The meaning of phytoplankton diversity within lake ecosystems

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften Dr. rer. nat. an der Fakultät für Biologie der Ludwig-Maximilians-Universität München

von

Sara Katharina Hammerstein

zur Beurteilung vorgelegt am 16.07.2019

1. Gutachter:	Prof. Dr. Herwig Stibor
2. Gutachterin:	Prof. Dr. Silke Werth
Tag der Abgabe:	16.07.2019
Tag der mündlichen Prüfung:	07.11.2019

Table of contents

Summary

Biodiversity loss is one of several anthropogenically driven factors severely influencing the functioning of the earth's ecosystems and with that also human wellbeing. Fisheries, agriculture, water usage and also tourism rely on ecosystem functioning in a way beneficial to mankind. Primary producer communities stand at the basis of terrestrial and aquatic ecosystems. Their contribution to ecosystem functioning is therefore increasingly focus of research in aquatic and terrestrial ecology. Phytoplankton communities - although only representing a small portion of the world's photosynthetic biomass - are responsible for around half of the carbon fixation by primary producers worldwide and thus drivers of global carbon and nutrient fluxes. Accordingly, investigating the mechanisms linking biodiversity and ecosystem functioning in aquatic habitats with phytoplankton as primary producers is of general importance.

Hence, my aim was to design experimental ways of manipulating natural phytoplankton communities in their diversity. As such studies were previously rare, my results should enable performing experimental studies close to realistic natural conditions to investigate mechanisms driving ecosystem functioning in aquatic habitats. Furthermore it was my aim to test whether such manipulated diversity gradients mimic naturally evolved diversity differences between water bodies in their responses to an additional stressor such as for example eutrophication.

Biodiversity loss is more and more seen in the context of functional diversity. Rather than only by species identity, organisms are categorized by traits that are decisive for ecosystem functioning, like for example pigment composition, size or temperature tolerance. To gain insight into the mechanisms linking functional diversity to ecosystem processes, I here present an example of such a trait-based approach. Studying the loss of diatoms – a phytoplankton group prone to environmental change - from the phytoplankton community, I analyzed it's consequences for light usage of the primary producers and corresponding ecosystem processes within lakes.

The assessment of functional phytoplankton community composition is crucial for basic research as described here as well as for environmental monitoring. Within the framework of my field study, I tested the applicability of two fast methods of assessing phytoplankton communities based on their pigment composition.

To tackle the described goals, I conducted small scale laboratory experiments as well as large scale mesocosm field studies in three temperate lakes. My results point out the meaning of diversity for the stable functioning of phytoplankton communities. I could show differences between naturally evolved diversity differences and short-term experimental manipulations of phytoplankton diversity. Additionally, I could connect the decrease of a functional group in the phytoplankton community to a decline of a characteristic photosynthetic pigment and subsequent changes in the community's light usage.

These results show the importance of an understanding of the mechanistic background of consequences of diversity loss. Considering the vital importance of marine primary producer communities for the world's carbon and nutrient cycles, I propose experiments to test whether results from my freshwater studies are also relevant for marine pelagic systems.

1. Introduction

1.1 Biodiversity

“”Biological diversity” means the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.” This very broad definition of biodiversity from the convention on biological diversity (CBD - UNEP) in 1992 covers genetic diversity within species, taxonomic diversity, ecosystem diversity and also functional diversity. So whether talk is about the several different species of Darwin’s finches on the Galápagos Islands, about different ecosystems ranging from deserts to rainforests or about the variety of functional groups within phytoplankton communities, all are encompassed in the term biodiversity. Although often the term biodiversity is used as a synonym for species richness, the above given definition shows that the meaning of biodiversity rather exceeds sole species numbers. After some controversy on the definition, scientists developed a more integrated point of view including traits influencing community performance (e.g. Naeem and Wright 2003, Mouillot et al. 2005, Naeem et al. 2012, Hillebrand et al. 2017) and paved the way for investigations on biodiversity as central point in ecological research. During the Millenium Ecosystem Assessment (MA, initiated in 2001 by the United Nations) a whole section was dedicated to biodiversity and its meaning for ecosystem functioning and human wellbeing, showing the importance of that topic in current debate in science as well as politics.

1.1.1 Biodiversity loss

Biodiversity on earth is facing a rapid decline. Concern is rising since it was noticed that species extinction rates within the last decades and even centuries are way higher than the background rates (estimated from rates between the five previous mass extinctions on earth; Ricciardi and Rasmussen 1999, Chapin et al. 2000, Sala et al. 2000, Barnosky et al. 2011). Ceballos et al. (2015) argued that even with very conservative calculation methods, the rates of species extinctions across different groups of vertebrates clearly indicate higher loss than compared to background rates and thereby are suggestive for a current sixth mass extinction event. Assessment of a loss of microbial species is somewhat more complicated due to their

microscopic nature and potential dispersion; nevertheless, concern of potential losses from the microbial realm is discussed. Microbial species for example largely influence the world's carbon and nutrient cycles and are also decisive for food web structures across all ecosystems, resulting in far-reaching consequences of such losses (Caviccioli et al. 2019). As microbial diversity is crucial for ecosystem functioning, more research on that topic is desirable (Bell et al. 2005, Weinbauer and Rassoulzadegan 2007).

Biodiversity loss can have several different reasons. First of all, it also is a natural process as part of evolution where species are forming new and others are disappearing again. But this process presently is outshone by the directly or indirectly human induced losses of diversity from different systems (Rockström et al. 2009). Diversity can be directly affected by intensifying agriculture and forestry (monocultures, use of fertilizers, etc.) or by overexploitation of environmental goods (Matson et al. 1997, Chapin et al. 2000, Tilman et al. 2001). Excessive fishing or hunting of certain species can lead to severe reductions in a population or even extinctions and with that trigger further changes in the systems they were part of. Another factor putting native species communities under pressure are non-indigenous species introduced into new habitats by humans and competing with the resident species pool (Vitousek et al. 1996, Shochat et al. 2010, Galiana et al. 2014). Not only do direct community changes as described above influence biodiversity. Also the environmental conditions do play a large role in maintaining diversity in a certain area. As all organisms have certain demands to their environments they can grow and develop under suitable conditions but get stressed or cannot exist when environmental conditions in a habitat do not match their demands. Habitat change, fragmentation or even destruction by human activities therefore is assumed to be one of the biggest threats to biodiversity (Ehrlich 1988, Brooks et al. 2002, Ceballos and Ehrlich 2002, Hanski 2011).

To assess human impact on environmental change and find a "safe" region for this change within to act, Rockström et al. (2009) defined planetary boundaries for nine processes or subsystems of the Earth. The processes were named climate change, ocean acidification, biogeochemical cycles of phosphorus and nitrogen, freshwater use, land use change, biodiversity loss, atmospheric aerosol loading, stratospheric ozone depletion and chemical

pollution. The authors tried to find certain threshold values (boundaries) within which the processes should stay to avoid "unacceptable environmental change" with regard to human wellbeing and evaluated the actual condition of the processes. Amongst climate change and interference with the nitrogen cycle, biodiversity loss was one of the processes already rated to be way above the safe operating space assigned to it.

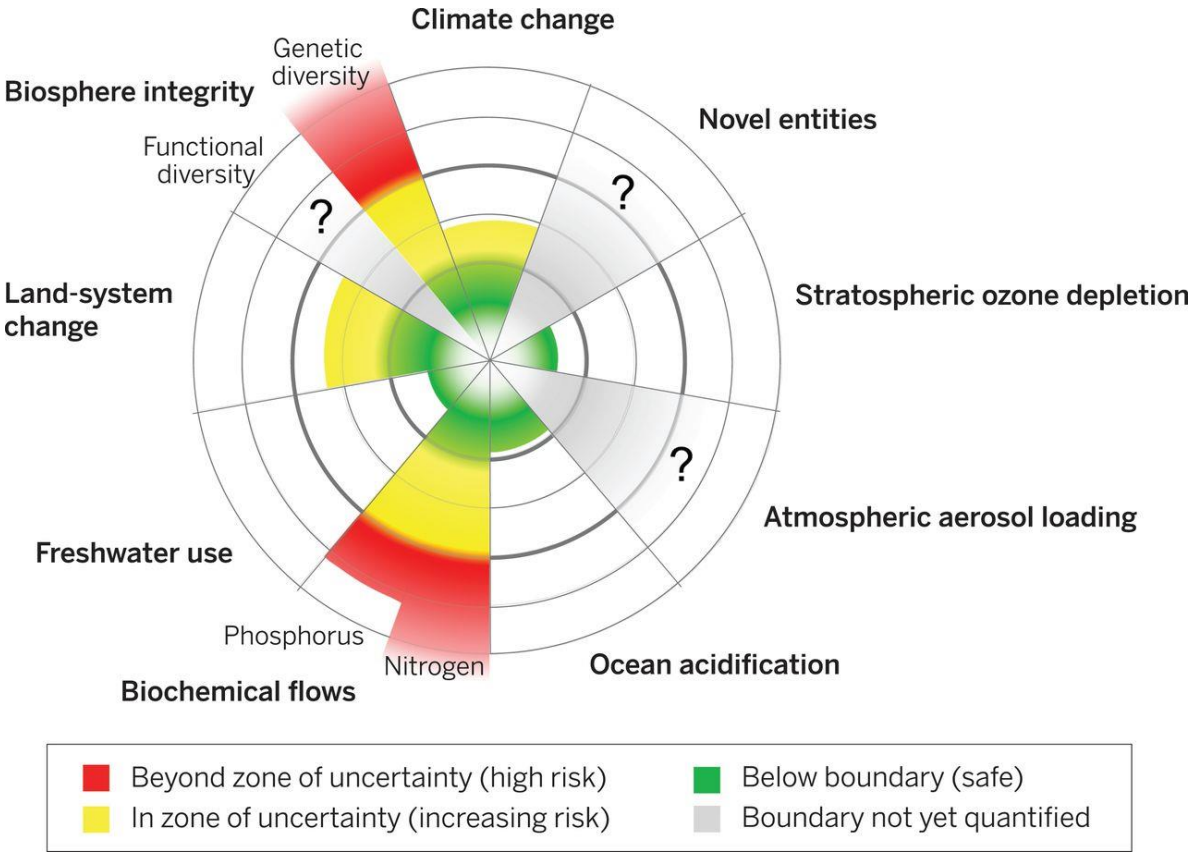

Figure 1: Control variables for nine processes important for the functioning of the Earth System and their respective planetary boundaries for a safe operating space for humanity. Green markings show the boundaries that are considered to be safe, yellow areas depict zones of increasing risk while the red marked processes are considered to be high risk. Grey areas show variables where boundaries have not yet been quantified. Within biosphere integrity – one of the core boundaries, genetic diversity was assessed as in an area of high risk and functional diversity could not yet be estimated (figure see Steffen et al. 2015).

In 2015 Steffen et al. revised and updated this work (see Fig. 1). They even identified two core boundaries which individually would have the potential to change the whole Earth System state when being exceeded: climate change and biosphere integrity. Biosphere integrity includes genetic as well as functional diversity. Genetic diversity was already outside the suggested safe boundary while functional diversity was marked with a question mark as a

global-level boundary could not yet be quantified. All of the boundaries mentioned were examined as single processes, yet, it is also pointed out, that they all act together as an integrated system on a global level. Scientists worry about the general biodiversity loss as it is assumed to have great impact on ecosystem functioning and with that on human wellbeing (Díaz et al. 2006, Rockström et al. 2009, Loreau 2010; Naeem et al. 2012). For this reason biodiversity ecosystem functioning (BEF) became a hot topic in ecological research.

1.1.2 Biodiversity and ecosystem functioning

Not only do human induced changes of ecosystems lead to a loss of biodiversity, but in return a diversity loss can result in changes in the functioning of ecosystems and the allocation of ecosystem services crucial to human wellbeing (Loreau et al. 2001, Balvanera et al. 2006, Díaz et al. 2006, Worm et al. 2006, Hautier et al. 2018, Lindegren et al. 2018). Biodiversity could therefore – via ecosystem functions – have feedback on its own persistence (Loreau 2010).

Ecosystem services are defined as all benefits mankind can obtain from any ecosystem. That comprises existential resources as freshwater or food, materials like wood for building as well as the recreational value of some landscapes. The allocation of such services depends on the functioning of the respective ecosystem and the condition it is in. As humanity depends on the allocation of specific ecosystem services and thereby on a favorable state of such a system, the understanding of ecosystem functioning became of great interest. (Millennium Ecosystem Assessment, Ecosystems and Human Well-Being (Island Press, Washington, DC, 2005)

A whole new field of ecology developed therefore to investigate the links between biodiversity and ecosystem functioning – BEF research. Focus thereby often lies on primary producer diversity and its influence on ecosystems as primary production builds the foundation for food webs and ecosystem processes. Several studies showed connections between biodiversity and productivity in terrestrial biomes as well as aquatic communities (Tilman et al. 2001, Ptacnik et al. 2008, Craven et al. 2016). Diversity was investigated as driver of productivity, stability under perturbations as well as nutrient cycling and other

ecosystem properties (Loreau et al. 2001, Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006, Worm et al. 2006, Isbell et al. 2015). Tilman and colleagues for example performed experiments in grasslands and found higher biomass production with increasing species richness of the primary producer community (Tilman et al. 1996, Tilman et al. 2006).

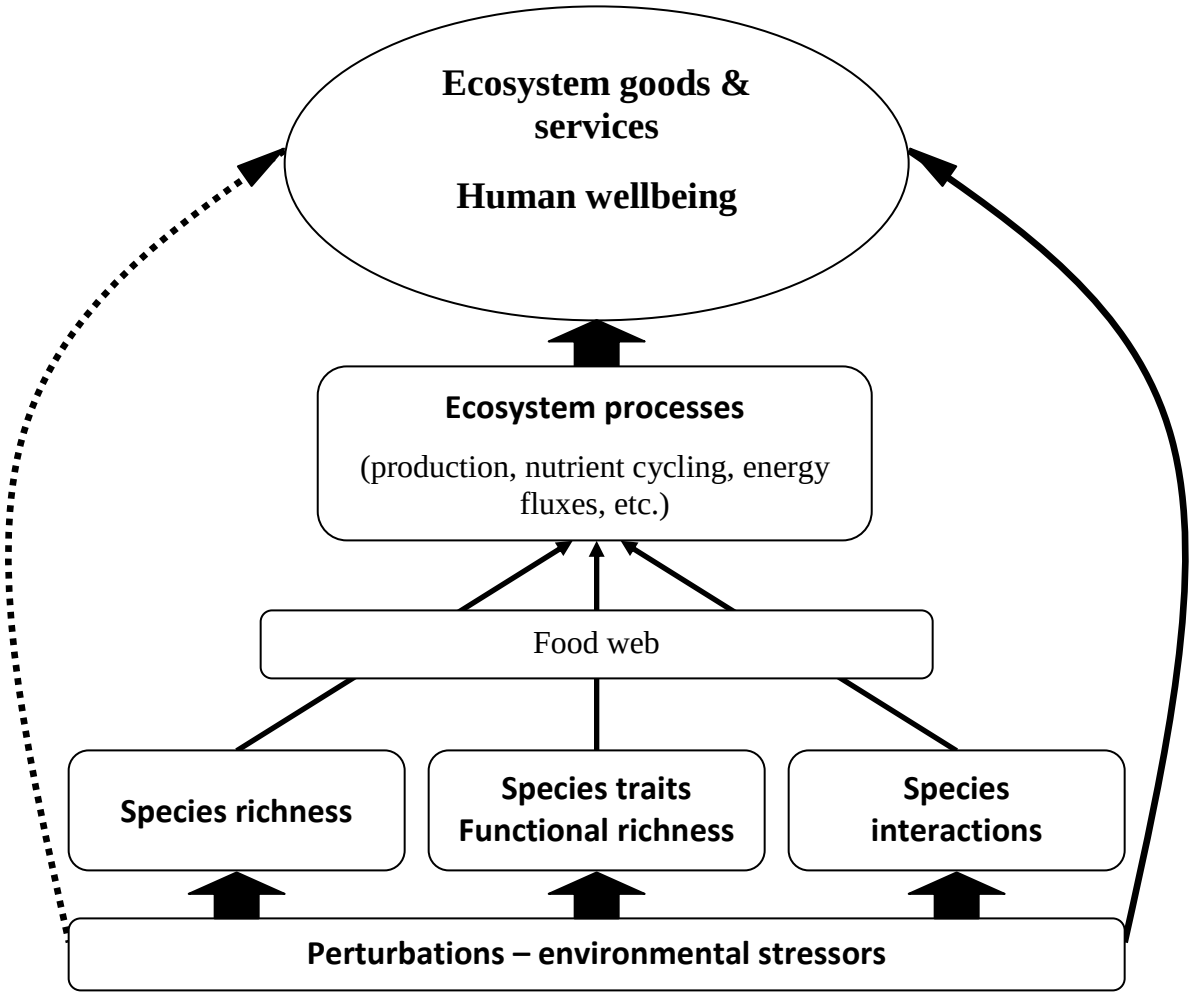

Figure 2: Diagram of links and dynamics in (freshwater) food webs. As represented by solid and dashed lines, stressors can have direct as well as indirect effects on ecosystem functioning (graphic modified after Woodward 2009).

Different explanations for a connection between biodiversity and productivity have been suggested. The sampling effect hypothesis describes the assumption that a community consisting of more species than another has a greater probability to contain a larger subset of all possible traits and with that performs better in the analyzed ecosystem processes (Huston 1997, Aarssen 1997, Tilman 1997). When facing perturbations, a high biodiversity is thought

to act as buffer, in the case of a loss of some species from the community, others with similar traits could take over their function in the collective and thereby keep system processes stable. As diversity functions like an insurance for ecosystem processes this is called “insurance hypothesis” (Yachi and Loreau 1999). Complementarity among species or functional groups could also lead to higher productivity (Loreau 2000). Especially the diversity of functional traits and the identity of single species within a community seem to be crucial for its performance in the ecosystem (for links between perturbations, diversity and ecosystem functioning via food webs see Fig. 2). To gain a deeper understanding of the role of biodiversity for ecosystem functioning and its interactions with abiotic factors is the aim of BEF research. Therefore, various experiments and analyses examining the mechanisms behind biodiversity ecosystem functioning relationships (Handa et al. 2014; Craven et al. 2016; Lewandowska et al. 2016) have been performed.

1.1.3 Phytoplankton

As primary producer diversity is tightly connected to the production of an ecosystem, this usually is a well explored example across different systems. For instance for grassland communities the influence of primary producer diversity on productivity is well studied (Tilman et al. 2001, Spehn et al. 2005). The function of plants in terrestrial systems is carried out by phytoplankton communities in aquatic systems. 71 % of the earth’s surface is covered in water (Wetzel 2001). Phytoplankton, although only representing around 1 % of the world’s photosynthetic biomass, are responsible for approximately 50 % of the fixation of inorganic carbon by primary producers and thereby driving major carbon and nutrient fluxes (Falkowski 2012).

Freshwater systems – with phytoplankton at the basis of their food webs – are also under increasing pressure of human induced change. Climate change with a following alteration in water temperatures can alter the stratification and mixing regimes of water bodies (Schindler et al. 1996, Livingstone 2008, Adrian et al. 2009). Nutrient inputs in catchment areas and water bodies due to for example use of fertilizers in agriculture additionally stress primary producer communities and change their environmental conditions (Carpenter 2005, Smith and

Schindler 2009) in freshwater and coastal waters. After increased nutrient input and rising water temperatures for instance the frequency of toxic algal blooms increased (Hallegraeff 1993, Anderson et al. 2002, Heisler et al. 2008, Paerl and Huisman 2008). Rivers, lakes and estuaries change their appearance due to the construction of dams, agricultural use of the catchment areas or river regulations. Perturbations such as described can lead to shifts in phytoplankton community composition and with that impact ecosystem functioning.

Microalgae suspended in the water column show an impressing variety of traits. Being described by their way of life rather than their evolutionary background, phytoplankton comprises organisms ranging from bacteria to eukaryotes. They are unified by their ability to perform photosynthesis but highly diverse in for example pigmentation, shapes and their demands to nutrient availability (Sommer 1994). To efficiently use light as a resource, phytoplankton has developed a particularly high diversity in photosynthetic active pigments. Thereby phytoplankton communities with species diverse in their pigment composition are able to use a wide range of wavelengths for primary production (Falkowski and Raven 2007, Litchman and Klausmeier 2008). Leading to a high functional diversity of phytoplankton communities, this shows their important role in the functioning of ecosystems and the interest of BEF research focusing on aquatic primary producer communities (e.g. Ptacnik et al. 2008, Vallina et al. 2017, Cavicchioli et al. 2019).

1.2 Motivation and research topics

Phytoplankton organisms show a diversity of functional traits like pigment composition or nutrient uptake rates as well as biochemical composition. Dynamics of such traits within a community are decisive for important ecosystem processes. With the above discussed decrease in biodiversity, a loss of functional traits is going hand in hand, which can alter ecosystem processes like productivity or stability under perturbations (Naeem et al. 2012). Biodiversity loss thereby becomes a major driver of ecosystem change (Hooper et al. 2012). Experiments investigating the mechanisms behind BEF (Tilman et al. 2001, Cardinale et al. 2006, Power and Cardinale 2009) showed that biodiversity loss resulting in a loss of functional traits connected with resource use will lead to a decrease in primary production. These experiments were mainly conducted with artificially assembled communities on a relatively small scale, allowing for high control over the diversity of experimental units as well as the experimental design. In phytoplankton biodiversity research experimental synthetic communities assembled from laboratory algal strains are most often used (Power and Cardinale 2009; Behl et al. 2011; Corcoran and Boeing 2012). Phytoplankton communities mainly consist of microscopic unicellular algae; this makes diversity manipulations of natural communities very difficult. Removal experiments that manipulate diversity by removing individual species such as done in terrestrial environments (Wardle et al. 1999, Symstad and Tilman 2001) are impossible to perform with plankton communities.

My aim was therefore to explore potential ways to perform diversity manipulations within natural phytoplankton communities. Thereby I wanted to gain insight into how a loss of diversity and with that a loss of functional traits affects ecosystem functioning. I was interested in how a change in diversity affects the dynamics of phytoplankton communities and how the reaction of communities to an external stressor (nutrient input) is connected to diversity. By scaling up from small scale laboratory experiments to larger mesocosm studies in the field, I investigated whether diversity manipulated phytoplankton communities show the same responses as communities sampled along naturally evolved diversity gradients.

Loss of a certain trait or functional group from a community might mechanistically link species loss to shifts in pelagic ecosystem processes. Therefore I also investigated in detail the

consequences of a loss of diatoms – an important functional group of phytoplankton organisms prone to environmental change – for light use efficiency in the primary producer community and corresponding processes within lake ecosystems.

Whether as part of large scale field experiments or for environmental monitoring, there is a need for techniques to assess phytoplankton community composition. Conventionally phytoplankton species identity is assessed by microscopy although this is a very time consuming technique. Hence, faster ways to analyze community composition were developed based on photosynthetic traits such as pigment composition. Therefore I was also interested in comparing two of those methods, namely spectrofluorometry by the AlgaeLabAnalyser (bbe Moldaenke, Kiel, Germany) and HPLC based analyses of pigment composition with CHEMTAX, to examine their sensitivity and handling in ecological research.

To assess the above stated research interests, I performed laboratory and mesocosm field studies based on phytoplankton communities from two ponds and three lakes in Upper Bavaria, covering a range of different trophic states. For the scope of this thesis I concentrated on the following four research topics:

1.2.1 Directed diversity manipulations of natural phytoplankton communities

To assess BEF relationships mechanistically, experiments including communities with artificially manipulated diversity are necessary. Experimental BEF studies often focus on the manipulation of primary producer communities and subsequent effects in food webs and ecosystem processes (Balvanera et al. 2006). So called removal experiments are a commonly used type of experimental manipulation of the diversity of primary producer communities (Wardle et al. 1999; Symstad and Tilman 2001). Primary producer communities are manipulated by removing single species or groups of species from a community and the consequences of these experimental changes in biodiversity and species composition are followed. In terrestrial primary producer studies this method is well established and experiments with natural or semi-natural communities are possible (Díaz et al. 2003).

Equivalent to plants in terrestrial habitats, phytoplankton represent the primary producers at the basis of aquatic food webs. Consisting mostly of microscopic unicellular algae, phytoplankton communities cannot be easily manipulated in species richness by methods such as individual species removal. Therefore phytoplankton BEF studies are usually based on synthetic combinations of laboratory strains without shared evolutionary history (e.g., Power and Cardinale 2009; Behl et al. 2011; Corcoran and Boeing 2012). However, the response of a community to environmental changes might also be determined by a shared evolutionary background. Competition, predation, niche partitioning and other interactions among organisms result in locally adapted communities. These natural communities might therefore respond to environmental factors or experimental conditions in a different way than laboratory communities would (Flombaum and Sala 2008). Additionally, artificial communities usually comprise a low number of species that is reflective of the lower end of naturally occurring biodiversity.

Finding an easily manageable tool to manipulate a natural community's diversity would be a further step to enable experiments on diversity gradients that closer represent natural conditions. As removal of single species is not possible, such manipulations must focus on a directed manipulation of the abundance of groups of species with certain characteristics.

Dilution and disturbance are two of several possible methods to change the diversity of natural algal communities in such a way.

Loss of rare species by dilution

With gradual dilution of a natural community rare species are expected to get lost and thereby a diversity gradient can be established with only the most common species being present at the highest dilution (Franklin et al. 2001, Giller et al. 2004). Dilution has been already applied in other fields to create species diversity gradients and was initially described as dilution to extinction approach to estimate structural diversity in microbial communities (Garland and Lehman 1999, Franklin et al. 2001). Similar approaches were used in other studies studying community dynamics related to diversity in a variety of systems (Taylor and Bruns 1999, Romanuk and Kolasa 2005, Vogt et al. 2006, Trommer et al. 2012). Giller et al. (2004) suggested using dilution as a method to manipulate communities in aquatic environments in a non-random way to address BEF relationships. This also provides the possibility to make use of natural communities in contrast to the widely used artificially assembled experimental communities. However, so far there were no detailed methodological descriptions for this method for natural freshwater phytoplankton communities.

Independent of their low abundance, rare species may have important community functions as they can potentially compensate a loss of more common species or provide unique functional traits to a system (Walker et al. 1999; Lyons et al. 2005; Mouillot et al. 2013; Jain et al. 2014). The experimental loss of rare species along a dilution gradient resembles the higher risk of rare species to be more prone to extinction, mainly due to stochastic processes that result in large effects on small populations (Pimm et al. 1988; Caughley 1994).

Loss of stress sensitive species by disturbance

On the other hand, experimental disturbance affects species that have narrow niche functions and are therefore very sensitive to certain stressors (Fisher 1977; Carpenter and Cottingham 1997; Elmqvist et al. 2003; Gallagher et al. 2015). This can result in a direct loss of species by stress and/or affect competition between species and thereby also competitive exclusion. Both, direct and indirect effects of disturbances can result in changes in species richness. It has been

shown that intermediate disturbance intensities and frequencies enable communities with high diversities to establish (Intermediate Disturbance Hypothesis, IDH; Connell 1978). By testing predictions of the IDH, Flöder and Sommer (1999) have already shown that diversity gradients can be established in natural phytoplankton communities (mesocosms) by gradual disturbance of the water column stratification. Mesocosms with an intermediate disturbance frequency showed the highest phytoplankton diversity levels. Changes in diversity were characterized by shifts in evenness of species distribution but also by a loss of species richness at both ends of the disturbance gradient.

To analyze the applicability of the above described possible experimental manipulations we tested both, disturbance and dilution to create diversity gradients within natural phytoplankton communities. To further compare effects of such diversity manipulations at contrasting trophic states, we used natural communities from two ponds showing low and high nutrient levels (oligotrophic, eutrophic). All experiments were performed at laboratory scale (microcosms < 1L), which represents a common scale for phytoplankton BEF research allowing for strictly controlled environmental conditions (Petersen et al. 2009). With adequate modifications both methods should be also applicable to large-scale field experiments such as performed in mesocosms.

1.2.2 Diversity gradients and nutrient enrichment – comparing effects of natural diversity differences with effects of short-term experimental manipulations of diversity

As pointed out in section 1.1 a loss of diversity from primary producer communities is considered to influence ecosystem services and the stability of ecosystems and food webs. Stability of an ecosystem or certain processes becomes noticeable in the reaction to environmental influences or stressors. Resistance and resilience are two terms tightly connected to the research focusing on stability in ecology. Resistance thereby describes the ability of a community or system to withstand a deflection from their present condition or state. By contrast, resilience would express the system's ability to recover after perturbation and would for example be shown in the return time a system needs to reach its initial state (Westman 1978, Webster et al. 1983, Tilman and Downing 1994). These terms have been subject to controversial discussion in the field resulting in numerous definitions connected to the resilience concept (see for example Holling 1973, Gunderson 2000, Carpenter et al. 2001, Scheffer et al. 2001, Ibelings et al. 2007, Lake 2013, Hillebrand et al. 2018). As my small-scale study setup does not allow for any far-ranging conclusions about state shifts in the ecosystem, I here refer to stability in the sense of a resistance to being deflected from the initial condition of a community under the influence of a certain stressor. Hence, I measure phytoplankton community reactions in growth and community composition to high nutrient input rates.

Increasing nutrient concentrations in aquatic systems could for example lead to higher community growth rates or a shift in community composition of primary producers (Leibold 1999, Carpenter 2005, Smith et al. 2006).

Several systems are known to have different stable states in which they can persist. Lakes can for example show regime shifts between a clear-water state with pronounced macrophyte communities and a microalgae dominated turbid state on the other hand (Scheffer et al. 1993). Depending on the system's characteristics, sudden switches between the different states can occur under the influence of slowly changing environmental factors. This is known as catastrophic regime shifts, tipping points or critical transitions between states (Scheffer and

Carpenter 2003, Barnosky et al. 2012, Oliver et al. 2015). For conservation issues it is desirable to develop techniques to anticipate such dramatic system changes and find reliable warning indicators for undesired regime shifts. For systems with several stable states where regime shifts can occur, phenomenons like critical slowing down (increasing return time to equilibrium state after a disturbance; Wissel 1984) or increasing variability have been described as potential early warning indicators previous to a transition. In detail such indicators could be changes in variance, autocorrelation, recovery rates and skewness of a measured variable (Guttal and Jayaprakash 2008, Lindegren et al. 2012, Scheffer et al. 2012, Dakos and Bascompte 2014). During a whole-lake experiment (adding piscivorous fish in a planktivore-dominated community to induce a trophic cascade), Batt et al. (2013, also see Carpenter et al. 2011) measured for example several basic variables like pH or chlorophyll a concentration and used these to compute resilience indicators and evaluated their ability to notify system changes in advance to a potential regime shift.

Stressors like nutrient input not only lead to visible changes such as described before but are also likely to influence diversity in primary producer communities (Leibold 1999, Smith et al. 1999, de Jonge et al. 2002). A loss of diversity under the influence of severe stressors can in turn lead to stronger reactions to stressors, potential feedbacks between biodiversity and ecosystem functioning could be a consequence (Chapin et al. 2000, Loreau 2010). Biodiversity has therefore been considered as a potential buffer against the impact of environmental stressors (Cardinale 2011). Hence, it is important to further investigate the causal mechanisms that link together biodiversity and a system's function and stability under the influence of environmental stressors. As previous studies mainly focused on artificially assembled communities, experiments closer reflecting naturally evolved microbial communities with a shared evolutionary background and the complex interactions between organisms are desirable. Recently some methods have been described to manipulate diversity of natural communities in small-scale studies (see research topic 1 above - Hammerstein et al. 2017, and Engel et al. 2017) that were up-scaled in this study to be also applicable with larger mesocosm experiments. Albeit the difficulties in setting up diversity gradients in natural communities, phytoplankton communities are - due to their fast growth responses - well suited to study diversity dependent effects of stressors on community dynamics.

However, experimental diversity manipulations reflect transient short-term changes of natural communities while diversity differences between communities from different environments are likely to evolve over longer periods shaped by ecological and evolutionary forces. Therefore the question arises whether such short-term experimental diversity shifts have comparable functional consequences on ecosystems as long-term evolved diversity differences between different ecosystems.

I therefore investigated the following questions:

1) Does experimentally reduced diversity in phytoplankton communities lead to lower community stability under the influence of a disturbance and is the community reaction to an environmental stressor (high nutrient input) stronger with a loss of diversity?

2) Do experimentally induced short-term changes in diversity of a natural community show the same direction and magnitude in the community's reaction to this stressor as communities with comparable natural diversity differences that developed by ecological and evolutionary processes?

To investigate these questions I performed nutrient enrichment experiments with natural phytoplankton communities from three lakes that were experimentally manipulated in diversity in large scale field studies using mesocosms.

1.2.3 Consequences of group specific trait losses from phytoplankton communities

As pointed out in section 1.1.3 pelagic primary producer communities are often directly affected by changing conditions. Modified nutrient availability or rising temperatures with following changes in stratification regimes notably affect growth conditions for phytoplankton. Shifts in primary producer community composition or changes in the timing of periods of growth can be the consequence (Cushing 1989, Edwards and Richardson 2004, Winder et al. 2009, Finkel et al. 2010, Morán et al. 2010, Thomas et al. 2012). Such changes at the basis of the food web can alter the availability of food for higher trophic levels or for example lead to trophic mismatches in the seasonal timing between trophic levels (e.g. Edwards and Richardson 2004, Thackeray et al. 2016). Thereby, shifts in primary producer communities can alter the structure and composition of whole food webs.

Bacillariophyceae represent an important group of phytoplankton that is generally prone to the addressed anthropogenic influences on their environment. Specific requirements to nutrient accessibility as well as to the depth of the mixed upper water layer lead to the diatom's susceptibility to climate change and eutrophication. Rising water temperatures lead to stronger stratification of the different water layers. As a result, due to the warmer conditions, the upper layer that is usually mixed by wind will be of lower depth (Livingstone 2003, Fang and Stefan 2009). Characterized by their silicon shells, diatoms have a higher specific density compared to other phytoplankton organisms and therefore show high sinking rates of up to several meters per day (Smayda 1970, Smol et al. 1984). With more pronounced stratification/shallower mixing depths, larger diatom species tend to sink out of the photic zone fast and are replaced by diatom species with smaller cell sizes or non-siliceous motile phytoplankton (Winder et al. 2009, Bramburger et al. 2017). A shift in phytoplankton community composition towards species that are not affected by high sinking rates, like smaller or motile species, would be the consequence.

