Climate and nutrient controls of cyanobacteria.

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Statement of Originality

I hereby confirm that this PhD thesis is an original piece of work conducted independently by the undersigned and all work contained herein has not been submitted for any other degree.

All research material has been duly acknowledged and cited.

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Date: 27/11/2018

Abstract

Cyanobacteria are a diverse group of primary producers that can form dense blooms which are a major threat to freshwater quality and global water security. While nutrient enrichment is a key driver of cyanobacteria abundance, there is a broad consensus that 'blooms like it hot' and that climate warming will promote the proliferation of cyanobacterial blooms. A highly cited hypothesis suggests that nutrients and temperature enhance cyanobacterial blooms synergistically, but only a few studies have tested this directly. Furthermore, while climate change is often treated as a single stressor – warming – the impact on cyanobacteria of other potentially interacting factors, such as seasonal or extreme rainfall patterns, also need to be understood. This thesis explores, the multiple stressor effects of global change factors – eutrophication, climate warming and changes in rainfall patterns – on cyanobacterial abundance. This extends our knowledge from simple single stressor studies to dynamic, multiple-stressor studies using a range of approaches and scales. This includes analysis of European scale observational data from 494 lakes (chapter two), a mesocosm experiment (chapter three) and a process-based phytoplankton community model, PROTECH (chapter four). Overall, it is hard to generalise cyanobacterial responses to multiple stressors; both synergistic and some surprising antagonistic relationships were observed influenced by: lake characteristics (chapter two); the gradient of the stressor tested (chapter three); the measure of the response (chapter three); the timing and magnitude of the stressor (chapter four) and the location of the waterbody (chapters two and four). Broad generalisations can be made within lake types, yet, despite the need for complex models to deliver improved understanding, complex solutions may not be required. While precise sensitivities to climate stressors may vary, nutrient control remains the clearest mitigation measure to reduce the abundance of cyanobacteria in freshwaters, and this becomes even more important in the face of climate warming.

Contents

Acknowledgments

Big up to my supervisors!

The best in the business, brilliant mentors -

hands down the best I could have hoped for.

Your individual characters and skills constantly gave me new perspectives.

I will keep you all with me eternally in my thinking and writing.

(*I'm sorry Stephen that these aren't real sentences…*)

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For creating an atmosphere of respect and collaboration

For being my friends – we had some great laughs.

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but you can keep your fermented trout and roast moose.

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It's over now!

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Acronyms and Abbreviations

WHO	World Health Organisation
TP	Total phosphorus
TN	Total nitrogen
SRP	Soluble reactive phosphorus
MARS	EU project – Managing Aquatic ecosystems and water Resources under multiple Stress
PROTECH	Process based phytoplankton community model – Phytoplankton RespOnses To Environmental CHange

Chapter 1

Introduction

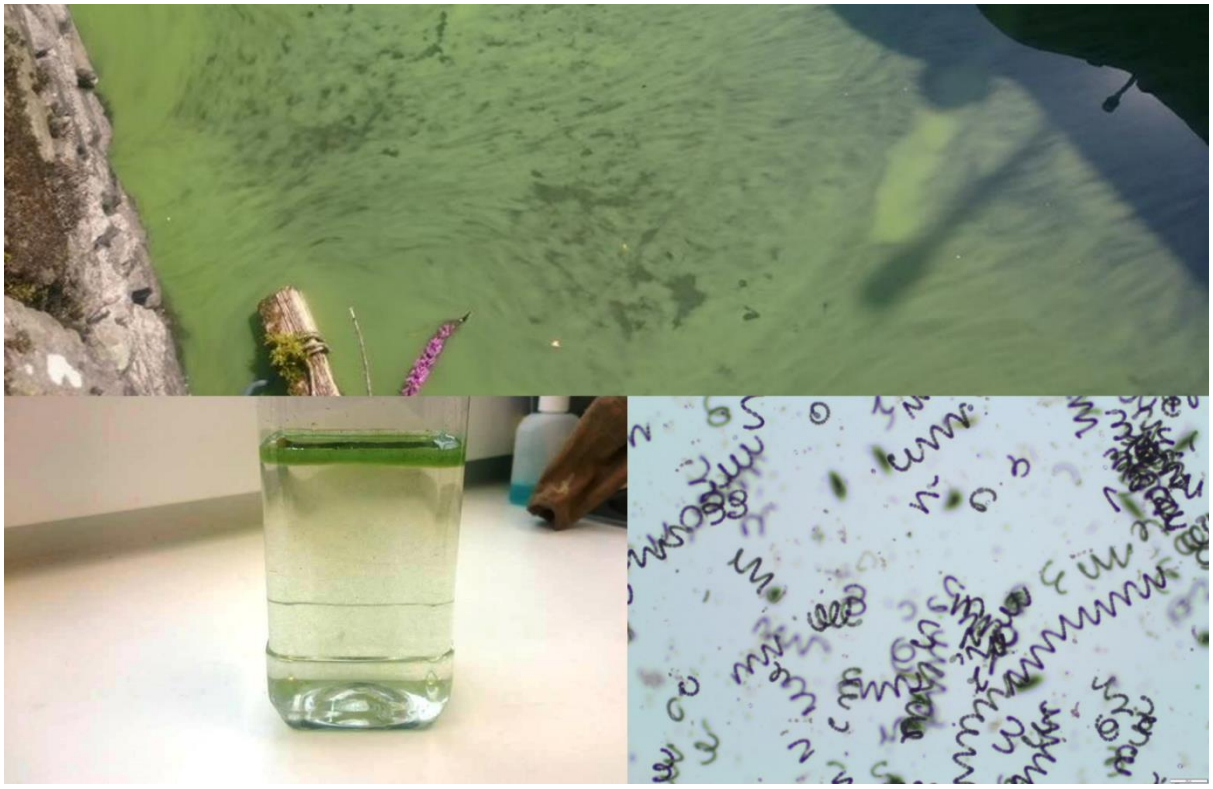


Fig. 1.1 Cyanobacteria in Windermere, the English Lake District in August 2015. Top, a bloom of *Dolichospermum* sp., in a sheltered bay of this large mesotrophic lake. Bottom left, a water sample from the bay left to stand to demonstrate buoyancy through the production of gas vesicles. Bottom right, the same sample under a microscope – the bloom is dominated by *Dolichospermum* sp., a nitrogen fixing genus, of which some species and variants within species can produce hepatoxins (cylindrospermopsin and microcystin) and neurotoxins (anatoxin-a, β-N-methylamino-L-alanine, neosaxitoxins and saxitoxins).

1.1 The global problem of cyanobacterial blooms

Cyanobacteria are microscopic, prokaryotic, phototrophic primary producers (blue-green algae) which evolved around 3.5 billion years ago (Schopf & Packer, 1987). They are a diverse, cosmopolitan group with eco-physiological characteristics that allow them to survive in a wide range of environmental niches (Dokulil & Teubner, 2000; Carey *et al*., 2012). These include: the ability to fix atmospheric nitrogen (Wood *et al*., 2010); the ability to regulate buoyancy through gas vesicle production (Ganf and Oliver, 1982); the production of resting cells, akinetes (Nichols & Adams, 1982; Adams & Duggan, 1999) allowing the survival of adverse/stressful conditions; higher temperature optima (Canale & Vogel, 1974; Reynolds, 2006); affinity for phosphorus and nitrogen (e.g. Takamur *et al*., 1987); the ability to sequester luxury P intracellularly (Jacobsen and Halmann, 1982; Isvánovics *et al*., 2000); superior CO_2 uptake kinetics (Shapiro 1997), high pH optima (Dokulil & Teubner, 2000), and resistance to grazing because of their size and morphology (Burns, 1987; Lampert, 1987; Gliwicz, 1990) and toxin production (Fulton 1988; Gobler *et al*., 2007). These traits are key to their success and dominance. Not all genera and species possess all these properties and further differences in morphologies affect functional traits. This functional diversity opens up the potential for dominance in many environmental settings, for example *Planktothrix* can dominate in low light environments (Reynolds, 1984) because of its morphology and accessory pigments (Reynolds , 1997) while *Dolichospermum* can dominate in thermally stable, nutrient limited environments because of its ability to regulate buoyancy, allowing access to hypolimentic nutrient stores (Wagner & Adrian, 2009) as well as its ability to fix nitrogen (Reynolds *et al*., 2002; Dokulil & Teubner, 2000).

Under certain environmental conditions cyanobacteria can dominate over other phytoplankton groups, forming dense blooms and surface scums. While blooms have been documented for centuries (e.g. Hayman, 1992; Francis, 1878), the incidence and intensity of cyanobacterial blooms has increased in recent decades (O'Neil *et al*., 2012; Taranu *et al*., 2015). The ecological consequences of cyanobacterial dominance, and the challenges they present, are of particular interest. Not only does their dominance and high biomass adversely affect biodiversity and aesthetics, reducing cultural, economic and functional ecosystem services (Steffensen 2008) but also their potential toxicity can pose serious health risks to aquatic organisms and humans (Chorus & Bartram, 1999; Codd *et al*., 2005). Considering the importance of inland waters for the benefits of ecosystem goods and services (Postel & Carpenter, 1997), the increased threat from cyanobacterial blooms is of great concern.

1.2 Anthropogenic drivers of cyanobacterial blooms

Human activities have been implicated in the rise in cyanobacterial blooms. At the centre of this is the over-enrichment of freshwaters which has increased as human populations have expanded. While nutrient enrichment is recognised as a key driver of cyanobacteria abundance (e.g. Taranu *et al*., 2015), there is also increasing interest in the role of climate change (Paerl & Huisman, 2008; Carey *et al*., 2012; O'Neil *et al*., 2012). This has mainly focused on global warming, as warmer temperatures suit the traits of many bloom forming, and often toxic cyanobacteria (Paerl & Huisman, 2008; Kosten *et al*., 2009; Carey *et al*., 2012).

1.2.1 Nutrient enrichment

Anthropogenic overenrichment of freshwaters comes from sewage, industrial activities, agricultural runoff and urbanisation. While many countries now regulate point sources of nutrients, non-point sources of nutrients such as runoff from agricultural or urban lands continues to be a major source of phosphorus and nitrogen to aquatic systems (Carpenter *et al*., 1998). Furthermore, the internal cycling of nutrients within lakes remains as a legacy of past activities (Nürnberg, 2009). It is estimated that net phosphorus storage in terrestrial and freshwater ecosystems is 75% greater than pre-industrial levels of storage (Bennet *et al*., 2001). Nutrient loading and in-lake concentrations are also influenced by climate change. Warming enhances the internal cycling of nutrients within lakes (e.g. Søndergaard *et al*., 2003; Jensen & Andersen, 1992) as well as affecting in-lake concentrations because of evaporative loss and reduced water inflow (Jepessen *et al*., 2009). It is expected that nutrient loading will also become increasingly variable because of modified hydrology - the extent of these change will vary among regions and seasons (Jeppesen *et al*., 2009; Jeppesen *et al*., 2011). For example, it is expected that there will be an increase in nitrogen loading in northern temperatre regions because of enhanced runoff (Sinha *et al*., 2017). In Europe, nutrient enrichment is considered the primary stressor in inland freshwater (Nõges *et al*., 2015).

Nutrient overenrichment leads to the eutrophication of freshwaters (Schindler 1977). This has become a global problem that will likely worsen as human populations expand and as countries develop (Millenium Ecosytem Assessment, 2005). While nutrient enrichment stimulates all algal growth (Reynolds, 1984), paleolimnological studies from the past two centuries show that cyanobacteria have increased at a rate greater than other phytoplankton in respone to enrichment (Moorhouse *et al*., 2014; Taranu *et al*., 2015). Cyanobacteria have traits which allow them to compete effectively for nutrients (Carey *et al*., 2012) such as the ability

to fix atmospheric nitrogen which can be advantageous under nitrogen deplete conditions (Smith 1983, Schindler *et al*., 2008; Vanni *et al*., 2011). While nutrients are clearly an important driver of large biomass events, the specifics of the role of nutrients in the development of blooms of cyanobacteria are still being studied. For example, whether phosphorus or nitrogen is the limiting nutrient (Smith, 1983; Elser *et al*., 2007; Paerl *et al*., 2016). Despite the advantages cyanobacteria have in the sequestration and storage of nutrients, being able to respond to nutrient enrichment depends on other factors and therefore while nutrient enrichment is a prerequiste for dominance, it is not necessarily predicted by it (Carvalho *et al*., 2013).

1.2.2 Climate Warming

In the past hundred years global temperatures have increased by 0.74 ± 0.18°C (Trenberth *et al*., 2007) and it is forecasted that this trend will continue with heatwaves predicted to be more intense, more frequent and lasting for longer (Meehl *et al*., 2007). Lakes are responding rapidly to global warming, with an average increase in water temperature of 0.34°C a decade during the summer (O'Reilly *et al*., 2015). Warming also influences the onset, strength and duration of the stratification of lakes, with stratification occurring earlier (McCormick 1990; Winder and Schindler, 2004) and for longer (Wagner and Adrian, 2009; Markensten *et al*., 2010).

There are two principal mechanisms by which warming may enhance cyanobacteria dominance: (a) directly through enhanced growth rates and (b) indirectly through enhanced lake thermal stability. Firstly, many common bloom forming cyanobacteria taxa, such as *Microcystis* and *Dolichospermum*, have growth optima at higher temperatures (Robarts and Zohary, 1987; Butterwick *et al*., 2005) which may provide them with a competitive advantage as water temperatures increase (De Senerpont Domis *et al*., 2007; Jöhnk *et al*., 2008). Studies of natural systems have found positive relationships between temperature and cyanobacteria abundance (e.g. Kosten *et al*., 2009; Taranu *et al*., 2012; Rigosi *et al*., 2014), as well as evidence for the expansion of the geographic range of tropical species to temperate lakes (Wiedner *et al*., 2007). Studies have also found evidence for the earlier onset of *Microcystis* growing season and the prolonged duration of blooms in response to elevated water temperatures (Zhang *et al*., 2012; Deng *et al*., 2014). Secondly, many bloom forming cyanobacteria can migrate vertically by altering their buoyancy. This trait becomes advantageous under stratified conditions as they can migrate down to access nutrient-rich waters (e.g. Ganf & Oliver, 1982; Wagner & Adrian, 2009) and upwards to access light near the surface (Ibelings *et al*., 1991). Other phytoplankton

taxa such as diatoms, cannot compete as well under these conditions because of increased sinking rates and nutrient limitation (Paerl & Huisman, 2009). Many studies have found stratification to be an important factor in explaining cyanobacterial bloom dynamics which is specifically linked to the ability of cyanobacteria to regulate buoyancy (Jöhnk *et al*, 2008; Wagner and Adrian, 2009). Furthermore, there is evidence that blooms of cyanobacteria can initiate a positive feedback to strengthen stratification (Kumagai *et al*., 2000; Jones *et al*., 2005; Rinke *et al*., 2010) which can potentially prolong blooms.

It is expected that these stimulatory, direct and indirect, effects of warming on cyanobacteria will proliferate the expansion of cyanobacterial blooms in nutrient enriched lakes. Specifically, it is hypothesised that there will be a synergistic interaction between temperature and nutrients (Paerl & Huisman, 2008; Moss *et al*., 2011; Havens & Paerl, 2015). While this is a popular hypothesis, until the past few years, very few studies have tested it. The evidence so far tells a complex story, suggesting that it may be hard to generalise the effects of temperature on cyanobacterial blooms. For example, evidence suggests that lake type plays an important role (e.g. Taranu *et al*., 2012; Rigosi *et al*., 2014) and that the response may change along stressor gradients (Rigosi *et al*., 2014; Adrian and Huber, 2009; Piggott *et al*., 2015).

1.3 Global change – a multiple stressor view

1.3.1 Other climate change effects

To date, most studies have incorporated climate change as a single stressor on the natural environment– warming. In reality, this masks a great deal of complexity as climate change is manifested as dynamic changes in the environment, incorporating '*press*' disturbances – warming – and '*pulse*' disturbances – extreme events (Lakes, 2000). Associated with climate warming are global changes in hydrology, the extent of which will vary on a regional (Milly *et al*., 2005) and catchment (Fowler & Kilsby, 2007) basis. Of particular interest, in terms of phytoplankton community dynamics, is the predicted (IPCC, 2013) and observed (Lehmann *et al*., 2015) increase in extreme rainfall and heatwave events. These events are predicted to occur in the summer (July-September) at mid to high latitudes (Christensen and Christensen, 2003). Modified hydrology is considered as one of the main stressors in inland freshwaters because of the large effect changes in flushing rates has on the physical and chemical environment (Nõges *et al*., 2015).

Water movement influences nutrient loading, the retention time of lakes, the thermal stability of the water column as well as turbidity/colour and thus affects the selection pressures on phytoplankton community composition. Extreme rainfall events result in sudden environmental changes in a lake (e.g. Sadro & Melack, 2012) – notably, an increased delivery of nutrients and organic material from the catchment, changes in the stability of the water column and losses of phytoplankton biomass as well as dissolved and biologically-bound nutrients through increased hydraulic flushing. These changes result in shifts in phytoplankton community composition and diversity, favouring ruderal taxa with morphological and functional traits that suit conditions of lower light, higher nutrients and greater mixing (Padisák *et al*., 1988; Hudnell *et al*., 2010; Reynolds *et al*., 2012). While some groups may be resilient to change, or even flourish, others will be excluded. Cyanobacterial blooms may be particularly sensitive to flushing events as most bloom forming genera are sensitive to turbulence, are slow growing and have functional traits suited to stable water columns (Steinberg & Hartmann, 1988; Perry *et al*., 1990; Jöhnk *et al*., 2008; Huber *et al*., 2012).

The extent of the impact of extreme events on phytoplankton assemblages will depend on many aspects relating to the event itself such as the timing (Verspagen *et al*., 2006; Padisák, 1993; Padisák *et al*., 1999; Elliot, 2010), intensity (Harris & Baxter, 1996; Oh and Kim, 1995; Ahn *et al*., 2002) and frequency (Padisák *et al*., 1988) but also on the characteristics of the lake, catchment (Reichtwaldt & Ghadouani, 2012) and other factors such as climate and nutrient source (Elliott *et al*., 2009). It is expected that disturbances will have the greatest effect at the time of seasonal succession when dominant taxa are more sensitive to the changes in light availability and mixing (Verspagen *et al*., 2006). The timing of the event may also affect whether lagged effects can occur. Spring extreme rainfall events could benefit summer blooms of cyanobacteria because of increased nutrient loading (Paerl & Huisman, 2008), and higher flushing through the winter can delay and reduce the vernal bloom with similar nutrient benefits for cyanobacteria later in the year (Moorhouse *et al*., 2014). Events at higher frequencies may be more disruptive to cyanobacteria than one-off events (Connell, 1978; Padisák *et al*., 1988) but this may also be seasonally dependent - increasing the frequency of events during spring may have less effect on community composition as dominant vernal bloom taxa are suited to these conditions (Elliott, 2010).

While the impact of extreme events are discussed as an important global change factor (Paerl & Huisman, 2008; Carey *et al*., 2012; O'Neil *et al*., 2012), pulse disturbances are poorly studied. The difficulty is that available observational data only rarely includes extreme flow

events, making it hard to compare across systems or temporal scales. Studies of single extreme events are highly system specific as the extent of physico-chemical change, and thus biological impacts, depends on event-, lake- and catchment characteristics. Greater understanding of the biological impacts of extreme climatic events can be gained by studying (a) the response to different events and (b) the response to one event but in different contexts of catchment, lake type and antecedent weather.

1.3.2 Global change – a multiple stressor view

The global reach of anthropogenic pressures means that many lakes are now exposed to multiple stress. In Europe, 45% of lakes are affected by multiple pressures such as agriculture, urbanisation and climate change. Specifically, nutrient enrichment, warming and modified hydrology are the most common stressor combinations in lakes (Nõges *et al*., 2015). Studies which have tested multiple stressor effects on cyanobacteria abundance have focused on the effects of nutrient enrichment and warming and show that the response may not be easily generalisable (Taranu *et al*., 2012; Rigosi *et al*., 2014). Variability in biological responses to multiple stressors in aquatic systems are found across scales of space and time (Crain *et al*., 2008; Côté *et al*., 2016; http://mars-project.eu/index.php/publications.html). These studies suggests that any inferences about the effect of multiple stressors that can be made from single-factor studies are limited (i.e. you cannot just add them up), emphasising the need for more studies focusing on the response of organisms, communities and ecosystems to multiple stressor combinations. Taking an inclusive, multiple stressor approach is increasingly necessary and should incorporate the full multiple stressor landscape, including press and pulse disturbances and realistic environmental change scenarios.

1.4 Thesis contribution

The overarching aim of this thesis is to contribute to our understanding of how the most important anthropogenic stressor that currently impact lakes – nutrient enrichment, warming and hydrological change – will affect cyanobacteria abundance and thus the future risk of cyanobacterial blooms. This is achieved by using large scale spatial data to explore the response to these environmental gradients in different types of lakes (**chapter two**), a mesocosm scale experiment (**chapter three**) to explore the response to extreme rainfall events in combination with a future step change in water temperature (+4°C) and nutrient enrichment

(eutrophic to hypertrophic) and finally, a process based phytoplankton community model (**chapter four**) to explore the effects of extreme flow events at different times of the year and of different magnitudes.

The first objective of this thesis is to incorporate changes in hydrology as an important stressor, which to date has received relatively little attention compared to the effects of climate warming. This factor will be incorporated into all chapters and will be explored as a press disturbance (retention time, on a continuum) and pulse disturbance (extreme rainfall events). The second objective is to move to a more multi-dimensional stressor space that is representative of the pressures that inland freshwaters are now experiencing, making use of advanced statistical methods, different approaches (observational, experimental and modelling) and different scales to disentangle the effects of multiple factors. Other specific objectives which sit within these are to: (a) test the synergistic interaction between temperature and nutrients (chapter two and three) and (b) assess the response of cyanobacteria to multiple stressors in different types of lakes (chapter two). Together, these objectives make a novel contribution which will contribute to our understanding of the risk of cyanobacterial blooms under future global change.

1.5 Outline of thesis chapters

Chapter two

Chapter two makes use of an existing large dataset of nearly 500 lakes. In this chapter the combined effect of temperature, nutrient enrichment and flow (retention time) on cyanobacteria biovolume was tested in different types of lakes which were defined by combinations of alkalinity, colour and mixing types. A lake type approach was taken in recognition of the differential sensitivity of lakes of different types to environmental stressors. While lake type has been considered by others in the context of cyanobacterial responses to nutrient enrichment and warming, these studies focus on single aspects of type – depth (Taranu *et al*., 2012) and nutrient trophic level (Rigosi *et al*., 2014) –this chapter is novel in incorporating multiple key environmental factors that shape phytoplankton community composition in lakes. Hydraulic flow was incorporated in this chapter as a continuous variable, rather than as extreme events.

Chapter three

Chapter three describes a shallow lake mesocosm experiment which assessed the potential interactions between warming, nutrient enrichment and extreme rainfall events on the abundance and composition of cyanobacteria. This study is novel in focusing on the impact and recovery of biomass loss from flushing events, but also for testing the combined effects of three major freshwater stressors at once. The response of cyanobacteria was compared to that of the whole phytoplankton community to assess whether there were any major compositional differences. The response of genera within the cyanobacteria community was also tested to assess for genera specific responses.

Chapter four

Chapter four takes advantage of the computational power and short run times of computer models to explore the effects of extreme events. Specifically, the phytoplankton community model PROTECH (Phytoplankton RespOnses to Environmental Change) was used to explore the sensitivity of cyanobacteria to one-off hydraulic flow events in a typical stratified lake. The focus was on how event characteristics, specifically the timing and intensity, may affect the impact and recovery from perturbation and how this may be shaped by other environmental factors, specifically nutrient loading, weather (temperature and wind) and the location of the lake. This chapter is novel in that it explores the effects of extreme events across temporal scales and stressor gradients (nutrients and flushing) while also incorporating other important environmental factors such as lake location and weather. This experiment was designed in recognition that the sensitivity to extreme climatic events will depend on the characteristics of the event but also on many attributes intrinsic to the lake and its catchment.

Chapter 2.

European scale study: an analysis of the combined effects of temperature, phosphorus and retention time in lake types across a large spatial scale.

Richardson, J., C. Miller, S. C. Maberly, P. Taylor, L. Globevnik, P. Hunter, E. Jeppesen, U. Mischke, S. J. Moe, A. Pasztaleniec, M. Søndergaard and L. Carvalho (2018). Effects of multiple stressors on cyanobacteria abundance vary with lake type. Global Change Biology **24**(11): 5044-5055.

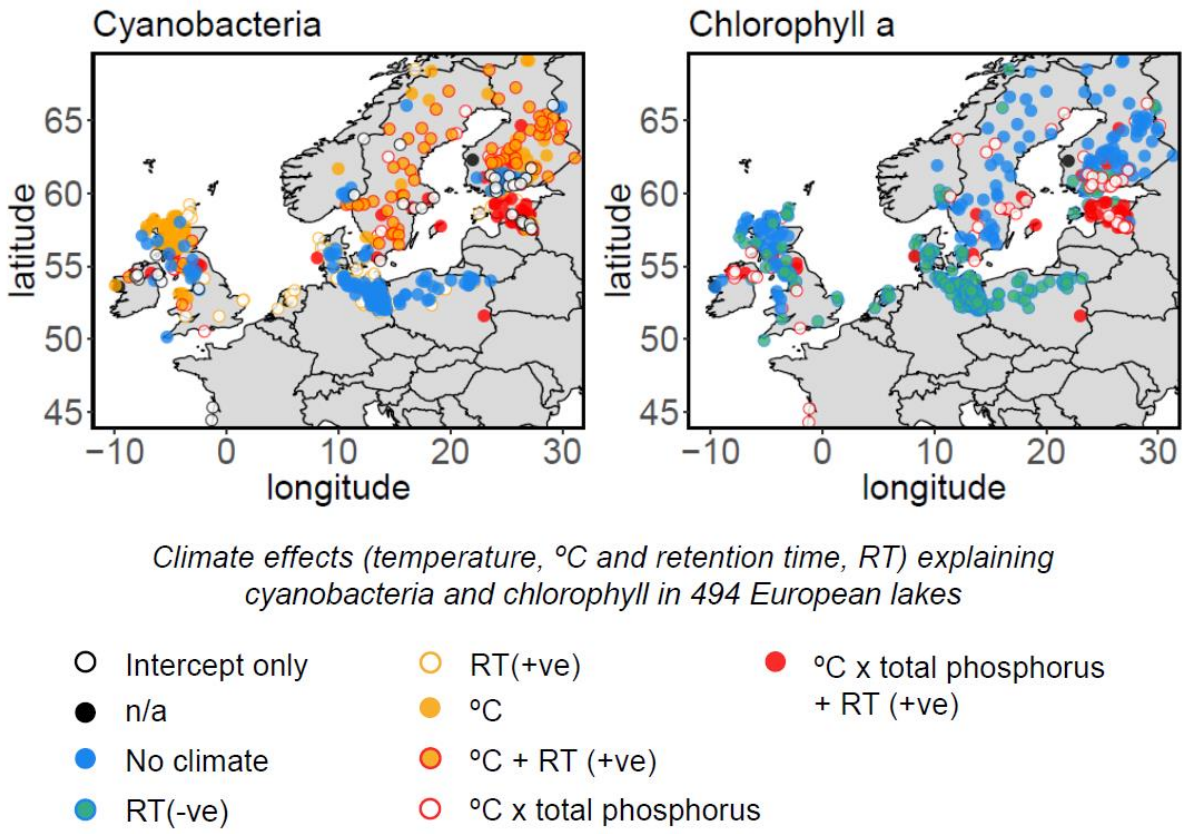


Fig. 2.1 Chapter two graphical abstract – climate effects explaining cyanobacteria and chlorophyll in 494 European lakes.

1.1 Abstract

Blooms of cyanobacteria are a current threat to global water security that is expected to increase in the future because of increasing nutrient enrichment, increasing temperature and prolonged drought. However, the responses to multiple stressors, such as those above, are often complex and there is contradictory evidence as to how they may interact. Here we used broad scale data from 494 lakes in central and northern Europe, to assess how cyanobacteria respond to nutrients (phosphorus), temperature and water retention time in different types of lakes. Eight lake types were examined based on combinations of major factors that determine phytoplankton composition and sensitivity to nutrients: alkalinity (low and medium-high), colour (clear and humic) and mixing intensity (polymictic and stratified). In line with expectations, cyanobacteria increased with temperature and retention time in five of the eight lake types. However, the sensitivity of cyanobacteria to temperature, retention time and phosphorus differed among types highlighting the complex response of lakes to multiple stressors. The analyses suggested that lake types currently not at risk could be affected by warming in the future, since temperature effects were greatest in lakes at higher latitudes. More work is needed to separate geographical from typological effects in order to provide advice for managers. It is already clear that climate change will need to be accounted for when managing risk of cyanobacteria in lakes and a 'one-size fits-all' approach is not appropriate. Our analysis shows that our understanding is greatly improved by considering how multiple stressors interact in a range of different lake types and that this approach could help better predict responses to future nutrient and climate changes.

1.2 Introduction

Blooms of cyanobacteria are becoming an increasing threat to global water security. Through anthropogenic activities we are not only enhancing but also combining some of the optimal conditions for the dominance of cyanobacteria. At the local scale, and despite remediation efforts, nutrient enrichment is hardly abating (Oliver *et al.*, 2017) as human populations grow and become more urbanised, requiring intensive agriculture to expand, while internal cycling of nutrients within lakes occurs as a legacy of past activities (Nürnberg 2009). At a global scale, and at the forefront of this paper, is the issue of climate change. In part, the recent rise in cyanobacteria has been attributed to climate warming (Paerl & Huisman, 2008; Kosten *et al.*, 2012). Increases in water temperature (O'Reilly *et al.*, 2015) alongside increases in the duration and strength of thermal stratification (Wagner & Adrian, 2009) create optimal conditions for the physiological and functional traits of many cyanobacteria taxa such as higher temperature growth optima and the ability to regulate buoyancy (Carey *et al.*, 2012). In combination with high nutrient concentrations, it is feared that warming will result in the accelerated deterioration of water quality (Paerl & Huisman, 2008; Jeppesen *et al.*, 2009; Moss *et al.*, 2011). This synergism is widely discussed as an important risk factor, however the evidence so far suggests that this will not be a generalisable response; others have found that the effect of temperature is dependent on other environmental factors such as trophic setting (Rigosi *et al.*, 2014) or by the mixing state of the lake (Taranu *et al.*, 2012).

Climate change also affects rainfall patterns (Milly *et al.*, 2005). Extreme rainfall events followed by prolonged periods of drought are expected to favour cyanobacteria because of the combined effects of elevated nutrients and stable physical conditions (Paerl & Huisman, 2008). Although, the benefits to cyanobacteria may depend on the frequency, duration, seasonal timing and intensity of rainfall events as well as other factors such as catchment land use and the ratio of catchment area to lake surface area (Padisák *et al.*, 1988; James *et al.*, 2008; Reichwaldt & Ghadouani, 2012). Studies exploring the effect of changes in flow on the abundance of cyanobacteria in combination with other anthropogenic stressors are limited, yet flow dynamics as a driver of the abundance, composition and succession of phytoplankton communities is well documented (e.g. Søballe & Kimmel, 1987; Tolotti *et al.*, 2010). In order to understand fully the effects of climate change on water quality in lakes, climate change effects other than that of incremental changes in temperature need to be incorporated. Although more challenging, the effects of extreme rainfall events, heatwave events and prolonged

periods of drought need to be understood and quantified in combination with anthropogenic nutrient enrichment (Michalak 2016).

The evidence so far indicates that the response of cyanobacteria to multiple anthropogenic stress may not be generalisable i.e. that a "one-size fits-all" approach is not appropriate across all lakes (e.g. Taranu *et al*., 2012). This is not surprising given that phytoplankton have varying sensitivities and tolerances to their physical and chemical environment (Reynolds *et al.*, 2012) and so many other factors, aside from temperature, nutrients and flushing rates, are involved in shaping phytoplankton biomass and community structure. Previous analyses have examined the effect of lake type on the sensitivity of cyanobacteria to nutrients and temperature in combination, focusing on the effect of trophic type (Rigosi *et al*., 2014), mixing type (Taranu *et al*., 2012) and depth x artificial *vs* natural lakes (Beaulieu *et al*., 2013). While they all highlight the importance of environmental context, they exclude other key environmental factors that shape community composition; for example, alkalinity (Ptacnik *et al*., 2008; Carvalho *et al*., 2011; Maileht *et al*., 2013), pH (Kosten *et al*., 2012; Beaulieu *et al*., 2013) and colour (Ptacnik *et al*., 2008; Maileht *et al*., 2013). Thus, when exploring how lake type might influence the response of cyanobacteria to multiple stressors such as eutrophication, climatic warming and changing rainfall patterns, including more types is necessary in order to provide robust information for the effective management of lakes.

Here, we took advantage of existing broad scale data from 494 natural European lakes to test whether eutrophication (phosphorus), temperature, and prolonged periods of drought (retention time) interact to exacerbate the problem of cyanobacteria. We modelled the response of chlorophyll-*a* concentration, as a proxy for total phytoplankton biomass, and cyanobacteria biovolume in eight different lake types which were defined by combinations of alkalinity (low and medium-high alkalinity), colour (clear and humic) and mixing types (polymictic and stratified). These types broadly match the common lake typologies which have been agreed across >25 European countries as part of the European Water Framework Directive (WFD, http://ec.europa.eu/environment/water/water-framework/) in recognition of the differential sensitivity of lakes of different types to environmental stressors. We hypothesised that elevated temperatures and increased retention time would have a greater positive effect on cyanobacteria than on total phytoplankton, and that their effect would be in synergy with phosphorus. We further hypothesised the sensitivity of these response variables to the interactions between multiple stressors would vary among lake types.

1.3 Methods

Data

i. Biological and chemical data

Data on cyanobacteria biovolume ($mm^3\ L^{-1}$), chlorophyll-*a* concentration ($\mu g\ L^{-1}$), total phosphorus concentration ($\mu g\ L^{-1}$) and lake type variables - altitude, depth, surface area, mixing status, humic content and alkalinity - were extracted from the WISER database (Moe *et al*., 2013) and supplemented by additional datasets. Total phosphorus was used as measure of nutrient enrichment as it is a robust indicator of eutrophication in freshwater systems (Howarth & Marino, 2006) and was also available for all lakes (whereas total nitrogen was not). Chlorophyll-*a* was used as a proxy for total phytoplankton abundance as this is the most widespread global measure of ecosystem quality used in lake management (OECD 1982); chlorophyll-*a* and total phytoplankton biovolume were strongly positively correlated (R^2 = 0.64, p <0.001). Biological and phosphorus data were summarised as monthly means for July, August and September; a period when cyanobacteria blooms are most reported in temperate, northern latitudes and when biological sampling fortunately is also most intense, thereby maximising data availability. Data were selected between 2000 and 2009 as sampling methods from this period were most standardised. Each lake contributed a variable number of observations; on average six monthly observations from different combinations of years (2000 – 2009) and months (July-September), Table S2.1 summarises the number of lake months for each year, month combination. The hierarchical structure of the statistical models accounts for differences in the number of observation per lakes, through the random effect error term.

ii. Catchment data

Catchment data – delineations and percent (%) CORINE land cover – were extracted from the MARS geodatabase (Globevnik *et al*., 2017).

iii. Climate data

Historical air temperature and effective rainfall data were downloaded from the Agri4Cast Data portal (Toreti, 2014) of the Joint Research Centre (JRC) which contains daily meteorological parameters from weather stations interpolated on a 25 x 25 km grid. Each lake was matched to the JRC square which contained the coordinates of the lake's sampling point. Mean monthly air temperature (°C) was used as a proxy for water temperature. For a subset of 299 lakes which had measurements of epilimnion temperature a significant linear relationship was found between mean monthly air and mean monthly water temperature with a slope of 0.89 ± 0.02

(R^2 of 0.59, p <0.001). Monthly effective rainfall was summed over the area of the catchment (catchment effective rainfall), correcting for the effect of different land cover types on evapotranspiration rates using correction coefficients adapted from Mircea-Mărgărit (2015). Catchment effective rainfall was then used as an estimate of the volume of water flowing into and out of the lake. To validate this estimate of outflow, measured outflow from a subset of 46 lakes from Norway and the UK were compared to the outflow estimated from effective rainfall. These countries were used as they had national datasets of flow gauge data for lake outflows. A significant positive linear relationship was found between measured and estimated outflow with a slope of 0.69 ± 0.02 (R^2 of 0.56, p <0.001) and this was used to adjust the outflow, estimated from the catchment effective rainfall. Lake volume was estimated by multiplying the mean depth by the area of the lake. The monthly flushing rate of the lake was estimated by dividing the adjusted outflow by the volume of the lake. The retention time, in days, was calculated from the monthly flushing rate divided by 30 days in all cases. Retention time was used because the expected response of cyanobacteria to all explanatory variables were then in the same direction and because intuitively it is a better representation of prolonged periods of drought.

iv. Defining lake types

The lake types defined in this study are based on common European typology schemes, used across all European countries in the European Water Framework Directive (WFD) (EC-JRC, 2014; Lyche Solheim *et al*., 2015). These lake types are based on geology, humic substances, mixing type/depth, altitude, size and region (Mediterranean). Modification to these types were made as some of the factors which define these types – altitude, depth and surface area – co-varied with the stressors (TP, temperature and retention time) and so their influence was retained through these variables (Fig. S2.1). Note that any additional lakes without information on these variables were then extracted from the WISER database (2 lakes). Alkalinity also positively co-varied with TP (Fig. S2.1) but was retained as this relationship showed some non-linearity; in low alkalinity lakes the relationship was not seen, yet in these lakes alkalinity and cyanobacteria showed statistically significant positive co-variation (R^2 = 0.17, p <0.001) in the lakes, supplementary material (Fig. S2.2 and S2.3), suggesting that alkalinity is an ecologically relevant type variable to include. Furthermore, others (e.g. Carvalho *et al*., 2011) have found alkalinity to be an important predictor of cyanobacteria.

Lake types were defined by combining the broad European type levels for alkalinity, humic substances and mixing to give 18 lake types. These lake characteristics are central to the European typology schemes, and have been shown by others (Ptacnik *et al.*, 2008; Maileht *et al*., 2013) to reflect ecologically meaningful characteristics that explain the distribution of phytoplankton and their response to eutrophication. Gower distance clustering (using the daisy function from the cluster package for R statistical software (Maechler *et* al., 2012) confirmed that these lake types sufficiently explained variation in cyanobacteria (Fig. S2.4 and Fig. S2.5). Although a large number of lakes were included in the dataset, imbalances in the data meant that 18 types could not be adequately modelled, therefore we further modified these types by combining ecologically similar levels of alkalinity and humic type. For alkalinity we retained 'low alkalinity' (<0.2 mEq L^{-1}) as a distinct level, and medium and high alkalinity (>0.2 mEq L^{-1}) were combined into a new level – 'medium-high alkalinity'. For humic type we retained 'low humic' as a distinct level (colour <30 mg Pt L^{-1}), renaming the level as 'clear', and medium and high humic (colour > 30 mg Pt L^{-1}) were combined into a new level – 'humic'. This merging of levels is consistent with the finding that bloom-forming cyanobacteria have a preference for neutral-alkaline lakes (Shapiro, 1984; Carvalho *et al.*, 2011; Maileht *et al*., 2013), and that cyanobacteria dominate more often in clear lakes than in humic lakes (Ptacnik *et al*., 2008). Furthermore, clusters formed from the Gower distance analysis also show a tendency for these levels to be grouped together (Fig. S2.6). The biovolume of cyanobacteria differed statistically significantly between levels of each lake type variable (Fig. S2.7): alkalinity (low *vs* med-high alkalinity, t = -22.5, df = 1574, p <0.001); humic (clear *vs* humic, t = 7.78, df = 1579.8, p <0.001) and mixing type (stratified *vs* polymictic, t = -7.03, df = 600.97, p <0.001). All combinations of these new levels gave eight types, Fig. 2.2 a shows the spatial distribution of the 494 lakes by type. A plot of the Silhouette width, Fig. S2.4 (used to determine the number of clusters) indicates that most of the differences between clusters are captured within 10 clusters and so reducing the clusters from 17 to 8 can be supported. Variation in cyanobacteria biovolume was explained by the types (Table S2.3), although differences between polymictic and stratified lakes were less clear when humic type and alkalinity type were taken into account (Fig. 2.2b, see also supporting information). The clearest difference in cyanobacteria biovolume was seen between levels of alkalinity, both as a single lake type variable but also in combination with other lake type variables (Fig. 2.2b and Fig. S2.7).

