Institut für Biochemie und Biologie

The effect of groundwater on benthic primary producers and their interaction

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von
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L’essentiel est invisible pour les yeux.

Antoine De Saint-Exupéry

Table of contents

Preface

This scientific work was conducted at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries Berlin (IGB), Department of Ecosystem Research, Müggelseedamm 301, 12587, Berlin, Germany. This work was carried out from 13.02.2013 until 21.06.2017 within the project AQUALINK. It was prepared independently and exclusively with the funding from the Leibniz association (SAW- 2012- IGB 4167).

Acknowledgements

This thesis has not been written by a little human living alone on its planet. Many great persons enabled this project, and must be acknowledged for it.

First I want to thank my supervisors, who made this work possible and were great source of help, all along the project. Sabine Hilt was a constant motor of motivation, she always gave the positive picture of the results, encouraged me to focus my research and was an invaluable help for writing. I received essential advice from Jörg Lewandowski and Michael Hupfer, and HP Grossart enabled the presentation of this thesis.

Soren has repaired my broken English with an astonishing celerity. Thanks again, and good luck in your new life! During my PhD time I had nice scientific and more personal conversations with Franzi, Nina, Max, Christian, Anne-Marie and Maria from the project Aqualink, and Marta, Bine, Garabet, Mikael and Alex from the working group. I wish you all the best success in your future

adventures and hope that this friendship will survive time and distance. I receiver great help and advice from the scientists and technicians of IGB in Berlin and Neuglobsow, they should be acknowledged for that. Especially Uschi has been a nice office neighbor, and Justyna’s team provided cake, good mood and motivation for running.

My parents created the favorable environment for studying and taught me how to keep the eyes and heart open to visible and invisible beauty, I owe them my frequent smile. François, Hélène and Juliette brought me open minds, laughs and supported my extra-long study time. Special thanks to my grandmothers, they inspired me a lot. Mame taught me to follow my dreams and work hard, and I guess Cécile appreciates reading her name in front of articles that are related to ecology! I want to thank the CA’s viola players and the members of Keller blues band, for their friendship and their confidence!

Regina and Thomas supported me greatly in the last years, letting me introduce some stress in their piece of paradise. Danke! Finally, Matthias cared for my health, fitness, brain, adventure, German grammar, happiness and soul, thank you for always supporting me!

List of publications

The present thesis is a publication-based dissertation. Four articles constitute the central part of the thesis, and are integrated as chapters, each with individual figures and tables numbering, and individual reference list. As all these articles have been elaborated with other authors, Cécile Périllon's share of work is explained for each.

Chapter 1

Périllon, C., and S. Hilt. 2016. Groundwater influence differentially affects periphyton and macrophyte production in lakes. Hydrobiologia **778**: 91–103.

Submitted: 2 May 2015; Revised: 20 July 2015; Accepted: 5 September 2015; Published online: 23 October 2015

I jointly developed the concept with SH, performed the literature research, wrote major parts of the manuscript

Chapter 2

Périllon, C., F. Pöschke, J. Lewandowski, M. Hupfer, and S. Hilt. 2017a. Stimulation of epiphyton growth by lacustrine groundwater discharge to an oligo-mesotrophic. Freshw. Sci. **36**, doi:10.1086/692832.

Submitted: 13 July 2016; Revised: 3 March 2017; Accepted: 30 March 2017; Published online: 25 May 2017

I designed and ran the epiphyton survey, part of pore water and groundwater sampling as well as the whole field experiments. I carried out the analyses and wrote of the manuscript, with contributions of all coauthors.

Chapter 3

Périllon, C., K. van de Weyer, J. Päzolt, P. Kasprzak, and S. Hilt. 2017b. Changes in submerged macrophyte colonization in shallow littoral areas of an oligo-mesotrophic lake and the potential role of groundwater-mobilized nutrients. Limnologica. doi: 10.1016/j.limno.2017.03.002

Submitted: 15 June 2016; Revised: 7 March 2017; Accepted: 21 March 2017; Published online: 24 April 2017

I analyzed the macrophytes mapping data, sampled and analyzed macrophytes and periphyton in Lake Stechlin. I jointly designed and wrote the manuscript with SH.

Chapter 4

Périllon, C., and S. Hilt. Groundwater discharge gives periphyton a competitive advantage over macrophytes.

The text present in the dissertation will be submitted in July 2017 to PlosOne (open access journal).

I designed and ran all the experiments, carried out analyses, and wrote the manuscript, with the help of SH.

Summary

In littoral zones of lakes, multiple processes determine lake ecology and water quality. Lacustrine groundwater discharge (LGD), most frequently taking place in littoral zones, can transport or mobilize nutrients from the sediments and thus contribute significantly to lake eutrophication. Furthermore, lake littoral zones are the habitat of benthic primary producers, namely submerged macrophytes and periphyton, which play a key role in lake food webs and influence lake water quality. Groundwater-mediated nutrient-influx can potentially affect the asymmetric competition between submerged macrophytes and periphyton for light and nutrients. While rooted macrophytes have superior access to sediment nutrients, periphyton can negatively affect macrophytes by shading. LGD may thus facilitate periphyton production at the expense of macrophyte production, although studies on this hypothesized effect are missing.

The research presented in this thesis is aimed at determining how LGD influences periphyton, macrophytes, and the interactions between these benthic producers. Laboratory experiments were

combined with field experiments and measurements in an oligo-mesotrophic hard water lake.

In the first study, a general concept was developed based on a literature review of the existing knowledge regarding the potential effects of LGD on nutrients and inorganic and organic carbon loads to lakes, and the effect of these loads on periphyton and macrophytes. The second study includes a field survey and experiment examining the effects of LGD on periphyton in an oligotrophic, stratified hard water lake (Lake Stechlin). This study shows that LGD, by mobilizing phosphorus from the sediments, significantly promotes epiphyton growth, especially at the end of the summer season when epilimnetic phosphorus concentrations are low. The third study focuses on the potential effects of LGD on submerged macrophytes in Lake Stechlin. This study revealed that LGD may have contributed to an observed change in macrophyte community composition and abundance in the shallow littoral areas of the lake. Finally, a laboratory experiment was conducted which mimicked the conditions of a seepage lake. Groundwater circulation was shown to mobilize nutrients from the sediments, which significantly promoted periphyton growth. Macrophyte growth was negatively affected at high periphyton biomasses, confirming the initial hypothesis.

More generally, this thesis shows that groundwater flowing into nutrient-limited lakes may import or mobilize nutrients. These nutrients first promote periphyton, and subsequently provoke radical changes in macrophyte populations before finally having a possible influence on the lake’s trophic state. Hence, the eutrophying effect of groundwater is delayed and, at moderate nutrient loading rates, partly dampened by benthic primary producers. The present research emphasizes the importance and complexity of littoral processes, and the need to further investigate and monitor the benthic environment. As present and future global changes can significantly affect LGD, the understanding of these complex interactions is required for the sustainable management of lake water quality.

Zusammenfassung

Im Uferbereich von Seen bestimmen eine Vielzahl von Prozessen das ökologische Gefüge und die Wasserqualität. Grundwasserzustrom, welcher häufig im Uferbereich eines Sees auftritt, kann zum Import von Nährstoffen führen und so signifikant zur Eutrophierung eines Gewässers beitragen. Darüber hinaus bildet der Uferbereich von Seen das Habitat für benthische Primärproduzenten wie Makrophyten (Wasserpflanzen) und Periphyton (Aufwuchs), welche eine Schlüsselrolle im Nahrungsnetz von Seen einnehmen und deren Wasserqualität beeinflussen können. Der durch Grundwasser gesteuerte Eintrag von Nährstoffen kann sich unterschiedlich auf die um Licht und Nährstoffe konkurrierenden Makrophyten und Periphyton auswirken. Während Makrophyten häufig über Wurzeln verfügen und damit Nährstoffe aus dem Sediment aufnehmen, kann Periphyton zu einer Beschattung der Makrophyten beitragen. Grundwasserzustrom könnte deshalb durch Nährstoffzufuhr das Wachstum von Periphyton fördern und damit zu einer Abnahme der Makrophytenabundanz führen.

Die in dieser Doktorarbeit vorgestellten Forschungsergebnisse zeigen den Einfluss von einströmendem Grundwasser in Seen auf Makrophyten und Periphyton, und insbesondere die Interaktionen

zwischen diesen beiden benthischen Primärproduzenten. Dafür wurden Laborexperimente, sowie Feldexperimente und Messungen in einem oligo-mesotrophen, kalkreichen See miteinander kombiniert.

In der ersten Studie wurden im Rahmen einer Literaturrecherche die Auswirkungen des Einstroms von Grundwasser auf das Wachstum von Makrophyten und Periphyton untersucht. Dafür wurden Einträge von Nährstoffen sowie anorganischem und organischem Kohlenstoff berücksichtigt und abschließend ein Konzept entwickelt, das die Interaktion zwischen benthischen Primärproduzenten betrachtet. Die zweite Studie zeigt den Einfluss von Grundwasser auf das Wachstum von Periphyton im geschichteten, oligo-mesotrophen, kalkreichen Stechlinsee (Brandenburg) auf der Basis von Freilanduntersuchungen und -experimenten. Es konnte nachgewiesen werden, dass einströmendes Grundwasser Phosphor aus dem Sediment mobilisiert und so das Wachstum von Periphyton signifikant fördert. Dies war insbesondere am Ende des Sommers relevant, wenn Phosphor im Epilimnion nur noch in sehr geringer Konzentration vorlag. Der Fokus der dritten Studie liegt auf den potenziellen Auswirkungen des Einstroms von Grundwasser auf die Makrophyten in flachen Litoralbereichen des Stechlinsees. Die in den letzten Jahrzehnten beobachteten Veränderungen in der Abundanz und Artenzusammensetzung der Makrophyten, insbesondere der Rückgang der Armleuchteralgen, könnten auch auf Veränderungen im Einstrom von Grundwasser zurückzuführen sein. In der letzten Studie wurden in einem Laborexperiment der Grundwasserzustrom ins Litoral simuliert, um die kombinierte Auswirkung auf Makrophyten- und Periphytonentwicklung unter kontrollierten Umweltbedingungen zu testen. Die Ergebnisse bestätigen die Hypothese, dass die durch den Grundwasserzustrom mobilisierten Nährstoffe aus dem Sediment das Wachstum von Periphyton fördern. Oberhalb eines Grenzwertes der Periphytonbiomasse wird die Entwicklung von Makrophyten behindert.

Die vorliegende Arbeit zeigt, dass einströmendes Grundwasser zur Mobilisierung und zum Import von Nährstoffen in Seen führen kann und damit weitreichende Konsequenzen für das ökologische Gefüge und die Wasserqualität haben kann. Die grundwassergesteuerte Nährstoffzufuhr fördert das Wachstum von Periphyton und führt bei genügend großer Periphytonbiomasse zu Änderungen der Makrophytenpopulation bis hin zum Verlust. Die Arbeit verdeutlicht die Relevanz und Komplexität von Prozessen im Litoral von Seen und zeigt zugleich die Notwendigkeit auf, diese benthische Habitate tiefgreifender zu untersuchen. Da globale Veränderungen des Klimas einen weitreichenden Einfluss auf den Grundwassereinstrom in Seen haben können, ist es von entscheidender Bedeutung, die komplexen Auswirkungen dieser Prozesse zu verstehen, um einen nachhaltigen Schutz dieser Ökosysteme zu gewährleisten.

Abbreviations

C	Carbon
chl-*a*	Chlorophyll-*a*
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
DSi	Dissolved silica
EC	Electro Conductivity
LGD	Lacustrine Groundwater Discharge
N	Nitrogen
P	Phosphorous
Si	Silica
SRP	Soluble Reactive Phosphorous

Table of figures

Introduction

Eutrophication is one of the most common and challenging problems for lakes in the 20th and 21st century (Smith 2003). It compromises their utility as sources of drinking water, recreation, fishery production, and other ecosystem services.

Numerous processes that govern lake trophic states have been revealed in recent decades. However, most studies of such processes have been conducted in the water column (Fig. 1). There remains less knowledge regarding processes at lake boundaries, such as the benthic habitat, although several key studies have indicated the key role of this habitat for whole-lake ecology (Sand-Jensen and Borum 1991; Hecky and Hesslein 1995). The present thesis aims to fill part of this knowledge gap by focusing on the groundwater-sediment-water interface in lakes. Specifically, it aims to unravel the influence of groundwater-borne nutrients on benthic primary producers and their subsequent interactions with other lake processes.

Benthic primary producers in lakes

Primary producers in lakes

Primary producers are at the basis of the food web and require inorganic nutrients, carbon dioxide, and light for their growth (bottom-up control factors). Although their production and biomass can also be top-down controlled by grazing, the present research focuses on bottom-up control processes.

The primary producers present in the water column (mostly phytoplankton) have traditionally been a major focus of research for limnologists (Vadeboncoeur et al. 2003, Fig. 1). Eutrophication can strongly facilitate phytoplankton production and lead to potentially harmful algal blooms. These blooms can severely deteriorate the water quality of lakes, and remain an important area of limnological research (Smith and Schindler 2009; Moss et al. 2011).

In contrast, much less research has been carried out on benthic primary producers (Fig. 1). Both submerged macrophytes and periphyton compete with phytoplankton for nutrients and light (Jäger and Diehl 2014). However, macrophytes and periphyton also asymmetrically compete against one another for nutrients and light, with rooted macrophytes having greater access to sediment nutrients and periphyton being able to shade out macrophytes These latter interactions, however, have received little research attention (Fig. 1).

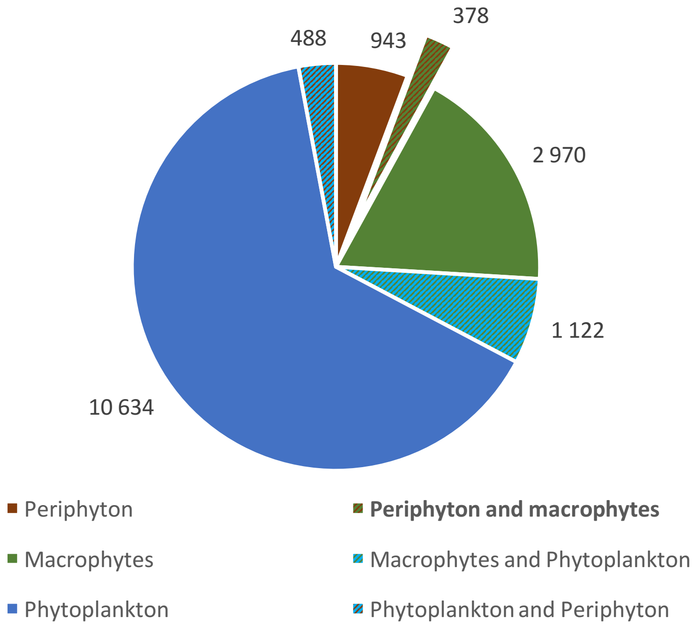

Figure 1. Studies of water column and benthic primary producers and their interactions in lakes (1945-April 2017, ISI Web Of Science database, search terms "lakes" AND periphyton ("periphyton", "epiphyton", "biofilm", "aufwuchs"), macrophytes ("macrophytes", "water plants"), phytoplankton, and their combinations). Only 170 articles focused on all three primary producer categories.

Macrophytes and periphyton

Aquatic macrophytes (from Greek phytón, plant) are comprised of a group of autotrophic organisms that are large ("macro") enough to be visible to the naked eye. They can be submerged, emergent, or floating, and include flowering plants, algae, mosses, and ferns (Hickey and King 2000). In this thesis, only submerged vascular macrophytes (charophytes or angiosperms) are considered. Submerged macrophytes are often anchored or even rooted in the lake sediments and their vegetative tissues grow exclusively in the sediments and water column.