Although diatoms tend to grow well at constantly low phosphorus (P) concentrations, they depend on silicon (Si) as an essential nutrient in the water column for their growth. Si commonly enters water bodies through weathering processes and consequently reaches lakes

and finally coastal areas by rivers that transport it from their catchment areas. Anthropogenic influences at the catchment areas therefore also indirectly affect nutrient composition and concentration in lakes or coastal areas where a relative decrease of Si concentrations compared to other nutrients was monitored over the last decades (Schelske et al. 1983, Justić et al. 1995, Humborg et al. 2002, Laruelle et al. 2009). Nitrogen (N) and P input to riverine systems increased due to human activities (e. g. Smith 2003). Si on the other hand is usually not added to aquatic systems by anthropogenic influences. The resulting change in nutrient ratios from Si to N or P can affect phytoplankton community composition and aquatic food web structures (Conley et al. 1993, Justić et al. 1995). In lakes and coastal areas, increased N and P input first leads to increased diatom growth and a subsequent Si burial in the sediments. This in turn can lead to a depletion of biogenic Si in the water column and a following switch to non-siliceous algae (Admiraal et al. 1990, Conley et al. 1993).

Additionally, the Si content in downstream regions and coastal areas is affected by the construction of dams. By blocking rivers and retaining water in artificial lakes or sedimentation areas, Si concentrations in the streams feeding lakes and seas are modified (Admiraal et al. 1990, Humborg et al. 1997, Humborg et al. 2002). In such artificial lakes, environmental conditions (deep mixing) favor growth of diatom communities that probably sink out before the retained water is let into the ongoing river. Thereby Si is bound in the sediments with the diatoms that sank out and cannot be washed into the coastal areas by the river. This effect has been observed in various systems around the world and leads to an increased silicon deficiency impacting phytoplankton communities (Humborg et al. 1997, Humborg et al. 2000, Conley et al. 2000). Following eutrophication and rising temperatures, it has already been observed in some marine habitats that systems formerly characterized by diatoms, fish and other predator species switched to a state dominated by more flagellate plankton species and higher numbers of jellyfish (Richardson et al. 2009).

With the above described anthropogenic impacts affecting mixing regimes and Si concentration, diatoms are expected to decrease in both, species richness and abundance. Hence, such changing environmental factors could lead to a shift in phytoplankton species composition with fewer diatoms and a simultaneous increase of non-siliceous phytoplankton

species. Other phytoplankton groups could benefit from the new conditions and show enhanced growth. Hence, the question arises whether species replacing diatoms are able to fulfill the same functional properties in the community as their predecessors. Diatoms have special functional traits, for example containing fucoxanthin as one of the main light harvesting pigments (Jeffrey and Vesk 1997, Gelzinis et al. 2015) and thereby covering parts of the green and yellow gap in light utilization that other species cannot use for photosynthesis (Kuczynksa et al. 2015). Additionally, diatoms contain for example a relatively high amount of polyunsaturated fatty acids (PUFAs) that can be essential components of food for higher trophic levels (Brett and Müller-Navarra 1997, Li et al. 2014). With diatoms being partially lost from phytoplankton communities, a concomitant functional change of communities and food webs would be expected.

My aim was therefore to study the consequences of a decrease or loss of diatoms in natural phytoplankton communities. I manipulated the stratification of water columns including natural phytoplankton communities from different lakes; thereby changing the abundance of diatoms within phytoplankton. I analyzed community responses in light use efficiency and pigment composition. Main question of my analyses was to investigate the consequences of a loss of diatoms in phytoplankton communities in terms of important functional traits and whether the communities reorganizing after a disturbance can compensate a potential loss of such traits.

1.2.4 Comparison of different techniques to assess phytoplankton diversity

To ensure the sustainable use of aquatic ecosystems and to manage the water treatment operations, the European Community legislation has introduced the EU Water Framework Directive (WFD, Directive 2000/60/EC). The WFD defines the composition of the phytoplankton community as one of the most important biological parameters that determine the quality and ecological status of surface water bodies (Catherine et al. 2012, Izydorczyk et al. 2009, Escoffier et al. 2015, Sarmento and Descy 2008). Beyond issues of water quality management, phytoplankton community composition has also often been used as an indicator of food quality for e.g. herbivorous zooplankton (Behl and Stibor 2015) and of the ecosystem productivity and trophic status of lakes (Reynolds and Petersen 2000).

Because of the rapid response of the phytoplankton community to environmental changes (Richardson et al. 2010) and a usually high number of samples that have to be processed (due to samples from different communities as well as high numbers of replicates in phytoplankton biodiversity experiments), it is necessary to use fast and time saving methods for the assessment of phytoplankton community composition. Traditionally, phytoplankton community composition is estimated via microscopic counting. However, this method is quite time consuming and depends on the taxonomic knowledge of the person identifying the phytoplankton taxa. Also, phytoplankton of very small size (picoplankton; 0.2 - 2 μm) are hard to be differentiated accurately (Booth 1993). Another approach, which is new and not yet completely implemented, is DNA metabarcoding, e.g. using 18S rDNA. Although this method could help to distinguish between small phytoplankton species and would provide a very high resolution, it is still very costly and limited by available databases that still lack many reference sequences (Simmons et al. 2016, Groendahl et al. 2017). Thus, chemotaxonomic alternatives have been proposed, such as pigment-based spectrofluorometry and high performance liquid chromatography (HPLC) of pigments. Both approaches are based on the differences in pigment composition of the main phytoplankton groups. For example, dinoflagellates contain the pigment peridinin, which is specific to them, while alloxanthin and lutein are pigments that are group-specific for cryptophytes and chlorophytes respectively

(Gieskes and Kraay 1983, Jeffrey et al. 2011). Thus, the pigment composition of the phytoplankton is commonly used to assess phytoplankton community composition.

Fluorescence-based chlorophyll a (Chl a) quantification methods were applied for both *in vitro* (Yentsch and Menzel 1963) and *in vivo* (Yentsch and Yentsch 1979, Yentsch and Phinney 1985) measurements of Chl a. Subsequently, spectrofluorometric methods that use multiple excitation and/or emission wavelengths were developed and became the standard application for phytoplankton monitoring (Beutler et al. 2002, Richardson et al. 2010, MacIntyre et al. 2010). The Chl a fluorescence is mostly determined by the peripheral and core antennae of photosystem II (Beutler et al. 2002). While the evolutionarily conserved core antenna contains the Chl a molecules in all phytoplankton taxa, the peripheral antenna includes species-dependent light-absorbing accessory pigments, which are responsible for selective excitation spectra and thus represent the fundament of the spectrofluorometric differentiation of phytoplankton groups (Rowan 1989). Based on these observations, Beutler et al. (2002) introduced the AlgaeLabAnalyser (bbe Moldaenke, Kiel, Germany) as a bench-top device which is commonly used by water authorities and routine laboratories. *In vivo* measurements with this device are very fast (< 2 min.) and based on group-specific excitation spectra Chl a content is assorted to the respective algal groups. This allows a swift monitoring and assessment of the phytoplankton community composition.

Another commonly used method for the assessment of the phytoplankton community composition is the HPLC analysis of photopigments, combined with the matrix factorization programme CHEMTAX. HPLC also allows for identification of small phytoplankton that is hard to detect by microscopic counting, as the detection limits of diagnostic pigments are usually low (Schlüter et al. 2016). The HPLC approach is nowadays often combined with the matrix factorization programme CHEMTAX, which was developed in 1996 by Mackey et al. for marine systems, but has been used and updated since then for both marine and freshwater ecosystems (Armbrecht et al. 2015, Buchaca et al. 2005, Descy et al. 2000, Descy et al. 2009, Lauridsen et al. 2011, Lewitus et al. 2005, Sarmento and Descy 2008, Tamm et al. 2015, Schlüter et al. 2016). The CHEMTAX approach is based on group-specific pigments (e.g. peridinin, lutein etc. as mentioned above) and uses input ratio matrices containing ratios of

such pigments to Chl a, which can be found in the literature. Depending on the pigment ratio matrix and observed concentrations of the pigments, CHEMTAX provides the best fit of contributions of the predefined phytoplankton groups to the total Chl a concentration. The number of groups discernible by CHEMTAX depends on the number of analyzed diagnostic pigments and the previous knowledge about the phytoplankton community composition of the water body of interest (Mackey et al. 1996).

Both the AlgaeLabAnalyser and the HPLC/CHEMTAX approach are limited in the taxonomic resolution, as they only allow a differentiation on functional group level. Yet, they offer a trait-based approach of community characterization leading to an assessment of functional diversity. Assessment of this functional diversity is increasingly interesting in phytoplankton studies, as functional phytoplankton diversity has been shown to be an important predictor of ecosystem functioning (Behl et al. 2011, Stockenreiter et al. 2013, Striebel et al. 2009).

Here, both methods are compared with samples from lakes varying in trophic status; the following method-based hypotheses were tested:

1) Both methods give a good representation of the phytoplankton community composition.

2) HPLC/CHEMTAX allows for a higher resolution of the phytoplankton biodiversity as it has low detection limits for rare and small phytoplankton.

3) The AlgaeLabAnalyser allows a more accurate determination of cyanobacteria, as the lipophilic solvent extraction of pigments used for the HPLC method does not capture the water-soluble dominant cyanobacterial pigments phycocyanin and phycoerythrin.

2. Material and Methods

2.1 Laboratory experiments – research topic 1

Phytoplankton communities

Phytoplankton communities originated from two ponds of different trophic status. Both are situated in Upper Bavaria, close to the Ludwig-Maximilians-University, Department Biology II in Martinsried, Germany. The first pond, BZ (N 48.109399 E 11.459073), is oligotroph (TP <10μg/L) mainly dominated by Bacillariophyta. The second pond, IZB (N 48.107114 E 11.457619), is highly eutroph (TP > 300 μg/L) and mainly dominated by Cyanobacteria and Chlorophyta.

Diversity manipulation by dilution

For the dilution experiment we used spring phytoplankton communities from both ponds. To exclude small rotifers and mesozooplankton, the water was filtered through 60 μm gauze. Additionally, 24 L were filtered through glass fiber filters to remove all algae. This algae free filtrate was used as medium for the experiment. To establish dilution gradients, 650 mL - cultivation bottles (Cell star, Greiner Bio One, Germany) were filled with 400 mL of the algae free filtrate. 1 μL, 10 μL, 100 μL, 1 mL and 10 mL of 60 μm – filtered pond water were added to the 400mL. Additionally, an undiluted control with 400 mL 60 μm-filtered pond water was prepared. Each dilution step (except for the control) was replicated three times per pond (32 flasks in total). All experimental units were kept in a 20 ° C temperature controlled – climate chamber. Light conditions were 12:12 hours light - dark cycle with a PAR (photosynthetic active radiation) intensity of 100 μmol photons m^{-2} sec^{-1}, which was determined by a LI-250A Light Meter (LI-COR Biosciences, USA). Bottles were randomly changed in position every day to reduce stochastic influence of the position on the shelf. Additionally, the bottles were shaken lightly every day. After an initial growth period (11 days) and the establishment of measurable biomass, every three to four days 10 % of each treatment was replaced by algae free filtrate.

To survey species composition, subsamples were taken at day 17 and 24 after inoculation and fixed in Lugol's iodine solution. 10 mL subsamples (2.5 % of the total experimental volume) were counted using an inverse microscope following the standard Utermöhl technique (Utermöhl 1958). Species presence was recorded by scanning the samples at 100 and 400-

times magnification. To ensure that also rare species are taken into account, the complete surface area of the bottom of the sedimentation chamber was screened. Algae were determined to species level where possible.

Diversity manipulation by disturbance

Phytoplankton communities from the same ponds as described above for the dilution experiment were sampled in spring and filtered through 60 μm gauze to exclude small rotifers and mesozooplankton. 650 mL – cultivation bottles (Cell Star, Greiner Bio One, Germany) were filled with 400 mL of the 60 μm filtered pond water. Additionally, 7 L were filtered through glass fiber filters as above to produce an algae free filtrate serving as medium for regular water exchange. Five different disturbance treatments were assigned to the phytoplankton communities, namely 1, 2, 3, 5 and 10 disturbances per week. Each disturbance treatment was replicated three times with an additional set of three undisturbed control replicates per pond (36 experimental units in total). All treatments were kept in a climate chamber under the same controlled conditions as described for the dilution experiment. For disturbances, bottles were manually shaken for one minute. In field experiments one way of disturbance is to manipulate water column stratification and thereby resource distribution and mixing (like for example in Flöder and Sommer 1999). Stratification manipulations are not reasonable in small-scale bottle experiments. Hence, shaking was chosen as an intense disturbance mode (Hu et al. 2009) which will also alter nutrient distribution and shearing forces. Controls were not shaken at all. Once a week 10 % of each treatment was replaced by algae free filtrate. At day 11, 25 and 39 samples from each treatment were taken and fixed with Lugol's iodine solution and afterwards analyzed microscopically such as described above.

Data from both parts of the experiments were analyzed using SigmaPlot 11.0 (Systat Software 2008) performing nonlinear and linear regression analyses.

Treatments were compared by effect sizes, namely the relative differences in species richness between the tested levels of dilution or disturbance.

Community similarity among treatments

To assess the similarity among replicates from the same experimental treatments (same dilution steps, same disturbance steps), species composition of all samples were pairwise compared to each other based on presence/absence data using the Sørensen similarity index (1; Clarke et al. 2014) in PRIMER 7 (Primer-e, New Zealand):

$$S = 100 \times \frac{2a}{2a+b+c} \quad (1)$$

With a being the number of species present in both samples, b being the number of species only present in the first sample but not in the second sample and c being the number of species only present in the second sample but not in the first. Means and standard errors of similarity indices between replicates were calculated from all pairwise comparisons within replicates of the same treatment and analyzed using regressions and ANOVAs in SigmaPlot 11.0 (Systat Software 2008).

2.2 Field mesocosms: setting up diversity gradients

Diversity gradients were established in natural freshwater phytoplankton communities in mesocosms in three lakes of differing nutrient status. This set-up was carried out twice in June and July of 2014 and 2015. All three lakes are situated in Upper Bavaria close to the Limnological Station Seeon of the LMU Munich and range from oligotrophic to mesotrophic status. Brunnensee = BS (N 47.984170 E 12.436148) is an oligotrophic lake fed by groundwater from subsurface springs with a maximum depth of 18.6 m and an area of 5.88 ha. Klostersee = KS is - with a maximum depth of 16 m and approximately 47 ha area - the largest of the three and has a meso-oligotrophic status (N 47.973492 E 12.455118). Thalersee = TS is a mesotrophic lake southwestward from the other two lakes with a maximum depth of 7 m and an area of 3.79 ha (N 47.906127 E 12.339043). Both Klostersee and Thalersee are not dominated by springs but have small inflows.

In all three lakes 20/10 (2014/2015) mesocosms of transparent low density polyethylene foil were installed around an anchored raft at least 15 m from the shoreline. Mesocosms were cylindrical, 6 m (5 m in Lake Thalersee, due to its shallowness) deep and had a diameter of 0.95 m, resulting in an approximate filling capacity of 4.2 x 10^3 L (3.5 x 10^3 L). On the top mescosoms were open to the atmosphere. They were filled with lakewater through 250 μm gauze to exclude meso- and makrozooplankton. Afterwards, a five week phase of regular disturbances followed (see table 1 for disturbance treatments) to establish phytoplankton communities of differing diversity within the mesocosms (also see Flöder & Sommer 1999, Hammerstein et al. 2017). All five treatments were replicated four times/twice (2014/2015) per lake. As disturbance, the stratified water column was perturbed for ten minutes using compressed air that was introduced into the mesocosms at the bottom.

Table 1: Disturbance treatments during mesocosm field experiments (2014/2015)

Treatment	1	2	3	4	5
Disturbances per week	7	3.5	2.33	1.4	1

2.2.1 Mesocosm sampling – Research topics 3 & 4

During the phase of disturbance treatments at least once a week samples from every enclosure and lake were taken for analysis. The sampling comprised approximately 1 L of water taken from 0.5 m to 2.5 m depth (2 L integrated water sampler, KC Denmark, Denmark) and was split up for microscopy samples as well as light measurements. Additional water samples were taken in the same way for pigment analysis (see section 2.2.3). All samples were kept dark till processing.

Phytoplankton microscopic identification

Samples for microscopic identification were fixed with Lugol's iodine and kept in brown glass bottles and stored in a dark place. For analyzes, phytoplankton was counted following standard Utermöhl procedure (Utermöhl 1958). Phytoplankton were determined on genus level and recorded by scanning two perpendicular transects (about 200 fields of view) of the

counting chamber. Thereby genera were counted to a minimum of 100 individuals to minimize counting error (Lund et al. 1958). Subsequently biovolume of cells was estimated according to Kremer et al. (2014) or estimated from earlier own measurements on algae from the same water bodies and calculations based on Hillebrand et al. (1999).

Chlorophyll a fluorescence and light measurements

Respective amounts of each sample were used for the following light measurements. After exciting the sample (25 mL) with differently coloured LEDs (λ: 450 nm, 525 nm, 570 nm, 610 nm), the AlgaeLabAnalyser (spectrofluorometer, bbe Moldaenke, Germany) determines the chlorophyll a (Chl a) content of the sample and assigns it to different algal groups ("green": Chlorophyta; "blue/bluegreen": Cyanobacteria; "brown/chromophytes": Bacillariophyceae, Chrysophyceae, Dinophyceae, "mixed": Cryptophyta and phycoerythrin-containing algae). This is done based on group-specific excitation spectra (norm spectra, see Beutler et al. 2002).Detailed information on photosynthetic parameters was gained by measurements with four AquaPen-C AP-C 100 (Photon Systems Instruments, Czech Republic) devices. These provide Chl a fluorescence transient data (including minimal fluorescence, F0) for excitation at eight different wavelengths (455 nm, 470 nm, 505 nm, 530 nm, 590 nm, 620 nm, 630 nm, white light). Light intensity of the light transmitted through a water column was measured in tubes of 0.01 m (I_S) and 1 m (I_L) length with a spectrometer (SpectraPen SP 100, Photon System Instruments). Thereby samples were illuminated by white light and the light intensity was measured over a spectrum from 325 nm to 790 nm in 2 nm steps. From these light intensities, the light attenuation coefficient k was calculated for each wavelength λ (1) and normalized to their maximum (2) to allow for the calculation of the area under the curve for the range of the photosynthetic active radiation spectrum (here 325 nm to 790 nm) AUC(PAR) (3).

$$k(\lambda) = (\ln(I_L(\lambda)) - \ln(I_S(\lambda))) / 0.99 \quad (1)$$

$$k'(\lambda) = k(\lambda) / \max[k(\lambda)] \quad (2)$$

$$\text{AUC(PAR) } (k') = \sum_{325}^{790} (\lambda_2 - \lambda_1) \left[\frac{k(\lambda_1) + k(\lambda_2)}{2}\right] \quad (3)$$

Additionally, the area under the curve was separately calculated for the absorption range of Fucoxanthin AUC(Fuco) (4) and the ratio between this range and the total spectrum was analyzed.

$$\text{AUC(Fuco) (k')} = \sum_{500}^{540} (\lambda_2 - \lambda_1) \left[\frac{k(\lambda_1) + k(\lambda_2)}{2}\right] \quad (4)$$

Data analysis for research topic 3 (loss of a trait)

Pigment contents were analyzed as described in section 2.2.3. Data were analyzed in SigmaPlot 11.0 (Systat Software 2008) or in R (R Core Team 2015) using linear and nonlinear regressions and R-figures were saved using the devEMF package (Johnson 2017). For each regression, all samples with the respective data available were considered.

2.2.2 Nutrient enrichment on diversity gradients – Research topic 2

After diversity gradients were established in the mesocosms described in section 2.2, subsamples of the mesocosm communities were brought to the lab for a small-scale nutrient addition experiment under controlled conditions.

Lab microcosms: nutrient enrichment

After the disturbance phase, 200 mL from the surface water of each mesocosm were filled into cell culture flasks (Cell Star, Greiner Bio One, Germany) and transferred to a climate chamber. Samples were kept under permanent light with a PAR (photosynthetic active radiation) intensity of 60 μmol photons m^{-2} s^{-1} (determined by a LI-250A Light Meter; LI-COR Biosciences, U.S.A.) and 20 °C for two weeks. Daily, Chl a concentration of all samples was measured by a Trilogy© Laboratory Fluorometer (Turner Designs, USA). Additionally, 1 mL nutrient solution per sample was added, resulting in a daily nutrient input of 1 μmol x L^{-1} phosphorus, 16 μmol x L^{-1}nitrogen and silicon, approximately equaling Redfield-Brzezinski nutrient ratio (Redfield 1934, Brzezinski 1985). Nutrient solution was prepared by dissolving sodium nitrate ($NaNO_3$), potassium dihydrogen phosphate (KH_2PO_4) and sodium metasilicate ($Na_2SiO_3 \times 5\ H_2O$) in ultrapure water. At the beginning and at the end of the phase in the

climate chamber 10 mL subsamples fixed in Lugol's iodine solution were gathered for microscopical analyses. These samples were analysed using an inverse microscope, following the Utermöhl method (Utermöhl 1958). Samples were scanned completely for species composition at 200 and 400 times magnification. Phytoplankton were determined at genus level.

Data analysis

Results from the microscopic counting were further processed to analyze the community composition in the course of the nutrient enrichment. Therefore the Sørensen similarity index between beginning and end samples was calculated with the same formula as described in section 2.1, this time using the designdist function from the vegan package (Oksanen et al. 2017) in R (R Core Team 2015). Additionally, with the same function, a presence-absence based species-exchange ratio (1; after Hillebrand et al. 2017) between beginning and end of nutrient enrichment in the experiment was calculated:

$$SER = \frac{S_n + S_l}{S_t} \quad (1)$$

with S_n equaling the number of species new to the second sample (present only in end sample), S_l being the number of genera lost from the first sample (uniquely in starting sample) and S_t depicting the total number of genera across both samples.

From Chl a measurements the coefficient of variation (CV) was calculated for every sample over the time of nutrient enrichment as stability indicator. CVs were gained by dividing the standard deviation by the mean calculated from all Chl a values during the two week nutrient addition phase of each community.

To assess the relationship between response variables like the CV, the species exchange ratio or similarity and possible explanatory variables, linear mixed effects analyses were performed in R (R Core Team 2015), using the lmer function of the lme4 package (Bates et al. 2015). Initial genus richness and disturbance frequency were considered as fixed effects while the originating lake, the year in which the experiment took place, relative and absolute genus loss during nutrient enrichment and maximum Chl a content were entered as random variables. To

obtain p-values, models with the effect in question were compared to models without the effect in question by likelihood ratio tests. For an overview over relationships, data were analyzed (scatter plots and linear regressions) with SigmaPlot 11.0 (Systat Software 2008) or R (R Core Team 2015) using again the devEMF package (Johnson 2017) to export graphs.

2.2.3 Pigment based assessment of phytoplankton community composition - Research topic 4

For research topic 4 samples were analyzed from the phase of disturbance within the mesocosms in summer 2014 as well as samples that were taken from the same mesocosms after the disturbances were finished and zooplankton was introduced in half of the enclosures for additional experiments (between July and September 2014). Samples were taken and analyzed in the same way as described in section 2.2.1. In addition to the above described fluorometric and microscopical analyses, HPLC and CHEMTAX were used to assess phytoplankton community composition as described below. Based thereupon fluorometric measurements from the AlgaeLabAnalyser were compared to the results from HPLC and CHEMTAX analyses.

In vitro chromatographic analysis (HPLC)

For the HPLC analyses, up to 1000 mL of the water samples from the lakes were filtered onto precombusted glass fiber filters (Ø 25 mm, GF/F, VWR, Germany). The filters were wrapped in aluminium foil and stored at -20 °C until analysis. Seston samples were extracted with 3.5 mL 100% acetone (HPLC grade) each, sonicated for 2 min and then placed on ice for 1 min. This was repeated five times, resulting in a total of 10 min sonication and extraction time. Subsequently, the filters were kept at 4 °C over night to allow for further extraction. On the following day, the filters were removed from the tubes and the extracts were centrifuged for 15 min at 4500 x g (Eppendorf Centrifuge 5804, Eppendorf, Germany) to remove cell and filter debris. 1 ml of the extracts were transferred to new tubes, evaporated to dryness under a gentle stream of nitrogen gas, re-dissolved in 100 μl acetone and transferred to HPLC vials. To correct for sample loss during the evaporation, we used trans-β-apo-8'-carotenal as an

internal standard (ISTD). 100 ng of ISTD were added to 1 ml of extract prior to evaporation. 25 - 50 μl per sample were injected into the HPLC system. All samples were measured within 72 hours after extraction.

A Prominence HPLC System from Shimadzu (Japan) equipped with a binary pump (LC-20AB), an autosampler SIL-A20C, a column oven CTO-10AC set at 40°C and a diode array detector (PDA) SPD-M20A was used for the analysis of phytoplankton pigments. A reverse phase Spherisorb ODS2 column was used (stationary octadecyl-phase (C_{18}), dimensions: 25 cm x 4.6 mm, particle size: 5 μm). Pigments were separated with a method modified after Garrido and Zapata (1993): The solvents used were methanol : 1 M ammonium acetate : acetonitrile (50:20:30, v/v, Solvent A) and acetonitrile : ethyl acetate (50:50, v/v, Solvent B). The gradient system used was as follows: 0 min: A: 90%, B: 10%; 2 min: A: 90%, B: 10%; 26 min: A: 40%, B: 60%; 28 min: A: 10%, B: 90%; 30 min: A: 10%, B: 90%. The composition of the solvents was returned to initial conditions over a 1 min gradient, followed by 2 min of system re-equilibration before the next sample was injected. The flow rate was 1 ml min^{-1}.

Absorbance was recorded in the PDA from 350 to 700 nm. Pigments were identified by the retention times and the absorption spectra, which were obtained from previous measurements of the pure pigment standards. Peak areas were integrated at 436 nm and corrected for internal standard. For the quantification of the pigments, calibration curves were estimated by measuring at least five different amounts of each pigment standard in triplicates and fitting a linear regression between the amount of the pigment and the observed peak area at 436 nm. Limit of detection and limit of quantification were determined as described in Hooker et al. (2005).

Based on phytoplankton groups usually present in the examined lakes (data from long-term monitoring), ten pigment standards were chosen, of which nine were obtained from DHI Water (Hoersholm, Denmark): alloxanthin (marker pigment for cryptophytes), β-carotene, Chl a, chlorophyll b (marker pigment for chlorophytes), diatoxanthin, echinenone (marker pigment for cyanobacteria), fucoxanthin (marker pigment for chrysophytes and diatoms), lutein (another marker pigment for chlorophytes) and zeaxanthin (usually used as the only marker pigment for cyanobacteria (Havskum et al. 2004, Lewitus et al. 2005, Llewellyn et al. 2005),

but also shared with other groups like chlorophytes). Peridinin (marker pigment for dinoflagellates, extracted from *Symbiodinium spp*. following the protocol from Rogers and Marcovich 2007) was kindly provided by D. Langenbach from the group of M. Melkonian at the University of Cologne. With the solvent gradient described above, all pigment peaks could be separated to the baseline except for lutein and zeaxanthin. Although well separated, diatoxanthin was excluded from the subsequent CHEMTAX analysis as it was detected in very low amounts and only in few samples. Also, β-carotene was excluded as it did not have any effect on the output data (previous CHEMTAX runs, data not shown).

CHEMTAX and data analysis

The recently published (Schlüter et al. 2016) pigment : Chl a ratio matrices had been established for lakes from oligotrophic to eutrophic status, including the three lakes from this study. These ratio matrices should thus be highly suitable for our study and were therefore used to calculate the contribution of six phytoplankton groups (chlorophytes, cryptophytes, cyanobacteria, chrysophytes, diatoms and dinoflagellates) to the total Chl a via CHEMTAX (Mackey et al. 1996; version 1.95 provided by S. Wright).

For CHEMTAX calculations, 60 different ratio matrices were generated from the initial ratio matrices (separately for the oligotrophic lake and for the meso-oligotrophic/mesotrophic lakes). 10 % (n = 6) of the matrices with the lowest residual root mean square (RMS) were averaged and used as new input ratio matrices. Runs were repeated using final ratio matrices from every previous run as input ratio matrix for the next run. This was repeated until the ratios became stable. For details on this procedure, see Latasa (2007) and Higgins et al. (2011). Parameters used within CHEMTAX were set as recommended by Mackey et al. (1996) and S. Wright (pers. comm.): ratio limits: 500 (this allowed initial pigment ratios to vary from r/6 to 6r, in total a 36-fold change), weighting: bounded relative (error by pigment, see Latasa 2007), iteration limit: 100, epsilon limit: 0.0001, initial step size: 10, step ratio: 1.3, cutoff step: 100, elements varied: 5, subiterations: 1, weight bound: 30. For explanations, see Mackey et al. (1996).

Subsequently, the HPLC derived pigment concentrations and the CHEMTAX derived biomasses of the phytoplankton groups (in units of Chl a) were used to calculate Shannon-Diversity Indices (1) as estimates of pigment and phytoplankton diversity (Shannon and Weaver, 1949) with the following equation:

$$H' = -\sum p_i \times \ln(p_i) \quad (1)$$

with p_i being the proportion of the pigment or phytoplankton class relative to the total amount of the pigments or the total biomass, respectively.

Shannon-Diversity Indices were calculated from the CHEMTAX data, following two approaches: first, the contributions of chrysophytes, diatoms and dinoflagellates to the total Chl a were summed up to one single data point per sample, to be comparable to biomass estimates for the brown group as assigned by the AlgaeLabAnalyser. Thereby, we were able to calculate and compare the Shannon-Indices based on the biomass estimates of the four groups (chlorophytes, chromophytes, cryptophytes and cyanobacteria) from both methods (spectrofluorometrically and chromatographically estimated biomass). The second approach was to calculate the Shannon-Indices using the biomass estimates for all six phytoplankton groups, as CHEMTAX was able to discriminate between the subgroups of the chromophytes (see above).

To compare the biomass (given as total Chl a, in the following abbreviated as TChl a) and biodiversity estimates from the AlgaeLabAnalyser with those from HPLC and CHEMTAX, we estimated the Spearman correlation coefficient, r_s, as the data were not normally distributed. Additionally, the ratio between the estimates from the AlgaeLabAnalyser and CHEMTAX was calculated: $R_{LAB/CHEM}$. The ratios and the biodiversity estimates were tested for normality with the Shapiro-Wilk test, while the homogeneity of variances was tested with Levene's test. One-Way ANOVAs were performed for all four phytoplankton groups, with the ratio $R_{LAB/CHEM}$ as the dependent variable and trophic status of the lakes as the independent variable, followed by the post-hoc Tukey HSD-test ($\alpha = 0.05$). Alternatively, when the data was not normally distributed and variances were heterogeneous, the nonparametric Kruskal-Wallis test was applied on both the ratios $R_{LAB/CHEM}$ and the biodiversity estimates, followed

by the post-hoc Dunn's test. All calculations, statistics and figures were performed in R (version 3.3.1, R Core Team, 2016), using the packages agricolae (version 1.2.4, de Mendiburu 2016), car (version 2.1.3, Fox and Weisberg 2011), ggplot2 (version 2.1.0, Wickham 2009), PMCMR (version 4.1, Pohlert 2014) and vegan (version 2.4.1, Oksanen et al. 2016).

3. Results

3.1 Directed diversity manipulations of natural phytoplankton communities

Diversity manipulation by dilution

Manipulation of primary producer communities by dilution resulted in distinct diversity gradients in both, the oligotrophic and the eutrophic pond (Fig. 1). After 17 days a diversity gradient following a nonlinear, logarithmic regression between dilution and species richness had formed in phytoplankton communities from both ponds. These gradients could also be found one week later at day 24. In the oligotrophic pond (day 17) per each experimental step of dilution on average 2.59 species more were found in the treatments (Fig. 1a, Table 1). A similar relationship was seen at day 24 (Fig. 1b, Table 1). While the lowest dilution for the oligotrophic pond resulted in a species richness between 4 and 9, the eutrophic treatments at the same dilution had 9 to 18 species. Although on a different level of species richness, the eutrophic pond showed the same pattern between dilution and species richness as the oligotrophic pond. After 17 days there were around 2.16 species difference between two exponential dilution steps (Fig. 1c, Table 1). A similar relationship was also seen at day 24 (Fig. 1d, Table 1).