Table 2.1. Response and explanatory variables included in the analysis. Means ± standard deviations and minimum and maximum values in parentheses, are summarised by each lake type. Total number of lakes in the analysis was 494.

		Phytoplankton parameters		**Stressors**		
Lake type	Number of lakes	Total cyanobacterial biovolume (mm^3 L^{-1})	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Mean monthly total phosphorus ($\mu g\ L^{-1}$)	Mean monthly air temperature (°C)	Monthly retention time (days)
Polymictic						
low alkalinity, clear	3	0.005 ± 0.01 (0 – 0.02)	3.21 ± 1.8 (1.2 – 5.6)	9.6 ± 5.1 (4 – 15)	15.7 ± 1.9 (13.6 – 18.6)	21.7 ± 22.8 (7.6 – 61)
low alkalinity, humic	15	3.1 ± 17 (0 – 114)	10.1 ± 12.4 (1.2 – 61)	21.4 ± 17.5 (3.6 – 91)	14.6 ± 1.9 (9.1- 18)	17.3 ± 29.6 (1.7 – 207.7)
med-high alkalinity, clear	89	7.9 ± 21 (0 – 224)	34 ± 33 (2 – 238)	50.1 ± 25.8 (10 – 100)	17 ± 2.9 (9.1 – 24.0)	48 ± 68.6 (0.2 – 339.7)
med-high alkalinity, humic	45	1.0 ± 2.0 (0 – 11)	20.1 ± 22.1 (1 – 120)	35.8 ± 20.6 (2 – 98)	16.2 ± 2 (10.6 – 20)	32.9 ± 53.7 (0.6 – 351)
Stratified						
low alkalinity, clear	70	0.05 ± 0.3 (0 – 5.3)	3.3 ± 2.6 (0.2 – 21.5)	8.2 ± 4.9 (1 – 37.6)	14.0 ± 2.6 (6.6 – 19.9)	82.3 ± 86.6 (2.9 – 363.2)
low alkalinity, humic	70	0.17 ± 0.9 (0 – 12.1)	8 ± 11.8 (0.3 – 110.3)	14.5 ± 11.8 (2 – 97)	14.8 ± 2.4 (6.2 – 20.2)	63.3 ± 74.2 (1.8 – 359.9)
med-high alkalinity, clear	163	1.9 ± 3.7 (0 – 31)	16.5 ± 54 (0.7 – 1025)	31.7 ± 20.1 (2 – 99)	17.1 ± 2.7 (5.5 – 24)	83.0 ± 81.7 (2.5 – 360)
med-high alkalinity, humic	39	1.0 ± 2.6 (0 – 26)	16.0 ± 22.3 (1.4 - 185.8)	33.2 ± 28.3 (2 – 100)	15.6 ± 3.0 (5.3 – 20.6)	82.5 ± 96.6 (3.6 – 356)

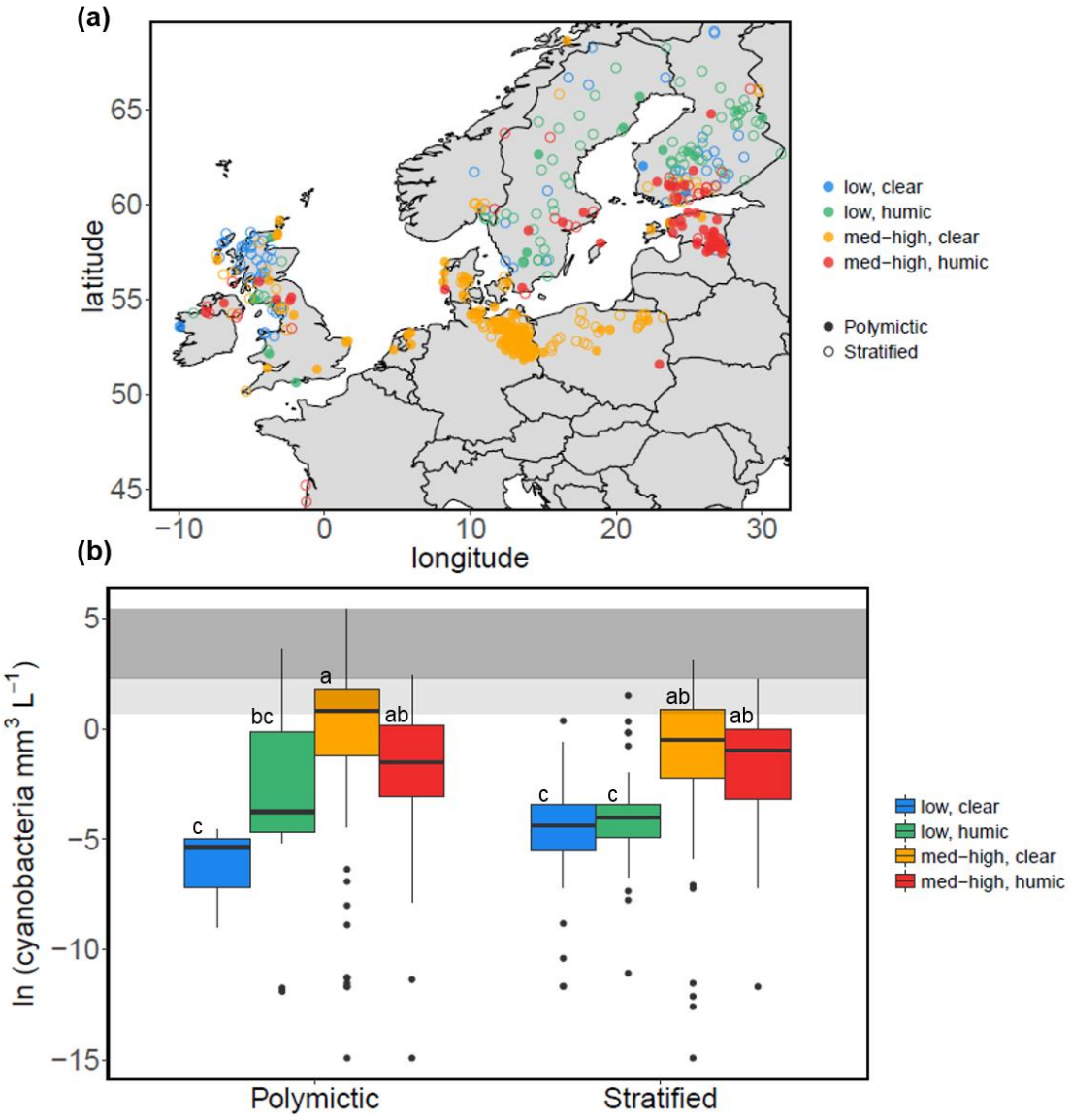


Fig. 2.2 Distribution of lake location (a) and cyanobacteria biovolume (b) by lake type. Lake types are combinations of: alkalinity, low (<0.2 mEq L^{-1}) and med-high (>0.2 mEq L^{-1}); humic content, clear (colour <30 mg Pt L^{-1}) and humic (colour > 30 mg Pt L^{-1}); and mixing type, stratified and polymictic. In (b) the shaded areas are for exceedance of low, 2 mm^3 L^{-1}, (light grey) and medium, 10 mm^3 L^{-1}, (dark grey) WHO (World Health Organisation) recommended threshold values for drinking and bathing (Chorus & Bartram, 1999), the conversion of WHO cell number guidelines to biovolume was taken from Carvalho *et al*., 2013. Cyanobacteria biovolume (mm^3 L^{-1}) is log transformed and averaged for each individual lake. Letters (a, ab, bc and c) indicate significant differences (at p <0.05) in mean cyanobacteria between groupings of lake types, Tukeys test for multiple comparison following an ANOVA (supplementary material). Note that observations of cyanobacteria biovolume in polymictic, low, clear lakes are from three lakes only, this lake type is not subsequently modelled as there is insufficient data for more complex multi variable modelling.

Statistical analysis

i. Relationships between variables

Prior to the analysis, relationships between variables were investigated using pairwise scatterplots, inspecting for co-variation between explanatory variables and also for potentially non – linear responses using LOESS regression (Cleveland & Devlin, 1988). Experimental studies have shown that interactions can change along the stressor gradient when the response to single stressors are non-linear (Piggott *et al.*, 2015), therefore we chose to restrict the regression to the range of each stressor where the data were linearly related. This was only relevant for the response to TP in which no relationship was found at high concentrations. See '*exploratory analysis*' in the results section for more details.

We found that TP and retention time negatively co-varied (Fig. S2.1), this relationship was influenced by lakes with very long retention times i.e. greater than a year. To minimise potential issues with this co-variation confounding the response, as well as the potential of outliers skewing the response, we limited the data to lakes with monthly retention times of ≤365 days (1 year). This selection reduced the co-variation between retention time and TP (Fig. S2.9) while still representing 90% of the data

ii. Lake type models

Linear mixed effects models were fitted using the lme4 package for R statistical software (Bates *et al.*, 2015) R, Version 3.4.1 (R Core Team (2017). To make distributions more symmetric, and assumptions of normality and homoscedasticity for error terms appropriate, cyanobacterial biovolume ($mm^3 L^{-1}$), chlorophyll-*a* ($\mu g L^{-1}$), retention time (days) and TP ($\mu g L^{-1}$) were ln-transformed. All stressor variables were then standardised (mean centred and divided by the standard deviation) so that the size effect of single stressor effects (when no interaction terms were present) could be compared within models. The potential interactive effects of TP, temperature and retention time on the biovolume of cyanobacteria and the concentration of chlorophyll-*a* were modelled in each lake type separately (seven models for cyanobacteria and seven models for chlorophyll-*a*). For each lake type the following model was fitted:

Lake type model e.g. polymictic, medium-high alkalinity, clear lakes

$$\gamma = \beta_0 + \beta_1 X_{TP} + \beta_2 X_{Temp} + \beta_3 X_{Retention} + \beta_4 X_{TP\ x\ Temp} + \beta_5 X_{TP\ x\ Retention} + \beta_6 X_{Temp\ x\ Retention} + \beta_7 X_{TP\ x\ Temp\ x\ Retention} + \delta_{lakeID} + \varepsilon, \quad \gamma \sim (0, \sigma_l^2), \quad \varepsilon \sim (0, \sigma_r^2) \tag{2.1}$$

where γ is the log response of interest (cyanobacteria biovolume, $mm^3\ L^{-1}$ and chlorophyll-*a*, $\mu g\ L^{-1}$), β_0 is the intercept term, β_1, β_2, and β_3 are model parameters for the TP term, temperature term and retention time term, respectively. The model parameters for the interactions are β_4 (TP and temperature), β_5 (TP and retention time), β_6 (temperature and retention time) and β_7 (TP, temperature and retention time). δ is the random effect term for lake ID which allows the response to vary on the intercept for individual lakes and ε is the overall error term, both with a mean of zero and unknown variance. Initially, year and month were also incorporated into the model as random terms to account for sampling within lakes over multiple months and years but this did not explain additional variance so were removed from the final models for parsimony. This model was then simplified by removing higher order interaction terms in turn, comparing simplified and more complex models using AIC and BIC, favouring simpler models when retaining more complex terms did not improve the model. Degrees of freedom and *p* values were approximated using the lmerTest package (Kuznetsova *et al*., 2015) . The variance explained by the model is reported as marginal R^2 which describes the proportion of variance explained by the fixed factor(s) alone and conditional R^2 which describes the proportion of variance explained by both the fixed and random factors (Nakagawa & Schielzeth, 2013).

1.4 Results

Exploratory analysis

Of the 572 lakes initially identified as being suitable for analysis i.e. lakes with complementary biological, climatic and typology data, 78 had mean monthly TP concentrations which exceeded 100 μg L^{-1} and therefore were omitted from the multiple stressor analysis as at high concentrations, TP explained little additional variation in the biovolume of cyanobacteria (Fig. S2.10). Piecewise regression analysis (Muggeo, 2008) of the data (n = 2900) identified a break point of 4.1 natural log TP, or 60 μg L^{-1} (standard error = 0.16, R^2 = 0.29). However, to avoid potential biases of the dataset and to limit the number of lakes removed from the analysis we restricted regression to data where TP ≤ 100 μg L^{-1}, which is also a more typical turning point identified in the literature for the widely reported asymptotic behaviours of chlorophyll-*a* and cyanobacteria to TP (McCauley *et* al., 1989; Watson *et* al., 1992; Phillips *et* al., 2008; Carvalho *et al*., 2013). The biovolume of cyanobacteria in these lakes was on average higher (mean 9.3 mm^3 L^{-1}) than in lakes with TP concentrations below 100 μg L^{-1} (mean 1.9 mm^3 L^{-1}); t = -4.1, df = 277.9, p <0.001.

In the 494 lakes analysed for the interactive effects of phosphorus, temperature and retention time, the mean monthly biovolume of cyanobacteria ranged from 0 to 225 mm^3 L^{-1}, while chlorophyll-*a* ranged from 0.2 - 1025 μg L^{-1}. 23% of these lakes had an average cyanobacteria biovolume that exceed the WHO low risk threshold of 2 mm^3 L^{-1} (Chorus & Bartram, 1999), the conversion of WHO cell number guidelines to biovolume was taken from Carvalho *et al* (2013). These lakes were predominantly located in central Europe while lakes with lower cyanobacteria biovolume were located in northern regions (Fig. S2.11). This spatial distribution of cyanobacterial abundance followed a pattern of decreasing temperature and decreasing TP concentrations with increasing latitude (R^2 = 0.20, p <0.001 and R^2 = 0.28, p <0.001 respectively). Latitudinal patterns in TP concentrations also corresponded to a decrease in percentage arable land in the catchment with increasing latitude (Fig. S2.12).

Multiple nutrient and climate effects on the abundance of cyanobacteria and phytoplankton

Climate and phosphorus relationships varied across the different lake types and the response of cyanobacteria and chlorophyll-*a* differed (Table 2.2, Fig. 2.3).

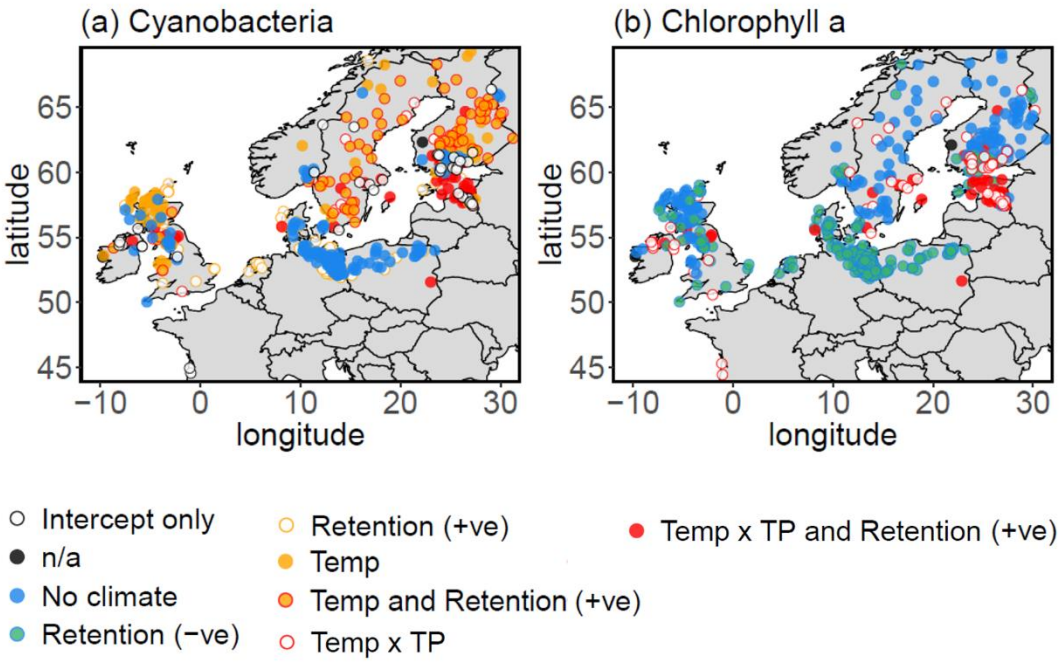


Fig. 2.3 Model summaries highlighting climate effects (temperature and retention time) for the response of (a) cyanobacteria and (b) chlorophyll-*a*. Each lake (point) is coloured according to statistically significant climate effects estimated for lake type to which the lake belongs. Warmer colours represent positive climate effects, cooler colours represent either no climate effect or a negative climate effect (only applicable for retention time in chlorophyll-*a* models). n/a are polymictic, low alkalinity, clear lakes (n = 3) which had insufficient data for analysis. See Fig. 2.2 for the spatial distribution of lake types.

We found that temperature and retention time had a stronger effect for cyanobacteria than for chlorophyll-*a* (Table 2.2, Fig. 2.3), being always positive for cyanobacteria, while we found negative retention time effects for chlorophyll-*a* in two of the lake types: polymictic, medium-high alkalinity, clear lakes and stratified, medium-high alkalinity, clear lakes (Fig. S2.13). Total phosphorus was a significant predictor of chlorophyll-*a* in all lake types, while this was not the case for cyanobacteria: in some lake types retention time and temperature were identified as better explanatory variables. Statistically significant effects of temperature showed a spatial pattern, with most temperature effects (independent effects and synergistic interactions with phosphorus) in lakes at Northern latitudes (> 55° N). The temperature gradient above this latitude ranged from 5.3 – 20.4 ºC (mean 14.8 ºC) while the gradient below this latitude ranged from 11.5 – 24 ºC (mean of 17.7 ºC).

Table 2.2. Linear regression mixed effect models explaining cyanobacteria biovolume and chlorophyll-*a* concentration. The models explain cyanobacterial biovolume (natural log, $mm^3 L^{-1}$) and chlorophyll-*a* concentration (natural log, $\mu g L^{-1}$) in different lake types and result from backward stepwise selection, starting with a model with full interactions between the independent variables: mean monthly total phosphorus (TP, $\mu g L^{-1}$), mean monthly air temperature (°C) and monthly retention time (days). TP and retention time are log transformed and all explanatory variables are standardised (mean centred and divided by the standard deviation) for comparability. Lakes are split into polymictic and stratified lakes (average conditions) and within each mixing regime into a further four types defined by combinations of alkalinity (low, med-high) and colour (clear, humic). Each model has an additional error term which accounts for differences between individual lakes, after accounting for the fixed effects, this is the random intercept term. The variance explained by the models is presented as marginal R^2 which describes the proportion of variance explained by the fixed factor(s) alone and conditional R^2 which describes the proportion of variance explained by both the fixed and random factors. The significance level is denoted as ***$p < 0.001$; ** $p < 0.01$; *$p < 0.05$, ·$p < 0.1$

			Model coefficients (standard error)				R^2	
Model	Lakes	Lake Type	TP	Temp	Retention	TP x Temp	Marginal	Conditional
	Cyanobacteria							
1a	3	polymictic, low Alk., clear	*Insufficient data*					
2a	15	polymictic, low Alk., humic	1.25 (0.65)·	1.15 (0.58)·		1.71 (0.73)*	0.07	0.77
3a	89	polymictic med-high Alk., clear			0.74 (0.27)**		0.05	0.69
4a	45	polymictic med-high Alk., humic	-0.05 (0.54)	-0.22 (0.61)	0.78 (0.34)*	1.82 (0.73)*	0.16	0.61
5a	70	stratified, low Alk., clear	0.54 (0.25)*	0.49 (0.16)**			0.05	0.63
6a	70	stratified, low Alk., humic		0.29 (0.12)*	0.41 (0.19)*		0.03	0.61
7a	163	stratified, med-high Alk., clear	0.77 (0.23)***				0.03	0.54
8a	39	stratified, med-high Alk., humic					0.00	0.80
	Chlorophyll-*a*							
1b	3	polymictic, low Alk., clear	*Insufficient data*					
2b	15	polymictic, low Alk., humic	0.61 (0.17)***	0.45 (0.16)**		0.84 (0.20)***	0.28	0.61
3b	89	polymictic med-high Alk., clear	0.70 (0.10)***		-0.15 (0.06)*		0.21	0.78
4b	45	polymictic med-high Alk., humic	0.32 (0.16)*	-0.71 (0.19)***	0.30 (0.09)**	1.03 (0.22)***	0.43	0.55
5b	70	stratified, low Alk., clear	0.31 (0.07)***				0.09	0.58
6b	70	stratified, low Alk., humic	0.35 (0.07)***				0.09	0.67
7b	163	stratified, med-high Alk., clear	0.65 (0.07)***		-0.19 (0.06)**		0.29	0.63
8b	39	stratified, med-high Alk., humic	0.51 (0.08)***	0.03 (0.04)		0.08 (0.04)*	0.35	0.81

There were synergistic interactions between temperature and TP in some lake types. However, unexpectedly, this interaction was not restricted to the response of cyanobacteria: in polymictic humic lakes, warming exacerbated the effect of TP on both the biovolume of cyanobacteria and chlorophyll-*a* concentration (Table 2.2, models 2 a, b and models 4 a, b; Fig. S2.14). A statistically significant positive interaction was also found in stratified, medium-high alkalinity, humic lakes but this was only significant for the response of chlorophyll-*a* and much smaller in size effect than the interactions found in polymictic, humic lakes (Table 2.2, model 8b). We did not find statistically significant evidence of interactive effects between retention time and phosphorus, nor between retention time and temperature, in any of the lake types for either response.

The fixed effects of the regression models for chlorophyll-*a* concentration explained more variance than regression models for cyanobacteria biovolume (marginal R^2, i.e. the proportion of variance explained by the fixed factor(s) alone, Table 2.2, Fig. 2.4a). The percentage of cyanobacteria biovolume explained by TP concentration and climate effects (temperature and retention time) was less than 7% in all lake types, with the exception of polymictic, medium-high alkalinity, humic lakes in which 16% of variance was explained. The variance of chlorophyll-*a* explained by stressors ranged between 9 – 43%, with most models explaining over 20% of the variance (Fig. 2.4a).

Although significant stressor relationships were detected, the natural variability between lakes was much larger. As an example, Fig. 2.4b shows that despite the interaction between TP and temperature being the same in all polymictic, low alkalinity humic lakes for any given TP – temperature combination, the average biovolume of cyanobacteria varied among individual lakes. The variance in the random intercept for each lake within each type is shown in Fig. S2.15.

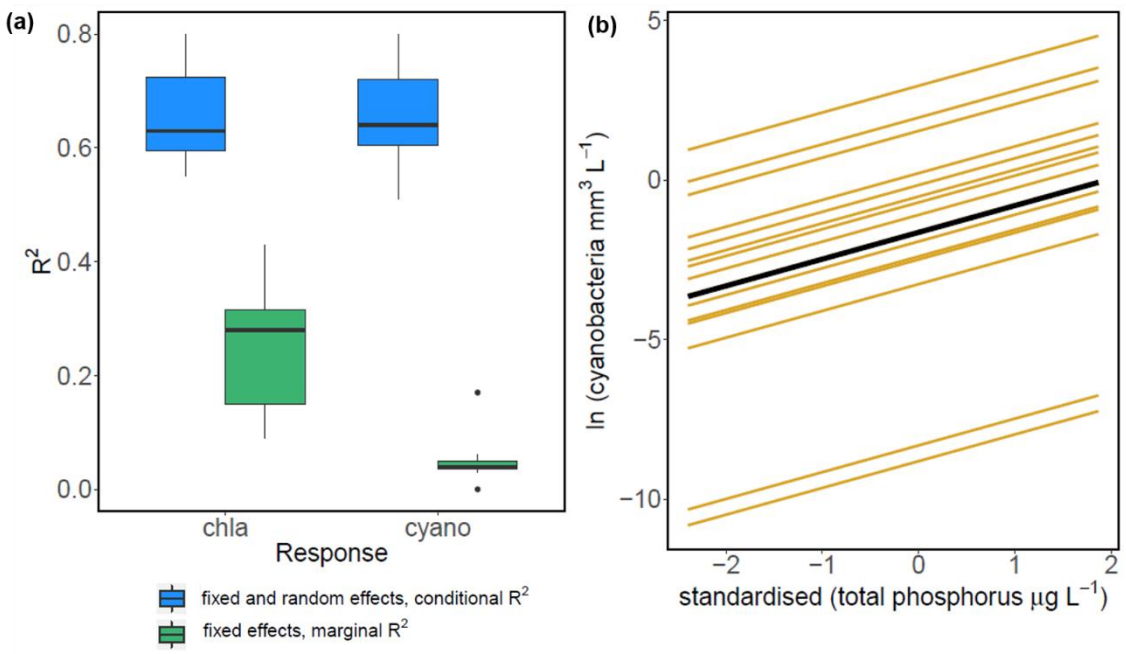


Fig. 2.4 Marginal and conditional variance explained by the models. (a) Boxplot of conditional R^2 (blue) and marginal R^2 (green) from all lake type models (n = 7 lake types) for chlorophyll-*a* and cyanobacteria responses. (b) Random effect plot of the response of cyanobacteria to TP in polymictic, low alkalinity, humic lakes (while keeping temperature constant). The fixed response is shown by the bold black line, individual lake responses are shown by the orange lines (i.e. differences in the intercept).

1.5 Discussion

The sensitivity of cyanobacteria to multiple stressors varies with lake type

Our results are consistent with previous work which suggests that the response of cyanobacteria to environmental change will be shaped by other environmental factors (Taranu, Zurawell et al. 2012, Beaulieu, Pick et al. 2013, Rigosi, Carey et al. 2014, Haakonsson, Rodríguez-Gallego et al. 2017). Unlike these studies, which mainly focused on one lake type factor, we combined a wider set of lake type variables that are also likely to shape community composition. We found that the sensitivity of cyanobacteria to temperature, retention time and phosphorus varied between lake types, suggesting that these additional lake typology factors are important in shaping the response of cyanobacteria to environmental change and could help better predict responses to future nutrient and climate changes. This is not surprising as the abundance of cyanobacteria is not just affected by factors that affect the amount of phytoplankton such as phosphorus, temperature and retention time but also by factors that shape community composition such as alkalinity, colour and mixing depth (Ptacnik, Lepistö et al. 2008, Maileht, Nõges et al. 2013, Lenard and Ejankowski 2017). Our results corroborate other studies that show the importance of allowing for interactions between multiple lake type factors; for example, interactions between mixing regime and colour (Havens and Nürnberg 2004), alkalinity and colour (Ptacnik, Lepistö et al. 2008), depth and alkalinity (Phillips, Pietiläinen et al. 2008) have been shown to shape phytoplankton nutrient relationships. Comparison of the sensitivity of chlorophyll-*a* and cyanobacteria to the effects of phosphorus, temperature and retention time among lake types suggests that chlorophyll-*a* may be less influenced by type (the response was similar between some lake types). This is consistent with Phillips, Pietiläinen et al. (2008) who found that nutrient chlorophyll-*a* relationships could be grouped into fewer groups than the eighteen WFD types that they tested, reducing the number of types to three. Our results suggest that more detailed groupings of lake types may be required to capture sensitivities of a community structure response, whereas chlorophyll-*a*, as a proxy for total biomass, appears to be less influenced by these finer details.

Colour as an additional lake type factor is an important inclusion, not only because changes in colour can strongly alter phytoplankton biomass and community structure (e.g. Lenard & Ejankowski, 2017) but also because humic substances have increased in lakes in past decades (Monteith *et al*., 2007). It is interesting that synergistic effects of temperature and phosphorus

were only detected in humic lakes (polymictic, humic types for cyanobacteria and chlorophyll-*a* as well as stratified, medium-high alkalinity and humic type for chlorophyll-*a*). The abundance of cyanobacteria is most often associated with clear lakes (data presented here, and e.g. Carvalho *et al*., 2011 and Ptacnik *et al*., 2008), consequently humic lakes are currently the least at risk (do not exceed WHO thresholds, Fig. S2.11), yet this interaction indicates that the deterioration of water quality may be accelerated in these lake types. This synergism could be caused by enhanced heat absorption in the lake surface caused by humic substances, a process that also increases thermal stratification (Kirillin & Shatwell, 2016) . It should be stressed that these relationships are for the levels of humic substances derived from the WFD European lake types, but it should be noted that many studies have demonstrated non-linear effects of colour on total biomass (Seekell *et al*., 2015) and cyanobacteria/composition (Carvalho *et al*., 2011; Rasconi *et al*., 2015; Urrutia-Cordero *et al*., 2016), adding further complexity. Nevertheless, our results show the importance of colour as a lake type factor and emphasises that other environmental factors may alter our expectations of multiple stressor interactions.

There is a risk that co-variation between environmental factors may lead to incorrect attribution of the processes behind a relationship. In particular, the striking spatial pattern of statistically significant temperature effects on cyanobacteria and chlorophyll-*a* in lakes at more northern latitudes coincides with the distribution of polymictic humic lakes (in which interactive temperature effects were found for both cyanobacteria and chlorophyll-*a*). The responses to changes in temperature have been shown to be greatest at lower latitudes because of larger shifts in metabolic rate which increases exponentially with temperature (Dillon, Wang et al. 2010, Kraemer, Mehner et al. 2017). However, our results show a different picture with greatest effects, particularly for cyanobacteria biovolume, at higher latitudes, which suggests that this is a sensitive part of the temperature gradient for cyanobacteria, or that other latitudinal effects such as longer summer photoperiod at higher latitudes (Nicklisch *et al*., 2008) or the effect of lake type may enhance the temperature effect. Another potential issue is the co-variation between alkalinity and TP. This co-variation is seen because many medium-high alkalinity lakes are located in central regions where the percentage arable land in the catchment and TP concentrations are higher. At higher latitudes, in contrast, there were a larger number of humic, low alkalinity lakes reflecting the tendency for acidic, humic and forested catchments in Fenno-Scandian areas (Maileht *et al*., 2013), in which TP concentrations were lower. Nevertheless, although average differences in the abundance of cyanobacteria among types may be attributed to average differences in TP (Fig. 2.2b and Fig. S2.16), most lakes types

were modelled over similar TP gradients, and so differences between lake type models are likely caused by other factors. The use of alkalinity as a type factor is both supported in the literature (e.g. Carvalho *et al.*, 2011; Phillips *et al.*, 2008 and Ptacnik *et al.*, 2008) but also from an exploratory analysis of the relationships between alkalinity, cyanobacteria and TP in low *vs* medium-high alkalinity lakes (supporting information).

Although we found statistically significant stressor relationships within lake types, in many cases the variation these explained was low and the natural variability among lakes within a lake type was much larger than the variance explained by the stressor effects. Phosphorus, temperature and retention time are important drivers, but they are not the only factors which influence phytoplankton biomass. Potential sources of variability can occur because of measurement error or missing covariate information e.g. other limiting nutrients (e.g. TN, (Downing *et al.*, 2001; Dolman *et al.*, 2012) grazer densities (Jeppesen *et al.*, 2000), competition with macrophytes (Phillips, 2005), light climate (Mischke, 2003) and past events such as remediation and associated hysteresis (Scheffer 1998, França *et al.*, 2016). Furthermore, the use of lake types as categorical variables may have reduced their explanatory power. In the future, it might be possible to incorporate sampling event-specific values that might also take account of within-year variation as can occur for the presence and duration of stratification (Jöhnk, Huisman et al. 2008, Wagner and Adrian 2009, Huber, Wagner et al. 2012), especially in polymictic lakes (Taranu, Zurawell et al. 2012) but also for colour variation (Lenard and Ejankowski 2017). Nevertheless, the use of lake types is an efficient means of simplifying statistical models and of providing information for managers on types of lakes at risk of generating algal blooms. It is possible that idiosyncratic responses to environmental change at the individual lake level could arise from interactions with other chemical, physical and biological environmental factors. A way to account for this would be to allow slopes of individual lakes to vary in the model structure, but due to limited data points within a lake we were unable to do this; further exploration using long-term datasets would be informative.

Implications for managing the risk of cyanobacteria in the future

The first take-home message for management is that the sensitivity of cyanobacteria to multiple anthropogenic stressors, and consequently the risk of water quality issues, will not be the same for all lakes. Thus, some lake types may require greater management intervention than others, and lakes that are currently not at risk (i.e. do not exceed WHO guideline thresholds) may develop problems in the future e.g. polymictic humic lakes. The broad typologies used are

similarly adopted (e.g. Havens & Nürnberg, 2004), and relevant, outside of Europe although some regions globally may have additional lake types that would need considering (e.g. endorheic lakes in North America and Africa). The second take home message, and perhaps a more generalisable outcome, is that our results suggest that in most lake types, management will become increasingly necessary because of the additional effects of climate change (temperature and retention time) on cyanobacterial abundance. As climate effects cannot be locally controlled, this means that existing models detailing phosphorus targets needed to minimise harmful algal blooms (Carvalho *et al.*, 2013) may have to be revised to mitigate these effects (Jeppesen *et al*., 2009). We do not make any quantitative recommendations here but indicate that this will be a likely management scenario for most lakes. It should be emphasised that we make reference here to the effects and control of phosphorus as it is often considered the limiting nutrient in lakes (Phillips *et al*., 2008; Schindler *et al*., 2008), however nitrogen can also play a key role (Maberly *et al*., 2002; Conley *et al*., 2009; Beaulieu *et al*., 2013; Paerl *et al*., 2016). Under projected climate scenarios, it is expected that there will be an increase in nitrogen loading because of enhanced runoff in the north temperate region (Sinha *et al*., 2017), the effects of which may also depend on ecosystem type. For example, shallow lakes are likely often nitrogen limited during the summer (Dolman *et al*., 2016; Søngergaard *et al*., 2017) and so enhanced loading could increase the carrying capacity in lakes with sufficient phosphorus. Furthermore, an increase in nitrogen could trigger a shift from a macrophyte, clear water state to a turbid phytoplankton dominated state (e.g. Olsen *et al*., 2015).

It should be emphasised that this is a broad view of management at a lake type level; the relationships that we present within lake types describe the generalised response for this population of lakes. However, we found that the natural variability among lakes within a lake type was much larger than the variance explained by the stressor effects. The implications of this are that, for a given value of a stressor (or combination of stressors, depending on the model), the abundance of cyanobacteria may vary considerably among lakes of the same type (Fig. 2.4b). Thus, while these models can be used to assess potential risk across a population of lakes (within a specific lake type), and inform where to prioritise monitoring for risk management, they are not appropriate for decision-making at the individual lake level. This view reflects the perspective which warns of copy and paste management methods for different lakes (Lürling *et al*., 2016).

Final remarks

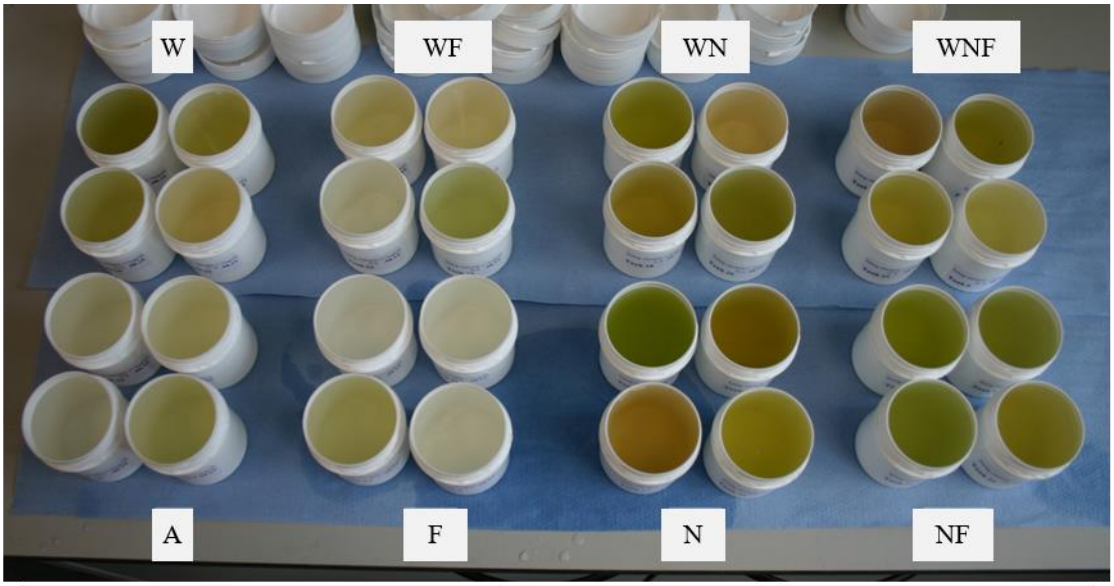
Our results indicate that the response of cyanobacteria to favourable future conditions of enhanced nutrient enrichment, elevated temperatures and prolonged periods of drought may not be the same in all lake types. While other studies have reached similar conclusions, here we provide evidence that these are not just limited to one type factor as has been explored before. We do not conclude that these are definitive 'end lake types', however we suggest that our ability to generalise and manage the response of cyanobacteria to multiple stress and future environmental change lies in defining the types of environment in which the risk/sensitivity differs.

Chapter 3.

Mesocosm experiment study: an experimental analysis of extreme rainfall events, warming and nutrient enrichment in a shallow lake mesocosm.

Richardson, J., H. Feuchtmayr, C. Miller, P. Hunter. S. C. Maberly and L. Carvalho (2018) The response of cyanobacteria to warming, extreme rainfall events and nutrient enrichment. *Under review in Global Change Biology.*

Fig. 3.1 Using a submersible fluorometer (bbe Moldaenke AlgaeTorch) to measure the fluorescence of phycocyanin, a quantitative biomarker for cyanobacteria.



Treatment	Response	Effect size (filled = sig)
Nutrient enrichment	chlorophyll-*a*, cyanobacteria	⬆
Warming (+4 ˚C)	chlorophyll-*a*,	⬆
	cyanobacteria	⬆
Extreme rainfall (flushing)	chlorophyll-*a*, cyanobacteria	⇩
Nutrient enrichment x warming	chlorophyll-*a*, cyanobacteria	⬇

Effects of nutrients, warming and flushing on phytoplankton and cyanobacterial abundance (May-August). A = ambient, W = warmed, F = flushed, N = nutrients.

Fig. 3.2 Chapter three graphical abstract – the effects of nutrients, warming and flushing on phytoplankton and cyanobacterial abundance.

3.1 Abstract

Cyanobacterial blooms are an increasing threat to water quality and global water security caused by the nutrient enrichment of freshwaters. There is also a broad consensus that blooms are increasing with global warming, but the impacts of other concomitant environmental changes, such as an increase in extreme rainfall events, may affect this response. Here we used a shallow lake mesocosm experiment to test the combined effects of warming (ambient *vs* +4°C increase) and extreme rainfall events (no events *vs* seasonal extreme events) in high nutrient environments (eutrophic *vs* hypertrophic) on cyanobacterial abundance and composition. We found that, as expected, warming stimulated both total phytoplankton and cyanobacterial growth, including the abundance of two of the most common bloom-forming harmful taxa: *Microcystis* spp. and *Dolichospermum* spp. Unexpectedly, there was an antagonistic interaction between warming and nutrient enrichment for total cyanobacteria and chlorophyll-*a*, indicating that at very high nutrient concentrations, other limiting factors may alter multiple stressor interactions and lead to ecological surprises. Extreme rainfall events only had short-term effects on phytoplankton abundance and composition during the growing season and the effects of nutrient addition and warming were not affected by these flushing events. During the winter months, extreme rainfall events resulted in a slower recovery of phytoplankton in general but also, unexpectedly, increased cyanobacterial dominance in ambient, nutrient enriched mesocosms. While this study highlights the clear need to mitigate against global warming, it also shows that ecological surprises can occur depending on the context of the multiple stressor environment. As such, over-simplification of global change effects on cyanobacterial blooms should be avoided; stressor gradients and temporal factors such as seasonality or the variance of extreme events should be considered as important factors shaping the response.

