms to how changes in biodiversity impact humans (Tilman 1999). Ecosystem functioning, which has strong connection to human well-being, is strongly linked to biodiversity (Burkhard *et al.* 2012). Over the past decades, several international initiatives and meta-analyses have shown that ecosystem functions, such as biomass production and nutrient cycling respond to changes in biodiversity (Balvanera *et al.* 2006; Cardinale *et al.* 2006; Cardinale *et al.* 2012). For example, a positive link between diversity and productivity has been often elucidated both on terrestrial ecosystems (Venail *et al.* 2015) and aquatic environments (Venail *et al.* 2008).

Freshwater ecosystems are essential and irreplaceable for humanity as they provide numerous valuable goods and services (Vanni *et al.* 2002; Geist 2011; Brooks *et al.* 2016). Despite this, freshwater ecosystems are threatened by pollution and climate change amongst other human derived activities (Dodds & Whiles 2010) and biodiversity loss is higher in freshwater than terrestrial ecosystems . Improving our understanding on the link between biodiversity and ecosystem functioning is important to evaluate the consequences of biodiversity decrease in these vital ecosystems.

With an increasing number of BEF studies, two important aspects shouldn't be ignored. First, numerous researches claim that biodiversity has a general positive effect on ecosystem functioning. This ignores a good portion of studies that may show null or negative effects of diversity on functioning. Second, we have limited knowledge about the underlying mechanism explaining biodiversity and ecosystem functioning relationships.

In this paper, we provide a detailed and comprehensive quantitative synthesis of BEF studies in freshwater phytoplankton. We first present the information from previous meta-analyses on BEF relationships in freshwater ecosystems. Then, we summarize the information from individual BEF studies with freshwater phytoplankton in controlled laboratory conditions and observational studies in the field. More importantly, we describe the mechanisms claimed to be affecting BEF relationships in freshwater phytoplankton. We also identify research gaps and



limitations. We finish by discussing some potential ways to improve BEF studies in freshwater ecosystems.

## Freshwater phytoplankton in BEF meta-analyses

Hundreds of BEF studies have been performed across all kinds of ecosystems during the last three decades. Some authors have collated and analyzed the information in these studies, resulting in the publication of several meta-analyses since 2006 (Balvanera *et al.* 2006; Cardinale *et al.* 2006; Cardinale *et al.* 2011; Hooper *et al.* 2012; Gross *et al.* 2014; O'Connor *et al.* 2017). However, most of these syntheses included a very limited number of studies on freshwater phytoplankton and reported almost exclusively studies under controlled laboratory conditions. For instance, Balvanera and colleagues collected 446 biodiversity effects on ecosystem functioning published in studies until 2004 (Balvanera *et al.* 2006) but only two of these studies were about freshwater phytoplankton. Cardinale and colleagues collated 111 experiments in total, but only one on freshwater phytoplankton (Cardinale *et al.* 2006). Five years later, Cardinale *et al.* (2011) updated their dataset by including a total of 640 entries, of which only 44 came from six studies with freshwater phytoplankton. Of the 44 relationships between phytoplankton diversity and functioning, seven were negative, 19 null and 18 were positive. Despite the large variation across individual studies on the type of relationship between diversity and functioning, they defined the overall biodiversity effect as significantly positive, which means that communities with more species produce more biomass than monocultures in controlled experiments (Cardinale *et al.* 2011). Only 41% of the individual studies matched their general conclusion. Later, phytoplankton studies became more popular, allowing to analyze them independently from other ecosystems. Cardinale *et al.* 2013 and Gross *et al.* 2014 compiled a set of eleven studies on phytoplankton and in addition to what was concluded in 2011, they were able to establish that the biomass of communities with more species was not more stable over time than the biomass of monocultures. Again, a large variation among studies was observed. In 2017, O'Connor and colleagues concluded that species richness and standing biomass were positively related via a power function in aquatic ecosystems under controlled experimental conditions (O'Connor *et al.* 2017). Finally, in 2017, Duffy and colleagues analyzed, for the very first time, studies conducted in natural conditions. They concluded that species richness and standing biomass are positively related in the wild and these effects were even stronger after correcting for environmental factors. However, the dataset allowing to get to these conclusions only included two studies on phytoplankton.

In conclusion, most previous meta-analyses did not include much information from freshwater phytoplankton and some did not treat the data from different ecosystems separately. Abundant data collected from terrestrial grasslands was often mixed and analyzed together with scarce data from freshwater phytoplankton. Often the authors considered the underlying processes for terrestrial plants and aquatic microorganisms as equivalent. Some evidence suggests that diversity influences functioning in different ways in different ecosystems (Cardinale *et al.* 2013, Gross *et al.* 2014, O'Connor *et al.* 2017) but a proper comparison is still to be assessed. These meta-analyses have played a major role in constructing the current understanding of BEF relationships in general. However, those synthesis have at least two other serious limitations. First, even very recent syntheses use an outdated database, ignoring studies conducted since 2010 (O'Connor *et al.* 2017). Second, species richness was the only aspect of diversity included, completely ignoring functional based diversity, which is expected to provide a better understanding of BEF relationships (Cadotte, Carscadden & Mirotchnick 2011).

Regarding the underlying mechanisms, less than half of the papers provide a mechanistic rationale for BEF relationships, and less than a quarter of the papers provide proper evidence about the suggested mechanisms. Most papers that explore the mechanisms use the relative yield to tell apart the relative contribution of selection complementarity effects (Loreau & Hector 2001). This limitation is present in both aquatic and terrestrial ecosystem's studies. Some data suggest that complementarity appears to play a primary role in aquatic ecosystems, whereas complementarity and selection effect appear equally important in terrestrial ecosystems (Cardinale *et al.* 2011). In conclusion, underlying mechanisms are not well explained in previous BEF researches.

## BEF relationships in freshwater ecosystems

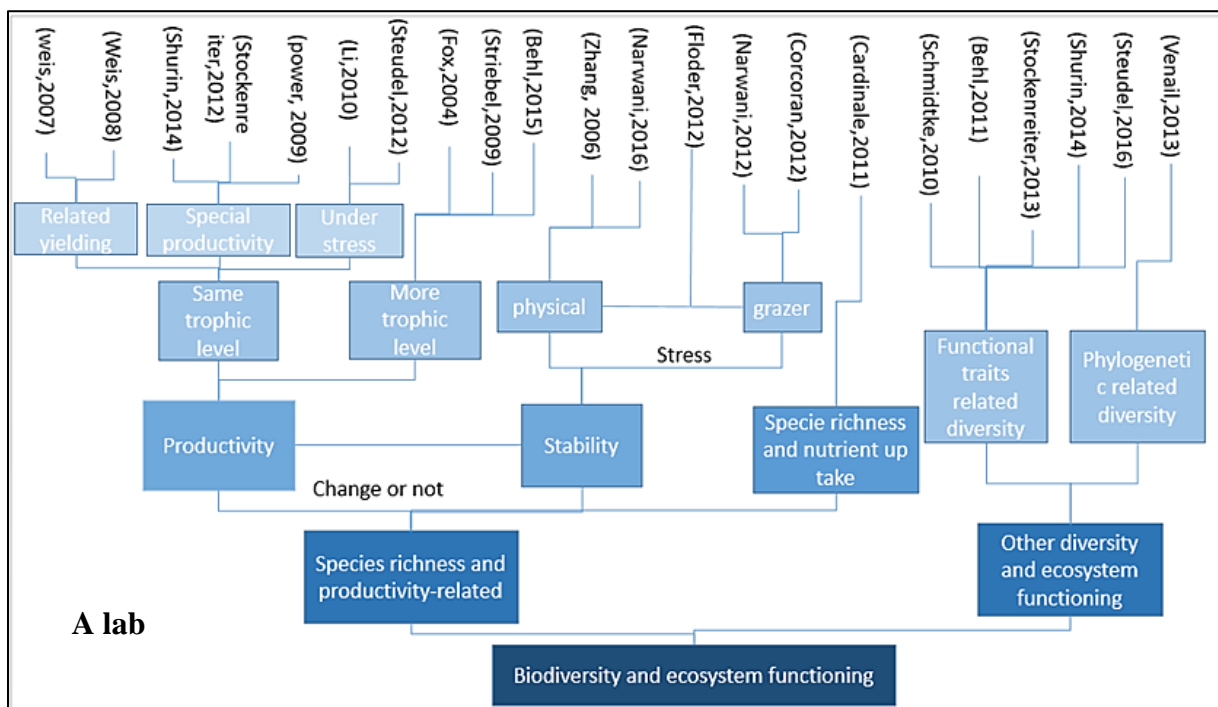
To better assess the impact of diversity on ecosystem functioning in freshwater phytoplankton, we performed a systematic review of the existing literature linking phytoplankton diversity to ecosystem functioning in freshwater lentic ecosystems. We collected over 2000 papers by searching for a series of keywords in Web of Science, including “phytoplankton diversity AND ecosystem function\*”, “alga\* AND ecosystem function\*” or “diatom\* AND ecosystem function\*” or “cyanobacteria\* AND ecosystem function\*” among others (**Table 2-1**). Across all search strings, and after reading the titles and abstracts, we collected 47 papers that we could use for our review based on the following criteria: 1) The study included phytoplankton

biodiversity (species richness; function diversity; genetic diversity; Shannon index...) and ecosystem functioning data (productivity as biomass or biovolume; resource use efficiency; water quality...). 2) The study included freshwater phytoplankton from lentic ecosystems (lakes, ponds and reservoirs). 3. The study statistically analyzed and discussed the relationship between biodiversity and ecosystem functioning. Only studies meeting the three criteria were included in this synthesis. We categorized the studies in two big groups: laboratory studies performed under controlled culture conditions where the authors directly manipulated diversity and observational field work performed in natural conditions. In this synthesis, we did not employ mathematical modelling to quantify the overall diversity effect, we rather tallied the numbers of experiments and their reported results of diversity and ecosystem functioning relationships.

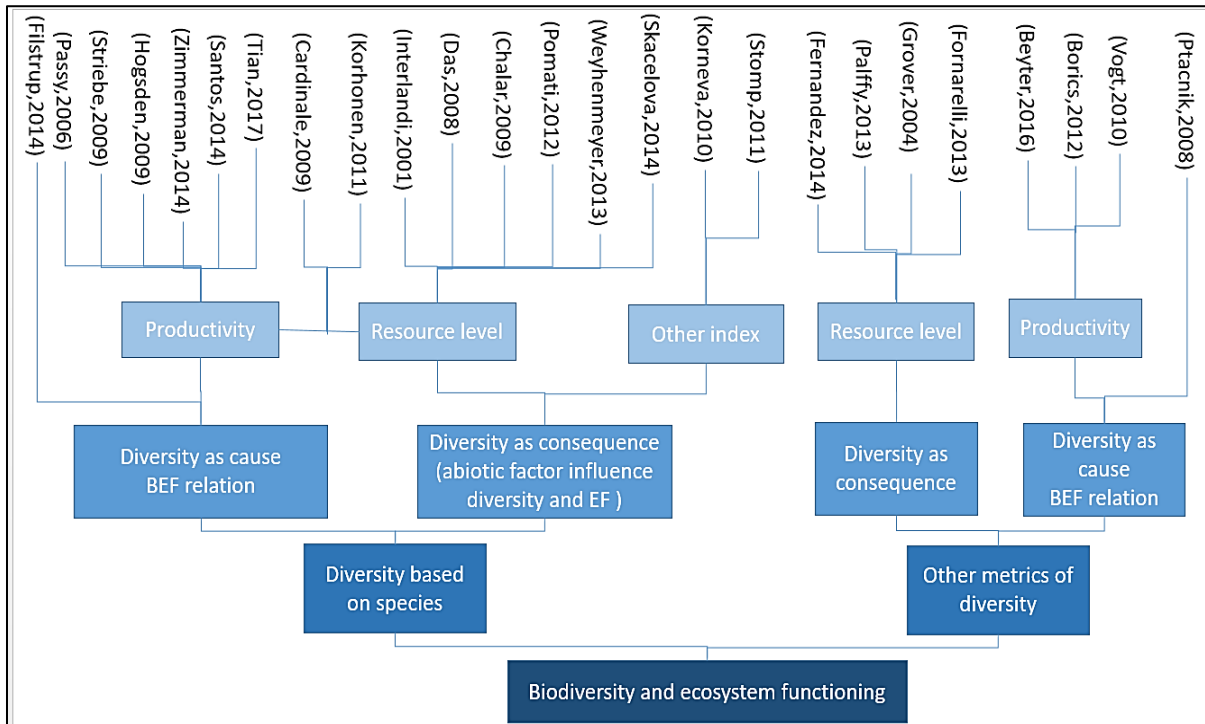
**Table 2-1.** Summary of the keywords used for the search in Web of Science and the number of papers resulted for each search. The results of each search included some repeated papers.

Examples of Key words used in Web of Science	Number of papers
Phytoplankton* diversity AND ecosystem function*	398
alga* diversity AND function*	1242
diatom* diversity AND function*	375
cyanobacteria* diversity AND function*	578

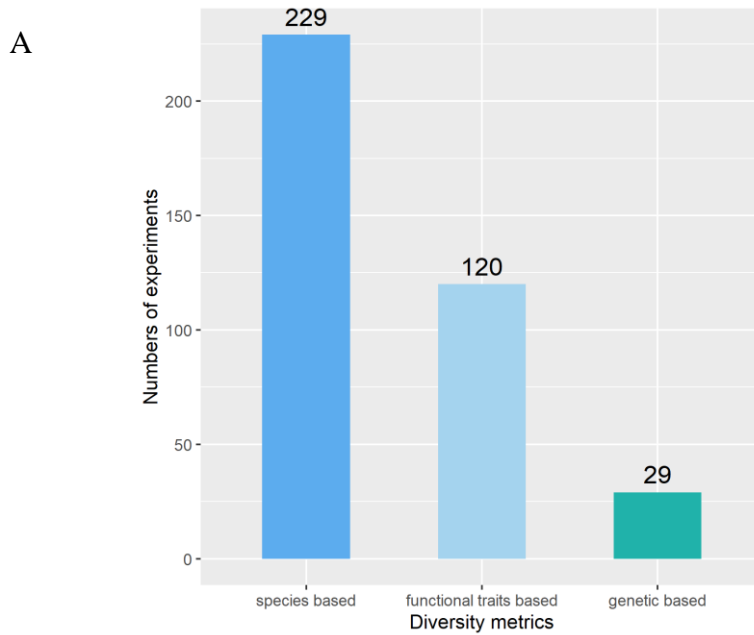
We found 22 studies published so far including a total of 378 experiments that directly manipulated any aspect of phytoplankton diversity and measured its influence on any aspect of ecosystem functioning in the laboratory under controlled environment. These studies covered a variety of BEF relationships, from producer-only to both producer and grazer functioning and from primary productivity per se to temporal stability of productivity (**Figure 2-1**). In lab studies, more than 60% of the experiments used a taxonomy-based quantification of diversity, with most studies using species richness (**Figure 2-2A**). 96% of lab studies focused on productivity as measure of ecosystem functioning (**Figure 2-2B**).



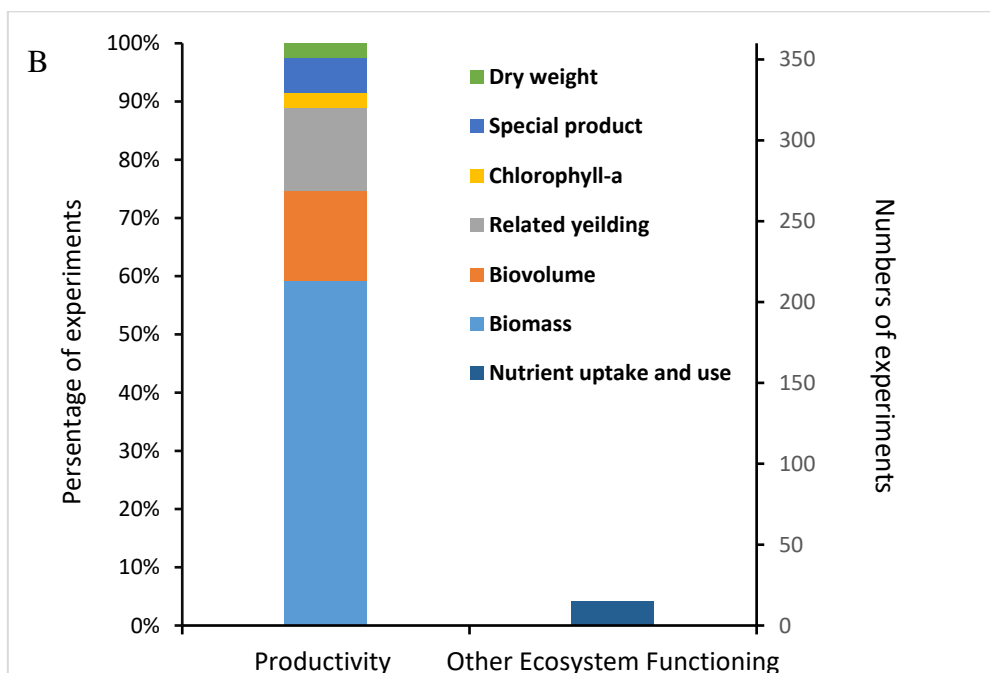
**Figure 2-1A** For laboratory studies, this figure clustered papers according to the topics included in the BEF papers. Studies can be classified according to the diversity metrics they used; species richness or other diversity metrics. The other diversity metrics included functional traits related diversity metrics and phylogenetic related diversity metrics. We classified papers using species richness into three groups based on different measures of functioning: productivity (10 studies), stability (5 studies) and nutrient uptake (1 paper). Three papers studied diversity effects on productivity across multiple trophic levels. Five papers studied the relative yield of biomass or biomass production under stress in the same trophic level. The stability experiments included two different kinds of stress, physical and grazing.



**Figure 2-1B.** Field studies can be distinguished by the diversity metrics into species-based studies and other diversity metrics. Most papers use species based diversity metrics. In field studies a clear distinction between studies in which diversity is considered a driver of functioning (diversity as cause) or the opposite (diversity as consequence). In each subgroup, studies differ in the type of ecosystem functioning considered.

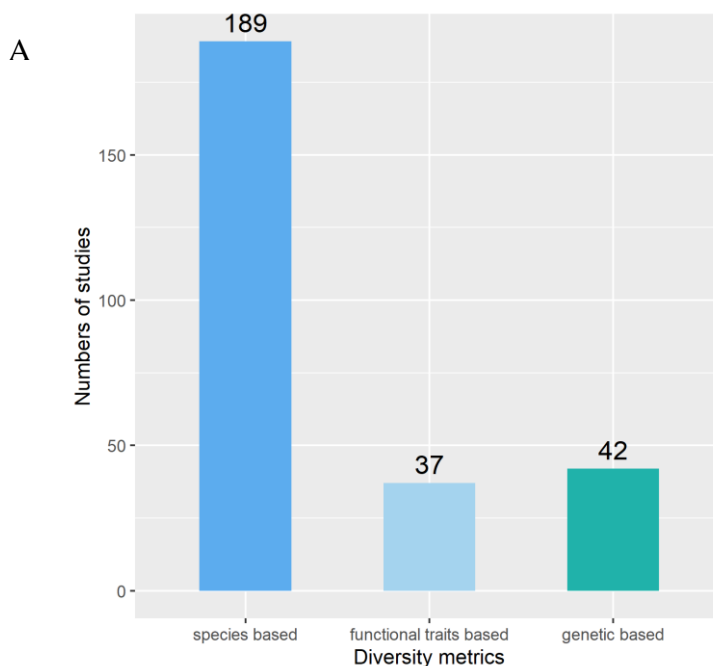


**Figure 2-2A.** Distribution of the biodiversity metrics used in lab experiments with freshwater phytoplankton: 229 experiments used species richness-based diversity, 120 experiments used functional trait-based diversity and 29 experiments used genetic-based diversity.

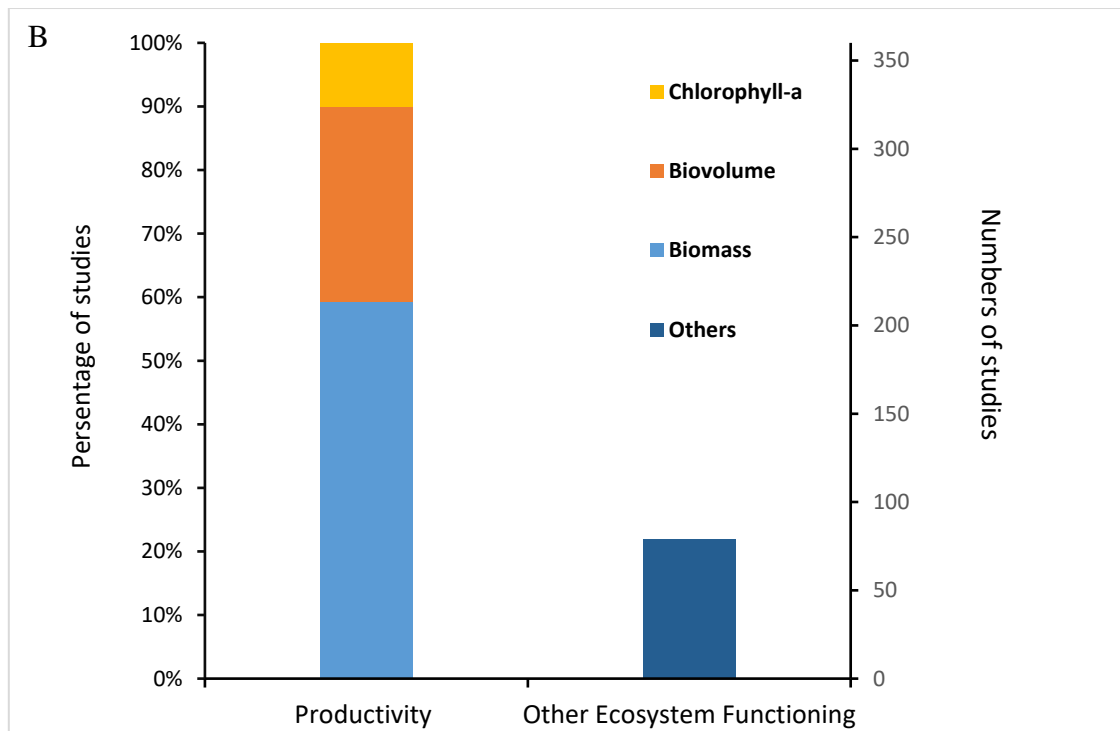


**Figure 2-2B:** Distribution of the ecosystem functioning variables used in lab experiments with freshwater phytoplankton: 96% of experiments focused on productivity, including dry weight, chlorophyll-a, biovolume, biomass and special products such as biofuel. Only 4% of experiments used a different ecosystem function such as nutrient uptake and use.

A similar trend was observed in the field studies. We collected 25 papers of freshwater phytoplankton under natural conditions that included a total of 268 reports of BEF relationships. Productivity and resource-use were the most common topic among field studies (**Figure 2-1B**). Most of them used taxonomy-based diversity, especially species richness that was present in 70% of the reports (**Figure 2-3A**). In 71% of field studies functioning was measured as productivity (**Figure 2-3B**). Together, the data gathered from the lab and field studies showed that taxonomy-based diversity, especially species richness was by far the dominated metric of diversity. Only a few papers included other metrics of diversity. In lab studies, functional based diversity and gene-based diversity metrics accounted for 32% and 8% of studies respectively (**Figure 2-2A**). In field studies, the proportions of functional-based and gene-based diversity were 14% and 16% respectively (**Figure 2-3A**). Productivity was the more common ecosystem function in the BEF studies with freshwater phytoplankton.



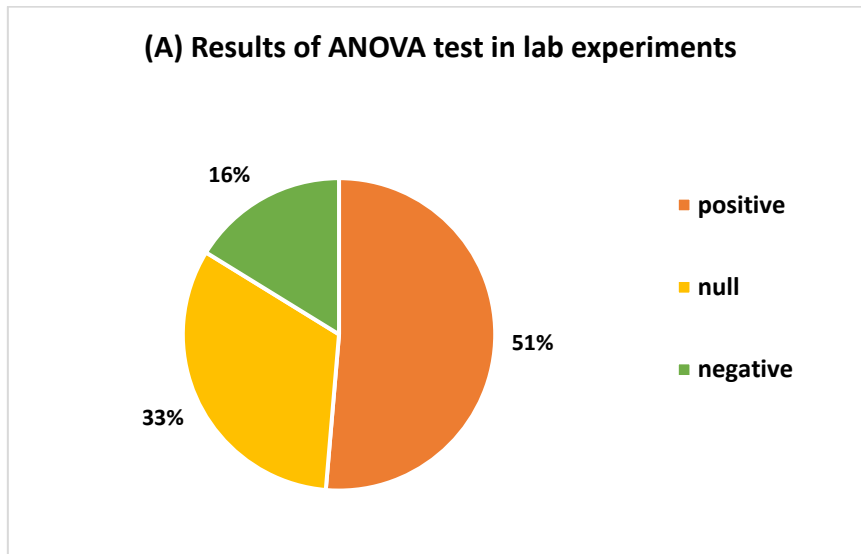
**Figure 2-3A.** *Distribution of biodiversity metrics used in field observational studies with freshwater phytoplankton: 189 experiments used species richness as measure of biodiversity, 37 experiments used functional-based diversity metrics such as functional diversity and functional trait diversity, 42 experiments used gene-based diversity.*



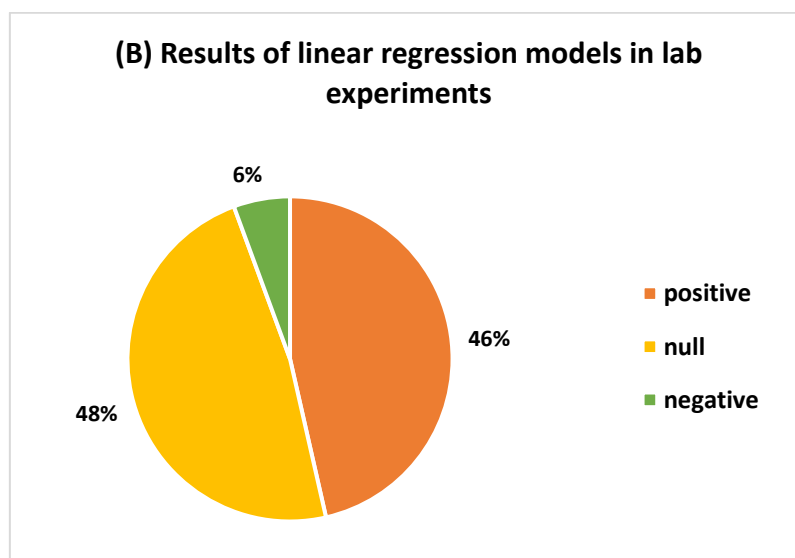
**Figure 2-3B.** Distribution of ecosystem functioning variables used in field studies with freshwater phytoplankton: 71% of experiments focused on phytoplankton's productivity, including chlorophyll-a, biovolume, biomass. The other 29% of experiments focused on other ecosystem functions such as resource use efficiency (RUE).

A variety of statistical tests are applied to establish the shape of the relationship between biodiversity and ecosystem functioning. The two more common are the analysis of variance (ANOVA) and the linear model fitting. The former is used to compare functioning levels across different levels of diversity which is presented as a categorical variable (e.g., 1 species, 2 species, 4 species, etc.). The latter is used when diversity is a continuous variable. We found that in laboratory studies using ANOVA, 51% of the experiments showed a positive relationship between biodiversity and functioning. This means that a decrease in biodiversity results in a reduced functioning. Only 16% of the experiment showed a negative relationship, meaning that functioning increased as diversity increased. Finally, 33% of the reported experiments showed a null relationship, meaning that functioning was not affected by changes in biodiversity (**Figure 2-4A**). In studies using linear fitting, the results show that in 46% of the experiments biodiversity has a positive effect on ecosystem functioning. The negative and null relationships represented 6% and 48% of studies respectively (**Figure 2-4B**).