Table 1: Species richness gradients - regression results for figures 1 - 3.

pond	treatment	day of exp.	n	p	R^2	df	equation	Fig.
BZ	dilution	17	16	< 0.0001	0.68	15	$y = 13.69 + 2.59 \times \log(x)$	1a
BZ	dilution	24	16	0.0018	0.51	15	$y = 11.33 + 1.47 \times \log(x)$	1b
IZB	dilution	17	16	0.0046	0.45	15	$y = 17.85 + 2.16 \times \log(x)$	1c
IZB	dilution	24	16	0.0006	0.59	15	$y = 21.42 + 2.88 \times \log(x)$	1d
BZ	disturbance	39	18	0.0088	0.47	17	$y = 31.17 \times e^{(-0.5 \times ((x-5.75)/6.22)^2)}$	2c
IZB	disturbance	25	18	< 0.0001	0.67	17	$y = 27.35 + 1.79 \times x$	3b
IZB	disturbance	39	18	< 0.0001	0.73	17	$y = 23.27 + 30.59 \times x / (4.06 + x)$	3c

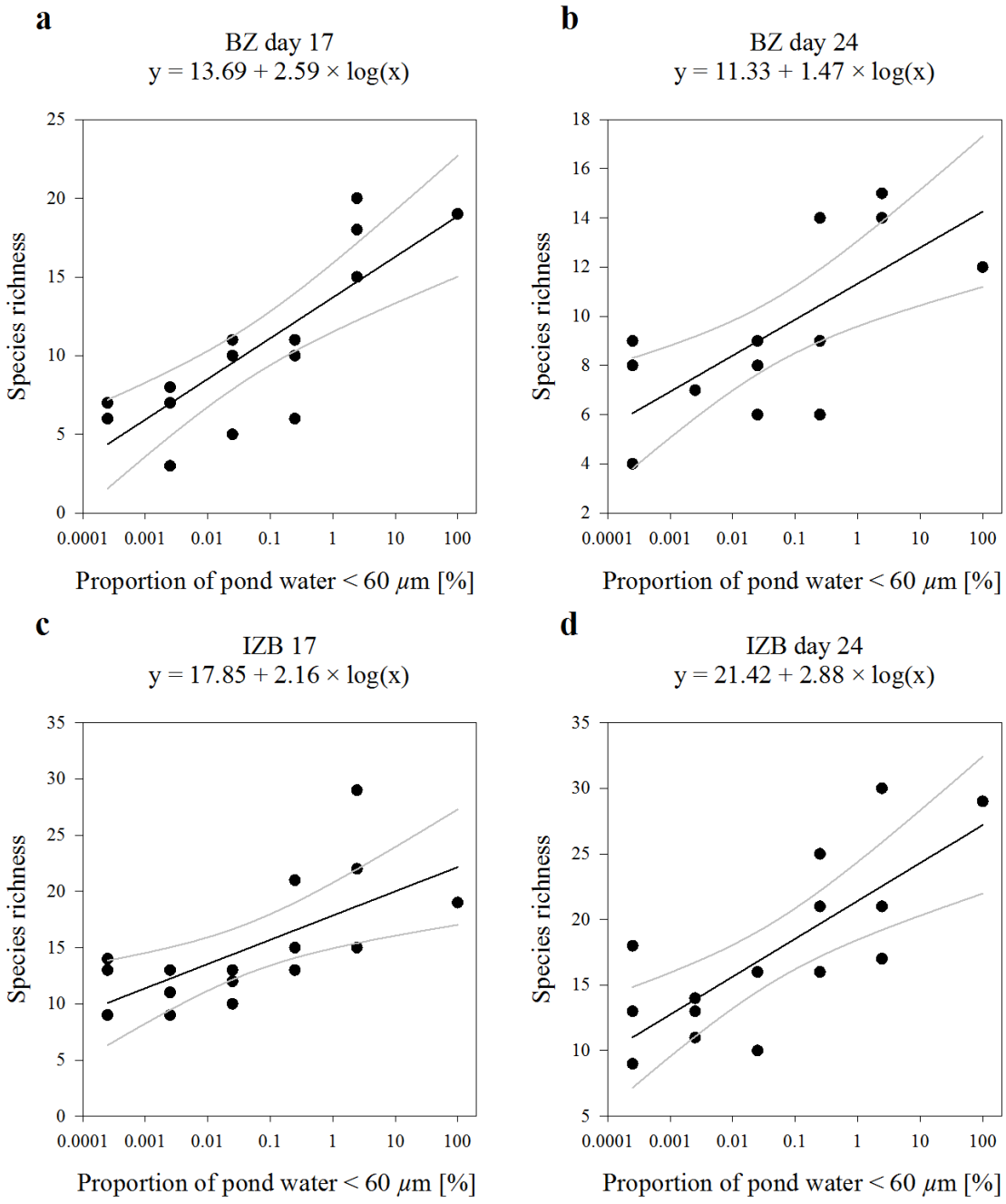

Figure 1: Species richness versus the proportion of pond water (< 60 µm) [%] at days 17 and 24 (in treatments with oligotrophic pond BZ and eutrophic pond IZB communities). Gray lines indicate 95 % confidence bands. a) Pond BZ at day 17. b) Pond BZ at day 24. c) Pond IZB at day 17. d) Pond IZB at day 24. For regression results see Table 1.

Diversity manipulation by disturbance

In the treatments originating from the oligotrophic pond we observed a distinct diversity gradient at day 39 of the experiment. After 11 and 25 days no distinct pattern between disturbance frequency and species numbers could be found (Fig. 2a and b). At day 39 however, species numbers showed a hump-shaped response to disturbance frequency. Moderate frequencies of three and five times per week resulted in the highest species numbers (Fig. 2c, Table 1).

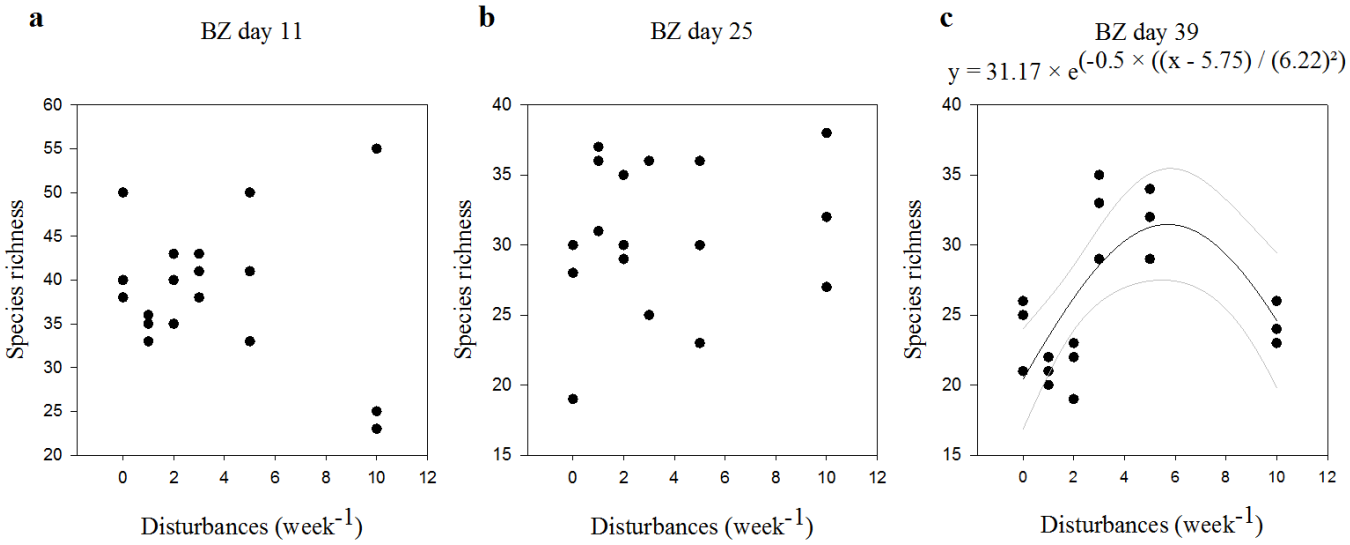

Figure 2: Species richness at days 11, 25 and 39 versus disturbance frequency in the oligotrophic pond BZ. a) Pond BZ at day 11. b) Pond BZ at day 25. c) Pond BZ at day 39, gray lines indicate the 95 % confidence bands. For regression results see Table 1.

In the eutrophic pond treatments a diversity gradient was visible after 25 days. At day 11 no distinct diversity pattern could be observed (Fig. 3a). At day 25 the diversity gradient followed a linear relationship between disturbance frequency and species richness. Each additional weekly disturbance event resulted on average in a diversity increase of about 1.8 species. (Fig. 3b, Table 1. After 39 days of disturbances a diversity gradient was still visible, following a hyperbolic function (Fig. 3c, Table 1).

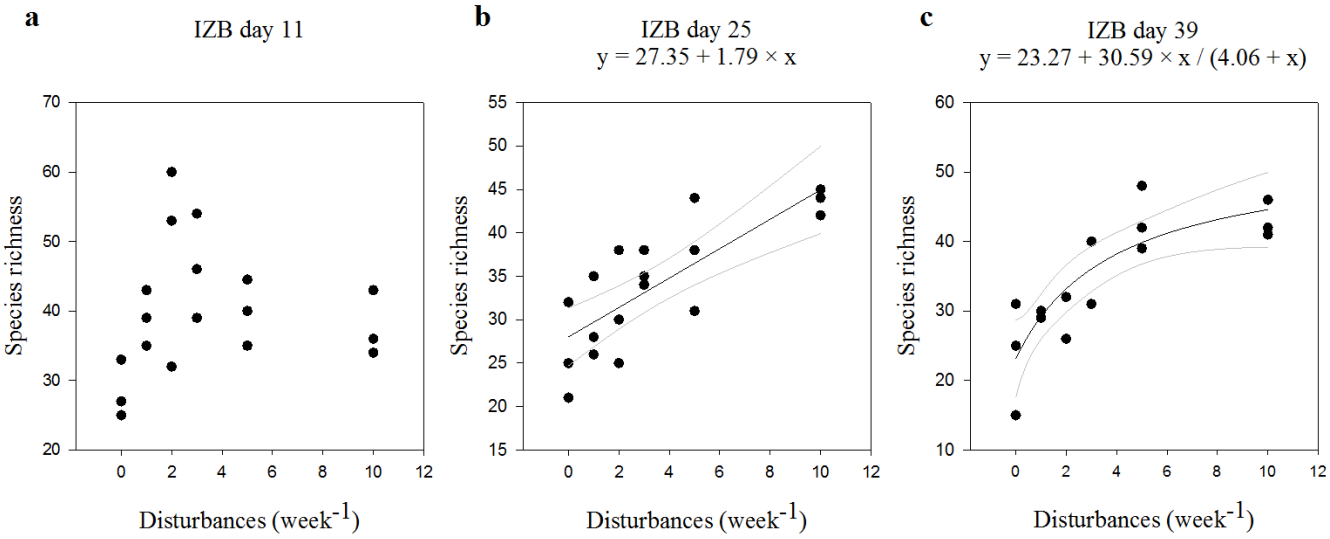

Figure 3: Species richness at days 11, 25 and 39 versus disturbance frequency for the eutrophic pond IZB; gray lines represent 95 % confidence bands. a) Pond IZB at day 11. b) Pond IZB at day 25. c) Pond IZB at day 39. For regression results see Table 1.

Similarity among treatment replicates

In the dilution experiment, community similarity among treatments changed with the different dilution steps. The high dilution treatments containing the lowest amount of 60 μm filtrate showed lower similarity than those at lower dilution steps. The treatments from the eutrophic pond showed a significant increase of similarity among replicates with decreasing dilution (Fig. 4b, Table 2) while the oligotrophic pond showed the same pattern not significant at 5% significance level (Fig. 4a, Table 2). Additionally, the variation in community similarity among replicates of the same dilution treatments gets smaller with decreasing dilution in the oligotrophic pond treatments (logarithmic regression of coefficients of variation of similarity values of each dilution level, $y = 0.06 - 0.15 \times \log(x)$, $n = 5$, $R^2 = 0.98$, $p = 0.0014$, $df = 4$).

Within disturbance treatments, no clear pattern between manipulations and community similarities could be found. Only the treatments from the eutrophic pond showed a linear increase of similarity between treatments with higher disturbance frequency (Fig. 4d, Table 2). In the treatments from the oligotrophic pond we could not find such a relationship between disturbances and community similarities (Fig. 4c).

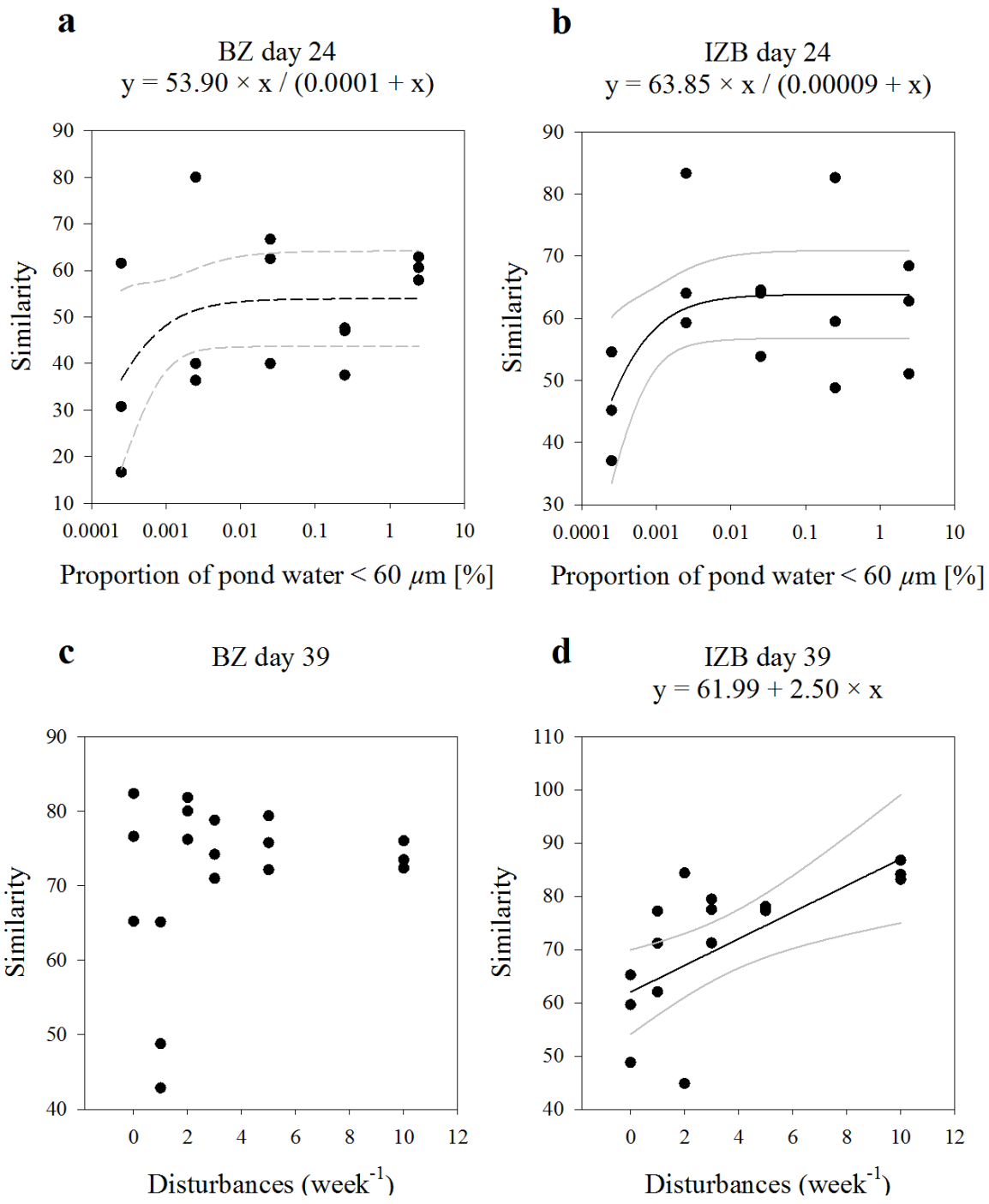

Figure 4: Similarity values (%) of species presence/absence data of all possible pairwise comparisons between the three replicates of each treatment in both ponds, gray lines indicating 95 % confidence intervals. a) Pond BZ at day 24 in the dilution experiment. b) Pond IZB at day 24 in the dilution experiment. c) Pond BZ at day 39 of the disturbance experiment. d) Pond IZB at day 39 of the disturbance experiment. For regression results see Table 2.

Table 2: Similarity - regression results for figure 4.

pond	treatment	day of exp.	n	p	R^2	df	equation	Fig.
BZ	dilution	24	15	0.12	0.18	14	$y = 53.90 \times x / (0.0001 + x)$	4a
IZB	dilution	24	15	0.031	0.31	14	$y = 63.85 \times x / (0.00009 + x)$	4b
IZB	disturbance	39	18	0.006	0.39	17	$y = 61.99 + 2.5 \times x$	4d

3.2 Diversity gradients and nutrient enrichment – comparing effects of natural diversity differences with effects of short-term experimental manipulations of diversity

In all three lakes and during both years, manipulation of phytoplankton communities by disturbance led to diversity gradients spanning six to 21 genera between lowest and highest diversity communities in each lake (Fig. 1). These communities were the basis for the second part of the experimental setup concerning nutrient enrichment.

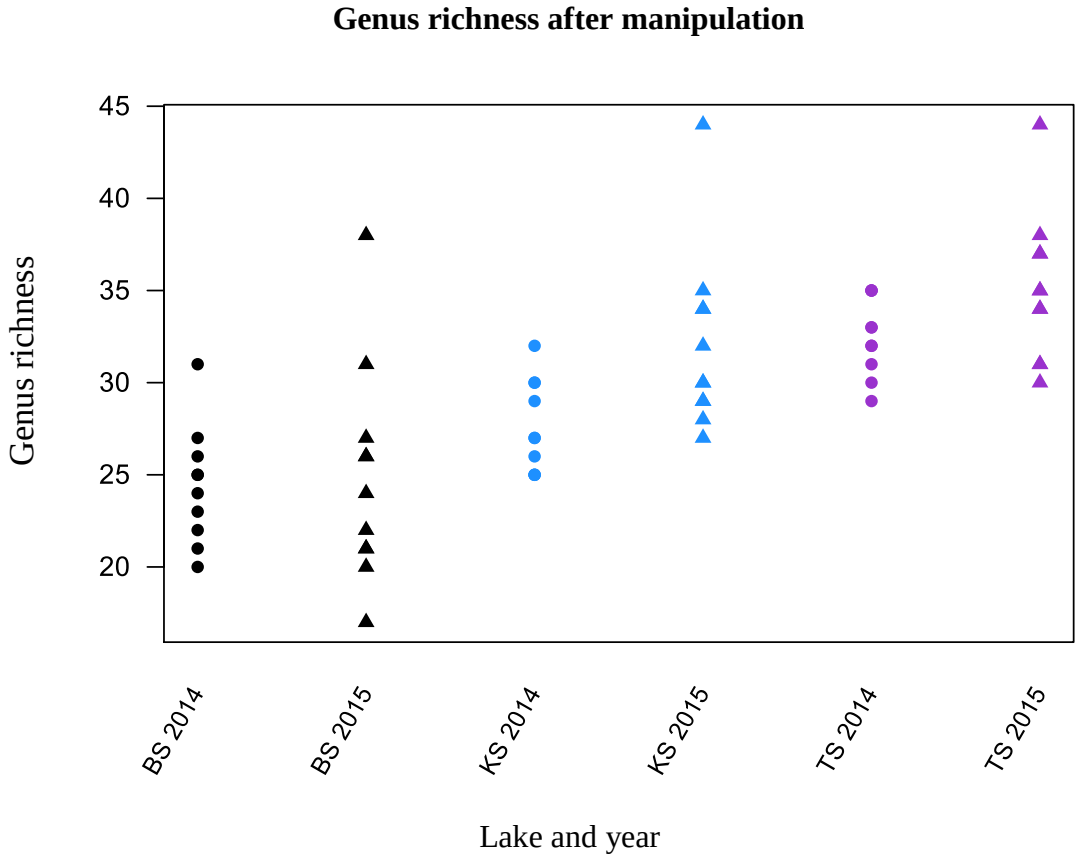

Figure 1: Genus richness of all communities after the disturbance phase and before nutrient enrichment in the lab started (black = BS, blue = KS, purple = TS, circles = 2014, triangles = 2015)

Community growth responses

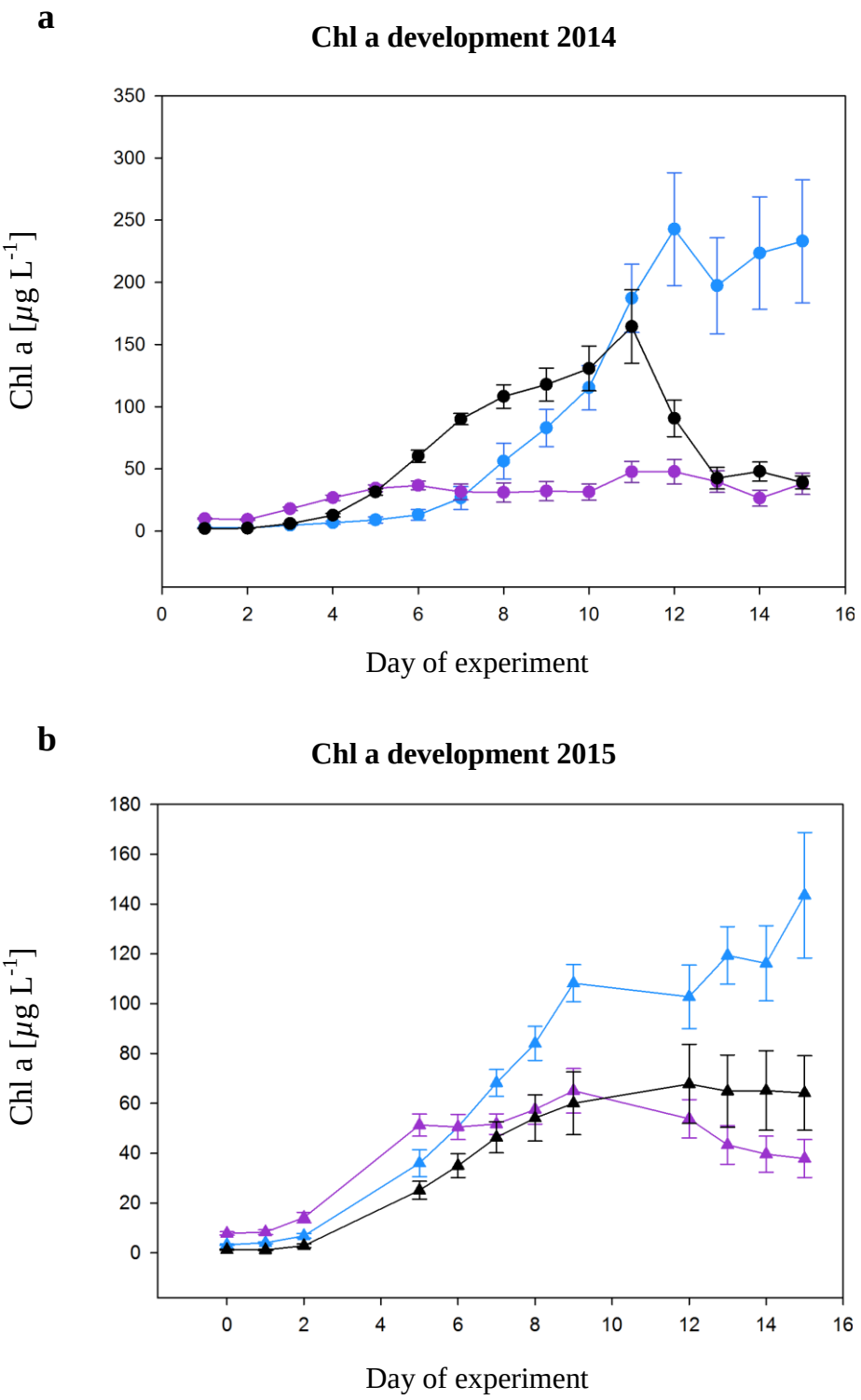

Figure 2: Chl a (μg L^{-1}) development during two weeks of nutrient enrichment in a) 2014 and b) 2015 (mean values of all communities from one lake are shown with standard error of the mean; black = BS, blue = KS, purple = TS, circles = 2014, triangles = 2015)

During the two weeks of nutrient enrichment phytoplankton communities overall showed enhanced biomass production (shown as Chl a content in μgL^{-1}) although communities originating from different lakes tended to respond on different scales (Fig. 2). Communities from the meso-oligotrophic lake showed the highest growth responses while Thalersee (mesotrophic) communities developed less pronounced responses on lower Chl a levels and with lower deviation amongst the different sample communities. Compared to the other two lakes, I could observe intermediate growth responses in the communities originating from oligotrophic Lake Brunnensee. During the experiment, some communities already showed a peak and subsequent decline in Chl a concentrations, indicating a breakdown of the phytoplankton community.

Community stability

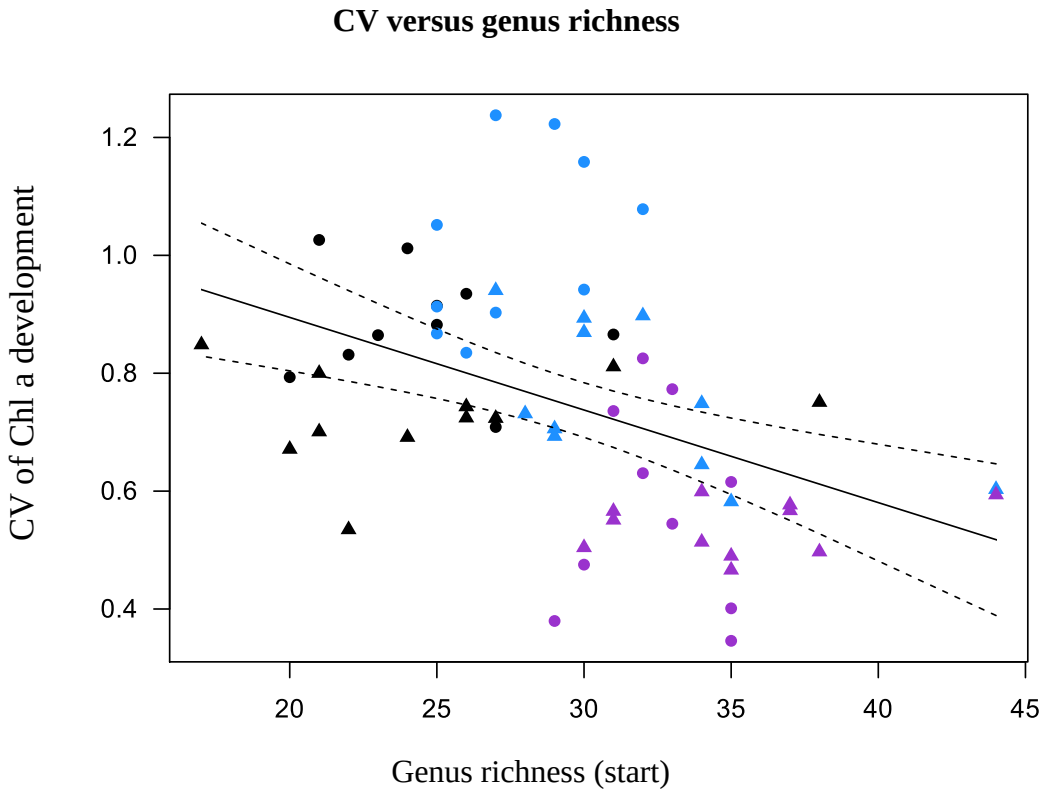

Figure 3: Coefficient of variation of the communities' Chl a development during nutrient enrichment versus initial genus richness (linear regression: $y = 1.21 – 0.016 \times x$, $R^2 = 0.19$, $p = 0.0003$, $n = 63$, $df = 62$, dashed lines indicate 95 % confidence interval; black = BS, blue = KS, purple = TS, circles = 2014, triangles = 2015)

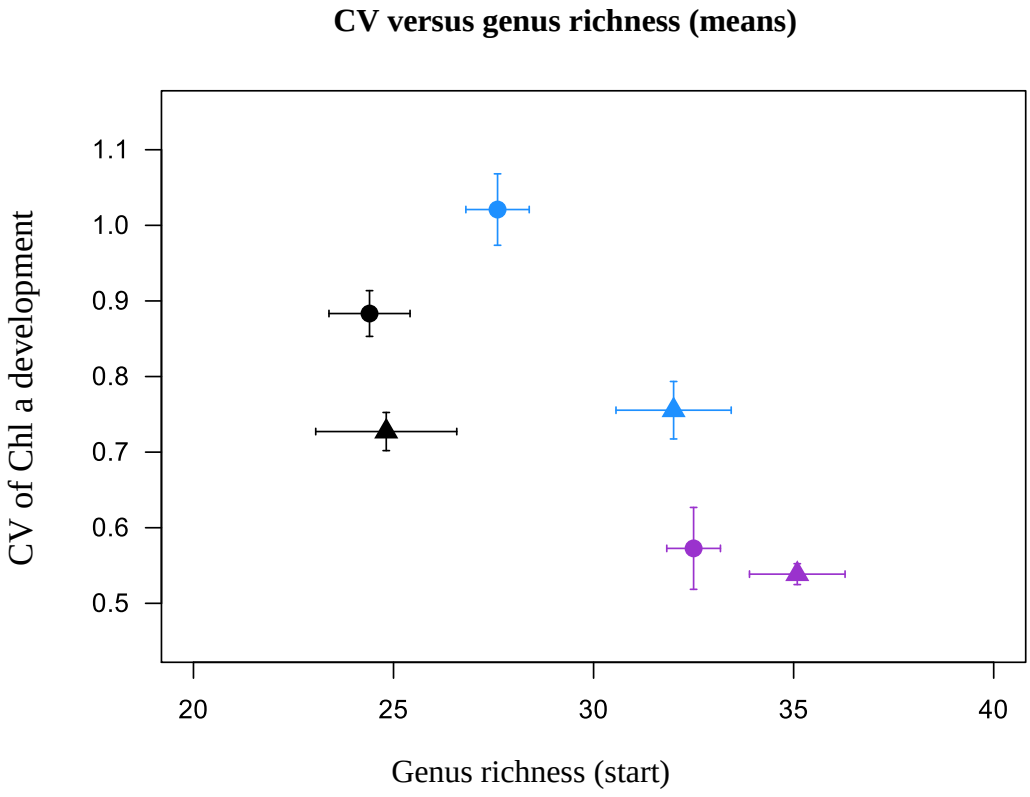

Figure 4: Coefficient of variation of the communities' Chl a development during the nutrient enrichment versus initial genus richness, means of all samples from one lake and one year (error bars depict the standard error of the mean of each group; black = BS, blue = KS, purple = TS, circles = 2014, triangles = 2015)

As an indicator for stability, the coefficient of variation of Chl a development during nutrient addition was calculated. Pooled analyses of all samples display a negative linear relationship between the coefficient of variation and the genus richness of a community at the start of the nutrient enrichment phase (Fig. 3, $y = 1.209 – 0.016 \times x$, $n = 63$, $R^2 = 0.19$, $p = 0.0003$, $df = 62$). Thus, with higher genus richness in the phytoplankton community, stability - in terms of a decreasing coefficient of variation – increases. Nonetheless, the samples group together by their respective lake of origin and the year in which the experiment took place (Fig. 3). When computing means of all samples from one lake in one year, the mesotrophic communities with the highest starting diversity also exhibited the lowest coefficient of variation and with that the highest stability (Fig. 4). The oligotrophic lake (black, Fig. 4) with the lowest genus richness in the beginning shows higher values of the coefficient of variation while the meso-oligotrophic lake communities seemed to react differently in the two years of the experiment.

The linear mixed effects analysis addressing the relationship between the coefficient of variation as response variable and genus richness lead to a model including initial genus richness as fixed effect and lake and year as random effects. Thereby lake (χ^2 (df = 1) = 30.13, $p < 0.0001$) and year (χ^2 (df = 1) = 11.67, p = 0.00064) affected the coefficient of variation in addition to the start genus richness (as discussed above). This suggests that, apart from the genus richness, the lake of origin and the time the experiment took place influence the stability during nutrient enrichment. Thereby the influence of the lake "identity" seemed to be stronger than that of the experimental year. When analyzing the samples separately for each lake and year with regressions, I did not find overall comparable patterns but different, mostly non-significant reactions within each lake (see colors and symbols in Fig. 3).

Community integrity

When looking at the relative genus loss in the samples that occurred during the nutrient enrichment phase (Fig. 5), the higher diverse communities relatively lost fewer genera then communities with an already lower diversity in the beginning. Taking absolute loss values instead, there was no apparent difference between the samples (data not shown). A one way ANOVA of the relative genus losses between the lakes showed significant differences between the mean values of the different groups ($F(5,62) = 3.6$, $p = 0.007$). Specifically the datasets of Lake Klostersee depicted the highest differences between 2014 and 2015 when compared pairwise. A one way ANOVA of the absolute genus losses between the lakes on the contrary did not show any significant differences between the 6 groups.

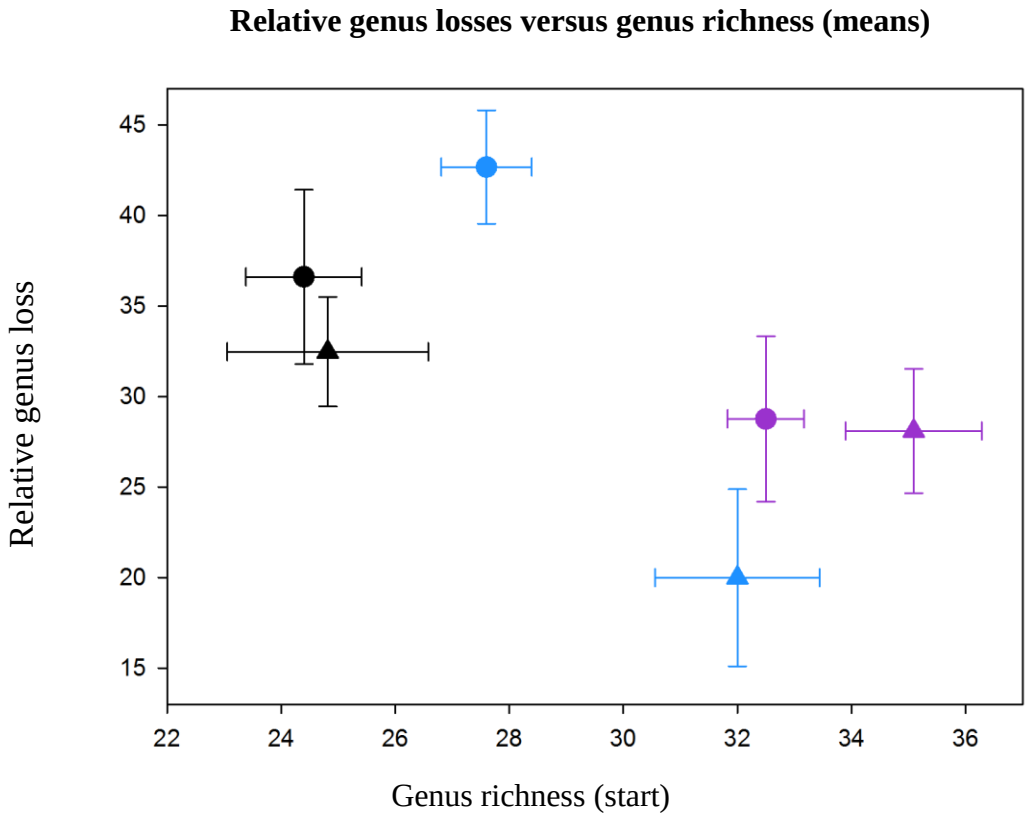

Figure 5: Relative genus losses during the nutrient enrichment versus initial genus richness, means of all samples from one lake and one year (error bars depict the standard error of the mean of each group; legend to symbols: black = BS, blue = KS, purple = TS, circles = 2014, triangles = 2015)

Similarity between the samples from beginning and end of the nutrient enrichment phase increased with rising initial genus richness (Fig. 6, linear regression: $y = 32.5 + 0.65 \times x$, $n = 63$, $R^2 = 0.11$, $p = 0.0068$, $df = 62$). Again, this was observed when analyzing the pooled data from all lakes and years together, single lakes showed varying responses. Linear mixed effects analysis addressing the relationship between similarity as response variable and genus richness lead to a model including the same variables as already in the coefficient of variation model. Lake (χ^2 ($df = 1$) = 7.29, $p = 0.0069$) and year (χ^2 ($df = 1$) = 17.95, $p < 0.0001$) affected the similarity in addition to genus richness at start (see results for Fig. 6). Opposing the first model for CVs, the influence of the year seemed to be stronger than that of the lake identity in this analysis.