3.2 Introduction

Blooms of cyanobacteria are produced primarily in response to the eutrophication of lakes and reservoirs (Taranu *et al*., 2015) and are a major threat to freshwater quality and global water security (Codd *et al*., 2005, Steffensen *et al*., 2008). However, there is a broad consensus that elevated water temperatures also promote the proliferation of cyanobacterial blooms (Paerl & Huisman, 2008) because cyanobacteria have a number of traits which provide them with an advantage under warmer conditions (Carey *et al*., 2012). For example, many bloom-forming cyanobacteria reach their maximum growth rate at higher temperatures than other phytoplankton (Butterwick *et al*., 2005; Reynolds, 2006; De Senerpont Domis *et al*., 2007), benefit from warming-enhanced internal cycling of nutrients (McKee *et al*., 2003) and greater water column stability (Huber *et al*., 2012; Jöhnk *et al*., 2008 also see Carey *et al*., 2012). Studies over a range of scales: experimental (Lürling *et al*., 2017), single water body (Taranu *et al*., 2012; Zhang *et al*., 2012) and regional (Kosten *et al.*, 2012; Beaulieu *et al*., 2013), provide ample evidence that higher temperatures promote higher cyanobacterial abundance and thus severely affect our ability to control blooms (Havens & Paerl, 2015). The threat of cyanobacterial blooms is, therefore, expected to be increasing in response to rapid global warming.

The response of cyanobacteria to warming may, however, be complicated by other large-scale environmental changes which can alter phytoplankton growth and community structure. This includes the predicted increase in extreme stormy weather (IPCC, 2013). More extreme rainfall events are now being observed globally (Lehmann *et al*., 2015) and, in particular, are predicted to increase during the summer months at mid- to high-latitudes (Christensen & Christensen, 2003). These events result in sudden environmental changes (e.g. Sadro & Melack, 2012), with an increased delivery of nutrients and organic material from the catchment, changes in the stability of the water column and losses of phytoplankton biomass, dissolved and biologically-bound nutrients through increased hydraulic flushing. While hydraulic flow is recognised to be an important factor in phytoplankton dynamics (e.g. Søballe & Kimmel, 1987; Tolotti *et al*., 2010) the physical effect of extreme rainfall events as a 'climate change stressor' on phytoplankton biomass and community composition has received relatively little attention. The effect of climate warming on cyanobacterial bloom dynamics and species dominance is, therefore, likely to be complex, depending on how it interacts with other global factors such as extreme rainfall events and local factors such as nutrient availability.

To improve our ability to forecast the effect of global warming on cyanobacteria, we need to take a more complete view of future conditions, incorporating 'event-focused' pulse events as well as 'trend-focused' press climate effects (Jentsch *et al*., 2007; Michalak, 2016). Mechanistic insight of complex interactions can be gained through experimental manipulation. Lake mesocosms are useful to explore the effects of multiple stressors, by allowing environmental conditions to be manipulated while retaining ecosystem complexity (Fordham, 2015; Stewart *et al*., 2013). Small, shallow lakes are of particular interest as they are numerically dominant globally (Messager *et al*., 2016; Verpoorter *et al*., 2014), are especially sensitive to changes in air temperature (Butcher *et al*., 2015), have a higher exposure to nutrient pressures because of their abundance in lowland, impacted landscapes (Nõges, 2009) and a higher sensitivity to extreme rainfall events because of their smaller volume. Here we describe a shallow lake mesocosm experiment that assessed the potential interactions between warming, nutrient enrichment and extreme rainfall events on the abundance and composition of cyanobacteria. We hypothesised that warming would favour the growth of cyanobacteria over other phytoplankton, in particular taxa with higher temperature growth optima such as *Microcystis* spp. and *Dolichospermum* spp. (previously *Anabaena*), and that the effect would be synergistic with nutrient addition. We expected the effects of extreme flushing events to broadly be negative but likely to differ among treatments and phytoplankton taxa. For example, we hypothesised that the effects of flushing would be less prolonged in warmed, high nutrient mesocosms during the growing period when the conditions for growth – and recovery – would be optimal.

3.3 Methods

A fully factorial experiment combining two temperature treatments, two nutrient treatments and two extreme rainfall treatments was performed in 32 outdoor mesocosms from July 2014 to August 2015 at the Centre for Ecology & Hydrology's Aquatic Mesocosm Facility located in the North West of England (54°1'N, 2°46'W) (https://www.ceh.ac.uk/our-science/research-facility/aquatic-mesocosm-facility). The levels of each treatment were chosen to simulate current and future scenarios. The eight treatments (the full cross of each factor) were replicated four times, one replicate randomly assigned to a mesocosm in each experimental block of eight mesocosms (Fig. S3.1 and S3.2).

Description of mesocosms

The mesocosms are free-standing, open-topped, non-transparent and insulated cylinders, measuring one metre in depth and two metres in diameter (3000 L capacity), Fig. S3.2. Each contains a heating element (Fig. S3.2c), located 14 - 15 cm above a 5 - 6 cm deep mixture of washed sand and lake sediment (in equal proportion), taken from Windermere, a large mesotrophic lake in the English Lake District, UK. Mesocosms were filled with an equal volume of rain water and water from Windermere and were inoculated with phytoplankton, zooplankton and macroinvertebrates, also from Windermere, to simulate realistic natural community compositions (Reynolds & Irish, 2000). Mesocosms were allowed to settle for 13 months; during which, macroinvertebrates were re-stocked twice and also cross-mixed twice to ensure similar starting conditions. At the start of the experiment there was no statistically significant difference in chlorophyll-*a* concentrations between the eight treatments (Table S1). Four adult three-spined sticklebacks (*Gasterosteus aculeatus*), two of each gender, were sourced from local streams (New Draught and Barton Brook, Lancashire) and were added to each mesocosm. Between capture and inoculation, fish were kept in 30 L glass aquaria, containing untreated water from Blea Tarn Reservoir. Macrophyte populations established from natural seed-banks within the sediment. Any water losses from evaporation were monitored and rectified by the addition of deionised water.

Treatments

Warming

Half the mesocosms were left at ambient temperature, while the other half were warmed to 4 °C above ambient using electric heating elements (Fig. 3.2c). Water temperature (°C) was recorded every minute by sensors located 40 cm horizontally and vertically (mid-depth) within

each mesocosm and then stored on a data logger. A computer program adjusted the water temperature in warmed mesocosms so that it tracked changes in temperature in ambient mesocosms (Fig. S3.3). Daily mean temperatures in ambient mesocosms followed a seasonal cycle typical of temperate regions, varying between 2.4 °C in January and 23.4 °C at the end of July. Each mesocosm contained an automatic mixer to prevent thermal stratification, so that direct effects of temperature could be assessed (Fig. S3.4).

Nutrient enrichment

The mesocosms were enriched with nitrogen and phosphorus, half of the mesocosms at high concentrations to create 'nutrient enriched' conditions and half at lower concentrations to create 'ambient nutrient' conditions. A fortnightly load of nitrogen and phosphorus was added to nutrient enriched mesocosms to produce final Redfield ratio concentrations (Redfield, 1958) in each mesocosm equivalent to 510 μg L^{-1} nitrogen (sodium nitrate) and 70 μg L^{-1} phosphorus (trisodium phosphate). Over the course of the experiment, this resulted in average nutrient concentrations of 314 ± 86 μg L^{-1} for total phosphorus (TP) and 1576 ± 298 μg L^{-1} for total nitrogen (TN) which is similar to the upper range of concentrations recorded in natural lakes in agricultural catchments in Europe (Moe *et al*., 2013). In the 16 ambient nutrient mesocosms, a fortnightly load equivalent to 73 μg L^{-1} of nitrogen and 10 μg L^{-1} of phosphorus was added until the 17th of December, after which any nutrients were derived from the lake water and from the sediment. Over the course of the experiment, the average TP concentration in the ambient nutrient addition mesocosms was 100 μg L^{-1} ± 47 μg L^{-1} and the average TN concentration was 692 μg L^{-1} ± 218 μg L^{-1}. Based on average TP concentrations over the duration of the experiment, nutrient enriched mesocosms were classified as being hypertrophic while ambient nutrient addition mesocosms were on the eutrophic-hypertrophic boundary (OECD 1982).

Extreme rainfall events

Half the mesocosms were exposed to extreme rainfall (flushing) simulations every twelve weeks – five events on the: 3rd of September 2014; 24th of November 2014; 17th of February 2015; 12th of May 2015 and 4th of August 2015. During each event, 1,500 litres of water (50% of the capacity of a mesocosm) was pumped into each treated mesocosm at a flow rate of 70 – 100 L min^{-1} (duration of 15 – 21 minutes), taking care not to disturb the sediment while ensuring homogenous mixing; water was lost by overflowing the top of the mesocosms. Water was sourced from Blea Tarn Reservoir, Hazelrigg, Lancaster, which was low in nutrients, phytoplankton and total suspended material. Any dissolved nutrients lost during the flushing

event were replaced so that the effects of biomass loss could be isolated from other effects of extreme rainfall events such as increases in nutrient loading and other allochthonous material. This was calculated from the amount of nutrients lost by dilution (see supplementary methods for details) and that added by the water used for flushing. Overall, there was no statistically significant effect of flushing on the concentration of SRP (soluble reactive phosphorus) or nitrate (NO_3-N; Fig. 3.3, Table 3.1).

Sampling

Water samples were taken once every four weeks (regular sampling). During extreme rainfall events, additional samples were collected immediately after the event, one week after the event and three weeks after the event, before returning to a four-weekly schedule (Fig. S3.5). Samples were collected using a one meter long plastic tube which integrated the whole water column. Water samples were thoroughly mixed before further processing.

Abiotic measurements

TP and TN concentrations were measured following Johnes & Heathwaite (1992) and nitrate and SRP concentrations were measured following Mackereth *et al*. (1987). Photosynthetic active radiation was measured every minute by sensors located 40 cm horizontally and vertically (mid-depth) within each mesocosm.

Biotic measurements

i. Phytoplankton and cyanobacterial abundance

Chlorophyll-*a* concentration (μg L^{-1}) was used as an estimate of total phytoplankton biomass. Known volumes (0.03 – 1 L, depending on the mesocosm) of the integrated water samples were filtered onto Whatman GF/C filters. Concentrations of the pigment were determined spectrophotometrically after cold ethanol extraction (96 %) in darkness overnight (Jespersen 1987); absorption was measured at 750 nm and at 665 nm.

The proportion of total chlorophyll-*a* (μg L^{-1}) assigned to cyanobacteria was measured using a submersible fluorometer (bbe Moldaenke AlgaeTorch) which measured the fluorescence of phycocyanin, a quantitative biomarker for cyanobacteria. Measurements of cyanobacteria chlorophyll-*a* began in November 2014. In some mesocosms, chlorophyll-*a* concentrations exceeded the calibrated range of the fluorimeter but because of an error at the user interface of the AlgaeTorch, these exceedances were undetected until the start of May

2015. Data prior to May are presented (for measurements below the manufacture's threshold of 200 μg L^{-1}) and are discussed but are not statistically analysed. From May the 5th onwards, mesocosms with chlorophyll-*a* concentrations that exceeded the manufacture's threshold (200 μg L^{-1}) were diluted with deionised water before measurement.

ii. Phytoplankton species composition

Phytoplankton composition was identified and enumerated the week before and three weeks after the flushing event in May 2015 and August 2015 using the Utermöhl technique (Utermöhl 1958, CEN 2014). For each sample at least 400 phytoplankton units (single cell, filament or colony) were counted according to phytoplankton size classes in the whole chamber (x10), in transects (x100) or fields of view (x400 and occasionally x630 for pico cyanobacteria). A minimum of ten measurements of key geometric dimensions were measured for each species from images taken on a digital camera (AxioCam MRc) attached to a Zeiss Axiovert 40 CFL inverted microscope using Zen software (2012 (blue edition) version 1.1.2.0. The mean of these dimensions was used to calculate biovolume (organism mm^3 L^{-1}), following Brierley *et al* (2007). Where distinguishing features were present, organisms were identified to species, while the remainder were identified to genus, class or were unidentified.

Statistical analysis

Changes in chlorophyll-*a*, cyanobacteria chlorophyll-*a*, TP, TN, SRP, NO_3-N and the biovolume of dominant cyanobacteria genera were analysed with mixed models using R version 3.2.2, R Core Team (2017). The trend over time (for chlorophyll-*a* and nutrients) and relationships with treatments (for all response variables) was tested while accounting for the random variation induced by the repeated measurements for each of the multiple mesocosms.

Trends in chlorophyll-*a* and nutrient concentrations were modelled over the duration of the experiment, between July 2014 and August 2015. Linear mixed models (LMM), using the lme4 package (Bates *et al.*, 2015) were used for temporal trends which could be fitted using a quadratic shape while additive mixed models (AMM) were used for more complex trends, using the gamm4 package (Wood & Scheipl, 2013), in addition to treatment covariates. Sampling date was converted into a decimal date and mean centred (mean of zero) so that the intercept related to the mid-point of the sampling period, mid-February (end of the northern hemisphere meteorological winter).

Measurements of cyanobacteria chlorophyll-*a* were restricted to a shorter time period - May 5th to August 26th 2015 - so no time component was included in the model i.e. the relationship between treatments and the average response was modelled. For comparison, as cyanobacteria is a component of the whole phytoplankton community, the relationship of chlorophyll-*a* to the treatments was modelled for the same time period as cyanobacteria chlorophyll-*a*.

Cyanobacteria genus biovolume data were zero-inflated and so the analysis followed a two-step process. First, the effect of treatment on the probability of occurrence (presence/absence) of the dominant cyanobacteria genera (*Aphanizomenon* sp.; *Microcystis* sp.; *Dolichospermum* sp.; and *Pseudanabaena* sp.) was tested using a Generalised Linear Mixed Model (GLMM) with a binomial distribution, then the effect of treatment on the biovolume of genera (for non-zero data) was tested using a LMM. Sampling date, as a categorical variable (four sampling dates), was included in the model as a potential co-variate; this was based on exploratory analyses of the data.

To stabilise the variability, all response variables were natural log transformed, with the exception of genus presence/absence data. As a result, the assumptions of normality and homogeneity of variance were appropriate for model error terms. To account for the repeated measures within mesocosms, a random effect term was included in both the linear and additive models which allowed the intercept to vary at the mesocosm level. This additional error term appropriately adjusts the coefficients and standard errors of the treatments but is also informative in quantifying additional among-mesocosm variance which cannot be explained by the fixed effects in the model (see, for example Bolker *et al.*, 2009)

Models were simplified by removing non-significant higher order interaction terms in turn. Simplified models were compared with more complex models using AIC and BIC and favoured, when retaining more complex terms did not improve the model. Satterthwaite approximations of degrees of freedom were used to obtain estimated *p* values (Gaylor, 2014). The variance explained by each model is reported as marginal R^2 which describes the proportion of variance explained by the fixed factor(s) alone and conditional R^2 which describes the proportion of variance explained by both the fixed and random factors (Nakagawa & Schielzeth, 2013).

3.4 Results

Treatment effects on nutrient concentrations

The effect of treatments on nutrient concentrations (total and biologically available) were statistically significant over the course of the experiment (Fig. 3.3, Table 3.1). The concentration of TP increased from spring (March) onwards, in particular in nutrient enriched mesocosms and warmed mesocosms, in the latter case including both ambient-nutrient and nutrient enriched mesocosms (Fig. 3.3a). The concentration of SRP decreased from spring onwards in all treatments except for warmed, nutrient enriched mesocosms in which concentrations increased (Fig. 3.3b). The concentration of TN increased in ambient, nutrient enriched mesocosms but also in warmed, ambient-nutrient mesocosms (Fig. 3.3c). During summer, spring and autumn, nitrate concentrations were low in all treatments (Fig. 3.3d). The increase in concentrations during the winter was statistically significant in nutrient enriched mesocosms, and also, but to a lesser extent in warmed, ambient-nutrient mesocosms (Table 3.2). Flushing had no effect on nitrate concentrations.

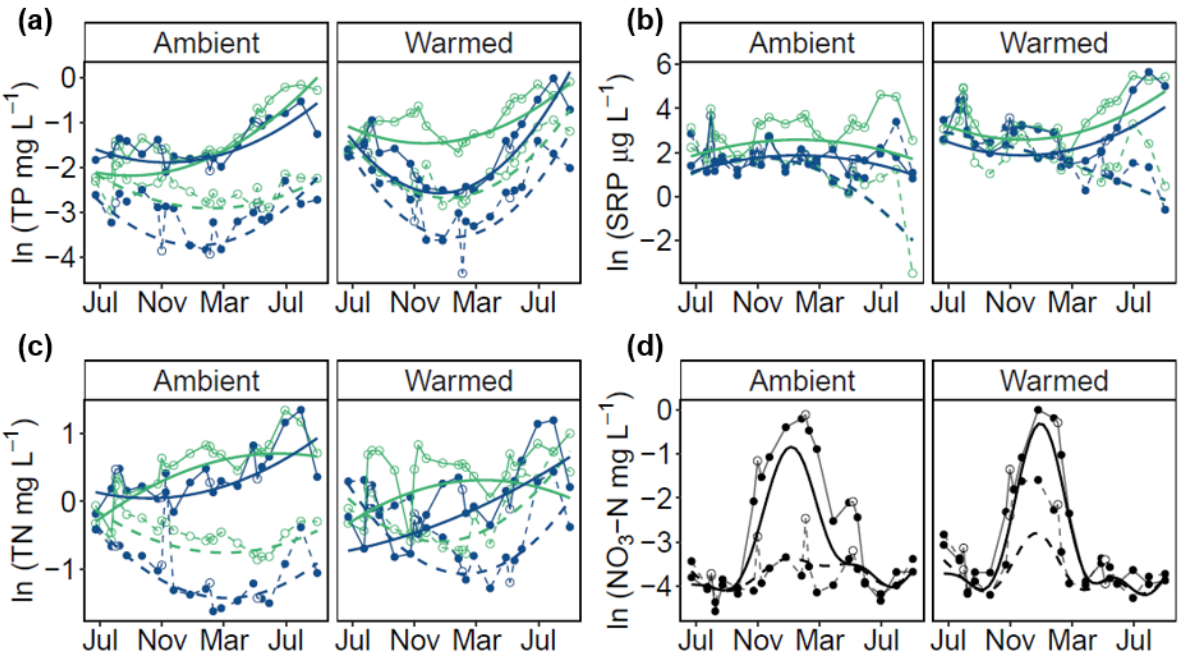


Fig. 3.3 Effect of nutrient enrichment and extreme rainfall events on the concentration of (a) TP (marginal R^2 = 0.55), (b) SRP (marginal R^2 = 0.29), (c) TN (marginal R^2 = 0.37) and (d) NO_3-N (R^2 adjusted = 0.59) in ambient and warmed mesocosms over time (July 2014 – August 2015). The response of each measured variable is ln transformed (note differences in the original scale); data points are mean responses for the treatment plotted. Smooth lines in panels (a – c) are the predicted fitted responses from the best fitting LMM model (Table 3.1): blue, flushed; green, unflushed; solid line, nutrient enriched; dashed line, ambient-nutrient. The smooth black lines in panel (d) are predicted fitted responses from the best fitting AMM (Table 3.2): solid line, nutrient enriched; dashed line, ambient-nutrient addition. For extreme rainfall treatments (blue lines in panels (a-c), all treatments in panel (d)) white data points show data from the sampling events the day immediately after an extreme rainfall event.

Table 3.1. Summary of ANOVA tables of type III for responses fitted with LMMs. For all measured variables, time is a second order quadratic term in the model. F values are presented with p-values based on Satterthwaite approximation for degrees of freedom. Significant effects at the $p < 0.05$ level are highlighted in bold and at the $p < 0.1$ level are underlined. Variance explained by each model is given by marginal R^2 for the fixed effects only and conditional R^2 for fixed and random effects. N, nutrient enriched; W, warmed; F, flushed.

	Chlorophyll-*a* F value	TP F value	SRP F value	TN F value
time	**216.5**	**166.5**	**19.3**	**20.8**
time x N	**21.2**	**24.9**	**45.9**	**38.1**
time x W	**5.3**	**12.7**	**13.3**	2.4
time x F	**4.3**	**6.7**	n.a	**5.9**
time x N x W	**14.9**	2.9	**4.3**	**3.8**
time x N x F	0.1	1.1	n.a	**4.7**
time x W x F	**5.0**	2.3	n.a	0.2
time x N x W x F	**10.1**	**8.2**	n.a	**3.3**
N	**59.8**	**55.8**	**33.8**	**38.6**
W	0.2	3.3	**18.4**	0.0
F	**4.4**	**10.2**	4.0	**7.3**
N x W	**4.9**	1.8	0	**8.6**
N x F	1.7	1.0	**4.3**	0.9
W x F	**7.2**	1.9	n.a	0.0
N x W x F	3.4	1.4	n.a	0.4
$R^2_{marginal}$	0.57	0.55	0.29	0.37
$R^2_{conditional}$	0.70	0.69	0.34	0.50

Table 3.2. GAMM results for log nitrate (NO_3-N, mg L^{-1}) response (July 2014 – August 2015). Significant effects ($p < 0.05$) are highlighted in bold. R^2 adjusted = 0.59

a. Parametric coefficients. Changes on the intercept (end of February) after removing non-significant terms sequentially.

	(Intercept)	Nutrient enriched
estimate	**-3.71**	**0.67**

b. Estimated degrees of freedom (edf) for approximately significant time smooth terms for nutrient treatment and warming treatment.

	Ambient-nutrient	Nutrient enriched	Ambient	Warmed
edf	**6.34**	**7.61**	0.75	**6.35**

Treatment effects on total phytoplankton

The concentration of chlorophyll-*a* showed statistically significant variation over time and with treatments (Table 3.1, Fig. 3.4a), with trends generally following changes in TP (Table 3.1, Fig. 3.3a). Chlorophyll-*a* concentrations increased linearly with time in ambient, nutrient enriched mesocosms while the response in all other treatments showed different time-dependent responses. In warmed mesocosms, the greatest increases in chlorophyll *a* occurred from around March onwards in all treatments except for warmed, unflushed, nutrient enriched mesocosms in which concentrations remained broadly constant from this point. After accounting for the effects of treatment and time, an additional 14% of variance was explained by between-mesocosm differences (conditional $R^2 = 0.72$, Fig. S3.6).

Treatment effects on total cyanobacteria

During the period of sampling (December 2014 – August 2015) the abundance of cyanobacteria generally followed a seasonal pattern observed in many shallow, temperate lakes, with highest values in summer (Fig. 3.4b). However, in nutrient enriched, flushed mesocosms, cyanobacterial dominance and abundance extended beyond the typical season (Fig. 3.4b). In this treatment, on average 55% of winter (December 2014 – February 2015) phytoplankton abundance was accounted for by cyanobacteria, while the average percentage cyanobacteria during the same period in all other treatments was 15% ± 14%.

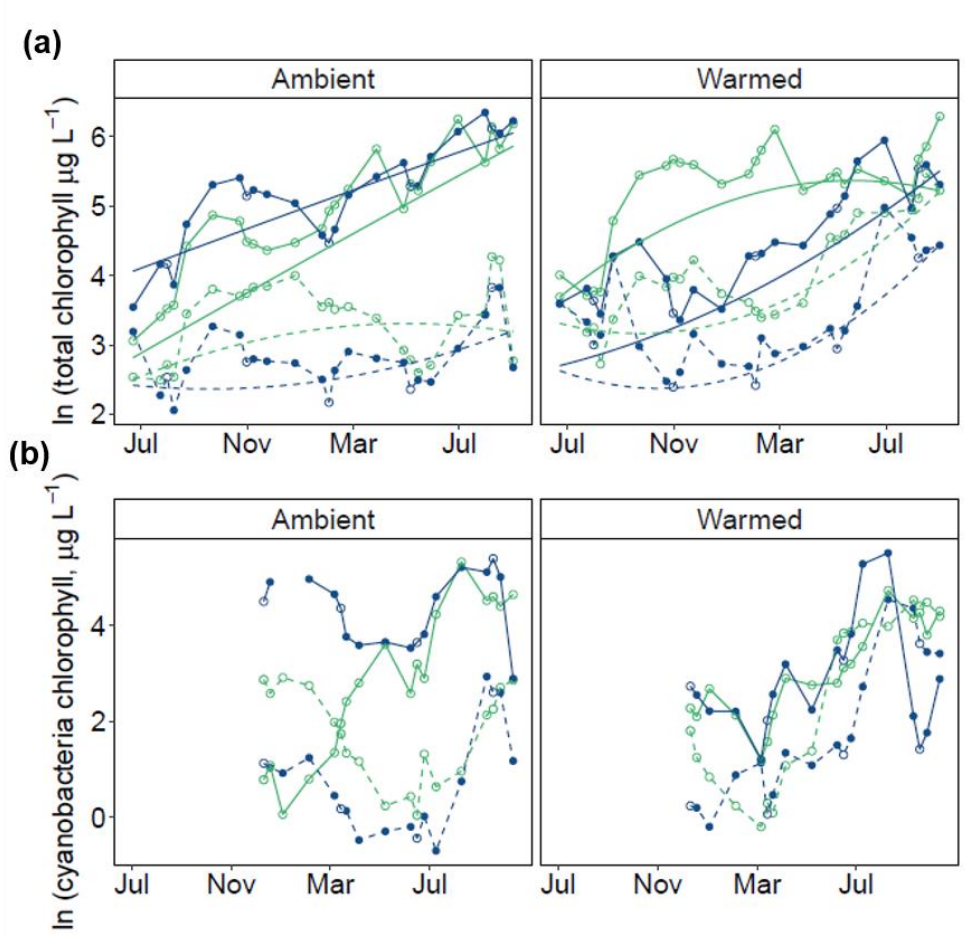


Fig. 3.4 Effect of warming, nutrient addition and extreme rainfall (flushing) events on the concentration of (a) ln total chlorophyll-*a* (µg L^{-1}) and (b) ln cyanobacteria chlorophyll-*a* (µg L^{-1}) over the duration of the experiment. Data points are ln transformed mean responses: blue, flushed; green, unflushed; solid line, nutrient enriched; dashed line, ambient-nutrient; left hand side, ambient treatments; right hand side, warmed treatments. For flushed treatments (blue lines), white data points are sampling events the day immediately after an extreme flushing event. The smooth lines in panel (a) are the fitted response from the best fitting LMM (marginal R^2 = 0.57). In panel (b), cyanobacteria chlorophyll-*a* data is only presented qualitatively as, prior to May, in some treatments (nutrient enriched – flushed, warming –nutrient enriched and warming –nutrient enriched – flushed), replicates varied between n = 0 (missing data point) and n = 4). These data were not missing at random and so the data were not statistically modelled over this period.

The abundance of cyanobacteria, between May 2015 and August 2015, depended on a negative interaction between warming and nutrient enrichment. Warming and nutrient enrichment, as single stressors, resulted in statistically significantly higher cyanobacteria than in ambient mesocosms. However, in combination, these stressors dampened the effect of one another, resulting in a weak antagonism (negative interaction). The size effect of warming and nutrient enrichment as single stressors was similar (Fig. 3.5a, Table 3.3). During the same period, the abundance of total phytoplankton also depended on a negative interaction between warming and nutrient enrichment. In contrast to the response of cyanobacteria, the size effect of nutrient enrichment as a single stressor was greater than the effect of warming as a single stressor (Fig. 3.5b, Table 3.3). The extreme rainfall events – in May and August - had no statistically significant effects on total chlorophyll-*a* or on cyanobacterial chlorophyll-*a*; although, extreme rainfall events did result in a statistically significant reduction in phytoplankton and cyanobacterial abundance (Table S3.2), i.e. short-term effects. After accounting for the effects of treatment, an additional 28% and 14% of variance was explained by between-mesocosm differences for the response of cyanobacterial chlorophyll-*a* and total chlorophyll-*a*, respectively.

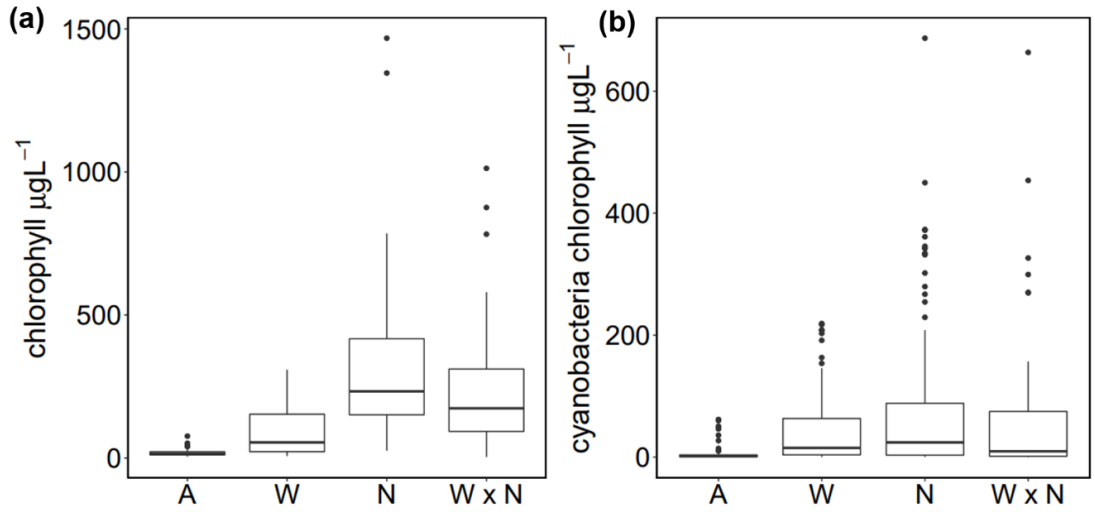


Fig. 3.5 Total chlorophyll-*a* (µg L^{-1}) and cyanobacteria chlorophyll-*a* (µg L^{-1}) between the 5th of May 2015 and 26th of August 2015. Data are plotted by the statistically significant treatment effects from the best fitting LMM for each response (Table 3). A, ambient (without warming or nutrient enrichment); W, warming only; N, nutrient enrichment only and WN, warming and nutrient enrichment together. The lower and upper hinges correspond to the 25th and 75th percentiles, the whiskers extend to 1.5x the interquartile range.

Table 3.3. LMM coefficients (± standard error) for ln total chlorophyll-*a* (μg L^{-1}) and ln cyanobacteria chlorophyll-*a* (μg L^{-1}) relationship to treatments between May and August 2015. Significant effects ($p < 0.05$) are highlighted in bold. The variance explained by each model is given by marginal R^2m for the fixed effects only and conditional R^2c for the fixed and random effects.

	(Intercept)	N	W	N x W	R^2m	R^2c
ln total chlorophyll-*a* (μg L^{-1})	**3.0 ± 0.2**	**2.6 ± 0.3**	**1.1 ± 0.3**	**-1.6 ± 0.4**	0.53	0.67
ln cyanobacteria chlorophyll-*a* (μg L^{-1})	0.2 ± 0.6	**2.5 ± 0.8**	**2.5 ± 0.8**	**-3.4 ± 1.1**	0.15	0.43

Treatment effects on the composition of cyanobacteria

Cyanobacterial biovolume was mainly composed of nitrogen-fixing cyanobacteria (68%), in particular *Aphanizomenon* spp. (51%) but also *Dolichospermum* spp. (17%). Other notable contributions to cyanobacterial composition were from *Microcystis* spp. (13%) and *Pseudanabaena* sp. (13%), Fig. S3.7, Table S3.3.

At the genus level, the occurrence and abundance of the dominant genera - *Aphanizomenon* spp., *Dolichospermum* spp., *Microcystis* spp. and *Pseudanabaena* spp. (Table 3.4) - were explained by single stressor effects only; no statistically significant interactive effects of stressors were detected. *Aphanizomenon* spp. was fairly ubiquitous, although its abundance was statistically significantly higher in nutrient enriched mesocosms (Table 3.4, Fig. 3.6b). *Dolichospermum* spp. occurrence was statistically significantly higher in nutrient enriched mesocosms and in samples taken later in the summer (July and August) while biovolume was statistically significantly higher in warmed mesocosms (Table 3.4, Fig. 3.6c). *Microcystis* spp. occurrence and biovolume was strikingly related to warming: 94% of occurrences were in warmed mesocosms, although overall this genus was only present in 25% of the samples. The occurrence of *Microcystis* spp. also depended on the time of the year, with statistically significantly higher occurrence during July and August compared to May (Table 3.4, Fig. 3.6d). The occurrence and abundance of *Pseudanabaena* spp. was positively explained by nutrient enrichment (Table 3.4, Fig. 3.6a).

At a higher taxonomic grouping, statistically significant treatment interactions were detected for biovolume of the group Nostocales (*Aphanizomenon* spp. and *Dolichospermum* spp.). The response at this higher grouping reflects the results obtained at the cyanobacterial community level (cyanobacterial chlorophyll-*a*) with positive effects of nutrient enrichment

and warming alone and a negative interaction together. Most *Aphanizomenon* spp. and *Dolichospermum* spp. filaments contained specialised heterocyte cells that are involved in the fixation of nitrogen (Fig. S3.7).

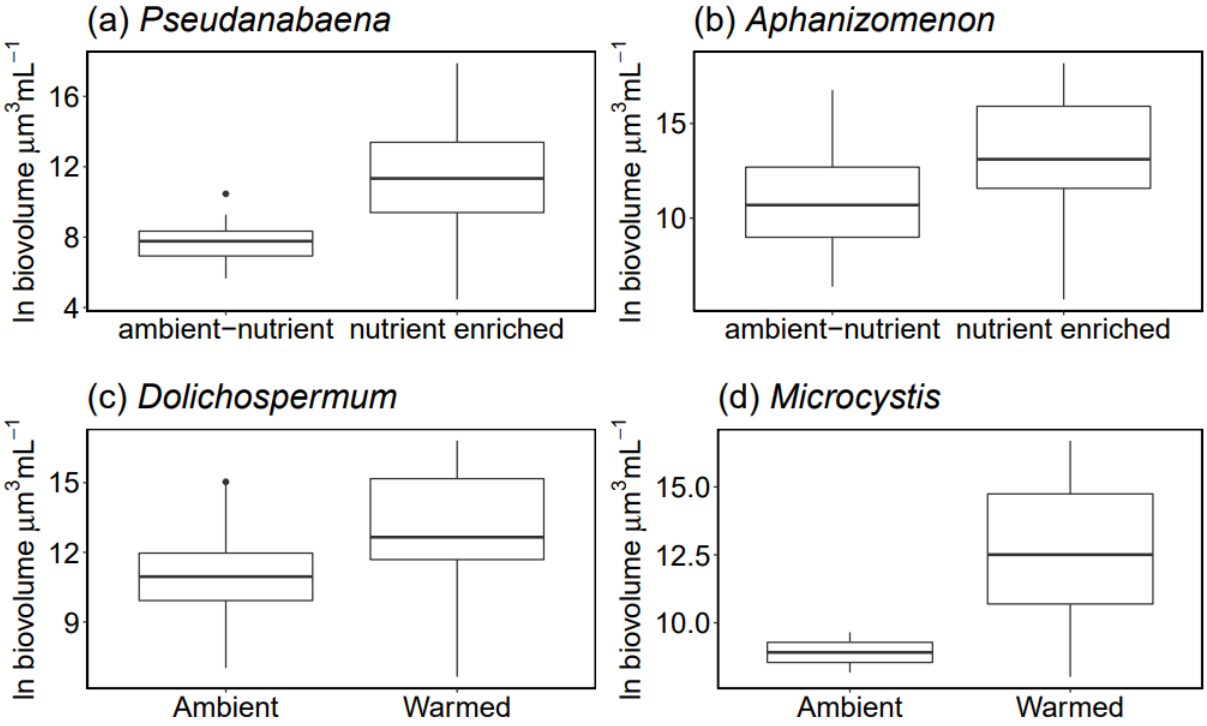


Fig. 3.6 Natural log biovolume ($\mu m^3 mL^{-1}$) of the dominant genera of cyanobacteria observed in May, June, July and August 2015, plotted by the statistically significant treatment effects from the best fitting LMM for each genus. The lower and upper hinges correspond to the 25th and 75th percentiles, the whiskers extend to 1.5x the interquartile range.

Table 3.4 Summary (coefficient and standard error) of best fit GLMMs explaining the probability of the presence of dominant cyanobacteria taxa (present/absent) and LMMs explaining taxa biovolume (natural log $mm^3 L^{-1}$), when present – i.e. non-zero data. The variance explained by each model is given by marginal R^2m for the fixed effects only and conditional R^2c for the fixed and random effects. Date is a factor and relates to the sampling date (n = 4): 6th of May, 3rd of June, 29th of July and 26th of August 2015. In models where date is significant, the intercept relates to the 6th of May and all other levels of date are compared to data from this date. Significant effects ($p < 0.05$) are highlighted in bold, blanks signify that these terms did not significantly improve the model.

Taxa	Intercept	Warming	Nutrient enrichment	Warming x Nutrient enrichment	Date			R^2m	R^2c
					3rd June	29th July	26th August		
Nostocales									
presence	**1.50 ± 0.32**							0.00	0.16
biovolume	**11.10 ± 0.57**	**2.15 ± 0.76**	**2.59 ± 0.79**	**-2.42 ± 1.12**				0.13	0.18
Aphanizomenon									
presence	**0.62 ± 0.27**							0.00	0.22
biovolume	**10.89 ± 0.52**		**2.30 ± 0.72**					0.15	0.30
Dolichospermum									
presence	-1.1 ± 0.66		**-2.41 ± 0.78**		**1.43 ± 0.73**	**3.05 ± 0.83**	**2.43 ± 0.83**	0.36	0.58
biovolume	**11.10 ± 0.56**	**1.72 ± 0.75**						0.11	0.29
Other genera									
Microcystis									
presence	**-9.57 ± 2.72**	**7.27 ± 2.33**			0.57 ± 1.09	**2.53 ± 1.17**	**4.94 ± 1.63**	0.36	0.68
biovolume	12.17 ± 0.63							0.00	0.58
Pseudanabaena									
presence	**-1.37 ± 0.31**		**1.49 ± 0.40**					0.15	0.15
biovolume	**7.77 ± 0.78**		**3.45 ± 0.95**					0.26	0.51

3.5 Discussion

Climate change is often considered as a single stressor on the natural environment. In reality, this masks a great deal of complexity with changes in the timing and extremity of weather events often ignored in favour of responses to general climate trends that are more straightforward to analyse. Experimental mesocosm studies offer an approach to investigate this complexity in order to develop a clearer mechanistic understanding of the interactions between multiple stressors, allowing quantification and comparison of individual stressor effects and their interactions (Crain *et al*., 2008; Feuchtmayr *et al*., 2009; Piggott *et al*., 2015).