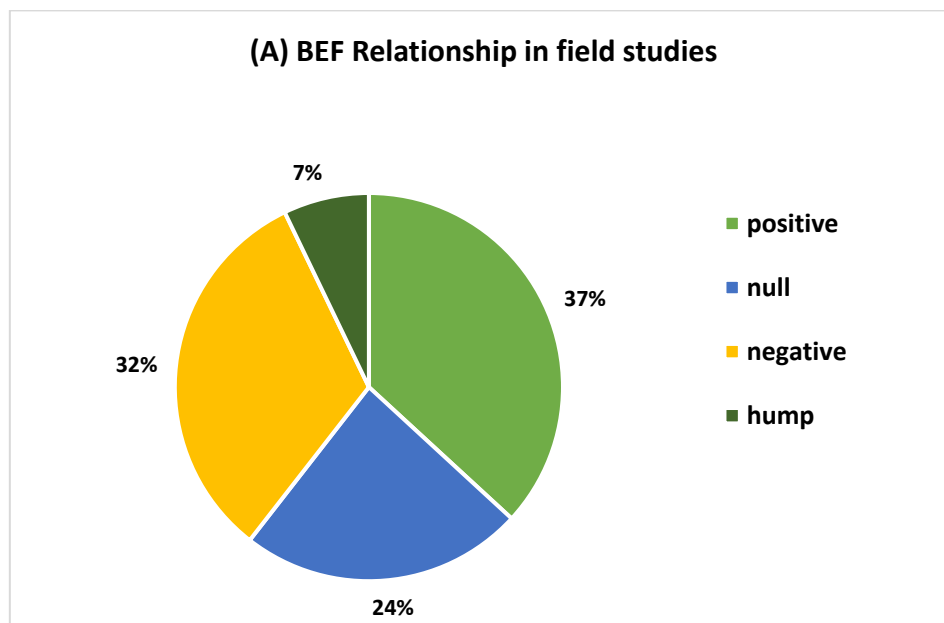


*Figure 2-4A. Types of relationships between biodiversity and ecosystem functioning in laboratory studies using ANOVA. There are 51% of laboratory experiments showing positive, 16% negative and 33% of null relationships.*

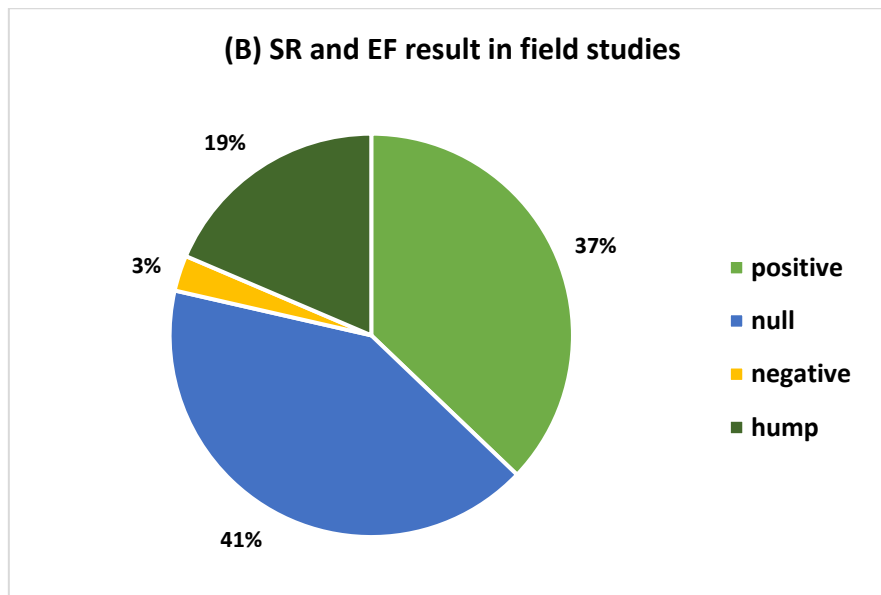


*Figure 2-4B. Types of relationships between biodiversity and ecosystem functioning using linear regression models in laboratory experiments. There are 46% of experiments showing positive, 6% negative and 48% null relationships.*

In the observational field studies, the BEF relationships are more complex than under controlled laboratory conditions. None of field studies used ANOVA to test for the effects of diversity. Instead, all of them used a model fitting approach. In that case, positive relationships included positive linear and positive nonlinear effects. Overall, 37% of field studies found a positive relationship between diversity and functioning. Negative and null relationships represented 32% of the results. Also, 7% of the experiments showed a hump-shaped relationship. Only 24% of the experiments showed a null relationship (**Figure 2-5A**). The results can also be split into different diversity metrics and ecosystem functioning variables. When species richness was used as a diversity measure, the positive relationships represented 37%, the negative ones represented 3%, and the hump-shaped relations 19% (**Figure 2-5B**)



*Figure 2-5A. Types of relationships between biodiversity and ecosystem functioning in observational field studies. There are 37% of studies showing positive, 32% of studies negative and 24% of studies null relationships. The other 7% of field studies showed a hump-shape relationship between biodiversity and ecosystem functioning.*

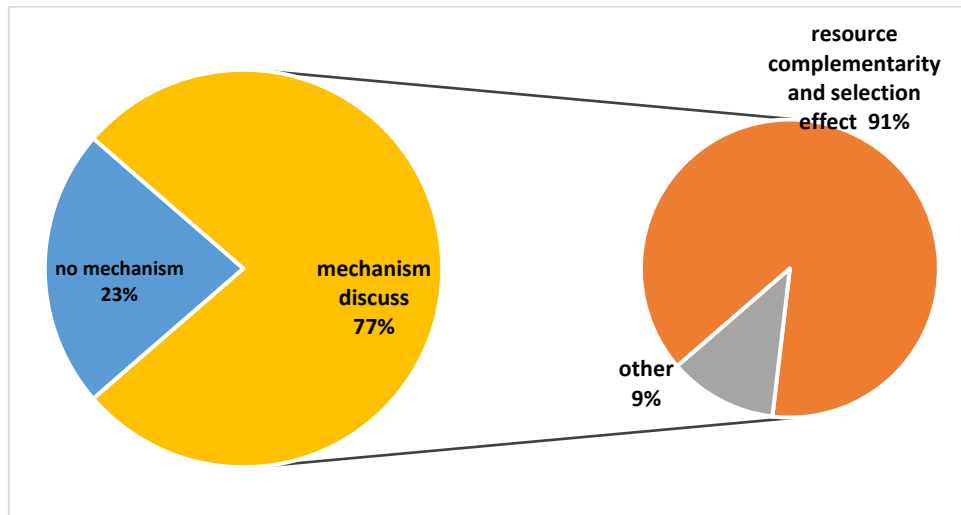


*Figure 2-5B. Types of relationships between species richness and ecosystem functioning in field studies. There are 37% of studies showing positive, 3% of studies show negative and 41% showing null relationships. The other 19% of field studies show a hump-shape relationship between species richness and ecosystem functioning.*

Taking all the relationship described in both lab experiments and field studies, we found that the BEF relationship in freshwater ecosystems are far from a general positive effect of diversity on ecosystem functioning as has been stated in previous studies.

## Underlying mechanisms

In accordance with previous meta-analysis on BEF studies across ecosystems (Cardinale *et al.* 2006) and despite the majority field studies do not discuss any underlying mechanisms, claims of complementarity and/or selection effect are frequent in the literature (**Figure 2-6**). However, we collected some evidence that questions their authenticity.



*Figure 2-6. The mechanistic explanation for diversity effects from the lab studies with freshwater phytoplankton. In 23% of lab studies no mentions of any mechanism was present but in the other 77% mechanisms are discussed. Within that 77% of lab studies, 91% speculate that complementarity and selection effect are the underlying mechanisms of biodiversity effects on ecosystem functioning.*

The first issue is that most of the papers that claim about mechanisms are simply incorporating a statistical approach to calculate the relative contribution of complementarity and selection effect (Loreau 1998; Loreau & Hector 2001; Barry *et al.* 2019). Such statistical methods do not represent by themselves proper evidence of actual mechanisms (Barry *et al.* 2019). We checked all the papers that are supposedly providing support for any mechanism and we found that almost all of them used the analytical method for calculating complementarity and selection effects based on the polyculture vs. monoculture comparison. Moreover, almost all those papers just calculated the effects mathematically and claimed that this is enough to support the complementarity or selection effects but did not analyze the real underlying mechanisms. This shows that the purely analytical method is overused and the way BEF studies have explored the underlying mechanisms is inconsistent. It was clearly mentioned by Loreau and Hector (Loreau

& Hector 2001) that this method cannot be used as unequivocal evidence of any mechanism *per se*. The authors also declared that their method is based on relative yield, which is used in short-term plant competition experiments, and the results may depend on the initial densities of the different species. Moreover, even if the result of complementarity effect or selection effect is null, it doesn't mean there are no complementarity or selection effects. Both effects can cancel each other. The Loreau and Hector approach can be divided into three different mechanisms (Loreau 1998; Loreau & Hector 2001): resource partitioning, facilitation (together as complementarity) and dominating effects (also referred to as sampling effect), all of which were developed for plant studies. Whether these mechanisms can be applied and extrapolated to freshwater ecosystems is not clear.

Resource partitioning is based on niche complementarity theory, which describes well the nutrient conditions of terrestrial ecosystems. One key aspect of niche complementarity is that the environment is heterogeneous. However, studies in freshwater ecosystem have explored whether freshwater ecosystems are heterogeneous enough to allow niche complementarity and compared biodiversity effect in homogenous and heterogeneous conditions (Weis, Madrigal & Cardinale 2008; Cardinale 2011). The results of these papers are contradictory. One popular way to test for niche partitioning is to check for the presence ofoveryielding, which occurs when the functioning of a polyculture is higher than the monoculture with higher functioning levels cultured alone. Overyielding is not frequent in freshwater ecosystems, undermining the use of relative yielding as evidence of niche complementarity (Weis *et al.* 2007).

Selection effects cannot be used to reveal the underlying mechanisms of BEF relationships in natural conditions. The selection effect is based on the resource competition theory from terrestrial ecosystems (Tilman, Lehman & Thomson 1997) and is not very frequent in freshwater ecosystems (Cardinale *et al.* 2006). Another synthesis, 5 years later, claimed that the selection effects do not differ from zero (Cardinale *et al.* 2011). After looking for the claims from the papers that we collected, we found no clear answer to whether selection effect are present in freshwater studies or not.

To date, only a few papers focused on facilitation in freshwater ecosystem. One reason is that it is very hard to distinguish facilitation from niche complementarity (Loreau & Hector 2001). Experiments with the largest diversity effect include species that can facilitate with other species, such as legumes in terrestrial ecosystems and cyanobacteria in aquatic ecosystem.

Briefly, BEF studies in freshwater ecosystem conducted so far do not provide a proper mechanistic understanding about the effects of biodiversity on functioning. The traditional method of using complementarity and selection effect as mechanism is inconsistent. New mechanistic approaches are urgently needed.

## Research gaps

Although there is strong empirical evidence suggesting that the relationship between biodiversity and ecosystem functioning in freshwater phytoplankton is generally but not always positive, several questions remain unanswered. Hereafter, we list and discuss some major research gaps in BEF research in general.

First, species richness is a poor predictor of ecosystem functioning, even it is the most widely used diversity metric in BEF studies. Our results showed that the majority of BEF studied in freshwater both in lab and field used species richness as the main biodiversity metric. Even if species richness has some benefits like being easy to measure it also has some defaults. Species are defined by interpretations of species identity; it is simply influenced by human concept. More importantly, species richness may not be linked with functional story means that sometimes species cannot explain the mechanism of ecosystem functioning (Ackerly & Cornwell 2007). Nowadays, the technology of gene sequencing is well developed, gene based diversity like the gene diversity (GD) and phylogenetic diversity (PD) are more presented in researches (Gravel *et al.* 2012). Additionally, with the developing of ecological consciousness, increasing number of ecologists pay attention in functional traits and have found that functionally similar morphotypes exhibit dynamics that are more synchronized (Rocha, Gaedke & Vasseur 2011). If we look at the time of all studies we collected, it is obvious prompted that after 2010, scientists are notices the drawback of species richness and divert their attention to functional traits-based diversity (**Figure 2-1A and 2-1B**). More importantly, it has been proved recently that individual-level trait diversity predicts phytoplankton community properties better than species richness or evenness (Fontana *et al.* 2017). Although more and more attention of functional traits related diversity is attracted, compare the number of studies used species richness, the number of these studies still less. So that more studies that adopt functional trait based and gene-based diversity in urgently needed.

Additionally, the shape of BEF relationship are presented different between Lab studies and field studies. The positive linear is dominant relationship in the lab studies. On the contrary, in

the field studies, hump-shaped relationship is exhibited much more in the field studies than in the lab studies. Another difference of BEF relations is that negative relation, both negative linear and negative nonlinear appeared more in field studies. One reason for the higher frequency of hump-shaped shown in field studies is that the different distinct attitude to examine their data. More than half of lab test are chosen ANOVA test that cannot give more detail of BEF relationship. Moreover, for the papers tested function model, they do not test other kind of function model than linear model. However, in field studies, authors tend to try as much function models as possible to describe the data in most acceptable way. Some studies do have test different function model, but since the diversity grade are not big enough to make the model presenting big difference in explained variance index. This situation reminds us the limitation of design and data examination in lab experiment, which could be a crucial opportunity for future research. Additional data assessment will be needed to better character shape of BEF relationship in lab studies. Besides the man-made bias, in our opinion, this is also because the huge disparity between diversity manipulated in lab and existed in natural environment. Although a total of 64 species for most are manipulated in lab, the average diversity of lab experiment are still far less than of field (Steudel *et al.* 2012). Also, both the timescale and spatial scale are divergence. For example, in lab, experiments cannot last for a long time, but the field expectation can continue years. This limits the ability to determine which shape of BEF relations, and researchers should document more effects to collect more data and draw conclusions.

Notably, one obvious aspect of field study is that special attention paid to nutrient resource as well as linking nutrient resource related functioning such as resource uptake and resource use efficiency to productivity. Conversely, lab experiments primarily engage in biodiversity's outcome on single functions. However, growth and development of researches suggest that effect of diversity is at least different when considered together more than one ecosystem functions (Maestre *et al.* 2012; Lefcheck *et al.* 2015; Daam *et al.* 2019). Several ecosystem processes simultaneously function together we call it "multifunction". The stark contrast that multifunction is valued in field studies but ignored in lab experiments not only decrease the usability of lab result to natural environment but also produce the bias in mechanism exploration.

## Prospect for original research

While the definition of multifunction was reported more than ten years ago (Rosenfeld 2002; Gamfeldt, Hillebrand & Jonsson 2008), our understanding of how diversity impact multifunction is still limited. As shown by our data, it is recognizable that most BEF relationships in freshwater ecosystem as well as other ecosystem result from individual ecosystem functioning especially in lab studies, syntheses typically focus on productivity related functioning. Linking this phenomenon with the fact that real ecosystems are composed by many interplayed processes, we can easily say that some underlying questions of summary the biodiversity influence in ecosystem functioning are exiting. Two kind of mistake will be happened when we only consider ecosystem function separately. One possibility is that we neglect biodiversity effect. Considering more functions superpose together, effect of diversity should be stronger than single function (Maestre *et al.* 2012). Other possibility is that effect of biodiversity in multifunction will be smaller than individual function, because of the trade-off among different functions. Even there is increasing number of papers is disseminating the importance to study it more, Disappointing, multifunction is completely ignored when we are designing BEF experiment. If we are not going to paying more attention in multifunction of ecosystem, we not only cannot solve the problem we have now, but also will not extra multiple goods and service form high-functioning ecosystem (Byrnes *et al.* 2014).

One main reason why researchers give up calculating multifunction when design the experiment is that multifunction is not as directly as single function and it is difficult to quantify the multifunctioning (Lefcheck *et al.* 2015). However, whining the effort made by numbers of ecologists, approach to measure multifunction is more and more comprehensive and informative. In 2014, Jarrent et al compare four basic approaches to explore quantify the multifunction, then propose a new approach (Byrnes *et al.* 2014). One year later, by using the new approach, their team prove that biodiversity enhances ecosystem multifunctionality across trophic levels and habitats (Lefcheck *et al.* 2015).

Furthermore, early in the end of last century, pioneers of BEF studies have propagated the idea that trait related functional diversity can influence the ecosystem processes (Tilman *et al.* 1997). Along this line of consideration, more review have been done to validate how extensive the functional diversity or the value and range of species traits, rather than species richness per se,



is to determine the ecosystem functioning in terrestrial ecosystem (Díaz & Cabido 2001) and marine ecosystems (Hood *et al.* 2006). There is a growing consensus, that trait-based diversity or functional diversity are better predictors for BEF studies in freshwater ecosystems, as the consequence of the publication of several groundbreaking theoretical development and experiment (Padisák, Crossetti & Naselli-Flores 2008; Stockenreiter *et al.* 2013). The most persuasive evidence are shown in our data that after 2010, the majority of BEF papers in freshwater ecosystem are applying trait related diversity as diversity metric. Even so, compare to the empirical afforded by species richness as diversity measurement, more experiments using trait-based diversity are need.

Meanwhile, in 2001, Mulder's group tested the value of high diversity under environmental variability on productivity. They found that facilitative interactions, rather than sampling effects (higher probability of sampling the most frequent species i.e. the most productive) or niche complementarity (success of species in a habitat relies on use of different resources), could best explain increased survivorship for almost all species and positive biodiversity effects on biomass production (Mulder, Uliassi & Doak 2001). As the idea, which we argued in the mechanism part of our paper, facilitation can be a reasonable and considerable direction we can set to.

## Conclusion

After hundreds of individual studies and a half dozen syntheses, it is today well accepted that biodiversity improves ecosystem functioning. However, the evidence to support such a claim comes mostly from controlled experiments with terrestrial plants and has been well supported for species richness and biomass production only. Because of the biotic and abiotic characteristics of freshwater ecosystems, the shape of the relationship between diversity and functioning and the underlying mechanisms maybe different from terrestrial ecosystems. The quantity and quality of theoretical and empirical data for phytoplankton are not enough to make a clear conclusion. Further experiments should be designed to overcome some limitations of previous studies such as the use of a single measure of diversity or functioning. Special attention should be given to the mechanistic interpretation of diversity effects. New hypotheses about the underlying mechanism by which diversity influences ecosystem functioning are needed, and properly designed studies are much needed.

## Acknowledgements

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## Authors' Contributions

Z.G., P.V. and B.I. conceived of the project. Z.G. collected all the data and do all the analysis. Z.G. wrote the papers. P.V. and B.I. contributed constructive comments and revisions. All authors contributed substantially to drafts and gave approval for publication.

## Supplementary Information

*Table2-S1: Collected Papers in Lab studies*

<b>Collected papers in lab studies</b>	<b>Number of experiments</b>
(Fox 2004)	1
(Zhang & Zhang 2006)	7
(Weis <i>et al.</i> 2007)	20
(Weis, Madrigal & Cardinale 2008)	3
(Power & Cardinale 2009)	16
(Striebel, Behl & Stibor 2009)	6
(Li <i>et al.</i> 2010)	9
(Schmidtke, Gaedke & Weithoff 2010)	1
(Behl, Donval & Stibor 2011)	36
(Cardinale 2011)	3
(Stockenreiter <i>et al.</i> 2011)	1
(Corcoran & Boeing 2012)	7
(Flöder & Hillebrand 2012)	39
(Narwani & Mazumder 2012)	28
(Steudel <i>et al.</i> 2012)	14
(Shurin <i>et al.</i> 2013)	16
(Stockenreiter <i>et al.</i> 2013)	12
(Venail <i>et al.</i> 2013)	4
(Shurin <i>et al.</i> 2014)	3
(Behl & Stibor 2015)	15
(Narwani <i>et al.</i> 2016)	3
(Steudel <i>et al.</i> 2016)	132
total	378

**Table 2-S2: Collected Papers in field studies**

<b>Collected papers in field studies</b>	<b>Number of experiments</b>
(Interlandi & Kilham 2001)	5
(Grover & Chrzanowski 2004)	4
(Passy & Legendre 2006)	9
(Das, Nordin & Mazumder 2008)	3
(Ptacnik <i>et al.</i> 2008)	8
(Cardinale <i>et al.</i> 2009)	1
(Chalar 2009)	4
(Hogsden, Xenopoulos & Rusak 2009)	2
(Striebel, Behl & Stibor 2009)	2
(Korneva 2010)	46
(Vogt, Beisner & Prairie 2010)	33
(Korhonen, Wang & Soininen 2011)	1
(Stomp <i>et al.</i> 2011)	1
(Borics <i>et al.</i> 2012)	2
(Fornarelli, Antenucci & Marti 2012)	6
(Pomati <i>et al.</i> 2012)	5
(Pálffy, Présing & Vörös 2013)	8
(Skácelová & Lepš 2013)	4
(Weyhenmeyer, Peter & Willen 2013)	1
(Fernández, Cáceres & Parodi 2014)	4
(Filstrup <i>et al.</i> 2014)	68
(Zimmerman & Cardinale 2014)	1
(Santos, Carneiro & Cianciaruso 2015)	10
(Beyter <i>et al.</i> 2016)	24
(Tian <i>et al.</i> 2017)	16
<b>Total</b>	<b>268</b>



## CHAPTER 3

# Resource uptake benefits from the presence of smaller species in experimental phytoplankton assemblages

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## Abstract

Cell size is a key trait affecting several physiological processes in phytoplankton. Similarly, population and community level processes such as abundance, biomass and primary production correlate with changes in the cell size composition of phytoplankton assemblages. However, the direct influence of cell size on functioning remains empirically underexplored and we know little about the ecosystem level consequences of changes in phytoplankton's cell size composition. Here, we manipulated the species composition of forty cultures of pairs of freshwater green algae, hence generating gradients in four different cell size variables but keeping species richness constant. We cultured the algae for twelve days in microcosms, under controlled laboratory conditions and measured the impacts of each of the cell size variables on four different functions: nitrogen uptake, phosphate uptake, light attenuation and biovolume production. We evaluated the relative contribution of species interactions to functioning by comparing the observed to the expected levels of each function based on the constituent species as monocultures. We found that the three functions related to resource uptake benefited from the presence of smaller species with larger surface area to volume ratios. This was driven by smaller species having higher resource uptake levels, not by changes in the strength or nature of species interactions. As the exception, biovolume production of the bi-cultures did not relate to cell size or to the production of the component species, suggesting an impact of interspecific interactions in this function. Our results offer original empirical evidence on how changes in the cell size composition of phytoplankton communities can directly influence different functions. Our findings suggest that in the current environmental context, with smaller taxa increasingly dominating phytoplankton, this might lead to an improvement in resource acquisition but without necessarily affecting biovolume production.

## Introduction

In phytoplankton, cell size varies over several orders of magnitude and is considered a key trait influencing a large variety of biological processes, such as nutrient uptake, light absorption and growth, amongst others (Litchman & Klausmeier 2008; Finkel *et al.* 2009; Edwards, Klausmeier & Litchman 2011b; Litchman *et al.* 2015; Maranon 2015; Kremer, Thomas & Litchman 2017; Sommer *et al.* 2017). It is well established that, in general but not always, smaller phytoplankton cells thrive in lower nutrient concentrations but have lower per-cell maximum nutrient storage capacities than larger cells (Litchman *et al.* 2010a; Litchman *et al.* 2015). In marine phytoplankton, the nitrogen and phosphorous competitive abilities tend to decrease with cell size, meaning that smaller taxa tend to outcompete larger ones at low nutrient concentrations (Edwards, Klausmeier & Litchman 2011b). Smaller phytoplankton cells also tend to have higher light absorption capacities, higher metabolic rates and faster growth rates than larger cells. However, some evidence also suggests that cells of intermediate size (i.e., around  $100 \mu\text{m}^3$ ) have the highest biomass-specific metabolic rates and the best capacity to convert nutrients into biomass (Maranon 2015). With respect to loss processes, smaller cells demonstrate lower sinking rates but are more susceptible to grazing compared to larger cells (Litchman & Klausmeier 2008; Litchman *et al.* 2010a).

Driven by these physiological and ecological constraints related to cell size, spatial (e.g., biogeographical) and temporal (e.g., seasonal) changes in the environmental conditions could generate changes in the cell size composition of phytoplankton assemblages in marine and freshwater ecosystems. For instance, larger cells are expected to dominate phytoplankton communities when nutrients are abundant, under fluctuating nutrient conditions or high grazing pressure (Litchman *et al.* 2010a; Acevedo-Trejos *et al.* 2015; Sommer *et al.* 2017). On the contrary, smaller cells should have a competitive advantage under more constant and nutrient-limited conditions, higher water column stability or in the absence of grazers (Litchman *et al.* 2010a; Acevedo-Trejos *et al.* 2015; Sommer *et al.* 2017). Variation in the cell size structure of phytoplankton communities also relates to changes in other ecosystem level processes including abundance, primary production, biomass production, biogeochemical cycles and energy fluxes (Cermeño & Figueiras 2008; Litchman & Klausmeier 2008; Litchman, Klausmeier & Yoshiyama 2009). As for many different organisms, the abundance of phytoplankton populations and communities are negatively related to cell size, with smaller taxa reaching higher abundances than larger ones (i.e., cross-community scaling relationships, (Cermeño &



Figueiras 2008; Maranon 2015; Sommer *et al.* 2017). In the Atlantic Ocean, primary production and energy export to higher trophic levels positively relate to the average cell size in phytoplankton communities (Acevedo-Trejos, Maranon & Merico 2018). In assemblages dominated by smaller phytoplankton, the energy transfer fluxes are mostly restricted to the surface microbial food web, whereas in communities of larger phytoplankton the energy export fluxes to the deep water via sedimentation increase and also involve more other trophic levels such as zooplankton and fish (Finkel *et al.* 2009; Peter & Sommer 2013; Litchman *et al.* 2015; Malerba *et al.* 2016). Consequently, it has been suggested that overall CO<sub>2</sub> fixation might decrease as smaller cells become dominant in marine phytoplankton (Finkel *et al.* 2009), which is a tendency supported by observations on shifts in the size structure of phytoplankton communities (e.g., (Winder & Sommer 2012).

Despite being a key trait influencing the ecology of phytoplankton, with possible links with carbon fixation and ultimately global climate, the direct effect of cell size on ecosystem functioning remains empirically underexplored (Venail 2017). This strongly limits our understanding and predicting capacities about the consequences of changes in the cell size composition of phytoplankton, as driven by climate warming and other processes of environmental change (Finkel *et al.* 2009; Peter & Sommer 2013; Litchman *et al.* 2015; Maranon 2015; Malerba *et al.* 2016). To our knowledge, only one study has directly manipulated the cell size composition of phytoplankton assemblages and evaluated its impact on one single function (Shurin *et al.* 2014). The authors found that the average cell volume had a positive impact on the biomass yield of phytoplankton assemblages of two species, meaning that assemblages composed of larger cells yielded more biomass. Increasing the difference in cell volume between the two species in the assemblages had the opposite effect, as assemblages with a combination of large and small taxa yielded less biomass. Based upon the large variety of impacts that cell size can have on different biological processes and considering the possible synergies or trade-offs amongst functions (Venail & Vives 2013; Krause *et al.* 2014), it is expected that changes in the cell size composition of phytoplankton assemblages does not influence all functions in the same direction and/or with the same strength. Thus, exploring multiple functions simultaneously and in conjunction (i.e., multifunctionality), rather than only one function at a time, would improve our understanding of the importance of cell size composition for the functioning of phytoplankton assemblages. We are unaware of any empirical study testing the influence of phytoplankton's cell size community composition on multiple functions. We performed a controlled laboratory experiment in which we manipulated

the cell size composition of two-species assemblages of freshwater green algae and measured its effects on four functions.

## Methods

### Algae

We purchased twelve different species of freshwater microscopic green algae from the culture collection of algae and protozoa (CCAP, United Kingdom) that were originally collected in Switzerland or nearby countries (i.e., France, Italy or Germany). After a series of pilot experiments, we selected eleven seemingly axenic species that: 1) were easily distinguishable under the microscope, 2) grew well under laboratory conditions and 3) covered a broad range of cell sizes (i.e., cell biovolume and surface area to volume ratios, **Supplementary Table 1**), so that when combined in pairs resulted in bi-cultures (mixtures of two species) generating considerable variation in cell size variables (**Supplementary Table 2**).

### Culture conditions

We grew two types of algae cultures under identical controlled conditions: 1) the eleven species as monocultures, and 2) all 55 possible combinations of two species (bi-cultures hereafter). We grew all cultures in triplicate, resulting in 198 cultures in total. Considering the broad set of variables measured afterwards for each culture (see below), including more taxa and consequently more bi-cultures was technically unfeasible. We opted for a substitutive design for the inoculation of bi-cultures. This means that the total initial biovolume in the bi-cultures was equivalent to the initial biovolume in the two monocultures, with a 50:50 biovolume proportion of each species. This design differs from an additive design in which the total initial biovolumes used to start the bi-cultures would be equivalent to the addition of the biovolumes used in the respective monocultures with a 50:50 biovolume proportion of each species. This would result in a double biovolume for initiating the bi-cultures. Both experimental designs offer advantages and disadvantages depending on the questions being addressed, but in experiments with fast growing populations (e.g., bacteria, microalgae) this distinction might not be as important as for slower growing organisms (e.g., terrestrial plants; see (Weis *et al.* 2007; Little *et al.* 2008; Foster & Bell 2012) for some comparisons among both experimental designs). Hence, we inoculated all 165 bi-cultures (55 combinations x 3 replicates) with the same initial biovolumes. This resulted in adding different volumes and thus different cell counts

of the two species. For culturing the algae, we used flat transparent 50 ml cell culture flasks filled with 40 ml of BG-11 media diluted twenty times and with 0.5 mg/L additional phosphate (potassium phosphate monobasic). We added this extra phosphate because a pilot study revealed that almost no phosphate was present in the media after ten days using diluted BG-11. Thus, the initial nitrate and phosphate concentrations were 50 mg/L ( $\sim 80.6 \times 10^{-5}$  mol/L) and 2 mg/L ( $\sim 2.1 \times 10^{-5}$  mol/L) respectively (i.e., N:P ratio of  $\sim 38$ ). The cultures were grown at  $\sim 22.5^\circ\text{C}$  under  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and a 16:8 h light dark cycle in a Multitron pro incubator (INFORS HT) with shaking speed set at 100 rpm. Flask caps included a breathable membrane to allow gas exchange. The interior of the incubator was saturated with a 2%  $\text{CO}_2$  - 98% air gas mixture to avoid  $\text{CO}_2$  growth limitation. After twelve days, we collected samples from all the 198 cultures for nutrient, light attenuation and biovolume quantification analyses (described below). A pilot study showed that after twelve days, all the cultures were still in the exponential phase of growth and that dissolved nutrients (nitrates and phosphates) were still available. We verified the presence of one single species in all the monocultures and of two species in the bi-cultures at the end of the experiment using a microscope. From the initial fifty-five possible two-species mixtures we retained for further analyses forty bi-cultures, in which both species persisted for the duration of the experiment in the three replicates. In the other fifteen bi-cultures, not cells of one of the species was observed at the end of the experiment after counting randomly five-hundred cells from the mixture under the microscope. Exploring patterns of coexistence or competitive exclusion amongst pairs of species of different cell size was out of the scope of this study. We previously addressed that topic in a recent study properly designed for that purpose .