Periphyton is a community of living organisms (algae, bacteria, fungi, grazers) and detritus (organic and inorganic) that are attached to a substratum (Wetzel 1983) or grow around solid particles (from Greek perí, around) in the water. The name periphyton might be misleading since autotrophic algae may only account for a small part of periphyton biomass (Frost et al. 2005). A related term is “biofilm”, yet in this case the focus is more often on the bacterial community and the non-living elements of the periphyton. Periphyton may grow on different substrata which may in turn influence its productivity (Vadeboncoeur et al. 2003): on macrophytes (epiphyton (Fig. 2)), on sediments (epipelon), or on rocks (epilithon). Periphyton may account for more than 75% of primary production, especially in clear-water systems (Burkholder and Wetzel 1989; Liboriussen and Jeppesen 2003; Vadeboncoeur et al. 2014), but has recently been shown to also play a significant role in highly humic lakes (Vesterinen et al. 2016).

Figure 2. Macrophyte (*Myriophyllum spicatum*) and periphyton in the littoral zone of Lake Stechlin, photographed on 17th June 2015

Benthic primary producers and lake trophy

Both periphyton and submerged macrophytes may stabilize clear water conditions in shallow as well as deep lakes (Scheffer et al. 1993; Genkai-Kato et al. 2012; Sachse et al. 2014) by promoting the sedimentation of suspended particles, by taking up nutrients that are no longer available to phytoplankton, and by slowing down nutrient release from the sediments (Carpenter and Lodge 1986; Kufel and Kufel 2002; Dodds 2003).

In addition, submerged macrophytes may provide a refuge for zooplankton (Timms and Moss 1984), habitat for piscivorous fish (Jacobsen and Perrow 1998), and release allelochemicals, inhibiting phytoplankton growth (Hilt and Gross 2008). However, macrophytes may also contribute to nutrient enrichment in the water column, as they are able to extract nutrients from the sediments (especially phosphorus (P)), which are subsequently released to the water column upon their decay (Barko and Smart 1980).

Epiphyton, however, impairs the development of macrophytes via shading (Sand-Jensen and Søndergaard 1981) and by slowing down the supply of inorganic carbon (Jones et al. 2000). Epiphyton shading can be a major driver of submerged macrophyte declines, shifting lakes to turbid states during the process of eutrophication (Jones and Sayer 2003; Phillips et al. 2016).

Temporal and spatial variation of benthic primary producers

Benthic primary producers have high temporal and spatial variation in productivity, nutrient content, growth forms, and diversity, even within a single waterbody. In temperate lakes, the primary growing season for annual submerged macrophytes is in the summer, between June and August. The field studies of the effects of LGD on periphyton-macrophyte interactions presented in this thesis were thus conducted in summer. Periphyton growth (Schroeder et al. 2012), its algal community diversity (Szabó et al. 2017), and the dominant algal groups (Liboriussen and Jeppesen 2006) are all temporally variable. The field campaigns which are part of this thesis were therefore repeated several times throughout the summer in order to account for these changes.

The coverage of benthic primary producers can be extremely heterogeneous, especially in the littoral areas. This patchiness has been attributed to variation in depth (Kahlert et al. (2002) for periphyton, Torn et al. (2015) for macrophytes), wind (Cattaneo (1990) for periphyton, Schutten et al. (2004) for macrophytes), and grazing (Vadeboncoeur and Steinman (2002) for periphyton, Bakker et al. (2016) for macrophytes). However, high uncertainty still remains in relation to the spatial variability of benthic primary producers (Chappuis et al. 2014).

Groundwater discharge in lakes

Terminology and basic processes in aquifers

Groundwater is the subsurface water in soil/sediment pores and rock fractures. The depth at which the pores, fractures, and voids are saturated with water is called water table. Above the water table is the unsaturated zone where the pores are filled with air and water. Water in the unsaturated zone is often called soil water and is not considered to be groundwater. An aquifer is an underground layer located in the saturated zone from which groundwater can be extracted using wells.

Groundwater is recharged from the surface by precipitation (rain or melting snow) or by infiltration from surface water bodies. During the percolation processes, but also during lateral transport in the aquifer, the groundwater is enriched with ions released from the soil/sediment matrix. The composition of groundwater therefore depends on the soil and aquifer characteristics, as well as the land use in its watershed. In unconfined aquifers, groundwater flows downward (as does surface water), from high to low altitude areas of the water table. In general, groundwater flow is driven by pressure heads. Water and solutes may, however, flow with different velocities since adsorption, desorption, chemical, and biological reactions might cause the retardation of solutes (Hölting and Coldewey 2008).

Groundwater flow in lakes

For a lake embedded in an aquifer, groundwater will exfiltrate into the lake if the water table in the aquifer is higher than the water level of the lake. This process is called "lacustrine groundwater discharge" (LGD). At sites where the water table of the aquifer is lower than the water level of the lake, the lake water infiltrates into the aquifer and recharges the groundwater. Often, groundwater tables are slightly higher than lake levels in some sections along the shoreline, while this relationship is reversed for the rest of the shoreline, in- and exfiltration can thus occur at the same lake. Lakes in which the water budget is mostly controlled by exchange with groundwater are named "seepage lakes".

While water tables vary geographically, the water level of a lake is spatially constant. Because of this, most LGD occurs close to the shore, at least when considering the aquifer to be homogenous. Groundwater approaching a lake flows in the direction of the steepest hydraulic gradient, with flow lines bending upwards as they approach the shore (McBride and Pfannkuch 1975; Belanger et al. 1985).

Importance of LGD for water and nutrient budgets of lakes

LGD is often not considered when the reasons for eutrophication are studied in lakes (Fig. 4). This is most probably due to difficulties in evaluating groundwater flows within a lake because these fluxes are invisible, diffuse, and highly heterogeneous (Rosenberry et al. 2015). Evaluating nutrient fluxes due to LGD is even more challenging (Lewandowski et al. 2015). However, groundwater may account for up to 95% of lake water budgets (with a median of 31% in 110 studies reviewed by Rosenberry et al. (2015)) and often provide more than 50% of the total P load (Lewandowski et al. 2015). As a consequence, LGD may greatly influence the pore water physico-chemical composition (Schafran and Driscoll 1990) and nutrient availability in the water column (Hayashi and Rosenberry 2002). LGD may thus significantly affect the trophic state of lakes (Holman et al. 2008; Meinikmann et al. 2015). Most previous studies, however, did not take into account any benthic processes which may be responsible for the retention and/or transformation of nutrients entering lakes via LGD.

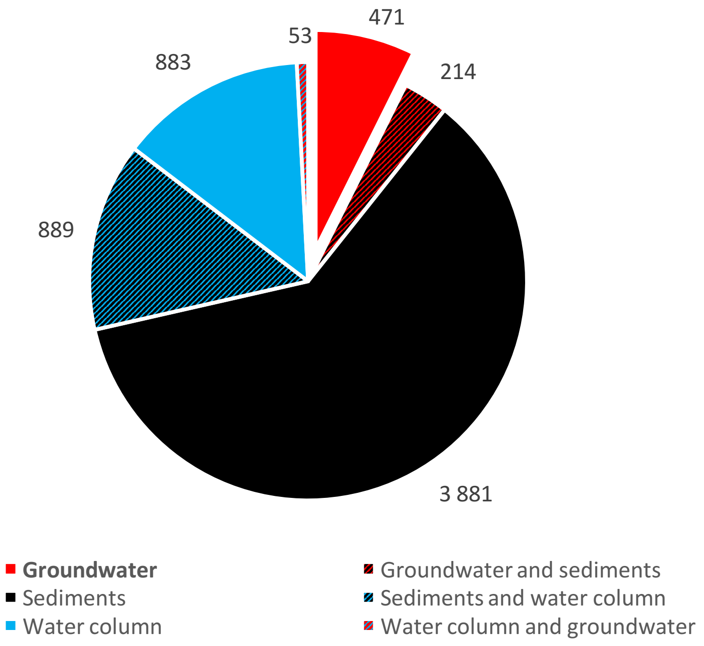

Figure 3. Studies of eutrophication which contain groundwater, sediments, or water column and/or their interaction (1945-April 2017, ISI Web Of Science database, search terms eutrophication" AND groundwater ("groundwater", "seepage"),

sediments, water column (“water column”, “pelagic”). Only 32 articles focused on all three categories.

The littoral zone, an important lake boundary

Littoral benthic primary producers grow in a boundary habitat. In this environment, the gradients of light and nutrients governing primary production are particularly strong. These gradients can be attributed to three main interfaces: (1) the shore-lake interface, (2) the sediment-water interface, and (3) the groundwater-surface water interface.

Shore-lake interface

Lakes are water bodies surrounded by land. In lakes with a high shoreline to surface area ratio, the shore may be an especially significant source of shade due to shoreline trees (Köhler et al. 2010; Ali et al. 2011), nutrients and organic matter entering by runoff and litterfall (Gasith and Hasler 1976; Rösel et al. 2012), and physical disturbance by humans and animals. All of these shading effects could potentially affect benthic primary producers.

At close proximity to the shore, wave turbulence influences macrophyte (Kautsky 1987) and periphyton growth (Strand and Weisner 1996). On the other hand, macrophytes and periphyton may buffer the shore against wave action (Dodds and Biggs (2002) for periphyton, Carpenter and Lodge (1986); Tugend et al. (2004) for macrophytes).

Sediment-water interface

The sediment-water interface is the site of strong physico-chemical gradients for factors such as temperature, oxygen, and pH. The concentration gradient of nutrients across this interface is controlled by diffusive and advective transport, adsorption and desorption to particles, and local decomposition processes (Heinen and McManus 2004; Spears et al. 2008). Rich microbial populations participate in and benefit from this environment, making the sediment surface a biological hotspot.

Benthic primary producers may depend upon pore water chemistry (Hansson (1992) for periphyton, Sebestyen and Schneider (2004), for macrophytes), and may in turn influence nutrient concentrations and binding forms in the sediments (Jaynes and Carpenter (1986); Laskov et al. (2007) for macrophytes, Dodds (2003); Zhang et al. (2012) for periphyton). Other sediment characteristics such as substrate properties may also be important for the colonization of benthic primary producers (Idestam-Almquist and Kautsky (1995) for macrophytes, Vadeboncoeur et al. (2003) for periphyton). Benthic primary producers may in turn stabilize the sediments and promote sediment formation (Neumeier et al. 2006 for periphyton, Carpenter and Lodge (1986); Madsen et al. (2001) for macrophytes).

Groundwater-surface water interface

Even though the water and nutrient budget of a lake can be primarily controlled by groundwater, the characteristics of lake water and groundwater can be very different. LGD can either provoke enrichment or dilution of lake water solutes because groundwater first flows through the sediments and interacts with biota before entering the water column. Other processes which may additionally change the water characteristics in a lake include nutrient uptake through primary producers, solute precipitation, and evaporation.

Studying LGD poses unique challenges that are inherently coupled to aquifer characteristics: fluxes are diffuse (Lee et al. 1980) and may be highly temporally and spatially variable (Rosenberry et al. 2015), affecting solute loads (Lewandowski et al. 2015). Another challenge in studying the groundwater-surface water interface is to disentangle the influences of groundwater, sediments, and the shoreline ecotone, since those factors all occupy the same space, have an impact on nutrient fluxes, and influence benthic primary producers.

Benthic primary producers are potentially affected by nutrients as well as inorganic and organic carbon (C) fluxes from groundwater [1]. They may in turn influence LGD, as they can clog up the upper sediments layers (Karan et al. (2014) for macrophytes, Salant (2011) for periphyton).

Objectives and study approach

In this thesis the influence of LGD on benthic primary producers has been investigated using laboratory experiments and field investigations and experiments in an oligotrophic hard water lake. The following research questions were addressed in four consecutive scientific papers, that will be presented in the subsequent chapters:

- Chapter 1: How can LGD influence benthic primary producers and their interactions?
- Chapter 2: Can LGD facilitate epiphyton growth in an oligotrophic hard water lake?
- Chapter 3: How can LGD affect submerged macrophyte communities in the shallow littoral areas of an oligotrophic hard water lake?
- Chapter 4: Can LGD give epiphyton a competitive advantage over macrophytes?

In order to answer these questions, a multi-scale approach was adopted, permitting different levels of external parameter control and system complexity (Fig. 4). After an initial overview of the existing literature and the development of a concept based on the potential effects of LGD on epiphyton-macrophyte interactions [chapter 1], field sampling [chapters 2 & 3], field experiments [chapter 2], and laboratory experiments [chapter 4] were conducted. Together, these provide a comprehensive assessment of the research questions by balancing the advantages and disadvantages of each respective approach.

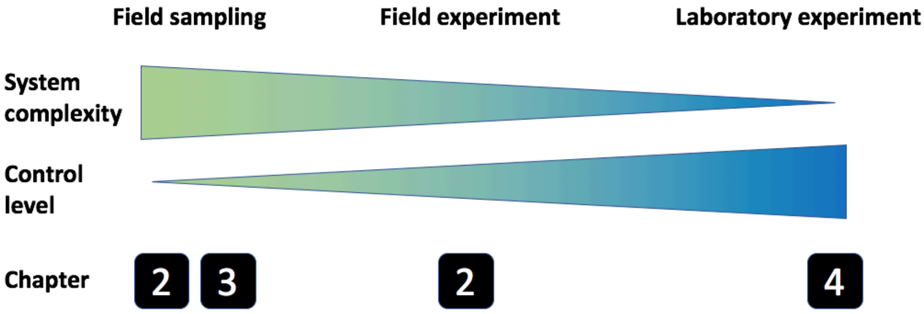

Figure 4. Schematic presentation of the multi-scale approach for this research

Chapter 1

Groundwater influence differentially affects periphyton and macrophyte production in lakes

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Abstract

Groundwater influx can significantly con- tribute to nutrient and carbon budgets of lakes, and its influence is the strongest in littoral areas dominated by macrophytes and periphyton. We have reviewed the effects of groundwater-borne nitrogen and phosphorus and dissolved inorganic and organic carbon (DIC, DOC) on these benthic primary producers in lakes. We develop a hypothesis for groundwater effects including the less studied impacts of periphyton shading on macrophytes. Groundwater-borne nutrients and DIC promote both macrophytes and periphyton. Direct studies on ground- water-borne DOC effects are lacking, but coloured DOC contributes to light attenuation and thus can restrict the growth of benthic primary producers. We predict that above certain threshold levels of nutrient influx by groundwater, periphyton and macrophyte biomass should decline owing to shading by phytoplankton and periphyton, respectively. However, because of their higher light requirements, those thresholds should be lower for macrophytes. For macrophytes, a threshold level is also predicted for a shift from DIC limitation to light limitation. Differences in light requirements are expected to result in lower thresholds of DOC loading for declines of macrophytes than periphyton.

Key words: Dissolved inorganic carbon, Dissolved organic carbon, Light, Macrophytes Nutrients, Periphyton

Introduction

Submerged macrophytes and periphyton play central roles as benthic primary producers in lake ecosystems. Submerged

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macrophytes are habitat for numerous organisms and can stabilize sediment and clear-water conditions in shallow (Scheffer et al. 1993) and deeper lakes (Sachse et al. 2014). Benthic primary production can dominate whole-lake primary production both in small shallow lakes, as well as in deep oligotrophic lakes (Vadeboncoeur and Steinman 2002; Vadeboncoeur et al. 2003), and the majority of fish species depend on this resource (Vadeboncoeur et al. 2014). Growth of benthic primary producers requires nutrients [crucially phosphorus (P) and nitrogen (N), silica (Si) for diatoms, inorganic dissolved carbon (DIC)] and light.

An important source of nutrients and DIC in lakes is influxes by surface and groundwater. They also transport coloured dissolved organic carbon (DOC) into lakes (Loeb and Goldman 1979). Groundwater influx usually is greatest near the shore and exponentially decreases with distance offshore (McBride and Pfannkuch 1975; Shaw and Prepas 1990). It passes the upper sediment layers with nutrient pore water concentrations often higher than in lake water (Hagerthey and Kerfoot 1998). Macrophytes and periphyton may dominate these littoral areas and consequently are supposed to be particularly influenced by groundwater. In general, groundwater plays a major role for the water balance of seepage and groundwater drainage lakes, but may also be relevant in other types of lakes fed by rain and snowmelt runoff through streams and rivers.