The effects of warming on cyanobacteria

a. Nutrient enrichment

In ambient-nutrient mesocosms, as expected, warming increased the abundance of cyanobacteria and phytoplankton. The overall increase in phytoplankton abundance in these mesocosms can be explained by both direct effects of temperature on growth rates (Reynolds 2006) and indirect effects of temperature on the release of phosphorus from the sediment (Jensen & Andersen, 1992). The latter process, at least, seemed to be important from late spring into the summer in warmed mesocosms (Fig. 3.3a). The direct effects of temperature on growth rates was particularly important for the abundance of cyanobacteria, for which we observed statistically significant increases in common bloom-forming taxa, *Microcystis* spp. and *Dolichospermum* spp. - the maximum growth rates of these genera are generally reached at higher temperatures compared to other cyanobacteria and phytoplankton (e.g. Lürling *et al*., 2017). This result supports the expectation that changes in water temperature will drive shifts in phytoplankton composition, with higher temperatures not only favouring cyanobacteria in general but, in particular, those genera that commonly form dense blooms in freshwaters and are known toxin producers (De Senerpont Domis *et al*., 2007; Jöhnk *et al*., 2008). It should be emphasised that these effects of warming were observed in nutrient rich systems. As has been discussed by others (Elliott, 2012; Lürling *et al*., 2017), the stimulatory effect of temperature depends on the carrying capacity of the system, thus warming in sites with low nutrient availability has less potential to increase biomass.

Unexpectedly, we found that warming in combination with high nutrient enrichment can reduce the abundance of cyanobacteria. This result is striking because it contrasts with the widely hypothesised (Paerl & Huisman, 2008) and observed (Taranu *et al*., 2012; Rigosi *et al*., 2014; Lürling *et al*., 2017) synergistic interaction between warming and nutrient enrichment

on cyanobacterial abundance. However, although we detected a statistically significant negative interaction, it is important to point out that the effect size was small - cyanobacterial and total phytoplankton abundance were still generally higher than the ambient (control) mesocosms (Fig. 3.5) and still led to chlorophyll concentrations that exceeded WHO (World Health Organisation) threshold guidelines for drinking and bathing waters (Chorus & Bartram, 1999). The mechanism for the antagonism that we observed is unclear but is probably linked to the very high productivity of the mesocosms. An antagonism between warming and nutrient enrichment was also detected for total phytoplankton, indicating that another factor(s) was limiting phytoplankton growth in general, not only cyanobacteria. Under these conditions, and in contrast to all other treatments, SRP was plentiful indicating that phosphorus limitation was not responsible for this response. Nitrogen and light limitation are also excluded as mechanisms since nitrate concentrations were similar (and low) in all treatments during the summer and light attenuation was no higher in warmed, high nutrient addition mesocosms than in high nutrient addition mesocosms (supplementary material, light attenuation analysis). A plausible explanation could be inorganic depletion of carbon that can lead to carbon limitation (Jansson *et al*., 2012), which has been shown to occur under nutrient enriched conditions (Maberly, 1996) and which may be exacerbated by warming (Yvon-Durocher *et al*., 2017). Unfortunately, available carbon was not measured, nor could it be estimated from the available data, and so this explanation cannot be tested.

b. Composition

The antagonistic effects of warming and nutrient enrichment was only detected at the community level (total cyanobacteria). At the genus level, no statistically significant treatment interactions were found, rather warming resulted in the increased abundance of *Dolichospermum* spp. and *Microcystis* spp. and nutrient enrichment resulted in the increased abundance of *Aphanizomenon* spp. and *Pseudanabaena* spp. Differences in the sensitivity of genera to anthropogenic stressors have been found before (Ekvall *et al*., 2013; Rigosi *et al*., 2014), and should be expected as cyanobacteria are a diverse group with a wide range of eco-physiological characteristics that will lead to varying responses (Carey *et al*., 2012; Reynolds *et al*., 2002). Differences in community and population level responses (for a variety of biological responses) to multiple stressors have also been found by others, as reviewed in Crain *et al* (2008) and Côté *et al* (2016).

The effects of extreme rainfall events on cyanobacteria

The effects of extreme rainfall events as a 'climate change stressor' on phytoplankton biomass and community composition has received relatively little attention compared to the effects of climate warming. Of these studies, most discuss the effects of enhanced nutrient loading (e.g. Paerl & Huisman, 2008) and terrestrial inputs such as an increase in water colour (e.g. Graham & Vinebrooke, 2009; Urrutia-Cordero *et al*., 2017) while the effect of losses of biomass from the outflow during flow events has been understudied. Two aspects of recovery of biomass from high flow events need to be considered: (a) the recovery of total phytoplankton abundance and (b) the recovery of community composition.

It is expected that the general recovery of phytoplankton biomass after a large flow event will depend on the factors that limit growth; these may be influenced naturally by season, such as light and temperature or influenced by anthropogenic pressures on the system, such as land use (nutrient loading) and climate change e.g. warming. In the spring and summer we found that extreme rainfall events only had short-term effects on phytoplankton abundance in all treatments while in the autumn and winter the effects of flushing were more prolonged. It is likely that the conditions for growth (nutrients, light and temperature) during the spring and summer were suitable in all treatments to allow for rapid recovery while seasonally limiting factors such as light and temperature would have slowed down the recovery outside of the main growing season.

The effect of flushing events on community composition is likely to be more influenced by the timing, intensity and frequency of the event (e.g. Padisák *et al*., 1999). Other studies have shown that in highly flushed lakes the seasonal succession of phytoplankton is suppressed (Brook & Woodward, 1956; Margalef, 1978), this is because taxa, such as cyanobacteria, are slow-growing and thus more sensitive to flushing (Reynolds, Huszar et al. 2002). However, we found no evidence that cyanobacteria, as a broad group or at the level of individual genera, were supressed in flushed mesocosms; this may be explained by the frequency of the events. The Intermediate Disturbance Hypothesis (Connell 1978) suggests that the frequency of disturbance influences the diversity of the phytoplankton community, with intermediate disturbances resulting in the highest diversity and infrequent disturbance resulting in competitive exclusion. In lakes, this competitive exclusion in stable summer conditions is often produced by taxa such as cyanobacteria that may lose their competitive advantage as flushing rate increases (Carvalho et al., 2011). For example, Padisák *et al*., (1999) found that

Aphanizomenon blooms were supressed when flushing rates were increased to a frequency of every 20 – 30 days and Wood *et al.*, (2017) found that multiple heavy rainfall events weakened the ability of cyanobacteria to recover from further events. The flushing events were likely too infrequent in the mesocosms for the competitive exclusion of cyanobacteria.

Many studies relating to the effects of flushing on phytoplankton have focused on the response during the growing season, as this is when it is expected that the effects will be greatest. Unexpectedly, we found that a flushing event increased cyanobacterial abundance and dominance in nutrient enriched mesocosms during the winter months. Although uncommon, winter blooms of cyanobacteria can occur naturally, comprising taxa such as *Planktothrix rubescens* (Naselli-Flores *et al*., 2007) and *Aphanizomenon* spp. (Reynolds *et al*., 2002) which are efficient at harvesting light under limiting conditions. This striking response was observed consistently among the replicates suggesting that the conditions within these mesocosms strongly favoured the dominance of cyanobacteria. The high abundance of winter cyanobacteria did not occur in unflushed, nutrient enriched mesocosms, indicating that short flushing events were key to this response. Overall these results suggest that changes in the phytoplankton community in response to flushing may depend on seasonal timing as well as the frequency of the event(s).

Management implications

Our results suggest that under future climate and nutrient scenarios, nutrients may need to be substantially reduced in shallow lakes in order to: (a) mitigate against the indirect effects of warming through enhanced nutrient cycling, especially in previously impacted lakes, and (b) mitigate against the direct effects of enhanced growth rates of common bloom-forming species of cyanobacteria, that are widely recognised for their potential to produce harmful toxins (Codd *et al*., 2005).

It should be stressed that the combined effects of warming, nutrient enrichment and extreme rainfall events on cyanobacterial abundance are only relevant to these conditions i.e. both the lake environment - shallow, low humic lakes -and the stressor gradients tested. Thus, these results should be applied with care. For example, several studies have shown that the relationship between chlorophyll-*a* (McCauley *et al*., 1989) and cyanobacteria (Carvalho *et al*., 2013) to TP concentrations, plateaus at high concentrations because of other limiting factors. This non-linear response means that the form of stressor interactions are likely to change along the nutrient gradient (Piggott *et al*., 2015; Rigosi *et al*., 2014). Likewise, while we found that

short-lived, one-off extreme events are unlikely to reduce algal blooms, this may not be the case if the frequency or intensity of the flushing event is greater. There may also be an important interaction between flushing and stratification that would not be captured in these well mixed mescoms. Furthermore, concomitant changes in the environment as a result of high rainfall such as an increase in turbidity/colour (e.g. Urrutia-Cordero *et al*., 2017) and an increase in nutrient loading, especially N (e.g. Sinha *et al*., 2017; Wood et al., 2017) may further complicate this response. The effects of warming may also differ between shallow and deep lakes (Richardson *et al*., 2018). In shallow lakes, as observed in our shallow mesocosms, warming may benefit cyanobacteria through enhanced internal loading of P (Dolman *et al*., 2016; Søndergaard *et al*., 2017) and potential increased benefits for N-fixers caused by increased denitrification rates (Veraart *et al*., 2011) while in deeper lakes, the benefits may emerge because of increased stability in the physical structure of the lake (Taranu *et al.*, 2012). Trophic interactions such as the effects of macrophytes in shallow lakes may also alter the effects of warming (Feuchtmayr *et al*., 2009; McKee *et al*., 2003 and Moss *et al*., 2003). Our study builds a foundation for understanding the complexity of how global climate change may impact on freshwater resources. It highlights the clear need to mitigate against global warming, but indicates that ecological surprises may occur depending on the lake characteristics and landscape context (low or high nutrient loading).

Chapter 4.

PROTECH modelling study: a process based model study of the effects of the timing and magnitude of one-off flow events under different nutrient loading and climate scenarios in a modelled stratified lake.

1.1 Abstract

There is great interest in the impact of climate change on the development and duration of harmful cyanobacterial blooms. This has mostly focused on global warming as a '*press*' disturbance (trends), however, associated with climate change are '*pulse*' disturbances such as increases in extreme rainfall. Pulse events are by definition short-lived, making their impacts difficult to assess using routine monitoring data. Here, a phytoplankton community model, Phytoplankton RespOnses To Environmental Change (PROTECH), was used to explore the impact and recovery of cyanobacteria from one-off extreme flow events of different magnitude and timing. The model was used to examine the responses of a typical deep, stratified lake in four different climate zones (Boreal, Continental, Atlantic and Mediterranean) and three nutrient load scenarios (baseline, +50% and -50% baseline nitrogen and phosphorus inflow concentrations), resulting in 64,800 model runs. As expected, the major effect of high flow events was the loss of phytoplankton biomass and this increased with flow velocity and the productivity of the system at the time of the event. Recovery of total and cyanobacterial chlorophyll-*a* from hydraulic loss depended on: (a) the magnitude of the event- the more biomass lost, the longer the recovery time; (b) the timing of the event– recovery took longer in winter when growth rates were limited by environmental conditions and (c) the climate region – higher latitude regions were least resilient to disturbance during the winter, when day length was short. Recovery time did not depend on nutrient load since although biomass loss was greater at high nutrient loading, high nutrient availability allowed more rapid recovery. There were no compositional differences in recovery, suggesting no major compositional shifts as a result of perturbation. This study suggests that the response to single events (in clear water systems), even if extreme, are generally short-lived.

1.2 Introduction

Harmful algal blooms (HABs) of cyanobacteria are a global water quality problem driven by widespread nutrient enrichment of fresh waters. There has been increasing interest in the role of climate change in the development and duration of blooms because of their potential to produce potent toxins that can severely affect the health of people and animals (Codd *et al*., 2005; WHO, 2011). This interest has focused principally on the potential stimulatory effects of increasing lake water temperature on cyanobacteria (Paerl & Huisman, 2008; O'Neil *et al*., 2012) especially those impacted by nutrient enrichment (Moss *et al*., 2011). While there is evidence for the effects of warming on cyanobacteria (Elliott *et al*., 2006; Jöhnk *et al*., 2008; Kosten *et al*., 2012), in reality climate change is manifested as dynamic changes in the environment, incorporating press and pulse disturbances, such as heatwaves and extreme rainfall events (Lakes, 2000). These short-lived disturbances can impact phytoplankton communities (James *et al*., 2008) which, depending on the characteristics of the event, could increase (Paerl & Huisman, 2008; Markensten *et al*., 2010) or decrease the impacts of HABs (Padisák *et al*., 1999; James *et al*., 2008). Despite recognition of the potential impact of these events on phytoplankton dynamics, extreme climatic events have been poorly studied, in favour of responses to annual or seasonal 'average' climate trends that are more straightforward to analyse using available monitoring data (e.g. Kosten *et al*., 2012; Taranu *et al*., 2012; Rigosi *et al*., 2014). Inclusion of short-term pulse events as well as longer-term press or ramp climate effects is needed for our ability to forecast cyanobacterial blooms under future climates/conditions (Jentsch *et al*., 2007; Michalak, 2016).

Extreme rainfall events are increasingly being observed (Lehmann *et al*., 2015) and are predicted to increase in the future (IPCC, 2013), in particular during the summer months at mid- to high-latitudes (Christensen & Christensen, 2003). These events result in large and sudden changes in the physico-chemical environment of lakes (Sadro & Melack, 2012) which can have large impacts on phytoplankton communities. Firstly, increased hydraulic flushing impacts individuals and populations through loss to the outflow (Dickman, 1969; Sadro & Melack, 2012). Secondly, changes to the environment along gradients of light availability, nutrient availability and mixing/turbulence can result in shifts in phytoplankton community composition and diversity (Padisák, 1988; Padisák, 1993; Bailey-Watts *et al.*, 1990), driven by differences in morphology and functional traits among phytoplankton (Reynolds *et al*., 2002). So, while some groups may be resilient to change or flourish under the new conditions, others will be excluded. Cyanobacterial blooms may be particularly sensitive to flushing events as

most bloom forming genera are sensitive to turbulence, are slow growing and have functional traits suited to stable water columns (Sherman *et al*., 1988; Reynolds *et al*., 2002; Hudnell *et al*., 2010; Cross *et al*., 2014).

The extent of the impact of extreme events on phytoplankton assemblages will depend on many aspects relating to the event itself such as the timing (Verspagen *et al*., 2006; Padisák, 1993; Padisák *et al*., 1999; Elliot, 2010), intensity (Harris & Baxter, 1996; Oh and Kim, 1995; Ahn *et al*., 2002) and frequency (Padisák, 1988) but also on the characteristics of the lake, catchment (Reichtwaldt & Ghadouani, 2012) and other factors such as climate and nutrient source (Elliott & Jones, 2009). This interconnectedness makes studies of single extreme events very system specific and while the response measured gives insight into some of the effects of extreme rainfall events, the biological outcome may be just one of many. An improved understanding of how extreme climatic events will impact cyanobacterial abundance can be achieved through (a) examining the effects of different events in the same lake and (b) examining the effects of one event given different lake- and catchment- characteristics, spatial variables and antecedent weather. Experimental studies can offer an approach to explore some of this complexity (e.g. Bækkelie *et al*., 2017; Zingel *et al*., 2018; Richardson *et al*., in review). In particular, computer modelling is a useful tool as the effects of many factors can be modelled separately and in combination, giving the scope to increase the complexity of experimental design (e.g. Elliott & Jones, 2009). In this study, the phytoplankton community model PROTECH (Phytoplankton RespOnses to Environmental Change) was used to explore the sensitivity of cyanobacteria to one-off hydraulic flow events in a typical stratified lake. The focus was on how event attributes, specifically the timing and intensity may affect the impact and recovery from perturbation and how this may be shaped by other environmental factors, specifically nutrient loading, weather (temperature and wind) and the location of the lake.

1.3 Methods

Overview of the experiment

A fully factorial modelling experiment was performed, combining one hundred and eighty different timings of the event (day of the year), six different magnitudes of the event, four climate zones, five climate models and three nutrient scenarios (Table 4.1). This resulted in 64,800 model runs. For each model run, ten different genera of phytoplankton (Table S4.2), with different growth rates, morphologies and traits, competed against each other over 365 days in a typical stratified lake (maximum depth of 26 meters) with an average retention time equivalent to 240 days. On one day of the year, the flushing was increased to simulate a hydraulic flow event (Table 4.1: retention times from 2.5 to 80 days). The response to the flow event was measured as: (a) the difference in total chlorophyll-*a* and cyanobacterial chlorophyll-*a* between the experimental and control run on the day of the event; (b) the number of days to recover from any changes in biomass and (c) the rate of recovery.

Table 4.1. Experimental factors and levels. Flow rate is given as the equivalent retention time in days of the lake.

Factor	Levels	Description
Timing of event (day of year)	180	Moving window of 2 days
Flow intensity (retention time in days)	5	2.5, 5, 10, 20, 40, 80
Climate zone	4	Boreal, Continental, Atlantic, Mediterranean
Climate models	5	GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM and NorESM1-M.
Nutrient scenarios (N and P)	3	Baseline, +50%, -50%

PROTECH

PROTECH simulates the response of phytoplankton in a 1D vertical water column at daily time steps. The daily change in the chlorophyll *a* concentration ($\Delta X/\Delta t$) of each phytoplankton taxon depends on the growth rate (r', this is a proportional increase over 24 hours), the loss due to sinking (*S*), the loss due to grazing by *Daphnia* (*G*, it is assumed any phytoplankton >50 μm diameter are not grazed - Table S4.1) and hydraulic loss (*D*, dilution):

$$\Delta X/\Delta t = (r' - S - G - D)\, X \tag{4.1}$$

The growth rate (r') is further defined by:

$$r' = \min \{r'_{(\theta,I)}, r'_{P}, r'_{N}, r'_{Si}\} \tag{4.2}$$

where $r'_{(\theta,I)}$ is the growth rate at a given temperature and daily photoperiod and r'_P, r'_N, r'_{Si} are the growth rates determined by phosphorus, nitrogen and silicon concentrations below these respective threshold concentrations: < 3, 80 and 500 mg m^{-3} (Reynolds, 2006). The *r'* values depend on phytoplankton morphology but also other taxon specific traits. For example, non-diatom taxa are not limitied by silica concentrations below 500 mg m^3 and nitrogen-fixing cyanobacteria are not limited by nitrogen. Nutrient loading remains constant for each daily time-step (expect for on the day of the event when nutrient loading increases) but availability varies with biological demand and mixed depth. Temperature and light change at each time-step throughut the simulated water column and depend on air temperature (modelled climate data) and the day of the year and latitude, respectively.

The value of change in chlorophyll-*a*, $\Delta X/\Delta t$ (Equation 4.1), is modified on a daily time-step for each algal taxon in each 0.1m layer of the water column. The top 5 meters of the water column is sampled on each day. For a full description of the model's equations and concepts see Reynolds et al (2001) and Elliott et al (2010).

Driving data

The hypothetical stratified lake

Data from a set of European lakes (Moe *et al*., 2013) was used to specify the dimension of the hypothetical lake. Median lake area, 1.67 km^2, and median annual retention time, 240 days, of 912 and 712 lakes, respectively, was used for the area and 712 lakes for the retention time of the experimental lake. The median maximum depth, 26 meters, of 379 lakes classified as stratified was used for the depth of the lake. A cone shaped bathymetry was assumed from which the volume of the lake, 3,620,000 m^3, was calculated. The volume was used to calculate the required inflow and outflow rate, 0.174 $m^3\ s^{-1}$, to achieve a retention time of 240 days (Supplementary Box 1).

Phytoplankton

Ten phytoplankton were selected for the simulations: *Chlorella, Staurastrum, Asterionella, Cyclotella, Plagioselmis, Cryptomonas, Ceratium, Planktothrix, Dolichospermum (*previously named *Anabaena*) *and Dinobryon.* These were selected to represent the main taxonomic classes and a range of functional groups (Reynolds *et al*., 2002) in the community (Table S4.1). Each taxonomic class was restricted to two genera to avoid bias. Two common bloom forming

filamentous cyanobacteria were selected that are known to perform well in the model: *Planktothrix* which is tolerant of low light and *Dolichospermum* which can regulate buoyancy and fix atmospheric nitrogen (Reynolds *et al*., 2002). In the model, neither genera are grazed. Validation simulations were run to check that changes in the community over the year were realistic for a stratified lakes, specifically characterised by two peaks of biomass, one in the spring and one in the autumn, with a spring bloom of diatoms (Sommer *et al*., 1986; Sommer *et al*., 2012).

Nutrients

Inflow nutrient loads – N (nitrogen) and P (phosphorus) - were calibrated to create baseline conditions in which modelled annual mean chlorophyll-*a* and modelled proportion cyanobacteria were validated against observed data which were sourced from the WISER data set (Moe *et al.*, 2013). WISER lakes that were classified as stratified were assigned to each climate zone based on the grid reference of the lake sampling point.

Average winter (December – February) total nitrogen, 660 µg L^{-1}, and total phosphorus, 16 µg L^{-1}, from 262 and 265 Boreal stratified lakes, respectively, was used as the starting point for calibration. Incremental multipication factors, from 1.10 to 1.40 at 0.05 intervals, was applied to these starting concentrations of N and P and the model was run for 365 days for each climate zone. The nutrient load which minimised the sum of the combined square root differences between modelled and observed chlorophyll-*a* and proportion cyanobacteria across all climate zones was selected (Supplementary material, Table S4.2, Fig S4.1). The same driving nutrients were used for all climate zones as chlorophyll-*a* and proportion cyanobacteria were similar among these zones (Table S4.3).

Simulations were run for three different nutrient scenarios: baseline nutrient load, low nutrient load (-50% of the baseline concentrations) and high nutrient load (+50% of the baseline concenrations). As all nutrients loads were diffuse (non-point source), the load increased with increasing flow rates.

Climate

Temperature and wind data were obtained from an ensemble of five present day (2006-2015) RCP 4.5 climate models: GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM and NorESM1-M. Multiple models were used for a measure of uncertainty. These data were then summarised as daily means from four broad climate zones - Boreal, Atlantic,

Continental and Mediterranean - which were selected to represent the majority of Europe in terms of latitudinal and longitudinal gradients of weather and seasons i.e. photoperiod (Fig. 4.1, Table S4.4). Delineations of the climate zones were obtained from the European Environment Agency biogeographical regions (2015, https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3). The centroid of the climate zone polygon (ArcGIS method) was used as the representative latitude for the zone (Fig. 4.1) which was used in the model to estimate daily changes in solar radiation.

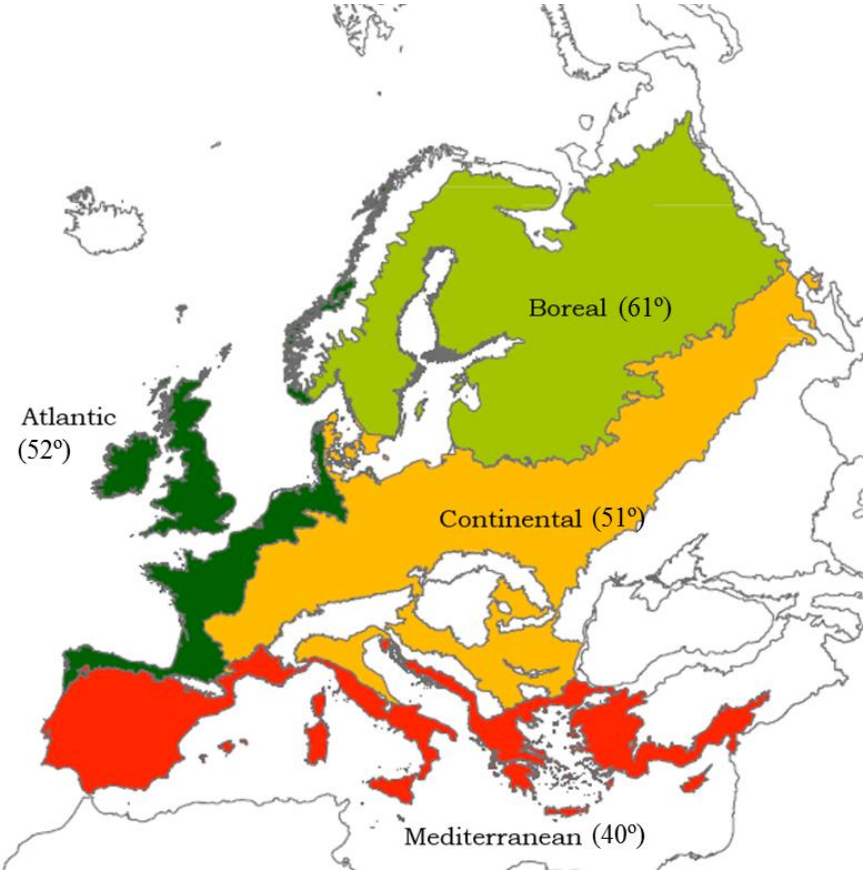


Fig. 4.1 Map of 'climate zones'. Delineations are from the European Environment Agency biogeographical regions (2015, https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3). The latitudinal centroid of each zone is given in parentheses.

Simulations

i. Control simulations

For each nutrient scenario (baseline, low and high) the model was run twenty times, one run for each combination of climate zone (n = 4) and climate model (n = 5). In the control simulations the daily flow rate was constant, at 0.174 $m^3 s^{-1}$, equivalent to a retention time of 240 days.

ii. Extreme event simulations

In the experimental simulations, the daily flow rate was also 0.174 m^3 s^{-1} (equivalent to a retention time of 240 days) with exception of one day in the year when the rate of the inflow and outflow increased. Different magnitudes of flow were applied which were equivalent to reducing the retention time to: 2.5, 5, 10, 20, 40 and 80 days with 2.5 days being the most extreme event and 80 days being the least extreme (Table S4.5). Only one event occurred per year, the timing of which changed on a two day moving window starting from the 2nd of January and finishing on the 26th of December. Model runs were executed to cover all combinations of the experimental factors listed in Table 4.1, to give a total of 64,800 simulations.

Responses measured

i. Immediate effects of the flushing event

The effect of flushing on chlorophyll–*a* (µg L^{-1}) and cyanobacterial chlorophyll-*a* (µg L^{-1}) on the day of the event was calculated by taking the difference between biological responses in the control and the experimental run.

ii. Number of days for cyanobacteria to recover

The difference between cyanobacterial chlorophyll-*a* in the extreme event run and the control run was calculated for each consecutive day after the event until the difference was equal to, or greater than zero – the 'recovery day'. The day of the flow event was subtracted from this 'recovery day' to give the number of days it took for chlorophyll-*a* and cyanobacteria chlorophyll-*a* to recover.

iii. Rate of recovery

The rate of recovery, chlorophyll-*a* (µg L^{-1} day^{-1}), was calculated using the growth rate equation (4.3):

$$\frac{\log(chlorophyll_{day\ of\ recovery}) - \log(chlorophyll_{day\ of\ event})}{\text{number of days to recover}} \qquad (4.3)$$

As changes in chlorophyll-*a* and cyanobacteria chlorophyll-*a* over time in the control and experimental simulations were not linear and were not always positive; the difference in the

growth rate between the experimental and control recovery period was calculated to give the difference in the rate of recovery. This ensured that the growth rate was always positive.

Statistical analysis

All analyses was performed in R version 3.2.2, R Core Team (2017).

Validation and control simulations

In the validation/calibration step, differences in modelled and observed total chlorophyll-*a* and proportion cyanobacteria were tested using an independent t-test under standard assumptions. In the control simulations, differences in total chlorophyll µg L^{-1} and cyanobacteria chlorophyll µg L^{-1} among nutrient scenarios were tested using a linear model, with the difference as the response and nutrient scenario as a factor. Temporal trends of chlorophyll-*a* and cyanobacteria chlorophyll-*a* in control simulations were plotted as the average and standard deviation from each biogeographic zone – nutrient scenario combination (average of the five climate models).

Flushing simulations

For the analysis of the biological response to extreme events – change on the day of the event, number of days to recover and the recovery rate – the day of the event was treated as a continuous variable while nutrient scenario, flow magnitude and biogeographic zone were retained as factors. The shape of these responses over time was first explored using the ggplot loess smoother function in R. Linear models were used for responses in which interactions among temporal trends and multiple factors were to be retained: (a) change of biomass on the day of the event and (b) the number of days to recover. An additive model was used for the rate of recovery as exploratory analysis showed that higher order interactions were not important. Models were simplified by removing non-significant higher order interaction terms in turn. Simplified models were compared with more complex models using AIC and favoured, when retaining more complex terms did not significantly improve the model.

i. Change in biomass on the day of the event

To account for temporal auto-correlation the response was modelled at a bi-monthly resolution using the following model:

$$Y = \beta_0 + \beta_1 X_{dayofevent} * \beta_2 X^2{}_{dayofevent} * \beta_3 X^3{}_{dayofevent} * \beta_4 X^4{}_{dayofevent} * \delta_{FlushingRate} * \zeta_{ClimateZone} * \eta_{NutrientScenario} + \varepsilon,\ \varepsilon \sim (0, \sigma_r{}^2) \quad (4.4)$$

where Y is the response of interest: change in chlorophyll-*a* on the day of the event; change in cyanobacteria chlorophyll-*a* on the day of the event. β_0 is the intercept term $\beta_{1\text{-}4}$ are model parameters for the response to events on different days of the year (fourth order polynomial), $\delta_{FlushingRate}$, $\zeta_{ClimateZone}$ and $\eta_{NutrientScenario}$ are the model parameters for each factor (Table 4.1 for factor levels). ε is the overall error term, with a mean of zero and unknown variance.

ii. The number of days to recover

To account for temporal auto-correlation the response was modelled at a bi-monthly resolution using the following model:

$$Y = \beta_0 + \beta_1 X_{dayofevent} * \beta_2 X^2{}_{dayofevent} * \delta_{FlushingRate} * \zeta_{ClimateZone} * \eta_{NutrientScenario} + \varepsilon, \quad \varepsilon \sim (0, \sigma_r{}^2) \tag{4.5}$$

where Y is the response of interest: number of days for chlorophyll-*a* to recover and number of days for cyanobacteria chlorophyll-*a* to recover. β_0 is the intercept term $\beta_{1\text{-}2}$ are model parameters for the response to events on different days of the year (second order polynomial), $\delta_{FlushingRate}$, $\zeta_{ClimateZone}$ and $\eta_{NutrientScenario}$ are the model parameters for each factor (Table 4.1 for factor levels). ε is the overall error term, with a mean of zero and unknown variance.

iii. Recovery rate

The rate of recovery was modelled using the following additive model:

$$Y = \beta_0 + s(dayofEvent) + \delta_{FlushingRate} \quad \varepsilon, \quad \varepsilon \sim (0, \sigma_r{}^2) \tag{4.6}$$

where Y is the response of interest, *s*(dayofEvent) is the smoothed term for the change in the response over time and $\delta_{FlushingRate}$ is the model parameters for flushing rate (Table 4.1 for factor levels). ε is the overall error term, with a mean of zero and unknown variance. Climate zone and nutrient scenario were excluded for parsimony as retaining them did not visibly alter temporal trends.

1.4 Results

Validation (calibrated baseline nutrient scenario)

Nutrient concentrations in the baseline scenario were calibrated so that modelled (9.9 µg L^{-1}) and observed (10 µg L^{-1}) annual mean chlorophyll-*a* and modelled (0.21) and observed (0.20) annual mean proportion cyanobacteria were not statistically significantly different (t = 0.12, df = 6, p-value = 0.92 and t = -0.45, df = 6, p-value = 0.66, respectively). Using the same nutrient load but altering the climate drivers resulted in modelled annual mean chlorophyll-*a* and modelled annual mean proportion of cyanobacteria that were representative of observed data within each climate zone (Fig. 4.2).

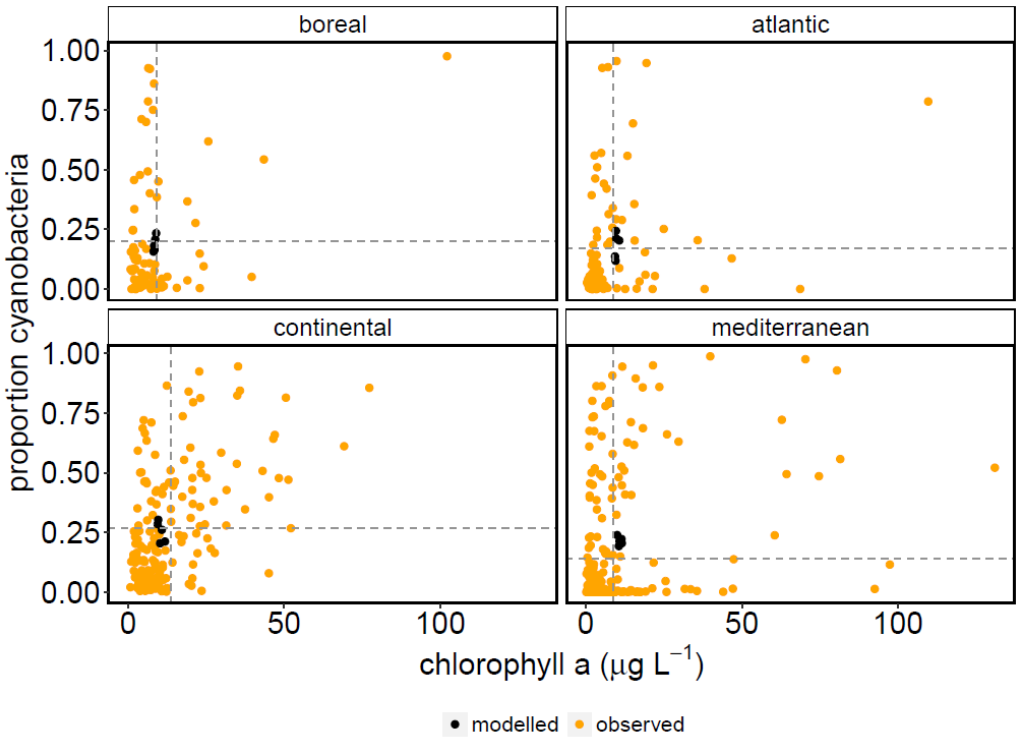


Fig. 4.2 Modelled and observed chlorophyll-*a* and proportion of cyanobacteria (annual averages). Orange dots are mean annual observed data for lakes classified as stratified in each of the climatic zones. Dashed lines are the mean annual cyanobacteria proportion and mean annual chlorophyll a from the observed data for each climate zone. Black dots are modelled chlorophyll-*a* and modelled proportion of cyanobacteria from the best calibrated model. There are 5 points for each climate zone, one for each climate model (Table 4.1).

Control simulations (no extreme event)

a. Water temperature and mixed depth varied among climate zones

Temporal trends in water temperature and mixed depth were similar in all climate zones (Fig. S4.2), with the highest temperatures during the summer (mid-July) and stratification of the water column occurring during the growing season (spring – autumn). Average water temperatures and the average duration of stratification (mixed depth ≤ 5 meters) generally increased with decreasing latitude, with lowest mean temperatures (9.6 ˚C) and the shortest duration of stratification (216 days) in Boreal simulations and highest mean temperatures (13.3 ˚C) and the longest duration of stratification (333 days) in Mediterranean simulations (Table S4.6). At similar latitudes i.e. Atlantic and Continental the differences were less distinct (see Fig. 4.1 for overlapping regions).

b. Chlorophyll-a and cyanobacteria chlorophyll-a amount varied with nutrient load and phenology with climate zone

Increasing the nutrient load resulted in statistically significant increases in total chlorophyll-*a* and cyanobacteria chlorophyll-*a* (Fig. S4.3, Table S4.7 and S4.8). General temporal trends were similar among climate zones with a spring bloom composed of diatoms (*Asterionella*) and flagellates (*Plagioselmis*) and a later summer/autumn bloom of flagellates (*Plagioselmis*) and cyanobacteria (*Planktothrix*). However, the phenology of these biomass events varied among zones, with earlier onset and later breakdown in Mediterranean lakes compared to Boreal lakes (Fig. 4.3). This reflected differences in the timing of stratification among climate zones (Fig. S4.2). The proportions of dominant taxa within these bloom events also varied among climate zones and among nutrient scenarios e.g. *Planktothrix* was more abundant in the Continental high nutrient scenario than the Continental low nutrient scenario (Fig. S4.4).

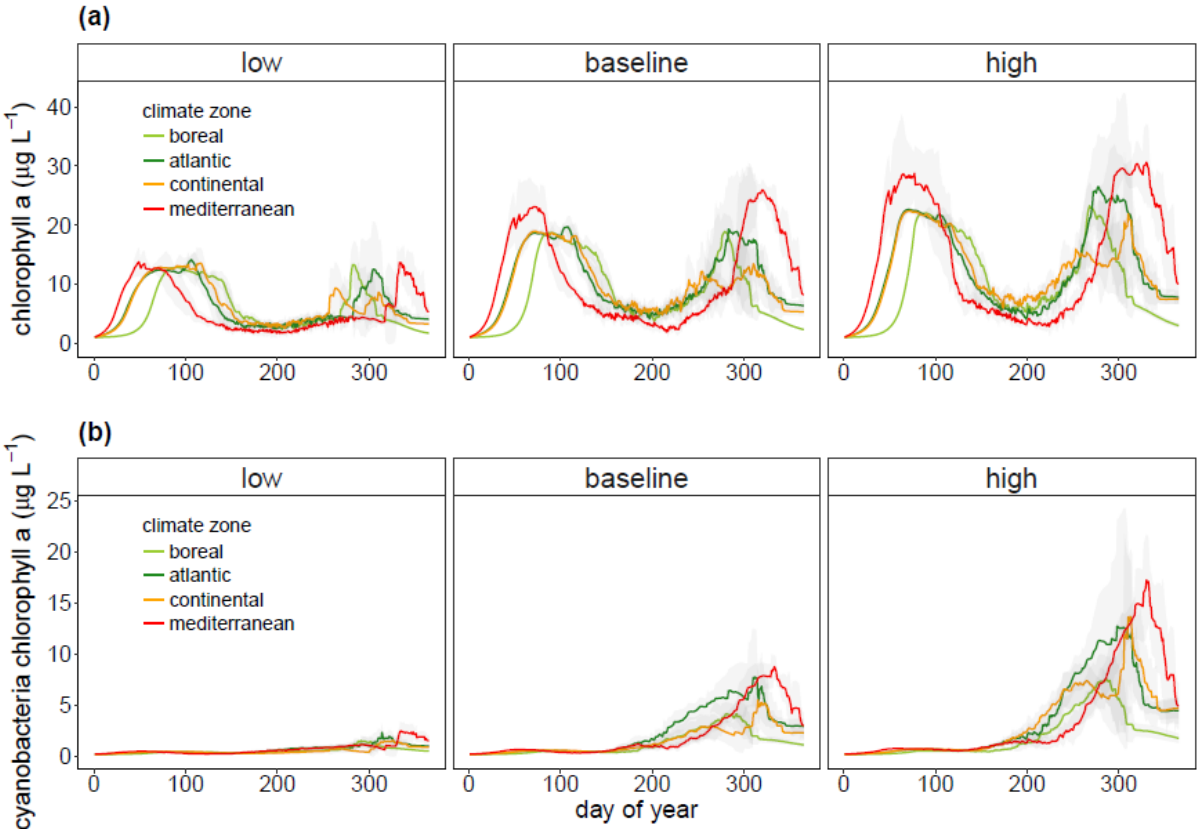


Fig. 4.3 Chlorophyll-*a* and cyanobacteria chlorophyll-*a* over a year simulation with no flushing events (control) for different climate zones and nutrient loads. The coloured lines are the average of the 5 climate models for each climate zone-nutrient load combination, the shaded area is the standard deviation.

Short term impacts of hydraulic flow events

Extreme flushing events predominantly resulted in net losses of chlorophyll-*a* (Fig. S4.5) and net losses of cyanobacteria chlorophyll-*a* (Fig. 4.4), although some gains were occassionally observed. Overall, changes in total and cyanobacterial chlorophyll-*a* increased with increasing nutrient loading (productivity) and increasing flushing rates (event magnitude). Temporal patterns of impact depended on the timing of the event, the climate zone and the response measured (total chlorophyll or cyanobacterial chlorophyll-*a*), Table 4.2 and Table S4.9).