### **Cell size variables**

Before starting the experiment, we collected information on two continuous traits related to cell size from each of the eleven taxa used in this study: 1) average cell biovolume (*BV*) and 2) average surface area to volume ratio (*S/V*). We quantified the average cell biovolume of each of the eleven species grown as monocultures using a CASY counter (INNOVATIS, Switzerland), allowing us measuring over 100 000 individual units (cells or colonies) for smaller species and over 10 000 individual units for larger ones. We estimated the surface area to volume ratios for each of the eleven taxa grown as monocultures based on cell shapes using pictures taken with the microscope (over 100 cells per species) and the average cell biovolumes mentioned before. For all taxa, the cell biovolumes measured under the microscope and with

the CASY counter differed by less than 2%. Average cell biovolume and average surface area to biovolume ratios of the individual taxa were related but poorly in our species pool ( $\rho = -0,521$ ,  $P = 0,099$ ,  $n = 11$ ) and because the  $S/V$  ratio also depends on cell shape, both traits may provide complementary information on the influence of cell size on functioning. For both  $BV$  and  $S/V$ , we calculated two variables describing the cell size composition of the forty bi-cultures: *mean*, which corresponds to the average trait value of the two species and *sd*, the standard deviation among the average cell size of the two species as a measure of its variability. Thus, the forty bi-cultures included in the analysis covered a wide range of variation related to cell size composition (**Supplementary Table 2**). The four cell size variables for the bi-cultures,  $BV_{mean}$ ,  $BV_{sd}$ ,  $S/V_{mean}$  and  $S/V_{sd}$ , showed either positive, negative or no correlations among each other. Consequently, some of them would provide complementary information on the influence of cell size composition on functioning (**Supplementary Table 3**).

### Functioning

After twelve days of growth, we quantified four different functions in each replicate of the eleven monocultures and the forty bi-cultures. These were; 1) nitrate uptake, 2) phosphate uptake, 3) light attenuation and 4) total biovolume production. Thus overall, a total of 1836 functioning values were measured (i.e., 153 cultures \* 3 replicates \* 4 functions). We estimated the nitrate and phosphate uptakes as the difference in nitrate and phosphate concentrations in the culture media within the 50 ml flasks from the beginning to the end of the twelve-day experiment. For this, we filtered 2 ml samples through a 0.2  $\mu\text{m}$  pore-size filter to discard the algae. We quantified dissolved nitrates and phosphates in the filtered media with an AQ2 analyzer (SEAL Analytical, UK). In all cultures, at least 51% of the initial nitrates (25.5 mg/L or  $41.1 * 10^{-5}$  mol/L) and 15% of the initial phosphates (0.3 mg/L or  $3.16 * 10^{-6}$  mol/L) remained in the media after day twelve. We calculated light attenuation, a rough estimate of light use, as the coefficient of vertical light extinction at the end of the experiment as follows:

$$\text{Light attenuation} = -\ln(I_{out}/I_{in})$$

With  $I_{in}$  being the incident light intensity at the top of the culture flask and  $I_{out}$  the light intensity leaving the flat culture flask placed in a horizontal position. This simplified measure of light attenuation is to be influenced by both algal biomass and pigmentation (Wágner, Valverde-Pérez & Plósz 2018). Samples with higher algal biomass or pigment concentration would result in higher light attenuation values. We measured light intensity using a LI-COR, LI-250A light

meter. As calculated here, this measure of light attenuation has no units. We estimated total biovolume in all cultures as the product of the average cell biovolume measured beforehand (see above) multiplied by the cell abundance data obtained using the CASY counter. The difference in total biovolume from the beginning to the end of the experiment represented the total biovolume production of each culture during the twelve-day period. From this point, for each monoculture and bi-culture, we averaged the functioning values over the three replicates. Such averaged functioning levels were used for all subsequent analyses and plots.

### **Expected and observed functioning levels**

For each of the forty bi-cultures and for each function, we calculated two levels of functioning: the observed and the expected levels. We measured the observed levels of functioning directly from the actual bi-cultures, representing a direct quantification. We estimated the expected levels of functioning for each bi-culture by averaging the observed levels of functioning of both constituent species as monocultures. This assumes that because each species in the bi-culture was inoculated at half of its initial biovolume in the monoculture (i.e., a substitutive design), its level of functioning in the bi-culture should be close to half of its level of functioning as monoculture. This estimation also assumes that the two species contribute equally (i.e., in 50:50 proportions) to functioning in the bi-cultures, at least initially.

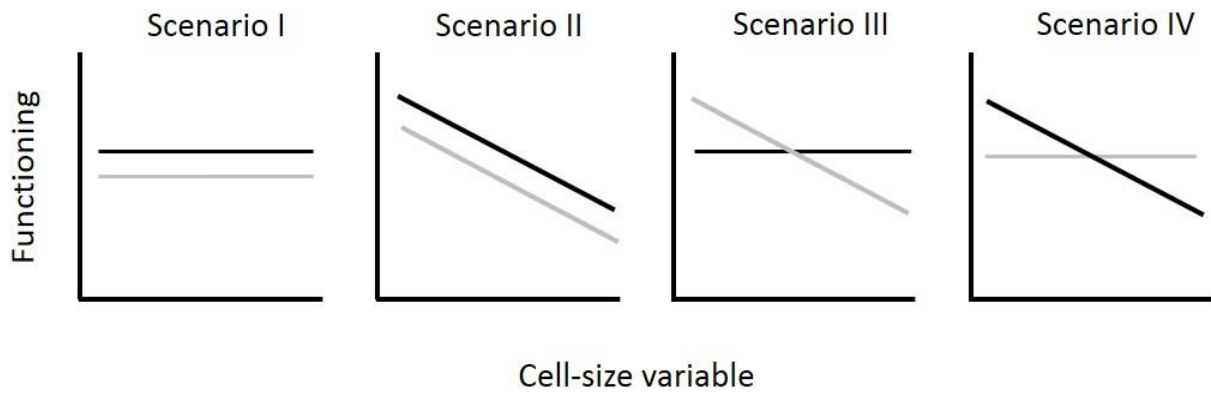
### **Effect of species interactions on functioning**

We explored the influence of species interactions on functioning by comparing the observed against the expected levels of functioning of the bi-cultures. For this, we propose four different hypothetical scenarios (**Figure 3-1**). In scenario I, cell size has no effect on the expected or the observed levels of functioning. This is, neither the functioning capabilities of the constituent species nor the nature and/or strength of species interactions varies along the cell size gradient. In scenario II, cell size influences both the expected and the observed levels of functioning. This suggests that the effect of cell size on the observed levels of functioning is due to changes in the functioning capabilities of the component species but not to changes in species interactions. In scenarios I and II, any changes in species interactions along the cell size gradient had no influence on functioning. Scenario III includes an effect of cell size on the expected but not on the observed levels of functioning. Finally, in scenario IV, cell size influences the observed but not the expected levels of functioning of the bi-cultures. In scenarios III and IV, changes in species interactions along the cell size gradient have consequences on functioning.

Overall, if the expected levels of functioning based on the constituent species as monocultures relate well to the observed levels of functioning of the actual bi-cultures, it suggests that species interactions (either positive or negative) had a limited impact on functioning. This is, species in the bi-cultures would reach functioning levels close to half their levels as monocultures. However, it is also possible that at least one of the species in the mixture is contributing less or more than expected, and that the resulting functioning level of the bi-culture is still near the expected level based upon the averaging assumption. One limitation of our approach is that we could not estimate the relative contribution of each component species to the functioning levels measured in the bi-cultures. Consequently, we could not determine exactly how the functioning of a species was influenced by the presence of the other species in the bi-cultures relative to its functioning as monoculture (i.e., *relative yield*). Nevertheless, fitting the expected versus observed levels of functioning would provide insightful information on whether species interactions alter these functions in the bi-cultures. In this study, we exclusively focused on assemblages of two species to be able to explore the influence of species interactions on functioning under the simplest scenario, without including potential confounding effects of species richness. Including more species in the mixtures would make such a detailed examination much more difficult and was outside the scope of this study.

### **Multifunctionality**

Multifunctionality is a consolidated measure of multiple ecosystem functions (Mokany, Ash & Roxburgh 2008). Here, we used two complementary approaches for estimating multifunctionality, the averaging approach and the multiple threshold approach (Mokany, Ash & Roxburgh 2008). For the averaging approach, we first standardized each of the four functions (i.e., nitrate uptake, phosphate uptake, light attenuation and biovolume production) by dividing it by its maximum value. This maximum value is the highest level of functioning observed amongst the forty bi-cultures. Then, we calculated the average of the four standardized functions for each bi-culture. The multiple threshold approach consisted in calculating for each bi-culture the number of functions (from 0 to 4) that were beyond a series of thresholds ranging from 5% to 95% of the maximum value of a function (as defined before). This approach examines the change of the shape of the relationship between the variables related to cell size and the number of functions beyond each threshold.



Scenario	I	II	III	IV
Effect of cell size on observed functioning	NO	YES	YES	NO
Effect of species interactions on observed functioning	NO	NO	YES	YES

**Figure 3-1:** Four hypothetical scenarios contrasting the effects of cell size (x-axis) on the observed (black line) and the expected (gray line) levels of functioning of bi-cultures (y-axis). The expected levels of functioning are based on the observed levels of functioning of the constitutive species as monocultures under a substitutive design. Lines represent linear regressions over the data (dots not shown for clarity). In scenario I, the cell size variable has no effect on the expected or the observed levels of functioning of the bi-cultures. In scenario II, the cell size variable has a strong negative effect (the same logic applies for a positive effect) on both the expected and the observed levels of functioning of bi-cultures. In scenarios I and II, any change in the strength and/or nature of species interactions along the cell size gradient had no influence on functioning (i.e., both lines have no differences in their slopes). In scenarios III and IV, the cell size variable has different effects on the expected and the observed levels of functioning (i.e., the two lines have different slopes). Scenario III shows a negative (but could also be positive) effect of the cell size variable on the expected levels of functioning but not on the observed levels of functioning. The opposite is represented in scenario IV, where the cell size variable has a negative effect on the observed but not on the expected levels of functioning of the bi-cultures. Scenarios III and IV suggest that the nature and/or strength of species interactions change along the cell size gradient, with direct consequences on functioning.

## Statistical analyses

For all the statistical analyses, we averaged the data over the three replicates for each bi-culture and each monoculture. We fitted linear models with either one or multiple factors to determine which variables described better the observed levels of functioning in the bi-cultures. In these linear models, we included as factors the expected levels of functioning based on the constituent monocultures and each of the four variables related to cell size. We then used the AIC (Akaike information criterion) to determine which model described the data better. We used simple linear regressions to assess the influence of the four variables related to cell size on multifunctionality. To depict the influence of species interactions on functioning along the cell size gradients, we compared the slopes of the linear models linking each cell size variable to the expected versus the observed levels of functioning. For this, we estimated the interaction term of a linear model that included each cell size variable as a continuous variable and *expected* or *observed* as a categorical variable. A significant interaction term would show that the slopes of the two linear models are different and consequently suggest that the nature or strength of species interactions had an influence on functioning along the cell size variable. We performed all statistical tests with JMP (SAS, version 13.2.1).

## Results

We found that the observed levels of nitrate uptake, phosphate uptake and light attenuation of the experimental bi-cultures were well related to the functioning levels of their constituent species as monocultures (i.e., expected functioning, **Table 3-1, Figure 3-2a-c**). This suggests two things. First, that the levels of nutrient uptake and light attenuation of the bi-cultures can be largely determined by the functioning capacities of the constituent species. Second, that species interactions had a limited impact on these three functions. Despite the overall strong positive correlations, some bi-cultures showed much lower or much higher functioning levels than expected from the constitutive monocultures (**Supplementary Figure 3-S1**). The observed levels of nitrate uptake ranged from -45% to +78% of the expected values. For phosphate uptake, the range was from -48% to +62%, whereas for light attenuation, they ranged from -29% to +61% of the expected values (**Supplementary Figure 3-S1**). On the other hand, we found no link between the expected and observed levels of biovolume production of the bi-cultures (**Table 3-1, Figure 3-2d**). This suggests that species interactions had a strong impact on this function by influencing the biovolume production of the constituent species (at least one



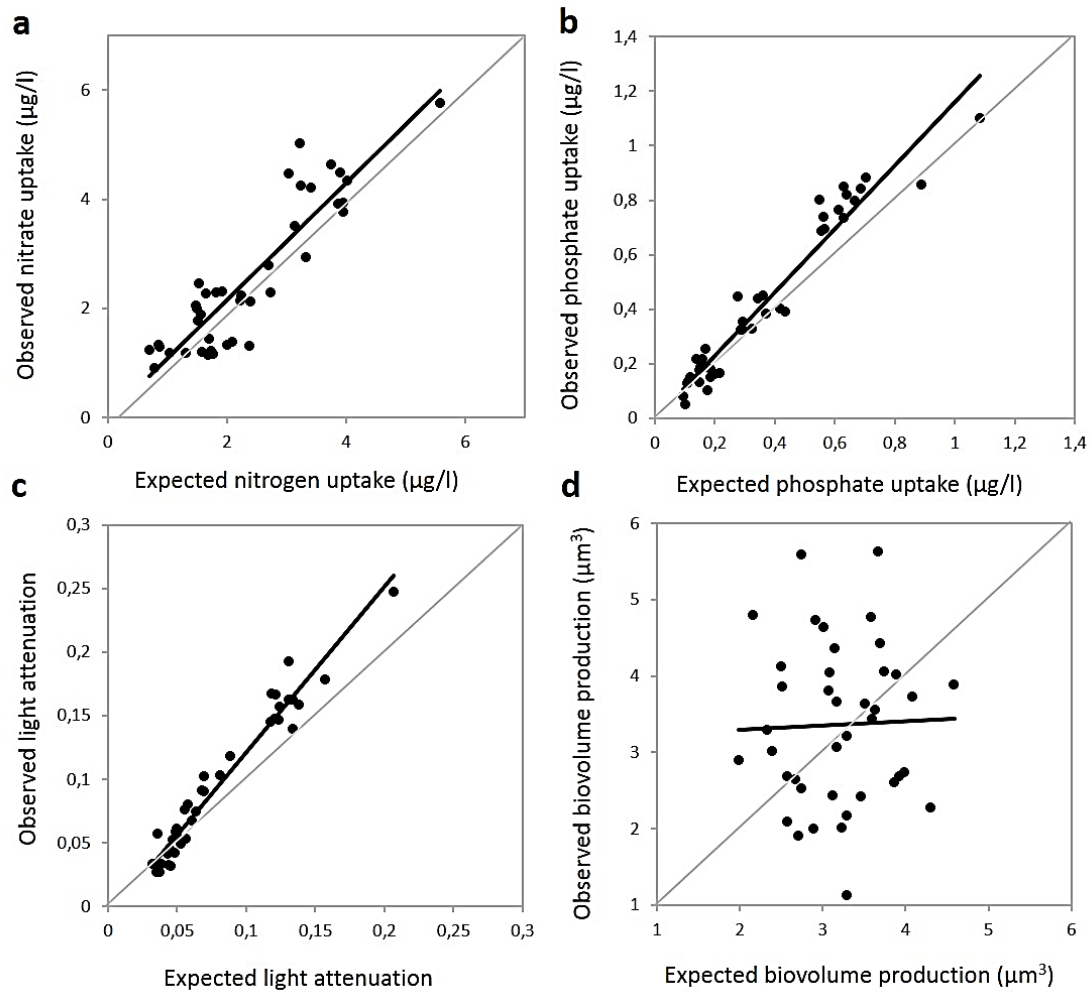
of them). Depending on the bi-culture, the impact of species interactions on biovolume production was either positive, negative or negligible, ranging from -66% to +122% (**Supplementary Figure 3-S1**). Moreover, the six bi-cultures that were expected to produce the least biovolume produced much more than expected (+59% on average) and the five bi-cultures that were expected to produce the most biovolume, produced much less than expected (-27% on average). This definitively contributed to the absence of correlation between the observed and expected biovolume production levels (**Figure 3-2d**).

Compared to the linear models that included the expected levels of functioning only, adding information about the cell size composition of the assemblages barely increased the capacity of the models to describe the observed levels of nitrate uptake, phosphate uptake and light attenuation of the bi-cultures (from 0.3 to 4.1% increase, **Table 3-1**). Overall,  $S/V_{mean}$  was the best single cell size predictor of nitrate uptake, phosphate uptake and light attenuation (**Table 3-1, Figure 3-3**), but it described only from 10 to 24% of their variation.  $BV_{mean}$  was also a meagre predictor of phosphate uptake and light attenuation, describing only 12 and 13% of their variation respectively (**Table 3-1**). Finally, the observed levels of biovolume production of the bi-cultures did not relate to any of the cell size variables included in our study (**Table 3-1, Figure 3-3**).

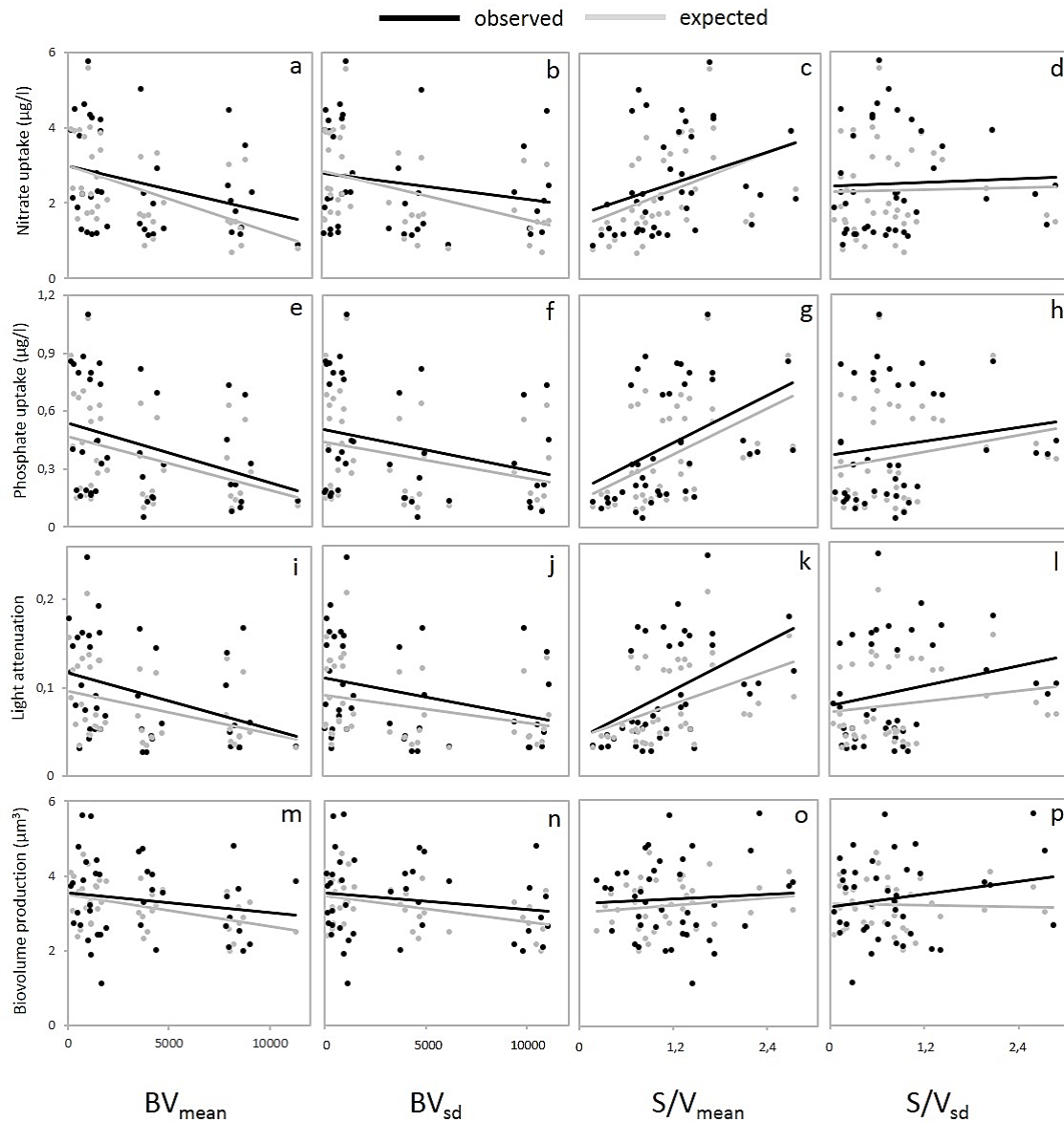
The data from this experiment matched two of the four hypothetical scenarios linking cell size to functioning described in **Figure 3-1**. Based on the linear models (**Table 3-1**) and the comparison of slopes of the observed versus the expected values of functioning along the cell size gradient (**Supplementary Table 3-S4**), we found scenario I in eight cases (**Figure 3-3**), meaning that cell size had no influence on the expected and observed functioning levels of the bi-cultures (i.e., no differences in regression slopes). This occurred in all cases linking  $S/V_{sd}$  to resource uptake related functions (panels d, h, and l). Similarly,  $BV_{sd}$  had no effect on either the expected or observed levels of nitrate uptake (panel b). Finally, none of the cell size variables included in our study had an impact on the expected or observed levels of biovolume production (panels m, n, o and p). We found support for scenario II in another eight cases (**Figure 3-3**), describing an effect (either negative or positive) of the size-related variable on both the expected and observed levels of functioning of the bi-cultures. The observed levels of nitrate uptake (panel a), phosphate uptake (panel e) and light attenuation (panel i) decreased as the average cell biovolume of the species in the bi-cultures ( $BV_{mean}$ ) increased. A similar pattern was observed regarding the effect of  $BV_{sd}$  on phosphate uptake (panel f) and light attenuation

(panel j). On the contrary, assemblages with relatively higher mean surface area to volume ratios ( $S/V_{\text{mean}}$ ) consumed more nitrates, more phosphates and attenuated more of the incident light (panel c, g and k respectively). Scenario II suggests that the observed negative or positive effects were all due to differences in the functioning levels of the species present in the assemblages along the cell size gradient. For instance, the positive impact of  $S/V_{\text{mean}}$  on phosphate uptake (panel g) occurred because species with larger  $S/V$  ratios had higher phosphate uptake levels, which resulted in assemblages with higher mean  $S/V$  ratios consuming more phosphates. Under scenario II, changes in the nature and/or strength of species interactions along the cell size gradient had no effect on the observed levels of functioning of the bi-cultures. Scenarios III and IV, describing unexpected effects (based on constitutive monocultures) of the cell size variable on the observed levels of functioning, were absent from our results. This suggests that in no case, the effect (or absence of effect) of a cell size variable on the observed levels of functioning resulted from changes in the nature and/or strength of species interactions along the cell size axis.

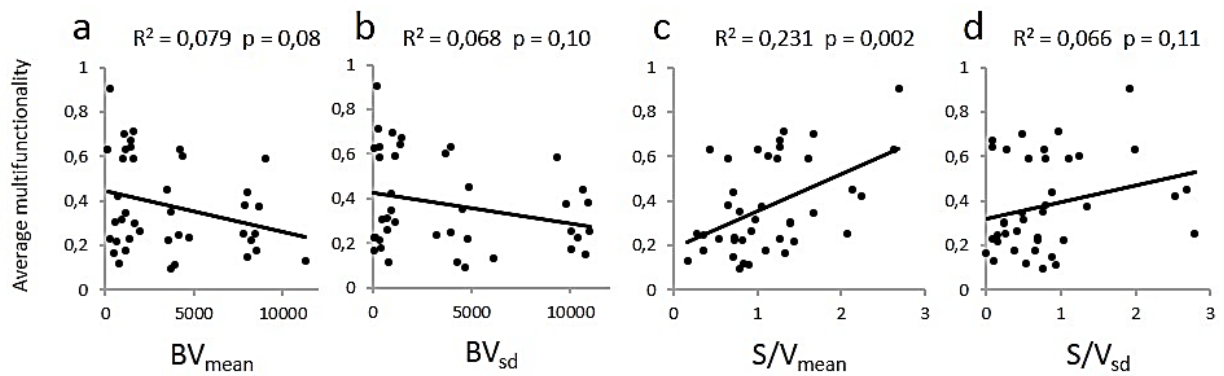
The above described negative impact of increasing  $BV_{\text{mean}}$  on three of the four individual functions lead to an overall negative effect on multifunctionality, as revealed by both the averaging and threshold approaches (**Figures 3-4a** and **Supplementary Figure 3-S2a**, respectively). This means that increasing the average cell volume of the species in our bi-cultures was detrimental for their overall functioning. By negatively influencing two of the four individual functions, increasing the variation in cell volume between the two species in the bi-cultures ( $BV_{\text{sd}}$ ) led to a slight negative impact on multifunctionality using the averaging approach (**Figure 3-4b**) and the threshold approach (**Supplementary Figure 3-S2b**). This means that increasing the variability in cell volume among species in our bi-cultures was also detrimental for their overall functioning. On the contrary, increasing the average surface area to volume ratio of the two species in the bi-cultures ( $S/V_{\text{mean}}$ ) influenced positively multifunctionality (using both the averaging and the threshold approaches) and was its best single predictor (**Figures 3-4c** and **Supplementary Figure 3-S2c**). This resulted from its positive effect on the three functions related to resource use (**Figure 3-3**). Finally, in accordance with a lack of effects on all four individual functions, increasing the variation in the surface area to volume ratio between the two species in the bi-cultures ( $S/V_{\text{sd}}$ ) did not affect much multifunctionality, using either the averaging (**Figure 3-4d**) or the threshold approach (**Supplementary Figure 3-S2d**).



**Figure 3-2:** Expected functioning levels of the bi-cultures based on the functioning of the constitutive species as monocultures (x-axis) versus their observed levels (y-axis) for a) nitrogen uptake, b) phosphate uptake, c) light attenuation and d) biovolume production. Each dot represents a bi-culture with a different species composition. Black lines represent linear fits. Grey diagonals represent the isoclines (i.e., equal values) between the two variables. Dots above the isocline show bi-cultures with observed levels of functioning higher than expected from the levels of functioning of their constitutive monocultures. Dots below the isocline show bi-cultures with observed levels of functioning lower than expected from the levels of functioning of their constitutive monocultures.



**Figure 3-3:** Effects of the four variables related to cell size on each individual function over the forty bi-cultures of green algae. Dots represent the observed levels of functioning in the experimental bi-cultures after twelve days with each dot representing a bi-culture with a different species composition. Black lines represent the simple linear regressions on the observed values of functioning (Table 1), whereas grey lines represent the simple linear regressions on the expected values of functioning. Panels b, d, h, l, m, n, o and p correspond to scenario I from Figure 1. Panels a, c, e, f, g, i, j and k correspond to scenario II. No panels represent scenarios III or IV (Supplementary Table 4).  $BV_{\text{mean}}$  = average cell biovolume of the two species,  $BV_{\text{sd}}$  = standard deviation of cell biovolume of the two species,  $S/V_{\text{mean}}$  = average surface area to volume ratios of the two species,  $S/V_{\text{sd}}$  = standard deviation of surface area to volume ratios of the two species. Units for  $BV$  and  $S/V$  variables are cubic micrometers ( $\mu\text{m}^3$ ) and  $\mu\text{m}^{-1}$  respectively.



**Figure 3-4:** Observed effects of the four variables related to cell size on multifunctionality based on the averaging approach in forty experimental bi-cultures of green algae. Lines represent simple linear regressions.  $BV_{mean}$  = average cell biovolume of species,  $BV_{sd}$  = standard deviation of cell biovolume of species,  $S/V_{mean}$  = average surface area to volume ratios of species,  $S/V_{sd}$  = standard deviation of surface area to volume ratios of species. Over each panel are shown the coefficients of determination  $R^2$  of the linear regressions and their  $p$ -values. Units for  $BV$  and  $S/V$  variables are cubic micrometers ( $\mu m^3$ ) and  $\mu m^{-1}$  respectively.