Numerous studies have shown that groundwater may significantly contribute to the nutrient and carbon budgets of lakes (references in Table 1). In the past, groundwater contributions of P to surface waters were often assumed unimportant because orthophosphate, as the most mobile P form, readily sorbs to soil particles, reducing its mobility (Hayashi and Rosenberry 2002). Other studies, however, have found that groundwater nutrient influx can significantly contribute to surface water eutrophication (e.g. Valiela et al. 1990; Burkart et al. 2004; Holman et al. 2008, 2010; Tomer et al. 2010). The influx of terrestrial DOC can lead to browning of lakes (Pace and Cole 2002).

This process restricts light availability at the sediment surface and thus potentially reduces benthic primary production (Ask et al. 2009), which can then affect production of benthic invertebrates and fish (Karlsson et al. 2009).

The available studies on effects of groundwater nutrients and DIC have either focussed on periphyton (Hagerthey and Kerfoot 1998, 2005; Roy et al. 2009) or on submerged macrophytes (Loeb and Hackley 1988; Lodge et al. 1989; Lillie and Barko 1990; Sebestyen and Schneider 2004; Frandsen et al. 2012) and generally describe positive impacts. Periphyton, however, can strongly impair submerged macrophyte growth by shading (Sand-Jensen 1977; Roberts et al. 2003) and is a main factor causing macrophyte declines with increasing eutrophication (Phillips et al. 1978; Jones and Sayer 2003). Groundwater solutes affecting periphyton growth are thus expected also to affect submerged macrophytes indirectly. Specific studies on the joint effect of groundwater on periphyton and macrophytes, however, are lacking.

Investigations on this issue seem particularly relevant to understanding the consequences of changing quality and quantity of groundwater seepage into lakes. During the last century, groundwater depletion from direct or indirect effects of climate change and/or human activities, such as groundwater pumping for irrigated agriculture or urban centres, has expanded from a local issue to one that affects large regions of the world (Green et al. 2011 and references therein). Natural lakes in north-eastern Germany, primarily fed by groundwater and precipitation, have shown periodic lake-level fluctuations with amplitudes of 1–2(– 3) m and declining depths during the last 20 years (Kaiser et al. 2014). Studies in fens have already shown the relevance of changing groundwater seepage for plant community composition (Grootjans et al. 1988; Wassen et al. 1989). Tremolieres et al. (1993) used changes in the aquatic macrophyte species composition to detect contamination of groundwater streams by P from River Rhine filtrate.

In this study, we have reviewed the available literature on the separate effects of groundwater-borne nutrients, DIC and DOC on periphyton and macrophytes in lakes. Based on this, we developed a qualitative, testable hypothesis for the joint effect of groundwater on periphyton and macrophytes with changing nutrient, DIC and DOC influx.

Literature review

Effects of nutrients in groundwater on macrophytes and periphyton

Groundwater influx can be responsible for up to 85% of the total phosphorus (TP), 67% of the total nitrogen (TN) and 100% of the total silica (TSi) influx to lakes (Table 1), at least for lakes in which groundwater is a major component of the water supply. It is, however, difficult to know how typical such lakes are of the world's inventory of lakes. Groundwater lakes, how- ever, are likely to be common. Groundwater does not only transport nutrients from the catchment to lakes but may also increase the flux of sediment pore water nutrients into the lake. Nutrient concentrations in pore water, especially that of soluble reactive phosphorus (SRP), can be substantially higher than in lake water (Hagerthey and Kerfoot 1998). In addition, groundwater may induce processes such as changes in redox conditions that result in the release of nutrients locally bound to sediments (Boström and Pettersson 1982). These may eventually be transported to the open water by groundwater influx, and the precise origin of these nutrients may be difficult to determine. For the influence of groundwater on benthic primary producers, however, the origin of the nutrients is of less importance than that they may be provided in increased supply through the movement of groundwater.

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Hydrobiologia. **778**: 91–103.

Table 1: Contribution (%) of groundwater to total phosphorus (TP), total nitrogen (TN), silica (Si), dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) loading of lakes.

TP	TN	Si	DIC	DOC	Lake	Reference
8.7	17.6				Tohopekaliga (USA)	Belanger et al. (1985)
12	2					Brock et al. (1982)
26-35					Little St. Germain (USA)	Robertson et al. (2005)
32					Ohrid	Matzinger et al. (2007)
0- 50					Mogan, Eymir (Turkey)	Özen et al. (2010)
	22 (NO_3)				Kasumigaura (JP)	(Nakayama and Watanabe (2008)
44	49				Tahoe (USA)	Loeb and Goldman (1979)
50 (SRP)	37				Sparkling Lake (USA)	Krabbenhoft et al. (1990)
50					Arendsee (Germany)	Meinikmann et al. (2013)
60					Narrow Lake (USA)	Shaw et al. (1990)
>66					Væng (DK)	Kidmose et al. (2013)
75	47				Mały Borek (Poland)	Jarosiewicz and Witek (2014)
85	67				Hampen (DK)	Ommen et al. (2012)
		100			Crystal Lake (USA)	Hurley et al. (1985)
			21-89	5-12	Black Hawk, Darling, Green Valley, Lizard, Prairie Rose (USA)	Pacheco et al. (2013)
			60-80	0	Williams, Shingobee (USA)	Striegl and Michmerhuizen (1998)
			80	n.d.	Williams (USA)	Mcconnaughey et al. (1994)
			100	42		Stets et al. (2009)

In oligotrophic systems, primary producers may be limited by nutrient supply, with P and/or N limiting algal biomass and growth in most freshwater habitats (Schindler 1977; Hansson 1992; Bergström and Jansson 2006; Elser et al. 2007). In lakes with low P concentrations in the surface water, pore water P might be more easily accessible for rooted macrophytes. Macrophytes with a high root-to-shoot ratio are assumed to have a competitive advantage (Barko and Smart 1980; Carignan and Kalff 1980; Roelofs et al. 1984). Macrophytes also store P, but with high interspecific variation (Thiébaut and Muller 2003; Garbey et al. 2004; Thiébaut 2005), and some species form symbioses with mycorrhizae that allow them to extract nutrients from sediments (Wigand et al. 1998; Andersen and Andersen 2006a)

P uptake rates in periphyton vary over five orders of magnitude in natural systems, and the area-specific P uptake is affected by several factors including grazing. Periphyton half-saturation constants for P uptake ranged from 0.62 to 1,271 µg P l^{-1}, with most values falling below 60 µg P l^{-1} (Dodds 2003 and references therein). In a direct comparison, both macrophytes and epiphytes removed P from the water of stream microcosms, but epiphyte P uptake was more rapid (Pelton et al. 1998). Nichols and Shaw (2002) did not find convincing evidence for an influence of groundwater flow on the distribution, abundance or biomass of aquatic plants in the lakes examined in their study, but in contrast a promotion of macrophytes through groundwater nutrients has been suggested by (Loeb and Hackley 1988) who measured higher seepage rates and pore water nutrient composition in macrophyte stands than in bare sediments.

Other field studies found a positive relationship between macrophyte biomass and seepage (Lodge et al. 1989; Lillie and Barko 1990) and have identified groundwater nutrients as drivers of macrophyte distribution. A field experiment in a softwater oligotrophic lake revealed that isoetid (*Littorella uniflora* (L.) Aschers.) biomass was 70% higher in groundwater seepage chambers as compared with solid bottom control chambers. Subsequent laboratory experiments revealed that the influence of seeping groundwater was partly related to improved nutrient availability (Frandsen et al. 2012). In addition, groundwater nutrients taken up by macrophytes accumulated in the plant material and thus may not have reached the surface water resulting in a filtration effect of macrophytes as suggested by Frandsen et al. (2012) and experimentally studied by (Christensen and Andersen 1996).

Frandsen et al. (2012), however, also suggested a potential direct influence of macrophytes on the groundwater flow path. Increased sedimentation in macrophyte stands could form a hydraulic barrier and lead to a displacement of the groundwater flow paths to areas outside the macrophyte stands. Compared with macrophytes, fewer studies are available on the effect of groundwater on benthic algae in oligotrophic lakes. However, a relatively dense benthic algal cover has been seen in groundwater discharge areas (Hagerthey and Kerfoot 1998; Roy et al. 2009). In an *in situ* experiment, Hagerthey and Kerfoot (1998) found positive correlations between groundwater influxes and benthic algal biomass, which was related to a higher advection flux of P through sediments. A significant promotion of algae by groundwater has been observed in a silica-limited oligotrophic lake, where Hurley et al. (1985) related blooms of diatoms to silica fluxes from groundwater. A potential impact of groundwater nutrient fluxes can also be assumed for studies that detected an increase in benthic algae on shallow shores of oligotrophic lakes that was not consistent with open-water concentrations of P and/ or N, e.g. in Lake Tahoe (Loeb 1986), Lake Taupo (Hawes and Smith 1993), Lake Huron (Barton et al. 2013) and Lake Ohrid (Schneider et al. 2014). Because of the high affinity of periphyton for P, it can scavenge excess P and thus contribute to the maintenance of low P concentrations in water (McCormick et al. 2001). With increasing nutrient loading, however, light limitation caused by phytoplankton rather than nutrient limitation will affect periphyton. Consequently, Hansson (1992) proposed a curvilinear relationship between the biomass of sediment-associated periphytic algae and lake productivity.

In addition, other variables may indicate a groundwater influence on benthic primary producers. First, the nutrient status of primary producers is reflected in their tissue stoichiometry (Gerloff and Krombholz 1966; Hillebrand and Sommer 1999). The influx of nutrients through groundwater has been found to affect nutrient ratios in benthic algae (Hagerthey and Kerfoot 1998, 2005) and macrophytes (Sebestyen and Schneider 2004). Stable isotope ratios in benthic primary producers may also contain information about ground- water sources. Bacchus and Barile (2005) used ^{15}N analyses of freshwater macrophytes to detect anthropogenic N contamination in groundwater. In addition, groundwater nutrient influx affected the species composition of benthic algae (Hawes and Smith 1993; Hagerthey and Kerfoot 2005; Roy et al. 2009). High groundwater discharge sites were dominated by diatoms and cyanobacteria characteristic of high water P concentrations, whereas low groundwater discharge sites were characterized by taxa associated with low P concentrations (Hagerthey and Kerfoot 2005).

Effects of DIC in groundwater on macrophytes and periphyton

Groundwater can sometimes be responsible for all of the DIC influx to lakes (Table 1). Groundwater obtains its DIC from a mixture of soil CO_2 and carbonate minerals (Deines et al. 1974). DIC concentrations in water comprise different inorganic carbon species: [CO_2^*] (=free CO_2 =dissolved CO_2 +H_2CO_3) + [HCO^-] + [CO_3^{2-}], which are linked by 33 chemical equilibria that are mainly determined by pH (Stumm and Morgan 1980). CO2 concentrations are greater for ombrotrophic (acidic) waters, and photo- synthesis of periphyton and macrophytes can cause strong CO_2 diurnal patterns, whereas HCO_3^- dominates minerotrophic (alkaline) waters with a greater buffering capacity (Hagerthey et al. 2011).

DIC acquisition mechanisms differ among algal species. They require an active mechanism (e.g. H^+- ATPase, carbonic anhydrase) to acquire HCO_3^-, whereas CO_2 is acquired passively or actively (Badger and Price 1992; Spijkerman et al. 2005). The same holds for submerged aquatic plants, as many species may use HCO_3^- in addition to CO_2 (Raven 1970; Bain and Proctor 1980; Maberly and Spence 1983). A comparative study showed a higher apparent affinity for HCO_3^- and CO_2 in microalgae than in macro- phytes, the latter having a larger diffusive resistance to CO_2 (Allen et al. 1981). However, some macrophyte species (isoetids) have a high root-to-shoot ratio and are able to exploit the carbon dioxide pool in the sediment (Brouwer et al. 2002; Murphy 2002; Pedersen et al. 2006).

Only a few studies have directly investigated the effect of groundwater DIC on benthic primary producers. Andersen and Andersen (2006b) tested the effect of increased CO_2 concentrations on the growth of the isoetid *L. uniflora* and filamentous algae (mainly *Zygnema* spp.) in a Danish softwater lake. Enclosures of 1.5 m diameter were enriched with free CO_2 to about 10 times atmospheric equilibrium (approximately 170 μM) and growth of plants and filamentous algae compared with that in control enclosures kept at atmospheric equilibrium. The biomass of filamentous algae was significantly higher (1.9–38 times) in the CO_2-enriched enclosures than in controls. *L. uniflora* leaf biomass increased from 75.0 ± 10.4 g dry weight m^{-2} in controls to 133.3 ± 42.5 g dry weight m^{-2} at increased CO_2 concentrations even though filamentous algal growth decreased the light intensity compared with controls (Andersen and Andersen 2006b). Frandsen et al. (2012) observed that groundwater discharge resulted in enhanced growth of isoetids and to some extent elodeids inhabiting a groundwater-fed softwater lake. The positive influence of seeping groundwater was related to increased inorganic carbon supply and, to a lesser degree, improved nutrient availability. A recent study by Takahashi and Asaeda (2014) indicated that the low pH in spring water increased the growth of *Egeria densa* by affecting free CO_2 concentration in the water. Indirect evidence for potential groundwater effects on macrophytes in a eutrophic hardwater lake was gained from an analysis of historical changes in species composition and abundance. *Fontinalis antipyretica* L. ex Hedw., a moss that prefers locations with groundwater discharge because of their high availability of free CO_2 (Melzer 1990), potentially disappeared from Lake Müggelsee in the early twentieth century after groundwater discharge to the lake was lost because groundwater was withdrawn from well galleries for drinking water production (Körner 2001).Other studies did not focus on groundwater, but indicate a potentially significant effect of changes in DIC availability on macrophytes and periphyton. Schippers et al. (2004) used a dynamic model to test the effect of an atmospheric CO_2 increase from 350 to 700 ppm on the growth of phytoplankton and submerged macrophytes in freshwater ecosystems. The increase in atmospheric CO_2 could affect submerged plant growth only under relatively eutrophic conditions and at a low community respiration rate. Under eutrophic conditions, algae and macrophytes using CO_2 and HCO_3^- may double their growth rate due to atmospheric CO_2 increase, while the growth of macrophytes restricted to CO_2 assimilation may be threefold (Schippers et al. 2004). Eusebio Malheiro et al. (2013), Hussner and Jahns (2015) and Hussner et al. (2015) showed that the competitive outcome between native and invasive submerged macrophytes was also affected by CO_2 availability. Species-specific differences in the growth rates under low CO_2 conditions partly explained the success of the alien over native species (Hussner et al. 2015). Marine periphyton sampled from artificial substrata exposed along a coastal CO_2 gradient at a shallow water cold vent system off the island of Vulcano (Italy) responded to raised CO_2 with significantly increased chlorophyll *a* concentrations (Johnson et al. 2013). Jones et al. (2002) tested the effect of increasing DIC concentrations (1.5, 2.5, 3.5 and 4.5 mM) on macrophytes and periphyton in mesocosm experiments. The biomass of *Elodea nuttallii* (Planch.) H. St John significantly increased with increasing DIC. This was assumed not to be a direct effect of carbon availability, because the same conditions did not

Hydrobiologia. **778**: 91–103.

affect plant growth in the previous year. A negative relationship between periphyton density and final plant density became significantly less steep with increasing DIC, indicating that periphyton and plants were competing for carbon as well as light (albeit asymmetrical competition, Jones et al. 2002).

Effects of DOC in groundwater on macrophytes and periphyton

Organic carbon concentrations in groundwater can vary considerably, with time and place. Analyses of about 250 groundwater samples from 4 European countries revealed DOC median concentrations of 2.2 mg C l^{-1} with a range from 0.2 to 58.9 mg C l^{-1}, showing that high organic carbon values can occasionally be found in some pristine aquifers (Gooddy and Hinsby 2009). In lakes, groundwater can be responsible for up to 42% of the DOC influx (Table 1). Natural DOC is mainly derived from decomposing vegetation and other organic matter in the soil and has a yellow/brown straw colour in shallow groundwater (Gooddy et al. 1995). Groundwater may also be affected by effluent from land-based wastewater septic systems that commonly contain high concentrations of DOC (Roy et al. 2009). Inputs of terrestrial DOC to surface waters have changed substantially over the past several decades in many north temperate and boreal regions (Hanson et al. 2007; Monteith et al. 2007). Many studies have focussed on the potential reasons for a widespread browning trend, whereas the consequences for recipient aquatic ecosystems have received less attention (Solomon et al. 2015).