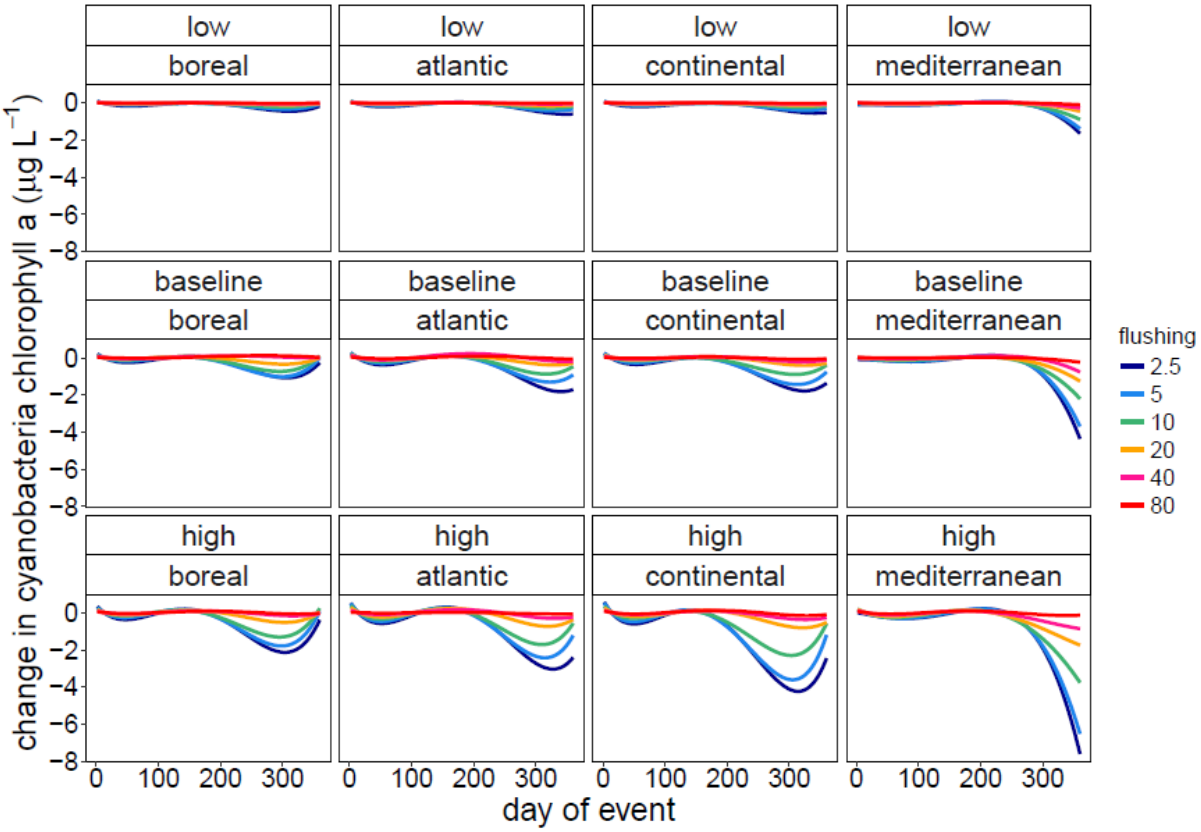


Fig.4.4 Short term impact of hydraulic flow events on cyanobacteria chlorophyll-*a* at different flushing magnitudes (rate in days), nutrient scenarios and climate zones. The coloured lines are the fitted response from the best fit linear model (Table 4.2).

Table 4.2. Analysis of Variance table of the best fit linear model for the change in cyanobacterial chlorophyll-*a* on the day of the event given different event timing, flushing rate, nutrient scenario and climate zone. The terms are ordered in descending sum of squares. $R^2 = 0.48$.

Term	df	sumsq	meansq	Statistic	p.value
Residuals	9360	4437	0.5		
poly(dayEvent, 4)	4	998	249.6	526.5	<0.001
poly(dayEvent, 4):flushing	20	593	29.7	62.6	<0.001
Flow	5	462	92.5	195.1	<0.001
poly(dayEvent, 4):climate_zone	12	404	33.7	71.0	<0.001
poly(dayEvent, 4):nutrient	8	387	48.3	101.9	<0.001
poly(dayEvent, 4):climate_zone:flushing	60	250	4.2	8.8	<0.001
poly(dayEvent, 4):flow:nutrient	40	235	5.9	12.4	<0.001
Nutrient	2	229	114.5	241.4	<0.001
flow:nutrient	10	162	16.2	34.2	<0.001
poly(dayEvent, 4):climate_zone:nutrient	24	132	5.5	11.6	<0.001
poly(dayEvent, 4):climate_zone:flushing:nutrient	120	109	0.9	1.9	<0.001
climate_zone	3	39	12.9	27.2	<0.001
climate_zone:nutrient	6	24	4.1	8.6	<0.001
climate_zone:flushing	15	22	1.5	3.1	<0.001
climate_zone:flushing:nutrient	30	22	0.7	1.5	<0.001

Recovery from extreme flow events

Recovery from hydraulic flow events depended on the timing of the event, the magnitude of the event, the climate zone/region and to a lesser extent the nutrient scenario (Tables 4.3-4.4). There were no clear differences in the shape of the recovery (days to recover or recovery rate) for chlorophyll-*a* (Fig. S4.5 and S4.6, Tables S4.10 and S4.11) and cyanobacteria chlorophyll-*a* with minimum recovery times occurring during the main summer period, around day 200 (Fig. 4.5a and Fig. 4.5b).

a. Pulse disturbances of different magnitudes

The number of days to recover from a flow event depended on the magnitude of the event - the greater the magnitude of the event, the longer it took to recover (Fig 4.5a, coloured lines, Table 4.3). Conversely the greater the magnitude of the event, the higher the rate of recovery (Fig 4.5b, coloured lines, Table 4.4).

b. Pulse disturbances at different times of the year

The number of days to recover and the rate of recovery also depended on the timing of the event. The rate of recovery was slowest when the event occurred at either end of the year (Fig. 4.5b), resulting in a longer time to recover (days to recover, Fig 4.5a). Conversely, the rate of recovery was fastest when flow events occurred during the summer. Recovery (days to recover and rate of recovery) depended on the daily solar energy input (J m^2 day^{-1}) so that when it increased, there was an increase in the rate of recovery (Fig. 4.6b, Table S4.12) and consequently a decrease in the number of days to recover (Fig. 4.7a, Table 4.8). Naturally, incoming energy showed a seasonal pattern, with higher amounts in the summer and lower amounts in the winter (Fig. S4.7).

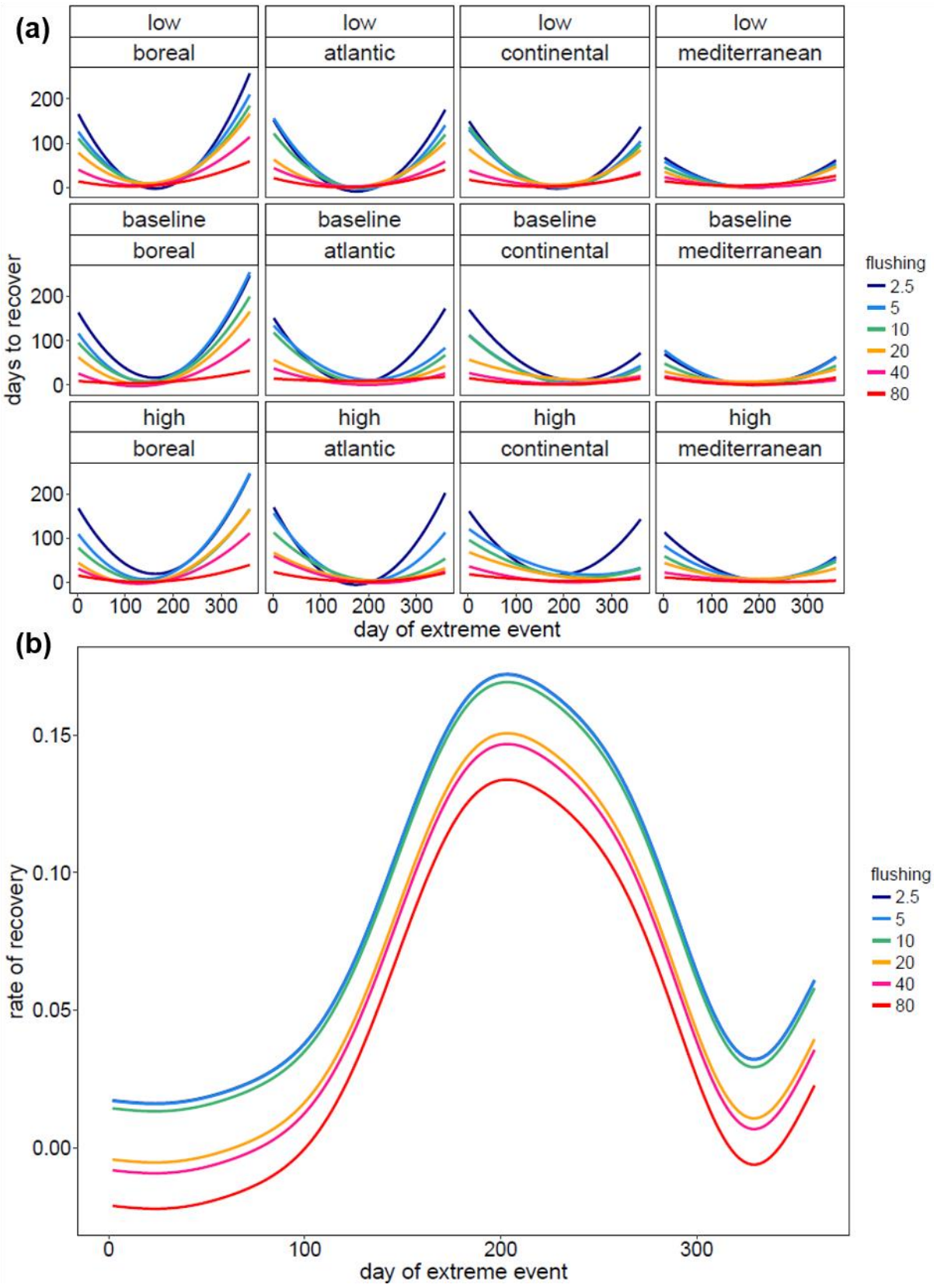


Fig. 4.5 Recovery of cyanobacteria chlorophyll-*a* from hydraulic flow events at different flushing magnitudes (rate in days), nutrient scenarios and climate zones. (a) the number of days to recover and (b) the rate of recovery. The coloured lines are the fitted response from the best fit linear model (Table 4.3 for (a) and Table 4.4 for (b)).

c. Pulse pressures in different climatic regions/across regions

Recovery also depended on climate zone/region where differences in recovery were most evident for flow events that occurred at either end of the year. Regions at higher latitudes, especially Boreal, took longer to recover during the winter than regions at lower latitudes, e.g. Mediterranean (Fig. 4.5a). Regional differences during the summer, if any, were not evident.

d. Effect of nutrients

Nutrient scenario was statistically significant in the explaining the number of days for total and cyanobacterial chlorophyll-*a* to recover but explained little additional variance in terms of temporal trends or overall effects.

Table 4.3. Analysis of variance table for best fit linear model for the number of days it took cyanobacterial chlorophyll-*a* to recover given the timing of the event, flushing rate, nutrient scenario and climate zone. The terms are ordered in descending sum of squares. $R^2 = 0.57$.

Term	Df	Sumsq	meansq	statistic	p.value
Residuals	9504	16879106	1776		
poly(dayEvent, 2)	2	5685633	2842816.3	1600.7	<0.001
Flushing	5	2648748	529749.7	298.3	<0.001
poly(dayEvent, 2)*climate_zone	6	2591536	431922.7	243.2	<0.001
poly(dayEvent, 2)*flushing	10	1822211	182221.1	102.6	<0.001
climate_zone	3	1414748	471582.6	265.5	<0.001
poly(dayEvent, 2)*climate_zone*flushing	30	616140	20538.0	11.6	<0.001
climate_zone*flushing	15	365737	24382.5	13.7	<0.001
poly(dayEvent, 2)*climate_zone*nutrient:flushing	60	173403	2890.0	1.6	<0.001
poly(dayEvent, 2)*nutrient	4	133330	33332.4	18.8	<0.001
poly(dayEvent, 2)*climate_zone*nutrient	12	125713	10476.1	5.9	<0.001
nutrient:flushing	10	81252	8125.2	4.6	<0.001
poly(dayEvent, 2)*nutrient*flushing	20	37357	1867.8	1.1	<0.001
climate_zone*nutrient*flushing	30	36459	1215.3	0.7	<0.001
climate_zone*nutrient	6	22922	3820.3	2.2	<0.001
Nutrient	2	9855	4927.7	2.8	<0.001

Table 4.4 Additive model results for the difference in the rate of recovery of cyanobacterial chlorphyll-*a* given the timing of the event and the flushing rate. Significant effects ($p < 0.05$) are highlighted in bold. R^2 adjusted = 0.28

Parametric coefficients. Changes on the intercept.						
	(Intercept)	Flush5	Flush10	Flush20	Flush40	Flush80
estimate	**0.07**	-0.00	-0.00	**-0.02**	**-0.03**	**-0.04**
Estimated degrees of freedom (edf) for approximately significant time smooth terms for the timing of the evnt						
Edf	**8.44**					

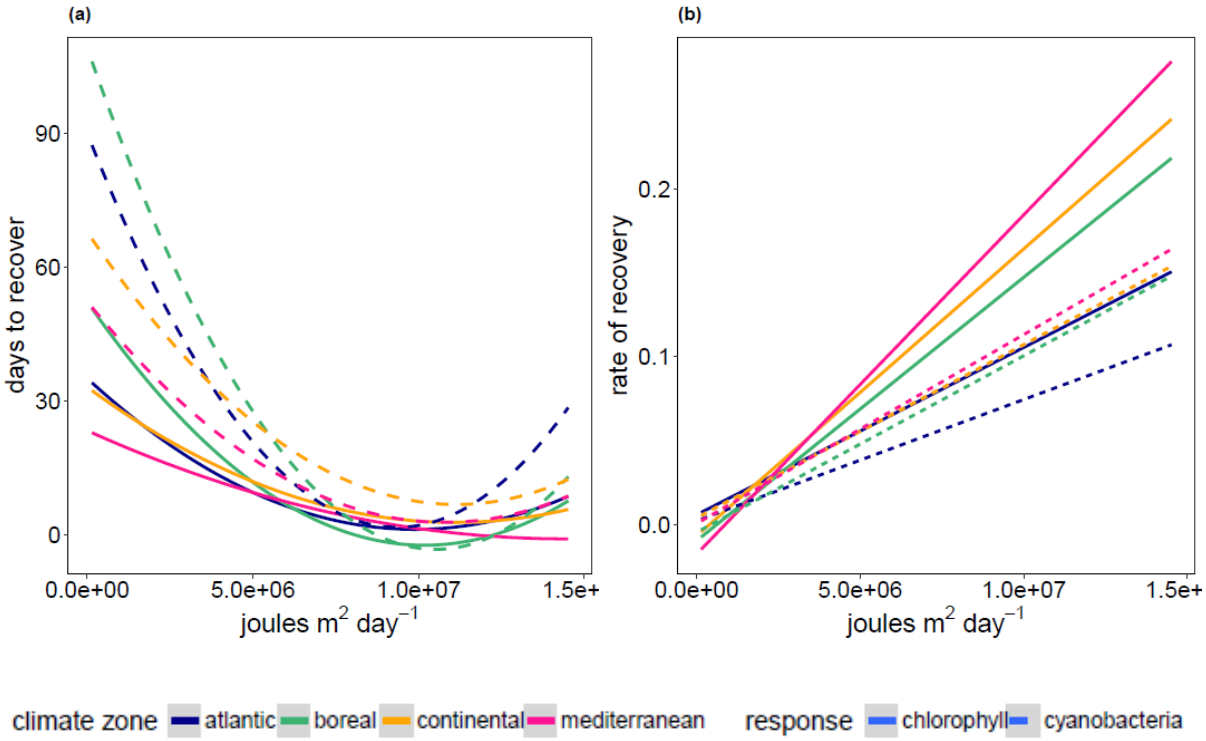


Fig. 4.6 Relationship between recovery (days to recover (a) and rate of recovery (b)) and incoming solar energy (joules m^2 day^{-1}). Lines are fitted values from the best fit linear model (Table S4.12 and S4.13, for (a) and (b), respectively).

1.5 Discussion

Associated with climate warming are changes in the hydrological cycle. Lake phytoplankton communities are impacted through more frequent and intense flushing events - pulse disturbances. The effect of these changes could have large direct effects on phytoplankton communities caused by increased loss rates and changing selection pressures on community composition. Because of their slow rate of growth and general preference for stratified conditions, extreme flushing events may have a particularly negative effect on cyanobacteria.

Control simulations

In control simulations, the timing of the vernal phytoplankton bloom and the breakdown of autumnal biomass varied among climate zones. This can be explained by the different onset and breakdown of thermal stratification among the zones. In stratified lakes, interannual variability in light availability – the "starter and terminator" of the phytoplankton growth season (Sommer *et al.*, 1986; Sommer *et al.*, 2012) - is driven by stratification. Earlier onset of thermal stratification results in earlier phytoplankton growth. Stratification varied among the zones because of differences in temperature. Thus, factors which alter physical forcing - weather and geographical location – affect the timing and duration of the phytoplankton growth season (Sommer *et al*., 2012). This demonstrates the influence of spatial and climate factors at broad scales in shaping biological responses, in the absence of disturbance. Nutrients defined the carrying capacity of phytoplankton biomass but did not alter phenology (Sommer *et al*., 2012).

Flushing events

Biological impacts of flushing events

The impact of flow events on biomass were predominantly negative. This was expected and can be explained by loss of biomass by hydraulic loss (Phlips *et al*., 2002; Jeong *et al*., 2011; Reynolds *et al*., 2012; Sadro & Melack, 2012). The amount of biomass lost was proportional to the magnitude of the disturbance but also to the productivity of the system. Productivity was determined by the nutrient carrying capacity but also on seasonally varying factors, specifically light and temperature; as a result there was a seasonal trend in impact which mirrored seasonal changes in total chlorophyll-*a* and cyanobacteria chlorophyll-*a*. For cyanobacteria, this will likely mean that flushing events will have the greatest short term impacts during the late summer to autumn when blooms most often occur. As the timing of

biomass events varied among biogeographical zones, so too did temporal variation of the event impact - a large storm affecting multiple regions may have different biological impacts.

Unexpectedly, in some scenarios there were small net increases in biomass as a result of flushing. These increases can be explained by the stimulatory effect of increased nutrient loading offsetting any losses. These gains wouldn't occur in lakes with predominantly point sources of nutrients as increasing flow would result in nutrient dilution (Elliott *et al*., 2009).

Recovery

a. Timing and magnitude of the event

Recovery from flow events depended both on the magnitude and the timing of the event. With increased flushing, more biomass (total and cyanobacterial chlorophyll-*a*) was lost and so, at any time of the year, it took longer to recover. This is expected, and is reported in the literature - severe events can have long term effects on phytoplankton biomass in the order of years (James *et al*., 2008; Harris and Baxter, 1996), while cyanobacteria can recover and even bloom preceding smaller precipitation events within days (Oh and Kim, 1995; Ahn *et al*., 2002). In natural systems the relationship between the event magnitude and recovery may not be linear. Firstly, heterogenous distributions of flow in natural lakes can create hydraulic storage zones which can maintain slow growing populations, decreasing the time needed for recovery and even sustaining blooms if the low flow zone is large enough (Grover *et al*., 2011; Michalak *et al*., 2013). Secondly, high flow events can result in increased delivery of terrestrial material, increasing turbidity, decreasing available light (Sadro & Melack, 2012; Zhou *et al*., 2012) and consequently prolonging recovery (James *et al*., 2008; Perga *et al*., 2018). Perga *et* al (2018) showed that the 'turbidity' of an event can be independent of the event magnitude, instead depending on antecedent weather and catchment geology.

Recovery was prolonged in the winter compared to the summer. Phytoplankton communities were less resilient to change during the winter months because of limited resources for growth. Seasonal differences in recovery can be expected and are reported elsewhere. For example, Richardson *et al* (in review, chapter three) observed that the effect of autumn and winter extreme rainfall (flushing) events were more prolonged than spring and summer rainfall events.

b. Regional differences – lake location

Recovery also depended on the climate zone - higher latitude regions were more seasonally sensitive to disturbance than lower latitude regions because of differences among regions in daily solar radiation. This latitudinal effect in recovery was most apparent at either end of the year when the tilt of the earth exacerbates differences in day length and energy load among latitudes.

Other spatial differences in recovery may arise from heterogeneous distributions of lake types (Nõges, 2009). Cyanobacterial responses to single (Phillips *et* al., 2008) and multiple stressors are shown to vary among lakes with different physical and chemical attributes (Richardson *et al*., 2018; Taranu *et al*., 2012). Differences in surface area (which influences fetch) and depth (which influences mixing) may be particularly important lake type factors, the former affecting the extent of wind induced mixing and the latter affecting species composition prior to the event. In fully mixed or polymictic lakes, conditions may likely already be tailored to taxa such as diatoms which can tolerate mixing (Carrick *et al*., 1993; Wagner & Adrian, 2011).

c. The role of nutrients in recovery

While increased nutrient loading stimulated higher growth rates, there was no difference among nutrient scenarios in terms of the time to recover because of increased biomass lost in systems of higher productivity. In natural systems, variation in nutrient loading may occur during high rainfall events depending on the season in which the event occurs (Donohue *et al*., 2005) which could alter recovery times. Nutrient source is also likely to be an important factor as changes in flushing rates will alter in-lake nutrient concentrations in different ways depending on whether the nutrient source is point or diffuse (Elliott *et al*., 2009). In point source systems, increased flow results in a loss of nutrients through the ouflow, without replacement, which could result in prolonged recovery.

d. Compositional differences in recovery

Unexpectedly, similar patterns of recovery were seen for total and cyanobacterial chlorophyll-*a*, suggesting no major compositional shifts occurred in response to one-off flow events. This is surprising because hydraulic flow is recognised as an important factor influencing phytoplankton composition, with different flow velocities suiting the tolerances of different functional groups (e.g. Harris & Baxter, 1996; Sherman *et al*., 1998; Katsiapi *et al*., 2011;

Bittencourt-Oliveira *et al*., 2012; Elliott & Defew, 2012; Cross *et al*., 2014). The extent of physical disturbance will likely affect community resilience. In this study the events were short lived, only temporarily changing the flow velocity, whereas rainfall events with changes in flushing rates as well as thorough wind-induced mixing (Woolway *et al*., 2018) may have greater impacts. The frequency of distrubance is also discussed widely as an important factor (Connell, 1978) with increased distrubance supresssing slow growing taxa like cyanobacteria (Padisak *et al*, 1999).

Alternative responses may occur depending on the dominant taxa in the system. In this study, *Planktothrix* and *Dolichospermum* were used, of which *Planktothrix* dominated under all climate and nutrient scenarios. *Planktothrix* is tolerant to mixing and light limitation (Reynolds *et al*., 2002) and so may not be as sensitive to flow disturbances as other taxa such as *Microcystis*. It should be expected that cyanobacteria genera with different functional traits and, therefore, different sensitivities and tolerances will respond to multiple stressors in different ways (Carey *et al*., 2012; Rigosi *et al*., 2014; Richardson *et al*., in review/chapter three).

Final remarks

This study is innovative and powerful in that it explores the effect of different extreme events in exactly the same lake under different climate and nutrient scenarios. Multi-factorial experimental designs, such as this one, are required to disentangle the complex nature of extreme climatic events and can be achieved through lake models such as PROTECH. Particular areas that deserve further consideration are: (a) the effect of other physico-chemical changes, in particular changes in turbidity and colour on recovery; (b) the influence of lake type, focusing on polymictic and stratified lakes to start; (c) species/genera specific responses and (d) other event attributes, in particular event frequency.

Chapter 5

General discussion

Understanding and quantifying the response of cyanobacteria to multiple stressors resulting from climate, land-use and population changes can be considered as a wicked problem (Churchman , 1967). With so many interdependent and changing factors at play, the outcome may be impossible to predict for any individual lake. Despite this complexity, improving our understanding of the role of climate change, in combination with nutrient enrichment, is clearly important so that we can make more informed decisions for risk assessment and management. So, how can we make sense of it? This thesis tackles some of the complexity of the response of cyanobacteria to a changing multiple stressor landscape by exploring combinations of stressors over multiple spatial and temporal scales. It does this by adopting three different scientific approaches: (1) analysis of empirical (monitoring) data from a large population of lakes across a climatically-variable continental-scale; (2) a mesocosm experiment to investigate responses to within-year weather dynamics and (3) process-based modelling of a representative system across several European climatic zones. These approaches provide independent lines of complementary evidence, incorporating multiple potentially interacting stressors and potentially confounding factors, such as lake type and geographical location and by incorporating the complex signature of pulse disturbances (i.e. different expressions of a stressor). A synthesis of the outcomes from these studies provides more robust evidence to consider what generalisations, if any, can be made and how the increased knowledge gained can be applied to the management of lakes to reduce the risk of HABs. Specifically, this discussion will focus on:

(a) Synthesis of multiple stressor effects across scales and study designs, including the wider literature – are the responses of cyanobacteria to climate and nutrient stressors generalisable? What factors need to be considered for predicting future change?
(b) Implications for management. Is it necessary to understand interactions? Is there a stressor hierarchy to prioritise in management?
(c) Future research directions – what gaps in our knowledge remain and what approaches could be used to fill these gaps?

5.1 A synthesis of multiple stressor effects across studies – are responses generalisable?

The range of responses observed to multiple stressors across the chapters highlight that it is difficult to predict how stressors may interact, especially at a given individual site. Additive, synergistic and antagonistic responses may be possible for the same stressor combination at sites with different characteristics or different levels of stress. For example, the hypothesised synergism between warming and nutrient enrichment was not always identified (chapter two) and was not always synergistic (chapter three). This suggests that the response to multiple stressors are context dependent. In particular, this thesis identified that the response can depend on: the waterbody type (chapter two); the stressor gradient (chapter three); the response level considered (chapter three) and the geographical location of the lake (chapter four and to some extent chapter two). Knowledge of how these factors shape variability in the response may provide some generalisations that may not be certain at an individual lake level, but provide a basis for more informed risk assessment and management of the pressures affecting lakes. These shaping factors are discussed in the following sub-sections in the context of the wider literature.

5.1.1 Environmental context – ecosystem type

Biological responses to environmental change can be shaped by their environment. The lake type analytical approach taken in chapter two highlights the importance of allowing for interactions between multiple lake type factors when defining environmental context. While other authors have identified trophic type (Rigosi *et al*., 2014), mixing type (Taranu *et al*., 2012) and depth x artificial *vs* natural lakes (Beaulieu *et al*., 2013) as being important in shaping the response of cyanobacteria to the combined effects of nutrients and temperature, chapter two includes other key environmental factors – mixing type, alkalinity and colour - that could further influence the response. 'Lake types' defined by multiple environmental factors may be especially relevant for capturing community structure response to change which may be sensitive to a greater number of interacting factors than general productivity (Phillips *et al*., 2008).

Findings from this chapter and the wider literature amount to convincing evidence that a lake type analytical approach could help better predict responses to future environmental change. This could allow clear generalisations of lake responses that can be used to assess

potential risk across a population of lakes and inform where to prioritise monitoring for risk management. However, a health warning comes with this approach because of the large natural variability found among lakes within a type. Variability may arise from missing covariate information such as other limiting nutrients (Downing *et al.*, 2001; Dolman *et al.*, 2012) or it is possible that idiosyncratic responses to environmental change at the individual lake level could arise. The latter can be explored with long-term datasets (e.g. MARS time series analysis). In this thesis, chapter three and chapter four tackle some of this unexplained variability by taking a more individual lake type approach. In chapter three, multiple stressor effects are explored in a shallow unstratified, clear water, high alkalinity system while in chapter four the analysis focuses on a typical stratified, clear water system.

5.1.2 Stressor gradients alter perspectives

Chapter three showed that stressor gradients are a key consideration in determining the response of cyanobacteria to multiple stress. A striking outcome of this study was the detection of an antagonistic, rather than synergistic (as widely hypothesised), interaction between warming and nutrient enrichment. This 'ecological surprise' warrants three key points of discussion: (a) the first is about the confidence of predicting outcomes from prior ecological knowledge; (b) the second is about ways in which we can sub set the problem to increase our confidence in predicting outcomes and (c) the third is a warning about the use and potential misinterpretation of multiple stressor terminology.

A major hypothesis in freshwater ecology is that cyanobacterial blooms will be enhanced because of a synergistic interaction between warming and nutrient enrichment. This thesis shows that this interaction is not always detected (chapter two) and not always synergistic (chapter three). Rigosi *et al.* (2014) also did not consistently detect this interaction among lakes of different nutrient trophy or among species responses. Unexpected multiple stressor responses have also been found by others e.g. between nutrient enrichment and acidification in Boreal lakes (Christensen *et al.*, 2006), emphasising the high degree of uncertainty of the expected impacts of global change on cyanobacterial blooms. This highlights the need for multiple stressor studies to identify and understand these unexpected outcomes.

The unforeseen antagonism can be explained by differences in the response along non-linear stressor gradients. This has been highlighted previously by Piggott *et al* (2015) in a stream mesocosm experiment in which the response to sediment and nutrient loading changed

along a non-linear nutrient gradient. Asymptotic behaviours of chlorophyll-*a* and cyanobacteria to TP are widely reported in the literature (Carvalho *et al.*, 2013; McCauley *et al*., 1989; Phillips *et al*., 2008; Watson *et al*., 1992) with the typical turning point occurring at 100 µg L^{-1}, above which TP no longer explains total or cyanobacterial chlorophyll-*a*. In chapter two, the regressions were restricted to the range of each stressor where the data were linearly related (this was only relevant for the response to TP). In chapter three the nutrient gradient between the treatments spanned this asymptotic point, with TP concentrations >300 µg L^{-1} in the high nutrient loading scenario. As systems move from eutrophic (positive nutrient relationship) to hypertrophic (no relationship) TP concentrations then it is not surprising that interactions may not be as expected. It is very likely then that the response will be different along the TP gradient especially for lakes at different ends of the gradient e.g. oligotrophic-mesotrophic compared to eutrophic-hypertrophic. One other study has explored the response of cyanobacteria to temperature and nutrient enrichment for lakes at different nutrient trophic levels. Contrary to results in chapter three, Rigosi *et al* (2014) detected synergisms to be more important in eutrophic and hypertrophic lakes. They suggest that these synergisms may occur because of enhanced light limitation in higher nutrient lakes because of increased shading which may give an advantage to cyanobacteria. The mechanisms for the antagonism detected in the mesocosm experiment is not clear but is hypothesised to be because of enhanced carbon limitation under warming and nutrient enrichment. It is difficult to make conclusions based on these studies when comparing different statistical approaches and over different scales. Specifically, Rigosi *et al* (2014) analysed the response over a large continental scale in which the effect of other lake type factors were not included, which, as highlighted in chapter two, is an important factor. Furthermore, the models presented in Rigosi *et al* (2014) explain very little variation, further limiting confidence in the comparison of effects.

Despite the detected antagonism, the effect size was small. This means that currently severely impacted lakes may not noticeably get any worse but, in terms of water quality metrics, they may not really get any better either. This leads onto the final point about terminology. In theoretical terms, an ‘antagonism’ means a response that is statistically significantly less than an additive response i.e. the combined effect of stressors acting on their own. While this terminology suggests a favourable outcome in terms of the risk of cyanobacterial blooms under future climate and nutrient conditions, in reality the size effect was small and the response was still greater than either individual stressor – just not quite additive. Thus, there is a potential risk of misinterpretation in using the term ‘antagonism’ and

so the inclusion of a size effect alongside the type of interaction is very important when communicating multiple stressor results.

5.1.3 Multiple stressor effects vary among response levels

Variability in the response to multiple stressors was also observed because of the response level measured. Specifically, differences in effects were seen when comparing the response at the producer (total phytoplankton), community (cyanobacteria) and population (genera or species) levels. In chapter two, the influence of lake type was more important at the community – cyanobacteria- level than for producers in general and interactions were more common for cyanobacteria. This is consistent with Phillips *et al* (2008) who found that nutrient – chlorophyll-*a* relationships could be grouped into fewer groups than the 18 WFD lake types that were tested, reducing the number of types to three. In chapter three, interactions were only detected at the community – cyanobacteria – level while no interactions were detected at the population level – cyanobacteria genera. Rigosi *et al* (2014) also found that interactions between warming and nutrient enrichment did not occur when testing genera specific responses while Ekvall *et al* (2013) found the opposite of interactions occurring at the species specific level but not at the whole community level.

Can generalisations be made about the type of interactions for different response levels? Christensen *et al*., 2006 suggested that higher taxonomic groups may be more susceptible to synergisms because reduced taxonomic-, physiological- and genetic- diversity may result in less 'biological insurance'. In this thesis, one instance of an antagonistic effects was detected at the producer level. However, this effect is difficult to fit into the hypothetical framework proposed by Christensen *et al*. (2006) because of difference in the use of multiple stressor terminology; in this thesis, and in the wider literature, synergisms for cyanobacteria are discussed in terms of success for cyanobacteria which translates to negative impacts on water quality while in other studies synergisms are interpreted as being negative for the response in question because of stressors being suppressants rather than stimulants. Such differences in the use of terminology could hamper our ability to generalise at this type of level (e.g. consumers *vs*. producers). Others have also summarised responses by responses levels - in a meta-analyses of 171 marine and coastal studies, Crain *et al*. (2008) found antagonistic effects for communities and autotrophs and synergistic effects for populations and heterotrophs. Mechanisms would need to be made clear for such generalisations of interaction types at different response levels.

Diverse taxonomic groups such as cyanobacteria are likely to respond in different ways to stressors (Carey *et al*., 2012) and so multiple stressor effects should be tested at the whole group, and functional type or genus level. Other authors also conclude that there is no support that cyanobacteria – as a group – will respond in a coherent way to environmental change (e.g. Ekvall *et al*., 2013; Rigosi *et al*., 2014). For example, in chapter three an antagonistic interaction between warming and nutrient enrichment was found for total cyanobacteria, yet underlying this response *Microcystis* sp. and *Dolichospermum* sp. (both common bloom forming, toxicogenic taxa) both increased in response to warming.

5.1.4 Spatial factors influence multiple stressor effects

Lake location is a key factor to consider when scaling up from single site studies. In this thesis, the clearest spatially related effect was that of latitude on the resilience of phytoplankton and cyanobacteria to extreme flushing events (chapter four). This was explained by differences in the extent of seasonal light and temperature limitation on growth rates among biogeographical zones at different latitudes. Latitudinal effects on the energy avaiable for growth, and recovery, may be less important for the recovery of cyanobacterial blooms which mostly occur during the summer and autumn.

Differences in responses to multiple stressors across landscapes can also occur due the heterogeneous distribution of lake types (Nõges, 2009) and pressures (MARS). In chapter two a striking spatial pattern of temperature effects was observed for cyanobacterial chlorophyll-*a* in lakes at more northern latitudes. This coincided with the clustered distribution of a lake types and so these latitudinal effects could be explained by the effect of lake type, the effect of latitude such as longer summer photoperiod at higher latitude (Nicklisch *et al*., 2008) or an interaction between both factors. This is an important consideration in generalising responses – the same lake type but different locations could result in different responses.

Other studies have identified other spatial factors that alter the response of cyanobacteria to local stressors, such as heterogeneous distribution of eco-regions (Beaver *et al*., 2014) and weather events (Mantzouki *et al*., in press). Different landscape factors such a geology can alter the shape of the non-linear relationship between chlorophyll and TP (Wagner *et al*., 2011; Filstrup *et al*., 2014) which could alter responses when introducing another stressor. The importance of multi-scale processes has been highlighted by others (Finley , 2011; Taranu *et al*,. 2017) and is especially important as studies move towards large scale analyses such as the National Lake Assessment (Beaver *et al*., 2013; Beaulieu *et al*.,2013 ;

Rigosi *et al*., 2014) and the European Multi Lake Survey (Mantzouki *et al*., 2018). Incorporating cross-scale interactions (Peters *et al*., 2007) into models can help us to understand the heterogeneous responses of cyanobacteria to environmental change.

5.1.5 Temporal factors

The temporal variability of disturbance events can also be a key driver of ecosystem responses. This can be expected because of inherent seasonal variation in phytoplankton abundance and composition (Sommer *et al*., 1986; Sommer *et al*., 2012) and consequent seasonal sensitivities to disturbance, depending on the type of disturbance and the sensitives and tolerances of impacted communities. The hypothesised effects of disturbances at different times, frequencies and intensities are deeply rooted in ecological theory in the form of the Intermediate Disturbance Hypothesis (Connell, 1978). To date, the effects of temporal variations in disturbance have come from single systems whereas this thesis explores different event characteristics in systematic ways in the same lake.

In chapter three and four, the effects of flushing events were tested at different times of the year. A clear response which emerged was that the recovery of cyanobacteria from perturbation (loss from the outflow) was limited by seasonally variable growth factors – recovery was slower in the winter when light and temperature were more limiting than during the summer (chapter three and four) and the extent of this limitation varied with latitude because of among latitude variations in solar input (chapter four). This resulted in flushing disturbances having greatest impact, in terms of recovery, during the winter. Unexpectedly, flushing events at times when cyanobacterial biomass was higher, did not have long term effects on cyanobacterial abundance, suggesting that one-off events will not impact blooms. This was unexpected as bloom forming genera are sensitive to higher flushing rates, having functional traits and slower growth rates which suit stable water columns (Sherman *et al*., 1998; Reynolds *et al*., 2002; Hudnell *et al*., 2010; Cross *et al*., 2014). The key aspect of both chapter two and three is that the effect of one-off short lived events were tested. Other authors have demonstrated that longer term increases in flushing rates can result in compositional shifts at times of the year when the dominant taxa are sensitive to alterations in flow or no change when the dominant taxa are not sensitive (Padisák, 1993; Padisák *et al*., 1999; Verspagen *et al*., 2006; Elliot, 2010). Alternatively, higher frequencies of events may be required to disrupt seasonal succession (Padisák *et al*., 1988; Chellappa & Costa, 2003; Xiao *et al*., 2011). Even greater complexity may arise from multiple pulse disturbances with different temporal patterns;

Molinos & Donohue (2010) show that interactions among temporal patterns of nutrient and sediment loading in streams varied the response of benthic communities.

While experiments, especially those using computer models, provide the scope to explore multi-factorial problems, some assumptions are made that may affect the results. For example, in terms of flow events, the model assumes homogenous distribution of flow whereas in reality heterogeneous flow can alter recovery from high rainfall events. More generally, process based models are simplifications and generalisations of a more complex ecosystem and even the niche of representative taxa may not be perfectly described in the model. Despite these caveats, modelling gives the scope to explore the variable signature of rare extreme events. Overall, this thesis and the wider literature highlight that temporal patterns of disturbances are important for reliable prediction of impacts from, and affect the management of, multiple stressors. This thesis also highlights the need to test stressor effects beyond the time of hypothesised greatest impact – recovery was longer from events which occurred in the winter (chapter three and four), but more striking and unexpected was the increase in cyanobacterial abundance and dominance after a winter flushing event in nutrient enriched mesocosms (chapter three).

5.2 Managing climate change effects

5.2.1 Do we need to generalise the impacts of climate change or is it all about nutrients?

Given a generally suitable environment for cyanobacteria (e.g. clear water, medium to high alkalinity lakes - Carvalho *et al*., 2011), nutrient enrichment is irrefutably the cause of enhanced cyanobacterial blooms. Given the complexity in generalising the response of cyanobacteria to multiple nutrient and climatic stressors we, therefore, need to ask whether we need to understand further. Are we over-complicating a simple situation when it is largely just about managing nutrients? In general qualitative terms, managing nutrients clearly remains as the principal management tool. However, it is important to get a more quantitative understanding of how climate may impact cyanobacterial abundance, to better understand where, and by how much, actions will require current nutrient targets to be amended (e.g. Carvalho *et al*., 2013) to mitigate against climate effects which are generally not controllable locally.