## Discussion

In this experiment, the performances of the constituent species as monocultures predicted well the levels of nitrate uptake, phosphate uptake and light attenuation observed in the bi-cultures, meaning that species interactions had a limited influence on these three functions. We hypothesize that this might be due to the short duration of the experiment and/or to the high levels of nutrients and light intensity in the cultures that created non-limiting conditions, allowing all species to meet their resource needs. The still unused amounts of nitrates, phosphates and the positive light transmittance values at the end of the experiment, in addition to the growth observed afterwards in all our cultures, are in line with the idea of an absence of resource limitation. However, the fact that several bi-cultures consumed much less nitrate and phosphate than expected, suggests that at least in some cases competition for resources may have occurred. Another possibility is that our simplified culture conditions limited the scope for species interactions to influence more the resource-use functions. On the contrary, the observed levels of biovolume production of the bi-cultures did not relate to the expected levels based on the production of the constituent species as monocultures, revealing changes in efficiency by which some species transformed resources into biovolume as consequence of interspecific interactions. Such a lack of correlation between the biovolume production of polycultures and the constituent monocultures is common in phytoplankton assemblages (e.g., (Fox 2004; Weis *et al.* 2007; Li *et al.* 2010). Previous similar empirical studies mixing pairs of phytoplankton species have described the variety of effects that interspecific interactions can have on biovolume production (Narwani *et al.* 2013; Venail & Vives 2013; Filstrup *et al.* 2014; Shurin *et al.* 2014; Venail *et al.* 2014; Gallego, Venail & Ibelings 2019). By comparing the biovolume yield of a species grown as monoculture versus in presence of a second species (i.e., relative yield, susceptibility), it has been shown that some algae reduce their biovolume production in the presence of another taxa, suggesting some form of competition or interference (i.e., underyielding; (Fox 2004; Schmidtke, Gaedke & Weithoff 2010; Behl, Donval & Stibor 2011; Fritschie *et al.* 2014; Steudel *et al.* 2016). In contrast, other algae benefit from the presence of a second species, suggesting facilitation (e.g., (Fox 2004; Behl, Donval & Stibor 2011; Venail & Vives 2013; Fritschie *et al.* 2014; Venail *et al.* 2014). We still poorly understand the details of such interspecific interactions in phytoplankton assemblages because studies to date have mainly focused on interpreting the outcome of interactions rather than on describing the actual underlying mechanisms. Some studies found that the nature and/or strength of species

interactions among phytoplankton taxa has no link with the evolutionary relatedness amongst them, but without providing a proper mechanistic rationale (Fritschie *et al.* 2014; Venail *et al.* 2014). Despite a general interest for positive interactions in ecology (Bruno, Stachowicz & Bertness 2003), mostly in terrestrial plants (Valiente-Banuet & Verdú 2007b; Brooker *et al.* 2008a; Verdú, Gómez-Aparicio & Valiente-Banuet 2012), a limited number of studies have explored the mechanism underlying facilitative or mutualistic interactions in phytoplankton. Some speculations include cross-feeding relationships, the improvement of water chemistry or light conditions as potential mechanisms of facilitation in phytoplankton (Venail *et al.* 2014). A recent experimental study established that facilitation in population growth was more common amongst species of green algae with more similar gene expression and suggested a boost in core metabolism as a potential underlying mechanism (Narwani *et al.* 2017).

Regarding the influence of cell size community composition on functioning, our results show that compared to bi-cultures composed of larger species, with lower surface area to volume ratios, those with smaller cells or with larger surface area to volume ratios consumed more nitrates, more phosphates and attenuated more of the incoming light. While some studies have found links between smaller phytoplankton species and higher nutrient uptake capacities (Litchman *et al.* 2007; Edwards *et al.* 2012; Maranon 2015; Sommer *et al.* 2017), our study directly links cell size composition to resource use in phytoplankton assemblages. The decrease in light attenuation with increasing cell size (or decreasing S/V ratio) can be attributed to more light passing unintercepted through larger cells (i.e., sieve effect; (Kirk 2001). Overall, our analysis suggests that the effects of cell size on resource use were purely compositional and were not much determined by changes in the nature or strength of species interactions.

Contrary to the three functions related to resource use, the observed biovolume production of our bi-cultures was unaffected by the mean cell biovolume or the mean surface area to volume ratio of their constituent species. This result contradicts a recent review suggesting that taxa around  $100 \mu\text{m}^3$  in cell volume (which corresponds to the smaller taxa used in this experiment) show the highest capacity to convert nutrients into biomass (Maranon 2015). Despite that some of our bi-cultures composed by the more voluminous species in our pool seemed to produce more biovolume than expected, perhaps due to a slight reduction in competition or interference amongst them, the high supply of nutrients in our experiment may have allowed assemblages of larger cells to be as productive as their smaller counterparts. Indeed, larger taxa have lower inner nitrogen and phosphate concentrations than smaller taxa (Maranon 2015) and under high

nutrient levels such as in this experiment, their metabolic rates can be similar, resulting in comparable biovolume production levels. The lack of effect of the average cell size on biovolume production observed here also challenges a previous study showing that the average cell volume had a positive impact in the yield of phytoplankton assemblages of two species (Shurin *et al.* 2014). Differences in the species pool used, in the culturing conditions and in how yield was quantified can explain the discrepancies between both studies. Changes in the average surface area to volume ratio in our bi-cultures also had no impact on their biovolume production. Again, according to the evidence collected here, changes in the nature and/or strength of species interactions along the  $S/V_{\text{mean}}$  gradient played no role on this as the observed null relation was expected based on the performances of the constituent species.

Multifunctionality benefited from the presence of species with higher surface area to volume ratios and with lower cell volumes. Our analysis suggests that the general negative effect of the average cell volume ( $BV_{\text{mean}}$ ) and the general positive effect of the average surface area to volume ( $S/V_{\text{mean}}$ ) on multifunctionality occurred mainly because more voluminous and lower  $S/V$  ratio's species had inferior resource use levels than species with lower cell volumes or higher  $S/V$  ratios. This compositional effect on functioning dominated over changes in the nature and/or strength of species interactions in the bi-cultures included in this study. The fact that increments in both  $BV_{\text{mean}}$  and  $BV_{\text{sd}}$  lead to similar reductions in multifunctionality suggests that the presence of voluminous, low functioning species in some bi-cultures was largely responsible for these trends. Again, the short time scale of our experiment may have reduced the possibilities for a stronger general impact of species interactions on functioning. In accordance with this hypothesis, studies linking biodiversity to ecosystem functioning suggest that the effects of species interactions on functioning (either positive or negative) intensify over time (Fox 2004; Cardinale *et al.* 2007; Weis *et al.* 2007; Li *et al.* 2010; Reich *et al.* 2012).

In addition to the importance of exploring multiple functions simultaneously, our findings advocate for incorporating multiple aspects of cell size community composition to improve our understanding of the importance of cell size in phytoplankton. Our results offer a cautionary tale when cell size is used to predict multifunctionality because a proper interpretation is only possible when information on individual functions is available. For instance, a positive (or negative) effect of cell size on multifunctionality can hide the absence of effect on some individual functions. The fact that our results show clear effects of cell size on some ecosystem functions but not on others, suggests that the biological mechanisms by which cell size



influences ecosystem functions are not universal and depend on the function considered. Interest in multifunctionality is growing fast as it may provide a more complete perspective on ecosystem functioning than focusing on single functions (Maestre *et al.* 2012). We offer unique empirical evidence of a direct impact of cell size on multifunctionality in phytoplankton. Prior analyses linking cell size to phytoplankton functioning included only one function (Shurin *et al.* 2014), which may considerably underestimate the importance of cell size community composition for functioning. Moreover, rather than establishing correlations from observational data between cell size community composition and functioning, like performed in some previous studies (Cermeño & Figueiras 2008; Litchman & Klausmeier 2008; Litchman, Klausmeier & Yoshiyama 2009), we managed to establish a direct link between some variables related to cell size and three different functions related to nutrient uptake. To corroborate our findings, we advocate for more empirical studies testing the influence of cell size composition on functioning in phytoplankton assemblages that include other functions, more taxa, in the presence of grazers, under a wider set of culture conditions and over longer periods.

In the current context of global environmental change, numerous studies suggest that phytoplankton communities will be increasingly dominated by smaller taxa as water gets warmer, more stratified and resource limited (Gaedke, Seifried & Adrian 2004; Cermeño & Figueiras 2008; Sommer & Lengfellner 2008; Finkel *et al.* 2009; Winder, Reuter & Schladow 2009; Huertas *et al.* 2011; Winder & Sommer 2012; Peter & Sommer 2013; Rossoll, Sommer & Winder 2013; Mousing, Ellegaard & Richardson 2014; Sommer *et al.* 2017; Iatskiu *et al.* 2018). Similarly, marine phytoplankton of different sizes are showing clear and distinct biogeographical distributions, with smaller cells dominating oligotrophic tropical waters near the equator and larger cells dominating nutrient-richer temperate and coastal waters (Cermeño & Figueiras 2008; Acevedo-Trejos *et al.* 2015). Such spatial and temporal changes in cell size composition may lead to major perturbations in ecosystem functioning that are still poorly understood and remain only correlational. By no means is this experiment's intention to depict or to recreate the complexity of natural phytoplankton assemblages. However, our original experimental setup, in which we directly manipulated the cell size community composition and measured its consequences on multiple functions, provides rare fundamental insight on the direct impact that changes in cell size can have on ecosystem functioning. While collected using a limited diversity of taxa and over a short timescale, our results suggest that phytoplankton communities composed of smaller taxa might outperform larger taxa in functions related to

resource uptake, even though this might not necessarily lead to increments in biovolume production.

**Table 3-1:** Basic statistics from the linear models relating the expected levels of functioning and the cell size variables with the observed levels of functioning. For each function, we fitted different models with one or multiple factors and ordered them according to increasing AICc values. The nine models with the lowest AICc values are shown.

<b>a) Observed nitrate uptake</b>			
<b>Factors</b>	<b>p-value</b>	<b>R<sup>2</sup></b>	<b>AICc</b>
Expected nitrate use; BV <sub>sd</sub>	<0.0001	0.856	67.61
Expected nitrate use; BV <sub>mean</sub>	<0.0001	0.838	72.38
Expected nitrate use	<0.0001	0.805	77.40
Expected nitrate use; S/V <sub>mean</sub>	<0.0001	0.814	77.81
Expected nitrate use; S/V <sub>sd</sub>	<0.0001	0.805	79.81
S/V <sub>mean</sub>	0.046	0.100	138.47
BV <sub>mean</sub>	0.060	0.090	138.93
BV <sub>sd</sub>	0.193	0.044	140.89
S/V <sub>sd</sub>	0.791	0.002	142.62

<b>b) Observed phosphate uptake</b>			
<b>Factors</b>	<b>p-value</b>	<b>R<sup>2</sup></b>	<b>AICc</b>
Expected phosphate use; S/V <sub>mean</sub>	<0.0001	0.942	-90.31
Expected phosphate use; S/V <sub>sd</sub>	<0.0001	0.942	-90.29
Expected phosphate use	<0.0001	0.939	-90.27
Expected phosphate use; BV <sub>mean</sub>	<0.0001	0.939	-87.98
Expected phosphate use; BV <sub>sd</sub>	<0.0001	0.805	-87.87
S/V <sub>mean</sub>	0.006	0.181	13.37
BV <sub>mean</sub>	0.029	0.118	16.34
BV <sub>sd</sub>	0.073	0.082	17.94
S/V <sub>sd</sub>	0.368	0.021	20.51

<b>c) Observed light attenuation</b>			
<b>Factors</b>	<b>p-value</b>	<b>R<sup>2</sup></b>	<b>AICc</b>
Expected light attenuation; $S/V_{sd}$	<0.0001	0.957	-232.41
Expected light attenuation; $S/V_{mean}$	<0.0001	0.956	-231.45
Expected light attenuation	<0.0001	0.953	-230.95
Expected phosphate use; $BV_{sd}$	<0.0001	0.952	-228.53
Expected phosphate use; $BV_{mean}$	<0.0001	0.952	-228.49
$S/V_{mean}$	0.001	0.236	-120
$BV_{mean}$	0.024	0.127	-114.69
$BV_{sd}$	0.066	0.086	-112.86
$S/V_{sd}$	0.151	0.054	-111.46

<b>d) Observed biovolume production</b>			
<b>Factors</b>	<b>p-value</b>	<b>R<sup>2</sup></b>	<b>AICc</b>
$S/V_{sd}$	0.244	0.036	121.74
$BV_{mean}$	0.302	0.028	122.05
$BV_{sd}$	0.306	0.028	122.07
$S/V_{mean}$	0.725	0.003	123.05
Expected biovolume production	0.840	0.001	123.14
Expected biovolume production; $S/V_{sd}$	0.496	0.037	124.14
Expected biovolume production; $BV_{mean}$	0.564	0.030	124.42
Expected biovolume production; $BV_{sd}$	0.569	0.030	124.44
Expected biovolume production; $S/V_{mean}$	0.931	0.004	125.51

## Acknowledgements

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## Authors' Contributions

Z.G., P.V. and B.I. conceived of the project. Z.G. and P.V. designed and finished both sampling and collecting the data. Z.G. and P.V. performed statistical analyses. Z.G. wrote the papers. P.V. contributed constructive comments and revisions. All authors contributed substantially to drafts and gave approval for publication.

## Supplementary Information

**Supplementary Table 3-S1:** CCAP (culture collection of algae and protozoa) numbers, species names and average cell biovolumes of the eleven green algae taxa used in this experiment; fl = femtoliter.

Taxa	CCAP number	Species name	Average Cell biovolume ( $\mu\text{m}^3$ or fl)
1	243/3	<i>Kirchneriella contorta</i>	60.5
2	202/2	<i>Ankistrodesmus angustus</i>	1775
3	379/1B	<i>Stichococcus bacillaris</i>	2418
4	216/14	<i>Coccomyxa viridis</i>	870
5	276/4E	<i>Scenedesmus armatus</i> var. <i>brevicaudatus</i>	499.5
6	11/25	<i>Chlamydomonas pulvinate</i>	1405.5
7	202/7A	<i>Ankistrodesmus brauni</i>	197
8	217/2	<i>Coelastrum proboscoides</i> var. <i>dilatatum</i>	1342
9	276/3C	<i>Scenedesmus obliquus</i>	390.5
10	34/1D	<i>Haematococcus pluvialis</i>	6961.5
11	612/15	<i>Cosmarium lundelli</i>	15578

**Supplementary Table 3-S2:** Basic descriptive statistics of the size-related variables used in this study.  $BV_{mean}$  = average cell biovolume of species,  $BV_{sd}$  = standard deviation of cell biovolume of species,  $S/V_{mean}$  = average surface area to volume ratios of species,  $S/V_{sd}$  = standard deviation of surface area to volume ratios of species. Units for BV variables are femtoliters (fl) or cubic micrometers ( $\mu m^3$ ). Units for S/V variables are  $\mu m^{-1}$ .

	minimum	maximum	mean	standard deviation
$BV_{mean}$	128	11270	3566	3232
$BV_{sd}$	45	10973	3740	3959
$S/V_{mean}$	0.168	2.682	1.131	0.606
$S/V_{sd}$	0	2.787	0.784	0.713

**Supplementary Table 3-S3:** Coefficients of correlation (upper right-side part of the table) and *P*-values (in italics, lower left-side part of the table) between the four cell-size related variables used in this study. *BV*<sub>mean</sub> = average cell biovolume of species, *BV*<sub>sd</sub> = standard deviation of cell biovolume of species, *S/V*<sub>mean</sub> = average surface area to volume ratio of species, *S/V*<sub>sd</sub> = standard deviation of surface area to volume ratios of species. Units for *BV* variables are femtoliters (fl) or cubic micrometers (μm<sup>3</sup>) and units for *S/V* variables are μm<sup>-1</sup>. Two variables are positively related, two are negatively related and two are unrelated to each other.

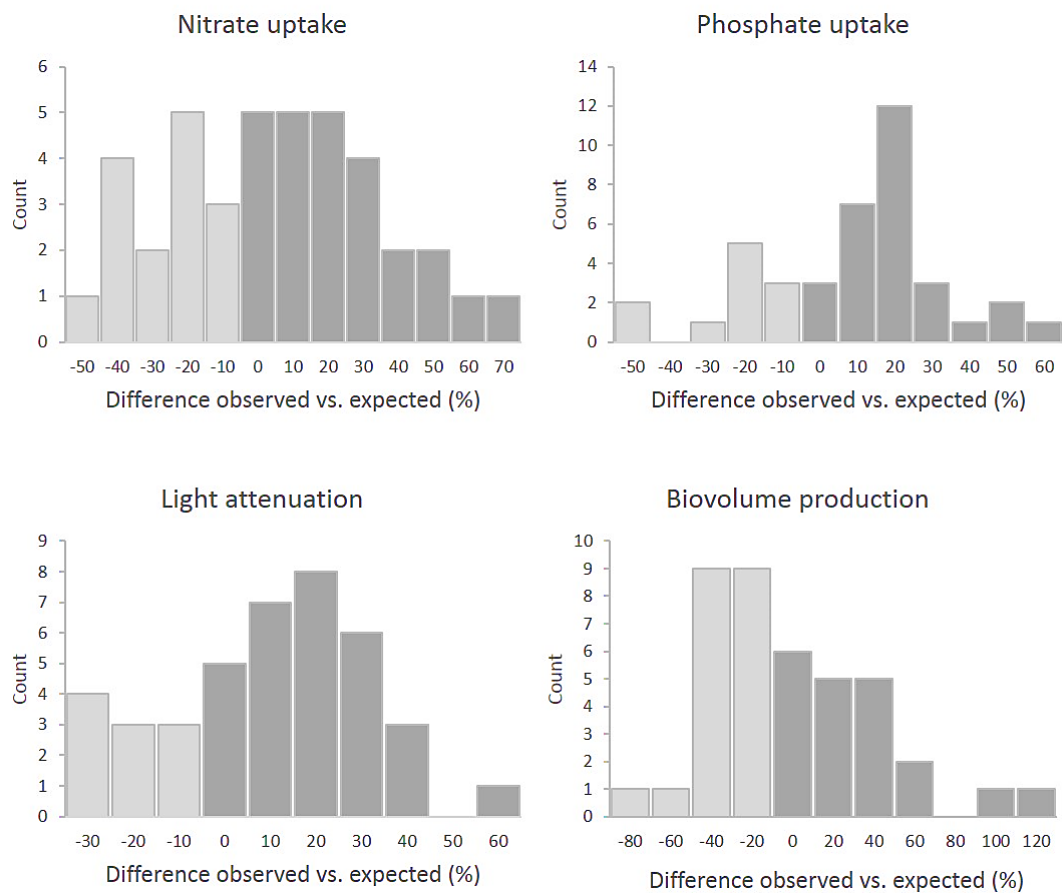
	<i>BV</i> <sub>mean</sub>	<i>BV</i> <sub>sd</sub>	<i>S/V</i> <sub>mean</sub>	<i>S/V</i> <sub>sd</sub>
<i>BV</i> <sub>mean</sub>		0.934	-0.547	0.084
<i>BV</i> <sub>sd</sub>	< 0.0001		-0.421	0.203
<i>S/V</i> <sub>mean</sub>	0.0003	0.0068		0.633
<i>S/V</i> <sub>sd</sub>	0.606	0.209	< 0.0001	

**Supplementary Table 3-S4:** Results of the influence of species interactions on functioning along the cell-size related gradients. Table shows *F* and *P*-values (in parenthesis) of the interaction term of a linear model that included each cell-size related variable as a continuous variable and expected or observed as a categorical variable on each ecosystem function. A significant interaction term would reveal that the slopes of the two linear models are different and consequently suggest that the nature or strength of species interactions had an influence on functioning along the cell-size related variable. A non-significant interaction term would mean that the slopes of the two linear models are not different from each other and consequently suggest that the nature or strength of species interactions had no impact on functioning along the cell-size related variable. *BV<sub>mean</sub>* = average cell biovolume of the two species, *BV<sub>sd</sub>* = standard deviation in cell biovolume of the two species, *S/V<sub>mean</sub>* = average surface area to volume ratios between the two species, *S/V<sub>sd</sub>* = standard deviation in surface area to volume ratios between the two species. Units for *BV* variables are cubic micrometers ( $\mu\text{m}^3$ ) and units for *S/V* variables are micrometers ( $\mu\text{m}^{-1}$ ).

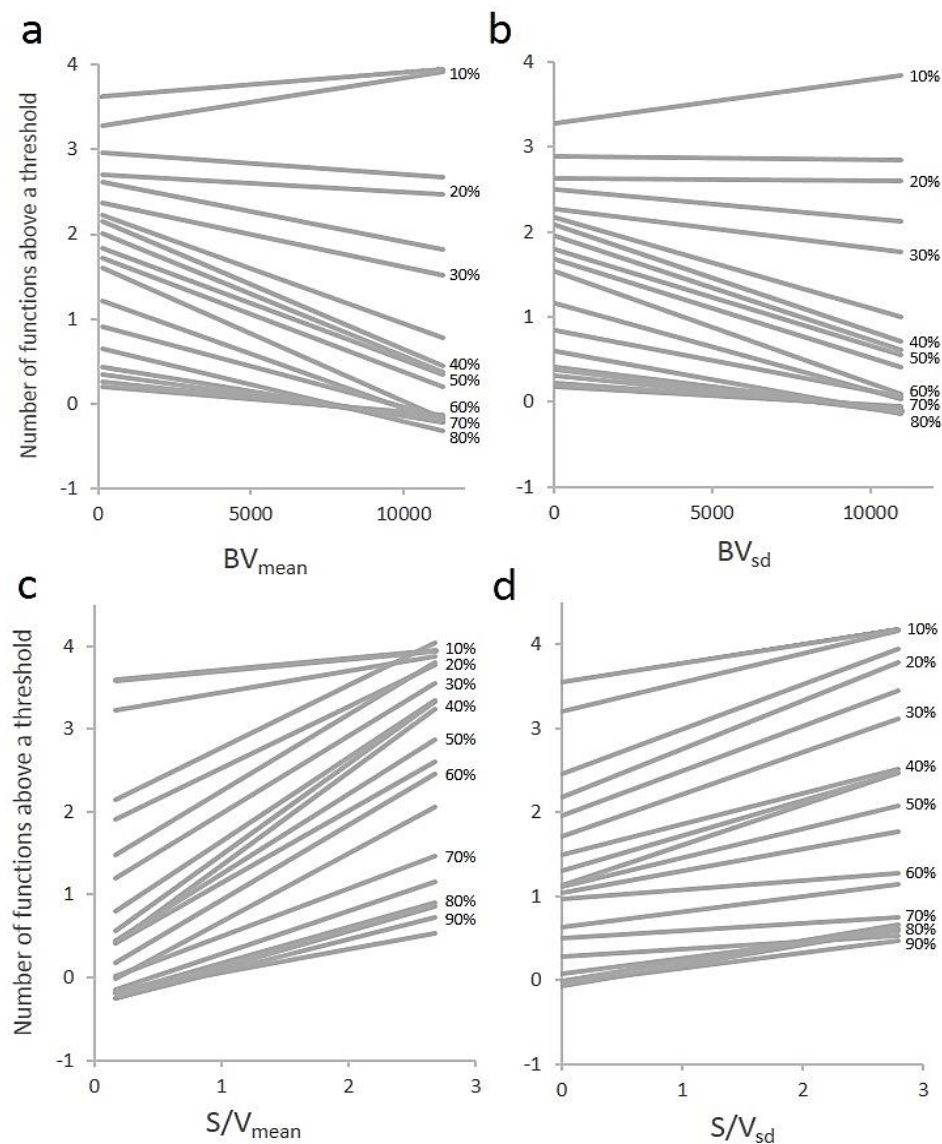
	<i>BV<sub>mean</sub></i>	<i>BV<sub>sd</sub></i>	<i>S/V<sub>mean</sub></i>	<i>S/V<sub>sd</sub></i>
Nitrogen uptake	0.4263 (0.5158)	0.7642 (0.3848)	0.0979 (0.7552)	0.0084 (0.927)
Phosphate uptake	0.0297 (0.8636)	0.0234 (0.879)	0.0049 (0.9445)	0.0224 (0.8814)
Light attenuation	0.2068 (0.6506)	0.1486 (0.7009)	0.7365 (0.3935)	0.2783 (0.5993)
Biovolume production	0.2917 (0.5907)	0.3108 (0.5788)	0.0389 (0.8442)	1.3344 (0.2516)



**Supplementary Figure 3-S1:** Distribution of the differences (as percentages) between the observed levels of functioning of the forty bicultures and their expected levels based on the average functioning levels of the constitutive monocultures. Light-grey bars represent negative values, meaning that some bicultures had functioning levels below the expectations. Dark-grey bars represent positive values, meaning that some bicultures had functioning levels above the expectations.



**Supplementary Figure 3-S2:** Multiple threshold plots of multifunctionality for each cell size variable. These graphs represent the number of functions (from 0 to 4) above a given threshold (from 5 to 95% of their maxima) as a function of cell size. Each line represents the linear fit over the actual data (not shown) for one percentage threshold. For clarity, only 5% increments of percentage thresholds are shown. Percentages on the right side of each graph represent the threshold percentages of the maxima. For  $BV_{mean}$  and  $BV_{sd}$ , the overall tendency is that the number of functions reaching a certain threshold decreases with increasing these two cell size variables. For  $S/V_{mean}$  and  $S/V_{sd}$ , the overall tendency is that the number of functions reaching a certain threshold increases with increasing these two cell size variables. Units for  $BV$  and  $S/V$  variables are cubic micrometers ( $\mu m^3$ ) and  $\mu m^{-1}$  respectively.





## CHAPTER 4

# Phosphate concentration determines the prevalence of facilitation in green algae assemblages

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*In preparation, data analysis in progress. This is a first draft written by Ziyu, partially revised by co-authors.*

## Abstract

Species interactions are considered as a key mechanism by which diversity influences ecosystem functioning. To date, studies on the influence of species interactions on ecosystem functioning have been largely focused on competition. Whereas recent evidence suggests that positive interactions are quite common among freshwater algae, the conditions under which these positive interactions emerge are unknown. We performed a controlled experiment using and additive design to test for the prevalence and magnitude of facilitation amongst green algal species under different resource conditions. We found that phosphate concentration influenced facilitation by changing its prevalence, but not its strength. Reciprocal facilitation was a rare outcome, with most of the time only one species showing evidence of facilitation.

## Introduction

After over two decades of intense empirical and theoretical research, it is now well established that changes in biodiversity can have an impact in many ecosystem functions. Extensive evidence shows that the loss of biodiversity negatively affects ecosystem functioning and the services they provide (Balvanera *et al.* 2006; Cardinale *et al.* 2006; Boyer, Kertesz & Bruno 2009). Historically, two main mechanisms have been proposed to explain biodiversity's influence on community functioning (Cardinale *et al.* 2007; O'Connor *et al.* 2017; Daam *et al.* 2019). The first mechanism called the sampling effect suggests that some species make a disproportionately high contribution to functioning. Increasing the number of species in a community increases the chances of including that species with high functioning levels. Thus, community functioning is not dependent on diversity per se but on a higher probability of the presence of that species (Cardinale *et al.* 2011). The second mechanism called complementarity effect is the result of interactions among species and their resulting impact on functioning (Barry *et al.* 2019). Species can either have negative interactions (e.g., competition) in which they interfere with each other's functioning, resulting in communities functioning less well than expected based on monocultures (negative complementarity effect); or positive interactions, in which species benefit from the presence of other species, resulting in communities performing better than the constitutive monocultures. Positive interactions include resource complementarity and facilitation (Wright *et al.* 2017). Complementarity posits that the addition of species to a community increases the average relative performance of each species because each one specializes on different resources and thus the whole community more thoroughly utilizes the available resources. Facilitation results when the functioning of one species benefits from the presence of another species (Wright *et al.* 2017). Most of the current mechanistic understanding of the effect of phytoplankton diversity on ecosystem functioning focuses on resource complementarity and selection effects. However, these effects do not necessarily correspond to real biological mechanisms (Loreau & Hector 2001; Venail 2017). Real improvements into the mechanistic understanding of the impact of diversity on ecosystem functioning will rely on the capacity to better depict the nature and strength of species interactions, either negative, positive or neutral (Cardinale, Palmer & Collins 2002).