In general, dissolved organic matter (DOM) can be divided into two categories: non-humic, labile and humic, refractory substances usually called coloured DOM (CDOM).

In lakes, the coloured DOM contributes to attenuation of light (Williamson et al. 1999) and thus potentially restricts the growth of benthic primary producers. Submerged macrophytes can grow down to 5 m at 60–70 mg Pt l^{-1}, but rarely exceed 2 m at colours above 100 mg Pt l^{-1} in lakes (Søndergaard et al. 2013). DOC concentrations were one of the most important variables driving aquatic macrophyte distribution in Mediterranean water bodies (Chappuis et al. 2014). Increased CDOM concentrations, resulting in browner water, were also found to reduce macrophyte abundance in softwater (McElarney et al. 2010) and hardwater lakes (Ejankowski and Lenard 2015).

Ask et al. (2009) investigated the benthic primary production of periphyton in 15 unproductive lakes ranging from clear-water to humic conditions in northern Sweden. They concluded that unproductive lakes are very sensitive to inputs of terrestrial DOC owing to the effects on basal energy mobilization. Vinebrooke and Leavitt (1998) experimentally tested the effect of increased DOC additions (3 mg l^{-1}). DOC amendments significantly increased the biomass of surface films on hard substrates (epilithon) but did not affect the abundance of either epipelon (on sediment) or phytoplankton.

Jones et al. (2012) developed a simple model to predict the response of primary producers to changes in DOC loading to lakes. Their model mainly accounted for the shading effect of terrestrial DOC on primary producers. Increased inputs of terrestrial DOC generally reduced phytoplankton and periphyton primary production and thus food for consumers. Only in very oligotrophic conditions (TP = 2 µg l^{-1}) did they observe an increase in phytoplankton production with raised DOC because P enters the lake along with C as part of the terrestrial dissolved organic matter. Benthic and pelagic primary production was reduced to 40% and 50%, respectively, at the highest tested DOC concentration (Jones et al. 2012). These theoretical results were confirmed by an experiment in a pond system with a gradient in terrestrial DOC supply (Jones and Lennon 2015) and by field observations in a small eutrophic shallow lake undergoing a strong increase in DOC and colour following a water level increase (Brothers et al. 2014). Leaching of DOC from flooded adjacent peatlands and internal browning via reduction of iron-bound DOC in the sediments resulted in an increase of DOC concentrations from a mean of 12 ± 1 mg l^{-1} to a maximum concentration of 53 mg l^{-1} in 18 months. Increasing water levels, DOC and phytoplankton concentrations reduced mean spring and summer Secchi depths from 1.5 to 0.7 m. As a consequence, periphyton growth was reduced by about 66% (Brothers et al. 2014).

In principle, CDOM may also hamper fish reliant on visual feeding and thus the fish community structure (Stasko et al. 2012). Such changes could potentially change top-down cascades suggested to affect periphyton and macrophytes (Jones and Sayer 2003), but studies on this question are not yet available. Apart from effects on light availability, there are also potential direct effects of groundwater- borne DOC on macrophytes and algae owing to interferences with photosynthesis and growth (Steinberg et al. 2006).

Hypothesis concerning the joint effect of groundwater on periphyton and macrophytes

Based on the available knowledge of the separate effects of groundwater on periphyton and macrophytes and the differential light requirements of these primary producers, we suggest a hypothesis for the qualitative joint effects of groundwater on both benthic primary producers (Fig. 1).

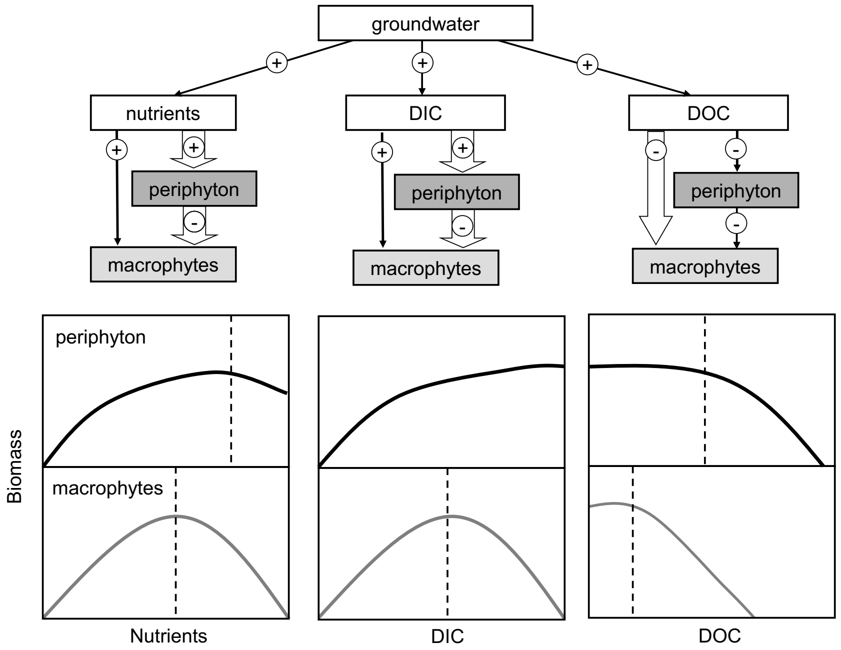

Figure 1. Conceptual model of potential effects of groundwater- borne nutrients, DIC and DOC on the biomass of periphyton and submerged macrophytes in the littoral of lakes. Dashed lines indicate the threshold level above which light limitation causes a decline in the biomass of the respective primary producer. The wider arrows indicate that the positive effects of increasing nutrients and DIC are stronger for periphyton growth than for macrophytes, whereas the negative effect of DOC is stronger on macrophytes than on periphyton

As outlined above, nutrient supply by groundwater is supposed to increase the biomass of periphyton and macrophytes in the littoral of oligo- and mesotrophic lakes (Fig. 1 left). However, above a certain threshold level, light will become limiting for periphyton due to increased growth of phytoplankton (Hansson 1992) and for macrophytes due to increased growth of periphyton and subsequent shading (Fig. 1 left). The minimum daily light supply for submersed macrophytes often ranges between 2 and 4 E m^{-2} (Sand-Jensen and Borum 1991). The mean light supply at the maximum depth of growth ranged between 1.6 E m^{-2} day^{-1} for caulescent and 5 E m^{-2} day^{-1} for rosette- type angiosperms in a wide range of lakes (Middelboe and Markager 1997). In contrast, unicellular algae require less light than rooted macrophytes (Sand-Jensen and Borum 1991). Tuji (2000) found half of the maximum growth rate of benthic diatoms at light intensities of 5–64 mE m^{-2} s^{-1} (0.25–3.2 E m^{-2} day^{-1} with 14 h of light per day). Rier et al. (2006) measured maximum growth of acclimated phytobenthos (mainly diatoms) at PAR > 2.5 E m^{-2} day^{-1}. We can therefore expect that both, macrophytes and periphyton, are promoted by groundwater-borne nutrients up to a threshold above which periphyton development limits light availability for macrophytes. Periphyton is only limited by phytoplankton shading at a higher threshold. With increasing nutrient loading, however, grazing becomes more relevant in controlling periphyton (Hillebrand 2002). The actual thresh- old levels for particular lakes will thus also depend on their particular trophic structure. Jones and Sayer (2003) have indicated a cascading effect of fish via periphyton grazing invertebrates to periphyton and macrophytes in lakes.

Aquatic macrophytes also frequently have carbon- limited photosynthesis at saturating irradiances owing to boundary-layer effects (Raven et al. 1982; Stevenson 1988); limited diffusion can induce the use of bicarbonate (Smith and Walker 1980). Significant boundary-layer effects have also been demonstrated for benthic algae (Turner et al. 1991). Results of Valiela et al. (1990) seem to support our concept. Groundwater-borne nutrients (specifically nitrate) were found to support the growth of light-intercepting epiphytes on eelgrass (*Zostera marina* L.) and thus were assumed to cause the decline of eelgrass in the studied coastal waters.

Groundwater-borne DIC is also assumed to pro- mote macrophyte and periphyton growth (see review above, Fig. 1, middle). However, as with nutrients, a threshold level for a shift from DIC limitation to light limitation is expected for macrophytes. Sand-Jensen (1977) found that periphyton reduced the photosynthesis of eelgrass (*Z. marina*), affecting both light and carbon supply. The underlying processes were investigated by (Jones et al. 2000): periphyton growth increased the boundary layer around macrophyte leaves, thus reducing CO_2 diffusion and availability. Boundary layer thickness increased linearly with periphyton thickness. Competition for carbon between benthic primary producers is, however, limited to environments with low DIC (Jones et al. 2002).

Our conceptual model is supported by findings of Jones et al. (2002). In their study, the concentration of DIC did have a significant influence on the interaction between benthic primary producers. Increased DIC resulted in a greater biomass of periphyton. Carbon competition between periphyton and macrophyte was higher at low DIC concentration, with macrophytes growing less well at the same density of periphyton. Lakes with low DIC concentration will be thus be more prone to plant loss with eutrophication than those with higher DIC. These effects indicate that changes of DIC concentrations because of changing ground- water influx can be very important to the functioning of lakes with low DIC. DIC concentrations in general are largely neglected and should be considered when comparing lakes (Jones et al. 2002).

The additional flux of coloured DOC to lakes through groundwater is supposed to disfavour both periphyton and macrophytes (see review above, Fig. 1 right). However, macrophytes will decline at a lower threshold of DOC loading than periphyton owing to their higher light requirements. Köhler et al. (2010) reported a decrease of macrophyte biomass, while periphyton remained unaffected when shaded by bank trees. Ask et al. (2009) calculated the light extinction coefficient kd in relation to the DOC concentrations in 15 unproductive Swedish lakes as $k_d = 0.2 \times e^{2.3 \times \log 10(DOC)}$, with DOC concentration in mg l^{-1}. These findings, together with a threshold level of 1.8 m^{-1} for k_d at which periphyton biomass (in 0.75 m water depth) was found to decline along a gradient of lakes with different turbidities (Hansson 1992), suggest that DOC threshold levels for periphyton might be around 9 mg l^{-1}. A study by Mormul et al. (2012) indicated a reduced growth of both, periphyton and native macrophytes with increasing DOC concentrations. However, this study also showed that there might be exceptions to our suggestion of macrophyte biomass decreasing at lower DOC thresholds than periphyton. Browner water treatments favoured the invasive *Elodea canadensis* by reducing the growth of periphyton and most native macrophyte competitors. No study, however, has been carried out on the effects of groundwater-borne DOC on periphyton and macrophytes in littoral areas of lakes.

Conclusions

The few available empirical case studies on effects of groundwater-borne nutrients, DIC and/or DOC on both, periphyton and macrophytes, seem to support our hypothesis suggesting a decline of macrophytes above certain threshold levels owing to their generally higher light requirements compared with periphyton. For a better description of these effects, more field and experimental studies along gradients of nutrient, DIC and DOC loading by groundwater are needed. These could help in interpreting the yet poorly known role of changes in groundwater fluxes and groundwater-borne nutrient, DIC and DOC loading on the benthic primary producer communities of lakes, and specifically the potential decline of submerged macrophytes in shallow littoral areas.

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References

Allen, A. E. D., D. H. N. Spence, and S. Url. 1981. The differential ability of aquatic plants to utilize the inorganic carbon supply in fresh waters. New Phytol. **87**: 269–283.

Andersen, F. Ø., and T. Andersen. 2006a. Effects of arbuscular mycorrhizae on biomass and nutrients in the aquatic plant *Littorella uniflora*. Freshw. Biol. **51**: 1623–1633.

Andersen, T., and F. Andersen. 2006b. Effects of CO2 concentration on growth of filamentous algae and *Littorella uniflora* in a Danish softwater lake. Aquat. Bot. **84**: 267–271.

Ask, J., J. Karlsson, L. Persson, P. Ask, P. Byström, and M. Jansson. 2009. Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. Limnol. Oceanogr. **54**: 2034–2040.

Bacchus, S. T., and P. J. Barile. 2005. Discriminating sources and flowpaths of anthropogenic nitrogen discharges to Florida springs, streams and lakes. Environ. Eng. Geosci. **11**: 347–369.

Badger, M. R., and G. D. Price. 1992. The CO2 concentrating mechanism in cyanobacteria and microalgae. Physiol. Plant. **84**: 606–615.

Bain, J. T., and M. C. F. Proctor. 1980. The requirement of aquatic bryophytes for free CO2 as an inorganic carbon source: some experimental evidence. New Phytol. **6**: 393–400.

Barko, J. W., and R. M. Smart. 1980. Mobilization of sediment phosphorus by submersed freshwater macrophytes. Freshw. Biol. **10**: 229–238.

Barton, D. R., E. T. Howell, and C. L. Fietsch. 2013. Ecosystem changes and nuisance benthic algae on the southeast shores of Lake Huron. J. Great Lakes Res. **39**: 602–611.

Hydrobiologia. **778**: 91–103.
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Belanger, T. V., D. F. Mikutel, and P. A. Churchill. 1985. Groundwater seepage nutrient loading in a Florida Lake. Water Res. **19**: 773–781.

Bergström, A. K., and M. Jansson. 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Glob. Chang. Biol. **12**: 635–643.

Boström, B., and K. Pettersson. 1982. Different patterns of phosphorus release from lake sediments in laboratory experiments. Hydrobiologia **92**: 415–429.

Brock, T. D., D. R. Lee, D. Janes, and D. Winek. 1982. Groundwater seepage as a nutrient source to a drainage lake; Lake Mendota, Wisconsin. Water Resour. **16**: 1255–1263.

Brothers, S., J. Köhler, N. Meyer, K. Attermeyer, H. P. Grossart, and S. Hilt. 2014. A feedback loop links brownification and anoxia in a temperate, shallow lake. Limnol. Oceanogr. **59**: 1388–1398.

Brouwer, E., R. Bobbink, and J. G. M. Roelofs. 2002. Restoration of aquatic vegetation in acidified and eutrophied softwater lakes: an overview. Aquat. Bot. **73**: 405–431.

Burkart, M. R., W. W. Simpkins, A. J. Morrow, and J. M. Gannon. 2004. Occurrence of total dissolved phosphorous in unconsolidated aquifers and aquitards in Iowa. J. Am. Water Resour. Assoc. **40**: 827–834.

Carignan, R., and J. Kalff. 1980. Phosphorus sources for aquatic weeds: water or sediments? Science (80-.). **207**: 987–989.

Chappuis, E., E. Gacia, and E. Ballesteros. 2014. Environmental factors explaining the distribution and diversity of vascular aquatic macrophytes in a highly heterogeneous Mediterranean region. Aquat. Bot. **113**: 72–82.

Christensen, K. K., and F. O. Andersen. 1996. Influence of *Littorella uniflora* on phosphorus retention in sediment supplied with artificial porewater. Aquat. Bot. **55**: 183–197.

Deines, P., D. Langmuir, and R. S. Harmon. 1974. Stable carbon isotope ratios and the existence of a gas phase in the evolution of carbonate ground waters. Geochim. Cosmochim. Acta **38**: 1147–1164.

Dodds, W. K. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J. Phycol. **39**: 840–849.

Ejankowski, W., and T. Lenard. 2015. Climate driven changes in the submerged macrophyte and phytoplankton community in a hard water lake. Limnologica **52**: 59–66.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. **10**: 1135–1142.

Eusebio Malheiro, A. C., P. Jahns, and A. Hussner. 2013. CO2 availability rather than light and temperature determines growth and phenotypical responses in submerged Myriophyllum aquaticum. Aquat. Bot. **110**: 31–37.