Significant effects of climatic variables are common, both in this thesis and in the wider literature, and so cannot be ignored. However, the variability in the response of cyanobacteria to climate change, with no clear pattern of additive or interactive effects, means that quantifying measures becomes challenging. Some lake types may require greater management intervention than others, and lakes that are currently not at risk (i.e. do not exceed WHO guideline thresholds for drinking water or recreational use) may develop problems in the future e.g. polymictic humic lakes (chapter two). The most effective management actions, in terms of outcome and cost, will come from identifying and quantifying interactions so that lakes at most risk can be identified in order to mitigate against extreme warming or drought years. Furthermore, understanding the response at multiple scales and gradients is needed so that important effects are not overlooked (chapter three).

5.2.2 Other management options

Depending on the suitability of the system, some climate effects can be managed locally. In chapter three, enhanced internal cycling of phosphorus was likely an important mechanism in the increase of algal biomass in warmed mesocosms. This could be counteracted by application of P-binding products e.g. Phoslock (Lang *et al*., 2016). While this is effective in reducing available phosphorus, the wider implications for the ecosystem are still being understood (e.g. Spears *et al*., 2016). In deep lakes management of indirect effects of warming through artificial

mixing to breakdown thermal stratification may be beneficial, but can have unintended consequences, such as mixing nutrient rich waters (Visser *et al*., 2016). Potential management options could be through limiting light availability (e.g. through solar panel farm schemes on lakes) or through better management of flushing rates (Carvalho *et al*., 2011). Although in the long run these options may be more costly than reducing nutrient load as they do not treat the main cause of enhanced blooms. The benefit of nutrient reductions is that multiple waterbodies within a catchment could benefit, including downstream lakes, estuaries and coastal waters. However, employing multiple management interventions in situations of synergisms may become useful.

5.2.3 Lakes should be managed as individuals

While stressor effects explained variation in cyanobacteria abundance, it is important to highlight that the natural variability among lakes and mesocosms of the same type/treatment was high. This emphasises an important point that management should be designed at a lake level and reflects the perspective which warns of copy and paste management methods for different lakes (Lürling *et al*., 2016). Although individual lakes tended to show idiosyncratic responses, the use of lake type categories or responses over different stressors gradients allows a clear generalisation of lake responses that are helpful to lake managers to prioritise which population of lakes to target measures to minimise risks.

5.3 Future research directions

What gaps in our knowledge remain and what approaches could be used to fill these gaps? There are two principal future research directions, one which focuses on furthering our understanding of multiple stressor effects for different contexts and the other which focuses on the trajectory of nutrient managed systems under multiple stress – is it what we expect?

i. Forward direction studies

A continuation of multiple stressor studies is clearly needed. These should specifically test multiple stressor effects over spatial and temporal scales, across stressor gradients and for different response types and lake types. Local factors such as nutrient source (point or diffuse), fish communities and macrophyte dominance should be incorporated to account for among lake variability and help strengthen our ability to develop a more predictable generalised framework. As demonstrated in this thesis, these factors can be explored using existing datasets (e.g. European WISER, Moe *et al*., 2013; US National Lake Assessment, e.g. Riosi *et*

al., 2014; Taranu *et al*., 2017), question specific continental scale sampling campaigns (European Multi Lake Survey dataset, Mantzouki *et al*., 2018) and experiments – both mesocosms and computer modelling. A particular focus of future work should be on the directionality of disturbances which has largely been ignored in favour of general trends. Other large scale environmental change, such as changes in lake colour (Monteith *et al*., 2007), should also be incorporated to test the whole suite of environmental change scenarios.

ii. Backward direction/remediation studies

As management becomes ever increasingly necessary, then it also becomes increasingly necessary for remediation studies. What trajectories will phytoplankton communities, and cyanobacteria in particular, take when nutrients are reduced in systems under multiple stress? This can inform at what scale restoration through nutrient load reduction is needed, for example greater reductions may be necessary to account for hysteresis (Jeppesen *et al*., 2005). Mesocosm and modelling experiments are useful tools to complement single site remediation case studies.

5.4 Summary

Multiple climatic and nutrient stressors are frequently present in lakes. Many climate factors such as warming, enhanced stratification, and drought can stimulate bloom formation and so mitigating the effects of climate change is a key area to future research – how far do we need to reduce nutrient loads to restore current impacted sites or deliver resilience to current unimpacted sites? The answer to this management question depends on the combined effects of nutrient and climatic stressors. This study and the wider literature highlights that multiple stress effects on cyanobacteria abundance are highly context-specific, precluding generalisation. With so many lakes under multiple stress (Nõges *et al.*, 2015) we could end up with inaction from overwhelming complexity. This thesis highlights demonstrable ways in which the problem can be subset to increase our understanding and identifies future directions of study.

Chapter 6.

Supplementary material

6.1 Chapter 2 supplementary material

6.1.1 Chapter 2 supplementary analyses

Relationships between TP, alkalinity and cyanobacteria

Lakes which had numerical alkalinity data were used to explore the relationship between TP, alkalinity and cyanobacteria (number of observations = 1246, number of lakes = 271).

Pairwise plots of alkalinity, TP and cyanobacteria show that they are all positively related (Fig. S2.2), although most paired relationships show some curvilinear tendency (quantification of these relationships are presented as Pearson correlation coefficients, which assume a linear relationship). It is possible that the relationships between cyanobacteria and alkalinity could be because of the co-variation between TP and alkalinity, in which case there would be a case for removing alkalinity from the types.

However, if we explore the relationship between the same variables but split between the two alkalinity types (low and medium-high) used in typology (Fig. S2.3) we can provide support for the inclusion of alkalinity:

a) The response of cyanobacteria to TP is steeper in medium-high alkalinity lakes over a very similar gradient (Table S2.2). This suggests that the response of cyanobacteria to TP may depend on alkalinity (Fig. S2.2a)
b) In low alkalinity lakes there is no longer a relationship between TP and alkalinity ($r = 0.04$, $p > 0.05$), yet in both low and high alkalinity lakes there's a positive relationship between cyanobacteria and alkalinity (low alkalinity, $r = 0.43$, $p < 0.001$; medium-high alkalinity, $r = 0.25$, $p < 0.001$). This provides further evidence that alkalinity explains variation in cyanobacteria independent of TP and so should be included to categorise lakes into types. Fig. S2.2b - c.

Gower distance clustering

Gower distance was calculated in R using the daisy function () from the cluster package with ln cyanobacteria biovolume ($mm^3 L^{-1}$) as the response and alkalinity (three levels: low, medium

and high), humic substances (three levels: low, medium and high) and mixing type (two levels: polymictic and stratified) as the categorical variables for clustering the data. As a visual check, we returned the most and least similar lakes. The two most similar lakes in term of cyanobacteria biovolume were both low alkalinity, medium humic and stratified lakes, the two least similar lakes were low alkalinity, medium humic and stratified *vs* medium alkalinity, low humic and polymictic. This initial check satisfied a basic expectation that cyanobacteria can be explained in part by combinations of these type variables, and that the most dissimilar values of cyanobacteria were from distinct lake types. We used the PAM algorithm (partitioning around medoids) for clustering and the silhouette width as the metric for helping to choose the number of clusters to be extracted (this is an aggregated measure of how similar an observation is to its own cluster compared to its closest neighbouring cluster, higher values are better). We calculated the silhouette width for clusters ranging from 2 to 30 using the PAM algorithm (Fig. S2.4) which suggests 17 clusters. These 17 clusters are broadly consistent with clustering cyanobacteria by a three way combination of alkalinity, humic and mixing types i.e. 18 types (Fig. S2.5). Because of imbalances in the data the 18 types could not be adequately modelled, therefore we further modified these types by combining ecologically similar levels of alkalinity and humic type, *see the manuscript methods*, resulting in 8 types which are broadly consistent with clustering the data by 8 groups using Gower based distance clustering (Fig. S2.6).

Differences in cyanobacteria among lake types

To test the differences in cyanobacteria biovolume among lake types, an ANOVA (Table S2.3) was fit with the response of natural log cyanobacteria biovolume ($mm^3\ L^{-1}$) and a factor of 'type' (n=8) which was then followed up with a Tukey test for the differences between each type (using the HSD.test function from the agricolae package in R). As some lakes had multiple data points, and thus violated the assumption of independence, one observation was randomly selected per lake. This random selection of observations was done ten times and the results were compared (Fig. S2.7). Six of the random draws resulted in the same groupings (Fig. S2.8 f-k), although there were some broad consistencies between these grouping and the groupings (Fig. S2.8 b-e) from the other four draws. In the paper we have presented the test based on the average response to complement what is presented in Fig. 2.2b (Fig. S2.7a).

6.1.2 Chapter 2 supplementary tables

Table S2.1. Number of monthly lake sample data for each year – month combination

	Month			
Year	**July**	**August**	**September**	**Grand Total**
2000	52	12	3	67
2001	23	58	11	92
2002	49	53	15	117
2003	19	56	14	89
2004	34	56	13	103
2005	32	83	21	136
2006	66	135	61	262
2007	104	153	75	332
2008	130	155	96	381
2009	3	2	3	8
Grand Total	**512**	**763**	**312**	**1587**

Table S2.2. Model summary for the linear relationship between cyanobacteria and TP in low and medium-high alkalinity lakes. The intercept is for medium-high alkalinity lakes.

Term	estimate	std.error	Statistic	*p* value
(Intercept)	-8.051	0.514	-15.652	<0.001
log(TP)	1.671	0.163	10.263	<0.001
AlkalinityType, low	0.560	0.630	0.889	0.374
log(TP):AlkalinityType, low	-0.762	0.226	-3.369	0.001

Table S2.3. ANOVA table for the difference in natural log cyanobacteria biovolume ($mm^3\ L^{-1}$) among lake types (n=8).

term	df	Sumsq	meansq	statistic	p.value
type	7	1245.059	177.8655	19.74748	<0.001
Residuals	486	4377.401	9.006998	NA	NA

6.1.3 Chapter 2 supplementary figures

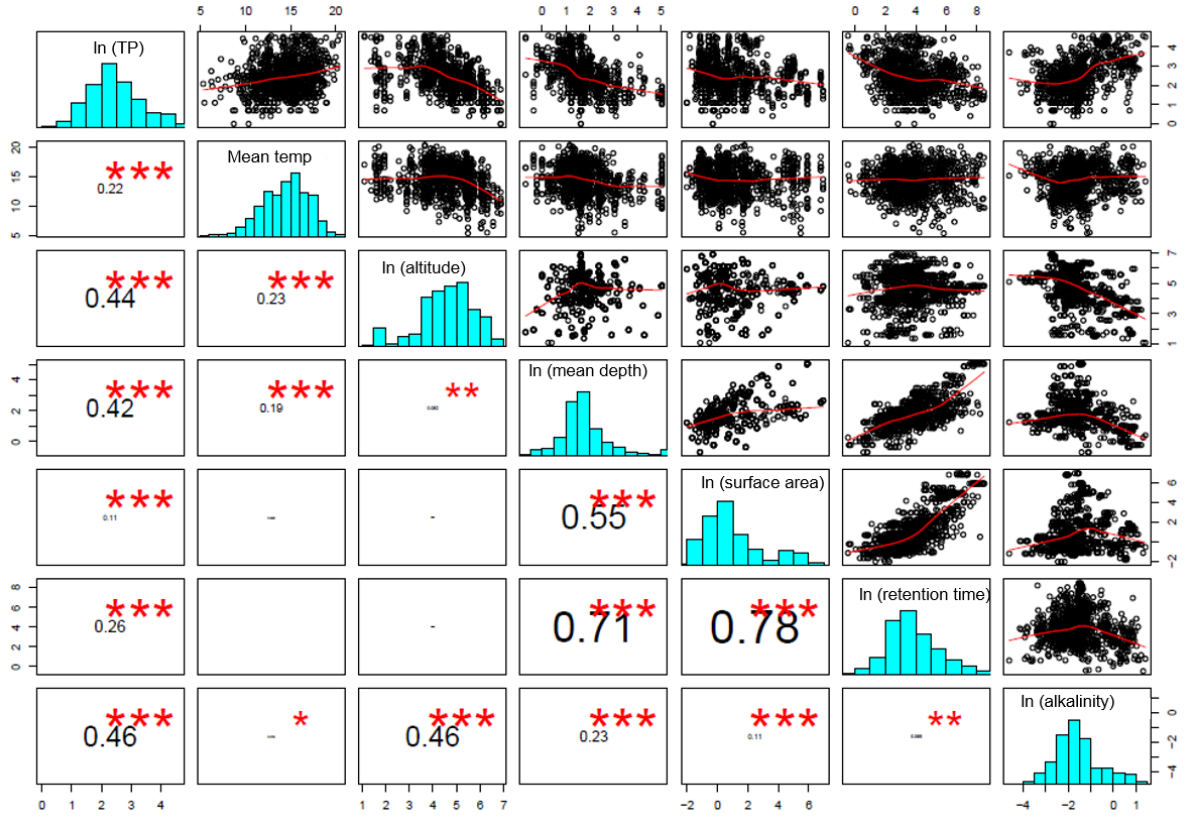


Fig. S2.1 Pair-wise plots showing the relationships between stressors (TP, temperature and retention time) and lake type variables (alkalinity, surface area, mean depth and altitude). The smooth red line in the upper diagonal panels shows the lowess (locally-weighted polynomial regression) fit, the middle diagonal plot shows a histogram of the distribution of the data and the lower diagonal panels shows the linear Pearson correlation coefficients – the size of the text is relative to the size of the correlation coefficient. Significance at the 0.05 level is denoted by *, at the 0.01 level by ** and <0.001 by ***. Relationships are for lakes in which TP was ≤ 100 µg L^{-1}. Where appropriate, variables were log transformed to make the distributions more symmetric.

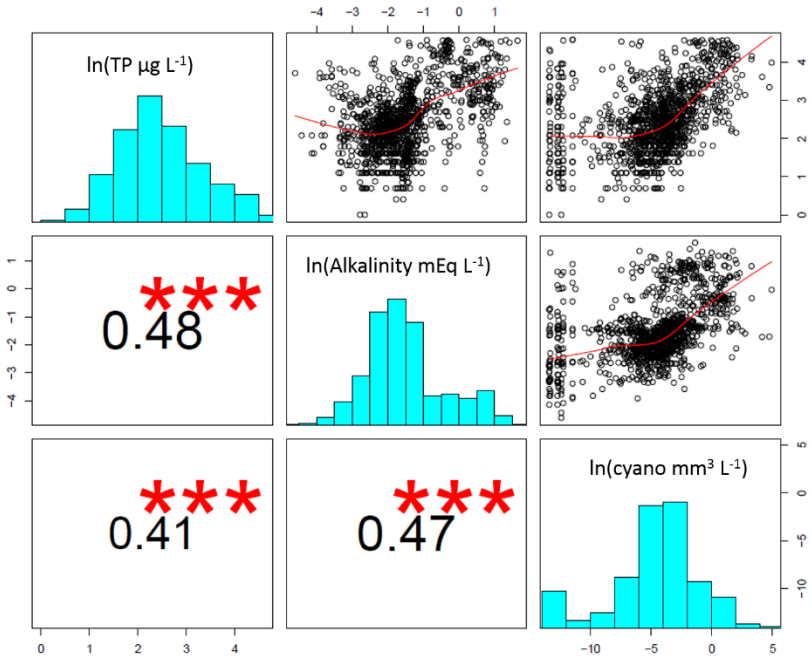


Fig. S2.2 Pair-wise plots showing the relationships between ln TP (µg L^{-1}), ln Alkalinity (mEq L^{-1}) and ln cyanobacteria (mm3 L^{-1}) (for 271 lakes, 1256 observations). The left horizontal panels show Pearson correlation coefficients and the *p* value associated with this relationship: ***, <0.001.

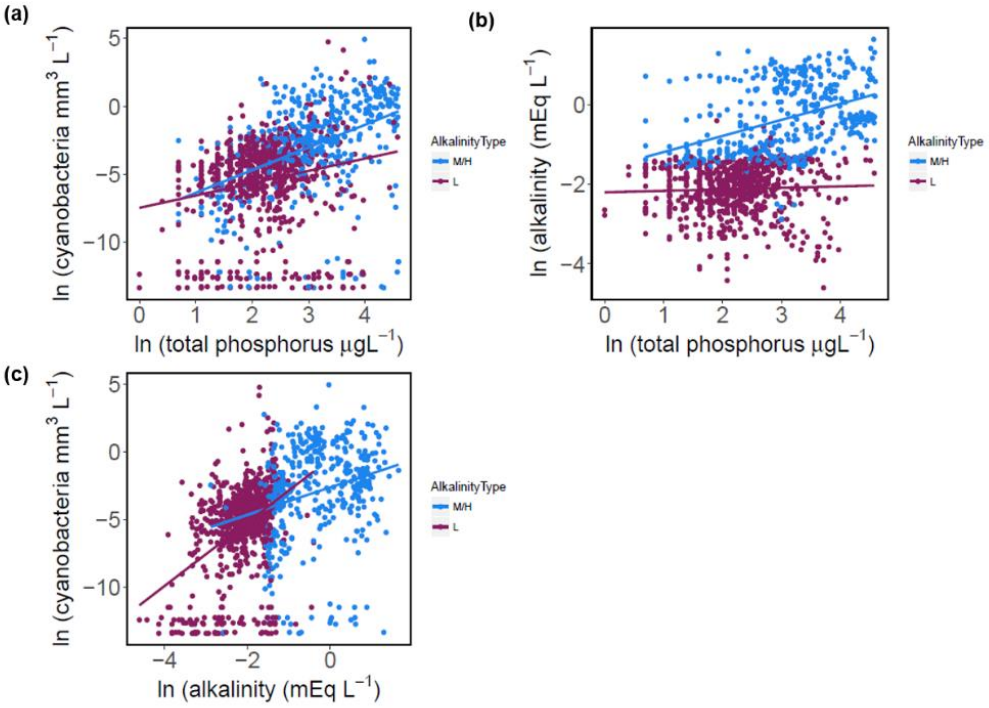


Fig. S2.3 Linear relationships between cyanobacteria, alkalinity and TP in low and medium-high alkalinity lakes. In (c) alkalinity shows some overlap over low and medium-high alkalinity lakes as the types are based on an average state whereas alkalinity is for a sampling date.

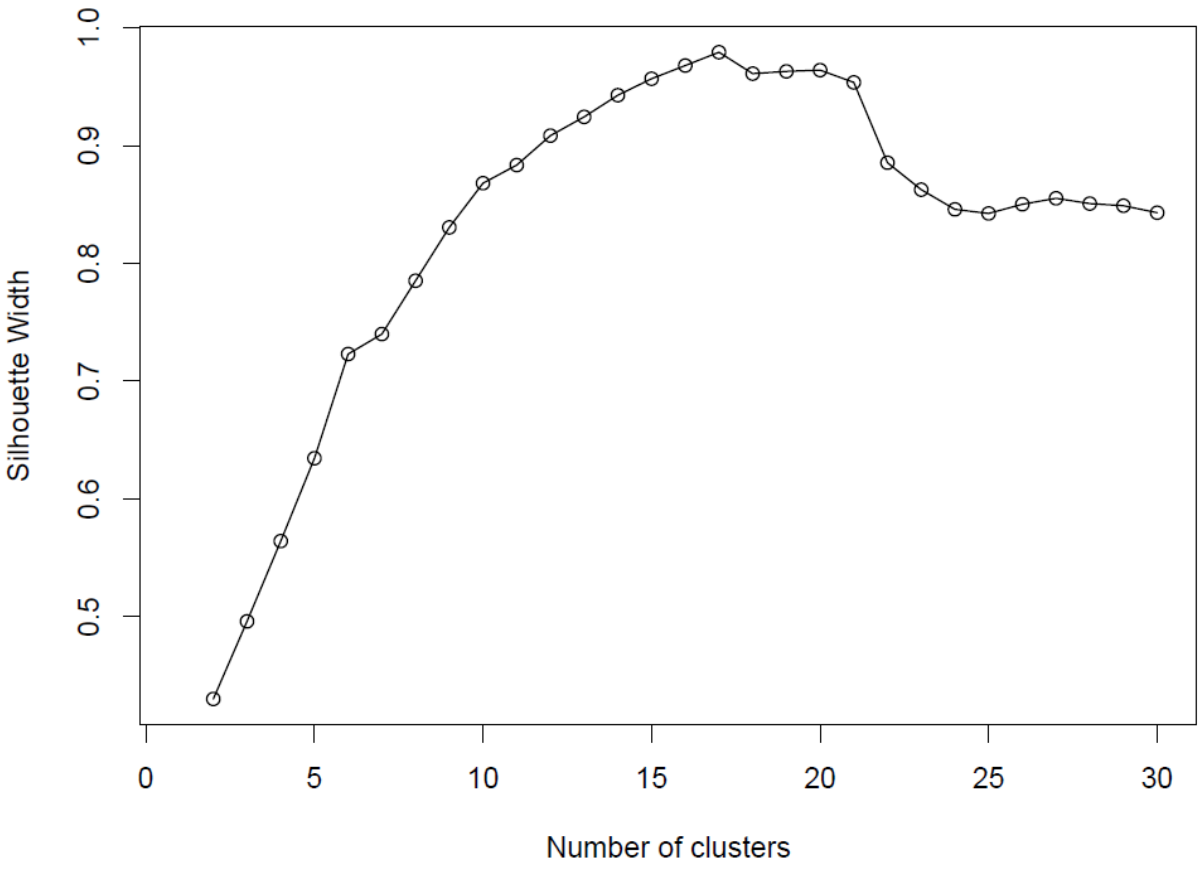
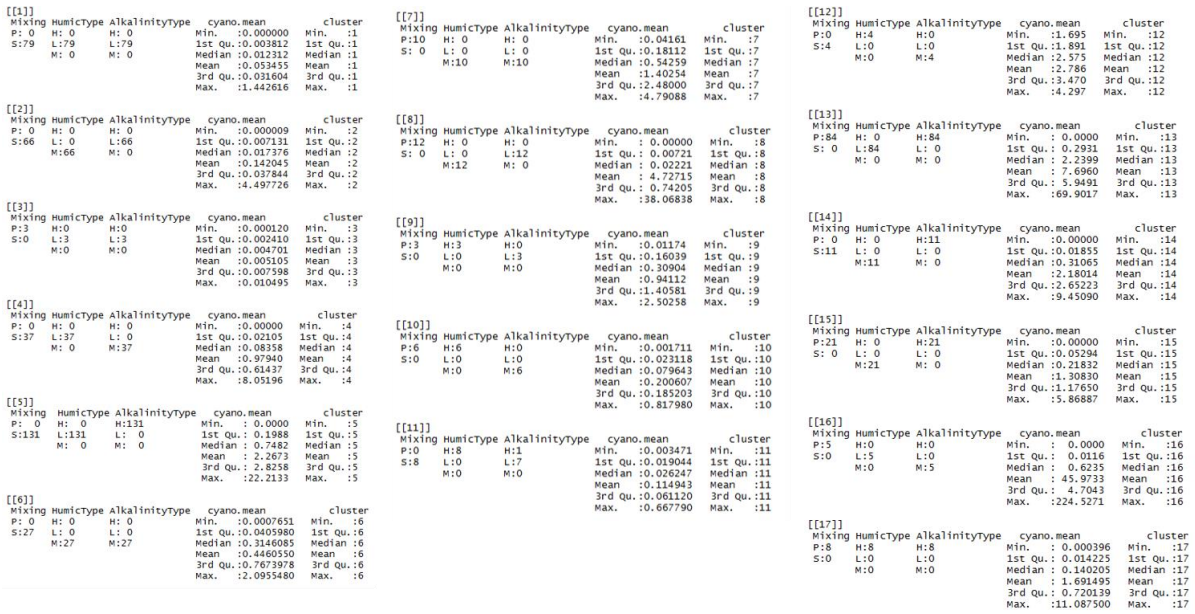


Fig. S2.4 Sillhoutte width for clusters ranging from 2-30 for the PAM algorithm. This suggests 17 clusters, based on the highest value being the best.



```
[[1]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P: 0   H: 0      H: 0             Min.   :0.000000   Min.   :1
 S:79   L:79     L:79             1st Qu.:0.003812   1st Qu.:1
        M: 0      M: 0            Median :0.012312   Median :1
                                  Mean   :0.053455   Mean   :1
                                  3rd Qu.:0.031604   3rd Qu.:1
                                  Max.   :1.442616   Max.   :1

[[2]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P: 0   H: 0      H: 0             Min.   :0.000009   Min.   :2
 S:66   L: 0      L:66             1st Qu.:0.007131   1st Qu.:2
        M:66      M: 0             Median :0.017376   Median :2
                                   Mean   :0.142045   Mean   :2
                                   3rd Qu.:0.037844   3rd Qu.:2
                                   Max.   :4.497726   Max.   :2

[[3]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:3    H:0       H:0             Min.   :0.000120   Min.   :3
 S:0    L:3       L:3             1st Qu.:0.002410   1st Qu.:3
        M:0       M:0             Median :0.004701   Median :3
                                   Mean   :0.005105   Mean   :3
                                   3rd Qu.:0.007598   3rd Qu.:3
                                   Max.   :0.010495   Max.   :3

[[4]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P: 0   H: 0      H: 0             Min.   :0.00000   Min.   :4
 S:37   L:37      L: 0             1st Qu.:0.02105   1st Qu.:4
        M: 0      M:37             Median :0.08358   Median :4
                                   Mean   :0.97940   Mean   :4
                                   3rd Qu.:0.61437   3rd Qu.:4
                                   Max.   :8.05196   Max.   :4

[[5]]
 Mixing  HumicType AlkalinityType   cyano.mean         cluster
 P:  0   H:  0     H:131           Min.   : 0.0000   Min.   :5
 S:131   L:131     L:  0           1st Qu.: 0.1988   1st Qu.:5
         M:  0     M:  0           Median : 0.7482   Median :5
                                   Mean   : 2.2673   Mean   :5
                                   3rd Qu.: 2.8258   3rd Qu.:5
                                   Max.   :22.2133   Max.   :5

[[6]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P: 0   H: 0      H: 0             Min.   :0.0007651   Min.   :6
 S:27   L: 0      L: 0             1st Qu.:0.0405980   1st Qu.:6
        M:27      M:27             Median :0.3146085   Median :6
                                   Mean   :0.4460550   Mean   :6
                                   3rd Qu.:0.7673978   3rd Qu.:6
                                   Max.   :2.0955480   Max.   :6

[[7]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:10   H: 0      H: 0             Min.   :0.04161   Min.   :7
 S: 0   L: 0      L: 0             1st Qu.:0.18112   1st Qu.:7
        M:10      M:10             Median :0.54259   Median :7
                                   Mean   :1.40254   Mean   :7
                                   3rd Qu.:2.48000   3rd Qu.:7
                                   Max.   :4.79088   Max.   :7

[[8]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:12   H: 0      H: 0             Min.   : 0.00000   Min.   :8
 S: 0   L: 0      L:12             1st Qu.: 0.00721   1st Qu.:8
        M:12      M: 0             Median : 0.02221   Median :8
                                   Mean   : 4.72715   Mean   :8
                                   3rd Qu.: 0.74205   3rd Qu.:8
                                   Max.   :38.06838   Max.   :8

[[9]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:3    H:3       H:0             Min.   :0.01174   Min.   :9
 S:0    L:0       L:3             1st Qu.:0.16039   1st Qu.:9
        M:0       M:0             Median :0.30904   Median :9
                                   Mean   :0.94112   Mean   :9
                                   3rd Qu.:1.40581   3rd Qu.:9
                                   Max.   :2.50258   Max.   :9

[[10]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:6    H:6       H:0             Min.   :0.001711   Min.   :10
 S:0    L:0       L:0             1st Qu.:0.023118   1st Qu.:10
        M:0       M:6             Median :0.079643   Median :10
                                   Mean   :0.200607   Mean   :10
                                   3rd Qu.:0.185203   3rd Qu.:10
                                   Max.   :0.817980   Max.   :10

[[11]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:0    H:8       H:1             Min.   :0.003471   Min.   :11
 S:8    L:0       L:7             1st Qu.:0.019044   1st Qu.:11
        M:0       M:0             Median :0.026247   Median :11
                                   Mean   :0.114943   Mean   :11
                                   3rd Qu.:0.061120   3rd Qu.:11
                                   Max.   :0.667790   Max.   :11

[[12]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:0    H:4       H:0             Min.   :1.695   Min.   :12
 S:4    L:0       L:0             1st Qu.:1.891   1st Qu.:12
        M:0       M:4             Median :2.575   Median :12
                                   Mean   :2.786   Mean   :12
                                   3rd Qu.:3.470   3rd Qu.:12
                                   Max.   :4.297   Max.   :12

[[13]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:84   H: 0      H:84             Min.   : 0.0000   Min.   :13
 S: 0   L:84      L: 0             1st Qu.: 0.2931   1st Qu.:13
        M: 0      M: 0             Median : 2.2399   Median :13
                                   Mean   : 7.6960   Mean   :13
                                   3rd Qu.: 5.9491   3rd Qu.:13
                                   Max.   :69.9017   Max.   :13

[[14]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P: 0   H: 0      H:11             Min.   :0.00000   Min.   :14
 S:11   L: 0      L: 0             1st Qu.:0.01855   1st Qu.:14
        M:11      M: 0             Median :0.31065   Median :14
                                   Mean   :2.18014   Mean   :14
                                   3rd Qu.:2.65223   3rd Qu.:14
                                   Max.   :9.45090   Max.   :14

[[15]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:21   H: 0      H:21             Min.   :0.00000   Min.   :15
 S: 0   L: 0      L: 0             1st Qu.:0.05294   1st Qu.:15
        M:21      M: 0             Median :0.21832   Median :15
                                   Mean   :1.30830   Mean   :15
                                   3rd Qu.:1.17650   3rd Qu.:15
                                   Max.   :5.86887   Max.   :15

[[16]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:5    H:0       H:0             Min.   : 0.0000   Min.   :16
 S:0    L:5       L:0             1st Qu.: 0.0116   1st Qu.:16
        M:0       M:5             Median : 0.6235   Median :16
                                   Mean   : 45.9733   Mean   :16
                                   3rd Qu.: 4.7043   3rd Qu.:16
                                   Max.   :224.5271   Max.   :16

[[17]]
 Mixing HumicType AlkalinityType   cyano.mean         cluster
 P:8    H:8       H:8             Min.   : 0.000396   Min.   :17
 S:0    L:0       L:0             1st Qu.: 0.014225   1st Qu.:17
        M:0       M:0             Median : 0.140205   Median :17
                                   Mean   : 1.691495   Mean   :17
                                   3rd Qu.: 0.720139   3rd Qu.:17
                                   Max.   :11.087500   Max.   :17
```

Fig. S2.5 Summary of Gower distance clustering based on 17 clusters.

```
[[1]]
 Mixing HumicType AlkalinityType   cyano.mean          cluster
 P: 3   H: 1      H: 0           Min.   :0.000000   Min.   :1
 S:80   L:82      L:83           1st Qu.:0.003812   1st Qu.:1
        M: 0      M: 0           Median :0.011164   Median :1
                                 Mean   :0.051176   Mean   :1
                                 3rd Qu.:0.031210   3rd Qu.:1
                                 Max.   :1.442616   Max.   :1

[[2]]
 Mixing HumicType AlkalinityType   cyano.mean          cluster
 P: 9   H: 6      H: 4           Min.   :0.000000   Min.   :2
 S:76   L: 0      L:81           1st Qu.:0.006308   1st Qu.:2
        M:79      M: 0           Median :0.018077   Median :2
                                 Mean   :0.123806   Mean   :2
                                 3rd Qu.:0.033181   3rd Qu.:2
                                 Max.   :4.497726   Max.   :2

[[3]]
 Mixing HumicType AlkalinityType   cyano.mean          cluster
 P: 3   H: 0      H: 0           Min.   :0.00000   Min.   :3
 S:37   L:40      L: 0           1st Qu.:0.01925   1st Qu.:3
        M: 0      M:40           Median :0.06868   Median :3
                                 Mean   :0.92182   Mean   :3
                                 3rd Qu.:0.61666   3rd Qu.:3
                                 Max.   :8.05196   Max.   :3

[[4]]
 Mixing  HumicType AlkalinityType   cyano.mean          cluster
 P:  0   H:  0     H:136          Min.   : 0.0000   Min.   :4
 S:136   L:131     L:  0          1st Qu.: 0.2082   1st Qu.:4
         M:  5     M:  0          Median : 0.8128   Median :4
                                  Mean   : 2.3564   Mean   :4
                                  3rd Qu.: 3.0722   3rd Qu.:4
                                  Max.   :22.2133   Max.   :4

[[5]]
 Mixing HumicType AlkalinityType   cyano.mean          cluster
 P: 5   H: 4      H: 1           Min.   :0.000765   Min.   :5
 S:32   L: 0      L: 0           1st Qu.:0.067879   1st Qu.:5
        M:33      M:36           Median :0.401012   Median :5
                                 Mean   :0.991770   Mean   :5
                                 3rd Qu.:1.393035   3rd Qu.:5
                                 Max.   :4.790878   Max.   :5

[[6]]
 Mixing HumicType AlkalinityType   cyano.mean          cluster
 P:29   H: 0      H:22           Min.   : 0.00000   Min.   :6
 S: 1   L: 0      L: 3           1st Qu.: 0.07574   1st Qu.:6
        M:30      M: 5           Median : 0.22096   Median :6
                                 Mean   : 2.83318   Mean   :6
                                 3rd Qu.: 2.29075   3rd Qu.:6
                                 Max.   :38.06838   Max.   :6

[[7]]
 Mixing HumicType AlkalinityType   cyano.mean          cluster
 P:17   H:18      H:9            Min.   : 0.000396   Min.   :7
 S: 1   L: 0      L:3            1st Qu.: 0.008655   1st Qu.:7
        M: 0      M:6            Median : 0.079643   Median :7
                                 Mean   : 0.975691   Mean   :7
                                 3rd Qu.: 0.355116   3rd Qu.:7
                                 Max.   :11.087500   Max.   :7

[[8]]
 Mixing HumicType AlkalinityType   cyano.mean          cluster
 P:86   H: 0      H:84           Min.   :  0.0000   Min.   :8
 S: 0   L:86      L: 0           1st Qu.:  0.3299   1st Qu.:8
        M: 0      M: 2           Median :  2.4152   Median :8
                                 Mean   : 10.1825   Mean   :8
                                 3rd Qu.:  6.0174   3rd Qu.:8
                                 Max.   :224.5271   Max.   :8
```

Fig. S2.6 Summary of Gower distance clustering based on 8 clusters.

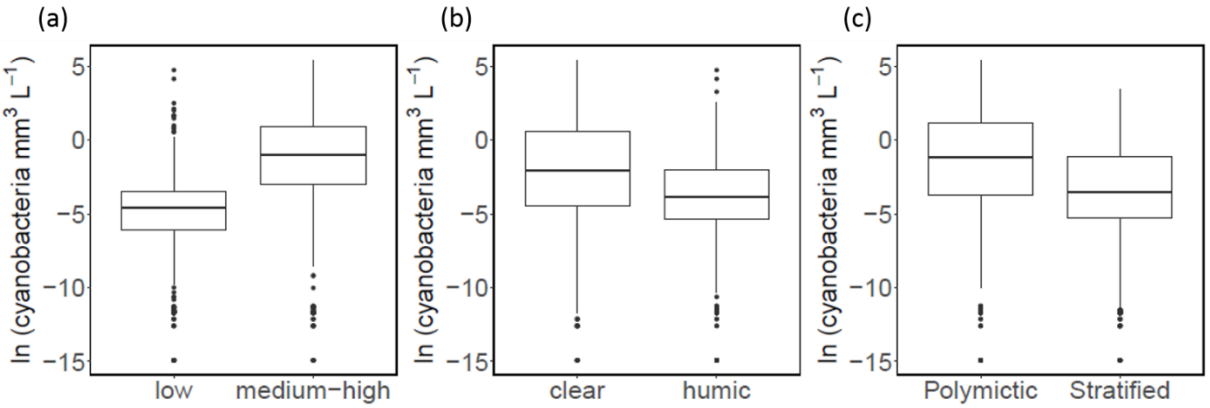


Fig. S2.7 Natural log cyanobacteria ($mm^3\ L^{-1}$) by lake type variables: (a) alkalinity, low (<0.2 mEq L^{-1}) and med-high (>0.2 mEq L^{-1}); (b) humic content, clear (colour <30 mg Pt L^{-1}) and humic (colour > 30 mg Pt L^{-1}); and (c) mixing type, stratified and polymictic. The biovolume of cyanobacteria was statistically significantly different, between levels of each lake type variable: alkalinity (low *vs* med-high alkalinity, t = -22.5, df = 1574, p <0.001); humic (clear *vs* humic, t = 7.78, df = 1579.8, p <0.001) and mixing type (stratified *vs* polymictic, t = -7.03, df = 600.97, p <0.001).

(a)

$groups

	log(mean.cyano)	groups
P.MH.C	-0.4528631	a
S.MH.C	-0.9668599	ab
S.MH.H	-1.7282288	ab
P.MH.H	-2.0502809	ab
P.L.H	-3.2630892	bc
S.L.H	-4.0905860	c
S.L.C	-4.6013979	c
P.L.C	-6.3101176	c

(b)

$groups

	log(cyano)	groups
P.MH.C	-0.8396646	a
S.MH.C	-1.2453362	a
S.MH.H	-1.9033005	ab
P.MH.H	-2.3659652	ab
P.L.H	-4.4983021	bc
S.L.H	-4.6188304	c
S.L.C	-5.0810253	c
P.L.C	-5.8528377	c

(c)

$groups

	log(cyano)	groups
P.MH.C	-0.9716285	a
S.MH.C	-1.2039664	a
S.MH.H	-2.1653940	ab
P.MH.H	-2.4781575	abc
P.L.H	-3.9599952	bcd
S.L.H	-4.2827655	cd
S.L.C	-5.2312347	d
P.L.C	-8.4611072	d

(d)

$groups

	log(cyano)	groups
P.MH.C	-0.9562831	a
S.MH.C	-1.1311709	a
S.MH.H	-2.3868179	ab
P.MH.H	-2.7752994	ab
P.L.H	-3.1097218	abc
S.L.H	-4.4231873	bc
S.L.C	-4.9422555	c
P.L.C	-7.2717922	c

(e)

$groups

	log(cyano)	groups
P.MH.C	-1.195927	a
S.MH.C	-1.288818	a
S.MH.H	-2.201160	ab
P.MH.H	-2.305551	ab
S.L.H	-4.287419	bc
P.L.H	-4.625247	bc
S.L.C	-5.133081	c
P.L.C	-8.461107	c

(f)

$groups

	log(cyano)	groups
P.MH.C	-0.7981028	a
S.MH.C	-1.1781571	a
S.MH.H	-1.9816787	a
P.MH.H	-2.5716012	a
S.L.H	-4.5196162	b
P.L.H	-4.6974963	b
S.L.C	-5.2220903	b
P.L.C	-5.8528377	b

(g)

$groups

	log(cyano)	groups
P.MH.C	-1.055097	a
S.MH.C	-1.220338	a
S.MH.H	-2.094450	a
P.MH.H	-2.137030	a
S.L.H	-4.699542	b
P.L.H	-4.875066	b
S.L.C	-5.001715	b
P.L.C	-9.880062	b

(h)

$groups

	log(cyano)	groups
P.MH.C	-0.6939997	a
S.MH.C	-1.2006531	a
S.MH.H	-2.0297497	a
P.MH.H	-2.3543383	a
S.L.H	-4.4106906	b
S.L.C	-5.1890980	b
P.L.H	-5.4768819	b
P.L.C	-7.2717922	b

(i)

$groups

	log(cyano)	groups
P.MH.C	-1.008945	a
S.MH.C	-1.259150	a
S.MH.H	-2.127377	a
P.MH.H	-2.239574	a
S.L.H	-4.627730	b
S.L.C	-5.261345	b
P.L.H	-5.338172	b
P.L.C	-5.852838	b

(j)

$groups

	log(cyano)	groups
P.MH.C	-0.9766831	a
S.MH.C	-1.1108065	a
S.MH.H	-2.2444006	a
P.MH.H	-2.5247675	a
S.L.H	-4.5882614	b
P.L.H	-4.9389261	b
S.L.C	-5.0403581	b
P.L.C	-9.8800617	b

(k)

$groups

	log(cyano)	groups
P.MH.C	-0.8868933	a
S.MH.C	-1.3977981	a
S.MH.H	-2.2902588	a
P.MH.H	-2.4548128	a
S.L.H	-4.7297934	b
S.L.C	-5.0440078	b
P.L.H	-5.0506019	b
P.L.C	-8.4611072	b

Fig. S2.8 Similarities in ln cyanobacertia biovolume (mm3 L^{-1}) among Gower lake types. Groupings are from Tukey test's for multiple comparison following an ANOVA (Table S3): (a) are groupings from a comparison of mean cyanobacteria for each lake, (b-k) are groupings based on one observation selected per lake (to meet the assumptions of independence), these were randomly selected 10 times.