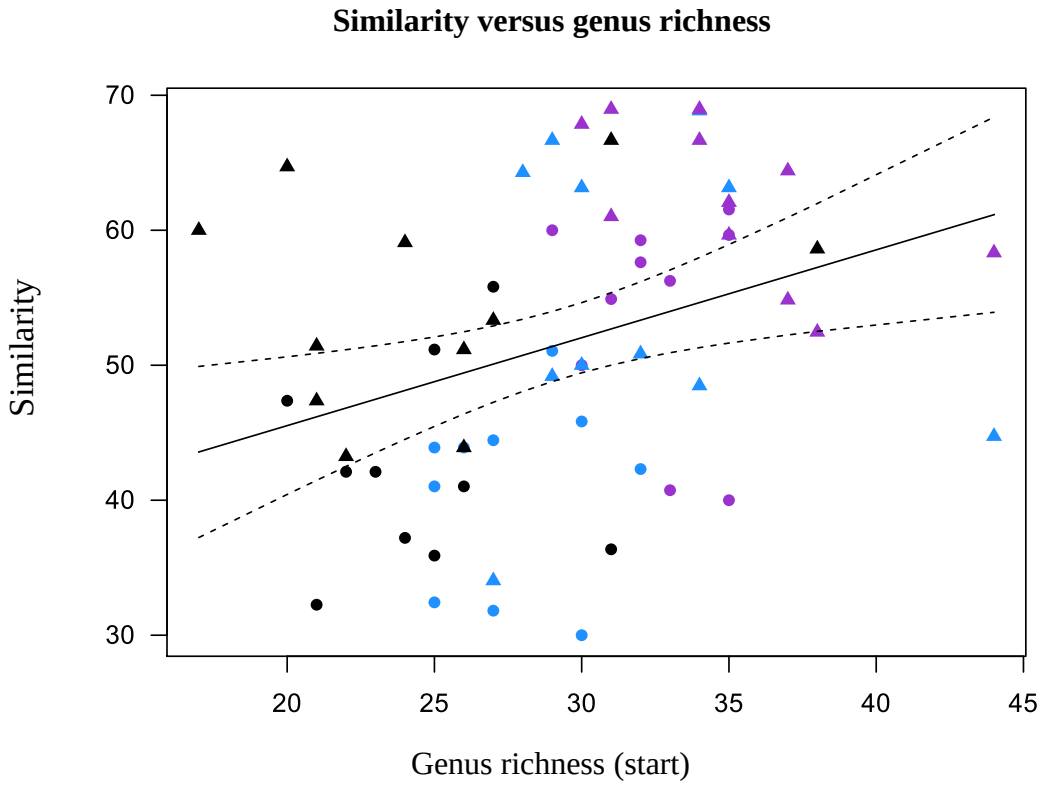

Figure 6: Similarity between the communities at start and end of the nutrient enrichment of each sample versus initial genus richness (linear regression: $y = 32.5 + 0.65 \times x$, $R^2 = 0.11$, $p = 0.0068$, $n = 63$, $df = 62$, dashed lines indicate 95 % confidence interval; black = BS, blue = KS, purple = TS, circles = 2014, triangles = 2015)

Consistent with the results for similarity, the species-exchange ratios between beginning and end showed a significant decline with higher starting genus richness (Fig. 7, $0.82 – 0.006 \times x$, $R^2 = 0.11$, $p = 0.0072$, $n = 63$, $df = 62$) in the pooled analysis. Again, single lakes showed varying responses. Similar to the linear mixed effects analysis for similarity indices, the analysis for species-exchange ratios resulted in a model including lake and year as random effects with year (χ^2 ($df = 1$) = 18.2, $p < 0.0001$) stronger impacting species-exchange ratios than lake (χ^2 ($df = 1$) = 7.26, $p = 0.007$).

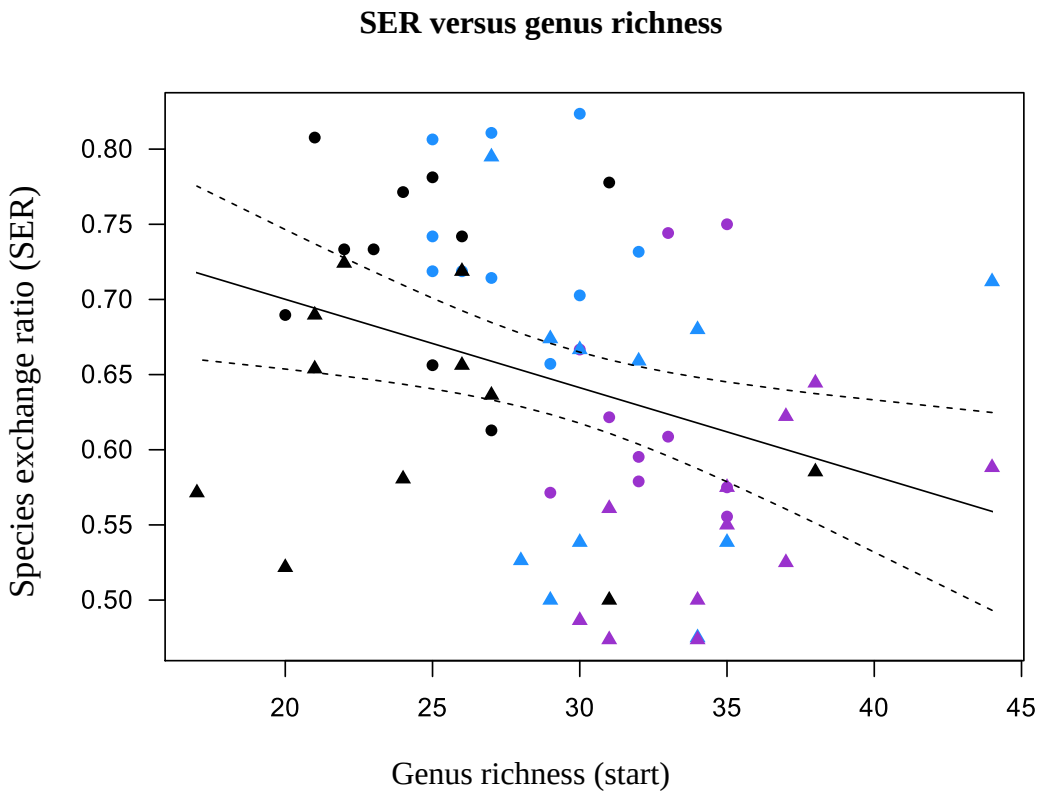

Figure 7: Presence-absence based species exchange ratio (SER) between the communities at start and end of the nutrient enrichment of each sample versus initial genus richness (linear regression: $y = 0.82 - 0.006 \times x$, $R^2 = 0.11$, $p = 0.0072$, $n = 63$, $df = 62$, dashed lines indicate 95 % confidence interval; legend to symbols: black = BS, blue = KS, purple = TS, circles = 2014, triangles = 2015)

3.3 Group specific trait losses from phytoplankton communities

Disturbance treatments in the mesocosms resulted in different phytoplankton communities between replicates. These different communities exhibited varying proportions of diatoms to total phytoplankton quantity. While the lakes themselves had diatom proportions of about 5 - 20 % (initially, at start of experiment), the manipulated enclosures ranged from 15 – 80 % diatom content (relating to biovolume; calculated after microscopic counting).

With an increasing abundance of diatoms in the experimental mesocosms (microscopic counting) the portion of fucoxanthin in relation to chlorophyll a (Chl a; pigment analysis) rose significantly (Fig. 1, linear regression, $y = 0.13 + 0.10 \times x$, $n = 63$, $R^2 = 0.19$, $p = 0.00033$, $df = 62$).

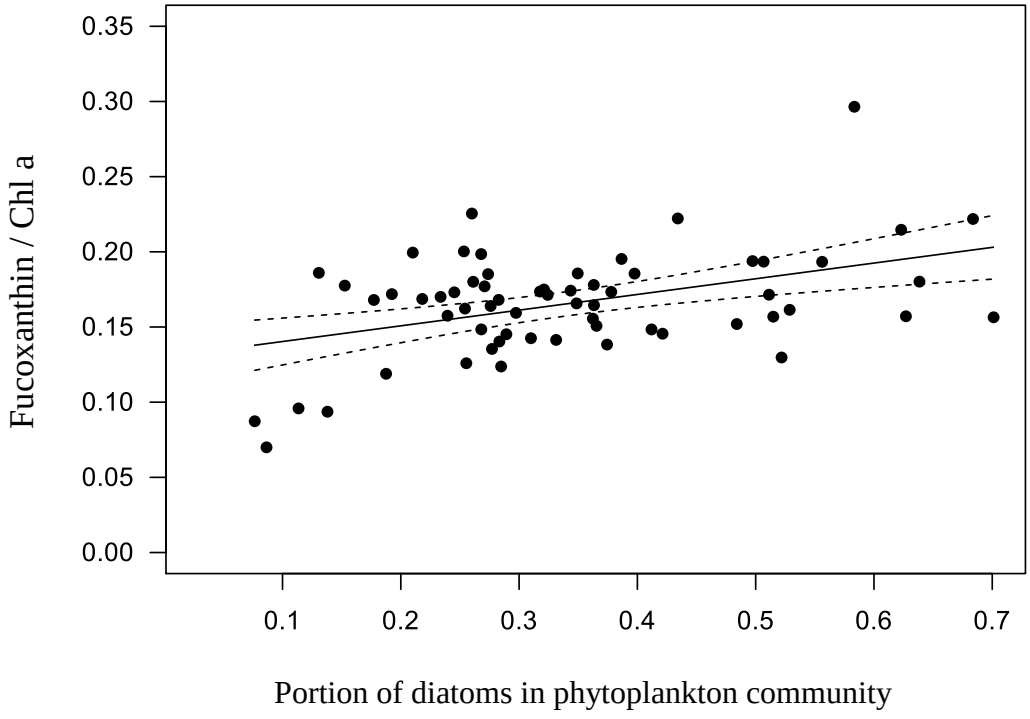

Figure 1: Fucoxanthin to Chl a ratio (pigment analysis) versus portion of diatom volume within the phytoplankton community (linear regression: $y = 0.13 + 0.10 \times x$, $R^2 = 0.19$, $p = 0.00033$, $n = 63$, $df = 62$, dashed lines indicate 95 % confidence interval)

Depending on the lake of origin of the different phytoplankton communities, different proportions of diatoms in the community could be found at diverse range of Chl a biomass. Noticeable is thereby, that communities from Lake Brunnensee span a range of diatom proportions while at the same time staying at relatively constant Chl a values per liter. Both, communities from Klostersee as well as Thalersee, show higher overall biomasses (Chl a values) and span a wider range of biomass content with varying diatom proportions (Fig. 2). To measure and compare community-dependent light attenuation, systems with similar biomass and thereby less confounding effects are of advantage. Such a situation is given in Lake Brunnensee, therefore communities from that lake were chosen for analysis of their light attenuation in dependence of diatom content.

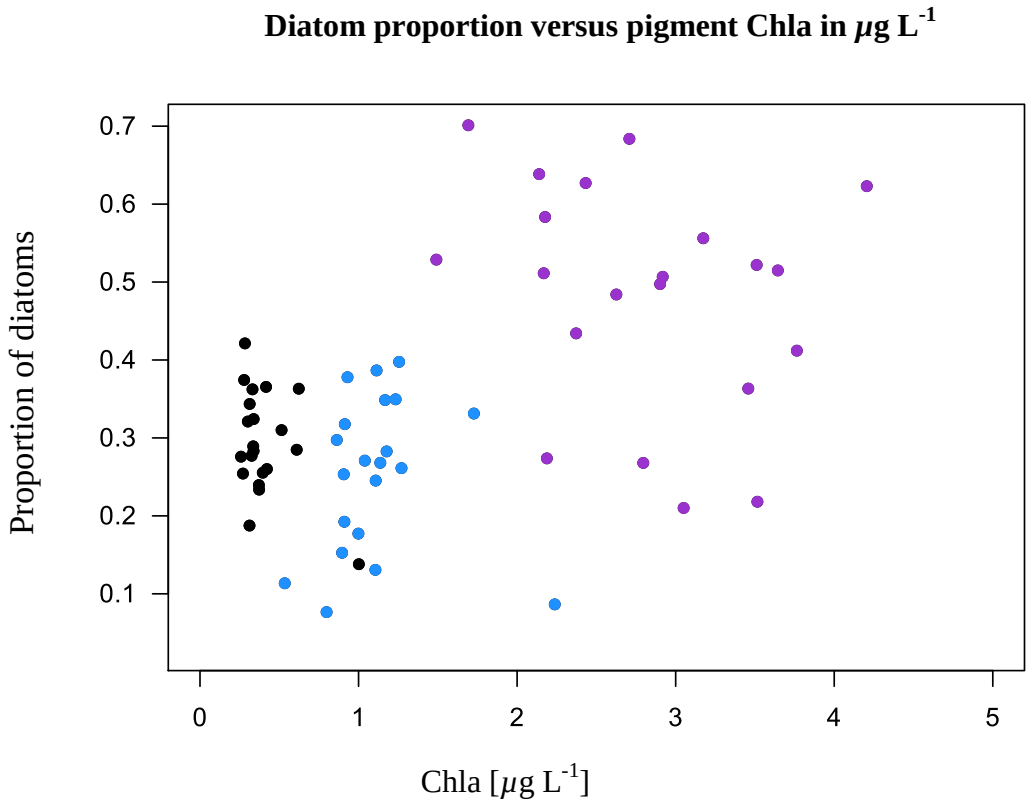

Figure 2: Proportion of diatoms in phytoplankton community versus Chla content [$\mu g \times L^{-1}$] (pigment analysis); black: mesocosms in Brunnensee, blue: mesocosms in Klostersee, purple: mesocosms in Thalersee

Additional to an increase of the pigment fucoxanthin relative to Chl a, the light absorbed by the water column containing the phytoplankton communities changed. Representing the absorbance maximum of fucoxanthin, the portion of light absorbed between 500 and 540 nm was divided by the whole of absorbed light between 325 and 790 nm. This ratio also increased significantly with a rising proportion of diatoms in the communities from Lake Brunnensee (Fig. 3, nonlinear regression, $y = 0.157 \times x / (0.001 + x)$, $n = 43$, $R^2 = 0.38$, $p < 0.0001$, $df = 41$)

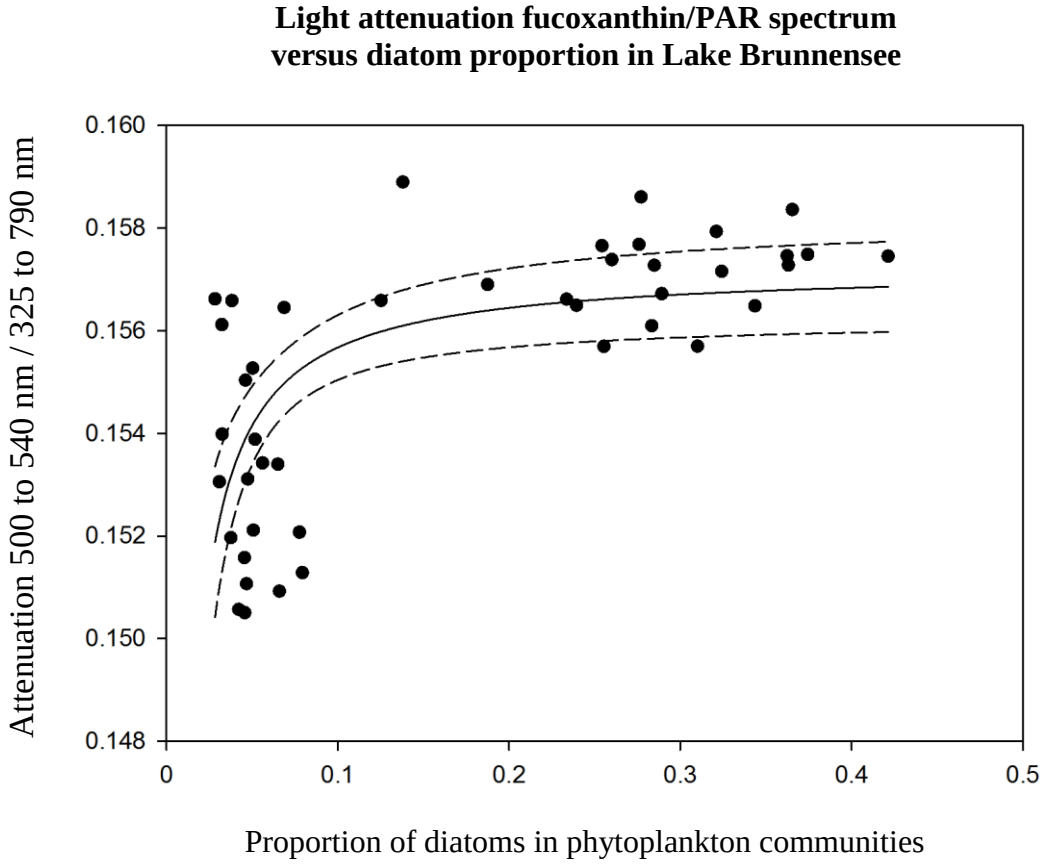

Figure 3: Light attenuation: ratio of 500 – 540 nm to 325 – 790 nm versus proportion of diatoms in phytoplankton communities originating from Lake Brunnensee (nonlinear regression: $y = 0.157 \times x / (0.001 + x)$, $R^2 = 0.38$, $p < 0.0001$, $n = 43$, $df = 41$, dashed lines indicate 95 % confidence interval)

Considering the fate of the light absorbed by different communities, Chl a fluorescence was examined in communities from all three lakes. Thereby the F0 of the photosystem II (PS II; minimal fluorescence; emitted with all reaction centers open) ratio of the mean of 505 nm and 530 nm to 455 nm was calculated to depict the light absorption allotted to the fucoxanthin

absorption maximum compared to the maximum value of fluorescence usually gained at 455 nm (Chla). These ratios also showed a significant positive relationship with increasing diatom content in the phytoplankton communities (Fig. 4, linear regression, $y = 0.24 + 0.05 \times x$, $n = 126$, $R^2 = 0.03$, $p = 0.049$, $df = 125$).

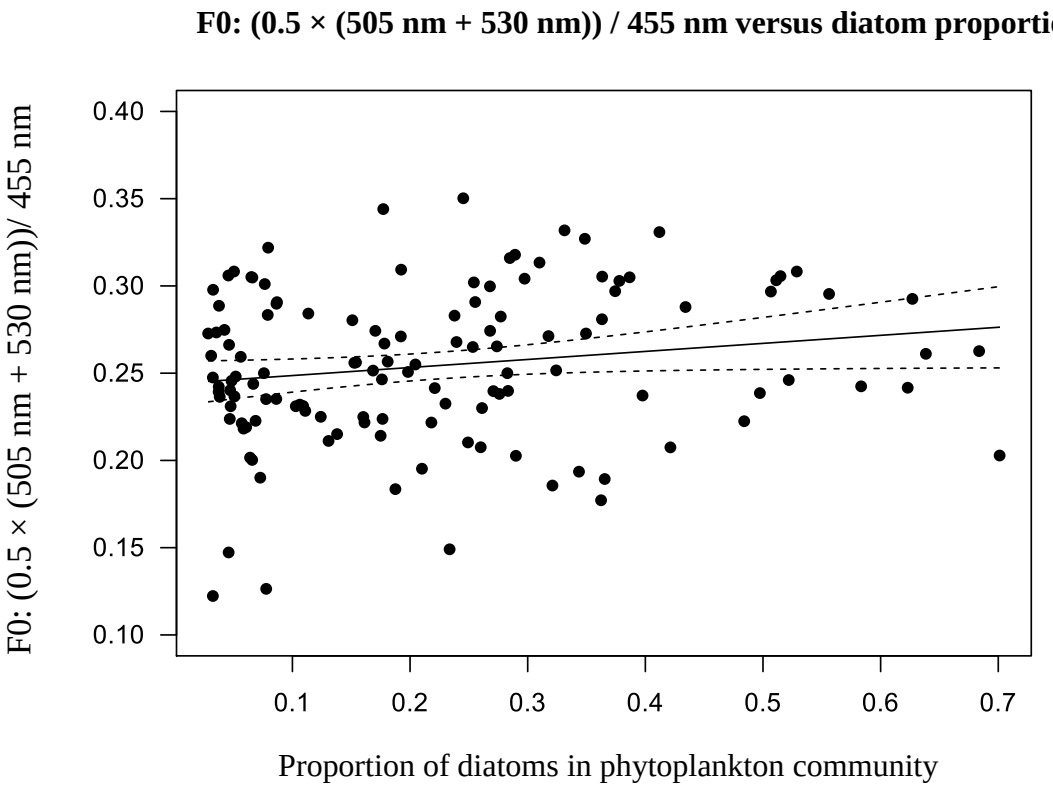

Figure 4: Chl a fluorescence: F0 (0.5 × (505 nm + 530 nm)) / 455 nm versus proportion of diatoms in phytoplankton community (linear regression: $y = 0.24 + 0.05 \times x$, $R^2 = 0.03$, $p = 0.04937$, $n = 126$, $df = 125$, dashed lines indicate 95 % confidence interval)

3.4 Comparison of different techniques to assess phytoplankton diversity

Pigment composition (HPLC)

The most abundant accessory pigment (all analyzed pigments - Chl a; given as an average over the whole duration of the experiment, including both mesocosms and the lake itself) in the oligotrophic lake was zeaxanthin (37 %), followed by fucoxanthin (36 %), while the relative abundance of alloxanthin (marker pigment for cryptophytes) was even below 0.5 %. In both the meso-oligotrophic and the mesotrophic lake, the most abundant accessory pigment was fucoxanthin (34 % and 30 %, respectively). Also, in both lakes, zeaxanthin (31 % and 18 %) and chlorophyll b (marker pigment for chlorophytes) were found in high relative abundances (zeaxanthin 31 %, 18 %; chlorophyll b 19 % and 22 % respectively). While peridinin (marker pigment for dinoflagellates) was moderately abundant in both the oligotrophic (14 %) and the mesotrophic (16 %) lake, only 1% was found in the meso-oligotrophic lake. The pigment diversity per sample ranged from 0.57 to 1.39 and was on average 1.11.

CHEMTAX final output ratio matrices

The final output ratio matrices from CHEMTAX calculations for all three lakes can be found in Table 1. Both the final output peridinin : Chl a and echinenone : Chl a ratios were lower in all three lakes compared to the input ratios from Schlüter et al. (2016).

Zeaxanthin : Chl a ratios for the cyanobacteria were found to be higher in the output ratio matrices from the oligotrophic and the meso-oligotrophic lake, while the final output zeaxanthin : Chl a and chlorophyll b: Chl a ratios for the chlorophytes were lower compared to the input ratios. In the mesotrophic lake, the opposite was the case.

While fucoxanthin : Chl a ratios between the diatoms and the chrysophytes were similar in both input ratio matrices, the ratios changed during the CHEMTAX calculations: in the oligotrophic lake, the final output fucoxanthin : Chl a ratio for chrysophytes was higher than the fucoxanthin : Chl a ratio for diatoms (0.463 and 0.104, respectively). Interestingly, in both the meso-oligotrophic and the mesotrophic lake, the final output fucoxanthin : Chl a ratio for

chrysophytes was found to be much lower than the fucoxanthin : Chl a ratio for diatoms (0.032 and 0.685 in the meso-oligotrophic lake and 0.044 and 0.399 in the mesotrophic lake, respectively).

Table 1: Final pigment : Chl a ratio matrices after CHEMTAX calculations for each of the lakes: oligotrophic (Brunnsee), meso-oligotrophic (Klostersee) and mesotrophic lake (Thalersee). Allo: alloxanthin, Chl b: chlorophyll b, Echi: echinenone, Fuco: fucoxanthin, Lut: lutein, Peri: peridinin, Zea: zeaxanthin.

	Allo	Chl b	Echi	Fuco	Lut	Peri	Zea
Oligotrophic lake							
Chlorophytes	0	0.276	0	0	0.131	0	0.002
Cryptophytes	0.228	0	0	0	0	0	0
Cyanobacteria	0	0	0.012	0	0	0	0.554
Chrysophytes	0	0	0	0.463	0	0	0.014
Diatoms	0	0	0	0.104	0	0	0.019
Dinoflagellates	0	0	0	0	0	0.340	0
Meso-oligotrophic lake							
Chlorophytes	0	0.264	0	0	0.139	0	<0.001
Cryptophytes	0.162	0	0	0	0	0	0
Cyanobacteria	0	0	0.024	0	0	0	0.538
Chrysophytes	0	0	0	0.032	0	0	<0.001
Diatoms	0	0	0	0.685	0	0	0.002
Dinoflagellates	0	0	0	0	0	0.367	0
Mesotrophic lake							
Chlorophytes	0	0.363	0	0	0.165	0	<0.001
Cryptophytes	0.147	0	0	0	0	0	0
Cyanobacteria	0	0	0.030	0	0	0	0.400
Chrysophytes	0	0	0	0.044	0	0	<0.001
Diatoms	0	0	0	0.399	0	0	<0.001
Dinoflagellates	0	0	0	0	0	0.401	0

Total biomass

The biomasses per sample, given as total chlorophyll a (TChl a), ranged between 0.01 and 11.51 μg TChl a L^{-1}, as determined with the AlgaeLabAnalyser, and between 0.22 and 12.92 μg TChl a L^{-1}, as determined via HPLC (Fig. 1a). Average TChl a per lake was higher when determined with AlgaeLabAnalyser (0.86 in the oligotrophic, 1.27 in the meso-oligotrophic and 3.19 μg L^{-1}in the mesotrophic lake) compared to the values determined via HPLC (0.53, 1.26 and 2.01 μg L^{-1}, respectively). Despite those differences, we found a high positive correlation for the estimated TChl a between the two methods (r_s = 0.82, Table 2) across all three lakes. The ratio $R_{LAB/CHEM}$ for TChl a was 1.47 and differed significantly from the 1:1 relationship (Table 3). The best match between the two methods was found in the meso-oligotrophic lake (Kruskal-Wallis test, $\chi^2_{2,559}$ = 144.57, p < 0.001, Table 4), where the ratio $R_{LAB/CHEM}$ was not significantly different from 1 (value of 1 included in the 95% confidence interval, Table 3).

Comparison TChl a and Phytoplankton diversity with both methods

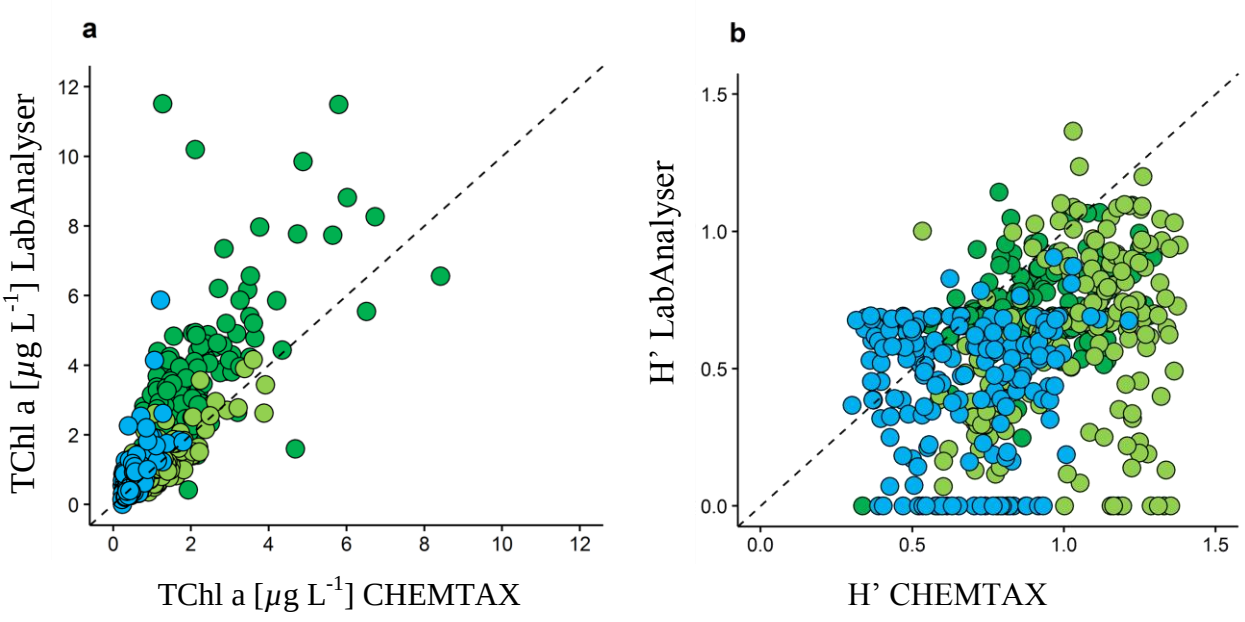

Figure 1: a) TChl a concentration (μg L^{-1}) and b) phytoplankton diversity H' (Shannon-Index) determined spectrofluorometrically *in vivo* with the AlgaeLabAnalyser (y-axis) and chromatographically *in vitro* via HPLC and CHEMTAX (x-axis). The dashed lines represent the 1:1 relationship. Color of the circles represents the trophic state of the lakes, blue: oligotrophic (n=186); light green: meso-oligotrophic (n=187); dark green: mesotrophic (n=189); n in parentheses indicates the number of water samples per lake used in the study.

Table 2: Spearman rank correlation coefficients (rs) between the Chl a concentrations (μg L^{-1}) determined fluorometrically with AlgaeLabAnalyser and chromatographically via HPLC and CHEMTAX estimated across all lakes and for each lake separately; significance levels are indicated with asterisks: * p < 0.05, *** p < 0.001.

Chlorophyll a (μg L^{-1})	All lakes	oligotrophic	meso-oligotrophic	mesotrophic
	r_s (n = 562)	r_s (n = 186)	r_s (n = 187)	r_s (n = 189)
Total	0.82***	0.56***	0.59***	0.71***
Chlorophytes	0.19***	-0.06	0.41***	0.26***
Chromophytes	0.77***	0.66***	0.72***	0.62***
Cryptophytes	0.62***	0.15*	0.33***	0.5***
Cyanobacteria	0.07	0.17*	0	-0.12

Phytoplankton community composition and biodiversity

The phytoplankton communities of all three lakes were strongly dominated by chromophytes, as determined by AlgaeLabAnalyser (given as an average over the whole duration of the experiment, including both mesocosms and the lake itself). Their relative abundance ranged from 55 % in the meso-oligotrophic lake to 76 % in the oligotrophic lake. The second most abundant group in the meso-oligotrophic as well as the mesotrophic lake were cryptophytes (32 % and 23 %, respectively), while the chlorophytes were the second most abundant group in the oligotrophic lake (16 %). Cyanobacteria were found only in very low abundances being even below 2 %.

With CHEMTAX, we were able to differentiate between the subgroups of chromophytes (chrysophytes, diatoms and dinoflagellates) and thus received a higher taxonomical resolution of the phytoplankton community composition as compared to results from the AlgaeLabAnalyser. According to CHEMTAX calculations, in the oligotrophic lake, diatoms were the most abundant phytoplankton group (46 %), followed by cyanobacteria (15 %), dinoflagellates (14 %) and chrysophytes (14 %), while the relative abundance of cryptophytes was below 1 %, as indicated by a very low amount of alloxanthin (0.36 %). In both the meso-

oligotrophic and the mesotrophic lake, we found high relative abundances of chrysophytes (43 % and 36 %, respectively). Indicated by high amounts of zeaxanthin, chlorophyll b and lutein, chlorophytes (22 %) formed the second most abundant phytoplankton group in the meso-oligotrophic lake, followed by cyanobacteria (16 %). In the mesotrophic lake which was dominated by chrysophytes and diatoms (in total 55 %), as indicated by high amounts of fucoxanthin, the other 4 phytoplankton groups were all present in relatively similar abundances, ranging from 8 % (cryptophytes) to 13 % (chlorophytes).

In almost 64 % of the samples, only one or two functional groups were found by the AlgaeLabAnalyser, while three or four groups were found in the remaining 36 % of the samples. With CHEMTAX, all four phytoplankton groups were found in 86 % of all samples, while the rest of the samples showed a functional richness of three.