The study of positive interactions between species of different functional groups started with manipulating functional groups or composition of functional traits on grassland models and measuring total net primary production (Tilman *et al.* 1997; Chapin *et al.* 1998). A mechanistic

definition of facilitation was first proposed by Vandermeer to describe the circumstances where a species modifies the environment in a way favorable to a co-occurring species (Vandermeer *et al.* 1998). In 2001, Mulder and colleagues tested the effect of diversity on productivity under environmental variability (Mulder, Uliassi & Doak 2001). They found that facilitative interactions, rather than sampling or niche complementarity effects better explained increased survivorship of almost all species and the positive biodiversity effects on biomass production. Scientists are increasingly interested in the positive interactions between species and propose that they may be an important but previously underemphasized mechanism in biodiversity and ecosystem functioning studies (Mulder, Uliassi & Doak 2001; Venail 2017; Wright *et al.* 2017). Understanding positive interactions in depth is quite complicated as positive interactions can occur either due to “niche complementarity” or “facilitation”, but separating the two is not always easy, and usually many studies end up determining positive interactions in general. A way to distinguish facilitation from positive interaction by looking at either the community overyielding or the species-specific overyielding has been recently proposed (Wright *et al.* 2017). Community overyielding occurs when communities perform better than the constitutive monocultures. Species-specific overyielding occurs when after accounting for differences in the proportion of each species in the community, a species grows more in a mixed culture than it does in a monoculture. According to this definition, facilitation can best explain species-specific overyielding.

Understanding better the facilitation mechanisms can shed light into the relationship between biodiversity and ecosystem functioning. Based on studies on the type of plant interaction in crops, facilitation can be classified in three different types: the indirect biotic facilitation, the abiotic facilitation via nutrient enrichment and the abiotic facilitation via microclimate amelioration (Wright *et al.* 2017). Indirect biotic facilitation occurs by diluting the effects of species-specific pathogen in diverse host communities when a pathogen decreases the yielding of one single species. In some cases, more diverse communities can dilute the absolute abundance of a certain pathogens species (Hendriks *et al.* 2013). Another example of indirect biotic facilitation occurs via a positive effect of belowground mycorrhizal fungi and rhizobacteria on plant yield. Studies have shown that the root microbiota of some plants can help other plant species to grow better. It can also be the result of complex indirect competitive interaction networks, when the community includes more than two species (Aschehoug & Callaway 2015). For example, species A might be a strong competitor and limit species B, at the same time, species B might limit the success of species C. Consequently, species A, B, C

form a complex competitive interaction network where it is also possible that there are, for example, indirect positive interactions between species A and C. Abiotic facilitation via nutrient enrichment is the most well-discussed and well-understood form of facilitation (Tilman *et al.* 2001). Certain species can benefit their neighbors due to increased nutrient availability. For example, legumes have the access to atmospheric nitrogen, which is an abundant resource that most plant species cannot directly use. So, when legumes are present, the resource availability for non-legume neighbors' increases, and thus results in species-specific overyielding. The same effect can also occur because of other nitrogen fixation species, such as feather mosses and cyanobacteria (Deluca *et al.* 2008). Abiotic facilitation via microclimate amelioration occurs when neighbor plants create microclimatic conditions that benefit other species. For example, when facing drought stress, increasing aboveground biomass in higher diversity plots increases shade, which in turn reduces drying and increases surface soil moisture (Wright, Schnitzer & Reich 2015). Additionally, increasing shade will decrease temperature and increase humidity that benefits the plants under shade. In this case, especially in severe climates, plants growth is often more limited by physiological strain instead of by competition with neighbors (Bertness & Callaway 1994).

Although research has shown that facilitative interactions between different species or different functional groups can positively influence resource uptake (Cardinale, Palmer & Collins 2002), there is almost no empirical evidence linking phytoplankton diversity to the underlying mechanisms of positive diversity effects in freshwater ecosystems. Furthermore, there is currently no consensus in the evidence about how phytoplankton diversity enhances the strength or frequency of facilitative interactions. In this paper, our goal is to perform a detailed characterization of the nature of positive species interactions among phytoplankton species and explore the conditions required for such positive interactions to emerge. In a recent study, we found additional evidence that several combinations of two species produced more biomass than it would be expected from the performances of individual species (Guan *et al.*, under review in *Freshwater Biology*). In addition, we found that the positive diversity effect might be linked to phosphate conditions. In this study, we performed an additive experiment in which we combined pairs of species in equal initial Chlorophyll-a as in the monocultures, and culture them in different phosphate conditions. Then we calculated the contribution of each species to community Chlorophyll-a production. We wanted to answer several questions. Do positive interactions between the tested species occur in our experiment? If yes, what kind of positive interactions are they? Is it niche differentiation or facilitation? Do these positive interactions



vary across different phosphate concentration? Last and most importantly, if yes, how does phosphate condition influence the positive interaction?

## Methods

### Species Selection

The freshwater green algae used in our experiment included nine species. We purchased them from the culture collection of algae and protozoa (CCAP, United Kingdom). They are *Ankistrodesmus angustus* CCAP 202/2, *Stichococcus bacillaris* CCAP 375/1B, *Coccomyxa vicidis* CCAP 216/14, *Scenedesmus armatus* var. *brevicaudatus* CCAP 276/4E, *Chlmydomonas pulvinata* CCAP 11/25, *Ankistrodesmus brauni* CCAP 202/7A, *Coelastrumpro boscodeum* var. *dilatatum* CCAP 217/2, *Scenedesmus obliquus* CCAP 276/3C, *Cosmarium lundelli* CCAP 612/15. These species are originally collected in Switzerland and nearby countries. They have different size and shapes that we can easy distinguish them under microscope (Chapter 3, Guan *et al.*, under review in Freshwater Biology).

### Experimental Design

This study was designed to compare the effect of species interactions under different phosphate concentrations. For this, we grew each of the nine algae species alone as monocultures and 29 pairwise combinations of two-species (bicultures). Different nutrient conditions were created by using different concentrations of BG-11 medium and adding extra  $\text{NaNO}_3^-$  and  $\text{K}_2\text{HPO}_4$ . We used three different phosphate concentrations: LowP, MidP and HighP (**Table 4-1**). Thus, the experiment included 39 different algal communities with different species compositions and three phosphate levels. Each of the 39 treatments was replicated at least three times for 3 condition group for a total of 351 microcosms.

**Table 4-1.** The nitrate and phosphate concentration in the medium prepared for each phosphate group.

Phosphate Group	Phosphate( $\text{mg L}^{-1}$ )	Nitrate( $\text{mg L}^{-1}$ )
MidP	1.7	13
LowP	0.35	13
HighP	7.12	13

The monocultures and bicultures were grown in 50mL cell-culture flasks with 40 mL of medium. All flasks were placed on a temperature-controlled incubator at 22.5 °C with a 16:8h (light/dark) photoperiod of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation (PAR). Culture media were sterilized on an autoclave at 15 lb inch<sup>-2</sup> pressure and 121 °C for 15 min. Algae were inoculated under sterile conditions into the flasks and were grown in an incubator as described above. We opted for an additive design for the inoculation of bicultures, where the total initial chlorophyll-a in the bicultures was equivalent to the sum of the chlorophyll-a of the two monocultures with a 50: 50 proportion of each species. By using this additive design, we can easily compare the production in biculture and in monoculture (Weis *et al.* 2007).

Algal cell numbers and chlorophyll-a concentrations for each individual cell were measured by flow cytometer (Cytobuoy bv. Netherlands). We took at least 10,000 cells for each species to measure their average cell size and chlorophyll-a concentrations. Each species of algae was taken a fixed chlorophyll-a value of 0.0015  $\mu\text{g}$  to transfer to new media. Thus the final concentration for monocultures was 0.0375  $\mu\text{g L}^{-1}$ . In bicultures the final concentration was 0.075  $\mu\text{g L}^{-1}$ . We collected 3 mL samples from each flask in days 7, 14, 21 and 28 to measure chlorophyll-a production and nutrient concentrations.

## Measurements

Chlorophyll-a concentration was measured with a Turner fluorometer (Trilogy ® USA) in the *in vivo* module. Every measurement was replicated three times. Phosphate (ortho-phosphate) and nitrate concentrations were measured using a discrete analyzer AQ2 (Seal Analytical Inc, Germany). The difference in nutrient concentrations between two measurements represented the uptake value of a culture. The cell ratio of each species in the bicultures was counted manually using a cell imaging multi-mode reader Cytation 5 (BioTek, Switzerland). Every cell count in the bicultures was replicated three times. The cell ratio of each species in polycultures was counted through upright automated fluorescence microscope (OLYMPUS Europa). For this, we counted at least 1000 cells in each polyculture.

The growth rate of every algae was determined as follows:

where  $\mu$  is the change of algae chlorophyll-a concentration per day,  $Y_0$  and  $Y_t$  are initial and final chlorophyll-a concentrations respectively, and  $t$  is the duration of incubation in days. (Davis *et al.* 2009)

$$\mu = \ln[Y_t/Y_0]/t$$

We defined the production of a species A in monoculture as  $Y_{EA}$  (yield expected species A), and the production of the same species in biculture as  $Y_{OA}$  (yield observed species A). And we defined production of species B in monoculture is  $Y_{EB}$  (yield expected species B), and the production of the same species in biculture is  $Y_{OB}$  (yield observed species B). The total production of biculture is  $Y_{total}$ . The biodiversity effect (BE) is the difference in the production of a biculture and the sum of production of the two species in monoculture (Weis, Madrigal & Cardinale 2008).

$$BE = Y_{total} - (Y_{OA} + Y_{OB})$$

We calculated the biodiversity effect based on chlorophyll-a concentrations and biovolume production. Because the biodiversity effect data were not normally distributed, we performed a Wilcoxon to test if BE was different from 0.

The resource use efficiency of phosphate ( $E_P$ ) and nitrite ( $E_N$ ) were defined as the chlorophyll-a production  $Y$  divided by the resource uptake during a certain period (Mandal *et al.* 2018).

$$E_P = Y/U_P$$

$$E_N = Y/U_N$$

We test the effect of species richness and phosphate concentration on BE and E by using a Kruskal-Wallis test. We used a Wilcoxon signed-rank test to determine differences in BE and E between measurement days.

### **Frequency and magnitude of facilitation**

To calculate the frequency of facilitation in the bicultures, we calculated the difference of the chlorophyll-a production for a species when grown in biculture  $Y_o$  to when grown alone in monoculture  $Y_E$ .

$$NBE_{spp} = Y_o - Y_E$$

Because the data were not normally distributed, we performed a Wilcoxon sign test to determine if NBE is significant from 0. For each biculture there are two  $NBE_{spp}$  values, one for each species. If the  $NBE_{spp}$  of both species in a biculture are positive, facilitation was reciprocal. If

only the  $NBE_{spp}$  of one species is positive, then facilitation is non-reciprocal. If both  $NBE_{spp}$  are negative, facilitation did not occur.

We defined the facilitation magnitude as the difference in chlorophyll-a production of a species in biculture and in monoculture.

$$\text{Facilitation strength} = (Y_o - Y_E) / Y_E \times 100\%$$

### Statistical Analyses

For the analyses, we calculated the mean and standard deviation of every measurement mentioned before. Species richness and phosphate concentration were treated as categorical variables. All statistical tests were performed using R.

## Results

We found that species richness (one, two and nine species) and days of culture influenced chlorophyll-a production ( $P < 0.001$  and  $P < 0.001$ ). Phosphate group had a marginal effect on chlorophyll-a too ( $P = 0.08$ ). When testing by using days as factor, the data showed that in Day 14 and Day 21, phosphate concentration influenced chlorophyll-a concentrations (**Table 4-1**). We found that besides species richness and culture time, phosphate group can also be a factor influencing chlorophyll production. However, it is interesting that the algae did not produce more chlorophyll at higher phosphate levels (**Figure 4-1**).

*Table 4-1, Kruskal-Wallis rank sum test of phosphate group for chlorophyll production at different culture times.*

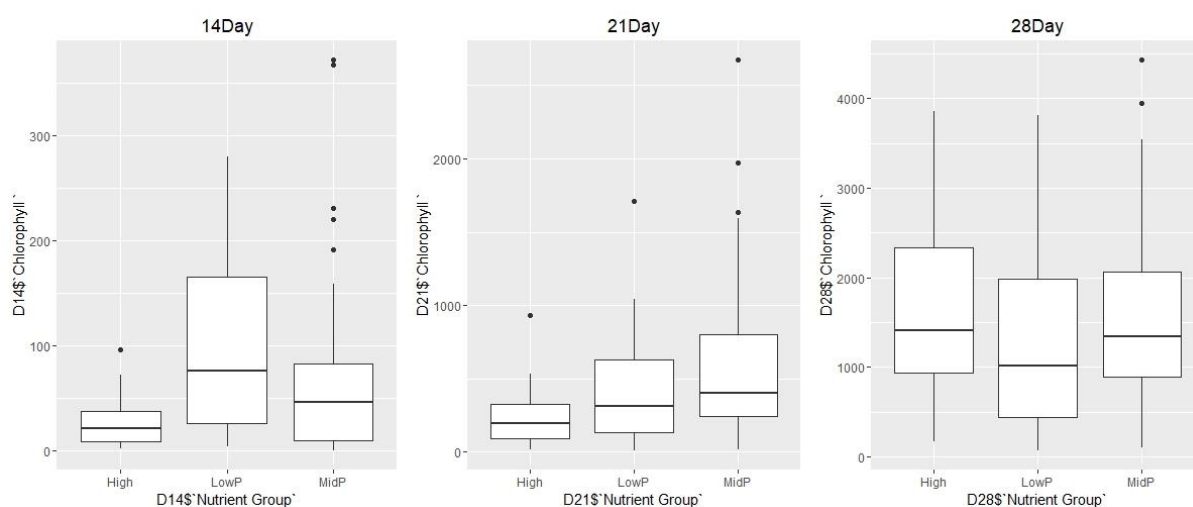
Culture days	Factor	Df	F value	p value
14Day	Phosphate Group	2	113.4	<0.001
21Day	Phosphate Group	2	113.4	0.015
28Day	Phosphate Group	2	134.4	0.3152

The chlorophyll growth rate decreased over time, presumably because of a reduction of the phosphate in the medium (**Figure 4-2**). Additionally, the interaction term of culture time and phosphate concentration also had an influence on chlorophyll-a (**Table 4-2**).

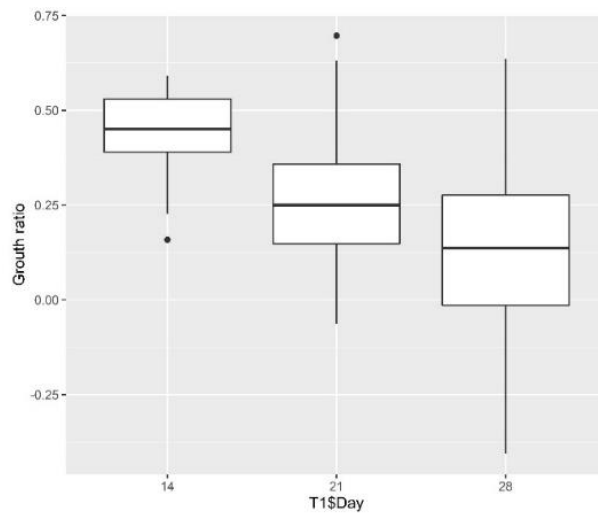
We calculate the biovolume equal to number of cell multiplied by a single cell volume in bicultures. And compared with the chlorophyll data in each bicultures, we found that there are no significant differences between the two data (paired test,  $p=0.8509$ , **Supplementary Figure 4-S1**).

**Table 4-2**, Two-way Anova with culture time and phosphate concentration for chlorophyll growth rate of all the cultures.

Factor	Df	F value	p value
Culture time	2	147.57	<0.001
Phosphate Group	2	25.80	<0.001
Culture time × Phosphate Group	4	54.35	<0.001

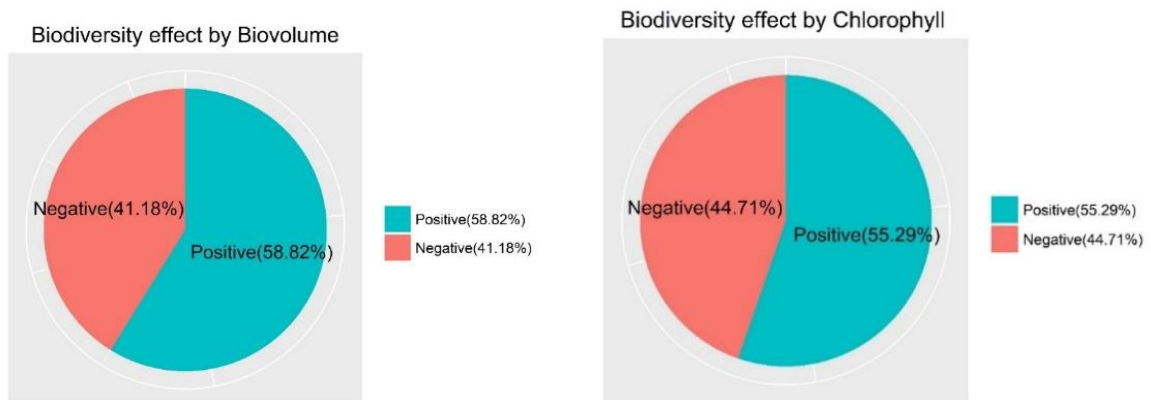


**Figure 4-1.** Box plot of chlorophyll production in bicultures at different phosphate concentrations and culture times. Left panel shows the chlorophyll-a levels at different phosphate concentrations after 14 days. Center panel shows the chlorophyll-a levels at different phosphate concentrations after 21 days. Right panel shows the chlorophyll-a levels at different phosphate concentrations after 28 days. Each dot represents one biculture.



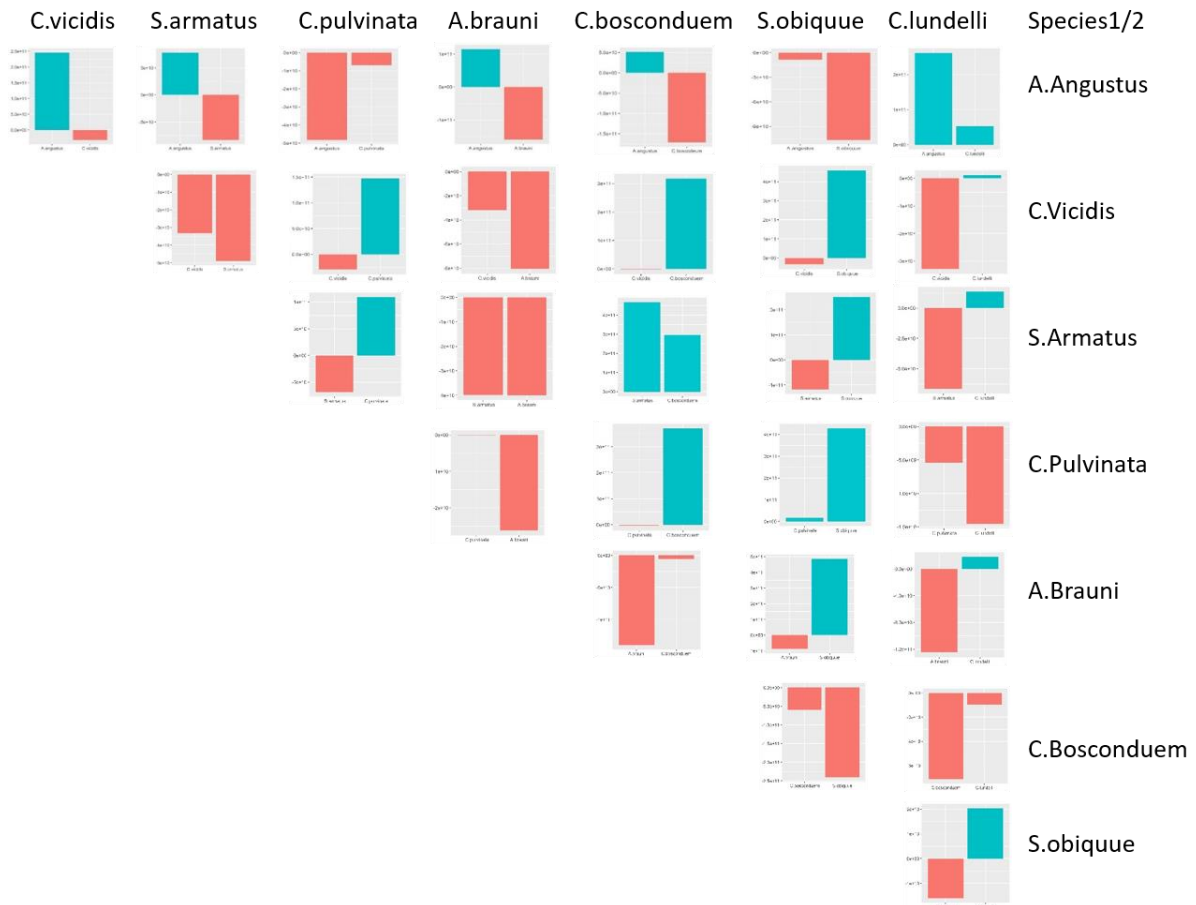
**Figure 4-2.** Box plot of chlorophyll growth rate of cultures at different culture times.

For clarity and to avoid confusion, we focused on the data from day 21th day for further analysis. Our results show that the biodiversity effects (BE) based on biovolume and chlorophyll-a were mostly positive (**Figure 4-3**). This suggests that positive diversity effects are common in phytoplankton communities, even when only two species are present.



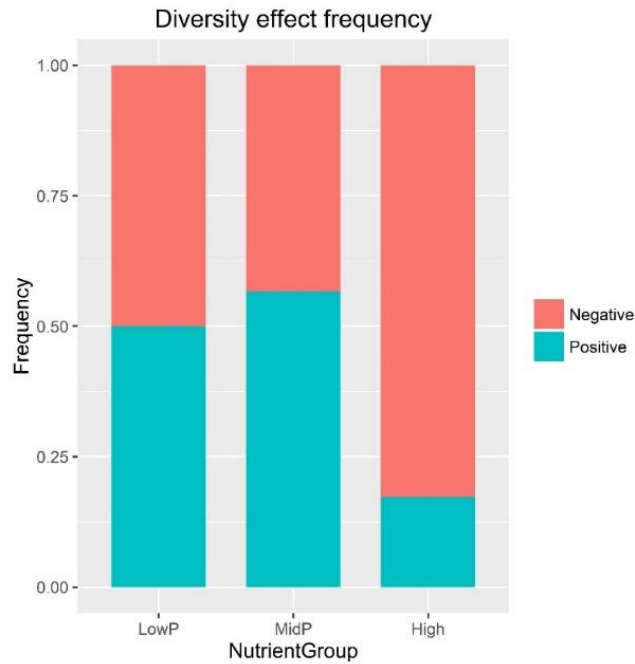
**Figure 4-3.** The biodiversity effect is the difference between the production of a biculture and the sum of production of the two species as monocultures. We calculated the biodiversity effect based by biovolume and chlorophyll-a production.

A variety of effects can generate positive diversity effects. Species-specific overyielding is one special situation of positive biodiversity effect and is a signature of facilitation. We found that species-specific overyielding occurred in 35.7% of the bicultures (**Figure 4-4**).



**Figure 4-4.** Chlorophyll-a production in each biculture at the intermediate phosphate level. In each panel, a column represents a species. Blue means that the algae produced more in the biculture than in the monoculture (species-specific overyielding occurred). Red means that algae produced less in biculture than in monoculture.

We found that over all the bicultures and all the phosphate concentrations, almost half (49.6%) of cultures included one species producing more than expected. None of the species showed facilitation in 25.0% of cases and facilitation was reciprocal in 25.4% of cultures. Thus, facilitation was present in 75% of the cultures.

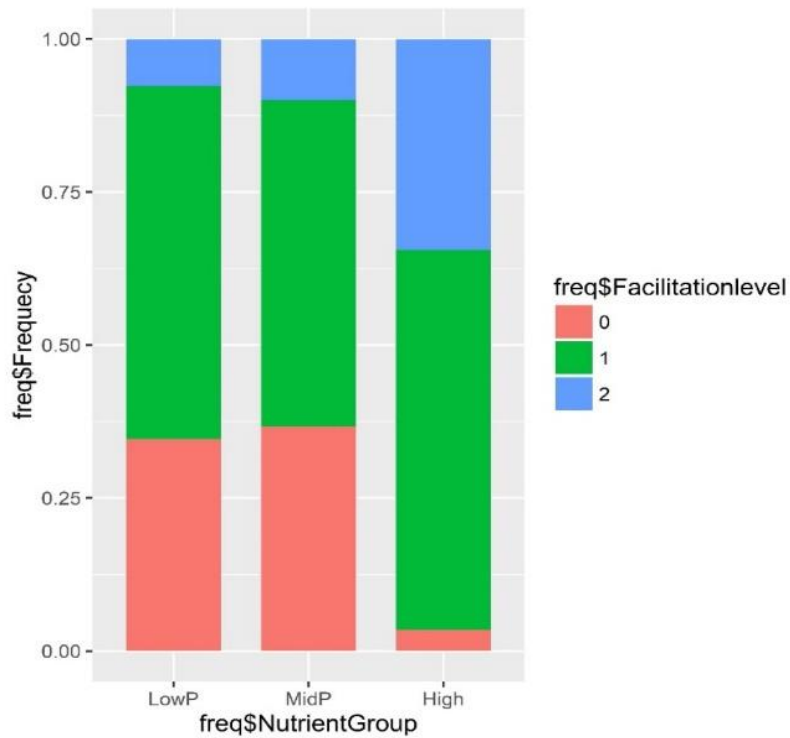


**Figure 4-5.** The frequency of positive and negative biodiversity effects at different phosphate concentrations. Phosphate concentration influenced the prevalence of positive and negative diversity effects in phytoplankton communities (Kruskal-Wallis rank sum test,  $P < 0.05$ )

Phosphate concentration had an influence on total biodiversity effects (**Figure 4-5**,  $P = 0.005$ ) and on the frequency of facilitation. The high phosphate treatment resulted in more frequent facilitation than the other two phosphate treatments (**Figure 4-6**,  $P = 0.0002$ ). On the contrary, we found that phosphate concentration had no impact on the magnitude of facilitation ( $P > 0.05$ ).

We use the phosphate and nitrate use efficiencies to understand why phosphate conditions influenced the biodiversity effects. We found that phosphate and nitrate use efficiency differed between monocultures and bicultures ( $P = 0.0014$  and  $P < 0.001$ , respectively). Furthermore, the initial phosphate concentrations also had an impact on phosphate and nitrate use efficiency. This suggests that the presence of a second species in the culture changed the way that algae used the nutrients.





**Figure4-6.** *Frequencies of facilitation along the three phosphate concentrations at day 21.*

*Red represents when no facilitation occurred. Green represents when facilitation was observed in only one species in a biculture. Blue represents reciprocal facilitation. The prevalence of the three types of scenarios was different in the high phosphate concentration from the other two phosphate concentrations.*

Overall, our results show that the initial phosphate concentration influence the prevalence of facilitation but not its magnitude.

## Discussion

It is now widely recognized that positive interactions are frequent in natural environment (Burns 2004; Brooker *et al.* 2008b). However, they have been relatively ignored compared to negative interactions such as competition. Facilitation has been defined as an interaction in which the presence of a second species alters the environment in a way that enhances growth, survival or reproduction of a second species. According to the definitions, facilitation can be mutualistic, antagonistic or commensal. In another words, facilitation can be beneficial, neutral or even harmful to the facilitator. Research to date has mostly focus on mutualistic facilitation (Callaway 2007) and facilitation has been identified among different tropic levels (Bracken, Gonzalez-Dorantes & Stachowicz 2007) but specially on plant-plant interactions.

In 2014, Venail and colleagues reported a laboratory experiment showing how phylogenetic relatedness of green algae influenced their competitive and facilitative interactions. They found evidence of facilitation in nearly one-quarter of the species interactions (Venail *et al.* 2014). A similar results also been found by Fritschie and colleagues who tested the phylogenetic limiting similarity hypothesis and found that 23% of algal populations were facilitated in the presence of other species (Fritschie *et al.* 2014). This proportion is similar to our result in middle phosphate concentration treatment (35.7%). In the high phosphate concentration treatment in our experiment, 75% of bicultures show evidence of facilitation. These results indicate that facilitation is very common in algae communities. One possible reason why our bicultures show a high frequency of facilitation occurred, is because our algal communities included species with large trait differences. By analyzing a large database of species, it is reported that nurse species facilitated more phylogenetic distantly related species than phylogenetic closely related species (Valiente-Banuet & Verdú 2007a). Together with the assumption that traits related to facilitation are evolutionarily conserved, it is acceptable that the frequency of facilitation will increase with trait differences. Furthermore, it is widely accepted that traits play an important role in facilitation, even though there still is unclear whether the prevalence of facilitation tends to be less in phylogenetic closely related communities (Litchman *et al.* 2010b; Fritschie *et al.* 2014; Venail *et al.* 2014).

It has been reported that *Scenedesmus acuminatus* and *Chlorella sorokiniana* are two species that can benefit from the presence of other algae (Venail *et al.* 2014). Our experiments show a similar result. The actual underlying mechanism behind facilitation still needs to be addressed.

One possible mechanism of facilitation in phytoplankton communities is that some species may provide resources such as vitamins or other metabolites. Another hypothesis is that some species can modify the physics and/or chemistry of water, such as pH, dissolved CO<sub>2</sub> or light availability (Venail *et al.* 2014).