Frandsen, M., B. Nilsson, P. Engesgaard, and O. Pedersen. 2012. Groundwater seepage stimulates the growth of aquatic macrophytes. Freshw. Biol. **57**: 907–921.

Garbey, C., G. Thiébaut, and S. Muller. 2004. Morphological plasticity of a spreading aquatic macrophyte, Ranunculus peltatus, in response to environmental variables. Plant Ecol. **173**: 125–137.

Gerloff, G. C., and P. H. Krombholz. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. **11**: 529–537.

Gooddy, D. C., and K. Hinsby. 2009. Organic quality of groundwaters, *In* W.M. Edmunds and P. Shand [eds.], Natural groundwater quality. Wiley-Blackwell.

Gooddy, D. C., P. Shand, D. G. Kinniburgh, and W. H. van Riemsdijk. 1995. Field-based partition coefficients for trace elements in soil

Hydrobiologia. **778**: 91–103.
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solutions. Eur. J. Soil Sci. **46**: 265–285.

Green, T. R., M. Taniguchi, H. Kooi, J. J. Gurdak, D. M. Allen, K. M. Hiscock, H. Treidel, and A. Aureli. 2011. Beneath the surface of global change: Impacts of climate change on groundwater. J. Hydrol. **405**: 532–560.

Grootjans, A. P., R. van Diggelen, M. J. Wassen, and W. A. Wiersinga. 1988. The effects of drainage on groundwater quality and plant species distribution in stream valley meadows. Vegetatio **75**: 37–48.

Hagerthey, S. E., B. J. Bellinger, K. Wheeler, M. Gantar, and E. Gaiser. 2011. Everglades periphyton: A biogeochemical perspective. Crit. Rev. Environ. Sci. Technol. **41**: 309–343.

Hagerthey, S. E., and W. C. Kerfoot. 1998. Groundwater flow influences the biomass and nutrient ratios of epibenthic algae in a north temperate seepage lake. Limnol. Oceanogr. **43**: 1127–1242.

Hagerthey, S. E., and W. C. Kerfoot. 2005. Spatial variation in groundwater-related resource supply influences freshwater benthic algal assemblage composition. J. North Am. Benthol. Soc. **24**: 807–819.

Hanson, P. C., S. R. Carpenter, J. A. Cardille, M. T. Coe, and L. A. Winslow. 2007. Small lakes dominate a random sample of regional lake characteristics. Freshw. Biol. **52**: 814–822.

Hansson, L.-A. 1992. Factors regulating periphytic algal biomass. Limnol. Oceanogr. **37**: 322–328.

Hawes, I., and R. Smith. 1993. Effect of localised nutrient enrichment on the shallow epilithic periphyton of oligotrophic Lake Taupo, New Zealand. New Zel. J. Mar. Freshw. Res. **27**: 365–372.

Hayashi, M., and D. O. Rosenberry. 2002. Effect of ground water exchange on the hydrology and ecology of surface water. Ground Water **40**: 309–316.

Hillebrand, H. 2002. Top-down versus bottom-up control of autotrophic biomass : a meta-analysis on experiments with periphyton. J. North Am. Benthol. Soc. **21**: 349–369.

Hillebrand, H., and U. Sommer. 1999. The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. Limnol. Oceanogr. **44**: 440–446.

Holman, I. P., N. J. K. Howden, P. Bellamy, N. Willby, M. J. Whelan, and M. Rivas-Casado. 2010. An assessment of the risk to surface water ecosystems of groundwater P in the UK and Ireland. Sci. Total Environ. **408**: 1847–1857.

Holman, I. P., M. J. Whelan, N. J. K. Howden, P. H. Bellamy, N. J. Willby, M. Rivas-Casado, and P. McConvey. 2008. Phosphorus in groundwater - an overlooked contributor to eutrophication? Hydrol. Process. **22**: 5121–5127.

Hurley, J. P., D. E. Armstrong, G. J. Kenoyer, and C. J. Bowser. 1985. Ground Water as a silica source for diatom production in a precipitation-dominated lake. Science (80-.). **227**: 1576–1578.

Hussner, A., D. Hofstra, P. Jahns, and J. Clayton. 2015. Response capacity to CO2 depletion rather than temperature and light effects explain the growth success of three alien *Hydrocharitaceae* compared with native *Myriophyllum triphyllum* in New Zealand. Aquat. Bot. **120**: 205–211.

Hussner, A., and P. Jahns. 2015. European native *Myriophyllum spicatum* showed a higher HCO_3 use capacity than alien invasive *Myriophyllum heterophyllum*. Hydrobiologia **746**: 171–182.

Jarosiewicz, A., and Z. Witek. 2014. Where do nutrients in an inlet-less lake come from? The water and nutrient balance of a small mesotrophic lake. Hydrobiologia **724**: 157–173.

Johnson, V. R., C. Brownlee, R. E. M. Rickaby, M. Graziano, M. Milazzo, and J. M. Hall-Spencer. 2013. Responses of marine benthic microalgae to elevated CO2. Mar. Biol. **160**: 1813–1824.

Hydrobiologia. **778**: 91–103.

Jones, J. I., J. W. Eaton, and K. Hardwick. 2000. The influence of periphyton on boundary layer conditions: A pH microelectrode investigation. Aquat. Bot. **67**: 191–206.

Jones, J. I., and C. D. Sayer. 2003. Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? Ecology **84**: 2155–2167.

Jones, J. I., J. O. Young, J. W. Eaton, and B. Moss. 2002. The influence of nutrient loading , dissolved inorganic carbon and higher trophic levels on the interaction between submerged plants and periphyton. J. Ecol. **90**: 12–24.

Jones, S. E., and J. T. Lennon. 2015. A test of the subsidy–stability hypothesis: the effects of terrestrial carbon in aquatic ecosystems. Ecology **96**: 1550–1560.

Jones, S. E., C. T. Solomon, and B. C. Weidel. 2012. Subsidy or substraction: how terrestrial inputs influence consumer production in lakes? Freshw. Rev. **5**: 33–49.

Kaiser, K., P. J. Koch, R. Mauersberger, P. Stüve, J. Dreibrodt, and O. Bens. 2014. Detection and attribution of lake-level dynamics in north-eastern central Europe in recent decades. Reg. Environ. Chang. **14**: 1587–1600.

Karlsson, J., P. Byström, J. Ask, P. Ask, L. Persson, and M. Jansson. 2009. Light limitation of nutrient-poor lake ecosystems. Nature **460**: 506–509.

Kidmose, J., B. Nilsson, P. Engesgaard, M. Frandsen, S. Karan, F. Landkildehus, M. Søndergaard, and E. Jeppesen. 2013. Focused groundwater discharge of phosphorus to a eutrophic seepage lake (Lake Væng, Denmark): implications for lake ecological state and restoration. Hydrogeol. J. **21**: 1787–1802.

Köhler, J., J. Hachoł, and S. Hilt. 2010. Regulation of submersed macrophyte biomass in a temperate lowland river: interactions between shading by bank vegetation, epiphyton and water turbidity.

Aquat. Bot. **92**: 129–136.

Körner, S. 2001. Development of submerged macrophytes in shallow Lake Müggelsee (Berlin, Germany) before and after its switch to the phytoplankton-dominated state. Arch. für Hydrobiol. **152**: 395–409.

Krabbenhoft, D. P., C. P. Bowser, M. P. Anderson, and J. W. Valley. 1990. Estimating groundwater exchange with lakes: 1. The stable isotope mass balance. Water Resour. Res. **26**: 2445–2454.

Lillie, R. A., and J. W. Barko. 1990. Influence of sediment and groundwater on the distribution and biomass of Myriophyllum spicatum L. in Deveil's Lake, Wisconsin. J. Freshw. Ecol. **5**: 417–426.

Lodge, D. M., D. P. Krabbenhoft, and R. G. Striegl. 1989. A positive relationship between groundwater velocity and submersed macrophyte biomass in Sparkling lake, Wisconsin. Limnol. Oceanogr. **34**: 235–239.

Loeb, S. L. 1986. Algal biofouling of oligotrophic lake Tahoe: causal factors affecting production, p. 159–173. *In* L.V. Evans and K.D. Hoagland [eds.], Algal Biofouling. Elsevier Science Publishers.

Loeb, S. L., and C. R. Goldman. 1979. Water and nutrient transport via groundwater from Ward Valley into Lake Tahoe. Limnol. Oceanogr. **24**: 1146–1154.

Loeb, S. L., and S. H. Hackley. 1988. The distribution of submerged macrophytes in Lake Tahoe, California and Nevada, and the possible influence of groundwater seepage. Verhandlungen der Int. Vereinigung für Theor. und Angewante Limnol. **23**: 1927–1933.

Maberly, A. S. C., and D. H. N. Spence. 1983. Photosynthetic Inorganic Carbon use by Freshwater Plants I. J. Ecol. **71**: 705–724.

Matzinger, A., M. Schmid, E. Veljanoska-Sarafiloska, S. Patceva, D. Guseska, B. Wagner, B. Muller, M. Sturm, and A. Wuest. 2007. Eutrophication of ancient Lake Ohrid: global warming amplifies detrimental effects of increased nutrient inputs. Limnol. Oceanogr. **52**: 338–353.

McBride, M. S., and H. O. Pfannkuch. 1975. The distribution of seepage within lakebeds. J. Res. USGS **3**: 505–512.

Mcconnaughey, T. a., J. W. Labaugh, D. O. Rosenberry, R. G. Striegl, M. M. Reddy, P. F. Schuster, and V. Carter. 1994. Carbon budget for a groundwater-fed lake: Calcification supports summer photosynthesis. Limnol. Oceanogr. **39**: 1319–1332.

McCormick, P. V., M. B. O'Dell, R. B. E. Shuford, J. G. Backus, and W. C. Kennedy. 2001. Periphyton responses to experimental phosphorus enrichment in a subtropical wetland. Aquat. Bot. **71**: 119–139.

McElarney, Y. R., P. Rasmussen, R. H. Foya, and N. J. Anderson. 2010. Response of aquatic macrophytes in Northern Irish softwater lakes to forestry management; eutrophication and dissolved organic carbon. Aquat. Bot. **93**: 227–236.

Meinikmann, K., J. Lewandowski, and G. Nützmann. 2013. Lacustrine groundwater discharge: combined determination of volumes and spatial patterns. J. Hydrol. **502**: 202–211.

Melzer, A. 1990. Die Makrophytenvegetation des Tegern-, Schlier- und Riegsees. Informationsberichte Bayerisches Landesamt für Wasserwirtschaft Heft 2.

Middelboe, A. L., and S. Markager. 1997. Depth limits and minimum light requirements of freshwater macrophytes. Freshw. Biol. **37**: 553–568.

Monteith, D. T., J. L. Stoddard, C. D. Evans, H. A. de Wit, M. Forsius, T. Hogasen, A. Wilander, B. L. Skjelkvale, D. S. Jeffries, J. Vuorenmaa, B. Keller, J. Kopacek, and J. Vesely. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature **450**: 537–539.

Mormul, R. P., J. Ahlgren, M. K. Ekvall, L. A. Hansson, and C.

Brönmark. 2012. Water brownification may increase the invisibility of a submerged non-native macrophyte. Biol. Invasions **14**: 2091–2099.

Murphy, K. J. 2002. Plant communities and plant diversity in softwater lakes of northern Europe. Aquat. Bot. **73**: 287–324.

Nakayama, T., and M. Watanabe. 2008. Missing role of groundwater in water and nutrient cycle in the shallow eutrophic Lake Kasumigaura, Japan. Hydrol. Process. **22**: 1150–1172.

Nichols, S. A., and B. Shaw. 2002. The influence of groundwater flow on the distribution and abundance of aquatic plants in some wisconsin lakes. J. Freshw. Ecol. **17**: 283–295.

Ommen, D. A. O., J. Kidmose, S. Karan, M. R. Flindt, P. Engesgaard, B. Nilsson, and F. Ø. Andersen. 2012. Importance of groundwater and macrophytes for the nutrient balance at oligotrophic Lake Hampen, Denmark. Ecohydrology **5**: 286–296.

Özen, A., B. Karapınar, I. Kucuk, E. Jeppesen, and M. Beklioglu. 2010. Drought-induced changes in nutrient concentrations and retention in two shallow Mediterranean lakes subjected to different degrees of management. Hydrobiologia **646**: 61–72.

Pace, M. L., and J. J. Cole. 2002. Synchronous variation of dissolved organic carbon and color in lakes. Limnol. Oceanogr. **47**: 333–342.

Pacheco, F. S., F. Roland, and J. A. Downing. 2013. Eutrophication reverses whole-lake carbon budgets. Inl. Waters **4**: 41–48.

Pedersen, O., T. Andersen, K. Ikejima, M. D. Z. Hossain, and F. Ø. Andersen. 2006. A multidisciplinary approach to under- standing the recent and historical occurrence of the freshwater plant, Littorella uniflora. Freshw. Biol. **51**: 865–877.

Pelton, D. K., S. N. Levine, and M. Braner. 1998. Measurements of phosphorus uptake by macrophytes and epiphytes from the Laplatte River using 32P in stream microcosms. Freshw. Biol. **39**: 285–299.

Hydrobiologia. **778**: 91–103.

Phillips, G. L., D. Eminson, and B. Moss. 1978. A mechanism to account for macrophytes decline in progressively eutrophicated freshwaters. Aquat. Bot. **4**: 103–126.

Raven, J. A. 1970. Exogenous inorganic carbon sources in plant photosynthesis. Biol. Rev. **45**: 167–221.

Raven, J. A., J. Beardall, and H. Griffiths. 1982. Inorganic C-sources for *Lemanea*, *Cladophora* and *Ranunculus* in a fast-flowing stream: measurements of gas exchange and of carbon isotope ratio and their ecological implications. Oecologia **53**: 68–78.

Rier, S. T., R. J. Stevenson, and G. D. LaLiberte. 2006. Photo-acclimation response of benthic stream algae across experimentally manipulated light gradients: a comparison of growth rates and net primary productivity. J. Phycol. **42**: 560–567.

Roberts, E., J. Kroker, S. Körner, and A. Nicklisch. 2003. The role of periphyton during the re-colonization of a shallow lake with submerged macrophytes. Hydrobiologia **506**–**509**: 525–530.

Robertson, D. M., W. J. Rose, and D. A. Saad. 2005. Water quality, hydrology, and phosphorus loading to Little St. Germain Lake, Wisconsin, with special emphasis on the effects of winter aeration and groundwater inputs. U.S. Geological Survey Scientific Investigations Report 01.

Roelofs, J. G. M., J. A. A. R. Schuurkes, and A. J. M. Smits. 1984. Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. Aquat. Bot. **18**: 389–411.

Roy, J. W., J. M. Robillard, S. B. Watson, and M. Hayashi. 2009. Non-intrusive characterization methods for wastewater-affected groundwater plumes discharging to an alpine lake. Environ. Monit. Assess. **149**: 201–11.

Sachse, R., T. Petzoldt, M. Blumstock, S. Moreira, J. Rücker, J. H. Janse, W. M. Mooij, S. Hilt, and P. Marlene. 2014. Extending one-dimensional models for deep lakes to simulate the impact of submerged macrophytes on water quality. Environ. Model. Softw. **61**: 410–423.

Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. Aquat. Bot. **3**: 55–63.

Sand-Jensen, K., and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. **41**: 137–175.

Scheffer, M., S. Hosper, M. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative equilibria in shalow lakes. Trends Ecol. Evol. **8**: 275–279.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science (80-.). **195**: 260–262.

Schippers, P., J. E. Vermaat, J. de Klein, and W. Mooij. 2004. The effect of atmospheric carbon dioxide elevation on plant growth in freshwater ecosystems. Ecosystems **7**: 63–74.

Schneider, S. C., M. Cara, T. E. Eriksen, B. Budzakoska Goreska, A. Imeri, L. Kupe, T. Lokoska, S. Patceva, S. Trajanovska, S. Trajanovski, M. Talevska, and E. Veljanoska Sarafiloska. 2014. Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low. Limnologica **44**: 90–97.