Phytoplankton diversity (Shannon-Diversity Index) based on the biomass estimates of four phytoplankton groups (chlorophytes, chromophytes, cryptophytes and cyanobacteria) from the AlgaeLabAnalyser ranged from 0 (only one group present) to 1.37 and was on average 0.59 across all three lakes. Average phytoplankton diversity based on CHEMTAX biomass estimates was higher (0.88) and ranged between 0.30 and 1.38 (Fig. 1b), resulting in a ratio significantly below 1 ($R_{LAB/CHEM} = 0.7 \pm 0.03$ (95% confidence interval), Table 3). The highest average diversity of the phytoplankton community was found in the meso-oligotrophic lake ($H' = 1.03 \pm 0.21$ (mean ± standard deviation); Kruskal-Wallis test, $X^2_{2,559} = 199.34$, $p < 0.001$) based on CHEMTAX biomass estimates, while the mesotrophic lake was the most diverse lake based on biomass estimates from AlgaeLabAnalyser ($H' = 0.72 \pm 0.15$; Kruskal-Wallis test, $X^2_{2,559} = 131.52$, $p < 0.001$). We found the lowest average phytoplankton diversity in the oligotrophic lake as indicated by both the AlgaeLabAnalyser and CHEMTAX biomass estimates (0.43 and 0.66, respectively).

Table 3: Average ratios RLAB/CHEM between the estimates from AlgaeLabAnalyser and CHEMTAX for the total biomass (TChl a, in μg L^{-1}), contribution of the four phytoplankton groups to the Chl a (μg L^{-1}) and the phytoplankton diversity (4: including only four groups, chlorophytes, chromophytes, cryptophytes and cyanobacteria; 6: including all 6 taxonomic groups determined via CHEMTAX, chlorophytes, cryptophytes, cyanobacteria, chrysophytes, diatoms and dinoflagellates). Given are average ratios ± 95% confidence intervals calculated across all lakes and for each lake separately.

Average ratio $R_{LAB/CHEM}$	all lakes	oligotrophic	meso-oligotrophic	mesotrophic
	n = 562	n = 186	n = 187	n = 189
TChl a	1.47 ± 0.06	1.65 ± 0.11	1.04 ± 0.06	1.72 ± 0.12
Chlorophytes	2.97 ± 0.49	5.57 ± 1.30	0.58 ± 0.16	2.79 ± 0.44
Chromophytes	1.47 ± 0.07	1.63 ± 0.10	1.17 ± 0.12	1.59 ± 0.12
Cryptophytes	6661.32 ±7618.32	10434.63 ± 8315.19	11017.03 ± 20647.46	23.69 ± 35.83
Cyanobacteria	0.100 ± 0.06	0.014 ± 0.01	0.168 ± 0.09	0.118 ± 0.15
Phytoplankton diversity 4	0.70 ± 0.03	0.73 ± 0.07	0.59 ± 0.04	0.79 ± 0.03
Phytoplankton diversity 6	0.44 ± 0.02	0.36 ± 0.04	0.47 ± 0.03	0.48 ± 0.02

Comparison of the biomass estimates from both methods

We found a very low, but nevertheless significant correlation between the two methods for the biomass estimates of chlorophytes (r_s= 0.19, $p < 0.001$, Table 2). As determined via the AlgaeLabAnalyser, the contribution of chlorophytes to TChl a was on average 0.36 μg L^{-1} and ranged from 0 to 8.05 μg L^{-1}. With CHEMTAX, we found a lower average contribution of chlorophytes to TChl a (0.20 μg L^{-1}), with a maximum value of only 3.21 μg L^{-1} in the mesotrophic lake (Fig. 2a), resulting in an average $R_{LAB/CHEM}$ ratio of 2.97 (Table 3). The best correlation for chlorophytes was found in the meso-oligotrophic lake (r_s = 0.41, $p < 0.001$, Table 2 and 4), which was also the only lake where the average contribution of chlorophytes

to TChl a was higher when determined via CHEMTAX than via AlgaeLabAnalyser ($R_{LAB/CHEM}$ = 0.58, Table 3 and Fig. 3).

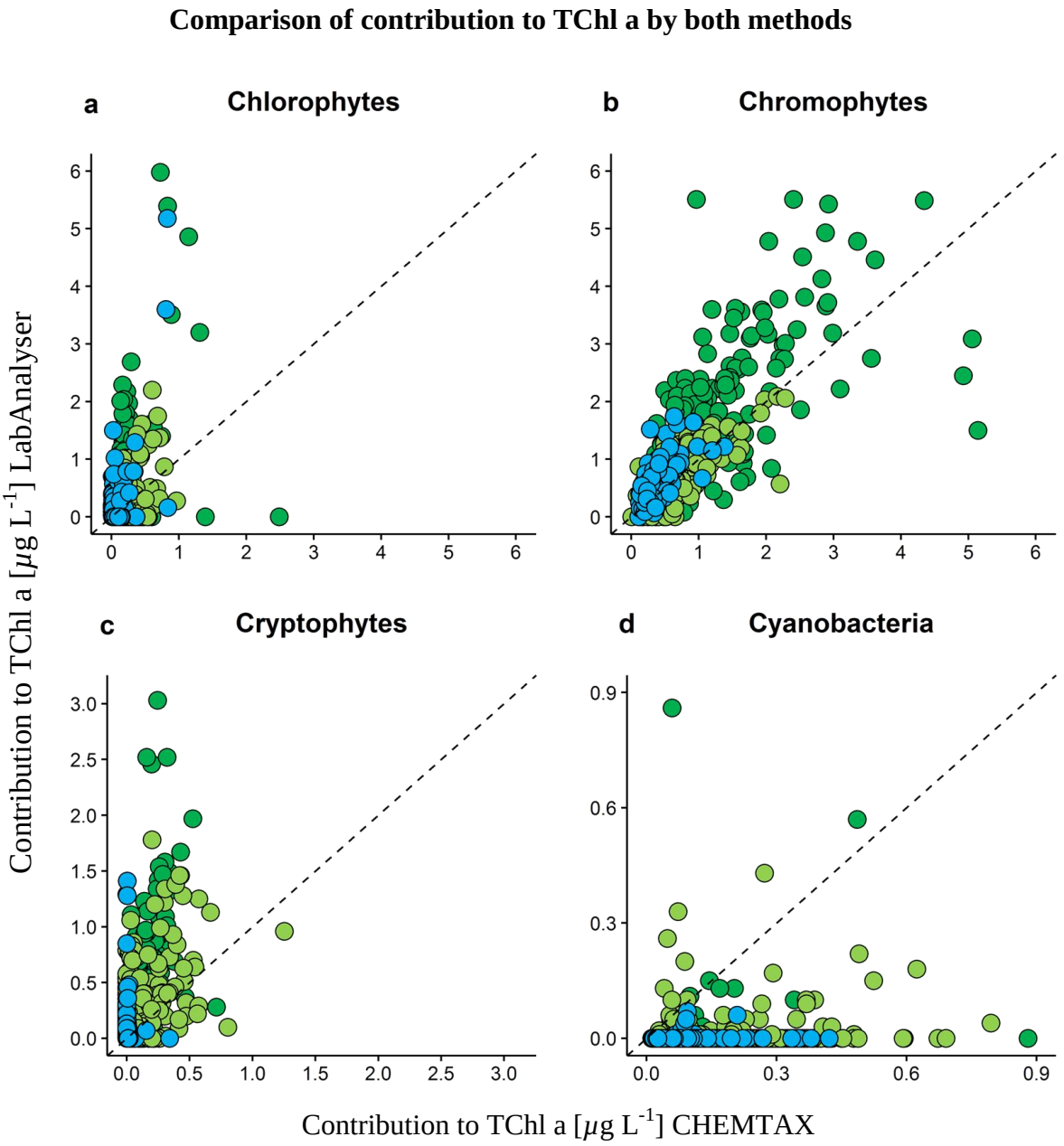

Figure 2: Contribution of a) chlorophytes, b) chromophytes (chrysophytes, diatoms and dinoflagellates), c) cryptophytes and d) cyanobacteria to the total Chl a concentration (μg L^{-1}) determined spectrofluorometrically *in vivo* with AlgaeLabAnalyser (y-axis) and chromatographically *in vitro* via HPLC and CHEMTAX (x-axis). Dashed lines represent the 1:1 relationship. Color of the circles represents the trophic state of the lakes, blue: oligotrophic (n=186); light green: meso-oligotrophic (n=187); dark green: mesotrophic (n=189); n in parentheses indicates the number of water samples per lake used in the study.

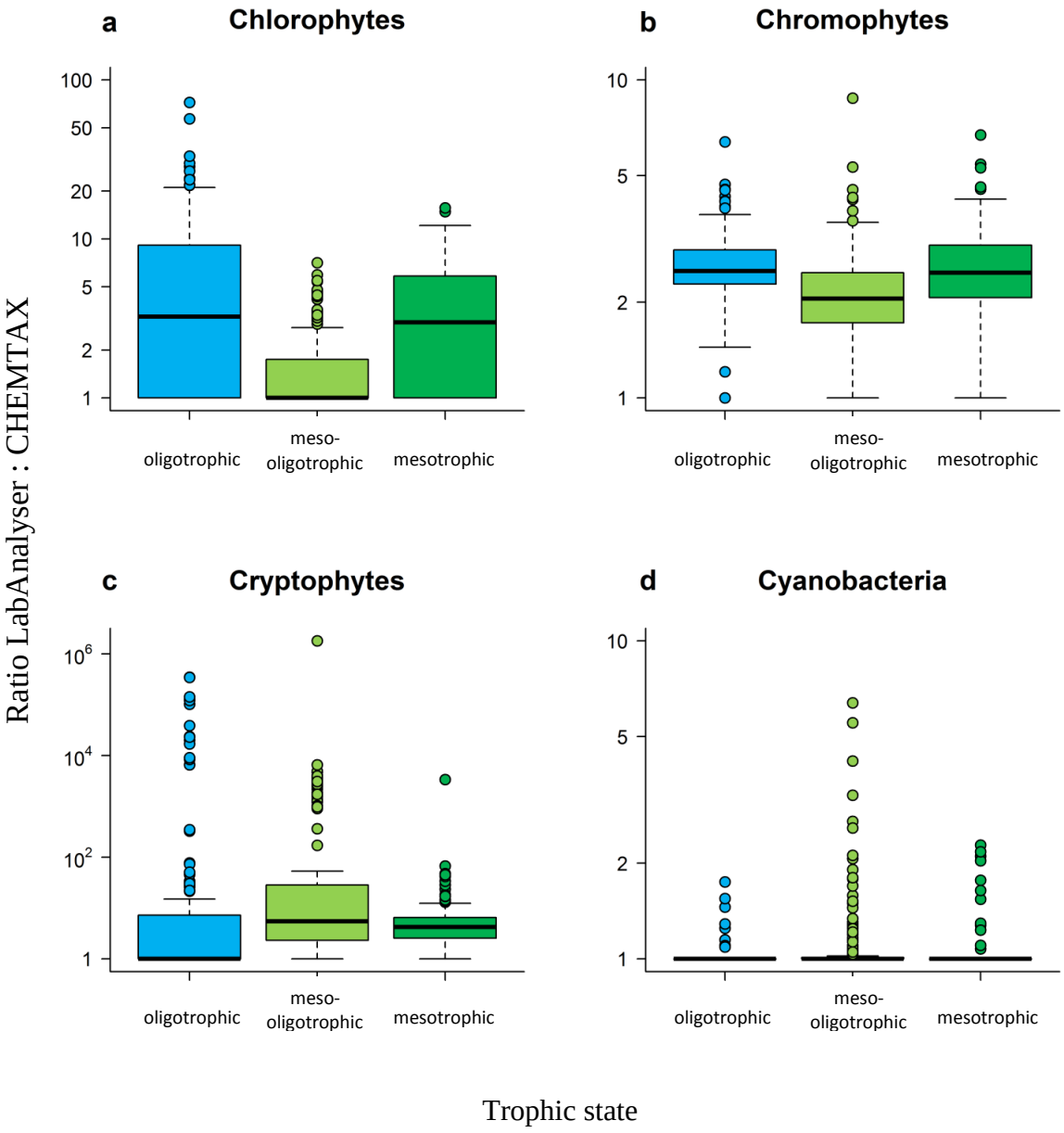

Figure 3: Distribution of the ratios (RLAB/CHEM + 1, to be able to use a logarithmic scale for the y-axis) between the contribution of the four phytoplankton groups a) chlorophytes, b) chromophytes (chrysophytes, diatoms and dinoflagellates), c) cryptophytes and d) cyanobacteria to the total Chl a determined spectrofluorometrically *in vivo* with the AlgaeLabAnalyser and chromatographically *in vitro* via HPLC and CHEMTAX for all three lakes.

Table 4: Effects of trophic status on the ratios RLAB/CHEM for a) TChl a, biomass estimates for b) chlorophytes, c) chromophytes, d) cryptophytes and e) cyanobacteria and for phytoplankton diversity (including either f) four or g) six groups from CHEMTAX). One-Way ANOVA was performed for homoscedastic data, while Kruskal-Wallis test (KW) was applied to heteroscedastic data. The different letters in the column "Group" are indicating significant differences between the trophic states (after Tukey HSD and Dunn's post-hoc tests following ANOVA and Kruskal-Wallis analyses, respectively).

$R_{LAB/CHEM}$	Group	Test	χ^2or F	p
a) TChl a				
Mesotrophic	a			
Oligotrophic	a	KW	$\chi^2_{2,559} = 144.57$	< 0.001
Meso-oligotrophic	b			
b) Chlorophytes				
Oligotrophic	a			
Mesotrophic	a	KW	$\chi^2_{2,559} = 64.579$	< 0.001
Meso-oligotrophic	b			
c) Chromophytes				
Oligotrophic	a			
Mesotrophic	a	KW	$\chi^2_{2,559} = 58.576$	< 0.001
Meso-oligotrophic	b			
d) Cryptophytes				
Meso-oligotrophic	a			
Oligotrophic	a	ANOVA	$F_{2,481} = 0.904$	0.406
Mesotrophic	a			
e) Cyanobacteria				
Meso-oligotrophic	a			
Mesotrophic	a	ANOVA	$F_{2,559} = 2.196$	0.112
Oligotrophic	a			
f) Phytoplankton diversity 4				
Mesotrophic	a			
Oligotrophic	b	KW	$\chi^2_{2,559} = 46.627$	< 0.001
Meso-oligotrophic	c			
g) Phytoplankton diversity 6				
Mesotrophic	a			
Meso-oligotrophic	a	KW	$\chi^2_{2,559} = 42.477$	< 0.001
Oligotrophic	b			

The highest correlation between biomass estimates from both methods was found for the chromophytes ($r_s = 0.77$, $p < 0.001$, Table 2). Average contribution of chromophytes to TChl a was 1.07 (AlgaeLabAnalyser) and 0.82 μg L^{-1} (CHEMTAX, Fig. 2b). Again, the best fit was found between the two methods in the meso-oligotrophic lake ($r_s = 0.72$, $p < 0.001$, Table 2 and 4), with an average $R_{LAB/CHEM}$ ratio of 1.17 (Table 3). Compared to the biomass estimates of the other phytoplankton groups, the $R_{LAB/CHEM}$ ratios for chromophytes were closest to 1 in all three lakes (Fig. 3).

Contribution of cryptophytes to TChl a ranged from 0 to 6.75 μg L^{-1} and from 0 to 1.25 μg L^{-1} as determined via AlgaeLabAnalyser and CHEMTAX, respectively (Fig. 2c). On average, only 0.09 μg L^{-1} of cryptophytes were found in our samples according to CHEMTAX calculations, while with the AlgaeLabAnalyser, the average biomass for cryptophytes was four times higher (0.36 μg L^{-1}). Still, there could be observed a highly significant positive correlation between the biomass estimates from the two methods across all lakes ($r_s = 0.62$, $p < 0.001$, Table 2), while the best fit was found in the mesotrophic lake ($r_s = 0.5$, $p < 0.001$, Table 2). The worst fit for cryptophytes was found in the oligotrophic lake, but was still significant ($r_s = 0.15$, $p < 0.05$, Table 2). $R_{LAB/CHEM}$ ratios of the biomass estimates for cryptophytes were in many cases very high and ranged up to about 2×10^6as found in the meso-oligotrophic lake (Fig. 3), which was due to very low concentrations of alloxanthin in the samples and thus, a very low contribution of cryptophytes to TChl a was determined via CHEMTAX.

The lowest correlation between the two methods was found for cyanobacteria ($r_s = 0.07$, $p > 0.05$, Table 2 and Fig. 2d). Although the biomass estimates for cyanobacteria from the AlgaeLabAnalyser and CHEMTAX were in a very similar range (0 to 0.86 μg L^{-1} and 0.01 to 0.88 μg L^{-1}), the overall $R_{LAB/CHEM}$ ratio was only 0.1 (Table 3 and Fig. 3). Interestingly, we found cyanobacteria in all samples as determined via CHEMTAX, but the same was the case only in 12 % of the samples when using AlgaeLabAnalyser. Consequential, in 88 % of all samples, cyanobacteria were not found at all according to the AlgaeLabAnalyser. In the oligotrophic and the mesotrophic lake, this was even the case in 96 % and 93 % of the samples. The only positive correlation between the two methods was found in the oligotrophic

lake, but was very low (r_s = 0.17, $p < 0.05$, Table 2). For both cryptophytes and cyanobacteria, no significant differences between the $R_{LAB/CHEM}$ ratios were found between lakes (Table 4).

CHEMTAX derived phytoplankton diversity including all 6 phytoplankton groups

The average ratio between the Shannon-Diversity Indices from the two methods was even lower when all six phytoplankton groups (chlorophytes, cryptophytes, cyanobacteria, chrysophytes, diatoms and dinoflagellates) were included into CHEMTAX derived phytoplankton diversity (Fig. 4a). Here, the correlation coefficient r_s between AlgaeLabAnalyser and CHEMTAX derived diversity was only 0.3, while the average ratio $R_{LAB/CHEM}$ across all lakes was 0.44 (Table 3).

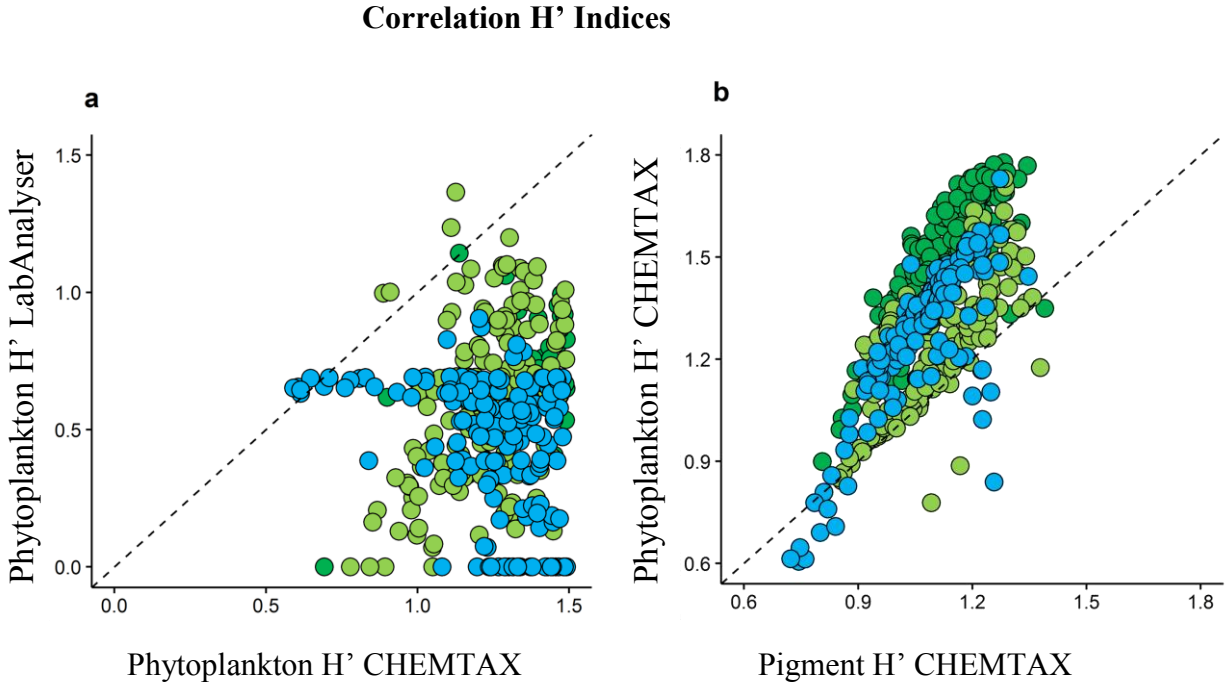

Figure 4: a) Correlation between the phytoplankton diversity *H'* (Shannon-Index) determined spectrofluorometrically *in vivo* with the AlgaeLabAnalyser (y-axis) and chromatographically *in vitro* via HPLC and CHEMTAX (x-axis, including all six taxonomic groups: chlorophytes, cryptophytes, cyanobacteria, chrysophytes, diatoms and dinoflagellates). b) Correlation between the phytoplankton diversity estimated with HPLC and CHEMTAX (including all six taxonomic groups) and pigment diversity (including Chl a). The dashed line represents the 1:1 relationship. For legend, see Fig. 1.

When we compared the HPLC derived pigment diversity (including Chl a) and phytoplankton diversity determined via CHEMTAX (all six groups included, Fig. 4b), we found a highly significant positive correlation ($r_s = 0.67$, $p < 0.001$), which was found to be the highest in the mesotrophic lake ($r_s = 0.82$, $p < 0.001$). The average ratio $R_{phytoplankton/pigments}$ was 1.23 across all lakes and ranged between 0.67 and 1.50. The ratio closest to 1 was found in the oligo-mesotrophic lake ($R_{phytoplankton/pigments} = 1.13$; Kruskal-Wallis test, $\chi^2_{2,559} = 290.9$, $p < 0.001$).

4. Discussion

4.1 Directed diversity manipulations of natural phytoplankton communities

Experimentally proofing realistic effects of species loss on BEF in natural unicellular freshwater and marine primary producer communities is still a challenge. Here we experimentally established diversity gradients within natural phytoplankton communities by using two different techniques. Both methods – dilution and disturbance – were appropriate to create diversity gradients in natural phytoplankton communities from ponds contrasting in nutrient availability. Dilution and disturbance were applicable methods in an oligotrophic as well as a eutrophic water body even though the effect sizes (relative species differences between dilution or disturbance levels) differed between systems. Dilution in the oligotrophic pond resulted in 64 % (day 17) and 51 % (day 24) of species loss between the highest and lowest dilution step while the communities originating from eutrophic water showed a species loss of about 46 % and 41 %. Disturbance manipulations of communities from the oligotrophic pond resulted in about 23 - 34 % lower species numbers for the low and high disturbance frequencies as for intermediate disturbance frequencies (at day 39). The eutrophic community on the other hand reached 45 % (day 39) lower species richness in the no-disturbance treatment when compared to the two highest frequency disturbance treatments. While for dilution treatments the higher effect sizes were reached for the oligotrophic water body, the opposite pattern was true for disturbance treatments communities.

Overall, dilution had species richness gradient effect sizes reaching from around 40 % to 65% species losses while disturbance generated a range of effect sizes from 33 % to 45 % species losses. Dilution thereby most likely acted on the loss of rare species in treatments containing only a very small volume of the original community. Eutrophic, highly productive communities often tend to be dominated by a few species that provide most of the community biomass (Hillebrand et al. 2007). Thereby the loss of species that were already very rare at the beginning of the experiment might not be recognized with our counting and enumeration methods (Utermöhl technique) which could explain the slightly lower effect sizes of disturbances in those treatments. Underestimating the total number of species in a community by not detecting in particular very rare species is considered a potential weakness of many

phytoplankton studies. Species are often highly diluted in open water environments. Therefore, species-accumulation curves or rarefaction analyses were assessed in several studies to value sampling sizes for meaningful comparison of different communities as is common practice in terrestrial species communities (Gotelli and Colwell 2001, Hughes et al. 2001, Rodríguez-Ramos et al. 2014). The above-named studies recommend higher sample-volumes than are commonly used in field studies to avoid undersampling of rare species. In our experiment we analyzed a rather high sample volume compared to the small overall treatment volume of 400 mL. Potential shortcomings by an undersampling of the rarest species in the communities should accordingly affect all treatments similarly and therefore still allow comparisons. Nevertheless it is advisable to follow the recommended techniques to avoid undersampling in potential larger mesocosm studies usually comprising treatment volumes of hundreds to thousands of liters.

Concerning biomass development or other ecosystem traits such as nutrient uptake and community composition, eutrophic communities are probably less sensitive to diversity manipulations by dilution compared to oligotrophic ones. Oligotrophic communities are rather characterized by a more even species distribution (Hillebrand et al. 2007). Accordingly, oligotrophic communities could therefore be more susceptible to dilution, as also shown by our data.

Disturbance on the other hand seemed to have affected phytoplankton communities following a pattern as predicted by the intermediate disturbance hypothesis (IDH, Connell 1978; Flöder and Sommer 1999). Although not all communities responded with the expected hump-shaped curves, species richness measurements indicated a distinct response to disturbance manipulations. While the oligotrophic treatments showed a hump shaped - IDH typical - response, the eutrophic pond treatments changed in their response of species richness to disturbance frequency from a linear relationship to a saturation curve over time. However, the response of diversity to disturbance should strongly depend on the productivity of the system (Huston 2014), therefore varying responses of different productive communities to the same levels of disturbances are to be expected. Applying disturbances as tool to manipulate diversity needs careful planning to find the “right” experimental duration and frequency of

disturbances to form distinct diversity gradients. Apart from the IDH as explanation, the probable direct loss of stress sensitive species in treatments with high disturbance frequencies of other, stronger types of disturbances such as heat (Engel et al. 2017) or freezing stress must be considered. Species loss under such stress scenarios should occur much faster.

One important aspect for performing experiments using diversity manipulated natural communities is the variation in species richness between replicates. Within artificially composed phytoplankton communities uncontrolled variation is usually zero as diversity is fully controlled by the investigator. Hence, in diversity manipulated natural communities there will be uncontrolled variation between replicates of disturbance or dilution treatments. Whereas species richness was similar between manipulation steps in our experiments, community similarities varied much more. However, this can be seen similar to establishing new species combinations at the same diversity levels as often performed in experiments investigating diversity ecosystem functioning relationships with artificial laboratory communities. Replicates can then be considered being real replicates for certain species richness levels (ranges) and not replicates of a certain community with identical species composition.

Decreasing similarity with increasing dilution can be explained by the mechanism of dilution. Low dilution results in a higher share of species of the initial community, whereas high dilutions result in a higher stochastic chance to create different subsets of the original communities of the initial species assemblages. All replicates of high dilution treatments showed lower species numbers, but differed substantially in their community composition, leading to lower similarity between replicates of the same treatment. Disturbance treatments on the contrary did not result in a clear pattern between experimental manipulation and similarity between treatments at the same manipulation level. Only in the treatments of the eutrophic pond the similarity between replicates of a treatment increased with disturbance frequencies. This indicates that in these treatments only the same few stress resistant species were able to grow under high disturbance, resulting in an increasing similarity with increasing disturbance frequencies. In opposite to dilution - which acts via the different abundance of phytoplankton species and addresses rare species - disturbance will act on specific traits of phytoplankton species and the effects will rather depend on the functional composition of the

initial community and the respective susceptibility to certain stressors. Choosing manipulations acting on different stress sensitive traits offers a portfolio of options to manipulate diversity given by different research questions (Engel et al. 2017).

Our experimental manipulations did not result in immediate visible diversity responses, diversity gradients established after certain time periods. Disturbance manipulations did not result in a sudden loss of species in our experiments; it took two weeks or more to see clear and distinct responses. Also dilution treatments could not be used immediately in our experiments, strong dilution results in initial very low biomasses whith most species most probably being present in numbers below microscopic detection levels. However, allowing manipulated treatments to grow for several generations did not only result in clear diversity gradients but also in more similar biomass concentrations as resource supplies to treatments were controlled and identical within experiments. Depending on the research question this can be a very useful characteristic of the above described protocols. If the created gradients are further used for experiments, it is recommendable to allow for a growing phase before the actual experiment or some other form of adjusting the biomass of the start communities such as suggested in other studies (Franklin et al. 2001, Giller et al. 2004).

Using artificial laboratory communities resulted already in important insights into phytoplankton diversity – ecosystem functioning relationships. The large advantages of such experiments, mainly full control over environmental parameters and diversity manipulations are allowing rigorous tests. For example, mechanisms of biodiversity related increases in productivity can be further characterized by variance partitioning (overyielding, etc.; Fox 2005) but for that the productivity of each species of diverse communities in monoculture is needed. Usually, that can only be done in artificially assembled phytoplankton communities.

However, artificially mixed laboratory communities consist of single species that have usually been cultured for hundreds of generations without the possibility of interaction with other species, laboratory cultures might have also lost trait flexibility through selection for standardized laboratory conditions. Additionally, these artificial communities rarely reach the natural richness level and only represent the lower end of the natural diversity scale (Ptacnik et al. 2008). Therefore, these approaches should be combined with experiments manipulating

diversity in natural communities including much higher complexity and the presence of key functional trait characteristics of "wild nature" (Naeem et al. 2012), even by sacrificing some experimental control. Regarding the need of high replication for a variety of several modern "data-hungry" statistical methods (Hector 2015) our results show that also high numbers of diversity treatments and replicates can be relatively easily established on a laboratory scale, still allowing controlled environmental conditions such as established in climate chambers or incubators. Additionally, being able to establish distinct diversity gradients such as seen in our experiments opens a large variety of experimental possibilities. We recommend dilution and disturbance manipulations as a feasible option to manipulate diversity of natural phytoplankton communities in a directed way. Depending on research questions diversity gradients can be established, potentially allowing detailed investigations and mathematical descriptions of diversity – ecosystem functioning relationships. However establishing these gradients took some time and it will depend on the constraints of experimental designs whether faster methods such as size selective filtration are more appropriate (Engel et al. 2017). Depending on the experimental needs it is possible to choose between a variety of methods to manipulate diversity in limnic and marine natural phytoplankton communities, each with its own advantages and constraints.

4.2 Diversity gradients and nutrient enrichment – comparing effects of natural diversity differences with effects of short-term experimental manipulations of diversity

In ecology there is persistent interest in understanding ecosystem responses to disturbances. Especially the mechanisms behind ecosystem functioning in regard to anthropogenic drivers are subject to numerous studies (Craven et al. 2016, Alexander et al. 2017, Cavicchioli et al. 2019). With my experiments I wanted to provide basic insight to this topic focusing on natural phytoplankton communities. Across the three lakes in my study, diversity gradients could successfully be established by my manipulations. These diversity gradients enabled me to compare short- and long-term reactions of primary producer communities to a stressor like nutrient enrichment.
Do the artificially manipulated diversity differences (short-term) result in responses of the same direction and magnitude as over a longer period of time evolved natural differences in diversity between water bodies?

As expected, nutrient enrichment did lead to an enhanced biomass production of the experimental communities from all lakes. Within my experiments, a lower overall community stability could be shown with an increasing loss of diversity. Nevertheless, the communities showed distinct differences in their reactions to additional nutrient input when considered lake-wise. Short- and long-term reactions of the communities did thus not show the same direction or magnitude of responses to nutrient addition. My data showed the expected higher stability with higher diversity when communities from all lakes were observed together. When separating the three lakes, the short-term manipulated communities showed more variable response patterns to the nutrient input but still clustered around their respective initial communities. Thereby the results were strongly depending on the lake of origin of the community as well as the year in which the experiment took place. A similar lake dependency of diversity responses to eutrophication could be monitored in experiments including communities from several tropical lakes (Soares et al. 2013). Also, Rigosi et al. (2014) observed a connection between the trophic states of freshwater lakes in the US and the

dependence of cyanobacterial blooms on the interplay between rising temperatures and eutrophication.

Apart from several studies showing an increasing stability in systems with higher species numbers (Balvanera et al. 2006, Isbell et al. 2009), the meaning of sole species number counts for ecosystem functioning is discussed controversially in ecological studies (Collins et al. 2008, Gotelli and Chao 2013). Species richness by itself is not seen as sufficing estimator for diversity anymore. Consequential, a more functional view on diversity issues (Weithoff et al. 2001, Vogt et al. 2010, Borges Machado et al. 2016) as well as additional estimators accounting for species identities and abundances like species turnover indices are suggested to give a better representation of a system's dynamic (Hallett et al. 2016). Hillebrand et al. (2017) for example proposed species exchange ratios to better capture temporal trends in community composition and give a more "management-relevant measure of change" decoupled from species richness itself. To account for the drawback of using species richness indices in this study, I also focused on analyzing the community integrity to get a better overall picture of the phytoplankton dynamics during the additional nutrient input. Similarity between pre- and post-nutrient addition was higher amongst communities with higher genus richness while the species exchange ratios were lower for the same communities. Both regressions are again lake and especially year dependent. Nevertheless they show that in my study higher genus richness seems to go hand in hand with a more stable community composition during the nutrient addition. This must not necessarily be the case as several other studies show a decoupling between species richness and other biodiversity indices (Collins et al. 2008, Hillebrand et al. 2017). Absolute losses in terms of genera lost from the community seem relatively equal across the experimental communities; relative values therefore fluctuate depending on the starting values. Communities starting with an already lower diversity therefore are prone to a higher relative loss which could lead to a downward spiral of diversity (Chapin et al. 2000, Loreau 2010). Hughes et al. (2007) also found reciprocal effects of diversity and disturbance in several systems when reviewing experiments published on the topic.