In this experiment, we found that phosphate concentration can influence the facilitation in green algae communities by changing its frequency. This result indicates that facilitative interactions between phytoplankton happen by changing the way algae use nutrients. It has been argued that when considering a large range of algal taxonomic groups, competitive abilities for nitrate and phosphate are negatively correlated, suggesting that species performing well under nitrate limited conditions perform badly under phosphate limited conditions and vice-versa (Edwards, Klausmeier & Litchman 2011a). Mandal and colleagues recently found that across different nitrogen treatments, the relationship between nutrient use efficiency and species richness was positive (Mandal *et al.* 2018). Thus, it is possible that nitrate also can influence the frequency of facilitation.

Studies on the relationship between biodiversity and ecosystem functioning are key to understand and predict the ecological consequences of diversity loss (Hooper *et al.* 2012). Despite being the largest contributor to global primary production and its crucial role in global energy fluxes and element cycles, biodiversity and ecosystem functioning studies in phytoplankton remain relatively scarce compared to studies in terrestrial plants. Some patterns obtained for terrestrial plants, such as the positive effects of species richness on biomass production, have also been observed in experiments using freshwater microalgae (Fox 2004; Power & Cardinale 2009; Narwani *et al.* 2016), but are far from universal. In natural phytoplankton communities, multiple interacting species are contained in a spatially heterogeneous and temporally fluctuating environment. It results in a complex interactive network in which the biodiversity effects on functioning can be hardly explained by one single mechanism (Fritschie *et al.* 2014). Simplified experiments such as the one presented here, allow to better depict those mechanisms but cannot capture the full diversity and dynamics of natural system. Our conclusions require further validation at higher levels of diversity and under more natural conditions.

In conclusion, it has been suggested that in addition to competition, positive species interactions such as facilitation might be very important for understanding the link between phytoplankton diversity and ecosystem functioning. By manipulating green algae assemblage, we found that

facilitation is the cause of positive diversity effect. Furthermore, initial phosphate concentration can influence the facilitation. More importantly, we found that phosphate concentration influenced the frequency of facilitation but not its magnitude.

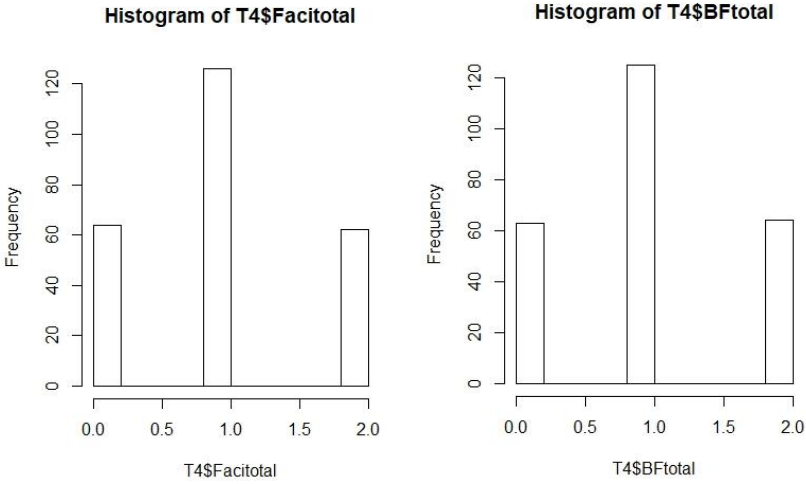
## Acknowledgements

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## Authors' Contributions

Z.G., P.V. and B.I. conceived of the project. Z.G. and P.V. designed and finished both sampling and collecting the data. Z.G. and P.V. performed statistical analyses. Z.G. wrote the papers. P.V. contributed constructive comments and revisions. All authors contributed substantially to drafts and gave approval for publication.

# Supplementary Information



*Supplementary Figure 4-S1 Facilitation frequency situation based on chlorophyll production and biovolume production have similar distribution*

## CHAPTER 5

Phytoplankton diversity relates negatively with productivity due to strong compositional effects in tropical high-altitude lakes from Southern Ecuador.

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## Abstract

Tropical high-altitude lakes are vital freshwater reservoirs in the Andean regions, heavily threatened by human activities that may alter their functioning and hamper the provisioning of key ecosystem services such as water supply. Despite their ecological and social relevance, we know little about these waterbodies, especially regarding the factors influencing their functioning. Here, we explored the links between several environmental variables and productivity, measured as chlorophyll-a concentration and total phytoplankton biovolume, across twenty-four tropical high-altitude lakes located over three-thousand meter above sea level in Southern Ecuador. We found that a combination of four abiotic factors explained over three quarters of the variation in chlorophyll-a concentration amongst lakes. Contrary to what studies from temperate regions suggest, taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, Shannon's diversity index was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume, presumably due to a strong compositional effect. We hope this study will help establishing a baseline for evaluating some of the future consequences of human activities in the ecology and functioning of these vital but fragile ecosystems. Our results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activities can indirectly impact their functioning and their capacity to provide vital ecosystem services.

## Keywords

Biodiversity; Ecuador; páramo; phytoplankton; productivity; tropical high-altitude lakes

## Introduction

High-altitude lakes, also called high-mountain lakes, are important natural freshwater reservoirs for human consumption, irrigation and hydropower production purposes in Andean regions (Buytaert *et al.* 2006; Buytaert, Cuesta-Camacho & Tobón 2011; Mosquera *et al.* 2017; Van Colen *et al.* 2017). Like high-altitude temperate lakes, tropical high-altitude lakes (hereafter TRHALs) have low average water temperatures that are negatively related to altitude and cloud cover as a determinant of solar incidence. TRHALs can have extreme diel water temperature variations, are submitted to strong winds and receive intense solar UV radiations. Generally, but not always, they are low in nutrients (Pérez & Restrepo; Miller, Kannan & Colinvaux 1984; Steinitz-Kannan 1997). Because of their low latitudes, TRHALs have some specific features not shared by high-altitude temperate lakes. This includes moderate or no seasonality, no ice cover, polymictic mixing regimes with often a complex thermal structure, intense UV radiation through the year, high dissolved organic carbon and low UV transparency, making them unique extreme freshwater ecosystems (Steinitz-Kannan 1997; Llames & Zagarese 2009; Aguilera, Lazzaro & Coronel 2013; Catalan & Rondón 2016; Michelutti *et al.* 2016; Mosquera *et al.* 2017; Barta *et al.* 2018). One type of TRHALs are the *páramo* lakes, located above the tree line between approximately from 3200 to 4500 meters above sea level (m.a.s.l.) and filled almost exclusively by rain and groundwater. Another type are the *glacial* lakes, located at even higher altitudes and are fed directly by glacier melting waters. These two types of lakes can show important differences in both abiotic and biotic characteristics (Barta *et al.* 2018). This study focuses in *páramo* lakes, located between 3288 and 3362 meters above sea level with no direct connection to glaciers.

TRHALs show a wide range of phytoplankton productivity levels, with chlorophyll-a concentrations ranging from below  $1 \mu\text{g l}^{-1}$  to values over  $8 \text{ mg l}^{-1}$  (Miller, Kannan & Colinvaux 1984; Dorador, Pardo & Vila 2003; Alcocer *et al.* 2004; Aguilera *et al.* 2006; Merchán Andrade & Sparer Larriva 2015; Van Colen *et al.* 2017). Their phytoplanktonic production has been negatively related to depth (Miller, Kannan & Colinvaux 1984), UV radiation (Kinzie, Banaszak & Lesser 1998) and positively to pH (Aguilera *et al.* 2006), total phosphates (Van Colen *et al.* 2017) and total nitrogen (Barta *et al.* 2018). Also, production in TRHALs has often been described as nutrient limited (Miller, Kannan & Colinvaux 1984; Alcocer *et al.* 2004; Van Colen *et al.* 2017). Studies of phytoplankton diversity in TRHALs reported over a hundred different genera in different regions of Ecuador (Van Colen *et al.* 2017; Barta *et al.* 2018).



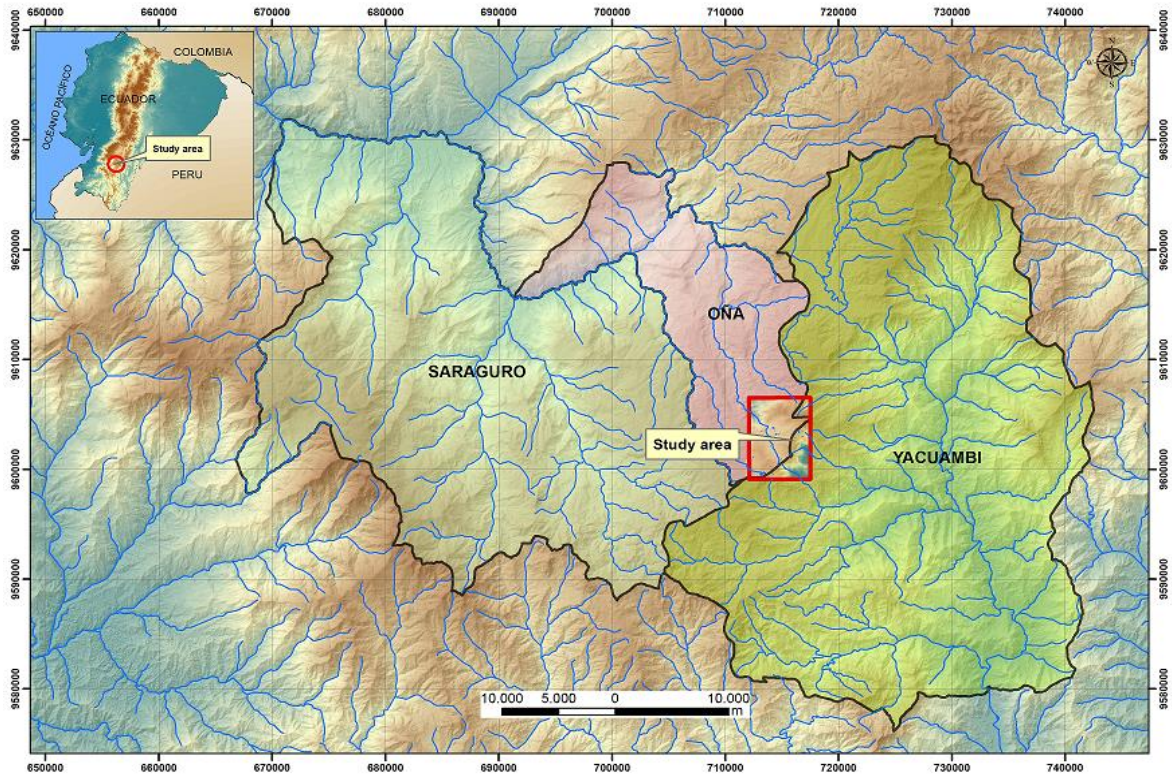
Regarding the phytoplankton diversity per lake, previous studies counted from 5 to 45 genera per lake in Southern Ecuador (Barta et al. 2018, Van Colen et al. 2017), with taxonomic richness decreasing with altitude and increasing with conductivity (Barta *et al.* 2018).

Despite their ecological and social relevance, we still know little about the abiotic or biotic factors influencing the functioning of tropical high-altitude lakes (TRHALs). It is well established that under controlled experimental conditions, phytoplankton communities with multiple species produce more biomass than monocultures (Cardinale et al. 2011, Cardinale et al. 2013, Gross et al. 2014) and that species richness and biomass are positively related (O'Connor et al. 2017). A recent meta-analysis on the importance of species richness for productivity suggests that the above-mentioned effects are even stronger in natural conditions (Duffy, Godwin & Cardinale 2017). However, this review only included information from two studies based on phytoplankton communities from temperate regions. To date, observational studies on the relationship between phytoplankton diversity and ecosystem functioning in freshwater lakes have completely ignored TRHALs (**Table 5-1**). The before-mentioned unique features of tropical high-altitude lakes suggest that previous findings on the relationship between phytoplankton diversity and ecosystem functioning on temperate or low-altitude lakes might not be extrapolated to TRHALs. The main purpose of the current study is to depict potential links between phytoplankton biodiversity and productivity, quantified as chlorophyll-*a* concentration and total phytoplankton biovolume. In addition, we aimed to explore if other abiotic variables, independently or in addition to biodiversity, relate to phytoplankton productivity. For this, we examined a set of twenty-four tropical high-altitude shallow lakes located over 3280 meters above sea level in Southern Ecuador.

## Methods

### Study system

*Tres Lagunas* belongs to a tropical high-altitude wetland ecosystem called *páramo* [1]. It is located at the eastern range of the southern Ecuadorian Andes, at approximately 20 km from Saraguro and 95 km from Loja, at the border of the Oña and Zamora-Chinchipe provinces (**Figure 5-1**).



**Figure 5-1.** Location of the Tres Lagunas wetland system in southern Ecuador.

This ecosystem harbors hundreds of small shallow freshwater lakes where rivers in the Amazon mountain range (heading to the Pacific Ocean) and the Andean mountain range (heading to the Atlantic Ocean) begin. The lake system *Tres Lagunas* consists of around 75 shallow lakes, including three larger ones: *Condorshillu* (6.3 ha), *Tres Lagunas* (8.5 ha) and *Laguna Grande* (12 ha). For the present study, we included the three larger lakes (as major freshwater reservoirs) and another 21 smaller lakes that were randomly selected in the map. The 24 lakes had areas ranging from 0.5 to 12 ha, maximum depths from 1 to 9 m and altitudes above sea level ranging from 3288 to 3362 m. Eleven of these lakes are in the Amazon (eastern) mountain range and thirteen in the Andes (western) range. The GPS coordinates of the center of the *Tres Lagunas* system are 3°35'50" S and 79°3'46" W.

### ***In situ* analyses and sampling**

The field work described below was performed in November 2016. *In situ*, we collected data on total chlorophyll-a concentration ( $\mu\text{g l}^{-1}$ ) with a BBE Moldaenke fluoroprobe. Dissolved oxygen ( $\text{mg l}^{-1}$ ), redox potential (mV), conductivity ( $\mu\text{S/cm}$ ), pH and water temperature ( $^{\circ}\text{C}$ ) were measured at the same locations with a HQ40D HACH® portable multiprobe. All the mentioned variables were measured once for each lake near its center. Water samples for

nutrient analyses were collected in 10 ml plastic acid-washed tubes at 0,5 m subsurface depth near the center of the lakes. We preserved the water samples with 98% sulfuric acid. For phytoplankton analyses, we collected samples in 100 ml acid-washed plastic bottles at 0,5 m subsurface depth near the center of the lakes and preserved them with glutaraldehyde. All samples were immediately stored in the dark, under cold conditions and sent by plane to the laboratory to be analyzed. Due to the harsh access conditions of the wetland and to the remoteness of some lakes, the *in situ* measurements and water samplings of the 24 lakes took four complete days (from 8<sup>th</sup> to 11<sup>th</sup> November 2016). All measurements and water samplings were performed from 10 AM to 3 PM.

### ***Ex situ* laboratory analyses**

Total phosphate ( $\mu\text{g l}^{-1}$ ) and dissolved nitrite/nitrate ( $\mu\text{g l}^{-1}$ ) quantifications were performed in the laboratory on an AQ2 discrete analyzer, based on EPA 365.1 version 2, EPA 353.2 version 2 and EPA 353.1 methods respectively. Phytoplankton abundances were determined based on Nietch et al. (2017) protocols using an inverted microscope with 40x magnification. Phytoplankton in water samples was first concentrated via sedimentation in 50 ml Falcon tubes for 24 hours. For cell counting, we used the Sedgwick-Rafter camera cell counter (S-R Camera) and included 100 fields of vision for each sample/lake. A field of vision measured  $0.38 \text{ mm}^2$ . We took digital pictures of each field of vision for phytoplankton identification and counting. For each taxon, we estimated the mean cell biovolume (in  $\mu\text{m}^3$ ) using at least fifty individuals. In each sample, the biovolume of each taxon was calculated as the product of the average cell biovolume by its cell density (in cells per ml). Total phytoplankton biovolume (biovolume of algae per volume of water,  $\mu\text{m}^3 \text{ ml}^{-1}$ ) was calculated as the sum of the biovolumes of all the taxa present in the sample. Phytoplankton richness in each sample was calculated by counting the number of different taxa at the genus level. In addition, as another measure of phytoplankton diversity, we estimated the Shannon's diversity index ( $H'$ ) based on biovolumes of each genus in each lake/sample. Thus, Shannon's diversity index was calculated with the following formula:

$$H' = - \sum_{i=1}^S p_i * \ln(p_i)$$

Where  $S$  is the number of genera in the sample and  $p_i$  is the relative biovolume of each taxon to total phytoplankton biovolume. The value of the Shannon's index increases with the number of genera and with the evenness in the contribution of each genus to total phytoplankton

biovolume. This is, samples with only a few genera contributing in large proportion to total biovolume would have a low Shannon's index value. Professor Miriam Steinitz-Kannan (Northern Kentucky University) and doctor Kalina Manoylov (Georgia College and State University) supervised and validated the taxonomic identification of the taxa.

## Data analyses

Our dataset included four geographic variables: lake surface (in hectares, ha), altitude (in meters above sea level), latitude (in degrees), longitude (in degrees); seven physico-chemical variables: water temperature (in °Celsius), pH, redox potential (in mV), conductivity (in S/m), oxygen concentration (in mg l<sup>-1</sup>), total phosphates (in µg l<sup>-1</sup>), dissolved nitrites/nitrates (in µg l<sup>-1</sup>) and four biological variables: taxonomic richness (number of genera), Shannon's-diversity index (no units), chlorophyll-a (in µg l<sup>-1</sup>) and total phytoplankton biovolume (in µm<sup>3</sup> ml<sup>-1</sup>, which represents the biovolume of phytoplankton in µm<sup>3</sup> per ml of lake water). We used chlorophyll-a concentrations and total phytoplankton biovolume as two proxies for phytoplankton production. We ran linear-models linking single or multiple of the two above-mentioned variables to phytoplankton production (i.e., chlorophyll-a and total biovolume). We then used the AIC (Akaike information criterion) to determine the abiotic (chemical, physical, geographic) or biotic variables that better described chlorophyll-a and total phytoplankton biovolume. We used JMP (SAS, version 14.0.0) for all statistical analyses. Total phytoplankton biovolume and surface of lakes were log transformed to improve the normality of the data.

## Results

Chlorophyll-a concentrations in the lakes from *Tres Lagunas* ranged from 1,49 to 5,05 µg l<sup>-1</sup>, with an average concentration of 3,01 µg l<sup>-1</sup>. Total phytoplankton biovolume ranged over four orders of magnitude, from 34,08.10<sup>3</sup> to 31,02.10<sup>7</sup> µm<sup>3</sup> ml<sup>-1</sup>, with an average value of 20,1.10<sup>7</sup> µm<sup>3</sup> ml<sup>-1</sup>. Chlorophyll-a and total phytoplankton biovolume (log transformed) were positively correlated across the lakes (*correlation coefficient* = 0,514, *P* = 0,01, *N* = 24). Genera richness ranged from 15 to 43 per lake with an average richness of 26,75 genera per lake. The less diverse lake had a Shannon index of 0,057 whereas the more diverse one had a Shannon index of 0,939. The average Shannon's diversity index of the lakes was 0,521. These two measures of

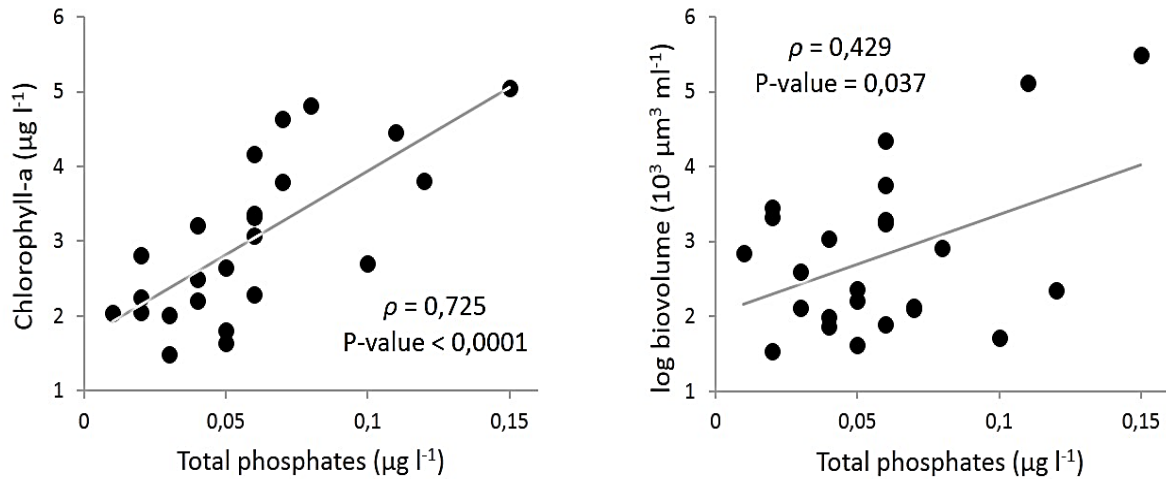
phytoplankton diversity were positively related (*correlation coefficient* = 0,343, *P* = 0,1, *N* = 24), but would still encompass different aspects of phytoplankton's diversity.

Four abiotic variables correlated well to chlorophyll-a concentration (**Table 5-2**). This included total phosphate concentration (**Figure 5-2**), oxygen concentration and altitude, that related all positively to chlorophyll-a concentrations. This means that lakes with higher phosphate concentrations, more dissolved oxygen levels and located at higher altitudes showed higher chlorophyll-a concentrations. The percentages of variance in chlorophyll-a concentrations explained by total phosphate, oxygen and altitude were 53%, 31% and 19% respectively. The surface of the lakes (log transformed) correlated negatively to chlorophyll-a, meaning that smaller lakes had higher chlorophyll-a concentrations than larger lakes. Only total phosphate concentrations related well and positively to total phytoplankton biovolumes (i.e., log biovolume, **Figure 5-2, Table 5-2**).

*Table 5-2. List of abiotic and biotic variables (related variables) that correlated significantly with either chlorophyll-a or total biovolume (response variable).*

*Variables are ranked from more positive to more negative correlation coefficients and includes only variables with p-values below 0.1.*

<b>Response Variable</b>	<b>Related Variable</b>	<b>Correlation Coefficient</b>	<b>p-Value</b>
Chlorophyll-a	Total Phosphate	0.725	<0.0001
Chlorophyll-a	Oxygen	0.559	0.004
Chlorophyll-a	Altitude	0.436	0.033
Log biovolume	Total Phosphate	0.429	0.037
Chlorophyll-a	Shannon (Biovolume)	-0.393	0.058
Chlorophyll-a	Log Surface	-0.504	0.012
Log biovolume	Shannon (Biovolume)	-0.658	<0.001



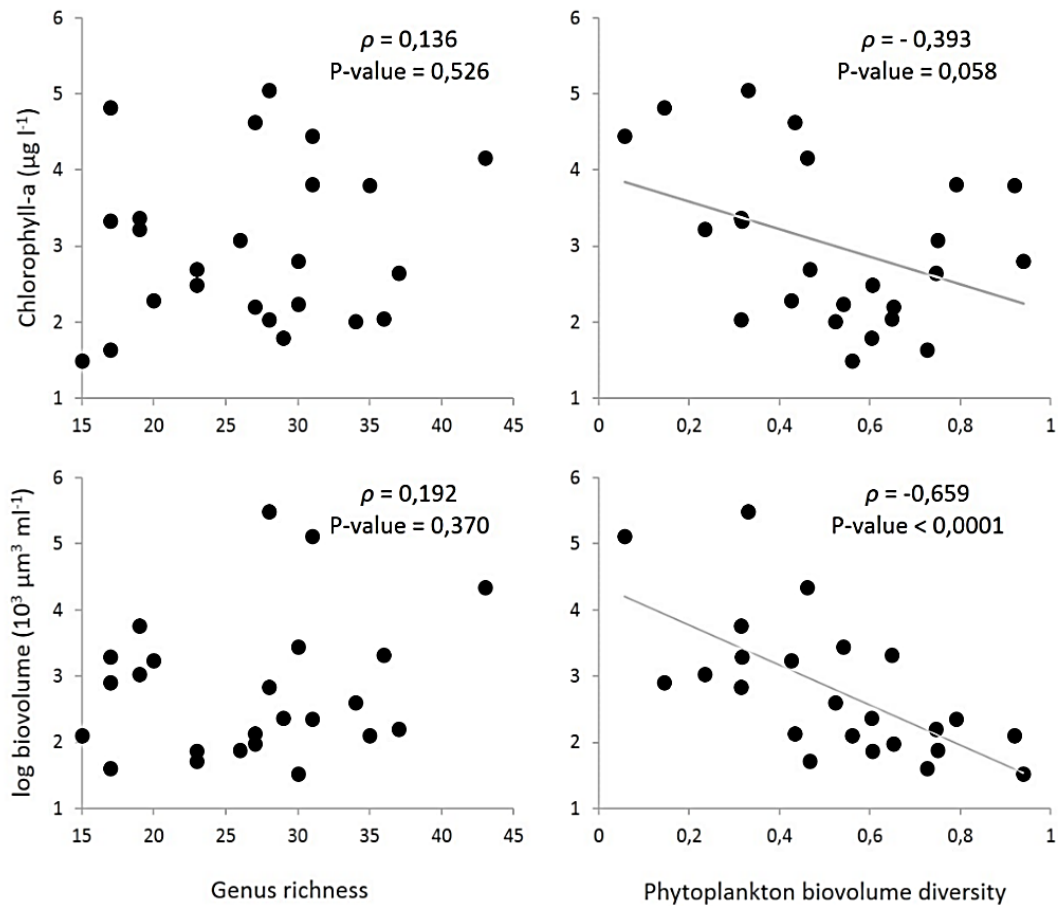
**Figure 5-2.** Correlations between total phosphates (phosphates, in µg L<sup>-1</sup>) and chlorophyll-a concentrations (µg L<sup>-1</sup>, left panel) and between total phosphates and total phytoplankton biovolume (log biovolume in 10<sup>3</sup> µm<sup>3</sup> mL<sup>-1</sup>, right panel). Indicated statistics are the correlation coefficients ( $\rho$ ) and p-values. Grey lines represent linear fits.

In other words, lakes with higher phosphate concentrations had also higher total phytoplankton biovolumes. Together, the correlation analyses suggest that phytoplankton production in the *Tres Lagunas* system, quantified as chlorophyll-a and total biovolume, might be partially phosphorous limited.

The two measures of phytoplankton biodiversity, genera richness (taxa richness) and Shannon's diversity index (phytoplankton biovolume diversity), showed different relationships with chlorophyll-a concentrations and total phytoplankton biovolumes (**Table 5-2, Figure 5-3**). Taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolumes. On the contrary, phytoplankton biovolume diversity (measured as Shannon's diversity index based on biovolumes) was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolumes (**Figure 5-3**). This suggests that lakes dominated by fewer genera had higher chlorophyll-a concentrations and total biovolume levels than lakes with more even distributions of biovolume amongst different taxa. Regarding the negative relationship between phytoplankton biovolume diversity and total biovolume (Figure 5-3, low right hand panel), a closer look at data allowed us to observe some interesting trends. We noticed the presence of three groups of lakes based on their total phytoplankton biovolumes. The first group includes three lakes with values of total phytoplankton biovolume above 1.10<sup>6</sup>

$\mu\text{m}^3 \text{mL}^{-1}$  and Shannon's diversity index values spanning from very low to intermediate. The analysis of the taxonomic composition of each lake showed that the communities of the two lakes with highest total biovolumes were largely dominated by *Mougeotia*, a filamentous alga (family: Zygnemataceae). This genus represented up to 96% of the total biovolume and 61% of the cell counts. The lake with the third highest total biovolume was largely dominated by *Peridinium*, a dinoflagellate representing 72% of the total biovolume and 74% of cell counts. This information is clearly showing that an extremely uneven distribution of biovolume amongst the different taxa explains the combined high total biovolume and low biovolume diversity values of these three lakes. The second group of lakes includes nine waterbodies with total phytoplankton biovolumes ranging from  $0.5 \cdot 10^4$  to  $1.10^6 \mu\text{m}^3 \text{mL}^{-1}$  and with Shannon indexes varying from low to intermediate/high. Overall, these lakes also reported a large dominance of biovolume production (up to 95%) by the dinoflagellate *Peridinium*, or by colonial diatoms such as *Synedra*, *Fragilaria* or *Asterionella*. However, these taxa did not overdominate cell counts, with percentages of total abundance ranging from 25 to 40%. Finally, a group of twelve lakes had biovolume values below  $0.5 \cdot 10^4 \mu\text{m}^3 \text{mL}^{-1}$  and phytoplankton biovolume diversities spanning from intermediate to high. In these lakes, the above-mentioned genera represented less than 70% of the total biovolume and less than 38% of the cell counts. In lakes from groups 2 and 3, the genus *Mougeotia* was not recorded at all.