Sebestyen, S. D., and R. L. Schneider. 2004. Seepage patterns, pore water, and aquatic plants: hydrological and biogeochemical relationships in lakes. Biogeochemistry **68**: 383–409.

Shaw, R. D., and E. E. Prepas. 1990. Groundwater-lake interactions: II. Nearshore seepage patterns and the contribution of ground water to lakes in central Alberta. J. Hydrol. **119**: 121–136.

Shaw, R. D., J. F. H. Shaw, H. Fricker, and E. E. Prepas. 1990. An integrated approach to quantify groundwater transport of phosphorus to Narrow Lake, Alberta. Limnol. Oceanogr. **35**: 870–886.

Smith, E. A., and N. A. Walker. 1980. Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO2 and HCO3- and to carbon isotopic discrimination. New Phytol. **86**: 245–259.

Solomon, C. T., S. E. Jones, B.C.Weidel, I.Buffam, M.L.Fork, J. Karlsson, S. Larsen, J. T. Lennon, J. S. Read, S. Sadro, and J. E. Saros. 2015. Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: current knowledge and future challenges. Ecosystems **18**: 376–389.

Søndergaard, M., G. Phillips, S. Hellsten, A. Kolada, F. Ecke, H. Mäemets, M. Mjelde, M. M. Azzella, and A. Oggioni. 2013. Maximum growing depth of submerged macrophytes in European lakes. Hydrobiologia **704**: 165–177.

Spijkerman, E., S. C. Maberly, and P. F. M. Coesel. 2005. Carbon acquisition mechanisms by planktonic desmids and their link to ecological distribution. Can. J. Bot. – Rev. Can. Bot. **83**: 850–858.

Stasko, A. D., J. M. Gunn, and T. A. Johnston. 2012. Role of ambient light in structuring north temperate fish communities: potential effects of increasing dissolved organic carbon concentration with a changing climate. Environ. Rev. **20**: 173–190.

Steinberg, C. E. W., S. Kamara, V. Y. Prokhotskaya, L. Manusadnianas, T. Karasyova, M. A. Timofeyev, J. Zhang, and B. K. B. & R. M. A. Paul, T. Meinelt, V. F. Farjalla, A. Y. O. Matsuo. 2006. Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? Freshw. Biol. **51**: 1189–1210.

Stets, E. G., R. G. Striegl, G. R. Aiken, D. O. Rosenberry, and T. C. Winter. 2009. Hydrologic support of carbon dioxide flux revealed by whole-lake carbon budgets. J. Geophys. Res. **114**, doi:10.1029/2008JG000783

Stevenson, C. 1988. Comparative ecology of submersed grass beds in freshwater, estuarine, and marine environments. Limnol. Oceanogr. **33**: 867–893.

Striegl, R. G., and C. M. Michmerhuizen. 1998. Hydrologic influence on methane and carbon dioxide dynamics at two north-central Minnesota lakes. Limnol. Oceanogr. **43**: 1519–1529.

Stumm, W., and J. J. Morgan. 1980. Aquatic Chemistry, 2nd ed., Wiley-Interscience.

Takahashi, K., and T. Asaeda. 2014. The effect of spring water on the growth of a submerged macrophyte *Egeria densa*. Landsc. Ecol. Eng. **10**: 99–107.

Thiébaut, G. 2005. Does competition for phosphate supply explain the invasion pattern of Elodea species? Water Res. **39**: 3385–3393.

Thiébaut, G., and S. Muller. 2003. Linking phosphorus pools of water , sediment and macrophytes in running waters. Ann. Limnol. - Int. J. Limnol. **39**: 307–316.

Tomer, M. D., K. E. Schilling, C. A. Cambardella, P. Jacobson, and P. Drobney. 2010. Groundwater nutrient concentrations during prairie reconstruction on an Iowa landscape. Agric. Ecosyst. Environ. **139**: 206–213.

Tremolieres, M., I. Eglin, U. Roeck, and R. Carbiener. 1993. The exchange process between river and groundwater in the floodplain of Alsace (Eastern France). 1. The case of the canalised river Rhine. Hydrobiologia **254**: 133–148.

Tuji, A. 2000. The effect of irradiance on the growth of different forms of freshwater diatoms: implications for succession in attached diatom communities. J. Phycol. **36**: 659–661.

Turner, M. A., E. T. Howell, M. Summerby, R. H. Hesslein, D. L. Findlay, and M. B. Jackson. 1991. Changes in epilithon and epiphyton associated with experimental acidification of a lake to pH 5. Limnol. Oceanogr. **36**: 1390–1405.

Vadeboncoeur, Y., S. P. Devlin, P. B. McIntyre, and M. J. Vander Zanden. 2014. Is there light after depth ? Distribution of periphyton chlorophyll and productivity in lake littoral zones. Freshw. Sci. **33**:

524–536.

Vadeboncoeur, Y., E. Jeppesen, M. J. Vander Zanden, H.-H. Schierup, K. Christoffersen, and D. M. Lodge. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnol. Oceanogr. **48**: 1408–1418.

Vadeboncoeur, Y., and A. D. Steinman. 2002. Periphyton Function in Lake Ecosystems. Sci. World J. **2**: 1449–1468.

Valiela, I., J. Costa, K. Foreman, J. M. Teal, B. Howes, and D. Aubrey. 1990. Transport of groundwater-borne nutrients from watershed and their effects on coastal waters. Biogeochemistry **10**: 177–197.

Vinebrooke, R. D., and P. R. Leavitt. 1998. Direct and interactive effects of allochthonous dissolved organic matter, inorganic nutrients, and ultraviolet radiation on an alpine littoral food web. Limnol. Oceanogr. **43**: 1065–1081.

Wassen, M. J., A. Barendregt, M. C. Bootsma, and P. P. Schot. 1989. Groundwater chemistry and vegetation of gradients from rich fen to poor fen in the Naardermeer (The Netherlands). Vegetatio **79**: 117–132.

Wigand, C., F. Ø. Andersen, K. K. Christensen, M. Holmer, and H. S. Jensen. 1998. Endomycorrhizae of isoetids along a biogeochemical gradient. Limnol. &Oceanography **43**: 508–515.

Williamson, C. E., D. P. Morris, M. L. Pace, and A. G. Olson. 1999. Dissolved organic carbon and nutrients as regulators of lake ecosystems: resurrection of a more integrated paradigm. Limnol. Oceanogr. **44**: 795–803.

Chapter 2

Stimulation of epiphyton growth by lacustrine groundwater discharge to an oligo-mesotrophic hard-water lake

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Abstract

Periphyton is a major contributor to aquatic primary production and often competes with phytoplankton and submerged macrophytes for resources. In nutrient-limited environments, mobilization of sediment nutrients by groundwater can significantly affect periphyton (including epiphyton) development in shallow littoral zones and may affect other lake primary producers. We hypothesized that epiphyton growth in the littoral zone of temperate oligomesotrophic hard-water lakes could be stimulated by nutrient (especially P) supply via lacustrine groundwater discharge (LGD). We compared the dry mass, chlorophyll *a* (chl *a*), and nutrient content of epiphyton grown on artificial substrates at different sites in a groundwater-fed lake and in experimental chambers with and without LGD. During the spring–summer periods, epiphyton accumulated more biomass, especially algae, in littoral LGD sites and in experimental chambers with LGD compared to controls without LGD. Epiphyton chl *a* accumulation reached up to 46 mg chl a/m^2 after 4 wk when exposed to LGD, compared to a maximum of 23 mg chl a/m^2 at control (C) sites. In the field survey, differences in epiphyton biomass between LGD and C sites were most pronounced at the end of summer, when epilimnetic P concentrations were lowest and epiphyton C∶P ratios indicated P limitation. Groundwater-borne P may have facilitated epiphyton growth on macrophytes and periphyton growth on littoral sediments. Epiphyton stored up to 35 mg P/m^2 in 4 wk (which corresponds to 13% of the total P content of the littoral waters), preventing its use by phytoplankton, and possibly contributing to the stabilization of a clear-water state. However, promotion of epiphyton growth by LGD may have contributed to an observed decline in

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macrophyte abundance caused by epiphyton shading and a decreased resilience of small charophytes to drag forces in shallow littoral areas of the studied lake in recent decades.

Key words: lacustrine groundwater discharge, periphyton, littoral, nutrients, benthic, macrophytes, seepage

Introduction

Groundwater-borne nutrients entering lakes can contribute significantly to their nutrient budgets (Belanger et al. 1985; Kidmose et al. 2013; Lewandowski et al. 2015) and to eutrophication processes (Valiela et al. 1990; Holman et al. 2008; Tomer et al. 2010; Meinikmann et al. 2015). Groundwater also may induce redox changes that result in a release of sediment-bound P (Boström and Pettersson 1982). However, the effect of groundwater on the trophic status of a lake, and especially on its benthic primary producers, has rarely been studied, and, thus, is potentially underestimated (Périllon and Hilt 2016).

Lacustrine groundwater discharge (LGD) comprises all groundwater flows from a lake bed into a lake, and is sometimes termed ‘groundwater influx’ or ‘groundwater exfiltration’. LGD is analogous to the marine term ‘submarine groundwater discharge’. For flow in the opposite direction, the terms ‘lake water infiltration’ or ‘groundwater recharge’ are used. LGD is usually greatest near the shoreline and decreases exponentially with distance from the shore (McBride and Pfannkuch 1975; Shaw and Prepas 1990). Thus, it may affect benthic primary producers, such as submerged macrophytes and periphyton, which predominantly colonize littoral zones.

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Periphyton is increasingly recognized as an important primary producer, even in oligotrophic and mesotrophic lakes (Schneider et al. 2014; Vadeboncoeur et al. 2014; Brothers et al. 2016). Periphyton is composed of algae and heterotrophic biota that are firmly or loosely attached to a submerged substrate. It is termed epiphyton when attached to submerged macrophytes. Periphyton can fix groundwater-borne P and, thus, prevent it from entering the water column. Furthermore, periphyton can prevent resuspension of particulate P into the water column (Neumeier et al. 2006), and O_2 production by benthic algal photosynthesis may retard sediment P release during the daytime (Zhang et al. 2012). These mechanisms lead to decreasing P availability for phytoplankton development. Periphyton may stabilize clear-water states in shallow lakes even in the absence of macrophytes (Genkai-Kato et al. 2012).

In oligotrophic lakes, periphyton biomass is controlled primarily by abiotic factors, such as light and nutrient availability (bottom-up control), rather than biotic factors, such as grazing (top-down control) (Hansson 1992; Schroeder et al. 2012; Vadeboncoeur et al. 2014), although both mechanisms may coexist (Hillebrand 2002). Groundwater-borne nutrients may significantly affect periphyton growth, but only a few studies have been done on the effects of LGD on periphyton. The only *in situ* experiment published to date revealed a direct positive effect of LGD on benthic algal biomass by advective fluxes of P through the sediments (Hagerthey and Kerfoot 1998). A field study in a temperate flow-through lake (Sparkling Lake, Wisconsin, USA) revealed that periphyton growing at sites of high LGD had lower N∶P and higher algal biovolumes than periphyton at sites with low LGD (Hagerthey and Kerfoot 2005). Roy et al. (2009) also linked relatively dense periphyton cover to the presence of groundwater discharge.

The available studies on the effects of LGD on periphyton have focused mainly on benthic algae growing at the sediment surface with direct access to nutrient-rich sediment pore water. The question of whether LGD also can facilitate epiphyton growth on submerged plants, thereby potentially affecting the competition between these primary producers, remains unresolved (Périllon and Hilt 2016).

We explored the potential link between LGD and epiphyton development in an oligomesotrophic hard-water lake that had experienced a decline in shallow charophyte meadows in recent decades. We tested the hypothesis that epiphyton biomass is higher at sites with additional nutrient (especially P) supply via LGD. We compared growth and nutrient accumulation of epiphyton on artificial substrates exposed at littoral sites with and without LGD. In addition, we conducted an *in situ* experiment in the littoral zone of our study lake and compared epiphyton growth in chambers that were open or closed to the LGD.

Methods

Study site

Lake Stechlin is a deep (69.5 m maximum depth) dimictic lake in northeastern Germany (lat 5379′5.59″N; long 137 1′34.22″E), with a surface area of 4.2 km^2 and a shoreline length of 16 km. Its water balance is dominated by precipitation and groundwater (G. Ginzel and U. Kaboth, Institut für Gewässerökologie und Binnenfischerei, unpublished data). Apart from 2 small ditches with negligible contribution to the water balance, the lake has no surface inflow or outflow. Hence, the lake is classified as a groundwater-dominated, flow-through lake. The zones of LGD and groundwater recharge vary according to long-term climatic conditions (Holzbecher 2001). In general, LGD occurs in the southeastern and northwestern parts of the lake, whereas groundwater recharge occurs in its northern bay (Fig. 1A). The subsurface watershed has been part of a natural protected area since 1938 and is dominated by mixed forests, representing 80% of the catchment area (Kirillin et al. 2013).

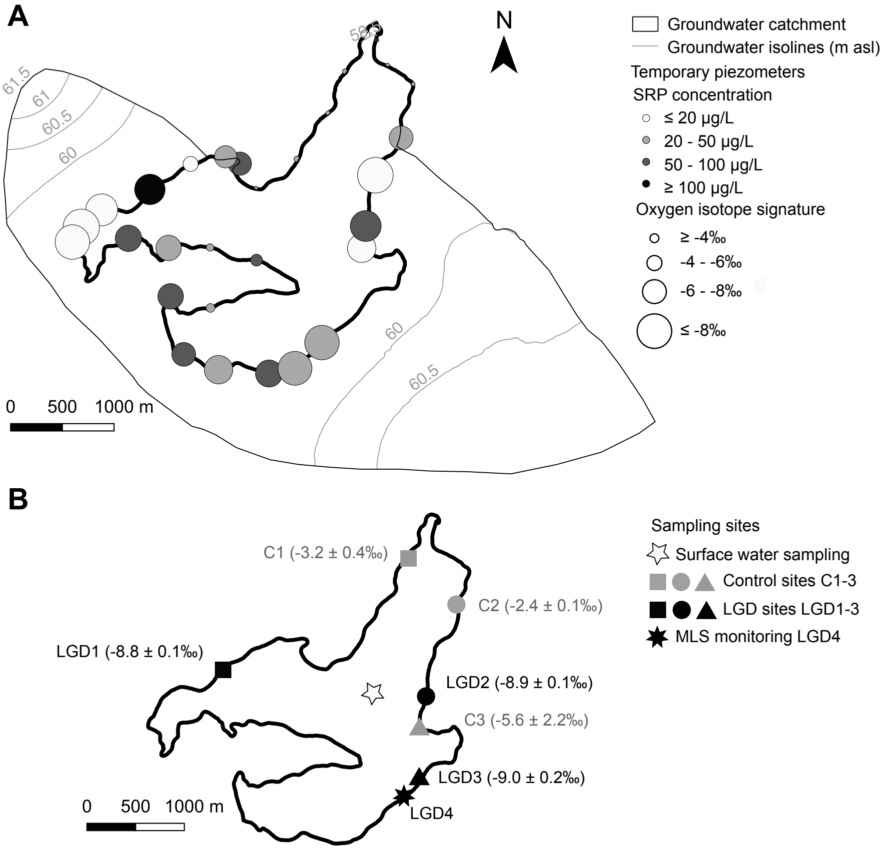

Figure 1. A.—Subsurface catchment of Lake Stechlin including groundwater table contour lines (after G. Ginzel [IGB] and U. Kaboth, brandenburgisches Landesamt für Bergbau, Geologie und Rohstoffe unpublished data) and groundwater screening sites. The points' opacity indicates soluble reactive P (SRP) concentration and the size of the circles indicates the stable O signature ($\delta^{18}O$). B.—Hydrological and ecological sampling sites. Mean (±SD) stable O signatures in sampled pore water are indicated next to epiphyton survey sites. LGD = lacustrine groundwater discharge, MLS = multilevel sampler.