Diversity seems to buffer against stressors, yet in my experiments there are diverse mechanisms acting on different timescales. While the ecological responses to nutrient input were rather unpredictable in the communities manipulated in diversity on a short timescale, the evolved differences in community composition between the three water bodies depicted the expected higher stability with higher diversity. An evolutionary adaptation of the phytoplankton community to conditions specific to one lake could explain the strong lake dependency in our data. Local adaptation was shown to strongly affect community assembly of zooplankton under the influence of several factors known to be decisive for the community structure (Pantel et al. 2015). It is therefore quite likely that evolutionary and ecological responses to a certain stressor act within similar time scales and are interdependent. Several studies from diverse systems show evolutionary processes to happen on time scales of ecological change and discuss a potential feedback between ecology and evolution (e. g. summed up in Fussmann et al. 2007 or Schoener 2011). Hence, the question arises whether a common evolutionary background history and the acclimatization to a certain stressor of a community over time could lead to a higher stability. Potentially, a community already used to certain conditions in the past could react more stable to a recurrence of the same stressor in the present. For multiple stressors occurring successively, such community adaptations via co-tolerance of species have been described (Vinebrooke et al. 2004). As the lakes in this study all represent different trophic states, reaching from oligotrophic to mesotrophic, an adaptation of the respective phytoplankton communities to the nutrient loading of their lake could be expected. It is for example noticeable that Lake Thalersee, whose communities responded to the nutrient input with the highest stability, has a background history of higher nutrient loading than found in the present. The lake was prone to nutrient inflow from agriculture in the surrounding area. After being subject to management actions for the last decades, the incoming nutrient amounts were reduced and the lake's trophic state recovered from eutrophic to a rather mesotrophic status. Eventually, the primary producer community therefore could still be adapted to higher nutrient content and respond to our experimental nutrient addition with a higher stability. Nevertheless, it should be considered that under the influence of stressors also threshold values could be reached. With ongoing change in for example nutrient enrichment, a certain amount of nutrient loading could suddenly lead to a shift from one stable

state to another in an ecosystem (e.g. Scheffer and Carpenter 2003). Such state shifts have for example been observed between clear water and a turbid state in shallow lakes (Scheffer et al. 1993) or also between different states in other ecosystems or scales (reviewed in Schröder et al. 2005). Albeit, the understanding of ecological stability and especially the prediction of a system's response under certain stressors are rather complicated. Hence, there have been calls for more holistic approaches in such studies (Donohue et al. 2016, Hillebrand et al. 2018). Hillebrand et al. (2018) tested several measurements of stability and their interrelations and concluded to consider several measurements for compositional as well as functional stability. Within their experiments some stability measures correlated between these two stability aspects while others did not show any relations between compositional and functional stability. The authors suggest that the time scale of recovery of a system as well as the chance for recovery at all could vary amongst compositional and functional aspects. Incorporating these different aspects should lead to a better understanding and predictability of ecosystem responses to disturbances. Similarly, another study concludes that several anthropogenically induced stressors can diversely affect ecosystem functioning with uncoupled responses in single functions but no consistent response in the overall multifunctionality of the system (Alberti et al. 2017). With respect to management and research, these findings advise to analyse multiple aspects and ecosystem functions in response to disturbances and change instead of focusing on single factors. Unique species compositions and interactions with the respective environmental conditions lead to complex dynamics and hamper simple general predictions and conclusions in respect of external forcing on a system's community. More insight on the mechanisms driving interactions between diversity and ecosystem functioning as well as relationships at the interface between evolutionary and ecological processes is therefore desirable (Oliver et al. 2015, Pelletier et al. 2009, Cavicchioli et al. 2019). Considering a functional approach, insight on mechanistic links could probably be gained by looking at the trait level rather than on the pure species richness. One example of such a functional approach is discussed in the next section.

4.3 Group specific trait losses from phytoplankton communities

Trending towards trait-based approaches, research in BEF tries to find mechanistic explanations for observed patterns in ecosystem functioning. I therefore investigated the subsequent trait changes in natural phytoplankton communities from lakes of different trophic status following alterations of the amount of one specific group of phytoplankton - namely diatoms. Except for some minor overlap with other phytoplankton groups, diatoms were chosen in this case due to their specific traits (e.g. pigment composition, light usage) and their susceptibility to a manipulation of the stratification regime.

My experiments clearly show a connection between the amount of diatoms in the community and the amount of the pigment fucoxanthin, resulting in a change in spectral light usage. A different portion of light of wavelengths in the absorption range of fucoxanthin was absorbed with changing diatom content in the communities. Furthermore, I could observe higher chlorophyll a F0 fluorescence of PS (photosystem) II when excited within the range of the fucoxanthin absorption maximum with rising diatom abundance. As a result, phytoplankton communities with fewer diatoms showed a reduction in their spectral light usage at those wavelengths. I could therefore observe the expected trait loss from the community linked to a previous loss of functional diversity in my data.

When looking in detail at the connection between light attenuation at fucoxanthin absorption maximum and the portion of diatoms in the community, I chose to restrict the analyzed communities to the ones originating from Lake Brunnensee. This is due to the lake containing a relatively high concentration of silicon and a low overall phytoplankton biomass. Communities therefore span a wide range of diatom contents at constantly low biomass levels when compared to the other two lakes in the study. Hence, communities from Lake Brunnensee do not show much shading by the silicon shells of the diatoms themselves (as could happen at high biomass values) or disturbances of the measurements by a high content of other algal groups, which also contain fucoxanthin like for example dinoflagellates. The results from Lake Brunnensee in this case show a strong increase of light attenuation in the fucoxanthin range with rising diatom content. With further increasing diatom contents, the light attenuation seems to reach a maximum value. As light of certain wavelengths is a finite

resource, this observation could mean that at low diatom concentrations additional diatoms in the community also mean a higher concentration of fucoxanthin and with that a higher light attenuation at its absorption maximum. At certain diatom concentrations a further increase in light use cannot be observed as a threshold value seems to be reached. Such a threshold could indicate a level of diatoms in the community, where the light of the respective wavelength is exhausted and additional amounts of fucoxanthin would not lead to a further increase of light usage. Instead, some diatoms are known to be able to shift their antenna-structure of the photosynthetic apparatus to adapt to certain light conditions (Herbstová et al. 2017), showing an alternative response possibility to the finite nature of light as a resource.

R^2 values indicate a proportion of 19 – 38 % of the variability in the data to be explained by the portion of diatoms in the community when looking at the fucoxanthin distribution or the light attenuation of the phytoplankton communities. Regarding the light used for photosynthesis (F0 proportion of PS II in the fucoxanthin absorption range), the proportion of variability explained by the diatom content in the community drops to only 3 %. Nonetheless this seemingly low value is not atypical for data concerning biodiversity-connected patterns in field experiments or observations (see for comparison Ptacnik et al. 2008). Natural communities are influenced by a range of factors that leave biodiversity as one of them and therefore result in lower variability explained by diversity parameters than could be expected in well-controlled experiments in laboratory environments.

Although I only picked one phytoplankton group (only making up a portion of the whole community) with specific features and investigated very detailed trait characteristics within natural communities in large field mesocosms, I could nevertheless find significant consequences of a decline of that group in the community pigment composition and light usage. This provokes the thought that these consequences might go beyond the light usage of the phytoplankton community.

Stockenreiter et al. (2013) observed a rising lipid production in phytoplankton communities comprising different functional groups. Within communities consisting of three or four functional groups (tested were one to four), they could also show an increase in microalgal neutral lipid content with higher light use (spectral absorption of photosynthetically active

radiation) of the community. In phytoplankton, the content of certain fatty acids is species or group specific and therefore also used as biomarker when analyzing aquatic food webs (Iverson 2009). The composition of essential polyunsaturated fatty acids is crucial to the nutritional value primary producers exhibit for consumers (e.g. Müller-Navarra 2008, Pajk et al. 2012). Hence, such characteristic fatty acids could link phytoplankton diversity to the productivity of higher trophic levels. This suggests the conclusion that a loss of a functional group like the diatoms would be relevant to the food quality of primary producers in the food web. The loss of one trait could consequently lead to a drop in the community's resource use efficiency, which could alter the whole food web performance.

Apart from their specific pigments and therewith their role in the phytoplankton communities' light usage, diatoms play a decisive role in the silicon cycle due to their silicon shells (Tréguer and De La Rocha 2013). Within riverine and lake systems, their growth and sinking rates as well as burial in the sediments are therefore decisive for the food web, material cycles, water chemistry and also for the amount of Si transported on to marine systems (Humborg et al. 2000, Wetzel 2001). Within these marine systems the availability of Si and the growth conditions for diatoms are of even higher significance. Diatoms are responsible for a large portion of the primary production in marine ecosystems and therefore are the basis for productive food webs (Nelson et al. 1995, Malviya et al. 2016). A loss of diatoms from the community or a switch to more non-siliceous algae communities consequently would have far-reaching consequences for the whole food web and fisheries, but also for the world carbon cycle (Richardson et al. 2009, Tréguer et al. 2017, Cavicchioli et al. 2019).

As a loss of diatoms has such sweeping effects in aquatic systems and beyond, the question arises, whether other organisms could fill the resulting gap. On a short timescale, some algal species are able to alter their pigment composition flexibly depending on light availability and the light usage of competitors (Stomp et al. 2004). Nevertheless, I could see a change in resource use efficiency during my experiments, showing that relatively short-term changes of conditions can lead to changes in the community that are not coped with in the same time frame. Therefore flexible use of pigments can be one response but in the long run phytoplankton groups are limited by their pigment repertory. On longer time scales one could

expect an evolutionary response of the community to a loss from one functional group. Potentially other phytoplankton species with overlapping pigment range could fill in the niches. Depending on the attendant circumstances some of the open niches could hence be taken over. However, if niche conditions are not suitable in all aspects (e.g. resources beside light etc.), lower species numbers and reduced functioning of the system would be maintained (Srivastava 1999, Mateo et al. 2017). In my case other groups like dinoflagellates that share the pigment fucoxanthin or other characteristics could probably increase when diatoms get lost from the phytoplankton community (Spilling et al. 2018). As discussed above, such changes in the community could also lead to state shifts in the whole system. Even so, it is hard to forecast precisely what will happen in a community undergoing change by a loss as described. Pigments do not only function as photosynthetic active pigments but can for example also be produced as protection against UV radiation (Falkowski and Raven 2007). Therefore it is rather complex to separate single mechanisms and it is not completely understood what the loss of a pigment trait means for the community and the functioning of the system.

4.4 Comparison of different techniques to assess phytoplankton diversity

Both the AlgaeLabAnalyser and CHEMTAX allowed for a good general overview of the composition of natural phytoplankton communities. Despite the somewhat limited taxonomic resolution of both methods, the overall patterns of phytoplankton group abundances matched well with both the *in vivo* and the *in vitro* assay. Further, phytoplankton biomass estimates (determined as total chlorophyll (Chl a)) were very similar with both methods. This demonstrates the general utility of both approaches, while other methods and devices such as FluoroProbe and Algae Online Analyser (both from bbe Moldaenke) are known to frequently underestimate the total Chl a (Gregor and Maršálek 2004, Harrison et al. 2016, Izydorczyk et al. 2009, Catherine et al. 2012). As both our methods require relatively little time in comparison to e.g. microscopic counts or DNA-metabarcoding approaches, this makes them highly suited for monitoring and routine phytoplankton analyses.

Despite their general comparability, both methods differed markedly in some important aspects. This applies in particular, but not exclusively, to the determination of cyanobacterial abundances, which are a major focus of phytoplankton community assessment in the context of water quality management. In our study, the AlgaeLabAnalyser was frequently unable to detect any cyanobacteria in the lakes' phytoplankton, even though the detection of echinenone in the HPLC gave clear indications of cyanobacterial presence. Microscopic observations of a subset of the samples also showed a presence of cyanobacteria in the majority of analyzed samples, supporting the results from HPLC. The manufacturer suggests calibrating the AlgaeLabAnalyser with phytoplankton species isolated from the water bodies of interest to get a more accurate assessment of the phytoplankton community composition. However, this seems unrealistic in practice, in particular for routine laboratories and water authorities that monitor numerous different lakes and other aquatic systems. Additionally, in this study, water samples were tested undiluted in the AlgaeLabAnalyser. At denser samples, this may cause the device to underestimate the signal actually belonging to cyanobacteria (pers. comm. H. Stibor).

An important aspect that might explain the observed differences between the two methods is the possibility to adjust the sensitivity of the HPLC/CHEMTAX method via the filtered volume of samples. While only 25 mL of the water samples are measured in the AlgaeLabAnalyser, 500 – 1000 mL of water were filtered for each sample for the pigment-analyses via HPLC. Thus, the concentration of the pigments extracted from the filters and detected via HPLC was higher compared to the pigment concentrations in the water sample measured *in vivo* with AlgaeLabAnalyser. This probably allowed for the higher sensitivity of the CHEMTAX method and its accuracy in the estimation of low cyanobacteria abundances.

Comparative assessment of methods

Three specific aspects in the comparative evaluation of the HPLC-based and the *in vivo* method merit particular attention: The first applies to the distinction between cryptophytes and cyanobacteria, which is of particular relevance for water quality assessment and monitoring (Catherine et al. 2012, Gregor et al. 2005, Izydorczyk et al. 2009): The detection of cryptophytes by the AlgaeLabAnalyser depends not only on the main cryptophyte marker pigment alloxanthin, but further on the specific absorption of phycoerythrin (Beutler et al. 2002, Beutler et al. 2004), which is also an important pigment for many "red" and "blue" cyanobacteria (Bryant 1982, Gregor et al. 2005, Haverkamp et al. 2009, Jasser et al. 2010).

As the lipophilic extraction commonly applied prior to the HPLC separation of pigments does not capture the hydrophilic pigment groups of phycoerythrins and phycocyanins, these pigments cannot be evaluated by the CHEMTAX approach. This led us to the assumption that CHEMTAX may underestimate the abundance of cyanobacteria in the lake phytoplankton, as this method does not consider these two main groups of pigments typical for cyanobacteria.

Interestingly, our data indicated quite the opposite, i.e. a much higher relative abundance of cyanobacteria in the phytoplankton community assessment via CHEMTAX as compared to the AlgaeLabAnalyser. Nonetheless, both methods could still underestimate the "real" abundance of cyanobacteria. Catherine et al. (2012) also reported a "potentially strong misattribution towards cryptophytes of "red" cyanobacteria" when they compared the biomass estimates of cryptophytes and cyanobacteria from FluoroProbe to the microscopic counts.

When examining cyanobacterial blooms in reservoirs, in some samples dominated by cyanobacteria, Gregor et al. (2005) detected certain amounts of cryptophytes (approx. 1 – 20 % of TChl a) via FluoroProbe, although microscopic counts revealed no cryptophytes. This may be explained by the inclusion of phycoerythrins into the detection of cryptophytes by the AlgaeLabAnalyser (and FluoroProbe). Admittedly, there have been attempts to account for this potential problem by the manufacturers of the AlgaeLabAnalyser (Beutler et al. 2003, Beutler et al. 2004). Nevertheless, our data indicate that under certain conditions, the CHEMTAX approach may be more sensitive to low cyanobacterial abundances when compared to the *in vivo* approach of the AlgaeLabAnalyser. Yet, in consequence of the working principle of the AlgaeLabAnalyser it seems reasonable to assume that some cyanobacteria with a rather atypical pigment composition could be sorted into the chlorophyte group. AlgaeLabAnalyser measures Chl a fluorescence while differently coloured LEDs enable the detection of an interaction of additional antennae pigments with Chl a. Thereby the device can "misinterpret" signals from atypically pigmented algae and sort them into other groups.

Beyond the distinction between cryptophytes and cyanobacteria, it may also be challenging to distinguish chlorophytes from cyanobacteria with the HPLC method under certain conditions. Most published HPLC gradients have difficulties in separating the peaks of lutein and zeaxanthin (Latasa et al. 1996, Ston-Egiert and Kosakowska 2005, Van Heukelem and Thomas 2001). This was also the case for our HPLC gradient. As a consequence, lutein may be frequently underestimated, which would lead to an underestimation of chlorophytes relative to cyanobacteria. Depending on the respective phytoplankton community, this could interact with the previous observation of an underestimation of cyanobacteria under certain circumstances. In our HPLC data, no lutein peak could be identified in some samples, although microscopic counts confirmed the presence of chlorophytes. Such an underestimation of chlorophyte abundances due to an insufficient separation of lutein and zeaxanthin may explain the lower chlorophytes : cyanobacteria ratio detected by CHEMTAX in comparison to the AlgaeLabAnalyser.

CHEMTAX estimates the relative abundance of chlorophytes mainly based on the occurrence of lutein and chlorophyll b. If chlorophyll b, but no lutein is detected, this is probably a consequence of the above mentioned weak separation of the lutein and zeaxanthin peaks in the HPLC. An alternative explanation could be the occurrence of euglenophytes that are characterized by the possession of chlorophyll b without a concomitant abundance in lutein (Fietz and Nicklisch 2004, Sarmento and Descy 2008, Schlüter et al. 2006). However, microscopic observations of our samples do not indicate frequent occurrences of euglenophytes in our study lakes.

The third important difference between the two methods is related to the distinction of diatoms and chrysophytes. As both share the characteristic pigment fucoxanthin, the AlgaeLabAnalyser does not allow for a distinction between these algal groups. Unfortunately these two algal groups often dominate in oligo- and mesotrophic lakes (Buchaca et al. 2005, Järvinen et al. 2013, Ptacnik et al. 2008, Poxleitner et al. 2016, Schlüter et al. 2016, Watson et al. 1997). CHEMTAX provides the distinct advantage of separating chrysophytes from diatoms based on their specific fucoxanthin : Chl a ratios. As mentioned before, the final output ratio of fucoxanthin : Chl a differed between the oligotrophic lake and the meso-oligotrophic and the mesotrophic lake, resulting in a switched dominance of either the diatoms or the chrysophytes comparing the lakes (oligotrophic lakes: diatoms more abundant than chrysophytes, while the opposite was the case in the meso-oligotrophic and the mesotrophic lake). In the case of the meso-oligotrophic lake, the microscopic counts indicated a dominance of diatoms rather than chrysophytes. One possible explanation might be the usage of a CHEMTAX ratio matrix established for meso- and eutrophic lakes (Schlüter et al. 2016). However, CHEMTAX calculations for the meso-oligotrophic lake with the ratio matrix established for oligotrophic lakes (Schlüter et al. 2016) yielded the same results (data not shown). This indicates that a differentiation between diatoms and chrysophytes based on their specific fucoxanthin : Chl a ratios is not sufficient to accurately discriminate these two phytoplankton groups. We thus suggest the inclusion of further pigments into the CHEMTAX approach for a more accurate differentiation of diatoms and chrysophytes, e.g. the inclusion of violaxanthin, which is a commonly used marker pigment for chrysophytes (Buchaca et al. 2005, Descy et al. 2000, Lauridsen et al. 2011, Schlüter et al. 2016).

Within the CHEMTAX analyses, the pigment : Chl a ratios are changed through a series of iterations until the RMS error is stable (Mackey et al. 1996). This means that depending on the data and parameters chosen, the ratios in the final matrix can be very different from the ones in the input matrix. This has positive aspects, as it indicates that the actual data (HPLC derived pigment concentrations) has the highest effect on the outcome of the CHEMTAX analysis. On the other hand, this means that under certain conditions, independently from the original input matrix, ratios can change in a wide range (here: 1/6 to 6 fold). When it comes to pigments that are shared between phytoplankton groups such as fucoxanthin, such an approach could shift the pigment : Chl a ratios between the groups in the opposite direction (input matrix: higher fucoxanthin : Chl a for group A compared to group B, output matrix: the other way around). This may be an additional explanation for the inconsistent relative abundance of diatoms versus chrysophytes in the meso-oligotrophic lake of our study. Similar results were found by Simmons et al. (2016), who compared the phytoplankton community composition via HPLC/CHEMTAX estimates to biovolume estimates derived from microscopic counts for the oligotrophic Lake Michigan. There, CHEMTAX overestimated chrysophytes versus diatoms. Interestingly, the input fucoxanthin : Chl a ratios for both groups of Simmons et al. (2016) were similar to the final output fucoxanthin : Chl a ratios for the meso-oligotrophic and the mesotrophic lake from our study, which leads to a consistent favoring of chrysophytes over diatoms in those CHEMTAX matrices. To overcome the observed mismatch between diatoms and chrysophytes, Simmons et al. (2016) suggested including chlorophyll c_1 and c_2 into CHEMTAX analyses as (freshwater) diatoms contain both chlorophyll c_1 and c_2, while most chrysophytes contain only chlorophyll c_2 (Jeffrey et al. 2011). Interestingly, when Simmons et al. (2016) combined the CHEMTAX-derived relative abundances of chrysophytes and diatoms, the match with the combined relative abundances of these two groups from the microscopic counts was much better, which was also observed in our study (personal communication M. Ilic, data not shown).

Phytoplankton biodiversity

Independent from the number of groups included into the calculation of the Shannon-Diversity Index, biodiversity of the phytoplankton community based on CHEMTAX was higher than

biodiversity calculated based on AlgaeLabAnalyser data. This indicates that CHEMTAX allows for a higher resolution of the phytoplankton community composition than the AlgaeLabAnalyser. The lower biodiversity estimates based on the data from the AlgaeLabAnalyser may be related to the observation, that in more than 63 % of the samples, the AlgaeLabAnalyser identified only one or two phytoplankton groups. This seems rather unlikely for samples from natural phytoplankton communities and shows a weakness of the AlgaeLabAnalyser in that case.

Another observation was the high positive correlation in our study between the pigment-based and the phytoplankton-based Shannon-Diversity. This indicates that even the pigment-based diversity can be used with these communities as a good proxy for the biodiversity of phytoplankton, without the necessity to perform CHEMTAX calculations.

Effects of trophic status (lake-dependent effects)

In some cases, the agreement of the two used methods seemed to depend on the trophic status of the lake. For example, the best agreement for TChl a, chlorophytes and chromophytes was found in the meso-oligotrophic Lake Klostersee (Table 4 in 3.4), while there were no lake-dependent effects on the agreement between AlgaeLabAnalyser and CHEMTAX for the biomass estimates of cryptophytes and cyanobacteria. This might indicate that the agreement between the two methods depends not only on the lake, its trophic status and phytoplankton community, but also on the overall biomass found in the lakes: too low or too high Chl a concentrations might be difficult to allocate accurately to the phytoplankton groups. Generally, lake identity and trophic status are two different effects, yet, in natural surroundings it is rather difficult to differentiate between these two, as “real” replicates are not possible. Nevertheless general effects of the trophic status like an increase in cyanobacteria with higher trophic status affect and interact with the respective lake identity. Therefore it can be assumed that the different trophic states of the investigated lakes certainly have an impact on our findings.

When differentiating between the chrysophytes and diatoms via CHEMTAX, we found higher abundances of diatoms in the oligotrophic lake compared to chrysophytes, while the opposite

was the case in the meso-oligotrophic and the mesotrophic lake. However, microscopic counts indicated a mismatch between the chrysophytes and diatoms in the meso-oligotrophic and the mesotrophic lake, while the abundances of those two groups, as determined with CHEMTAX, corresponded well to the cell counts in the oligotrophic lake. One explanation for such findings may be the usage of different input ratio matrices for the oligotrophic lake compared to the meso-oligotrophic and the mesotrophic lake. However, a repetition of CHEMTAX calculations for the meso-oligotrophic lake with the input ratio matrix for oligotrophic lakes (Schlüter et al. 2016) yielded unaltered results.

Interestingly, with both AlgaeLabAnalyser and CHEMTAX, we found the lowest average diversity in the oligotrophic Lake Brunnensee. This was surprising, as many studies claim that oligotrophic lakes usually harbor more diverse phytoplankton communities compared to mesotrophic or eutrophic lakes (Dodson et al. 2000, Leibold 1999). Our observations is probably due to a strong dominance of chromophytes and in particular diatoms in Lake Brunnensee. Nevertheless, we cannot exclude that despite the low functional diversity observed in Brunnensee, there may be an underlying high species richness within one functional group. Besides, due to the low overall biomass in the oligotrophic lake, rarely occurring phytoplankton species might be underestimated with both methods as too little individuals per liter are present in the samples.

Both the AlgaeLabAnalyser and HPLC/CHEMTAX are fast and useful tools for the assessment of the phytoplankton community composition. However, the agreement between the methods was not always completely satisfactory. This is similar to findings by Richardson et al. (2010) and may be due to different marker pigments utilized by the two methods. Also, more pigments should be included in the HPLC analysis, especially to be able to distinguish between diatoms and chrysophytes, e.g. violaxanthin and chlorophylls c1 and c2. As both methods have advantages and disadvantages, the method of choice depends on the aim of the study or the field of use. While the AlgaeLabAnalyser is more suitable for rapid monitoring, CHEMTAX provides a higher resolution of the biodiversity in the community and better estimates of cyanobacterial abundances.

4.5 Conclusions and outlook

With my laboratory experiments I could show that a diversity manipulation of natural phytoplankton communities is possible utilizing dilution or disturbance. Both tested techniques allowed to establish diversity gradients that could for instance be of further use in biodiversity ecosystem functioning (BEF) experiments. At the same time the experimental design can be upscaled to mesocosm experiments comprising several thousand liters of water. This provides the possibility of testing natural phytoplankton communities in set ups that closely resemble natural conditions. Such kinds of experiments could fill in the gap left by conventional studies using artificially assembled communities under laboratory conditions (Giller et al. 2004, Flombaum and Sala 2008).

After a successful diversity manipulation of the phytoplankton communities, the question arose, whether these manipulated communities show comparable reactions to environmental stressors and changes as naturally evolved communities that differ in diversity. My analyses with manipulated communities from a large scale mesocosm experiment under influence of nutrient enrichment thereby show differences in the performance of naturally evolved and short-term manipulated communities. It seems that short-term manipulations of diversity result in more variable responses while long-term evolved communities respond in a more directed way. Stabilizing effects of diversity could definitely be shown while it was difficult to separate single drivers operating. Yet, depending on the research question, the diversity gradients from these manipulated communities can provide good insight into the mechanisms behind biodiversity and ecosystem functioning. Therefore, the way of manipulating diversity (e. g. via dilution, different kinds of disturbances such as temperature, nutrients, manipulating stratification regimes, etc…) as well as the time scale should be attuned to the respective research question. Such diversity gradients should especially be useful in studies analyzing mechanisms acting on a trait and functional level as the suggested manipulations act on those levels in particular. Following a growing trend towards trait and functional based studies in ecology and evolution (Ackerly and Cornwell 2007, Smith et al. 2014, Kiørboe et al. 2018), studies as here presented should facilitate a better understanding of the mechanisms driving ecosystem functioning relationships.

A step towards such trait-based analyses is pursued by my experiments focusing on diatoms as a functional group and their traits connected to light usage. With a loss of diversity from this functional group, several associated traits also showed specific responses in the community. As these traits are closely connected to the functioning of the primary producer community in its ecosystem, a loss of traits here is directly connected to ecosystem functioning. Although this presents a first attempt of explaining mechanistic links at the basis of aquatic food webs, further insight into trait related functioning of ecosystems would be appreciated. For a more holistic approach, adding also higher trophic levels to experimental studies could provide a more comprehensive understanding (Duffy et al. 2007). In experimental setups as described here, a suggestion would be to include natural zooplankton communities in the study to analyze the consequences of a loss of diatoms from the community for organisms higher in the food chain. I would for example expect trait losses at the light usage level to affect higher trophic positions in the food chain via changes in food quality and quantity. With such approaches, an understanding of the system's functioning can be reached that enables better predictions and easier development of strategies in environmental management. Combining trait based functional research and multi-trophic experimental studies should thereby render valuable services.

Especially within environmental monitoring, in planning and deciding on management strategies, time is an important factor. Defining diversity and capturing species abundances in phytoplankton communities is still challenging. Apart from microscopy which still represents the gold standard and newer molecular tools, it is important to consider less time consuming techniques like the here described AlgaeLabAnalyser and HPLC measurements. Both methods have weaknesses considering a pure taxonomic assessment of the phytoplankton community. Yet, depending on the specific lab requirements and questions asked, they present the opportunity of fast analyses. While the HPLC method can give a very good trait based overview of the community, the AlgaeLabAnalyser results in a more functional classification of the community in a few minutes of time. For taxonomic analyses, molecular tools will probably evolve to be the method of choice in the future (e.g. Malviya et al. 2016), though implementing trait and functional based studies promises – like discussed above – progress in understanding the functioning of ecosystems.

A vast majority of water on earth is represented by oceans (> 97 %) and considerably less than 1 % are freshwater bodies like lakes and rivers (Wetzel 2001). Yet, marine and freshwater systems share similar structures and bottom up or top down mechanisms in the food web (Hessen and Kaartvedt 2014). Similar effects as those I observed would therefore be expected and should be tested in marine mesocosm experiments. Engel et al. (2017) have tested related ways of manipulating phytoplankton diversity as discussed here, yet in marine environments. Their findings propose a good transferability of experiments as presented in this study to marine phytoplankton communities. Experimental approaches like those frequently applied in freshwater habitats, become more common in marine studies, which have traditionally been of rather descriptive nature. Marine primary producer communities are of utmost significance to fishery yields as well as to cycles of matter and the stability of ecosystem functioning in oceans (Duffy and Stachowicz 2006). Besides academic interest, transferring the effects observed in my freshwater studies to marine systems should therefore provide relevant and applicable insight.

Loss of functional diversity is likely connected to anthropogenic factors influencing the environment, resulting in climate change, alteration of biogeochemical cycles and land usage or the dispersal patterns of species. Understanding the basic mechanisms driving major ecosystem functions can be gained by experiments as presented here. In a world undergoing constant change, such insights are important for future management actions and better predictability of ecosystem functioning.

5. References

Aarssen, L. W. 1997. High productivity in Grassland Ecosystems: Effected by Species Diversity or Productive Species? Oikos. 80: 183-184. doi: 10.2307/3546531

Ackerly, D. D., Cornwell, W. K. 2007. A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. Ecology Letters. 10: 135-145. doi: 10.1111/j.1461-0248.2007.01006.x

Admiraal, W., Breugem, P., Jacobs, D. M. L. H. A., de Ruyter van Steneninck, E. D. 1990. Fixation of dissolved silicate and sedimentation of biogenic silicate in the lower river Rhine during diatom blooms. Biogeochemistry. 9: 175-185. doi: 10.1007/BF00692170

Adrian, R., O'Reilly, C. M., Zagarese, H., Baines, S. B., Hessen D. O., Keller, W., Livingstone, D. M., Sommaruga, R., Straile, D., Van Donk, E., Weyhenmeyer, G. A., Winder, M. 2009. Lakes as sentinels of climate change. Limnology and Oceanography. 54: 2283-2297. doi: 10.4319/lo.2009.54.6_part_2.2283

Alberti, J., Cebrian, J., Alvarez, F., Escapa, M., Esquius, K. S., Fanjul, E., Sparks, E. L., Mortazavi, B., Iribarne, O. 2017. Nutrient and herbivore alterations cause uncoupled changes in producer diversity, biomass and ecosystem function, but not in overall multifunctionality. Scientific Reports. 7: 2639. doi: 10.1038/s41598-017-02764-3

Alexander, T. J., Vonlanthen, P., Seehausen, O. 2017. Does eutrophication-driven evolution change aquatic ecosystems? Philosophical Transactions of The Royal Society B. 372: 20160041. doi: 10.1098/rstb.2016.0041

Anderson, D. M., Glibert, P. M., Burkholder, J. M. 2002. Harmful Algal Blooms and Eutrophication: Nutrient Sources, Composition, and Consequences. Estuaries. 25: 704-726. doi: 10.1007/BF02804901

Armbrecht, L.H., Wright, S.W., Petocz, P., Armand, L.K. 2015. A new approach to testing the agreement of two phytoplankton quantification techniques: Microscopy and CHEMTAX: Agreement between microscopy and CHEMTAX. Limnology and Oceanography: Methods. 13: 425–437. doi:10.1002/lom3.10037

Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Nakashizuka, T., Raffaelli, D., Schmid, B. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters. 9: 1146-1156. doi: 10.1111/j.1461-0248.2006.00963.x

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T. B., Marshall, C., McGuire, J. L., Lindsey, E. L., Maguire, K. C., Mersey, B., Ferrer, E. A. 2011. Has the Earth's sixth mass extinction already arrived? Nature. 471: 51-57.

Barnosky, A. D., Hadly, E. A., Bascompte, J., Berlow, E. L., Brown, J. H., Fortelius, M., Getz, W. M., Harte, J., Hastings, A., Marquet, P. A., Martinez, N. D., Mooers, A., Roopnarine, P., Vermeij, G., William, J. W., Gillespie, R., Kitzes, J., Marshall, C., Matzke, N., Mindell, D. P., Revilla, E., Smith, A. B. 2012. Approaching a state shift in Earth's biosphere. Nature. 486: 52-58. doi: 10.1038/nature11018

Bates, D., Maechler, M., Bolker, B., Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 67: 1-48. doi:10.18637/jss.v067.i01

Batt, R. D., Carpenter, S. R., Cole, J. J., Pace, M. L., Johnson, R. A. 2013. Changes in ecosystem resilience detected in automated measures of ecosystem metabolism during a whole-lake manipulation. Proceedings of the National Acadamy of Sciences (USA). 110: 17398-17403. doi: 10.1073/pnas.1316721110

Behl, S., Donval, A., Stibor, H. 2011. The relative importance of species diversity and functional group diversity on carbon uptake in phytoplankton communities. Limnology and Oceanography. 56: 683-694. doi: 10.4319/lo.2011.56.2.0683

Behl, S., Stibor, H. 2015. Prey diversity and prey identity affect herbivore performance on different time scales in a long term aquatic food-web experiment. Oikos. 124: 1192–1202. doi:10.1111/oik.01463

Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L., Lilley, A.K. 2005. The contribution of species richness and composition to bacterial services. Nature. 436: 1157-1160. doi: 10.1038/nature03891

Beutler, M., Wiltshire, K.H., Meyer, B., Moldaenke, C., Lüring, C., Meyerhöfer, M., Hansen, U.-P., Dau, H. 2002. A fluorometric method for the differentiation of algal populations in vivo and in situ. Photosynthesis Research. 72: 39–53. doi:10.1023/A:1016026607048

Beutler, M., Wiltshire, K.H., Arp, M., Kruse, J., Reineke, C., Moldaenke, C., Hansen, U.-P. 2003. A reduced model of the fluorescence from the cyanobacterial photosynthetic apparatus designed for the in situ detection of cyanobacteria. Biochimica et Biophysica Acta (BBA) – Bioenergetics. 1604: 33–46. doi:10.1016/S0005-2728(03)00022-7

Beutler, M., Wiltshire, K.H., Reineke, C., Hansen, U.-P. 2004. Algorithms and practical fluorescence models of the photosynthetic apparatus of red cyanobacteria and Cryptophyta designed for the fluorescence detection of red cyanobacteria and cryptophytes. Aquatic microbial ecology. 35: 115–129. doi:10.3354/ame035115

Booth, B.C. 1993. Estimating cell concentration and biomass of autotrophic plankton using microscopy. Handbook of methods in aquatic microbial ecology. Lewis, pp. 199-205.