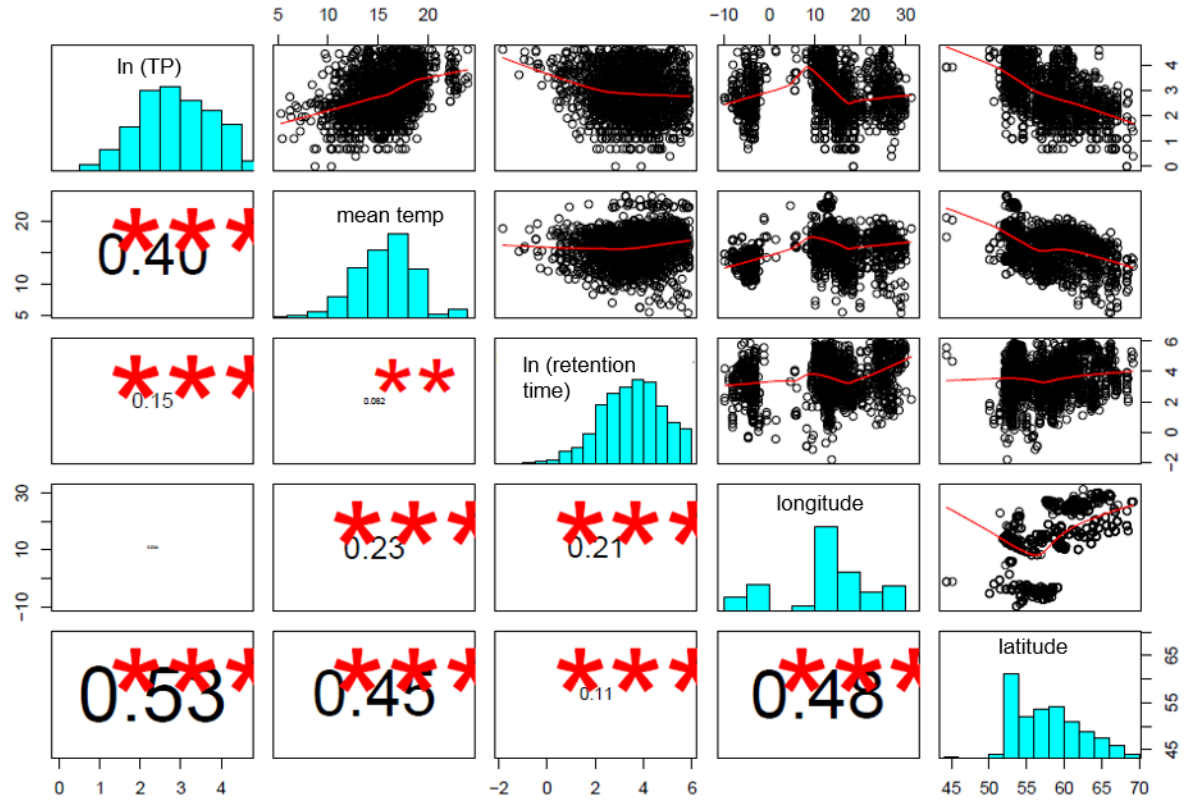


Fig. S2.9 Relationships between stressors (TP, temperature and retention time), longitude and latitude. The smooth red line in the upper diagonal panels shows the lowess (locally-weighted polynomial regression) fit, the middle diagonal plot shows a histogram of the distribution of the data and the lower diagonal panels shows the linear Pearson correlation coefficients – the size of the text is relative to the size of the correlation coefficient. Significance at the 0.05 level is denoted by *, at the 0.01 level by ** and <0.001 by ***. Relationships are for lakes in which TP was ≤ 100 µg L^{-1} and retention time was ≤365 days. Where appropriate, variables were log transformed to make the distributions more symmetric.

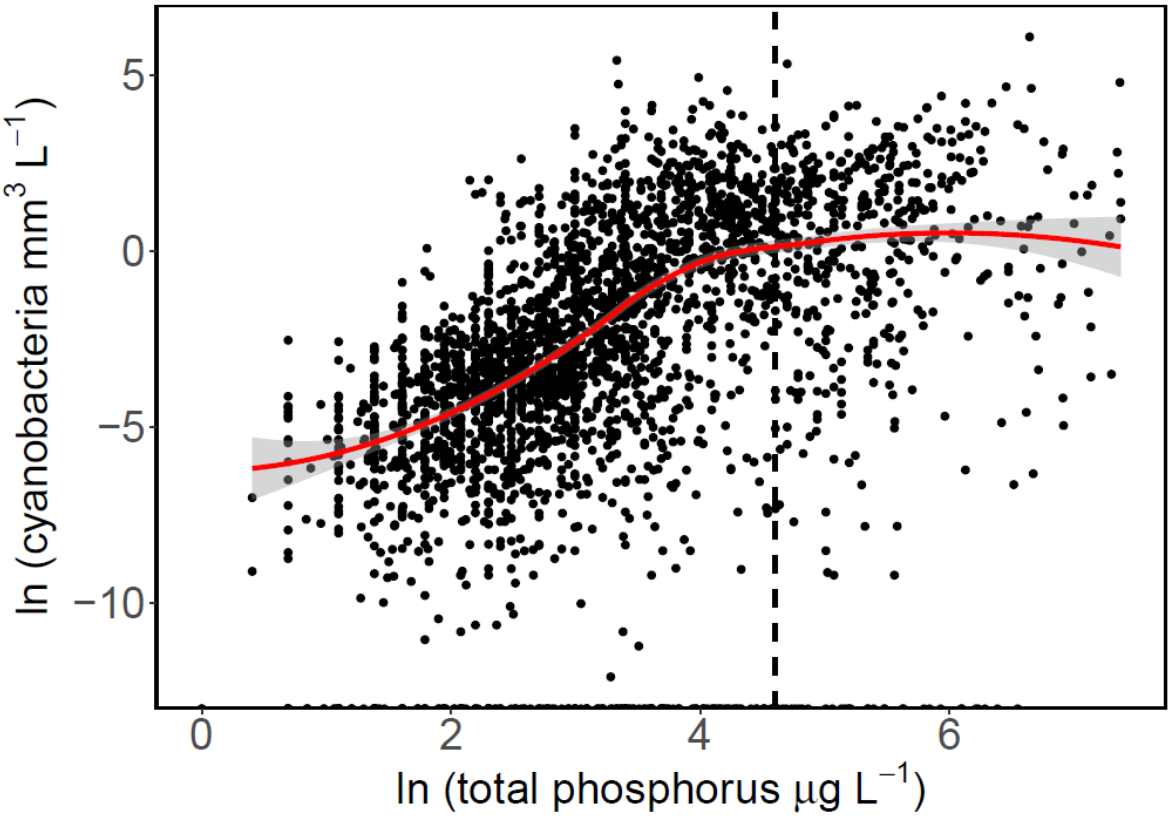


Fig. S2.10 Relationship between average monthly natural log cyanobacteria biovolume (mm^3 L^{-1}) and average monthly ln total phosphorus (µg L^{-1}) using the global dataset (n = 572 lakes, number of monthly observations = 2900). The red curve shows the smoothed area response of cyanobacteria. Smoothing was fitted using locally weighted polynomial regression (LOESS), the grey shaded area shows 95% confidence intervals. The dashed black line shows the TP concentration which we restricted the regression analysis to (≤100 µg L^{-1}).

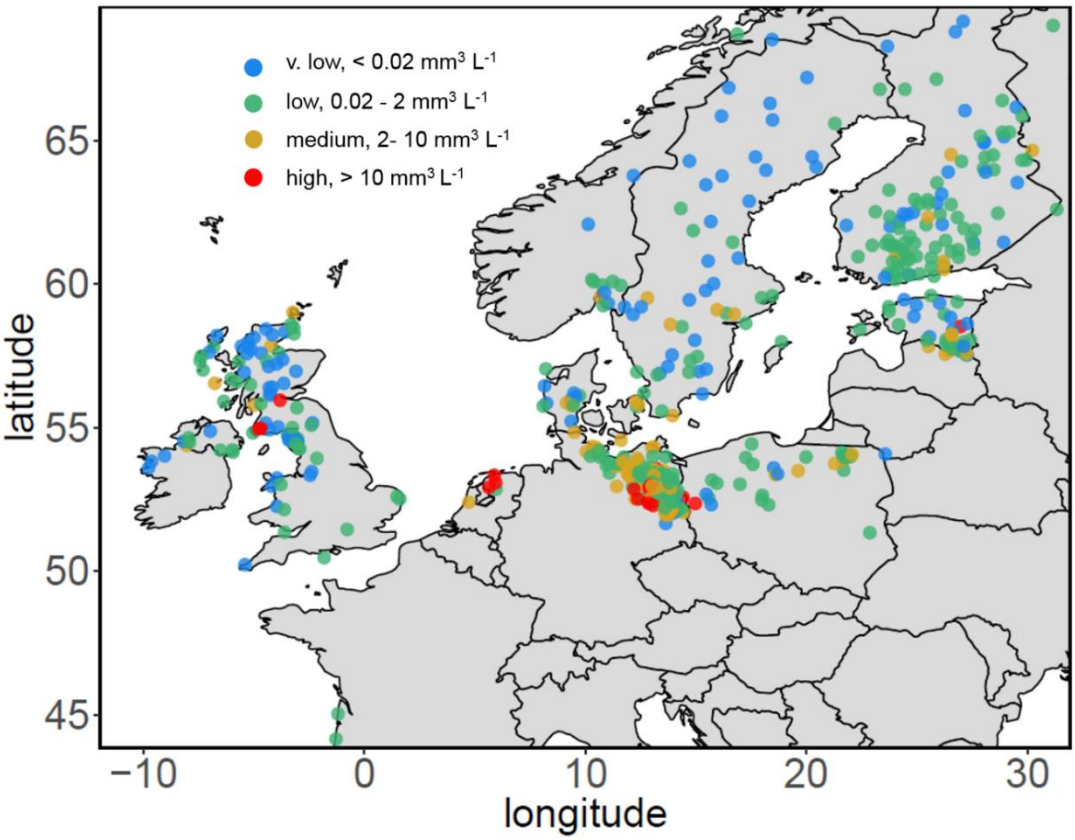


Fig. S2.11 Categories of average cyanobacteria biovolume ($mm^3\ L^{-1}$) in lakes included in the study (n = 494). Categories are based on World Health Organisation (WHO) recommended threshold values for drinking and bathing (Chorus & Bartram, 1999).

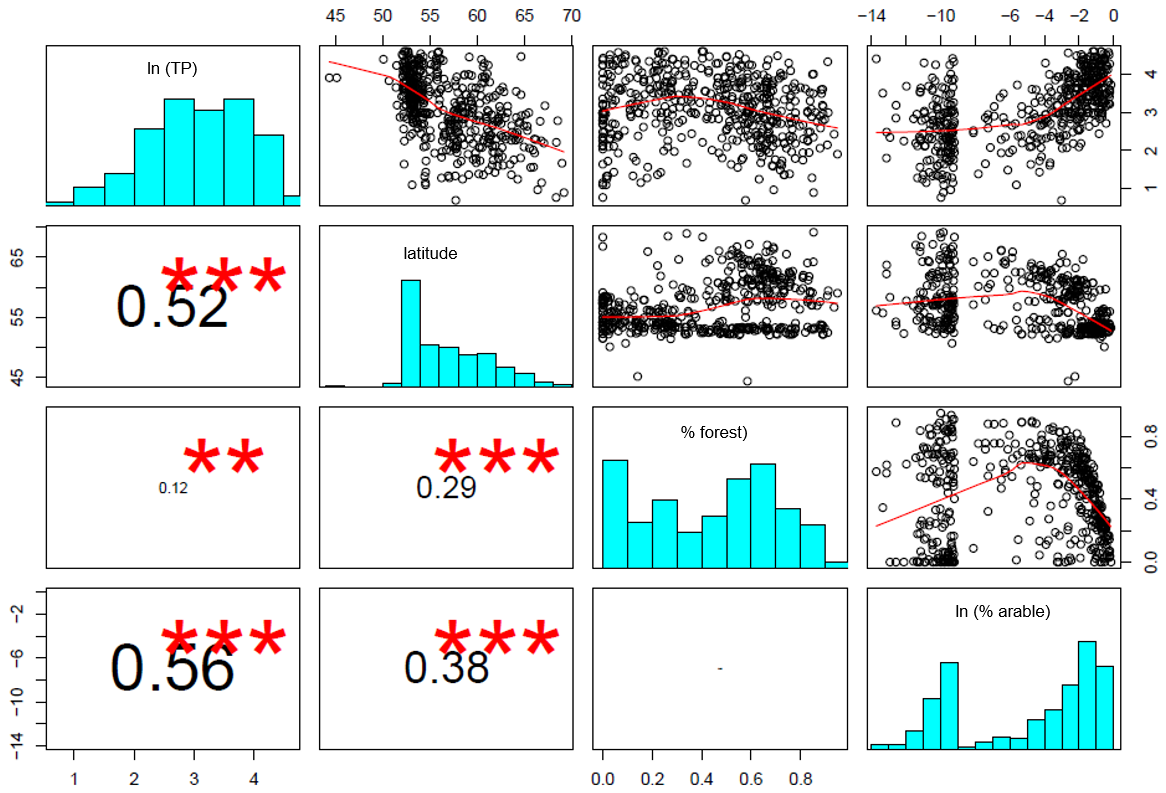


Fig S2.12 Relationships between TP, latitude, percent catchment forest land cover and percent catchment arable land cover. The smooth red line in the upper diagonal panels shows the lowess (locally-weighted polynomial regression) fit, the middle diagonal plot shows a histogram of the distribution of the data and the lower diagonal panels shows the linear Pearson correlation coefficients – the size of the text is relative to the size of the correlation coefficient. Significance at the 0.05 level is denoted by *, at the 0.01 level by ** and <0.001 by ***. Relationships are for lakes in which TP was ≤ 100 µg L^{-1} and retention time was ≤365 days. Where appropriate, variables were log transformed to make the distributions more symmetric. Note that a constant was added to percentage arable land so that the data could be log transformed, these were sampled from a random generation of data from the distribution of percentage arable land data.

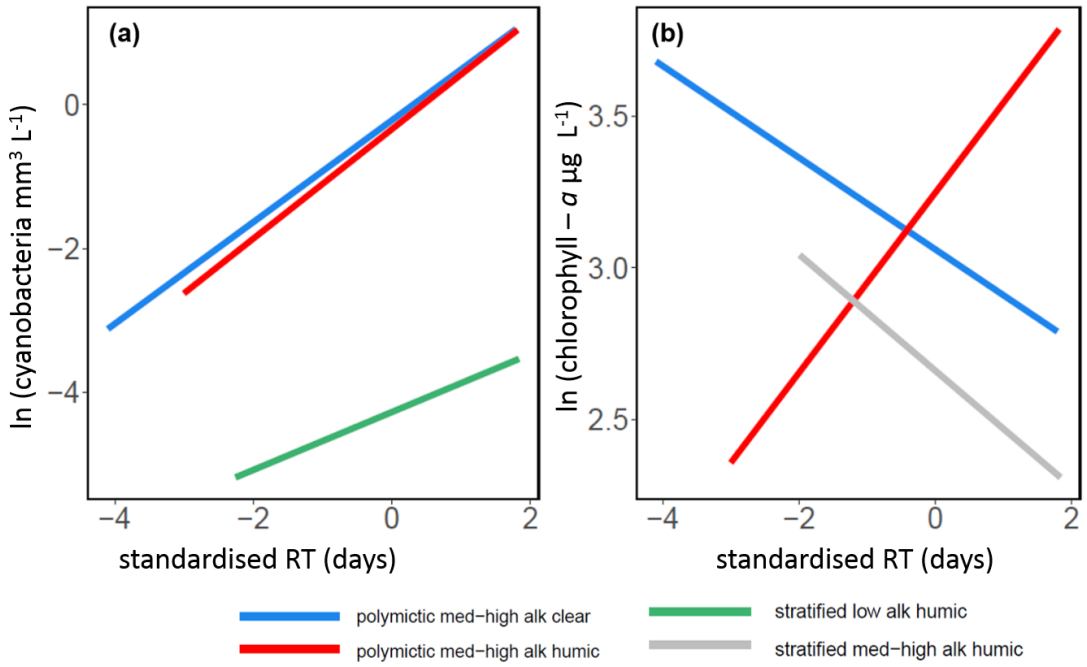


Fig. S2.13 The effect of retention time on (a) ln cyanobacteria biovolume (mm^3 L^{-1}) and (b) ln chlorophyll a (µg L^{-1}) for the lake types which retention time effects were statistically significant. The effects of retention time are fitted from the models presented in Table 2, keeping temperature and TP constant (for models where this applies). Retention time (days) is standardised (mean centred and divided by the standard deviation).

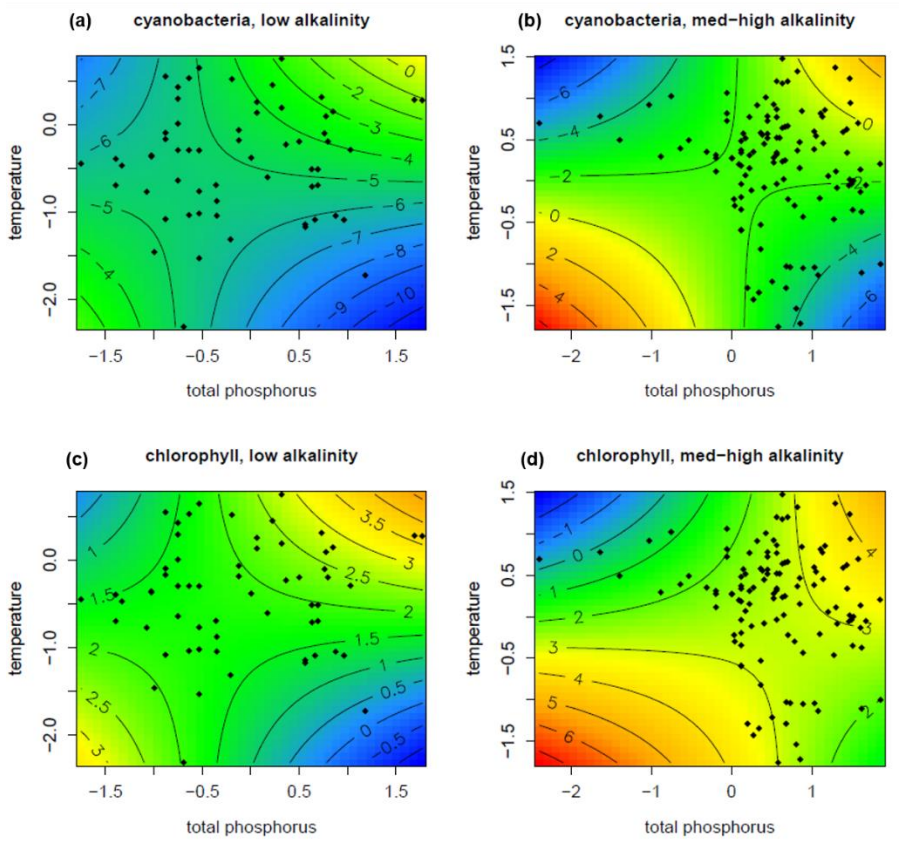


Fig. S2.14 Response of ln cyanobacteria biovolume (mm^3 L^{-1}) and ln chlorophyll a (µg L^{-1}) to the interaction between standardised temperature (°C), and standardised total phosphorus (µg L^{-1}) in polymictic, low alkalinity humic lakes (a and c) and polymictic medium-high alkalinity humic lakes (b and d). Temperature and total phosphorus are standardised (mean centred and divided by their standard deviation). Contour lines show the range of the response, colours show comparative differences: cooler colours are lower responses, warmer colours are higher responses. Points show the underlying data driving the model.

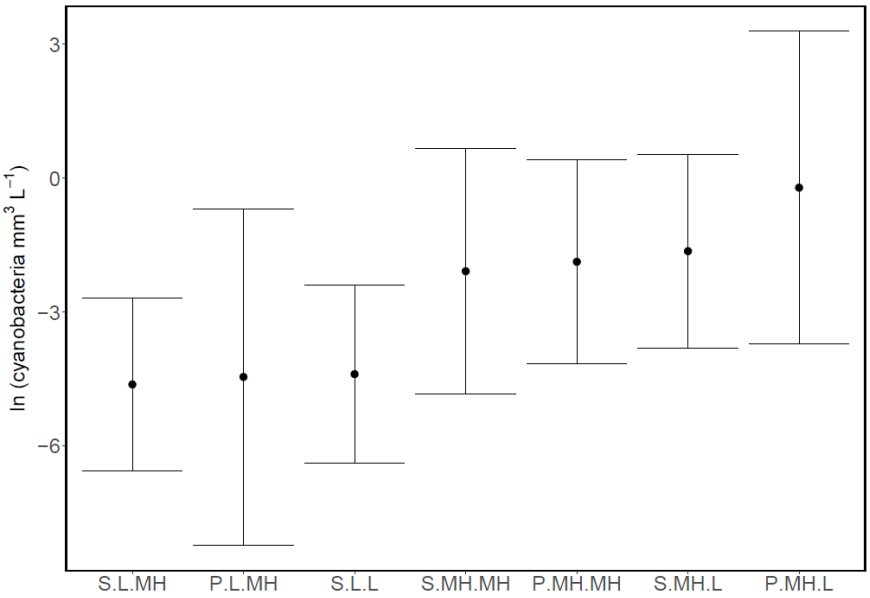


Fig. S2.15 Variance of the random effect for each lake type model. The point shows the intercept for each model and lake types are ordered from lowest to highest intercept. S.L.MH, stratified, low alkalinity, humic; P.L.MH, polymictic, low alkalinity, humic; S.L.L, stratified, low alkalinity, clear; S.MH.MH, stratified, medium-high alkalinity, humic; P.MH.MH, polymictic, medium-high alkalinity, humic; S.MH.L, stratified, medium-high alkalinity, clear; P.MH.L, polymictic, medium-high alkalinity, clear.

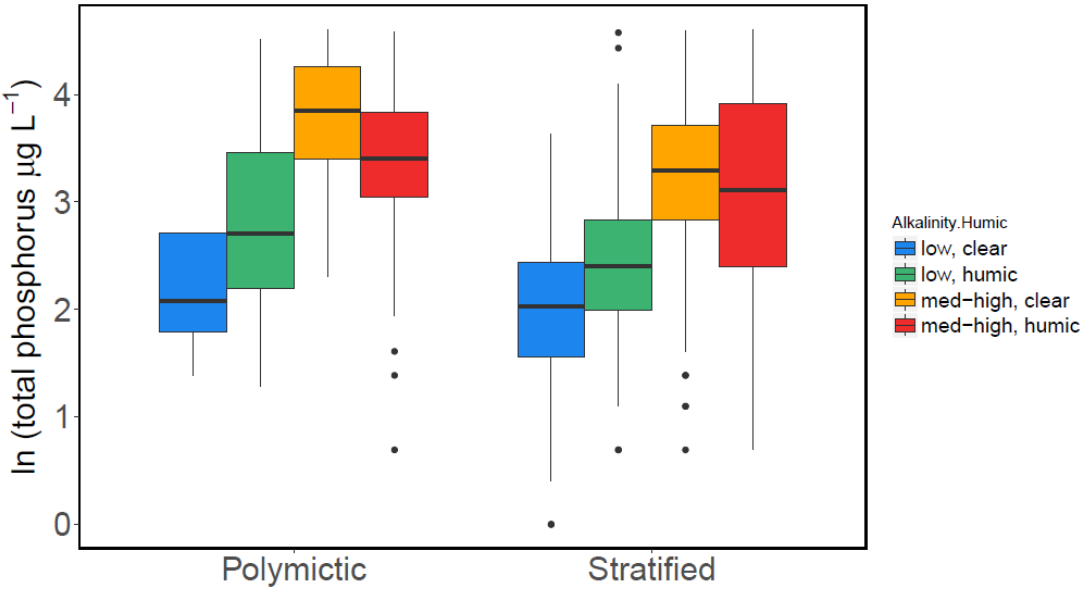


Fig. S2.16 Natural log total phosphorus (µg L^{-1}) by lake type. Lake type are combinations of: alkalinity, low (<0.2 mEq L^{-1}) and med-high (>0.2 mEq L^{-1}); humic content, clear (colour <30 mg Pt L^{-1}) and humic (colour > 30 mg Pt L^{-1}); and mixing type, stratified and polymictic.

6.2 Chapter 3 supplementary material

6.2.1 Chapter 3 supplementary analysis

Estimates of nutrient loss through extreme rainfall events.

To calculate the amount of nutrients lost during flushing events we used the following formula to calculate the dilution curve:

$$\frac{nutrient\ concentration\ at\ time\ n\ (minutes)}{initial\ volume} \times (initial\ volme - flow\ rate)$$

in which the initial volume was 3000 L and the flow rate was calculated from the time it took to pump 1500 L of water into the mesocosm; we used two different pumps, one with a flow rate of 71 L minute^{-1} and the other with a flow rate of 100 L minute^{-1}. Nutrient concentrations were calculated after one minute of the given flow rate from initial nutrient concentrations (µg mL^{-1}) within the mesocosm, these updated concentrations were then used to iteratively calculate the concentration after each successive minute until the total minutes of flushing was reached for each respective pump (15 minutes and 21 minutes). Initial nutrient concentrations were estimated from the preceding sampling event. The difference in nutrient concentrations between the initial concentration and the concentration lost was replaced to each treated mesocosm (after subtracting nutrient concentrations recorded in the water used for flushing).

Light attenuation analysis

A potential explanation for the antagonistic interaction between warming and nutrient enrichment is that potential treatment effects on phytoplankton composition could result in greater self-shading. To explore this we explored potential differences in light attenuation among treatments.

A light attenuation coefficient (k m^{-1}) was calculated from mean daily measurements of PAR (photosynthetically active radiation) using the following equation:

$$\mathrm{k\ m^{-1}} = \frac{\ln\left(\frac{\mathrm{PAR_{air}}}{\mathrm{PAR_{mesocosm}}}\right)}{0.45}$$

PAR was recorded every minute by sensors located 40cm horizontally and vertically (mid-depth) within each mesocosm. K was higher in nutrient enriched mesocosms (Fig. S8, S9), in which attenuation from algae was higher (because of higher biomass in these mesocosms, main text Fig. 2-3) however K was no higher in warmed mesocosms than ambient mesocosms or in warmed x nutrient enriched mesocosms. This indicates that the mechanisms of the antagonism is not light limitation through increased self-shading.

6.2.2 Chapter 3 supplementary tables

Table S3.1. Three way ANOVA of between treatment differences in chlorophyll-*a* at the first time point of the experiment.

Treatment	Df	Sum Sq	Mean Sq	F value	Pr(>F)
nutrient addition	1	583	583.2	0.48	0.50
Flushed	1	4	4.3	0.00	0.95
Warming	1	2817	2817.5	2.31	0.14
nutrient enriched x flushed	1	84	84.1	0.07	0.80
nutrient enriched x warming	1	7	6.9	0.00	0.94
flushed x warming	1	1115	1114.6	0.91	0.35
nutrient enriched x flushed x warming	1	133	1222.1	0.11	0.74

Table S3.2. Paired t-test - total chlorophyll-*a* and cyanobacteria chlorophyll-*a* concentration (μg L^{-1}) before and after flushing.

Variable	estimate	statistic	*p*-value	parameter	conf.low	conf.high
Cyanobacteria chlorophyll-*a*	23.46	2.97	**0.006**	31	7.36	39.56
Chlorophyll-*a*	142.91	2.67	**0.01**	31	33.93	251.90

Table S3.3. Percent (%) cyanobacteria genus biovolume of total cyanobacterial biovolume composition from four sampling events (May 5th, June 3rd, July 29th and August 26th 2015).

Order	*genus*	% biovolume
Nostocales		68
	Dolichospermum	*17*
	Aphanizomenon	*51*
Oscillatoriales		14
	Oscillatoria	*<0.1*
	Pseudanabaena	*13*
	Limnothrix	*0.4*
Chroococcales		18
	Aphanothece	*0.6*
	Cyanodictyon	*4*
	Microcystis	*13*
	Unidentified	*0.2*

6.2.3 Chapter 3 supplementary figures

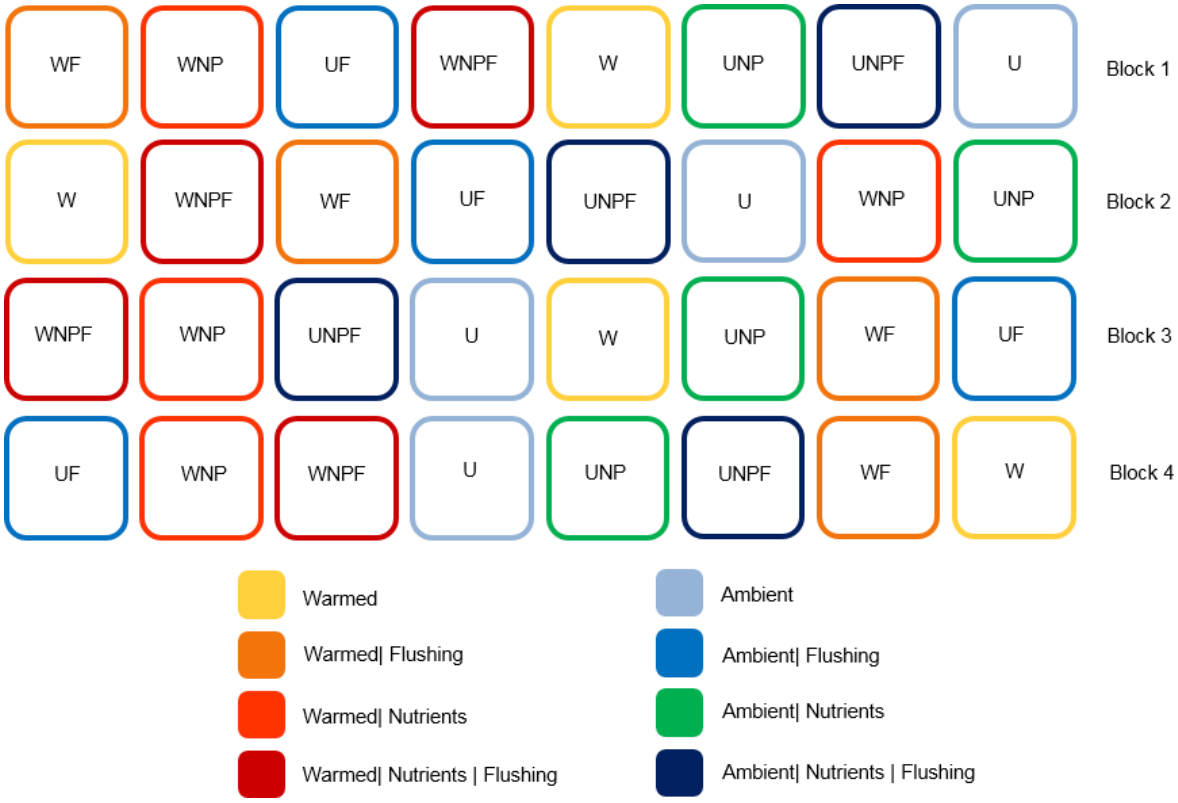


Fig. S3.1 Schematic of the experimental set-up. There were eight treatments in total, as represented by the different colours. Each treatment was repeated four times, one replicate randomly assigned to a mesocosm in each experimental block. U = unheated/ambient, W = warmed, N = nutrient enriched, F = flushed e.g. UNPF = unheated, nutrient enriched, flushed.

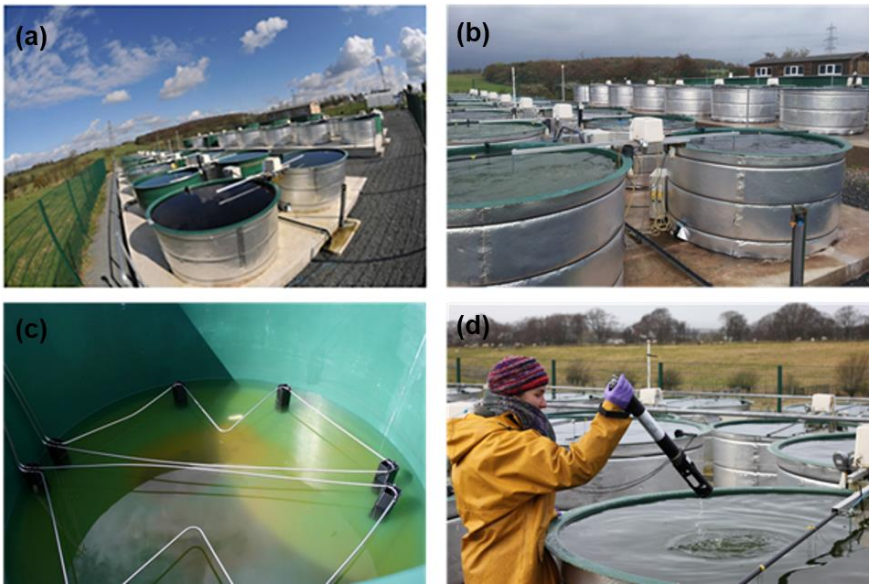


Fig. S3.2 Images of the mesocosm facility. The mesocosms are organised into four experimental blocks of eight mesocosms (a) and (b). Each mesocosm has a mechanical mixing system (white extended arm), a power supply (white box) for the heating system and a heating element (c) which sits above the sediment (the sediment is no shown in this image). Measurements of cyanobacteria chlorophyll-*a*, turbidity, pH and conductivity were measured from the middle of each mesocosm using submersible sondes. In (d) cyanobacteria chlorophyll-*a* μg L^{-1} is being measured using fluorescence, using a bbe Moldaenke Algae torch.

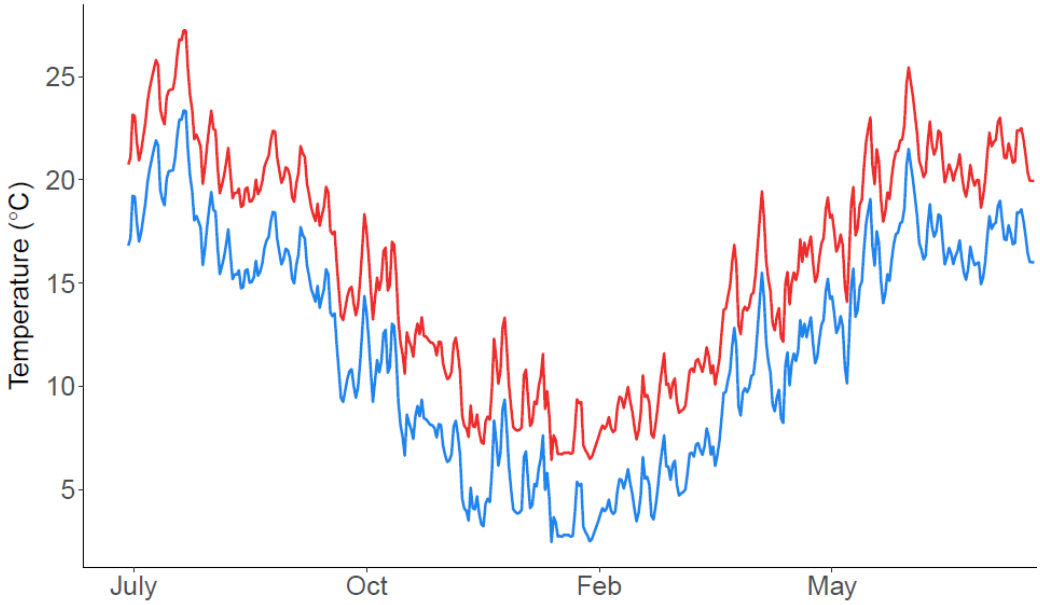


Fig. S3.3 Mean daily water temperatures (°C) in mesocosms between July 2014 and August 2015 in 16 mesocosms at ambient temperature (blue) and 16 mesocosms warmed to 4°C above ambient (red).

Fig. S3.4 Image showing mixers which are suspended in the middle of each mesocosm and move up and down to allow disruption of thermal stratification. The top and bottom of the mixers are hollow to increase mixing.

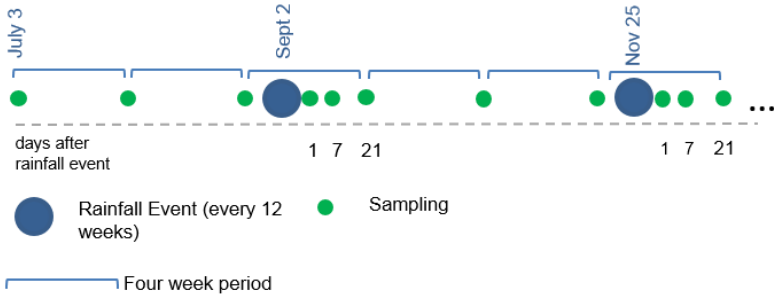


Fig. S3.5 Schematic of the sampling schedule. Water samples were taken once every four weeks (regular sampling, green circles), expect for when extreme rainfall events (blue circles) occurred, then sampling occurred the day after the event, one week after the event and three weeks after the event, then returning to a four weekly schedule. The schema shows sampling events from the start of the experiment in July 2014 until three weeks after the second rainfall event. This schedule was repeated until the last sampling event on the 26th of August, 2015.

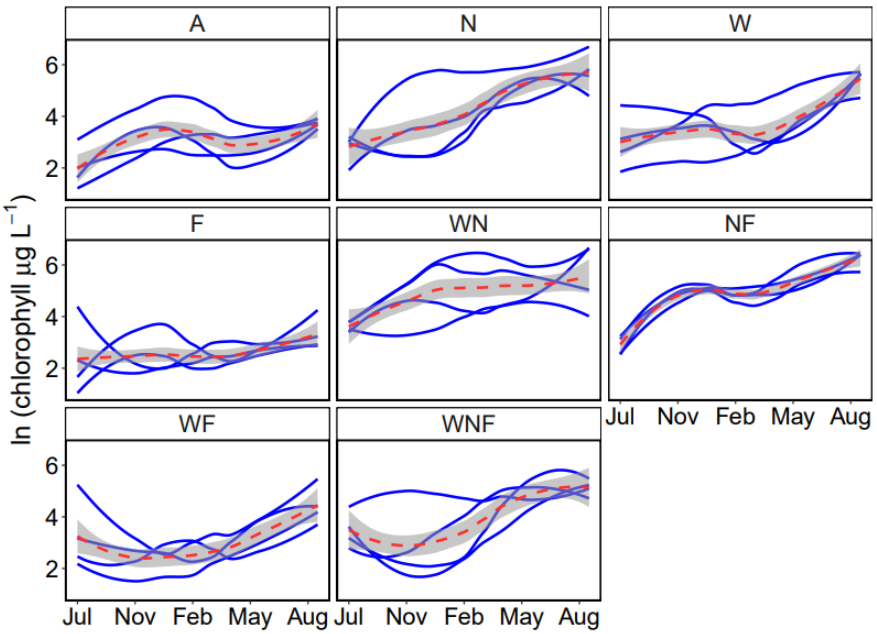


Fig. S3.6 Variation in log mean chlorophyll-a (µg L^{-1}) within and between treatments between July 2014 and August 2015. The dashed red line is the smoothed average response of chlorophyll-a in each treatment, the blue line is the smoothed response for each replicate within treatments. Smoothing is fitted using locally weighted polynomial regression (LOESS), the shaded area shows 95% confidence intervals. Treatments: A, ambient mesocosms; N, nutrient enriched; W, warmed; F, flushed; WN, warmed x nutrient enriched; NF, nutrient enriched x flushed; WF, warmed x flushed; WNF, warmed x nutrient enriched x flushed.