Overall, the analysis of the composition of phytoplankton communities suggests that the observed negative relationship between total biovolume and phytoplankton biovolume diversity (**Figure 5-3**) can be largely explained by a very uneven distribution of biovolume amongst taxa. Total phytoplankton biovolume in the samples from *Tres Lagunas* decreased as the dominance, both in biovolume and cell counts, of some taxa such as *Mougeotia* and *Peridinium* decreased. As the contribution of each taxa to total chlorophyll-a could not be determined, we can only speculate about the reasons why chlorophyll-a decreases as phytoplankton biovolume diversity increases. Based on the distribution of the data from the positive correlation between total phytoplankton biovolume and chlorophyll-a concentration, it is quite possible that the uneven contribution of taxa to total biovolume is at the origin of a negative correlation between biovolume diversity and chlorophyll-a concentration. Five lakes were characterized with low biovolume diversity but very high chlorophyll-a values, three of which also showed the highest biovolume levels that resulted from the dominance of one single taxon.



**Figure 5-3.** Correlations between genus richness (left panels), phytoplankton biovolume diversity (Shannon's diversity based on biovolume, right panels), chlorophyll-a (in µg L<sup>-1</sup>, upper panels) and total phytoplankton biovolume (log biovolume in 10<sup>3</sup> µm<sup>3</sup> mL<sup>-1</sup>, lower panels). Indicated statistics are the correlation coefficients ( $\rho$ ) and *p*-values of the correlation. Grey lines represent linear fits for *P* values below 0.1.

After fitting linear models with all possible combinations of single and multiple factors (both abiotic and biotic) to chlorophyll-a concentrations and total phytoplankton biovolumes, we ranked these models according to the Akaike criteria (AICc, **Table 5-3**).



**Table 5-3.** Summary table of the different linear models (single and multiple factor) linking abiotic and biotic variables to chlorophyll-a. Models are ranked by increasing AICc (Akaike information criterion) values. Oxygen stands for oxygen concentration, altitude is for altitude above sea level.

<b>Factors Included in Model</b>	<b>R<sup>2</sup></b>	<b>p-Value</b>	<b>AICc</b>
Total phosphate, oxygen, altitude, log surface	0.777	<0.0001	50.764
Total phosphate, oxygen, log Surface	0.724	<0.0001	52.249
Total phosphate, altitude	0.684	<0.0001	52.322
Total phosphate, oxygen, altitude	0.719	<0.0001	52.707
Total phosphate, altitude, log Surface	0.717	<0.0001	52.877
Total phosphate, oxygen	0.628	<0.0001	56.198
Total phosphate, log Surface	0.586	<0.0001	58.776
Total phosphate	0.525	<0.0001	59.166
Oxygen, log Surface	0.578	0.0001	59.244
Oxygen, altitude, log Surface	0.602	0.0003	61.099
Oxygen	0.312	0.0045	68.062
Altitude, log Surface	0.379	0.0067	68.52
Oxygen, altitude	0.375	0.0071	68.659
Log Surface	0.254	0.012	70.011
Altitude	0.190	0.0331	71.984

For chlorophyll-a concentration, the linear model that better described variation in the data (77.7%, with the lowest AICc value) included four abiotic factors: total phosphate, oxygen, altitude and log surface. As shown previously with the correlations, the best single abiotic predictor of chlorophyll-a concentration was total phosphate, explaining 52.5% of the variation amongst lakes. None of the biotic variables included in our study appeared in the models that better predicted chlorophyll-a concentrations. For total phytoplankton biovolume, the model that better described variation in the data (50.3%, with lowest AICc, **Table 5-4**) included total

phosphate concentration and phytoplankton biovolume diversity (Shannon’s index based on biovolumes). The best single predictor of total phytoplankton biovolume was phytoplankton biovolume diversity (Shannon’s diversity), explaining 43.3% of the variation in total biovolume among lakes.

*Table 5-4. Summary table of the different linear models (single and multiple factor) linking abiotic and biotic variables to total phytoplankton biovolume. Models are ranked by increasing AICc (Akaike information criterion) values. Only models with p-values below 0.05 are presented.*

<b>Factors Included in the Model</b>	<b>R<sup>2</sup></b>	<b>p-value</b>	<b>AICc</b>
Total phosphate, Shannon’s diversity	0.503	<0.001	63.223
Shannon’s diversity	0.433	<0.001	63.466
Total phosphate	0.184	0.0367	72.226

## Discussion

The levels of chlorophyll-a measured in this group of lakes from *Tres Lagunas* are comparable to most previous studies in tropical high-altitude lakes (TRHALs) from Mexico, Bolivia and other parts of Ecuador (Miller, Kannan & Colinvaux 1984; Alcocer *et al.* 2004; Aguilera *et al.* 2006; Merchán Andrade & Sparer Larriva 2015; Barta *et al.* 2018). Like in a recent study in the Cajas National Park in Southern Ecuador (Van Colen *et al.* 2017), chlorophyll-a values correlated positively with total phosphate concentrations. Another study involving TRHALs from Ecuador with similar levels of phytoplankton production found that total nitrogen concentration was the only variable that explained some variation in chlorophyll-a amongst lakes (Barta *et al.* 2018). Other abiotic variables such as pH (Aguilera *et al.* 2006) and UV radiation (Kinzie, Banaszak & Lesser 1998) have been related to chlorophyll-a concentrations in TRHALs as well. Such discrepancies amongst studies in terms of the abiotic determinants of phytoplankton’s productivity (with chlorophyll-a as a proxy) suggested that the large geographic variation in the productivity of TRHALs can hardly be predicted by one single abiotic factor. In line with this hypothesis, our analysis showed that chlorophyll-a

concentrations had simultaneous positive and negative links with several abiotic variables, including phosphate concentration, oxygen concentration, altitude and lake surface. According to the results of the linear models, these four abiotic variables together explained 78% of the variation in chlorophyll-a levels amongst the lakes from *Tres Lagunas*. In brief, our results showed smaller lakes located at higher altitudes, with higher concentrations of oxygen and total phosphates have a tendency to be more productive in terms of chlorophyll-a concentrations.

Despite the observed positive correlation of chlorophyll-a with total phytoplankton biovolume in our dataset, the links between the different abiotic variables and total biovolume were weak. Among all the abiotic variables included in this study, only total phosphate concentration related to total phytoplankton biovolume but explained only 18% of its variation (versus 52% for chlorophyll-a). This result suggests that other variables not included in our study (abiotic or biotic) might be more relevant as determinants of total phytoplankton biovolume. Previous studies about the determinants of phytoplankton production in tropical high-altitude lakes using total biovolume as proxy are rare. This can be explained because acquiring total biovolume information requires more sophisticated equipment (e.g., particle counter, cytometer) or time demanding techniques (e.g., microscopy). In a recent study, total phytoplankton biomass was measured along sixteen lakes in the Ecuadorian Andes, but none of the abiotic variables included related to phytoplankton biomass (Barta *et al.* 2017). In our study, only the Shannon's diversity index strongly improved the capacity of the linear models to predict total biovolume variation among lakes. Alone it was the best single predictor of total biovolume (43.3% of the variation) and together with total phosphate explained up to 50.3% of the variation.

So far, studies on the relationship between phytoplankton diversity and ecosystem functioning in freshwater lakes have overlooked tropical high-altitude lakes. To the best of our knowledge, this study represents the first attempt to link phytoplankton diversity to productivity in these extreme aquatic ecosystems. Our results revealed no relation between taxonomic richness and either chlorophyll-a or total phytoplankton biovolume. A similar null pattern between taxonomic richness and functioning was described before in temperate lakes from Finland (Ptacnik *et al.* 2008) but contradict most studies from temperate lakes showing a positive impact of taxonomic richness on phytoplankton productivity (Ptacnik *et al.* 2008; Striebel, Behl & Stibor 2009; Korhonen, Wang & Soininen 2011). Moreover, phytoplankton diversity measured as the Shannon's diversity index correlated negatively with both chlorophyll-a and total biovolume. Whereas not included as one of the factors explaining much variation in

chlorophyll-a amongst lakes, phytoplankton diversity turned out to be the best single predictor of total phytoplankton biovolume. The analysis of community composition revealed that this pattern was mainly due to changes in the dominance amongst lakes of a few taxa, both in terms of abundance and biovolume. A reduction in the prevalence of taxa such as *Mougeotia* (a filamentous algae) and *Peridinium* (a dinoflagellate) resulted in a concomitant increase of diversity and a decrease of total phytoplankton biovolume. Such negative links between diversity and productivity due to strong compositional effects are not very frequent but have been reported in temperate lakes (Korneva 2010; Pálffy, Présing & Vörös 2013; Filstrup *et al.* 2014; Fontana *et al.* 2017).

Phytoplankton productivity in the *Tres Lagunas* lakes showed large geographic variation, due in big part to variation in abiotic and biotic factors, as reported in previous studies from temperate regions (Cardinale *et al.* 2009; Korhonen, Wang & Soininen 2011; Stomp *et al.* 2011; Zimmerman & Cardinale 2014). Overall, chlorophyll-a was strongly related to four abiotic factors whereas total biovolume was strongly linked to phytoplankton diversity through compositional effects. However, the scope of these findings has at least two limitations. First, lakes were sampled only once, thus ignoring the spatial and temporal variability in the abiotic and biotic parameters considered. It is possible that the relationships among variables described in this study might vary when considering other spatiotemporal scales (Interlandi & Kilham 2001; Korhonen, Wang & Soininen 2011). Second, the patterns described here are purely correlational because no causal relationships amongst variables can be established using only observational data (Ptacnik *et al.* 2008, Cardinale *et al.* 2009). The current study addresses diversity as a possible determinant of phytoplankton productivity, but it is well known that the alternate perspective, with productivity as a determinant of diversity can be considered too (Interlandi and Kilham 2001, Cardinale *et al.* 2009, Stomp *et al.* 2011).

Tropical high-altitude lakes (TRHALs) are the major freshwater reservoirs in Andean regions but are also very vulnerable to human driven activities, putting at risk its own functioning and the provisioning of key ecosystem services such as water supply (Bradley *et al.* 2006; Buytaert *et al.* 2006; Buytaert, Cuesta-Camacho & Tobón 2011; Mosquera *et al.* 2017; Van Colen *et al.* 2017). As in other South American high-altitude ecosystems, road construction, controlled fires, agriculture, livestock and extreme sports are modifying the *Tres Lagunas* ecosystem. To our best knowledge, no environmental impact studies have ever been made in this region and no actions to mitigate their potential impacts have been considered. We hope this study will help

establishing a baseline for evaluating some of the future consequences of human activities in the ecology and functioning of this vital but fragile ecosystem. Our results suggest that by impacting abiotic and biotic parameters of these lakes, human driven activities can also have either positive or negative impacts on the functioning of tropical high-altitude lakes and the provisioning of ecosystem services.

## Acknowledgements

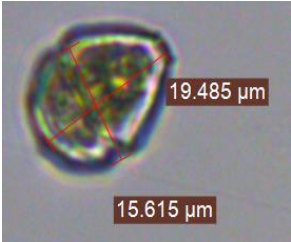
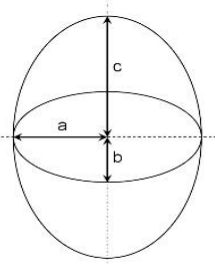
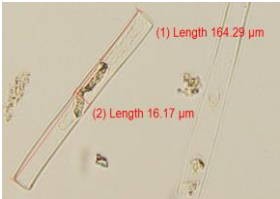
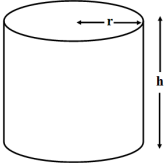
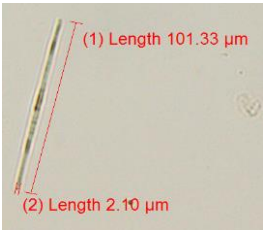
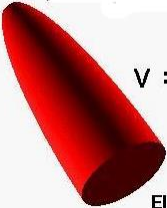
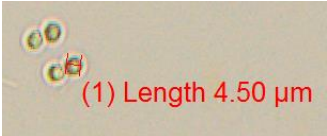
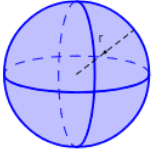
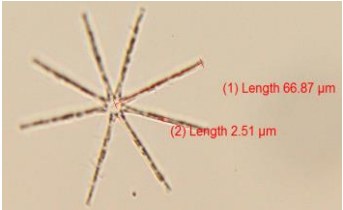
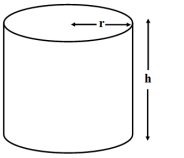
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## Authors' Contributions

Author Contributions: Conceptualization, P.V.; Methodology, A.C, Z.G. and P.V.; Formal Analysis, A.C. and P.V.; Writing – Original Draft Preparation, A.C.; Writing – Review & Editing, A.C., Z.G, B.I and P.V.; Funding Acquisition, P.V.

## Supplementary Material

Table 5-S1. Five examples on how the cell biovolume of different taxa were estimated.

Taxon	Example Picture and Measurements under the Microscope	Formula for Estimation of Cell Volume
<i>Peridinium</i>		 $V = \frac{4 \cdot \pi}{3} \cdot a \cdot b \cdot c$
<i>Mougeotia</i>		$V = \pi r^2 h$ 
<i>Fragilaria</i>		 $V = \frac{\pi d^2 h}{6}$ <p>Elliptical Cone</p>
<i>Chroococcus</i>		 <p>Volume of Sphere</p> $= \frac{4}{3} \pi r^3$
<i>Asterionella</i>		$V = \pi r^2 h$ 

# CHAPTER 6

## Applications and Perspectives

The relationship between biodiversity and the functioning of ecosystems has emerged as a central topic in ecological sciences during the last decade. It is now well acknowledged that, generally, the functioning of less diverse ecosystems is impaired, making them less stable than more diverse ones. A related body of research questions has begun to attract the attention of scientists. The emerging field is known as biodiversity and ecosystem services (BES) and is linked to the BEF studies treated in my thesis. Ecosystem services refer to the benefits that ecosystems provide to humanity. Generally, the main topic of BES studies has been focused more towards economic or cultural interests. As far as I can tell, BES studies is more akin an applied direction of BEF studies, although in essence they are comparable. A variety of benefits related to the natural environment and human society could be gained from the application of BEF and the related BES research, such as biodiversity conservation, habitat restoration, sustainable agriculture and (e.g. algal) biomass production. In this chapter, we will explore this applied side from our fundamental understanding resulting from BEF research and I will make clear how the knowledge developed in my thesis contributes to this.

Our research explores how biodiversity is linked to ecosystem functioning in freshwater ecosystems. In this chapter, we first introduce the topic of biodiversity conservation and habitat restoration in aquatic ecosystems in general. Secondly, we explore how the mechanisms of positive interactions as studied in Chapter 4 might be applied in aquatic ecosystem restoration and conservation. Thirdly, I expand on two examples, which could potentially benefit from our experimental results, i.e. wastewater treatment and algal biotechnology (biomass production).

### **Biodiversity conservation and habitat recovery in aquatic ecosystem**

Life evolved in water. Aquatic ecosystems include a rich diversity of habitats, ranging from lightless deep-sea trenches to extremely oligotrophic high altitude lakes, coastal wetlands, open oceans etc. Moreover, these systems support high phyletic biological diversity, containing various organisms such as the world's largest animal, deep-sea specialists, and photoautotrophic picoplankton, possibly the most abundant life form on earth. The abundant abiotic and biotic resources in aquatic ecosystems provide great value to human society. These



values can be classified into several categories. First, the values that we benefit from most obviously are the so-called “direct use values”, e.g., harvesting aquatic organisms for food. Or think off emerging technologies which are centered around using algae as a source of energy, fine chemicals or pharmaceuticals. The second type of value are the “indirect uses”, for example, the role that aquatic ecosystems play in moderating climate change, purifying the water etc. Finally we distinguish “non-use values” such as esthetic or religious values. Overall, we only are beginning to realize the enormous value of aquatic ecosystems and the essential services we obtain from them.

Despite the values offered by aquatic ecosystems, they may well be the most endangered ecosystems on the planet (Dudgeon *et al.* 2006). There are several threats to aquatic ecosystems. One of the main and most serious threats are various types of water pollution. Chemicals and heavy metals from industrial facilities are discharged in surface waters. After they enter the aquatic system, many contaminants prove to be toxic for the aquatic organisms, most often reducing an organism’s lifespan and ability to reproduce (Ormond, Gage & Angel 1998). What is more, some toxins, like PCBs, may bioaccumulate in the foodweb and cause toxic effects in particular at the higher trophic levels (Derraik 2002). Plastic debris is another kind of pollution that is swept from the land into storm drains and eventually reaches the sea (Derraik 2002). This type of pollution affects marine life at all trophic levels oceans. Discarded fishing gear, plastic bags, soda cans, and all this kind of rubbish can strangle, suffocate and starve animals. Meanwhile, the burning of fossil fuels leads to ocean acidification, making it tougher for shellfish and corals to survive (Doney *et al.*, 2009). Additionally, when water eutrophication causes algal blooms (Mantzouki *et al.* 2018) in lakes and oceans this may result in reduced oxygen availability in the water, creating dead zones that suffocate plants and animals. In some cases, harmful algal blooms produce different types of toxins that affect wildlife and even humans (Mantzouki *et al.* 2018).

One of the most damaging effects of water pollution are losses in biodiversity, sensitive species that disappear from aquatic ecosystem (Beatley 1991). Since scientists and the public are aware of the importance of biodiversity and the current threats to biodiversity, governments around the world have begun to formulate policies to help protect biodiversity. Here we summarize the main concepts and approaches for conservation and restoration of aquatic ecosystems, supported by recent advances in biodiversity and ecosystem studies.

The first thing that comes up in the discussion about the conservation of aquatic biodiversity is the need to remove or reduce current anthropogenic impacts on these ecosystems (Primack & Ralls 1995). The ideal template for action is to look for intact and undisturbed reference sites at the appropriate spatial scales. These reference sites will set the targets for ecosystem protection and preservation. However, in particular for larger, open water systems, it is usually impossible to stop all human impacts. The most feasible method then is to reduce the extent of the impacts. One example is in controlling water eutrophication, which has been partially successful in most industrial countries. By replacing, detergents containing phosphate, improving sewage collection, and doing tertiary treatment, the load of nitrogen and phosphate to most surface waters has been greatly reduced over the last thirty years (Geist 2015). Another successful example is that the acidification of water bodies has been much reduced thanks to using low sulphur fuels and filters in industry (Primack & Ralls 1995).

So disturbed ecosystems can be restored, to some extent. Still, preventing damage to aquatic ecosystems and maintaining the best quality habitats should remain the priority (Moilanen, Leathwick & Elith 2008). For some of the most critically endangered ecosystems, particularly streams and rivers, the problems involve changes to physical structures that have been done over the years, caused by river diversions and construction of dams, leaving insufficient natural habitat diversity (Allan 2004). In these cases, habitat restoration should be the first priority, which can rehabilitate the key physical structural and chemical properties of the system, which is instrumental for the recovery of biodiversity itself, but also for good water quality. For example, fish bypass channels or fish ladders can be used to restore the capability for fish to pass dams in their upstream journey to the spawning grounds and re-establish environmental flow dynamics (Myers *et al.* 2000). Nonetheless, some artificial constructions may come with great risks. They can result in very different effects, diverging from what was intended (Geist & Hawkins 2016). Furthermore, after studying stream restoration techniques, based upon four stream substrate restoration measures, it was found that generally the effects of stream reconstruction lasted only one year (Roni *et al.* 2002). A more complete set of restoration measures should have been taken, closely considering the more natural conditions at reference sites (Halme *et al.* 2013). As such a rethinking of the current restoration techniques is needed (Pander, Mueller & Geist 2015).

Artificial marine habitats are common worldwide. In recent decades many deliberate shipwrecks have been put in place. These are one of the main efforts to create artificial reefs to

enhance habitats for fish, supporting - diving - tourism and recreational angling. Intertidal seawalls and other similar structures provide new surfaces for colonization by benthic organisms and therefore provide new intertidal habitats for estuarine animals and plants. They have the potential to supplement natural habitat by supporting natural assemblages, in terms of species composition and enhanced abundances (Chapman & Bulleri 2003). This technique also supports better cover and higher densities of algae and invertebrate groups (Chapman & Browne 2014).

Many conservation actions also happen in an urban setting. When restoration to the original status is not possible, some kind of mitigation measures can reverse biodiversity losses and perhaps achieve a comparable level of ecosystem functioning as in a more natural state. Artificial buildings such as zoos or aquaria can maintain a number of endangered marine species to a certain extent, but in the long run, small scale reproduction that is typically linked to breeding in captivity is not good for the genetic diversity of these species. Additionally, these measures are not durable, but more of like a drop in the ocean (Geist & Hawkins 2016).

In addition to ecological aspects of restoration of aquatic ecosystems, technical and social aspects should also be considered, being equally important. Technical factors include the technical limitations of restoration measures that are taken, the requirement of skilled operators, as well as the time needed for implementation. Social factors are mostly an economic problem, such as the costs of restoration and temporal or more permanent reduced commercial profitability of systems where the emphasis is placed on ecological functioning rather than e.g. production of one or two species. Other factors exist as well, such as the need of a persistent support of the public for the restoration, the timescale needed to obtain the desired results, as well as the chances of success. To date, ecologists are – still - often the main parties who support restoration. For restoration to be more beneficial and acceptable in the future, transdisciplinary approaches should be considered. All ecological, technical and social factors need to be addressed in a coherent manner so that conservation and restoration are more likely to be successful.

In conclusion, pressures aquatic ecosystems threaten biodiversity in aquatic ecosystems. Meanwhile, I would like to put forward that ultimately conservation and restoration of ecosystems is rooted in BEF studies. Because, only if we understand clearly how biodiversity is linked to ecosystem functioning, is it possible to conserve the intact functioning of - in our case aquatic - ecosystems. Most of the current mechanistic understanding of the effect of

phytoplankton diversity on ecosystem functioning focuses on resource complementarity and selection effects. However, these effects do not necessarily correspond to real biological mechanisms (Loreau & Hector 2001; Venail 2017). Real improvements into the mechanistic understanding of the impact of diversity on ecosystem functioning will rely on the capacity to better depict the nature and strength of species interactions, either negative, positive or neutral (Cardinale, Palmer & Collins 2002). In my thesis, a main result we obtained is that positive interactions play an important role in BEF relationships (Chapter 4, Guan *et. al.*). In the next several paragraphs, I will introduce examples of the incorporation of positive interactions in aquatic conservation and restoration.

## Incorporating positive interactions in aquatic conservation and restoration

Positive interactions, which mean that one species benefits from the presence of another species, have traditionally been classified into mutualism, commensalism and facilitation. Mutualism means both species benefit from the interaction. Commensalism means one species benefits, with no effect on the other species. Facilitation means one species renders the conditions more favorable for another species. Although these interactions are very important for the establishment of natural communities, it is known that the ecological theory on positive interactions has been slow to develop in recent decades, and until now, the theory is not complete and falls behind the theory on negative interactions like competition, predation and parasitism. Consequently, while an outstanding body of work has been developed on the positive interactions in recent years, as yet it is unlikely to cover a broad range of ecosystems and interests.

In this part, we summarize several categories of positive interactions and explain how they can enhance the efficiency of conservation and restoration measures again with a focus on aquatic systems.

The first category I want to discuss is the most studied and widely accepted type of positive interactions, called “traditional interactions” (Table 6-1) (Brady *et al.* 2002). Foundation species, mutualism and commensalism, as well as facilitation all belong to this category. Foundation species - somewhat akin ecosystem engineers or keystone species - are species that define much of the structure of a community by creating locally stable conditions for other

species, and by modulating and stabilizing fundamental ecosystem processes (Ellison *et al.* 2005). They are essential for ecosystem restoration, because they can provide key habitats and food for the ecosystem (Brady *et al.* 2002). In fact, most restoration efforts manipulate traditional interactions, i.e. measures are taken to promote the occurrence of foundation species, so that the rest of the ecosystem will benefit from their key roles in the system. The typical example is the artificial coral reefs, as we have described above. When the target species that are in specific need of protection and conservation require other species for their recruitment, growth or survival, the roles of mutualism and commensalism should be taken into consideration in the ecosystem restoration process. A good example is the mutualism system of clownfish and sea anemones, which occurs on natural and artificial coral reefs. In this system, the clownfish feeds on invertebrates that can be harmful to the sea anemone and provides nutrients to the anemone. At the same time, the sea anemones protect clownfish from predators using stinging cells. Different from foundation species, mutualism, commensalism and facilitation are not all that common, or at least not commonly considered, in aquatic restoration and conservation. The history of studying facilitation began with plant-plant interactions. In some of the earliest research, scientists found that certain individuals demonstrate a better performance when a neighboring species is present. Since then, most facilitation studies have focused on plant-plant interactions as well. More importantly, facilitation was less studied at the community level. The resulting research gaps have caused an absence of facilitation in aquatic conservation and restoration. However, scientists have suggested that the application of facilitation has great potential to improve the quality and efficiency of aquatic conservation and restoration, because facilitation may occur more in aquatic ecosystems (Venail *et al.* 2014).

The second category of positive interactions is that of “within-population interactions”. Allee effects, density-dependent recruitment and reproduction all belong to this category. It is well known that that a high population density may lead to intraspecific competition for limiting resources. On the other hand, it is necessary for populations to have minimum densities to persist and to grow. Allee effects refer to a positive relationship between a component of individual fitness and either numbers or densities of conspecifics. The mechanisms of Allee effects include predator dilution, antipredator vigilance, social thermoregulation, and reduction of inbreeding, genetic drift, or loss of integrity by hybridization (Stephens & Sutherland 1999). Conservation scientists have recognized the significance of Allee effects for a long time, applying it as a common conservation strategy. The prohibition of the hunting of whales for commercial reasons and reduced fishing during spawning time are all examples of the

application of Allee effects in aquatic ecosystems. Another related concept is density-dependent recruitment. It means that conspecifics can create a more attractive or hospitable environment for later arrivals. One classic example for this type of restoration in a marine ecosystem is oyster conservation. That is, oysters themselves – when present in sufficient numbers - can provide structure and increase the later recruitment of the oyster population on the reef. (O'Beirn *et al.* 2000). Oysters themselves create the conditions for a healthy oyster population.

The third category involves “large-scale interactions”. In this category, we should consider broad scale interactions; including factors such as resource subsidies between ecosystems, and protection of neighboring ecosystems. The entire ecosystem on earth functions like a sophisticated machine. Each part of the ecosystem is like a part of such a machine. They have their own independent function. Meanwhile, they link with each other and influence one another. The transporting of resources is one of the most frequent interactions between ecosystems. Species migrations can transport organic nutrients. The nutrients that are released after the decomposition of dead bodies of freshwater organisms, for example, can raise the nutrient level of water in an estuary (Carpenter *et al.* 1998). One successful application of large scale interactions can be seen in the conservation and management of marine fishes and invertebrates by establishing nurseries in estuarine and mangrove ecosystems (Frusher & Hoenig 2001), that is by protecting the biodiversity in mangroves and seagrass beds to improve productivity of fishes and invertebrates. Oceanic salmon are marine animals that have several habitats across different life stages. Migratory species that connect different ecosystems should receive more attention when designing conservation measures. Some species can stabilize community dynamics and the structure of a neighboring ecosystem. For example, riparian ecosystems help to maintain clear water and stabilize stream and river shorelines, thus protecting neighboring marine and freshwater ecosystems (Naiman, Decamps & McClain 2010). Another example is seen when mangroves protect coastal habitats from the impact of storms. That is one reason why maintaining intact mangrove forests is a key target for the conservation of coastal ecosystems.

Understanding the significance of patterns within positive interactions will support conservation in restoration efforts. For example, if we understand the facilitation mechanism between two species, then we can perhaps expand facilitation among other, similarly related species. One possible application of a facilitation can be cordgrass stabilizing and shading the substrate of salt marshes, providing an advantage for both the invertebrate and the algal

communities. When cordgrass stabilizes and shades the substrate, ribbed mussels can colonize the area, which in return encourages invertebrates and algal communities to grow by offering a rock-like substrate for attachment (Acevedo-Trejos *et al.* 2015).

To summarize what we have discussed here, the positive interactions, which have been applied in aquatic conservation, and restoration can be classified to three categories. They are the “traditional interaction”, which is the interaction between different species; the “within-population interactions”, which is the interaction within the same species; and the “large-scale interactions”, which is the interaction between different ecosystems. In my thesis, I focused on the relationship between biodiversity and ecosystem functioning. In other words, among the three categories of interaction discussed above, I focused on interactions between different species, especially facilitation. Understanding how facilitation occurring in freshwater ecosystems can support future aquatic conservation and restoration efforts. For example, if we understand the facilitation mechanism between two species, then we can perhaps expand facilitation among other, similarly related species. In chapter 4, we found that phosphate concentration can influence the facilitation occurring in phytoplankton communities. This result may help us to create the right nutrient conditions to stimulate the occurrence of facilitation. Even though the results from our experiment remain rather theoretical and far from a practical application in field, at least we are offering an idea that may support facilitation as a new tool for aquatic conservation and restoration. The results from my research not only can offer potential support for aquatic conservation, but can also be applied in water treatment. I will introduce the possible application of my results in water treatment below.