Borges Machado, K., Teresa, F. B., Galli Vieira, L. C., de Moraes Huszar, V. L., Nabout, J. C. 2016. Comparing the effects of landscape and local environmental variables on taxonomic and functional composition of phytoplankton communities. Journal of Plankton Research. 38: 1334-1346. doi: 10.1093/plankt/fbu062

Bramburger, A. J., Reavie, E. D., Sgro, G. V., Estepp, L. R., Chraibi, V. L. S., Pillsbury, R. W. 2017. Decreases in diatom cell size during the 20th century in the Laurentian Great Lakes: a response to warming waters? Journal of Plankton Research. 39: 199-210. doi: 10.1093/plankt/fbx009

Brett, M. T., Müller-Navarra, D. C. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology. 38:483-499. doi: 10.1046/j.1365-2427.1997.00220.x

Brooks, T. M., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., Rylands, A. B., Konstant, W. R., Flick, P, Pilgrim, J., Oldfield, S., Magin, G., Hilton-Taylor, C. 2002. Habitat Loss and Extinction in the Hotspots of Biodiversity. Conservation Biology. 16: 9009-923. doi: 10.1046/j.1523-1739.2002.00530.x

Bryant, D.A. 1982. Phycoerythrocyanin and phycoerythrin: properties and occurrence in cyanobacteria. Microbiology. 128: 835–844.

Brzezinski, M. A. 1985. The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. Journal of Phycology. 21: 347-357. doi: 10.1111/j.0022-3646.1985.00347.x

Buchaca, T., Felip, M., Catalan, J. 2005. A comparison of HPLC pigment analyses and biovolume estimates of phytoplankton groups in an oligotrophic lake. Journal of Plankton Research. 27: 91–101. doi:10.1093/plankt/fbh154

Cardinale, B. J., Srivastava, D. S., Duffy, J. E., Wright, J. P., Downing, A. L., Sankaran, M., Jouseau, C. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature. 443: 989-992. doi: 10.1038/nature05202

Cardinale, B. J. 2011. Biodiversity improves water quality through niche partitioning. Nature. 472: 86-89. doi: 10.1038/nature09904

Carpenter, S. R., Cottingham, K. L. 1997. Resilience and restoration of lakes. Conservation Ecology[online]1(1): 2. Available from the Internet. URL: http://www.consecol.org/vol1/iss1/art2/

Carpenter, S., Walker, B., Anderies, J. M., Abel, N. 2001. From Methaphor to Measurement: Resilience of What to What? Ecosystems. 4: 765-781. doi: 10.1007/s10021-001-0045-9

Carpenter, S. R. 2005. Eutrophication of aquatic ecosystems: Bistability and soil phosphorus. Proceedings of the National Acadamy of Sciences (USA). 102: 10002-10005. doi: 10.1073/pnas.0503959102

Carpenter, S. R., Cole, J. J., Pace, M. L., Batt, R., Brock, W. A., Cline, T., Coloso, J., Hodgson, J. R., Kitchell, J. F., Seekell, D. A., Smith, L., Weidel, B. 2011. Early Warnings of Regime Shifts: A Whole-Ecosystem Eyperiment. Science. 332: 1079-1082. doi: 10.1126/science.1203672

Catherine, A., Escoffier, N., Belhocine, A., Nasri, A.B., Hamlaoui, S., Yéprémian, C., Bernard, C., Troussellier, M. 2012. On the use of the FluoroProbe®, a phytoplankton quantification method based on fluorescence excitation spectra for large-scale surveys of lakes and reservoirs. Water Research. 46: 1771–1784. doi:10.1016/j.watres.2011.12.056

Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology. 63(2): 215-244. doi: 10.2307/5542

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., Behrenfeld, M. J., Boetius, A., Boyd, P. W., Classen, A. T., Crowther, T. W., Danovaro, R., Foreman, C. M., Huisman, J., Hutchins, D. A., Jansson, J. K., Karl, D. M., Koskella, B., Welch, D. B. M., Martiny, J. B. H., Moran, M. A., Orphan, V. J., Reay, D. S., Remais, J. V., Rich, V. I., Singh, B. K., Stein, L. Y., Stewart, F. J., Sullivan, M. B., van Oppen, M. J. H., Weaver, S. C., Webb, E. A., Webster, N. S. 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews Microbiology. doi: 10.1038/s41579-019-0222-5

Ceballos, G., Ehrlich, P. R. 2002. Mammal Population Losses and the Extinction Crisis. Science. 296: 904-907. doi: 10.1126/science.1069349

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., Palmer, T. M. 2015. Accelerated modern human-induced species losses: Entering the sixth mass extinction. Science Advances. 1: e1400253. doi: 10.1126/sciadv.1400253

Chapin, F. S., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., Hooper, D. U., Lavorel, S., Sala, O. E., Hobbie, S. E., Mack, M. C., Díaz, S. 2000. Consequences of changing biodiversity. Nature. 405: 234-242. doi: 10.1038/35012241

Clarke, K. R., Gorley, R. N., Somerfield, P. J., Warwick, R. M. 2014. Change in marine communities: an approach to statistical analysis and interpretation. 3rd edition. PRIMER-E: Plymouth.

Collins, S. L., Suding, K. N., Cleland, E. E., Batty, M., Pennings, S. C., Gross, K. L., Grace, J. B., Gough, L., Fargione, J. E., Clark, C. M. 2008. Rank clocks and plant community dynamics. Ecology. 89: 3534-3541. doi: 10.1890/07-1646.1

Conley, D. J., Schelske, C. L., Stoermer, E. F. 1993. Modification of the biogeochemical cycle of silica with eutrophication. Marine Ecology Progress Series. 101: 179-192. doi: 10.3354/meps101179

Conley, D. J., Stålnacke, P., Pitkänen, H., Wilander, A. 2000. The transport and retention of dissolved silicate by rivers in Sweden and Finland. Limnology and Oceanography. 45 1850-1853. doi: 10.4319/lo.2000.45.8.1850

Connell, J. H. 1978. Diversity in Tropical Rain Forests and Coral Reefs. Science. 199: 1302-1310. doi: 10.1126/science.199.4335.1302

Corcoran, A. A., Boeing, W. J. 2012. Biodiversity Increases the Productivity and Stability of Phytoplankton Communities. PLOS ONE. 7: e49397. doi: 10.1371/journal.pone.0049397

Craven, D., Isbell, F., Manning, P., Connolly, J., Bruelheide, H., Ebeling, A., Roscher, C., Ruijven, J., Weigelt, A., Wilsey, B., Beierkuhnlein, C., de Luca, E., Griffin, J.N., Hautier, Y., Hector, A., Jentsch, A., Kreyling, J., Lanta, V., Loreau, M., Meyer, S.T., Mori, A.S., Naeem, S., Palmborg, C., Polley, H.W., Reich, P.B., Schmid, B., Siebenkäs, A., Seabloom, E., Thakur, M.P., Tilman, D., Vogel, A., Eisenhauer, N. 2016. Plant diversity effects on grassland productivity are robust to both nutrient enrichment and drought. Philosophical Transactions of the Royal Society B 371: 20150277. doi: 10.1098/rstb.2015.0277

Cushing, D. H. 1989. A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. Journal of Plankton Research. 11: 1-13. doi: 10.1093/plankt/11.1.1

Dakos, V., Bascompte, J. 2014. Critical slowing down as early warning for the onset of collapse in mutualistic communities. Proceedings of the National Acadamy of Sciences (USA). 111: 17546-17551. doi: 10.1073/pnas.1406326111

Descy, J.-P., Higgins, H.W., Mackey, D.J., Hurley, J.P., Frost, T.M. 2000. Pigment ratios and phytoplankton assessment in northern Wisconsin lakes. Journal of Phycology. 36: 274–286. doi: 10.1046/j.1529-8817.2000.99063.x

Descy, J.-P., Sarmento, H., Higgins, H.W. 2009. Variability of phytoplankton pigment ratios across aquatic environments. European Journal of Phycology. 44: 319–330. doi:10.1080/09670260802618942

Díaz, S., Symstad, A. J., Chapin, F. S., Wardle, D. A., Huenneke, L. F. 2003. Functional diversity revealed by removal experiments. TRENDS in Ecology and Evolution. 18: 140-146. doi: 10.1016/S0169-5347(03)00007-7

Díaz, S., Fargione, J., Chapin, F. S., Tilman, D. 2006. Biodiversity Loss Threatens Human Well-Being. PLOS Biology. 4:e277. doi: 10.1371/journal.pbio.0040277

Dodson, S.I., Arnott, S.E., Cottingham, K.L. 2000. The Relationship in Lake Communities between Primary Productivity and Species Richness. Ecology. 81: 2662. doi:10.2307/177332

Donohue I., Hillebrand, H., Montoya, J. M., Petchey, O. L., Pimm, S. L., Fowler, M. S., Healy, K., Jackson, A. L., Lurgi, M., McClean, D., O'Connor, N. E., O'Gorman, E. J., Yang, Q. 2016. Navigating the complexity of ecological stability. Ecology Letters. 19: 1172-1185. doi: 10.1111/ele.12648

Duffy, J. E., Stachowicz, J. J. 2006. Why biodiversity is important to oceanography: potential roles of genetic, species, and trophic diversity in pelagic ecosystem processes. Marine Ecology Progress Series. 311: 179-190. www.jstor.org/stable/24870044.

Duffy, J. E., Cardinale, B. J., France, K. E., McIntyre, P. B., Thébault, E., Loreau, M. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. Ecology Letters. 10: 522-538. doi: 10.1111/j.1461-0248.2007.01037.x

Edwards, M., Richardson, A. J. 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature. 430: 881-884. doi: 10.1038/nature02808

Ehrlich, P. R. 1988. The Loss of Diversity Causes and Consequences. In Biodiversity. Editor: Wilson, E. O. The National Academies Press. Washington, DC, USA. doi: 10.17226/989

Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B., Norberg, J. 2003. Response diversity, ecosystem change, and resilience. Frontiers in Ecology and the Environment. 1: 488-494. doi: 10.1890/1540-9295(2003)001[0488:RDECAR]2.0.CO;2

Engel, F. G., Lewandowska, A. M., Eggers, S. L., Matthiessen, B. 2017. Manipulation of Non-random Species Loss in Natural Phytoplankton: Qualitative and Quantitative Evaluation of Different Approaches. Frontiers in Marine Science. 4: 317. doi: 10.3389/fmars.2017.00317

Escoffier, N., Bernard, C., Hamlaoui, S., Groleau, A., Catherine, A. 2015. Quantifying phytoplankton communities using spectral fluorescence: the effects of species composition and physiological state. Journal of Plankton Research. 37: 233–247. doi:10.1093/plankt/fbu085

Falkowski, P. G., Raven, J. A. 2007. Aquatic Photosynthesis. 2nd edition. Princeton University Press. Princeton, USA.

Falkowski, P. 2012. The power of plankton. Nature. 483: 17-20. doi: 10.1038/483S17a

Fang, X., Stefan, H. G. 2009. Simulations of climate effects on water temperature, dissolved oxygen, and ice and snow covers in lakes of the contiguous United States under past and future climate scenarios. Limnology and Oceanography. 54: 2359-2370. doi: 10.4319/lo.2009.54.6_par_2.2359

Fietz, S., Nicklisch, A. 2004. An HPLC analysis of the summer phytoplankton assemblage in Lake Baikal. Freshwater Biology. 49: 332–345. doi: 10.1111/j.1365-2427.2004.01183.x

Finkel, Z. V., Beardall, J., Flynn, K. J., Quigg, A., Rees, T. A. V., Raven, J. A. 2010. Phytoplankton in a changing world: cell size and elemental stoichiometry. Journal of Plankton Research. 32: 119-137. doi: 10.1093/plankt/fbp098

Fisher N.S. 1977. On the Differential Sensitivity of Estuarine and Open-Ocean Diatoms to Exotic Chemical Stress. The American Naturalist. 111(981): 871-895. doi: 10.1086/283220

Flöder, S., Sommer, U. 1999. Diversity in planktonic communities: an experimental test of the intermediate disturbance hypothesis. Limnology and Oceanography. 44: 1114–1119. doi: 10.4319/lo.1999.44.4.1114

Flombaum, P., Sala, O. E. 2008. Higher effect of plant species diversity on productivity in natural than artificial ecosystems. Proceedings of the National Acadamy of Sciences (USA). 105: 6087-6090. doi: 10.1073/pnas.0704801105

Fox, J. W. 2005. Interpreting the 'selection effect' of biodiversity on ecosystem function. Ecology Letters. 8: 846-856. doi: 10.1111/j.1461-0248.2005.00795.x

Fox, J., Weisberg, S. 2011. Package "car": Companion to Regression, Second Edition, version 2.1.3. Thousand Oaks CA: Sage

Franklin, R. B., Garland, J. L., Bolster, C. H., Mills, A. L. 2001. Impact of dilution on microbial community structure and functional potential: Comparison of numerical simulations and batch culture experiments. Applied and Environmental Microbiology. 67:702-712. doi: 10.1128/AEM.67.2.702-712.2001

Fussmann, G. F., Loreau, M., Abrams, P. A. 2007. Eco-evolutionary dynamics of communities and ecosystems. Functional Ecology. 21: 465-477. doi: 10.1111/j.1365-2435.2007.01275.x

Galiana, N., Lurgi, M., Montoya, J. M., López, B. C. 2014. Invasions cause biodiversity loss and community simplification in vertebrate food webs. Oikos. 123: 721-728. doi: 10.1111/j.1600-0706.2013.00859.x

Gallagher A.J., Hammerschlag, N., Cooke, S.J., Costa, D.P., Irschick, D.J. 2015. Evolutionary theory as a tool for predicting extinction risk. Trends in Ecology & Evolution. 30: 61-65. doi: 10.1016/j.tree.2014.12.001

Garland, J. L., Lehman, R. M. 1999. Dilution/extinction of community phenotypic characters to estimate relative structural diversity in mixed communities. FEMS Microbiology Ecology. 30: 333-343. doi: 10.1111/j.15746941.1999.tb00661.x

Garrido, J.L., Zapata, M. 1993. High performance liquid chromatography of chlorophylls c 3, c 1, c 2 and a and of carotenoids of chromophyte algae on a polymeric octadecyl silica column. Chromatographia. 35: 543–547. doi: 10.1007/BF02267914

Gelzinis, A., Butkus, V., Songaila, E., Augulis, R., Gall, A., Büchel, C., Robert, B., Abramavicius, D., Zigmantas, D., Valkunas, L. 2015. Mapping energy transfer channels in fucoxanthin-chlorophyll protein complex. Biochimica et Biophysica Acta - Bioenergetics. 1847: 241-247. doi: 10.1016/j.bbabio.2014.11.004

Gieskes, W.W.C., Kraay, G.W. 1983. Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. Marine Biology. 75: 179–185. doi: 10.1007/BF00406000

Giller, P. S., Hillebrand, H., Berninger, U. G., Gessner, M. O., Hawkins, S., Inchausti, P., Inglis, C., Leslie, H., Malmqvist, B., Monaghan, M. T., Morin, P. J., O'Mullan, G. 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. OIKOS. 104: 423-436. doi: 10.1111/j.0030-1299.2004.13253.x

Gotelli, N. J., Colwell, R. K. 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters. 4: 379-391. doi: 10.1046/j.1461-0248.2001.00230.x

Gotelli, N. J., Chao, A. 2013. Measuring and Estimating Species Richness, Species Diversity, and Biotic Similarity from Sampling Data. In Encyclopedia of Biodiversity. 2nd edition. Editor: Levin, S. A. Elsevier Academic Press. Waltham. pp. 195-211

Gregor, J., Maršálek, B. 2004. Freshwater phytoplankton quantification by chlorophyll a: a comparative study of in vitro, in vivo and in situ methods. Water Research. 38: 517–522. doi:10.1016/j.watres.2003.10.033

Gregor, J., Geriš, R., Maršálek, B., Heteša, J., Marvan, P. 2005. In situ Quantification of Phytoplankton in Reservoirs Using a Submersible Spectrofluorometer. Hydrobiologia. 548: 141–151. doi:10.1007/s10750-005-4268-1

Groendahl, S., Kahlert, M., Fink, P. 2017. The best of both worlds: A combined approach for analyzing microalgal diversity via metabarcoding and morphology-based methods. PLOS ONE. 12: e0172808. doi:10.1371/journal.pone.0172808

Gunderson, L. H. 2000. Ecological Resilience – In Theory and Application. Annual Review of Ecology and Systematics. 31: 425-439. doi: 10.1146/annurev.ecolsys.31.1.425

Guttal, V., Jayaprakash, C. 2008. Changing skewness: an early warning signal of regime shifts in ecosystems. Ecology Letters. 11: 450-460. doi: 10.1111/j.1461-0248.2008.01160.x

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia. 32: 79-99. doi: 10.2216/i0031-8884-32-2-79.1

Hallett, L. M., Jones, S. K., MacDonald, A. A. M., Jones, M. B., Flynn, D. F. B., Ripplinger, J., Slaughter, P., Gries, C., Collins, S. L. 2016. CODYN: An R package of community dynamics metrics. Methods in Ecology and Evolution. 7: 1146-1151. doi: 10.1111/2041-210X.12569

Hammerstein, S. K., Stockenreiter, M., Stibor, H. 2017. Directed diversity manipulations within natural phytoplankton communities. Limnology and Oceanography Methods. 15: 653-662. doi: 10.1002/lom3.10190

Handa T., Aerts R., Berendse F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid B., Ruijven, J., Vos, V.C.A., Hättenschwiler, S. 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature. 509: 218-221. doi: 10.1038/nature13247

Hanski, I. 2011. Habitat Loss, the Dynamics of Biodiversity, and a Perspective on Conservation. AMBIO. 40: 248-255. doi: 10.1007/s13280-011-0147-3

Harrison, J.W., Howell, E.T., Watson, S.B., Smith, R.E.H. 2016: Improved estimates of phytoplankton community composition based on in situ spectral fluorescence: use of ordination and field-derived norm spectra for the bbe FluoroProbe. Canadian Journal of Fisheries and Aquatic Sciences. 73: 1472–1482. doi: 10.1139/cjfas-2015-0360

Haverkamp, T.H., Schouten, D., Doeleman, M., Wollenzien, U., Huisman, J., Stal, L.J. 2009. Colorful microdiversity of Synechococcus strains (picocyanobacteria) isolated from the Baltic Sea. The ISME journal. 3: 397–408. doi: 10.1038/ismej.2008.118

Havskum, H., Schlüter, L., Scharek, R., Berdalet, E., Jacquet, S. 2004. Routine quantification of phytoplankton groups microscopy or pigment analyses? Marine Ecology Progress Series. 273: 31–42. doi: 10.3354/meps273031

Hector, A. 2015. The new statistics with R, 1st ed. Oxford University Press

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C. J., Heil, C. A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H. G., Sellner, K., Stockwell, D. A., Stoecker, D.

K., Suddleson, M. 2008. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae. 8: 3-13. doi: 10.1016/j.hal.2008.08.006

Herbstová, M., Bína, D., Kaňa, R., Vácha, F., Litvín, R. 2017. Red-light phenotype in a marine diatom involves a specialized oligomeric red-shifted antenna and altered cell morphology. Scientific Reports. 7: 11976. doi: 10.1038/s41598-017-12247-0

Hessen, D. O., Kaartvedt, S. 2014. Top-down cascades in lakes and oceans: different perspectives but same story? Journal of Plankton Research. 36: 914-924. doi: 10.1093/plankt/fbu040

Van Heukelem, L., Thomas, C.S. 2001. Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. Journal of Chromatography A. 910: 31–49. doi: 10.1016/S0378-4347(00)00603-4

Higgins, H.W., Wright, S.W., Schlüter, L. 2011. Quantitative interpretation of chemotaxonomic pigment data, in Phytoplankton Pigments. Editors: Roy, S., Llewellyn, C., Egeland, E.S., Johnsen, G. Cambridge University Press, Cambridge. pp. 257–313.

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., Zohary, T. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology. 35:403–24. doi: 10.1046/j.1529-8817.1999.3520403.x

Hillebrand, H., Grunder, D. S., Borer, E. T., Bracken, M. E. S., Cleland, E. E., Elser, J. J., Harpole, W. S., Ngai, J. T., Seabloom, E. W., Shurin, J. B., Smith, J. E. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. Proceedings of the National Acadamy of Sciences (USA). 104: 10904-10909. doi: 10.1073/pnas.0701918104

Hillebrand, H., Blasius, B., Borer, E. T., Chase, J. M., Downing, J. A., Eriksson, B. K., Filstrup, C. T., Harpole, W. S., Hodapp, D., Larsen, S., Lewandowska, A. M., Seabloom, E. W., Van de Waal, D. B., Ryabov, A. B. 2017. Biodiversity change is uncoupled from species richness trends: consequences for conservation and monitoring. Journal of Applied Ecology. 55: 169-184. doi: 10.1111/1365-2664.12959

Hillebrand, H., Langenheder, S., Lebret, K., Lindström, E., Östman, Ö., Striebel, M. 2018. Decomposing multiple dimensions of stability in global change experiments. Ecology Letters. 21: 21-30. doi: 10.1111/ele.12867

Holling, C. S., 1973. Resilience and Stability of Ecological Systems. Annual Review of Ecology and Systematics. 4: 1-23. doi: 10.1146/annurev.es.04.110173.000245

Hooker, S.B., Van Heukelem, L., Thomas, C.S., Claustre, H., Ras, J., Barlow, R., Session, H., Schlüter, L., Perl, J., Trees, C., Stuart, V., Head, E., Clementson, L., Fishwick, J., Llewellyn, C., Aiken, J. 2005. The second SeaWiFS HPLC analysis round-robin experiment (SeaHARRE-2). NASA Tech. Memo, 212785, 124

Hooper, D. U., Chapin III, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A. J., Vandermeer, J., Wardle, D. A. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs. 75: 3-35. doi: 10.1890/04-0922

Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E. K., Hungate, B. A., Matulich, K. L., Gonzalez, A., Duffy, J. E., Gamfeldt, L., O'Connor, M. I. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature. 486: 105-108. doi: 10.1038/nature11118

Hu, W., Gladue, R., Hansen, J., Wojnar, C., Chalmers, J. J. 2009. Growth Inhibition of Dinoflagellate Algae in Shake Flasks: Not Due to Shear This Time! Biotechnology Progress. 26: 79-87. doi: 10.1002/btpr.301

Hughes, J. B., Hellmann, J. J., Ricketts, T. H., Bohannan, B. J. M. 2001. Counting the uncountable: Statistical approaches to estimating microbial diversity. Applied and Environmental Microbiology. 67: 4399-4406. doi: 10.1128/AEM.67.10.4399-4406.2001

Hughes, A. R., Byrnes, J. E., Kimbro, D. L., Stachowicz, J. J. 2007. Reciprocal relationships and potential feedbacks between biodiversity and disturbance. Ecology Letters. 10: 849-864. doi: 10.1111/j.1461-0248.2007.01075.x

Humborg, C., Ittekkot, V., Cociasu, A., v. Bodungen, B. 1997. Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. Nature. 386: 385-388. doi: 10.1038/386385a0

Humborg, C., Conley, D. J., Rahm, L., Wulff, F., Cociasu, A., Ittekkot, V. 2000. Silicon Retention in River Basins: Far-reaching Effects on Biogeochemistry and Aquatic Food Webs in Coastal Marine Environments. Ambio. 29: 45-50. doi: 10.1579/0044-7447-29.1.45

Humborg, C, Blomqvist, S., Avsan, E., Bergensund, Y., Smedberg, E., Brink, J., Mörth, C.-M. 2002. Hydrological alterations with river damming in northern Sweden: Implications for weathering and river biogeochemistry. Global Biogeochemical Cycles. 16: 1039. doi: 10.1029/2000GB001369

Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia. 110: 449-460. doi: 10.1007/s004420050180

Huston, M. A. 2014. Disturbance, productivity, and species diversity: empiricism vs. logic in ecological theory. Ecology. 95: 2382-2396. doi: 10.1890/13-1397.1

Ibelings, B. W., Portielje, R., Lammens, E. H. R. R., Noordhuis, R., van de Berg, M. S., Joosse, W., Meijer, M. L. 2007. Resilience of Alternative Stable States during the Recovery of Shallow Lakes from Eutrophication: Lake Veluwe as a Case Study. Ecosystems. 10: 4-16. doi: 10.1007/s10021-006-9009-4

Isbell, F. I., Polley, H. W., Wilsey, B. J. 2009. Biodiversity, productivity and the temporal stability of productivity: patterns and processes. Ecology Letters. 12: 443-451. doi: 10.1111/j.1461-0248.2009.01299.x

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., Bezemer, T. M., Bonin, C., Bruelheide, H., de Luca, E., Ebeling, A., Griffin, J. N., Guo, Q., Hautier, Y., Hector, A., Jentsch, A., Kreyling, J., Lanta, V., Manning, P., Meyer, S. T., Mori, A. S., Naeem, S., Niklaus, P. A., Polley, H. W., Reich, P. B., Roscher, C., Seabloom, E. W., Smith, M. D., Thakur, M. P., Tilman, D., Tracy, B. F., van der Putten, W. H., van Ruijven, J., Weigelt, A., Weisser, W. W., Wilsey, B., Eisenhauer, N. 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature. 526: 574-577. doi: 10.1038/nature15374

Iverson, S. J. 2009. Tracing Aquatic Food Webs Using Fatty Acids: From Qualitative Indicators to Quantitative Determination. In Lipids in Aquatic Ecosystems. Editors: Arts, M. T., Brett, M. T., Kainz, M . Springer Science + Business Media. pp. 281-308.

Izydorczyk, K., Carpentier, C., Mrówczyński, J., Wagenvoort, A., Jurczak, T., Tarczyńska, M. 2009. Establishment of an Alert Level Framework for cyanobacteria in drinking water resources by using the Algae Online Analyser for monitoring cyanobacterial chlorophyll a. Water Research. 43: 989–996. doi:10.1016/j.watres.2008.11.048

Jain, M., Flynn, D.F.B., Prager, C.M., Hart, G.M., DeVan, C.M., Ahrestani, F.S., Palmer, M.I., Bunker, D.E., Knops, J.M.H., Jouseau, C.F., Naeem, S. 2014. The importance of rare species: a trait-based assessment of rare species contributions to functional diversity and possible ecosystem function in tall-grass prairies. Ecology and Evolution 4(1): 104-112. doi: 10.1002/ece3.915

Järvinen, M., Drakare, S., Free, G., Lyche-Solheim, A., Phillips, G., Skjelbred, B., Mischke, U., Ott, I., Poikane, S., Søndergaard, M., Pasztaleniec, A., Van Wichelen, J., Portielje, R. 2013. Phytoplankton indicator taxa for reference conditions in Northern and Central European lowland lakes. Hydrobiologia. 704: 97–113. doi:10.1007/s10750-012-1315-6

Jasser, I., Karnkowska-Ishikawa, A., Kozłowska, E., Królicka, A., Łukomska-Kowalczyk, M. 2010. Composition of picocyanobacteria community in the Great Mazurian Lakes: isolation of phycoerythrin-rich and phycocyanin-rich ecotypes from the system-comparison of two methods. Polish Journal of Microbiology. 59: 21-31

Jeffrey, S.; Vesk, M. 1997. Introduction to marine phytoplankton and their pigment signatures. In Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods; Editors: Jeffrey, S., Mantoura, R., Wright, S. UNESCO Publishing: Paris, France. pp. 37–84.

Jeffrey, S.W., Wright, S.W., Zapata, M. 2011. Microalgal classes and their signature pigments, Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography. Editors: Roy, S., Llewellyn, C. A., Egeland, E. S., Johnsen, G. Cambridge University Press. United Kingdom. pp. 3-77

Johnson, P. 2017. devEMF: EMF Graphics Output Device. R package version 3.4

de Jonge, V. N., Elliott, M., Orive, E. 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. Hydrobiologia. 475: 1-19. doi: 10.1023/A:1020366418295

Justić, D., Rabalais, N. N., Turner, R. E., Dortch, Q. 1995. Changes in Nutrient Structure of River-dominated Coastal Waters: Stoichiometric Nutrient Balance and its Consequences. Estuarine, Coastal and Shelf Science. 40: 339-356. doi: 10.1016/S0272-7714(05)80014-9

Kiørbe, T., Visser, A., Andersen, K. H. 2018. A trait-based approach to ocean ecology. ICES Journal of Marine Science. 75: 1849-1863. doi: 10.1093/icesjms/fsy090

Kremer, C. T., Gillette, J. P., Rudstam, L. G., Brettum, P., Ptacnik, R. 2014. A compendium of cell and natural unit biovolumes for >1200 freshwater phytoplankton species. Ecological Archives. E095-257. doi: 10.1890/14-0603.1

Kuczynska, P., Jemioa-Rzeminska, M., Strzalka, K. 2015. Photosynthetic Pigments in Diatoms. Marine Drugs: 13: 5847-5881. doi: 10.3390/md13095847

Lake, P. S. 2013. Resistance, Resilience and Restoration. Ecological Management & Restoration. 14: 20-24. doi: 10.1111/emr.12016

Laruelle, G. G., Roueix, V., Sferratore, A., Brodherr, B., Ciuffa, D., Conley, D. J., Dürr, H. H., Garnier, J., Lancelot, C., Le Thi Phuong, Q., Meunier, J.-D., Meybeck, M., Michalopoulos, P., Moriceau, B., Ni Longphuirt, S., Loucaides, S., Papush L., Presti, M., Ragueneau, O., Regnier, P., Saccone, L., Slomp, C. P., Spiteri, C., Van Cappellen, P. 2009. Anthropogenic perturbations of the silicon cycle at the global scale: Key role of the land-ocean transition. Global Biogeochemical Cycles. 23: GB40301. doi: 10.129/2008GB003267

Latasa, M., Bidigare, R.R., Ondrusek, M.E., Kennicutt, M.C. 1996. HPLC analysis of algal pigments: A comparison exercise among laboratories and recommendations for improved analytical performance. Marine Chemistry. 51: 315–324. doi: 10.1016/0304-4203(95)00056-9

Latasa, M. 2007. Improving estimations of phytoplankton class abundances using CHEMTAX. Marine Ecology Progress Series. 329: 13–21. doi:10.3354/meps329013

Lauridsen, T.L., Schlüter, L., Johansson, L.S. 2011. Determining algal assemblages in oligotrophic lakes and streams: comparing information from newly developed pigment/chlorophyll a ratios with direct microscopy: Comparison between algal pigments and microscopy. Freshwater Biology. 56: 1638–1651. doi:10.1111/j.1365-2427.2011.02588.x

Leibold, M. A. 1999. Biodiversity and nutrient enrichment in pond plankton communities. Evolutionary Ecology Research. 1: 73-95.

Lewandowska, A.M., Biermann, A., Borer, E.T., Cebrián-Piqueras, M.A., Declerck, S.A.J., De Meester, L., Van Donk, E., Gamfeldt, L., Gruner, D.S., Hagenah, N., Harpole, W.S., Kirkman, K.P., Klausmeier, C.A., Kleyer, M., Knops, J.M.H., Lemmens, P., Lind, E.M., Litchman, E., Mantilla-Contreras, J., Martens, K., Meier, S., Minden, V., Moore, J.L., Venterink, H.O., Seabloom, E.W., Sommer, U., Striebel, M., Trenkamp, A, Trinogga, J., Urabe, J., Vyverman, W., Van de Waal, D.B., Widdicombe, C.E., Hillebrand, H. 2016. The influenced of balanced and imbalanced resource supply on biodiversity-functioning relationship across ecosystems. Philosophical Transactions of the Royal Society B. 371: 20150283. doi: 10.1098/rstb.2015.0283

Lewitus, A.J., White, D.L., Tymowski, R.G., Geesey, M.E., Hymel, S.N., Noble, P.A. 2005. Adapting the CHEMTAX method for assessing phytoplankton taxonomic composition in Southeastern U.S. estuaries. Estuaries. 28: 160. doi:10.1007/BF02732761

Li, H.-Y., Lu, Y., Zheng, J.-W., Yang, W.-D., Liu, J.-S. 2014. Biochemical and Genetic Engineering of Diatoms for Polyunsaturated Fatty Acid Biosynthesis. Marine Drugs. 12: 153-166. doi: 10.3390/md12010153

Lindegren, M., Dakos, V., Gröger, J. P., Gårdmark, A., Kornilovs, G., Otto, S. A., Möllmann, C. 2012. Early Detection of Ecosystem Regime Shifts: A Multiple Method Evaluation for Management Application. PLOS ONE. 7: e38410. doi: 10.1371/journal.pone.0038410

Litchman, E., Klausmeier, C. A. 2008. Trait-Based Community Ecology of Phytoplankton. Annual Reviwe of Ecology Evolution and Systematics. 39: 615-639. doi: 10.1146/annurev.ecolsys.39.110707.173549

Livingstone, D. M. 2003. Impact of Secular Climate Change on the Thermal Structure of a Large Temperate Central European Lake. Climatic Change. 57: 205-225. doi: 10.1023/A:1022119503144

Livingstone, D. M. 2008. A Change of Climate Provoke a Change of Paradigm: Taking Leave of Two Tacit Assumptions about Physical Lake Forcing. International Review of Hydrobiology. 93: 404-414. doi: 10.1002/iroh.200811061

Llewellyn, C.A., Fishwick, J. R., Blackford, J. C. 2005. Phytoplankton community assemblage in the English Channel: a comparison using chlorophyll a derived from HPLC-CHEMTAX and carbon derived from microscopy cell counts. Journal of Plankton Research. 27: 103–119. doi:10.1093/plankt/fbh158

Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. OIKOS. 91: 3-17. doi: 10.1034/j.1600-0706.2000.910101.x

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D. A. 2001. Science. 294: 804-808. doi: 10.1126/science.1064088

Loreau, M. 2010. Linking biodiversity and ecosystems: towards a unifying ecological theory. Philosophical Transactions of the Royal Society B. 365:49-60. doi: 10.098/rstb.2009.0155

Lund, J. W. G., Kipling, C., Le Cren, E. D. 1958. The Inverted Microscope Method of Estimating Algal Numbers and the Statistical Basis of Estimations by Counting. Hydrobiologia. 11:143-170. doi: 10.1007/BF00007865

Lyons, K.G., Brigham, C.A., Traut, B.H., Schwartz, M.W. 2005. Rare Species and Ecosystem Functioning. Conservation Biology. 19: 1019-1024. doi: 10.1111/j.1523-1739.2005.00106.x

MacIntyre, H.L., Lawrenz, E., Richardson, T.L. 2010. Taxonomic discrimination of phytoplankton by spectral fluorescence. In Chlorophyll a fluorescence in aquatic sciences: methods and applications. Springer Netherlands. pp. 129-169

Mackey, M.D., Mackey, D.J., Higgins, H.W., Wright, S.W. 1996. CHEMTAX-a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Marine Ecology Progress Series. 144: 265–283. doi: 10.3354/meps144265

Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., Wincker, P., Iudicone, D., de Vargas, C., Bittner, L., Zingone, A., Bowler, C. 2016. Insights into global diatom distribution and diversity in the world's ocean. Proceedings of the National Acadamy of Sciences (USA). 113: E1516-E1525. doi: 10.1073/pnas.1509523113

Mateo, R. G., Mokany, K., Guisan, A. 2017. Biodiversity Models: What If Unsaturation Is the Rule? Trends in Ecology & Evolution. 32: 556-566. doi: 10.1016/j.tree.2017.05.003

Matson, P. A., Parton, W. J., Power, A. G., Swift, M. J. 1997. Agricultural Intensification and Ecosystem Properties. Science. 277: 504-509. doi: 10.1126/science.277.5325.504

de Mendiburu, F. 2016. Package "agricolae": Statistical Procedures for Agricultural Research, version 1.2.4.