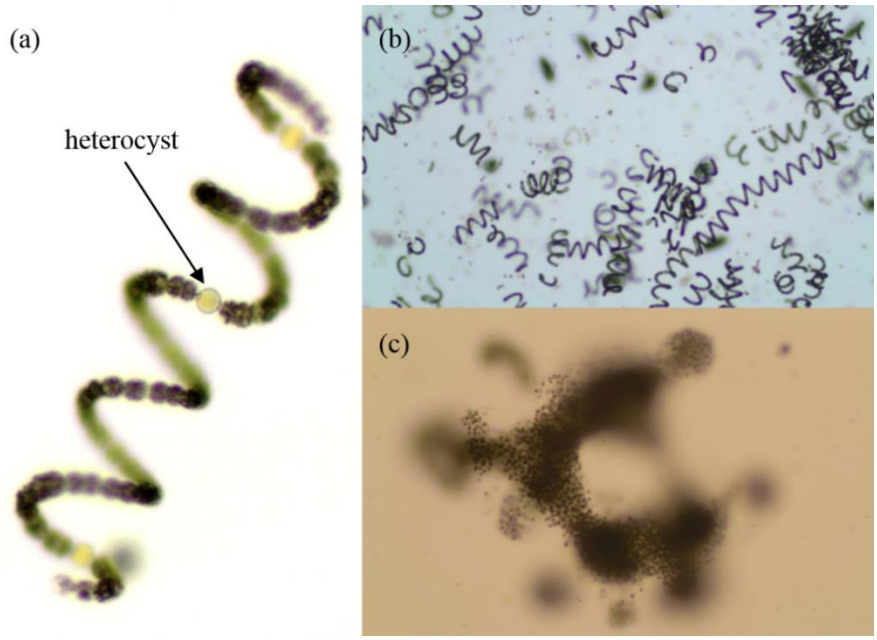


Fig. S3.7 Images of some of the dominant cyanobacteria genera observed between the 5th of May and 26th of August 2015. Image (a) and (b) are Dolichospermum spp.; (b) shows the high density of individual filaments seen within some of the samples; (c) shows a colony of Microcystis sp.

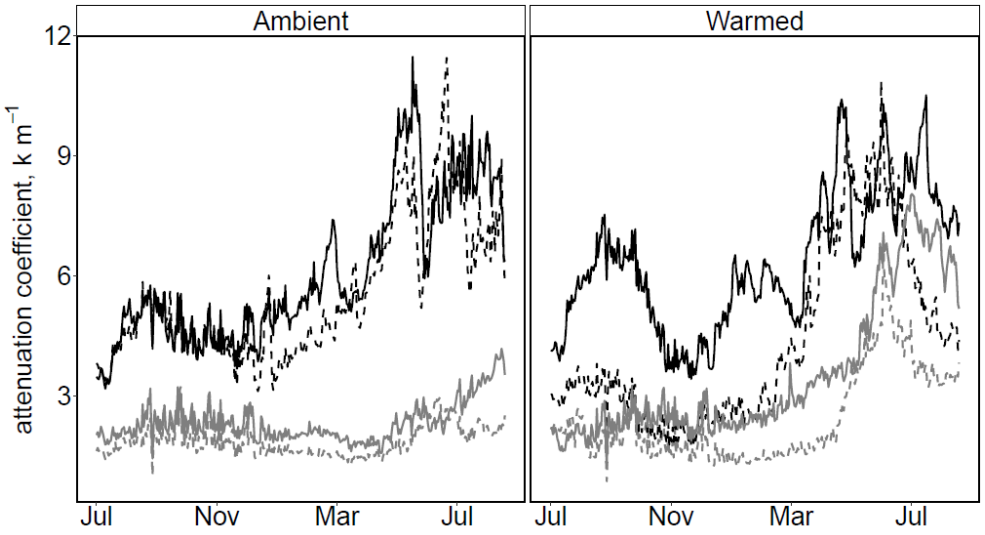


Fig. S3.8 Mean daily light attenuation coefficient (k m^{-1}) in different treatments over the duration of the experiment. Solid line, unflushed; dashed line, flushed; black line, nutrient enriched; grey line, ambient-nutrients; left hand side, ambient temperature; right hand side, warmed.

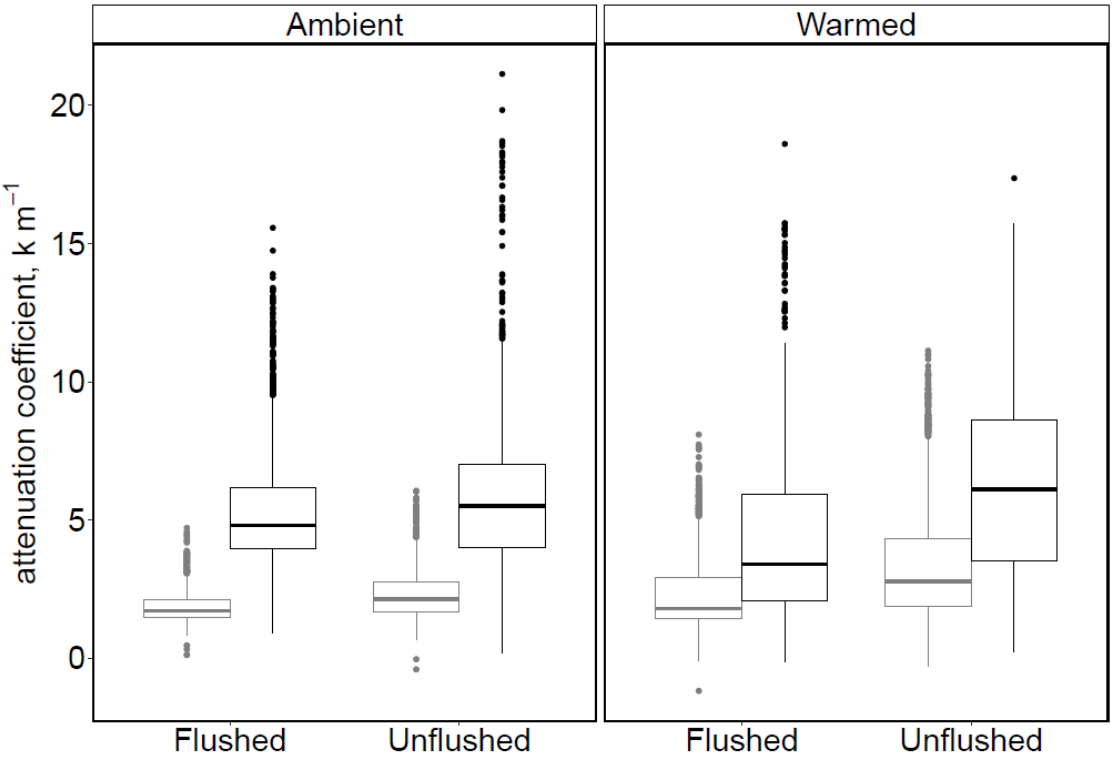


Fig. S3.9 Boxplot of mean daily attenuation of light (k m^{-1}) in different treatments. Black, nutrient enriched; grey, ambient-nutrients. The lower and upper hinges correspond to the 25th and 75th percentiles, the whiskers extend to 1.5x the interquartile range.

6.3 Chapter 4 supplementary material

6.3.1 Chapter 4 supplementary tables

Table S4.1. Competing phytoplankton genera in the model. Functional traits are according to Reynolds *et al*., 2011.

Genus	Class	Nitrogen-fixer	Grazed	Functional trait
Chlorella	Chlorophyte	No	Yes	X1
Staurastrum	Chlorophyte	No	No	N/P
Asterionella	Diatom	No	Yes	C
Cyclotella	Diatom	No	Yes	A
Plagioselmis	Cryptophyte	No	Yes	X2
Cryptomonas	Cryptophyte	No	Yes	Y
Ceratium	Dinophyte	No	No	Lm
Planktothrix	Cyanophyte	No	No	R/S1
Dolichospermum	Cyanophyte	Yes	No	
Dinobryon	Chrysophyte	No	Yes	E

Table S4.2. Sum of combined sum of square root difference of modelled cyanobacteria and chlorophyll for different nutrient calibration models compared to observed values. P start 16 μg L^{-1}, N start 660 μg L^{-1}, multiplication factors: model 1, 1.10; model 2, 1.15; model 3, 1.20; model 4, 1.25; model 5, 1.30; model 6, 1.35; model 7, 1.40. In bold is the best model, based on sum of square root differences, for each climate model. Highlighte is the final model which was selected to represent all climate zones.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
Atlantic	**2.89**	3.51	4.38	5.72	6.72	8.30	8.65
Boreal	4.90	4.32	3.36	2.59	**1.84**	1.95	1.96
Continental	21.42	20.67	19.45	17.41	16.65	**15.50**	15.52
Mediterranean	**6.80**	8.22	8.96	11.65	13.50	17.12	17.62

Table S4.3. Annual averages of chlorophyll-*a* and proportion cyanobacteria from stratified lakes with annual retention times greater than 120 days.

	Chlorophyll a	Proportion cyanobacteria
Boreal	8.9	0.20
Med	8.5	0.14
Continental	13.8	0.27
Atlantic	8.5	0.16

Table S4.4. Centroid of polygon (ArcGIS) latitude and longitude for each climate zone.

Zone	Latitude	Longitude
Boreal	61	35
Atlantic	52	1
Continental	51	26
Mediterranean	40	10

Table S4.5 Conversion between flow rate and retention time.

Flow rate (m^3 s^{-1})	Retention time (days)
0.010407	2.5
0.020813	5
0.041626	10
0.083252	20
0.166504	40
0.333008	80

Table S4.6. Temperature and mixed depth in baseline lakes from different climate zones. Mean, min and max of the means from the 5 climate models. Duration of stratification (days) is when the lake is not fully mixed. Mean (standard deviation of the means, n=5 means).

Zone	min	max	Mean	stratification
Boreal	3.9 (day 94)	19.56 (day 229)	9.6 (0.18)	216
Atlantic	4.5 (day 74)	19.26 (day 230)	10.9 (0.40)	257
Continental	4.95 (day 59)	20.98 (day 215)	11.8 (0.50)	260
Mediterranean	5.8 (day 21)	19.88 (day 245)	13.3 (0.60)	333

Table S4.7 Linear model summary of nutrient effects (cyanobacteria).

nutrient level	estimate	std.error	t value	p.value
(Intercept) low	-0.7001	0.011622	-60.2377	<0.001
baseline	0.728267	0.016437	44.30793	<0.001
high	1.092722	0.016437	66.48144	<0.001

Table S4.8 Linear model summary of nutrinet effects (chlorophyll-*a*).

nutrient level	estimate	std.error	t value	p.value
(Intercept)	1.564158	0.0089	175.7389	<0.001
baseline	0.472851	0.012587	37.56618	<0.001
high	0.673336	0.012587	53.4939	<0.001

Table S4.9 Analysis of Variance table of the best fit linear model for the change in chlorophyll-*a* on the day of the event given different event timing, flushing rate, nutrient scenario and climate zone. The terms are ordered in descending sum of squares. $R^2 = 0.51$

term	df	Sumsq	meansq	statistic	p.value
Residuals	9360	37809	4.0	NA	NA
flushing	5	13912	2782.5	688.8	<0.001
poly(dayEvent, 4)	4	9254	2313.5	572.7	<0.001
poly(dayEvent, 4)*flushing	20	5327	266.4	65.9	<0.001
poly(dayEvent, 4)*climate_zone	12	4267	355.6	88.0	<0.001
poly(dayEvent, 4)*flushing*climate_zone	60	2503	41.7	10.3	<0.001
nutrient	2	1066	533.0	132.0	<0.001
Flushing*nutrient	10	907	90.7	22.5	<0.001
poly(dayEvent, 4)*nutrient	8	627	78.4	19.4	<0.001
climate_zone	3	479	159.7	39.5	<0.001
poly(dayEvent, 4)*climate_zone:nutrient	24	407	17.0	4.2	<0.001
poly(dayEvent, 4)*flushing:nutrient	40	372	9.3	2.3	<0.001
poly(dayEvent, 4)*flushing:climate_zone*nutrient	120	301	2.5	0.6	<0.001
Flushing*climate_zone	15	286	19.1	4.7	<0.001
climate_zone*nutrient	6	81	13.4	3.3	<0.001
Flushing*climate_zone*nutrient	30	50	1.7	0.4	<0.001

Table S4.10 Analysis of variance table for best fit linear model for the number of days it took chlorophyll-*a* to recover given the timing of the event, flushing rate, nutrient scenario and climate zone. The terms are ordered in descending sum of squares. $R^2 = 0.58$

term	df	sumsq	meansq	statistic	p.value
Residuals	9504	3326101	350.0		
poly(dayStart, 2)	2	1556753	778376.7	2224.1	<0.001
poly(dayStart, 2)*climate_zone	6	675057	112509.6	321.5	<0.001
climate_zone	3	354560	118186.7	337.7	<0.001
Flushing	5	338336	67667.1	193.4	<0.001
poly(dayStart, 2)*flushing	10	284458	28445.8	81.3	<0.001
poly(dayStart, 2)*climate_zone*flushing	30	220350	7345.0	21.0	<0.001
poly(dayStart, 2)*climate_zone*nutrient	12	81033	6752.7	19.3	<0.001
climate_zone*flushing	15	68768	4584.5	13.1	<0.001
poly(dayStart, 2)*climate_zone*nutrient*flushing	60	60784	1013.1	2.9	<0.001
poly(dayStart, 2)*nutrient*flushing	20	24851	1242.5	3.6	<0.001
poly(dayStart, 2)*nutrient	4	20391	5097.7	14.6	<0.001
Nutrient*flushing	10	18816	1881.6	5.4	<0.001
climate_zone*nutrient*flushing	30	17357	578.6	1.7	<0.001
climate_zone*nutrient	6	11274	1879.0	5.4	<0.001
Nutrient	2	2524	1261.8	3.6	<0.001

Table S4.11 Additive model results for the difference in the rate of recovery of chlorphyll-*a* given the timing of the event and the flushing rate. Significant effects ($p < 0.05$) are highlighted in bold. R^2 adjusted = 0.37

Parametric coefficients. Changes on the intercept.						
	(Intercept)	Flush5	Flush10	Flush20	Flush40	Flush80
estimate	**0.09**	-0.00	**-0.02**	**-0.04**	**-0.05**	**-0.06**
Estimated degrees of freedom (edf) for approximately significant time smooth terms for nutrient treatment and warming treatment.						
edf	8**.44**					

Table S4.12 Analysis of variance table for linear model of number of days to recover given solar energy (J m^2 day^{-1}) and the response (chlorophyll-*a* and cyanobacterial chlorophyll-*a*). $R^2 = 0.31$.

Term	df	sumsq	meansq	statistic	p.value
Residuals	129576	170440263	1315.4	NA	NA
poly(joules, 2)	2	55060456	27530228.0	20929.7	<0.001
Response	1	9000600	9000600.0	6842.6	<0.001
poly(joules, 2):response	2	7496362	3748181.1	2849.5	<0.001
poly(joules, 2):climate_zone	6	4179527	696587.8	529.6	<0.001
climate_zone	3	1635073	545024.2	414.4	<0.001
poly(joules, 2):climate_zone:response	6	630998	105166.3	80.0	<0.001
climate_zone:response	3	289026	96341.9	73.2	<0.001

Table S4.13 Analysis of variance table for linear model of rate of recovery given solar energy (J m^2 day^{-1}), response (chlorophyll-*a* and cyanobacterial chlorophyll-*a*). R^2 = 0.19.

Term	df	sumsq	meansq	statistic	p.value
Residuals	102195	1178	0.0	NA	NA
jolues	1	236	236.2	20483.3	<0.001
Response	1	13	13.1	1136.4	<0.001
Jolues*climate_zone	3	11	3.8	331.2	<0.001
load.light*response	1	11	11.0	950.7	<0.001
climate_zone	3	8	2.8	243.3	<0.001
joules*climate_zone*response	3	2	0.6	53.1	<0.001
climate_zone*response	3	0	0.1	9.5	<0.001

6.3.2 Chapter 4 supplementary figures

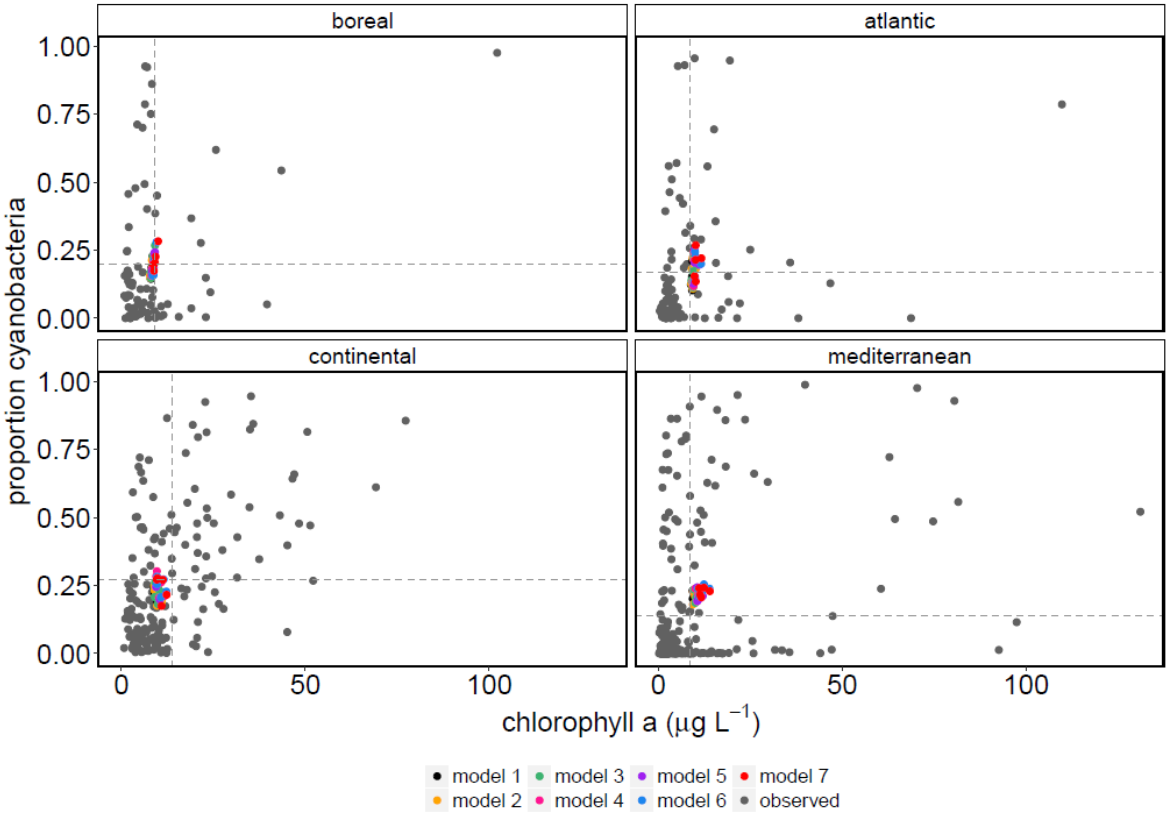


Fig. S4.1 Modelled chlorophyll-*a* and proportion cyanobacteria for different concentrations of inflowing nutrients. Dashed lines are the mean yearly cyanobacteria proportion and mean yearly chlorophyll a from the observed data. There are 5 dots for each nutrient model, one for each climate model (GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM and NorESM1-M).

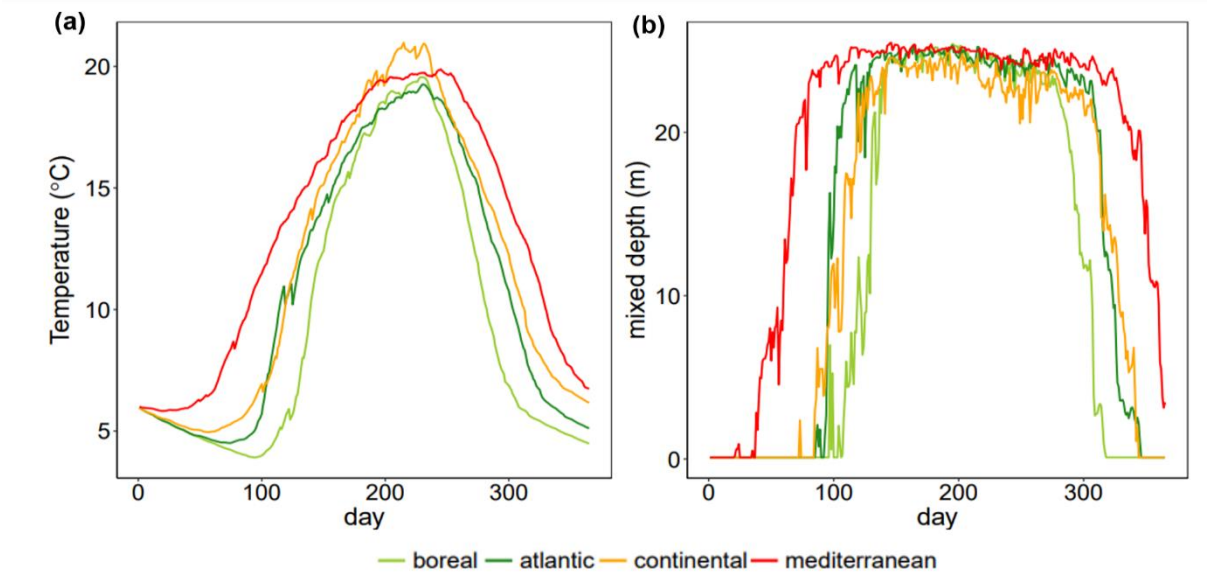


Fig. S4.2 Daily average water temperature and mixed depth in control simulations for different climate zones. The different colour lines are the mean water temperature, ˚C (a) and mean mixed depth, meters (b) over the duration of the year simulation for different climate zones. Means are calculated from daily output driven by the five different climate models.

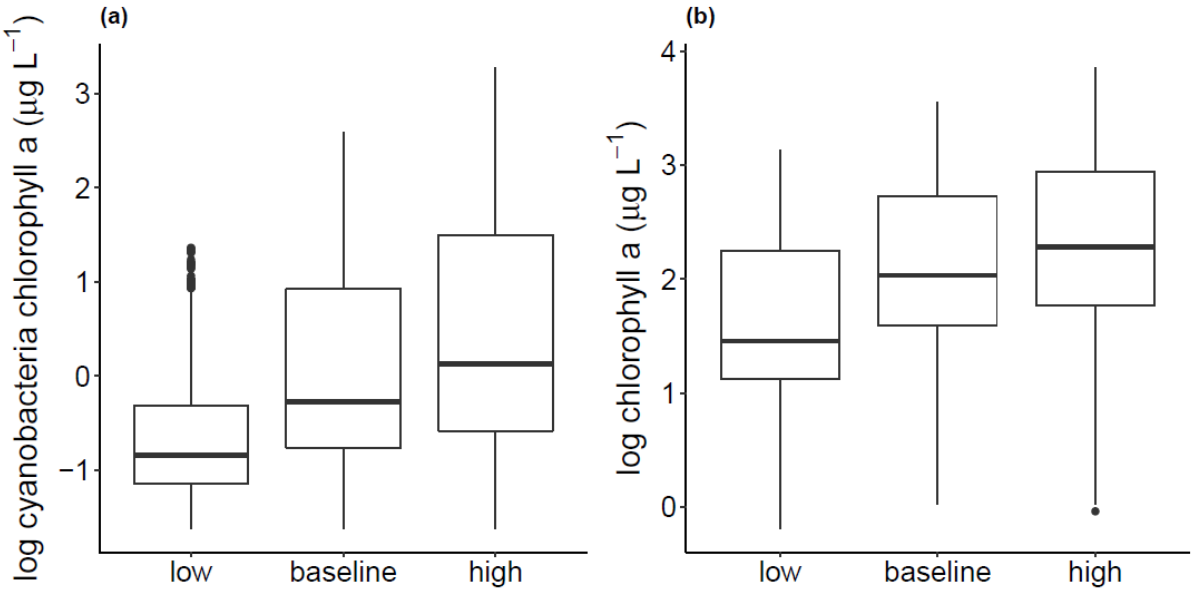


Fig. S4.3 Log cyanobacteria chlorophyll a (a) and total chlorophyll (b) for different nutrient scenarios. The lower and upper hinges correspond to the 25th and 75th percentiles, the whiskers extend to 1.5x the interquartile range.

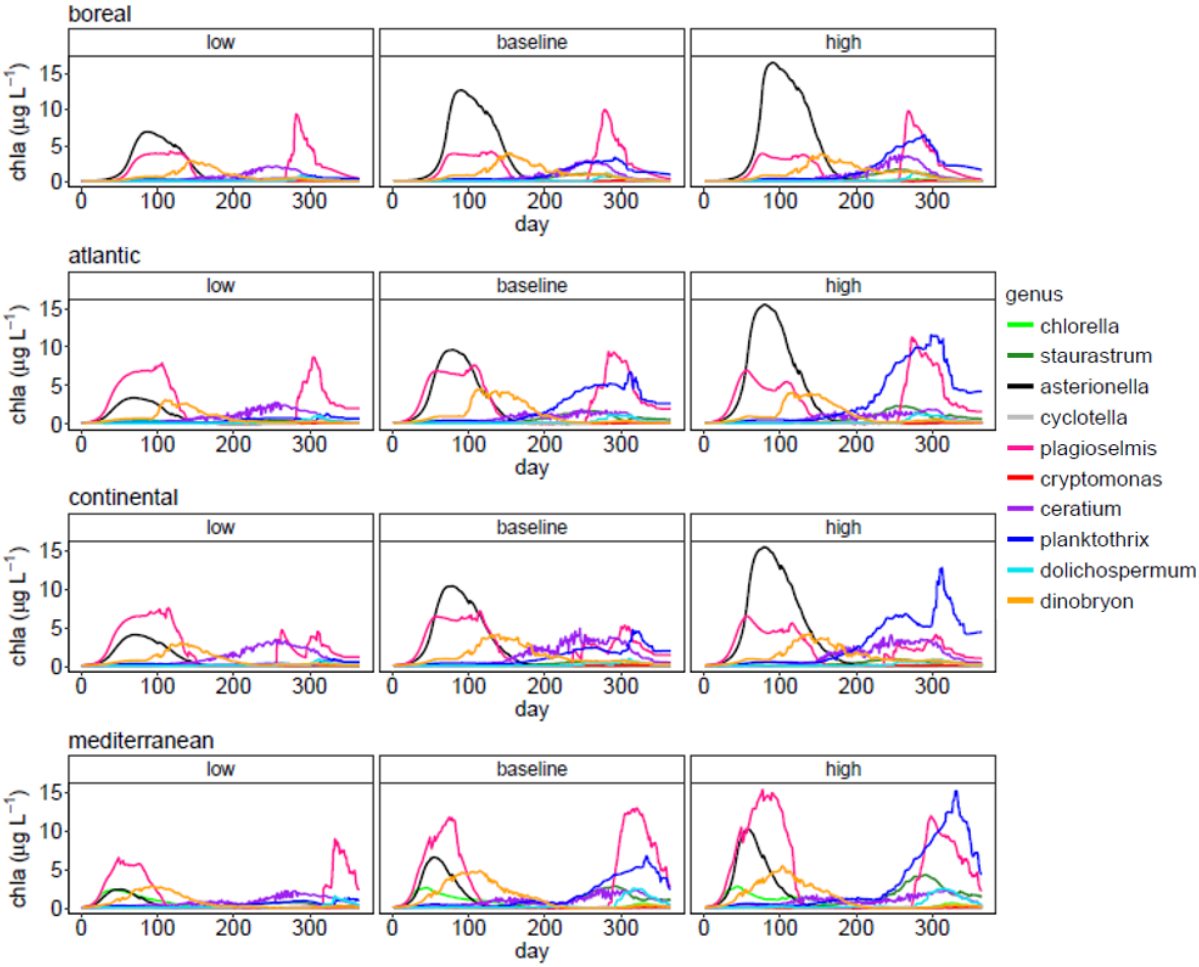


Fig. S4.4 Composition in control simulations for different climate zones and nutrient scenarios. The lines are the averaged response from five different climate models: GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM and NorESM1-M.

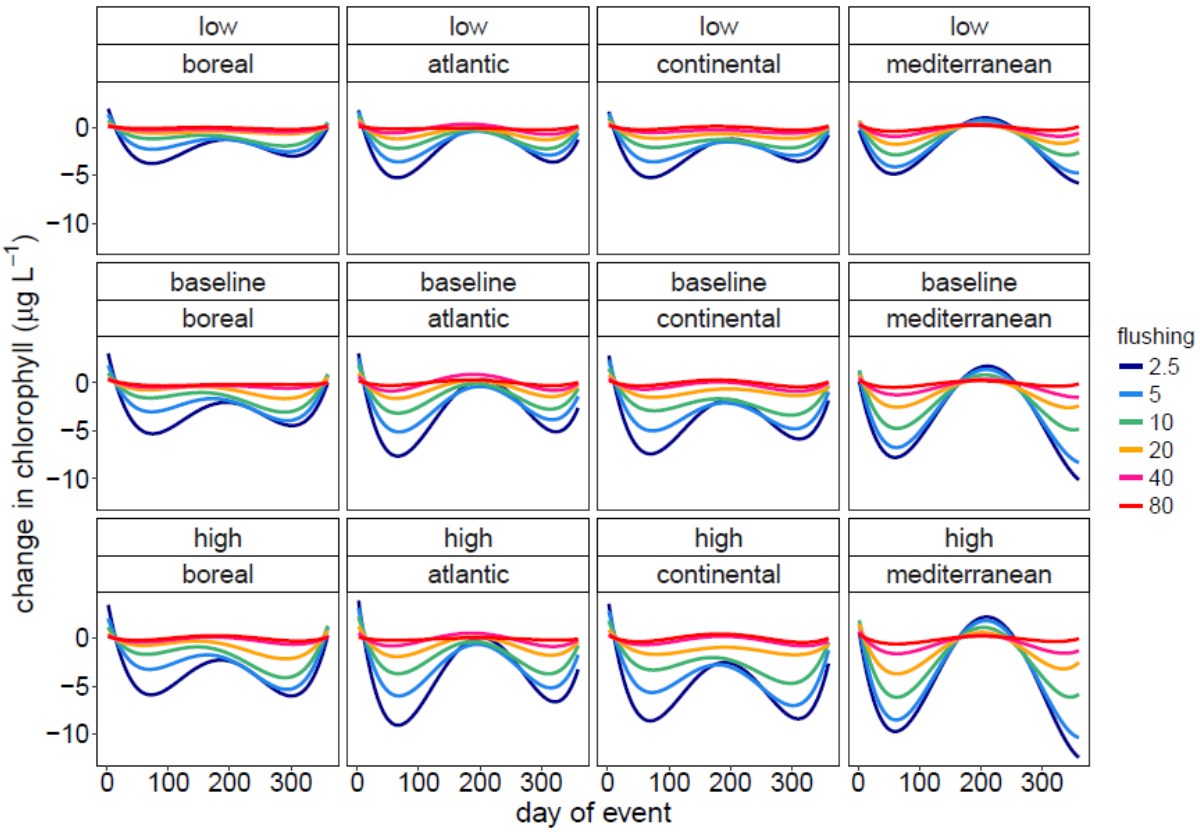


Fig. S4.5 Short term impact of hydraulic flow events on chlorophyll-*a* at different flushing magnitudes, nutrient scenarios and climate zones. The coloured lines are the fitted response from the best fit linear model (Table S8).

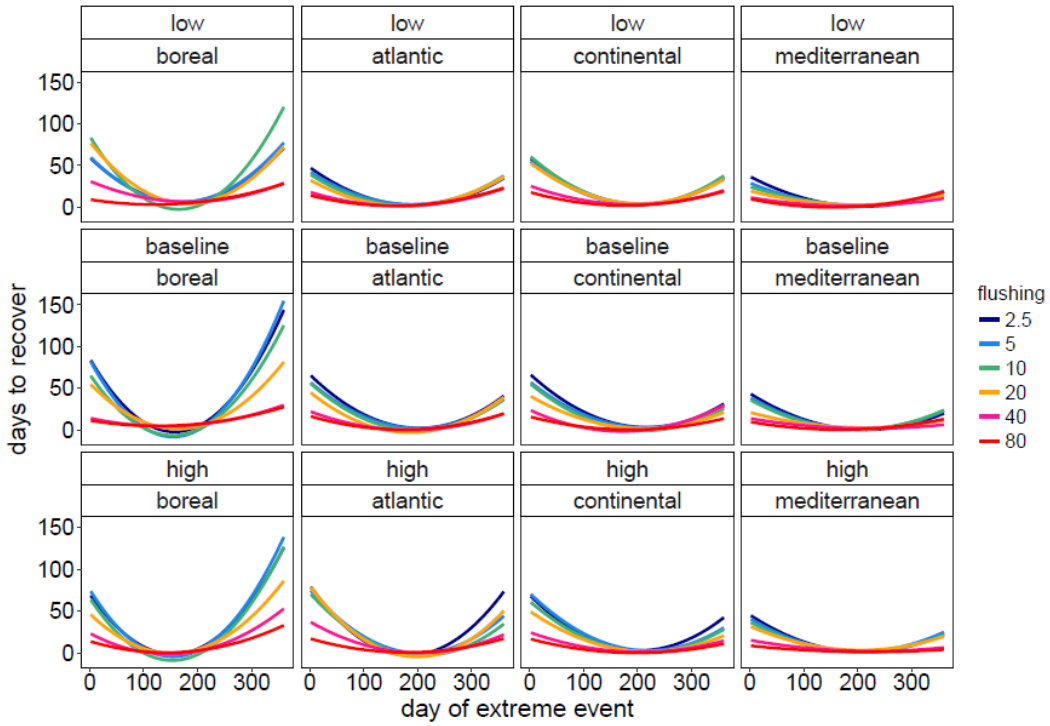


Fig. S4.6 Number of days for chlorophyll-*a* to recover from hydraulic flow events at different flushing magnitudes, nutrient scenarios and climate zones. The coloured lines are the fitted response from the best fit linear model (Table S4.10).

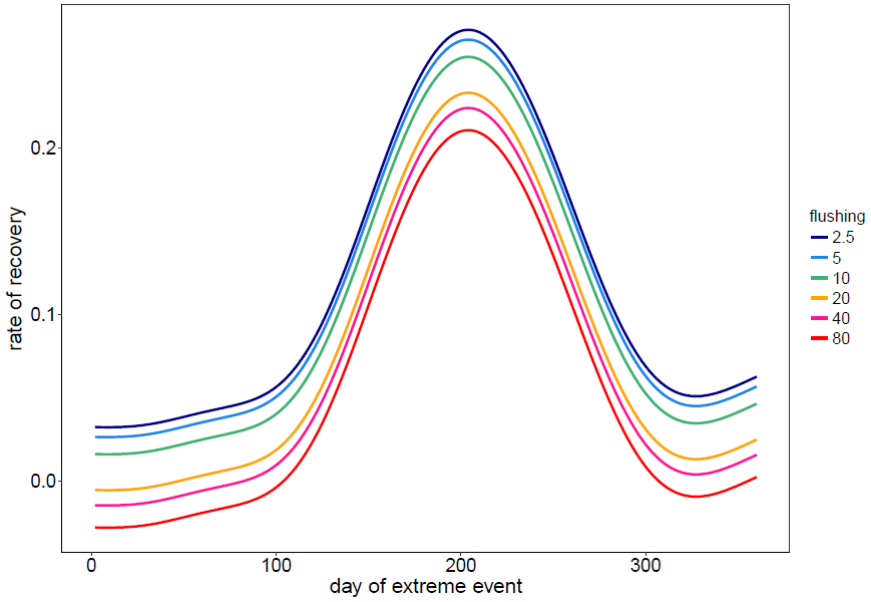


Fig. S4.7 The rate of recovery of total chlorophyll-*a* from hydraulic flow events at different flushing magnitudes, nutrient scenarios and climate zones. The coloured lines are the fitted response from the best fit linear model (Table S4.11).

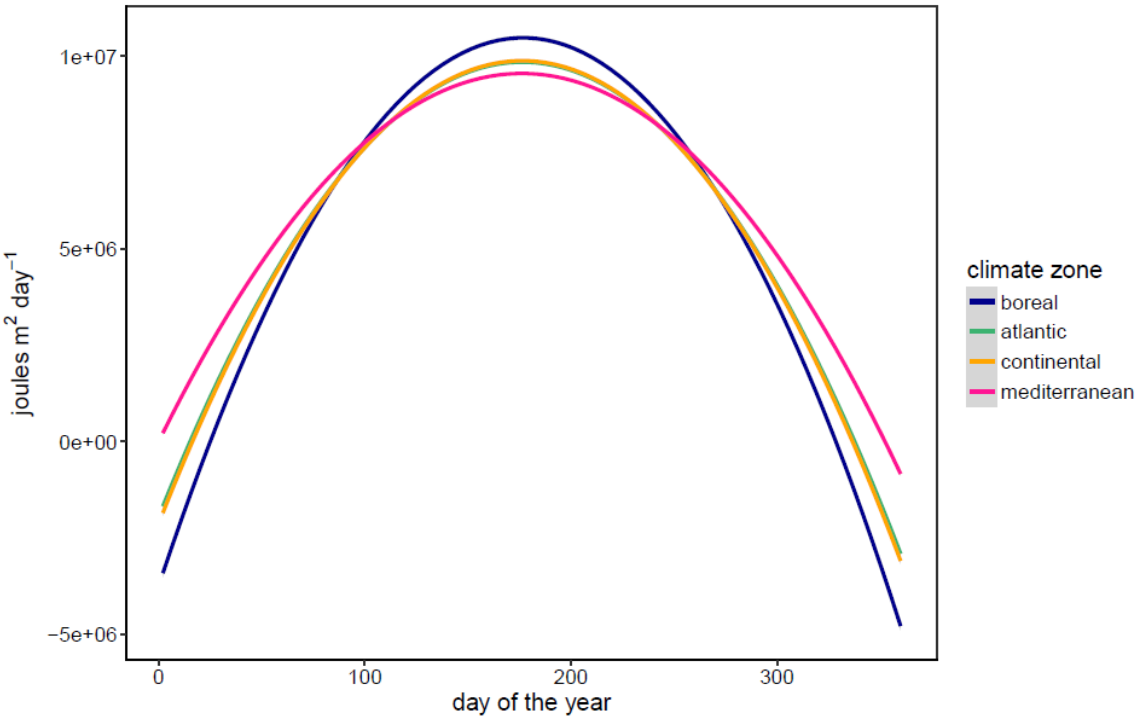


Fig. S4.8 Incoming solar energy (jolues m^2 day^{-1}) over the course of a year in different climate zones. Coloured lines are from the best fit model.

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