Based on long-term summer annual averages (May–September 1998–2006) of offshore total P (TP) concentrations (10–14 µg L^{-1}) and planktonic chlorophyll *a* (chl *a*) concentrations (2–4 µg L^{-1}), the lake has been classified as oligotrophic to weakly mesotrophic. Planktonic cyanobacterial populations have increased since the mid-1990s (Dadheech et al. 2014), but land use did not change in the watershed during that period. Submerged macrophytes reached maximum colonization depths between 7.7 and 18.9 m (spatial average: 14.0 m) in 2008 (van de Weyer et al. 2009). Shallow-water charophyte communities (mainly *Chara aspera*, *Chara filiformis*, and *Chara rudis*) declined strongly (by 93%) from 1962 to 2008. Shallow littoral areas are now covered by bare sand or are colonized with taller submerged macrophytes such as *Potamogeton pectinatus* (recently named *Stuckenia pectinata*), *Najas marina ssp. intermedia*, *Ceratophyllum demersum*, and *Myriophyllum spicatum*, indicating a deviation from the reference oligotrophic conditions (van de Weyer et al. 2009).

Study design

To test the influence of LGD on epiphyton, we combined an epiphyton survey and experiment with several measurements of nutrient concentrations in the groundwater, surface water, seepage, and pore water (Table 1). First, we screened groundwater flow directions and P concentrations around the lake in 2012 to localize LGD zones with potentially high P fluxes. In 2013, we surveyed epiphyton grown on artificial substrates at 3 sites with LGD and 3 C sites without LGD, but with otherwise similar conditions. In 2014, we performed an *in situ* experiment comparing epiphyton grown on artificial substrates in chambers with and without LGD at site LGD 3 (Fig. 1B, Table 1). In addition, we measured electrical conductivity (EC) and $\delta^{18}O$ values to confirm LGD at our study sites and nutrient concentrations in sediment pore waters at different depths and in seepage water. Nutrient concentrations in surface waters were monitored as part of the regular monthly lake sampling program.

Groundwater screening

Temporary piezometers (steel pipe, 1.3 cm in diameter, 4 slits with a length of 20 cm and a width of 0.5 mm) were placed ~2 m inland from the lake shore and 1 m below the groundwater level at 32 sites around the lake (Fig. 1A). Subsequently, we pumped water for 20 min with a peristaltic pump (0.3 L/min; Eijkelkamp, Giesbeek, The Netherlands) before collecting a sample. The sample was filtered (0.45 lm; Whatman, Maidstone, UK) and subdivided for stable-isotope ($\delta^{18}O$ and $\delta^{2}H$) and soluble reactive P (SRP) analyses (Table 1).

Surface-water monitoring

The surface-water quality of Lake Stechlin is measured every month at the deepest site (Fig. 1B) as part of a longterm monitoring program. Nutrient concentrations (Table 1) were measured following standard procedures (Giling et al. 2016). During the study, we verified that surface-water quality at the central lake station was not significantly different from that at our littoral survey sites (data not shown).

Table 1. Summary of methods used in our study. SRP = soluble reactive P, TP = total P, DIN = dissolved inorganic N, TN = total N, DSi = dissolved Si, DIC = dissolved inorganic C, EC = electrical conductivity, LGD = groundwater discharge, C = control, MLS = multilevel samplers (Rivett et al. 2008), Peeper = dialysis sampler (Hesslein 1976), Temp pie = temporary piezometer (Meinikmann et al. 2014), Seep chamb = seepage chambers (Frandsen et al. 2012), Art sub = Artificial substrates (4 wk exposure; Köhler et al. 2010).

No.	Substudy	Detail	Year	Period	Sites	Method	Measured variables
1	Groundwater screening		2012	June	32 (Fig. 1A)	Temp pie	$\delta^{18}O$, SRP
2	Surface water monitoring		2013, 2014	Monthly	Deepest point of lake		TP, SRP, TN, DIN, DSi, DIC
3	Pore and seepage water monitoring	Piezometer pore water at survey sites	2013, 2014	May–Sept	LGD1–3, C1–3	Temp pie	$\delta^{18}O$ (2013); EC, DIC, DSi, NO_2^-, NO_3^-, NH_4^+, SRP (2014)
		MLS pore water	2013–2014	Monthly (September 2013–2014)	LGD4 (Fig. 1B)	MLS	$\delta^{18}O$, SRP
		Peeper pore water	2013	June	LGD1, LGD3 (Fig. 1B)	Peeper	$\delta^{18}O$, EC, DIC, DSi, NO_2^-, NO_3^- NH_4^+, SRP
		Seepage as part of the epiphyton experiment	2014	7 flux measurements (Jun, Jul, Aug, Sept); 3 water sampling times (Jun, Aug, Sept)	LGD2	Seep chamb	Volume, $\delta^{18}O$, EC, SRP, DIN
4	Epiphyton survey		2013	May–Sept	LGD1–3, C1–3 (Fig. 1B)	Art sub	
5	Epiphyton experiment		2014	May–Aug	LGD1 (Fig. 1B)	Art sub	

Pore and seepage water monitoring

We sampled pore water at 6 survey sites to confirm the local pattern of groundwater fluxes based on electrical conductivity (EC) and $\delta^{18}O$ values and to evaluate nutrient fluxes (see Fig. 2A, B for sampling dates). For this purpose, we placed temporary piezometers identical to those used for groundwater screening several meters offshore in the lake sediments at a water depth of 50 cm (Fig. 1B), with filter screens situated 70 cm below the sediment surface. We pumped and discarded 500 mL of water, and retained the following 200 mL of pumped water. We measured electrical conductivity (EC) with a multisensor probe (Multi 340; Wissenschaftlich Technische Werkstätten, Weilheim, Germany) and filtered the samples (0.45-lm cellulose acetate filters; Sartorius, Göttingen, Germany) for analyses of stable isotopes, EC, dissolved inorganic C (DIC), dissolved Si (DSi), NO_3^-, NO_2^-, NH_4^+, and SRP (Table 1).

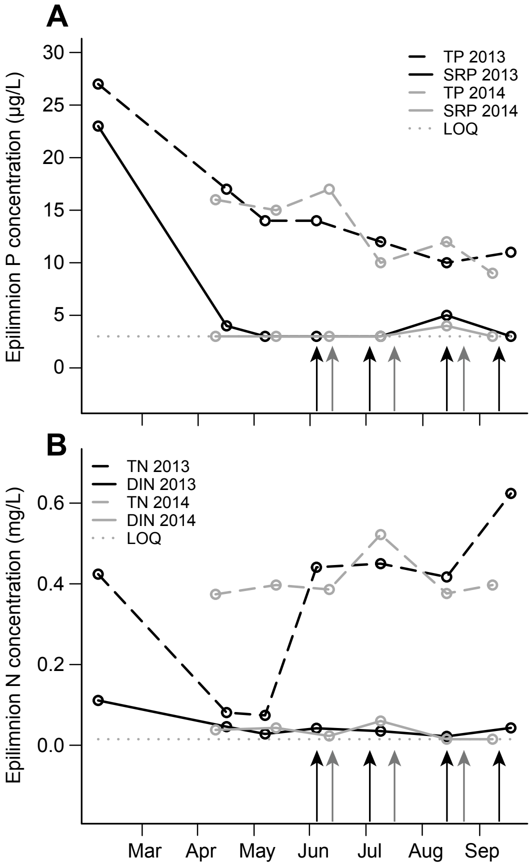

Figure 2. Concentrations of total P (TP) and soluble reactive P (SRP) (A), and of total N (TN) and dissolved inorganic N (DIN) (B) in the surface waters of Lake Stechlin between March and September 2013 and 2014. Epiphyton sampling dates are represented by black (2013) and grey (2014) arrows. LOQ = limit of quantification.

We installed 4 multilevel samplers (MLS) at 1 site along the eastern shore of the lake (2 m offshore, site LGD4; Fig. 1B) to confirm groundwater fluxes into the lake and to study the local and temporal variability of seepage and pore-water P concentrations. The samplers had sampling ports at 10-, 25-, 50-, and 100-cm depths below the sediment surface and were situated a few meters from each other. MLS pore water was sampled simultaneously from the specified depths with a multichannel peristaltic pump (9 mL/min; IPC24;

Ismatec, Wertheim, Germany). We discarded the first 10 mL from each port and analyzed the next 20 mL for SRP and stable isotopes (Table 1).

We used dialysis samplers (peepers) to observe the variability of seepage and pore-water P concentrations in the first centimeters below the sediment surface. The peepers (8.5 x 1.5 x 30 cm) were made of 2 Perspex® plates to form 6 chambers of 40-mL volume each. The chambers were filled initially with O2-free deionized water and separated from the environment by a 0.2-µm polysulfone membrane (Gelman HT 200 Tuffryn®; Pall, Port Washington, New York). Subsequently, we installed 5 peepers at 2 sites for 14 d (3 peepers at site LGD1 and 2 at site LGD3; Fig. 1B) with 2 chambers in the overlying water and 4 chambers in the sediments. Diffusion results in a concentration equilibrium between the water in the chambers and the pore water in front of the chambers. We analyzed the sampled water for stable isotopes, EC, and SRP (Table 1).

Water isotopic and chemical analysis

For all hydrological surveys (groundwater screening, pore and seepage water monitoring), we analyzed stable-isotope signatures of O and H ($\delta^{18}O$ and $\delta^{2}H$) in the water by cavity ring down spectroscopy, with an L2130-i-analyzer (Picarro, Santa Clara, California) with V-SMOW2 and SLAP2 as standards (IAEA 2009). The relative abundance of heavy (^{18}O and ^{2}H) and light (^{16}O and ^{1}H) isotopes does not depend much on the age of the water molecule (making them 'stable' isotopes), but instead reflects evaporation and biological processes because light isotopes are processed more readily than heavy ones. Therefore, heavy isotopes accumulate in lake waters (where both evaporation and biological processes take place), leading to a higher ratio of heavy to light isotopes than in rainwater or groundwater (Meinikmann et al. 2014). We present only $\delta^{18}O$ data because they were closely correlated with $\delta^{2}H$ results.

We measured SRP concentrations from groundwater screening and pore-water monitoring photometrically (LP2W photometer; Hach Lange GmbH, Düsseldorf, Germany) ≤12 h of on-site addition of molybdenum-blue-method reagents (limit of quantification [LOQ] = 10 µg L^{-1}; Murphy and Riley (1962). We analyzed SRP and TP concentrations in other water samples photometrically by the molybdenum-blue method, but the measurements were carried out in the laboratory with a flow-injection analyzer (FIA compact; Medizin- und Labortechnik Engineering GmbH, Dresden, Germany) and a lower LOQ (3 µg L^{-1}).

We analyzed dissolved inorganic C (DIC) with non-dispersive infrared techniques after combustion (samples taken in 2013; LOQ = 0.5 mg L^{-1}). Samples taken in 2014 and samples originating from surface-water monitoring were analyzed with a combustion catalytic oxidation method (LOQ = 0.1 mg L^{-1}; TOC-V CPH; Shimadzu, Tokyo, Japan).

In 2013, we measured concentrations of NO_3^-, NO_2^- and NH_4^+ with continuous flow analysis photometry (LOQ = 10 µg $L^{-1}$for NO_3^- and NO_2^- and 30 µg L^{-1} for NH_4^+; Skalar, Breda, The Netherlands). In 2014 and for all surface water monitoring, we analyzed dissolved inorganic N (DIN, as the sum of NO_3^-, NO_2^- and NH_4^+) photometrically (LOQ = 15 µg L^{-1} for NO_3^- and NO_2^- and 6 µg L^{-1} for NH_4^+; FIAstar; Foss, Hillerød, Denmark).

We measured dissolved Si (DSi) photometrically (2013: LOQ = 100 µg L^{-1}, Skalar; 2014: LOQ = 1 µg L^{-1}, FIAstar).

Epiphyton survey

Between June and September 2013, we collected monthly epiphyton samples from artificial substrates to compare growth between sites with and without exposure to groundwater (Table 1, Fig. 1B). For each of the 4 sampling campaigns, we exposed new substrates for 4 wk at 6 sites at a water depth of 50 cm (Fig. 1B). We grouped the sites into 3 pairs with comparable exposure to light and waves (Fig. 1B). For each pair, one site was situated in an LGD zone (LGD1– 3) and the other in an area without LGD (C1–3). We exposed pair 1 (C1 and LGD1) in a southeastern direction with a fetch of 0 km, pair 2 (C2 and LGD2) in a western direction with a fetch of 1.2 km, C3 in a southern direction with fetch of 0.7 km, LGD3 in a northwestern direction with a fetch of 1.1 km.

Epiphyton experiment

We verified the influence of groundwater on epiphyton by conducting an *in situ* experiment at site LGD1 during summer 2014 (Fig. 1B). We filled 10 experimental chambers (polyvinyl chloride [PVC] tubes, 15-cm diameter, 30-cm height) with aquarium gravel (2–4 mm diameter) that had been rinsed previously with deionized water. The calculated hydraulic conductivity of the gravel (0.1– 1 m/s) was higher than that of the natural lake sediments (~1024 m/s, measured by the falling-head method with a KSAT instrument; Meter Group, Munich, Germany). We buried the chambers in the sediments with the tops of the chambers at the sediment surface. All chambers were situated ~5 m offshore, separated by ≥1.5 m. The chambers were either closed with a PVC plate at the bottom or open to LGD with a net (Fig. 3). Epiphyton was sampled from artificial substrates (for details, see below), which were exposed above the gravel in the middle of each chamber. At each sampling occasion, we sampled 4 substrates from each chamber that had been exposed for 4 wk. In total, 3 samplings were carried out during summer 2014 (Fig. 2A, B).

To monitor and quantify the groundwater flow in the chambers, we measured seepage through the artificial gravel (as described by Frandsen et al. 2012) in all 10 experimental chambers during the period of the experiment (June–September 2014). Seepage chambers (30 cm long, 15-cm diameter, closed at the upper end) were connected to the experimental chambers via a rubber ring and fixed with 2 metal frames (Fig. 3). Thin-walled bags were emptied of air, prefilled with

200 mL lake water, protected from waves by a shelter, and connected to the seepage chambers. Bag-volume changes were measured after 2 to 4 h, and seepage was calculated in L m^{-2} h^{-1}. Five additional physicochemical variables (EC, SRP, DIC, DSi, and DIN were measured in the seepage water of the 10 bags during 3 dates within the sampling period (28 June, 22 August, 19 September). We installed the seepage chambers ≥24 h before installing the bags, so that the lake water in the seepage chambers was flushed out.

We measured bioavailable P in the gravel in open and closed chambers at the beginning (4 replicates) and at the end of the experiment (3 replicates/chamber). The gravel was exposed to HCl (0.5M), after a modification of a method published by Zak et al. (2014). Subsequently, the solution was analyzed for TP.

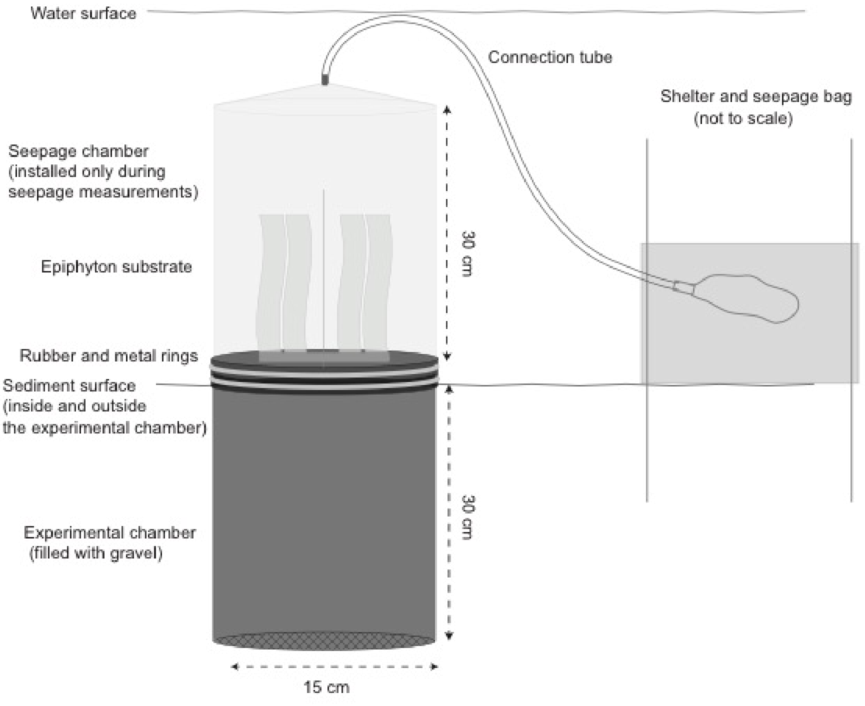

Figure 3. Schematic diagram of the set-up of the *in situ* experiment. Seepage chamber, connection tube, and seepage bags were present only during seepage measurements.