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute. Island Press. Washington, DC.

Morán, X. A. G., López-Urrutia, Á., Calvo-Díaz, A., Li, W. K. W. 2010. Increasing importance of small phytoplankton in a warmer ocean. Global Change Biology. 16: 1137-1144. doi: 10.1111/j.1365-2486.2009.01960.x

Mouillot, D., Mason, W. H. N., Dumay, O., Wilson, J. B. 2005. Functional regularity: a neglected aspect of functional diversity. Oecologia. 142: 353-359. doi: 10.1007/s00442-004-1744-7

Mouillot, D., Bellwood, D.R., Baraloto, C., Chave, J., Galzin, R., Harmelin-Viviens, M., Kulbicki, M., Lavergne, S., Lavorel, S., Mouquet, N., Paine, C.E.T., Renaud, J., Thuiller, W. 2013. Rare Species Support Vulnerable Functions in High-Diversity Ecosystems. PLOS Biol 11(5): e1001569. doi:10.1371/journal.pbio.1001569

Müller-Navarra, D. C. 2008. Food Web Paradigms: The Biochemmical View on Trophic Interactions. International Review of Hydrobiology. 93: 489-505. doi: 10.1002/iroh.200711046

Naeem, S., Wright, J. P. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecology Letters. 6: 567-579. doi: 10.1046/j.1461-0248.2003.00471.x

Naeem, S., Duffy, J. E., Zavaleta, E. 2012. The Functions of Biological Diversity in an Age of Extinction. Science. 336: 1401-1406. doi: 10.1126/science.1215855

Nelson, D. M., Tréguer, P., Brzezinski, M. A., Leynaert, A., Quéguiner, B. 1995. Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. Global Biogeochemical Cycle. 9: 359-372. doi: 10.1029/95GB01070

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H. 2016. Package "vegan": Community ecology package, version 2.4.1.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H. 2017. vegan: Community Ecology Package. R package version 2.4-3

Oliver, T. H., Heard, M. S., Isaac, N. J. B., Roy, D. B., Procter, D., Eigenbrod, F., Freccleton, R., Hector, A., Orme, C. D. L., Petchey, O. L., Proença, V., Raffaelli, D., Shuttle, K. B., Mace, G. M., Martín-López, B., Woodcock, B. A., Bullock, J. M. 2015. Biodiversity and Resilience of Ecosystem Functions. Trends in Ecology & Evolution. 30: 673-684. doi: 10.1016/j.tree.2015.08.009

Paerl, H. W., Huisman, J. 2008. Blooms Like It Hot. Science. 320: 57-58. doi: 10.1126/science.1155398

Pajk, F., von Elert, E., Fink, P. 2012. Interaction of changes in food quality and temperature reveals maternal fitness parameters of a keystone aquatic herbivore. Limnology and Oceanography. 57: 281-292. doi: 10.4319/lo.2012.57.1.0281

Pantel, J. H., Duvivier, C., De Meester, L. 2015. Rapid locl adaptation mediates zooplankton community assembly in experimental mesocosms. Ecology Letters. 18: 992-1000. doi: 10.1111/ele.12480

Pelletier, F., Garant, D., Hendry, A. P. 2009. Eco-evolutionary dynamics. Philosophical Transactions of The Royal Society B. 364: 1483-1489. doi: 10.1098/rstb.2009.0027

Petersen,J.E., Kennedy, V.S., Dennisen, W.C., Kemp, W.M. 2009. Enclosed experierimental ecosystems and scale. Tools for understanding and managing costal ecosystems. Springer-Verlag New York.

Pimm, S.L., Jones, H.L., Diamond, J. 1988. On the Risk of Extinction. The American Naturalist. 132(6): 757-785. doi: 10.1086/284889

Pohlert, T. 2014. Package "PMCMR": The Pairwise Multiple Comparison of Mean Ranks, version 4.1

Power, L. D., Cardinale, J. 2009. Species richness enhances both algal biomass and rates of oxygen production in aquatic microcosms. Oikos. 118: 1703-1711. doi: 10.1111/j.1600-0706.2009.17585.x

Poxleitner, M., Trommer, G., Lorenz, P., Stibor, H. 2016. The effect of increased nitrogen load on phytoplankton in a phosphorus-limited lake. Freshwater Biology. 61: 1966–1980. doi:10.1111/fwb.12829

Ptacnik, R., Solimini, A. G., Andersen T., Tamminen, T., Brettum, P., Lepistö, L., Willén, E., Rekolainen, S. 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. Proceedings of the National Acadamy of Sciences (USA). 105: 5134-5138. doi: 10.1073/pnas.0708328105

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Redfield, A.C. 1934. On the Proportions of Organic Derivatives in Sea Water and Their Relation to the Composition of Plankton. James Johnstone Memorial Volume, University Press of Liverpool, 176-192

Reynolds, C.S., Petersen, A.C. 2000. The distribution of planktonic Cyanobacteria in Irish lakes in relation to their trophic states, in: The Trophic Spectrum Revisited. Editors: Reynolds, C. S., Dokulil, M., Padisák, J. Springer, pp. 91–99.

Ricciardi, A., Rasmussen, J. B. 1999. Extinction Rates of North American Freshwater Fauna. Conservation Biology. 13: 1220-1222. doi: 10.1046/j.1523-1739.1999.98380.x

Richardson, A. J., Bakun, A., Hays, G. C., Gibbons, M. J. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. TRENDS in Ecology & Evolution. 24: 312-322. doi: 10.1016/j.tree.2009.01.010

Richardson, T.L., Lawrenz, E., Pinckney, J.L., Guajardo, R.C., Walker, E.A., Paerl, H.W., MacIntyre, H.L. 2010. Spectral fluorometric characterization of phytoplankton community composition using the Algae Online Analyser®. Water Research. 44: 2461–2472. doi:10.1016/j.watres.2010.01.012

Rigosi, A., Carey, C. C., Ibelings, B. W., Brookes, J. D. 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. Limnology and Oceanography. 59: 99-114. doi: 10.4319/lo.2014.59.01.0099

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H. J., Nykvist, B., de Wit, C. A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P. K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R. W., Fabry, V. J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley, J. 2009. Planetary Boundaries: Exploring the Safe Operating Space for Humanity. Ecology and Society. 14: 32. http://www.ecologyandsociety.org/vol14/iss2/art32/

Rodríguez-Ramos, T., Dornelas, M., Marañon, E., Cermeño, P. 2014. Conventional sampling methods severely underestimate phytoplankton species richness. Journal of Plankton Research. 36:334-343. doi: 10.1093/plankt/fbt115

Rogers, J.E., Marcovich, D. 2007. A simple method for the extraction and quantification of photopigments from Symbiodinium spp. Journal of Experimental Marine Biology and Ecology. 353: 191–197. doi:10.1016/j.jembe.2007.08.022

Romanuk, T. N., Kolasa, J. 2005. Resource limitation, biodiversity, and competitive effects interact to determine the invisibility of rock pool microcosms. Biological Invasions. 7: 711-722. doi: 10.1007/s10530-004-0997-8

Rowan, K. S. 1989. Photosynthetic pigments of algae. CUP Archive

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T.,

Walker, B. H., Walker, M., Wall, D. H. 2000. Global Biodiversity Scenarios for the Year 2100. Science. 278: 1770-1774.

Sarmento, H., Descy, J.-P. 2008. Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes. Journal of Applied Phycology. 20: 1001–1011. doi:10.1007/s10811-007-9294-0

Scheffer, M., Hosper, S. H., Meijer, M-L., Moss, B., Jeppesen, E. 1993. Alternative Equilibria in Shallow Lakes. TRENDS in Ecology & Evolution. 8: 275-279. doi: 10.1016/0169-5347(93)90254-M

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., Walker, B. 2001. Catastrophic shifts in ecosystems. Nature. 413: 591-596. doi: 10.1038/35098000

Scheffer, M., Carpenter, S. R. 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. TRENDS in Ecology & Evolution. 18: 648-656. doi: 10.1016/j.tree.2003.09.002

Scheffer, M., S. R. Carpenter, T. M. Lenton, J. Bascompte, W. Brock, V. Dakos, J. van de Koppel, I. A. van de Leemput, S. A. Levin, E. H. van Nes, M. Pascual, J. Vandermeer. 2012. Anticipating Critical Transitions. Science. 338: 344-348. doi: 10.1126/science.1225244

Schelske, C., Stoermer, E. F., Conley, D. J., Robbins, J. A., Glover, R. M.1983. Early Eutrophication in the Lower Great Lakes: New Evidence from Biogenic Silica in Sediments. Science. 222: 320-322. doi: 10.1126/science.222.4621.320

Schindler, D. W., Bayley, S. E., Parker, B. R., Beaty, K. G. Cruikshank, D. R., Fee, E. J., Schindler, E. U., Stainton, M. P. 1996. The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. Limnology and Oceanography. 41: 1004-1017. doi: 10.4319/lo.1996.41.5.1004

Schlüter, L., Lauridsen, T.L., Krogh, G., Jorgensen, T. 2006. Identification and quantification of phytoplankton groups in lakes using new pigment ratios – a comparison between pigment analysis by HPLC and microscopy. Freshwater Biology. 51: 1474–1485. doi:10.1111/j.1365-2427.2006.01582.x

Schlüter, L., Behl, S., Striebel, M., Stibor, H. 2016. Comparing microscopic counts and pigment analyses in 46 phytoplankton communities from lakes of different trophic state. Freshwater Biology. 61: 1627–1639. doi:10.1111/fwb.12803

Schoener, T. W. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. Science. 331: 426-942. doi: 10.1126/science.1193954

Schröder, A., Persson, L., De Roos, A. M. 2005. Direct experimental evidence for alternative stable states: a review. Oikos. 110: 3-19. doi: 10.1111/j.0030-1299.2005.13962.x

Shannon, C.E., Weaver, W. 1949. The Mathematical Theory of Information. University of Illinois Press, Urbana, Illinois

Shochat, E., Lerman, S. B., Anderies, J. M., Warren, P. S., Faeth, S. H., Nilon, C. H. 2010. Invasion, Competition, and Biodiversity Loss in Urban Ecosystems. BioScience. 60: 199-208. doi: 10.1525/bio.2010.60.3.6

Simmons, L.J., Sandgren, C.D., Berges, J.A. 2016. Problems and pitfalls in using HPLC pigment analysis to distinguish Lake Michigan phytoplankton taxa. Journal of Great Lakes Research. 42: 397-404. doi:10.1016/j.jglr.2015.12.006

Smayda, T. J. 1970. The suspension and sinking of phytoplankton in the sea.Oceanography and Marine Biology An Annual Review. 8: 353-414

Smith, S. L., Merigo, A., Wirtz, K. W., Pahlow, M. 2014. Leaving misleading legacies behind in plankton ecosystem modelling. Journal of Plankton Research. 36: 613-620. doi: 10.1093/plankt/fbu011

Smith, V. H., Tilman, G. D., Nekola, J. C. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution. 100: 179-196. doi: 10.1016/S0269-7491(99)00091-3

Smith, V. H. 2003. Eutrophication of Freshwater and Coastal Marine Ecosystems A Global Problem. Eutrophication. Environmental Science and Pollution Research. 10:126-139. doi: 10.1065/espr2002.12.142

Smith,V. H.,Joye, S. B., Howarth, R. W. 2006. Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography. 51: 351-355. doi: 10.4319/lo.2006.51.1_part_2.0351

Smith, V. H., Schindler, D. W. 2009. Eutrophication science: where do we go from here? TRENDS in Ecology & Evolution. 24: 201-207. doi: 10.1016/j.tree.2008.11.009

Smol, J. P., Brown, S. R., McIntosh, H. J. 1984. A hypothetical relationship between differential algal sedimentation and diatom succession. Verhandlungen des Internationalen Verein Limnolie. 22: 1361-1365. doi: 10.1080/03680770.1983.11897500

Soares, E. M., Figueredo, ,C. C., Gücker, B., Boëchat, I. G. 2013. Effects of growth condition on succession patterns in tropical phytoplankton assemblages subjected to experimental eutrophication. Journal of Plankton Research. 35: 1141-1153. doi: 10.1093/plankt/fbt061

Sommer, U.1994. Planktologie. Springer-Verlag. Berlin Heidelberg, Germany. doi: 10.1007/978-3-642-78804-8

Spehn, E. M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G., Finn, J. A., Freitas, H., Giller, P. S., Good, J., Harris, R., Högberg, P., Huss-Danell, K., Jumpponen, A., Koricheva, J., Leadley, P. W., Loreau, M., Minns, A., Mulder, C. P. H., O'Donovan, G., Otway, S. J., Palmborg, C., Pereira, J. S., Pfisterer, A. B., Prinz, A., Read, D. J., Schulze, E.-D., Siamantziouras, A.-S. D., Terry, A. C., Troumbis, A. Y., Woodward, F. I., Yachi, S., Lawton, J. H. 2005. Ecosystem effects of biodiversity manipulations in European grasslands. Ecological Monographs. 75: 37-63. doi: 10.1890/03-4101

Spilling, K., Olli, K., Lehtoranta, J., Kremp, A., Tedesco, L., Tamelander, T., Klais, R., Peltonen, H., Tamminen, T. 2018. Shifting Diatom-Dinoflagellate Dominance During Spring Bloom in the Baltc Sea and its Potential Effects on Biogeochemical Cycling. Frontiers in Marine Science. 5: 327. doi: 10.3389/fmars.2018.00327

Srivastava, D. S. 1999. Using local-regional richness plots to test for species saturation: pitfalls and potentials. Journal of Animal Ecology. 68: 1-16. doi: 10.1046/j.1365-2656.1999.00266.x

Steffen, W., Richardson, K., Rockström, J., Cornell, S. E., Fetzer, I., Bennett, E. M., Biggs, R., Carpenter, S. R., de Vries, W., de Wit, C. A., Folke, C., Gerten, D., Heinke, J., Mace, G. M., Persson, L. M., Ramanathan, V., Reyers, B:, Sörlin, S. 2015. Planetary boundaries: Guiding human development on a changing planet. Science. 347 (6223). doi: 10.1126/science.1259855

Stockenreiter, M., Haupt, F., Graber, A.-K., Seppälä, J., Spilling, K., Tamminen, T., Stibor, H. 2013. Functional Group Richness: Implications of Biodiversity for Light Use and Lipid Yield in Microalgae. Journal of Phycology. 49:838-847. doi: 10.1111/jpy.12092

Stomp, M., Huisman, J., de Jongh, F., Veraart, A. J., Gerla, D., Rijkeboer, M., Ibelings, B. W., Wollenzien, U. I. A., Stal, L. J. 2004. Adaptive divergence in pigment composition promotes phytoplankton biodiversity. Nature. 432: 104-107. doi: doi.org/10.1038/nature03044

Stoń-Egiert, J., Kosakowska, A. 2005. RP-HPLC determination of phytoplankton pigments—comparison of calibration results for two columns. Marine Biology. 147: 251–260. doi:10.1007/s00227-004-1551-z

Striebel, M., Behl, S., Diehl, S., Stibor, H. 2009. Spectral Niche Complementarity and Carbon Dynamics in Pelagic Ecosystems. The American Naturalist. 174: 141–147. doi:10.1086/599294

Symstad, A. J., Tilman, D. 2001. Diversity loss, recruitment limitation, and ecosystem functioning: lessons learned from a removal experiment. Oikos. 92: 424-435. doi: 10.1034/j.1600-0706.2001.920304.x

Tamm, M., Freiberg, R., Tõnno, I., Nõges, P., Nõges, T. 2015. Pigment-Based Chemotaxonomy - A Quick Alternative to Determine Algal Assemblages in Large Shallow Eutrophic Lake? PLOS ONE. 10: e0122526. doi:10.1371/journal.pone.0122526

Taylor, D. L., Bruns, T. D. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: Minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology. 8: 1837-1850. doi: 10.1046/j.1365-294x.1999.00773.x

Thackeray, S. J., Henrys, P. A., Hemming, D., Bell, J. R., Botham, M. S., Burthe S., Helaouet, P., Johns, D. G., Jones, I. D., Leech, D. I., Mackay, E. B., Massimino, D., Atkinson, S., Bacon, P. J., Brereton, T. M., Carvalho, L., Clutton-Brock, T. H., Duck, C., Edwards, M., Elliott, J. M., Hall, S. J. G., Harrington, R., Pierce-Higgins, J. W., Høye, T. T., Kruuk, L. E. B., Pemberton, J. M., Sparks, T. H., Thompson, P. M., White, I., Winfield, I. J., Wanless, S. 2016. Phenological sensitivity to climate across taxa and trophic levels. Nature. 535: 241-245. doi: 10.1038/nature18608

Thomas, M. K., Kremer, C. T., Klausmeier, C. A., Litchman, E. 2012. A Global Pattern of Thermal Adaptation in Marine Phytoplankton. Science. 338: 1085-1088. doi: 10.1126/science.1224836

Tilman, D., Downing, J. A. 1994. Biodiversity and stability in grasslands. Nature. 367: 363-365.

Tilman, D., Wedin, D., Knops, J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature. 379: 718-720. doi: 10.1038/379718a0

Tilman, D. 1997. Distinguishing between the Effects of Species Diversity an d Species Composition. Oikos. 80: 185. doi: 10.2307/3546532

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., Lehman, C. 2001. Diversity and Productivity in a Long-Term Grassland Experiment. Science. 294: 843-845.

Tilman, D., Reich, P. B., Knops, J. M. H. 2006. Biodiversity and eccosystem stability in a decade-long grassland experiment. Nature. 441: 629-631.

Tréguer, P., De La Rocha, C. L. 2013. The World Ocean Silica Cycle. Annual Review of Marine Science. 5: 477-501. doi: 10.1146/annrev-marine-121211-172346

Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., Bittner, L., Dugdale, R., Finkel, Z., Iudicone, D., Jahn, O., Guidi, L., Lasbleiz, M., Leblanc, K., Levy, M., Pondaven, P. 2017. Influence of diatom diversity on the ocean biological carbon pump. Nature Geoscience. 11: 27-37. doi: 10.1038/s41561-017-0028-x

Trommer, G., Pondaven, P., Siccha, M., Stibor, H. 2012. Zooplankton-mediated nutrient limitation patterns in marine phytoplankton: an experimental approach with natural communities. Marine Ecology Progress Series. 449: 83-94. doi: 10.3354/meps09508

UNEP (United Nations Environment Programme). 1992. Convention on biological diversity. United Nations Environment Programme. Nairobi, Kenya.

Utermöhl, H., 1958. Zur Vervollkomnung der quantitativen Phytoplankton-Methodik. Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie. 9: 1-38.

Vallina, S. M., Cermeno, P., Dutkiewicz, S., Loreau, M., Montoya, J. M. 2017. Phytoplankton functional diversity increases ecosystem productivity and stability. Ecological modelling. 361: 184-196. doi: 10.1016/j.ecolmodel.2017.06.020

Vinebrooke, R. D., Cottingham, K. L., Norberg, J., Scheffer, M., Dodson, S. I., Maberly, S. C., Sommer, U. 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos. 104: 451-457. doi: 10.1111/j.0030-1299.2004.13255.x

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., Westbrooks, R. 1996. Biological Invasions as Global Environmental Change. American Scientist. 84: 468-478

Vogt, R. J., Romanuk, T. N., Kolasa, J. 2006. Species richness-variability relationships in multi-trophic aquatic microcosms. Oikos. 113: 55-66. doi:10.1111/j.0030-1299.2006.14494.x

Vogt, R. J., Beisner, B. E., Prairie, Y. T. 2010. Functional diversity is positively associated with biomass for lake diatoms. Freshwater Biology. 55: 1636-1646. doi: 10.1111/j.1365-2427.2010.02397.x

Walker, B., Kinzig, A., Langridge, J. 1999. Plant Attribute Diversity, Resilience, and Ecosystem Function: The Nature and Significance of Dominant and Minor Species. Ecosystems. 2: 95-113. doi:10.1007/s100219900062

Wardle, D. A., Bonner, K. I., Barker, G. M., Yeates, G. W., Nicholson, K. S., Bardgett, R. D., Watson, R. N., Ghani, A. 1999. Plant removals in perennial grassland: Vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecological Monographs. 69: 535-568. doi: 10.1890/0012-9615(1999)069[0535:PRIPGV]2.0.CO;2

Watson, S.B., McCauley, E., Downing, J.A. 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnology and Oceanography. 42: 487–495. doi: 10.4319/lo.1997.42.3.0487

Webster, J. R., Gurtz, M. E., Hains, J. J., Meyer, J. L., Swank, W. T., Waide, J. B., Wallace, J. B. 1983. Stability of Stream Ecosystems. in: Stream Ecology: Application and Testing of General Ecological Theory. Editors: Barres, J. R., Minshall, G. W. pp. 355-395. Plenum Press. New York.

Weinbauer, M. G., Rassoulzadegan, F. 2007. Extinction of microbes: evidence and potential consequences. Endangered Species Research. 3: 205-215. doi: 10.3354/esr003205

Weithoff, G., Walz, N., Gaedke, U. 2001. The intermediate disturbance hypothesis – species diversity or functional diversity? Journal of Plankton Research. 23: 1147-1155. doi: 10.1093/plankt/23.10.1147

Westman, W. E. 1978. Measuring the Inertia and Resilience of Ecosystems. BioScience. 28: 705-710. doi: 10.2307/1307321

Wetzel, R. G. 2001. Limnology Lake and River Ecosystems. 3rd edition. Elsevier Academic Press. San Diego, USA.

Wickham, H. 2009. Package "ggplot2": Elegant Graphics for Data Analysis, version 2.1.0. Springer-Verlag New York

Winder, M., Reuter, J. E., Schladow, S. G. 2009. Lake warming favours small-sized planktonic diatom species. Proceedings of the Royal Society B. 276: 427-435. doi: 10.1098/rspb.2008.1200

Wissel, C. 1984. A universal law of the characteristic return time near thresholds. Oecologia. 65: 101-107. doi: 10.1007/BF00384470

Woodward, G. 2009. Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. Freshwater Biology. 54: 2171-2187. doi: 10.1111/j.1365-2427.2008.02081.x

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., Jackson, J. B. C., Lotze, H. K., Micheli, F., Palumbi, S. R., Sala, E., Selkoe, K. A., Stachowicz, J. J., Watson, R. 2006. Impacts of Biodiversity Loss on Ocean Ecosystem Services. Science. 314: 787-790. doi: 10.1126/science.1132294

Yachi, S., Loreau, M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proceedings of the National Acadamy of Sciences (USA). 96: 1463-1468. doi: 10.1073/pnas.96.4.1463

Yentsch, C.S., Menzel, D.W. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea Research and Oceanographic Abstracts. Elsevier, pp. 221–231.

Yentsch, C. S., Yentsch, C. M. 1979. Fluorescence spectral signatures: the characterization of phytoplankton populations by the use of excitation and emission spectra [Algae, diatoms]. Journal of Marine Research. 37: 471-483

Yentsch, C.S., Phinney, D.A. 1985. Spectral fluorescence: an ataxonomic tool for studying the structure of phytoplankton populations. Journal of Plankton Research. 7: 617–632. doi: 10.1093/plankt/7.5.617

6. Personal notes

6.1 Curriculum Vitae

Personal Data

Name	Sara Katharina Hammerstein
Date of birth	09.04.1988
Place of birth	München

Education

since 10/2012	Ludwig-Maximilians-Universität München Dissertation: "The meaning of phytoplankton diversity within lake ecosystems" Supervisor: Prof. Dr. Herwig Stibor
10/2010 – 09/2012	Ludwig-Maximilians-Universität München Master of Science (Evolution, Ecology and Systematics) Thesis: "Nestling provisioning behavior and parent personality in great tits (*Parus major*)." Supervisor: Prof. Dr. Niels Dingemanse
10/2007 – 09/2010	Ludwig-Maximilians-Universität München Bachelor of Science (Biology) Thesis: "Quartiernutzung von Fledermäusen in den Landkreisen Dachau, Erding, Fürstenfeldbruck und Freising" Supervisor: Dr. Andreas Zahn
09/1998 – 6/2007	Josef-Effner-Gymnasium Dachau Abitur

Work Experience

12/2012 – 07/2017	Ludwig-Maximilians-Universität München Research assistant; Aquatic Ecology Group, Prof. Dr. Herwig Stibor
04/2011 – 08/2011	Ludwig-Maximilians-Universität München Tutor; „Statistics for biologists"
07/2009 – 01/2010	Ludwig-Maximilians-Universität München

	Student assistant; Botany
09/2009 – 10/2009	Max-Planck-Institut für Ornithologie Seewiesen Internship; Behavioural Biology
10/2008 – 04/2009	Ludwig-Maximilians-Universität München Tutor; „Mathematics for biologists“

Award

02/2015	“Outstanding Student Presentation Award” Association for the Sciences of Limnology and Oceanography (ASLO) at the ASLO 2015 Aquatic Sciences Meeting

Additional Skills

Languages	German – first language English - fluent
Computing	Microsoft Word, Excel, PowerPoint R, SigmaPlot, CMS-Fiona
Workshops	Timeseries Analyses – DFG Dynatrait Project; 2016 Multivariate Statistics – LMU and Wassercluster Lunz; 2015 EES Summer School Aquatic Ecology – LMU; 2013 Leading and Promoting Discussions – Sprachraum LMU; 2010

6.2 Publications

Hammerstein, S. K., Stockenreiter, M., Stibor, H. 2017. Directed diversity manipulations within natural phytoplankton communities. Limnology and Oceanography Methods. 15: 653-662. doi: 10.1002/lom3.10190 – corresponding to research topic 1 – I conducted the experiments, analyses and wrote the manuscript and participated in designing the experiments.

Ilic, M., Walden, S., **Hammerstein, S. K.**, Stockenreiter, M., Stibor, H., Fink, P. Determination of phytoplankton biodiversity in lakes of different trophic state through pigment and fluorescence proxies. *in revision*, this thesis contains my suggestions for resubmitting – corresponding to research topic 4 – I conducted the field mesocosm experiments and the fluorescence measurements and participated in designing the experiments, analyses and writing the manuscript. M. Ilic conducted the HPLC and CHEMTAX analyses and wrote the manuscript.

6.3 Presentations

Talks

Stockenreiter, M., **Hammerstein, S. K.**, Ilic, M., Fink, P., Stibor, H. Phytoplankton – zooplankton interface in lake foodwebs: Consequences of a loss of phytoplankton traits. ASLO Aquatic Sciences Meeting. Honolulu, USA. 2017

Ilic, M., **Hammerstein, S. K.**, Stockenreiter, M., Stibor, H., Fink, P. Pigment composition of natural phytoplankton communities – Possible link between biodiversity loss and ecosystem functioning? ASLO Aquatic Sciences Meeting. Honolulu, USA. 2017

Hammerstein, S. K., Ilic, M., Fink, P., Stockenreiter, M., Stibor, H. Diversity loss and trait dynamics in natural phytoplankton communities. DFG Dynatrait Meeting. Potsdam, Germany. 2016

Hammerstein, S. K., Stockenreiter, M., Stibor, H. Testing techniques for establishing diversity gradients within natural phytoplankton communities. Fresh Blood for Fresh Water Meeting. Mondsee, Austria. 2015

Hammerstein, S. K., Stibor, H. Biodiversity and Ecosystem functioning in microbial communities. EES Summer School Aquatic Ecology. Frauenchiemsee, Germany. 2013

Posters

Hammerstein, S. K., Stockenreiter, M., Ilic, M., Fink, P., Stibor, H. Manipulating diversity in natural phytoplankton communities – a mesocosm field study. ASLO Aquatic Sciences Meeting. Honolulu, USA. 2017

Hammerstein, S. K., Stockenreiter, M., Fink, P., Stibor, H. Effects of nutrient enrichment along diversity gradients in natural phytoplankton communities. DFG Dynatrait Meeting, Potsdam, Germany. 2016

Hammerstein, S. K., Vogt, H., Büttner-Koch, C., Stockenreiter, M., Stibor, H. Testing techniques for establishing diversity gradients within natural phytoplankton communities. ASLO Aquatic Sciences Meeting. Granada, Spain. 2015

Hammerstein, S. K., Vogt, H., Stibor, H. Testing a new technique for establishing diversity gradients with natural algal communities. SIL Astria Meeting. Lunz am See, Austria. 2014

7. Acknowledgments

Lieben Dank an alle, die mich währen der letzten Jahre auf dem Weg zum Doktortitel unterstützt haben und alle Hochs und Tiefs mit durchgestanden haben!

- Prof. Dr. Herwig Stibor – Danke, Herwig für deine Unterstützung, deine Ratschläge und das teilhaben lassen an deinem unerschöpflichen Erfahrungsschatz während der Dr. Arbeit und danke dafür, dass du versucht hast mir die Angst vor dem Wasser zu nehmen! Wer weiß schon, was in unseren Seen für riesige Monster leben
- Dr. Maria Stockenreiter – Danke Mia für alles, was du mir vor allem in Seeon beigebracht hast und für deine unermüdliche Unterstützung beim Auswerten, Schreiben und Nicht-komplett-verrückt-werden! Ohne dich würde ich vermutlich heute noch zielgerichtet ins nächste Gebüsch rudern
- Moni, Feli, Katrin und Tine – Danke an die allerbesten Doktorandenschwestern (bzw. Ehrendoktorandenschwester ;)), die man sich wünschen kann. Mit euch war es eine tolle Zeit in Seeon, in der Uni oder auf Konferenzen. Danke fürs immer da sein und das gemeinsame Durchhangeln durch alle großen und kleinen Turbulenzen des Lebens und der Doktorarbeit!
- Margit – Danke für die seelische Unterstützung, den ein oder anderen Tritt in den Hintern und für all die kleinen (und großen) Dinge, die sich bei uns anscheinend von selbst erledigen, was irgendwie viel zu selten gewürdigt wird!
- Angelika – Danke für die großartige und kompetente Unterstützung im Labor in Seeon, deine gute Laune und die aufmunternden Kaffeepausen an den besonders langen Tagen!
- Achim – Danke für deine Hilfe bei allem in Seeon, deine großartigen Sprüche zwischendurch und vor allem für die musikalische Untermalung unzähliger Stunden im Labor in Seeon!
- Danke auch an alle anderen Kollegen aus der aquatischen Ökologie, die immer ein offenes Ohr hatten und für gute Atmosphäre gesorgt haben.

- Hannah, Claudi, Marc, Eva, Rachelle, Nicole, Jasmin und Raphi – Danke für eure Mitarbeit an den Versuchen und einige grandiose Augenblicke auf Station und in der Uni. Ohne so viele tatkräftige Helferlein hätte das hier alles nie so gut geklappt!
- sämtliche Süßwarenhersteller – Danke für den Zucker im Blut wenn er nötig war und die Extrakalorien, die das Schreiben einfacher gemacht haben.
- Team Alberti – Danke fürs Höhentraining, den sportlichen Ausgleich und das wieder loswerden der eben erwähnten Extrakalorien ;)
- Flo – Danke dafür, dass du da bist, für die außergewöhnlichste Freundschaft meines Lebens und dafür, dass du mein Leben in einen Tanz verwandelst! Danke, dass du so gut auf mich aufpasst, dass ich am Ende nicht komplett verrückt werden muss!
- Mama, Papa und Schwesterlein – Danke! Für alles! Fürs Aushalten meiner Launen durch alle Phasen der Arbeit und für eure Unterstützung in allen Lebenslagen wenn ich alleine nicht mehr recht weiter wusste. Danke, dass ihr immer für mich da seid! Ich hätte euch nicht besser aussuchen können, selbst wenn ich die Wahl gehabt hätte!

8. Declaration

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den 13.11.2019 .....

Sara Hammerstein

Erklärung

Hiermit erkläre ich, *

☒ dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

☒ dass ich mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen habe.

☐ dass ich mich mit Erfolg der Doktorprüfung im Hauptfach .....

und in den Nebenfächern .....

bei der Fakultät für ..... der .....

(Hochschule/Universität)

unterzogen habe.

☐ dass ich ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

München, den 13.11.2019 .....

Sara Hammerstein

*) Nichtzutreffendes streichen