<b>Category</b>	<b>Ecological concept</b>	<b>When important for restoration and conservation</b>	<b>applied successful examples</b>
Traditional interactions	Foundation species	Restoring species dependent on some foundation species	Artificial coral reef
	Mutualism and commensalism	Managing target species that require other species for recruitment, growth or survival	Clownfish and sea anemone in Artificial coral reef
	Facilitation	Species dependent on biotic or abiotic conditions to recruit	Less common
Within-population interactions	Allee effects	Target species have small population size	Prohibition of hunting whales
	Density-dependent recruitment and reproduction	Target species are recruitment limited	Oyster restoration in reef
Large-scale interactions	Resource subsidized between ecosystems	Choosing location for mitigation sites	Nursery marine fishes and vertebrate in estuarine and mangrove ecosystem
	Ontogenetic habitat shifts	Target species use multiple habitats during life history	Salmon conservation
	Protection of neighboring ecosystems	Managing ecosystem sensitive to external abiotic factors	Maintaining mangroves for coastal ecosystem

**Table 6-1** Positive interactions and their implication for restorations and conservations



## Possible application of our research results in wastewater treatment

Water treatment, as an important effort we make to reduce the impact on natural ecosystems, has been considered more and more important in sustainable development. The results obtained from my research can be communicated to the wastewater industry, where they could be used to help improve emergent technologies such as the use of algal photobioreactors in wastewater treatment. In an algal photobioreactor microalgae are grown on wastewater, and the microalgae can absorb useful substances, such as phosphate and nitrogen from the waste streams. At the same time, these microalgae also can take up CO<sub>2</sub>, which is the main reason for the greenhouse effect and produce oxygen. In general, only certain species of microalgae are chosen in the bioreactor, such as from the *Chlorella*-genus.

In wastewater treatment there are several key steps or processes, such as the break-down of organic matter or the removal of phosphate and nitrate. . In fact, these processes can all be regarded as a type of ecosystem functioning. It is widely accepted that biodiversity can improve ecosystem functioning in general (Cardinale 2011; Cardinale *et al.* 2013). In addition, it is also reported that biodiversity increases the productivity and stability of phytoplankton communities (Corcoran & Boeing 2012). Thus, using a diverse community of microalgae in wastewater treatment would seem to be a better choice than the use of only one single species. The efficiency of e.g. the uptake of nutrients from the waste is predicted to go up in a more diverse photobioreactor. The application of microalgal biodiversity for wastewater treatment makes the purification process more sustainable and enables a better quality of water. At the same time, valuable microalgae biomass is created, allowing for recycling of the nutrients therein and re-usage such as in cattle fodder and biofuel production. We provide the wastewater industry with new, relevant information. For example, in green algal communities, the average biovolume is a good predictor for phosphate uptake, and the average surface area-to-volume ratio of the algae has the strongest link to nitrate uptake. When considering several factors like biomass production, nitrate uptake, phosphate uptake, and light absorption, the best predictor for multifunctionality is the average surface-to-volume ratios of the algal community in the reactor. This information can help choose the proper species to compose microalgae communities in the reactor.

There are three main problems with wastewater: heavy metals, nutrients (nitrogen and phosphorus) and antibiotics (Chen 2004). Nutrients and heavy metals are the most common

problem in freshwater ecosystems (Fu & Wang 2011). Based on the results of our research and publications from other scientists, I designed a simple bioreactor for wastewater treatment. The bioreactor can have one or several chambers, depending on the purpose of treatment. Here we give an example of two of the main purposes, i.e., reducing heavy metal and nutrient loads in wastewater. Because of the two main application goals, the bioreactor in this case has two chambers, one for dealing with the heavy metals, and one for diminishing the nutrients. It is reported that *Chlorella* has a high efficiency in handling heavy metals in contaminated water (Muñoz et al. 2006). Therefore, in the first chamber, the *Chlorella* system, which is already applied in some wastewater treatment plants will be operated (Khosmanesh, Lawson & Prince 1996). The wastewater that needs to be treated flows into the first chamber, and the *Chlorella* system works until the concentration of heavy metal drops to the target level. Then the water flows to the second chamber. Even though some nutrient have been absorbed by microalgae in first chamber, it may be need to be reduced further to meet regulatory standards Thus the role of the second chamber is to further reduce the nutrient content in the wastewater – after uptake by *Chlorella* in the first chamber. There can be different requirements such as nitrogen removal only, phosphorus removal only, or removal of both. Algae communities of diverse species serve as a functional unit. Different combinations of algae species are selected for different purposes. For example, if the main goal is to remove high concentrations of nitrogen, our study shows that algae communities of *Stichococcus bacillaris* and *Coccomyxa vicidis* can take up most nitrogen in the first two weeks (Chapter 4, Guan *et al.*,.). Therefore we could advise to include these two species in our second reactor. But if we need to absorb both nitrogen and phosphorus, according to our results, it is a better option to choose algae communities with a higher average surface to volume ratio, which tend to be the case for the smaller species. In this way, using the best design and choosing the appropriate species, nutrients can be removed at a high constant rate until the concentration of nutrients reaches the required levels. Finally, clean(er) water flows out of our bioreactor. Another possibility is combining both heavy metals removing algae and nutrient reducing algae systems together, letting them function together in a single big chamber. Although we did not do any experiments considering heavy metal removal as a key ecosystem function of algae communities, we believe that our results predict that multifunctionality from morphological traits can be implemented to improve algae wastewater treatment systems.

The obtained nutrient-rich algal biomass can offer various further services. They can be used as source of bioenergy (Fargione et al. 2008). The algae that have not been used to remove

heavy metals, and are therefore of good quality, could be used to feed fish and livestock or serve as a natural fertilizer.

## Possible application of our research results in the production of algae biomass and their products

Algae contain a wide variety of high-quality nutritional compounds, such as peptides, lipids, carbohydrates, vitamins, trace elements, minerals and pigments. With the deepening of algae research and growing maturity of algae biotechnology, more and more people have begun to realize the commercial value of algae. This leads to the establishment and development of industries with algae as the core product. For example, companies such as Algaetech international from Malaysia and Algatechology from Isreal have entered the field of algae production and marketing.

There are three main types of commercial exploitation of algae. The first involves using algae as biofuel. Scientists have developed biofuels by combining species of algae (Mata, Martins & Caetano 2010). Compared to other energy resources, algae biofuel is sustainable and environmentally friendly, as well as cheaper to harvest (Mata, Martins & Caetano 2010). A second application is algae as animal food. Algae contain an ideal combination of nutrients for animals, such as ducks, chicken and pigs, that feed on them. Algae can provide higher nutrient quality than conventional animal food (Borowitzka 1999). More importantly, the current costs of conventional animal fodder - like soja - are low because of the substantial subsidies received by the crop industry. In other words, if those subsidies were removed, algae biofood would be the best choice to replace conventional animal food (Borowitzka 1999; Mulbry *et al.* 2005). The last and most popular commercial range of products obtained from algae are nutraceuticals for humans. The large amount of micronutrients, carotenoids and polyunsaturated fatty acids in algae cells are responsible for the popularity of nutraceuticals. These compounds are essential for human dietetics and therapeutics (Spolaore *et al.* 2006; Christenson & Sims 2011). Because consumers prefer popular compounds such as eicosatetraenoic, docosahexaenoic, arachidonic, astaxanthin, lutein, beta carotene, chlorophyll, phycobiliprotein, and beta-1,3-glucan, algae that contain large amounts of these compounds have become the primary species for industry production, such as *Clorella* and *Dunaliella salina* (Pulz & Gross 2004).

It is believed that algae bioproducts in future could be in even greater demand and the market may expand quickly as production costs come down. The main issue limiting commercial use of algae is how to produce more and higher quality biomass at lower costs. Based on our research results, we provide an innovative idea for the algae producing industry based on mixed cultures rather than the commonly used single species cultures.

First, mixed cultures could produce more compounds within a similar timeframe. As we described above, one crucial algae-derived commercial product serves as a nutritional supplement. Frequently, a variety of micronutrients and vitamins are contained in one pill. However, it is impossible to obtain all of these compounds from one algae species. Different algae can supply different nutrient compounds. Culturing different species of algae is the direct method to collect the different required compounds from a single reactor. The general concern about mixed cultures is that competition could negatively affect the productivity of algae growing, or even cause the loss of some species from the community (Foster & Bell 2012). However, our results show that positive interactions among species are more common than people think (Chapter 4, Guan *et. al.*). More importantly, as long as the right species are chosen to be cultured together, it is easy to procure positive interactions. In that way, diversity of compounds can be achieved at the same time by the appropriate mix culture.

Additionally, even for a single compound, mixed cultures have the possibility to produce higher yields with the same nutrient investment. We take astaxanthin as an example. Astaxanthin derived from *Haematococcus pluvialis*, is known as one of the most potent antioxidants in nature (Wu *et al.* 2014). Many companies such as Algatechnologies, market this product for food and beverages and cosmetics industries. The traditional way to obtain astaxanthin is to culture *Haematococcus pluvialis* alone in an algae farm, then extract astaxanthin. *H. pluvialis* is one of the algae species we applied in our experiments. By monoculturing *H. pluvialis* we notice that a high amount of astaxanthin is present in the resting cells, which are produced and rapidly accumulate when environmental conditions become unfavorable for normal cell growth. That means that it is hard to achieve a large amount of astaxanthin and keep biomass growing at the same time. If the culture conditions are good for algae growth and production of biomass, *H. pluvialis* will not accumulate astaxanthin. On the contrary, if the culture conditions are harsh for algae, although astaxanthin will be produced, the biomass may not be large, thus still resulting in a low amount of astaxanthin. Bright light, high salinity and low availability of nutrients cannot solve this problem (Limin, Quanling & Hailong 2008). However, in our

experiment, after we mixed *H.pluvialis* with other algae species, we found that a positive diversity effect happened, showing *H.pluvialis* to produce more biomass in mixed cultures than in monocultures, even under the poor nutrient conditions that could potentially trigger astaxanthin production. Even though we did not test the concentration of astaxanthin, the results imply that mixed cultures of *H.pluvialis* have a higher potential for astaxanthin production than monocultures, even under limiting growth conditions.

Mixed cultures can also have other benefits, such as a more stable culture system, a more sustainable culture, and reduced labor costs. Of course, achieving the desired effect requires a careful selection of algae species. More in-depth experiments are needed. Our research has just laid the foundation and merely points out the direction for further studies.

Concluding, the observations from my thesis – and others before me – that ecosystem functions like biomass production benefit from a diverse algal community and the positive interactions between members of this community, is not just of academic interest, but may well be worthy of further study for fields as wide apart as aquatic ecosystem conservation, waste water treatment and algal biotechnology.

# CHAPTER 7

## General Discussion and Conclusion

### BEF mechanisms

In the present alarming biodiversity context, BEF studies provide unique relevant information on the consequences of diversity loss (Chapin *et al.* 1998). The statement that less diverse ecosystems are generally less productive and less stable than ecosystems that are more diverse is now well acknowledged (Ellison *et al.* 2005; Maestre *et al.* 2012). Much of the value and significance of this message has been limited by the large amount of unexplained variance in ecosystem functioning, the lack of a convincing biological mechanistic rationale and the frequent consideration of single functions at a time (Cardinale *et al.* 2006; Byrnes *et al.* 2014). Such issues undermine our understanding of the role of biodiversity as a driver of ecological processes and impede BEF findings to be as relevant and informative for biodiversity conservation as they should be (Myers *et al.* 2000). The simplicity of our experimental setup, in which we directly manipulated community trait structure and combined trait information together with multiple functions, allowed addressing some of those issues. Our results show clear effects of species interactions on biovolume production, without affecting nutrients, indicating that species interactions may have an effect on one ecosystem function, but not on others (Chapter 3, Guan *et al.*). This at least brings with it the suggestion that the biological mechanisms by which diversity influences ecosystem functions are not universal and depend on the function being considered. Moreover, our results show that community trait structure had a strong influence on ecosystem functioning. By testing the impact of diversity based on one single trait on multifunctionality in phytoplankton, we found that certain traits can indeed be good predictors for certain types of ecosystem functioning as well as for multifunctionality (Chapter 3, Guan *et al.*). This may suggest that the maintenance of some ecosystem functions relies on the presence of a particular trait, rather than on traditional measures such as species diversity. In addition to the importance of exploring multiple functions and their possible positive or negative correlations, our findings advocate for incorporating multiple traits and exploring their independent effects on ecosystem functioning. Interest in multifunctionality in BEF studies is mounting. Our results offer a cautionary tale that even when traits predict

multifunctionality, a proper mechanistic interpretation is only possible when information on individual functions is available. We also provide clear evidence that different traits can influence different ecosystem functions and that different functions can be dependent on different traits, which also has direct implications for diversity conservation. Concluding, to preserve and insure multiple ecosystem functions, including a variety of traits (diversity among traits) might be more important than including a variety in each trait (diversity within traits).

As mentioned in Chapter 2, the mechanistic understanding of the effect of biodiversity on ecosystem functioning relies predominately on disputed claims regarding the effects colloquially known as “complementarity and selection effects” (Loreau & Hector 2001, Cardinale *et al.* 2011), which are generally studied using a variety of ad hoc statistical tests. While informative, these effects do not necessarily correspond to real biological mechanisms. Instead, significant advances regarding the mechanistic understanding of the impact of biodiversity on ecosystem functioning rely on the capacity to better depict the nature and strength of species interactions. These interactions, which can be, negative (i.e. competition), positive, (i.e. facilitation) or neutral (i.e. resource partitioning), largely determine the relevant functional traits and how they influence ecosystem functioning. Recently, some efforts have been made to establish a framework that combines resource partitioning, biotic feedback, and abiotic facilitation as interrelated explanations of mechanistic functioning.

In order to develop an understanding of the mechanism involved in the relationship between biodiversity and ecosystem functioning, we first need to clearly distinguish between potential mechanisms. We can identify several: Resource partitioning occurs when species use different portions of the available resource pool. The results of resource partitioning is that the existing resource pool is more completely used in higher-diversity communities compared with monocultures. (Finke & Snyder 2008). Abiotic facilitation occurs when an increase in the abundance of one species increases the relative performance of a different species via changes to the abiotic environment. Biotic feedbacks are narrowly defined as the amplifying (positive feedbacks) or dampening (negative feedbacks) effects on the performance of a species or community caused by another trophic level in response to changes in community diversity (Barry *et al.* 2019). The most important source of distinction between resource partition, abiotic facilitation, and biotic feedbacks is whether a single organism performs better when biodiversity increases. For example, if a single organism performs the same despite an increase in biodiversity; it suggests that the mechanism in play is more likely to be resource partition

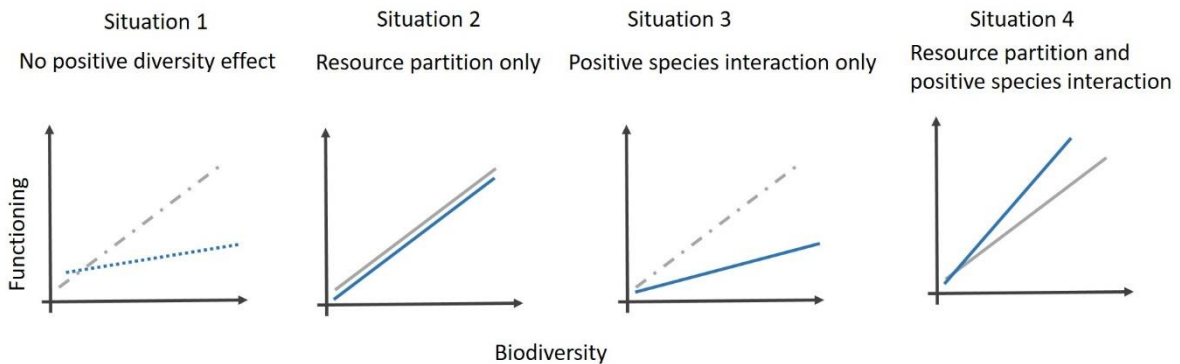
than biotic feedbacks or abiotic facilitation. If a community with higher biodiversity performs better because of the species in the community performing better than they are in monoculture. Then this suggests that abiotic facilitation or biotic feedbacks are more likely to be the real mechanism of positive diversity effect. Hence, by carefully studying the effects of abiotic facilitation and biotic feedbacks – which will both increase the performance of single organisms – on communities, one may be able to discern the roles played by different mechanisms.

In a natural environment, both the interaction between organisms and abiotic components of the environment (such as soil, water, and nutrients) and the biological interactions between different species or within the same species occur simultaneously and affect one another. Such simultaneity is impossible to avoid even under the controlled conditions of a laboratory study. This makes it difficult to explain the BEF relations using only one effect. Therefore, considering multiple mechanisms at the same time is the only way we can accurately explore the nature of BEF relations.

Last year, the Barry Group at the University of Leipzig in Germany, proposed that resource partitioning and biotic facilitation might be present at the same time (Barry *et al.* 2019). Inspired by the Barry *et al.* (2019) framework, we decided to define a positive species interaction framework that merges the effects of biotic feedback and abiotic facilitation. Moreover, we take biodiversity as the x-axis and ecosystem functioning as the y-axis, using linear relations to depict a simplified BEF relation. It is worth noting that BEF relations can exist in a variety of forms depending on the function measured. We chose to use a linear representation because it is the easiest to understand. However, if a different effect were to occur, the BEF relation could be drawn in different ways (Fig 7-1). For example, if only resource partitioning occurred, then the ecosystem functioning would be increased, but the functioning of each species would remain the same. In other words, the average of the functioning of the species would be the same in mixed cultures and monocultures, and the BEF relations could be predicted by the addition of each species. Conversely, both biotic feedback and abiotic facilitation can improve the performance of specific species, which results in a higher average functioning for all species. Therefore, if resource partitioning and species interactions occurred simultaneously, then the average functioning of the species' increases, but cannot be explained or predicted by any of the single effects. In order to explain my point of view more clearly, specific examples are provided below (Fig 7-1).



We use the simplest model, as it involves only two species and uses biomass production to represent ecosystem functioning, while species richness represents the biodiversity. Moreover, we assume that BEF relations are linear. Four possible situations can be drawn based upon the BEF relations. The expected levels of functioning are estimations for the mixed-culture based on the observed functioning of the species in the monoculture (grey line). In situation 1, as biodiversity increases, functioning of mixed-culture does not increase significantly (blue dashed line). This means that there is no positive diversity effect, so neither resource partition nor positive species interactions occur. In situation 2, functioning of mixed-culture increases as biodiversity increases, meaning that a positive diversity effect occurs (blue solid line), and that this positive diversity effect can be fully predicted by expected levels of functioning in the monoculture (grey solid line). In another words, resource partition is the only cause of this positive effect. In situation 3, a positive diversity effect exists (blue solid line), but the positive pattern cannot be explained by the expected functioning whatsoever (grey dashed line). This means that some sort of positive species interaction occurs, but that resource partitioning did not exist. In situation 4, a positive diversity effect exists, and positive diversity effect can be partially predicted by the expected level of functioning (grey solid line and blue solid line). This means that resource partition occurs and is one of the reasons for the positive effect. Simultaneously, the positive species interaction has superimposed on it, resulting in an even more powerful positive effect (blue solid line).



*Figure 7-1: Four possible situations of the biodiversity and ecosystem functioning relations drawn, comparing the expected and observed levels of functioning in mixed-culture. X-axis is one metrics of biodiversity, y-axis is certain ecosystem functioning. Grey line is the expected levels of functioning that are estimation for mixed-culture based on monoculture. Blue line is the observed levels of functioning that should be directly measured. Solid lines represent linear significant effects of the variable related to biodiversity on ecosystem functioning whereas dashed line represent non-significant relations.*

This model is the basic model for the four hypothetical scenarios discussed in Chapter 3. Considering cell-size is a key metric of biodiversity in phytoplankton communities, we adjusted the model to fit our experimental data analysis. In chapter 3, we explored the influence of species interactions on bi-culture's functioning along cell-size gradients by comparing the effects of the variables related to cell size on the observed levels of functioning against their effects on the expected levels of functioning based on the constituent monocultures. In addition, we found that that some changes in the nature and/or strength of species interactions counteracted the differences in the functioning capabilities of the species present in the assemblages along the cell-size related gradient. In other words, we found that the species interactions play a role in BEF relations in our experimental phytoplankton communities. To testify what kind of species interaction are occurring in our phytoplankton communities, we designed our second experiment, which is presented in chapter 4. Moreover, the results of chapter 4 proved that facilitation was occurring in our phytoplankton communities. This proves that the direction of our efforts is correct and our idea is feasible. Of course, the models also has its own shortcomings, including the risk of having oversimplified the issue. As a result, we

will continue to optimize the models through continued dedication to innovation and the exploration of the BEF mechanism.

## Ecosystem functioning good or bad?

The study of biodiversity and ecosystem functioning allows us to better understand how the ecosystem works, and how to better benefit from services provided by ecosystems. Given that ecosystem functions are ecological processes that control the fluxes of energy, nutrients and organic matter through an environment, the concept of ecosystem functioning is both sizeable and abstractive at the same time. Not only does it include primary production, which is the process by which plants use sunlight to convert inorganic matter into new biological issue; but it also involves nutrient cycling, which is the process by which biological essential nutrients are captured, released and then recaptured and so on . In my opinion, all ecological processes that happened in environment can be considered as ecosystem functioning.

Throughout my years of research on BEF relations, the broader public, who lack appropriate ecological knowledge, have approached me with questions as to whether this ecosystem functioning is good or bad? Additionally, because the most widely studied aspect of ecosystem functioning is biomass production; I frequently got questions as to whether increased biomass production is beneficial. In my opinion, these questions themselves are inaccurate and unanswerable.

To determine whether a certain aspect of ecosystem functioning is good or bad is a philosophical question rather than a scientific question. From my own point of view, all ecosystem functions are neutral. They are neither good nor bad, but rather, they simply exist. Imagine an ocean and a forest: we cannot say that an ocean is good or that a forest is bad. They both bring benefits to humans, such as providing food in the form of fish from the sea or fruit from the forest. Meanwhile, they both also have the potential to threaten the safety of human life, as floods from the sea and bush fires both contribute to human fatalities. Overall, we cannot define either an ocean or a forest as entirely good or bad entities, but instead simply as things that exist in a largely neutral state with regards to humans. Ecosystem functioning should be considered in a similar manner, as it is both impossible and unnecessary to define ecosystem functioning as good or bad. However, they matter greatly and we depend on them. From this point of view, they are good.

We can adjust ecosystem functioning to better meet our needs. This concept is referred to as “ecosystem services”. Ecosystem services are the suite of benefits that ecosystems provide to humanity, including the two primary types of services, provisioning and regulating. Provisioning services involve the production of renewable resources such as food, timber, fresh water. Regulating services are those that lessen the impact of environmental change such as climate regulation, pest or disease control . If we define “good” as a benefit to humans, then in some way, ecosystem services are the “good” aspects of ecosystem functions. However, even for ecosystem services, it is not true that more is always better. . For example, regarding the food provisioning in agriculture, if one were to solely consider current production and force the land to produced as much food as possible, it may result in increasingly barren land and crop production that decreases year by year. As a result of these sorts of dilemmas, more and more scientists have recently begun to think about the issue of sustainable development.

Looking back the history of BEF research, we know that the ecosystem functioning studies began in the 1990s and focused on terrestrial ecosystems. At that time, biomass production was at the core of ecosystems and served as an almost unique ecosystem functioning. With the subsequent development of BEF research, research a more comprehensive system has gradually formed. As discussed in Chapter 2, we believe that failing to consider ecosystem functions beyond productivity is a limiting factor of BEF studies in recent years. This is why we applied a multifunctionality analysis to our experiment in Chapter 3.

The intricate connections within ecosystems have caused difficulties not only for scientists involved in scientific research, but also for the public in understanding the importance of such research. However, I endeavor to spread the ecological point of view and explain our research results to public.

## Conclusion

The accelerated loss of biodiversity observed during the last few decades has generated great interest in understanding the consequences of its decline and assessing the associated risks for human societies(Hooper *et al.* 2005). Much of what is currently known about the consequences of diversity erosion comes from hundreds of studies on the relationship between biodiversity and ecosystem functioning. In most cases, the results show that increased biodiversity promotes better functioning of an ecosystem.

At the same time, population growth and human activities such as industrialization and agriculture are heavily disrupting freshwater ecosystems by polluting the water and degrading habitats, leading to a concomitant reduction of the biodiversity. Such alteration in the structure and diversity of ecological communities may have a strong effect on the functioning of the freshwater system as a whole. Despite the serious situation facing the freshwater ecosystems, limited studies have focused on the BEF relations of freshwater ecosystems. Even fewer have focused on phytoplankton diversity and basic functions such as nutrient uptake and its impact on biomass production. Overall, the main goal of my PhD research has been to explore the mechanisms influencing how the diversity of phytoplankton impacts ecosystem functioning in freshwater ecosystems.

Firstly, by reviewing all the meta-analysis in BEF studies, we found that the BEF data available for freshwater ecosystems is far less than that for terrestrial ecosystems, and that the use of a mechanistic explanation is even rarer. To correct this disparity, we collected data from BEF studies in freshwater ecosystems, after having reviewed the existing literature linking phytoplankton diversity to ecosystem functioning in freshwater lakes and ponds. Then, we summarized the published pattern of BEF studies in freshwater ecosystems. Both lab experiments and field studies mainly used species richness as the diversity metric and productivity as the measure of ecosystem functioning. Additionally, the pre-existing BEF data concerning freshwater ecosystems failed to sufficiently explain the real mechanism behind biodiversity effect. Furthermore, we addressed the research gaps by 1) utilizing functional related diversity metrics, which may be a better predictor than species richness for ecosystem functioning; and 2) exemplifying the ways in which BEF relations shown in lab experiments are different from those shown in the field. Based on the research gaps and limited data available, the prospect topics we derived for BEF studies were functionally related diversity metrics, multifunctionality and positive species interactions. As a result, we designed experiments based on these topics.

After recognizing the research gaps of the BEF studies in freshwater ecosystems, we decided to perform an experiment to study the relationship between traits related to cell size and ecosystem functioning, as cell size is the master trait in phytoplankton. We manipulated the composition of forty bi-cultures of freshwater green algae, hence generating gradients in four different cell-size related variables, but keeping species richness constant. We cultured the algae for twelve days under controlled laboratory conditions and measured the impacts of each of the

variables on nitrogen uptake, phosphate uptake, light attenuation and biomass production. We compared the observed levels of functioning of the bi-cultures with estimated levels based on the constitutive species as monocultures. By comparing the observed versus expected levels of each function along the cell-size related gradients, we established the relative contribution of species interactions to ecosystem functioning. We found that the three functions related to resource uptake benefited from the presence of smaller species with larger surface area to volume ratios. Our analyses revealed that these effects were mainly driven by smaller species having higher functioning levels, whereas interactions among species had little impact on these three functions. As an exception, biomass production did relate neither to cell size, nor to the production levels of the component species, suggesting that interspecific interactions strongly influenced this function. Changes in the cell size composition of phytoplankton assemblages can directly affect their functioning levels. The results from this controlled experiment suggest that in the current environmental context, with smaller taxa increasingly dominating phytoplankton (Huertas *et al.* 2011), this tendency might ensure higher levels of ecosystem functioning via an improvement in resource acquisition that avoids affecting biomass production resulting from a shift towards smaller taxa.

Through the first experiments, we noticed that positive interaction play an important role in BEF relations in phytoplankton communities. Thus, we designed our second experiment, with the aims to understand the detailed characterization of positive species interactions among phytoplankton species, and explore the conditions required for such positive interactions to emerge. We combined two green algae species equally together, and cultured them under different phosphate concentrations. Then we calculated the contribution of each species to community biovolume production, compared with monoculture, we found positive diversity effects occurred because of facilitation between algae species. Moreover, initial phosphate concentration can influence to what extend facilitation occurs by changing the frequency of facilitation rather than the strength of facilitation.

From the review results in chapter 2, we found that tropical high-altitude lakes represent a research gap in freshwater ecosystems studies. Thus, we did a field study on tropical high-altitude lakes in South America. We found that a combination of four abiotic factors explained over three quarters of the variation in chlorophyll-a concentration amongst lakes. Contrary to what studies from temperate regions suggest, taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, Shannon's diversity index was

negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume, presumably due to a strong compositional effect. Our results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activities can indirectly affect their functioning and their capacity to provide vital ecosystem services.

My PhD research has involved exploring the mechanisms of how biodiversity determines ecosystem functioning in freshwater ecosystems. Moreover, we put forward that the results of our studies can be used in both wastewater treatment and algal biotechnology. BEF relations are a classic and meaningful topic in ecology. It is important to explore the underlying mechanisms of BEF relationships, as well as to promote the importance of the ecosystem functioning and BEF relations. We began with an exploration of the current state of BEF studies in freshwater ecosystems. After that, we designed basic experiments to help fill some of the identified research gaps. Then we used the information we have obtained from our experiments to propose new hypotheses and conduct more in-depth understanding of underlying mechanisms. Our studies are a good example of systematic research on mechanisms that are less studied in biodiversity and ecosystem functioning.

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