Epiphyton sampling and analysis

In both the epiphyton survey and the epiphyton experiment, we grew epiphyton on artificial substrate strips (2 12 cm, transparent polypropylene; General Binding Corporation, Chicago, Illinois) fixed vertically ~5 cm above the sediment or gravel on a metal structure fixed in the gravel. We sampled 4 replicates per site and campaign. The strips were stored in open tubes, transported in a cool, humid, insulated box, and processed either the next (survey) or same (experiment) day.

First, we removed visible grazers (e.g., chironomids and snails) and mussels. We discarded substrate strips when one side of the strip was epiphyton-free because of intensive grazing. We removed epiphyton from strips with a toothbrush and filtered lake water. We measured epiphyton dry mass, ash mass (for half of the sampling campaigns), pigment (chl a, chl b, and fucoxanthin [fuco] measured via high-performance liquid chromatography [HPLC]), and nutrient (C, N, and P) concentrations as described by (Köhler et al. 2010). We used chl a concentrations from the HPLC measurements as a proxy for the total algal content of epiphyton. The chl b and fuco pigments indicated the share of green algae and diatoms, respectively. Concentrations (µg L^{-1}) for pigments and nutrients were converted to mass per unit area (accumulation, mg m^{-2}) based on the suspension volume and surface area of epiphyton strips or to content as dry mass (% dm) relative to the dry mass of a given replicate. Molar C∶N and C∶P ratios were used as indicators of N or P limitation in epiphyton samples (Kahlert 1998). The shading effects of epiphyton (absorption [A]) were calculated from epiphyton dry mass (dm) as:

$$A = 108 \times dm \div (9.2 + dm)$$

based on the relationship between dry mass and the light absorption spectra of periphyton grown on artificial substrates (Köhler et al. 2010).

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Statistics

We used Student's *t*-tests (for normally distributed data with equal variances), Welch's *t*-test (for normally distributed data without equal variances), or the nonparametric Mann–Whitney *U*-test to evaluate differences between LGD and C sites in hydrological and ecological experiments. We applied Spearman's rank correlation to test for a correlation between biomass and TP content in monitored epiphyton samples. Water data from lake surface, C, and LGD sites were compared using a Kruskal–Wallis test followed by a post hoc Dunn's test.

Epiphyton growth variables from epiphyton surveys were evaluated with a linear mixed model (LMM) with a random intercept. We applied direction of groundwater flux (n = 2), site (site pair, n = 3), and date (sampling date, n = 4) as fixed effects, and replicate (n = 4) as a random effect. The variance and standard error associated with the random effect were 0. Therefore, we applied another model with flow and site as fixed effects and date as a random effect. The results supported the use of date as a random effect, and the assumptions of homoscedasticity and normality of residuals were verified. With only site as a fixed effect, and date as a random effect, we made further verifications of assumptions. We compared the goodness of fit between models (with and without flow) with a log-likelihood ratio test to extract the particular influence of flow. We then applied LMM with site as a random effect by the procedure described above. The results were identical, validating the choice of model. We applied an LMM in a similar way to the experimental data. Data were log (x)-transformed to meet the necessary assumptions. All statistical analyses and graphical presentations were done with the software R (version 3.3.0; R Project for Statistical Computing, Vienna, Austria), and the LMM was created with the package *lme4* (Bates et al. 2014).

Results

Identification of lacustrine groundwater discharge zones, vertical and temporal patterns

The locations of the groundwater screening (Table 1) could be classified into 2 groups based upon their isotopic signatures: 18 locations in the southern and western bays had δ^{18}O values of 29.0 ± 0.6‰ (mean ± SD), whereas 14 locations in the northern area had δ^{18}O values of 23.5 ± 1.2‰ (Fig. 1A), which was close to values obtained from the lake surface, where δ^{18}O values averaged 22.4 ± 0.2‰.

The δ^{18}O values of pore water (piezometer measurements) at LGD 1–3 (Fig. 1B) were similar to those of the groundwater screening in these areas and were significantly lower than the values C1–3, whose signatures were similar to those of the lake water (Table 2). Little or no LGD was present at C3, even though this site was in a zone where lacustrine groundwater discharge generally occurs. This fact may be explained by the convex shape of the shoreline at this site, which diverts the groundwater approaching the interface toward both ends of the concave shoreline section.

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Table 2. A comparison of mean ± SD (n) values of selected variables in samples taken from the surface waters of Lake Stechlin, piezometer surveys, and seepage measurements (electrical conductivity [EC] measurements were included with the lake data) from monthly samplings in summer 2013 and 2014. Means were calculated by averaging across sampling dates and groundwater influence types. For data below the limit of quantification (LOQ), values = 0.5LOQ were used for calculations. DIC = dissolved organic C, DSi = dissolved Si, SRP = soluble reactive P, L = lake surface water, C = control sites, LGD = groundwater discharge sites. For parameters with significant differences (Kruskal–Wallis test, $p < 0.05$), the results of post hoc Dunn's tests are presented as "Ranking".

Parameter	Unit	Lake water	Control sites	LGD sites	Ranking
Conductivity	$\mu S\ cm^{-1}$	275 ± 12.4 (5)	452 ± 55 (8)	562 ± 52 (8)	L = C < LGD
$\delta^{18}O$	‰	–2.17 (2)	–2.9 ± 0.5 (6)	–8.3 ± 1.3 (7)	LGD < C = L
DIC	$mg\ L^{-1}$	23.0 ± 3.2 (10)	50.3 ± 9.1 (7)	52.7 ± 10.0 (7)	L < C = LGD
DSi	$mg\ L^{-1}$	0.23 ± 0.12 (9)	4.3 ± 1.1 (6)	5.9 ± 0.87 (6)	L < C =LGD
NO_3^-+NO_2^-	$\mu g\ L^{-1}$	16.5 ± 11.8 (10)	7.1 ± 3.6 (7)	4.9 ± 2.9 (7)	LGD ≤ C < L
NH_4^+	$\mu g\ L^{-1}$	18.8 ± 11 (8)	474 ± 225 (6)	115 ± 131 (6)	L < LGD ≤ C
SRP	$\mu g\ L^{-1}$	2.4 ± 1.3 (10)	17 ± 6.9 (3)	16 ± 22.5 (3)	L < LGD ≤ C

Pore-water monitoring with MLS revealed no significant variations in the $\delta^{18}O$ signatures between monthly samplings (Kruskal–Wallis test, $p > 0.05$). At all 4 depths of the MLS (10, 25, 50, and 100 cm below the sediment surface) pore-water $\delta^{18}O$ values were relatively low, and characteristic for groundwater (29.2‰ < $\delta^{18}O$ < 28.1‰). Slightly higher $\delta^{18}O$ values were detected only at the uppermost port (28.6 ± 0.3‰ in the uppermost port, 28.8 ± 0.2‰ in lower ports; Mann–Whitney *U*-test, $p < 0.001$).

Peepers revealed higher $\delta^{18}O$ values in the water overlying the sediment surface than in pore water within the sediments (22.3 ± 0.3‰ and 28.4 ± 0.3‰, respectively, Mann– Whitney U-test, $p <$

0.001; Fig. 4A). An inverse trend was seen in EC values (501 ± 45 µS cm^{-1} above, 293 ± 16 µS cm^{-1} below; Mann–Whitney *U*-test, $p <$ 0.001; Fig. 4B). No gradient was observed in the δ^{18}O signatures below the sediment surface (Kruskal–Wallis test, $p > 0.05$). EC values at LGD3 were highest in the first 2 chambers below the sediment surface, whereas no difference between chambers was observed at LGD1. EC and δ^{18}O did not differ between chambers at the same depth (Student's *t*-tests, $p > 0.1$).

The volume and EC of seepage measurements through artificial gravel (from the *in situ* epiphyton experiment) differed significantly between open and closed chambers. Seepage volumes in open chambers were 13.1 ± 8.7 L m^{-2} h^{-1}, without significant differences between chambers (Kruskal–Wallis test, $p > 0.1$), and were close to 0 in the closed chambers. EC values of water taken from closed chambers were 277 ± 10 µS cm^{-1} compared to 475 ± 70 µS cm^{-1} from open chambers receiving LGD (Mann– Whitney *U*-test $p < 0.001$; Kruskal–Wallis test, $p > 0.1$).

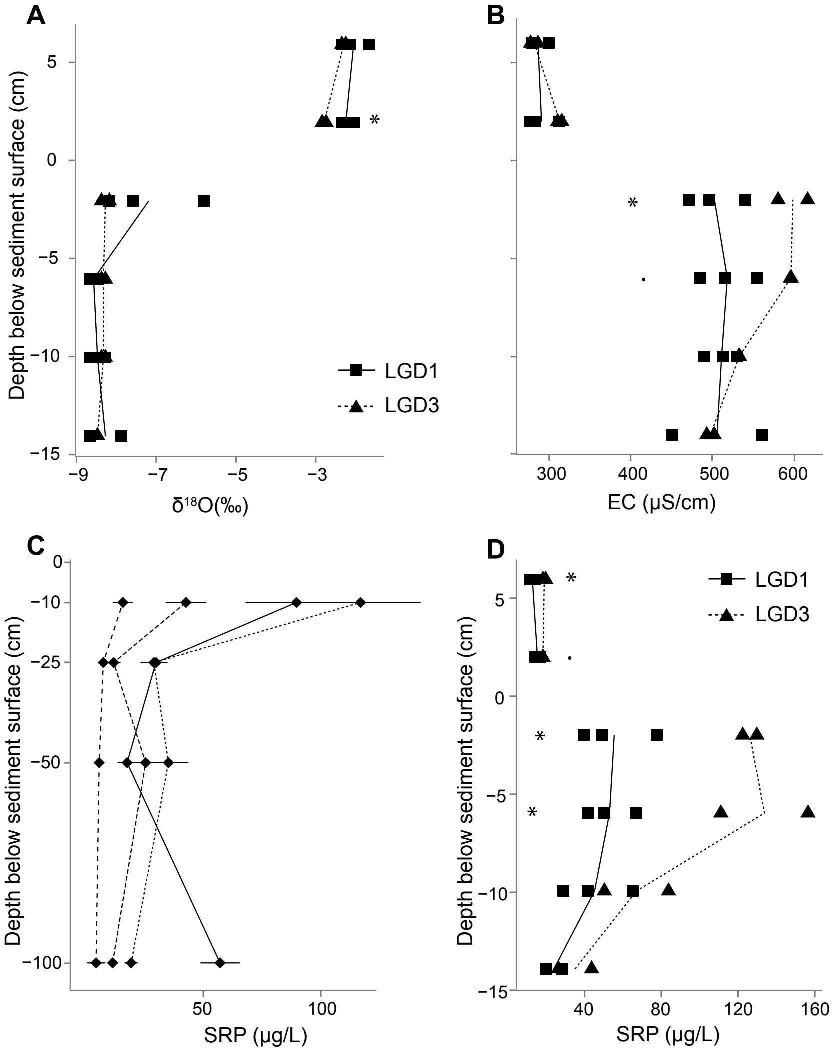

Figure 4. A, B, D.—Pore-water depth profiles in the littoral zone for samples collected in July 2013 by passive pore-water samplers (peepers) installed at sites LGD1 ($n = 3$) and LGD3 ($n = 2$). Profiles show means (lines) and single values for stable O2 isotopes (δ^{18}O) (A), electrical conductivity (EC) (B), and soluble reactive P (SRP) concentrations (D). C.— Pore-water depth profiles in the littoral zone for samples collected by 4 multilevel samplers from 4 depths (10, 25, 50, and 100 cm), 2 m offshore in the eastern littoral zone of Lake Stechlin (LGD4, Fig. 1B). Profile shows mean (±SE) SRP concentrations measured over 1 y ($n = 12$). * = $p < 0.05$, • = $p < 0.1$ (Welch's *t*-tests).

Nutrients in groundwater and pore water

SRP concentrations from groundwater screenings were very heterogeneous, ranging from <10 µg L^{-1} to 210 µg L^{-1} (Fig. 1A). They did not follow the same pattern as $\delta^{18}O$ signatures (Mann–Whitney *U*-test, $p > 0.05$).

Pore water sampled with piezometers at survey sites revealed low SRP concentrations that were not significantly different between C and LGD sites (Table 2). DIC and DSi concentrations were lower in lake-water samples than in pore waters at both LGD and C sites. Concentrations of NO_3^- and NO_2^- were higher in lake waters than in pore waters at LGD sites. Concentrations of NH_4^+ were higher at C sites than in lake-water samples (Table 2).

The MLS pore-water monitoring revealed a high spatial and temporal heterogeneity of SRP concentrations at a depth of 10 cm in littoral sediments (site LGD4; Fig. 4C). SRP concentrations decreased from 90 ± 84 µg L^{-1} in September 2013 to 16 ± 11 µg L^{-1} in April 2014, followed by an increase to 150 ± 83 µg L^{-1} by August 2014. In deeper zones, spatial and temporal variations were low and mean SRP concentrations were similar throughout the sampling period (25 cm: 26 ± 16 µg L^{-1}; 50 cm: 29 ± 20 µg L^{-1}; 100 cm: 29 ± 25 µg L^{-1}; Fig. 4C).

SRP concentrations in the peepers ranged from 15.9 ± 2.7 µg L^{-1} in the top 2 chambers (above the sediment surface) to 84 ± 42 µg L^{-1} in the first 2 chambers below the sediment surface. The SRP values in the latter chambers differed between LGD1 and LGD3 (Mann-Whitney *U*-test, $p < 0.01$; Fig. 4D). SRP concentrations were lower in the lowermost chambers than in those directly below the sediment surface (Student's *t*-test, $p < 0.05$). DSi and DIC concentrations were higher in chambers under (8.7 ± 2.4 mg L^{-1}, 55.8 ± 8.0 mg L^{-1}, respectively) than above sediments (0.51 ± 0.45 mg L^{-1}, 21.5 ± 2.2 mg L^{-1}, respectively; both Mann–Whitney *U*-tests, $p < 0.001$). No significant gradient for DSi and DIC was detected in chambers

doi: 10.1086/692832

situated under the sediment surface (both Kruskal–Wallis tests, $p > 0.1$).

All seepage bags from the epiphyton experiment contained very low concentrations of SRP and DIN (typically <3 µg L^{-1} and 50 µg L^{-1}, respectively). DSi and DIC concentrations were 3.5 ± 1.2 mg L^{-1} and 45.6 ± 7.5 mg L^{-1}, respectively.

Nutrients in epilimnetic lake water

P concentrations in the lake epilimnion showed a seasonal pattern: TP decreased during the stratification period (April–October 2013 and 2014; Fig. 2A) and SRP concentrations remained <LOQ (3 µg L^{-1}) from May onward (Fig. 2A). In 2013, DSi concentrations decreased gradually throughout the stratification period from >1 mg L^{-1} at the end of the winter to 0.7 mg L^{-1} by April and 0.15 mg L^{-1} by the end of the summer. In 2014, DSi decreased from 0.8 to 0.2 mg L^{-1} in April followed by relatively constant concentrations throughout the remainder of the year. No recurrent seasonal patterns were observed for TN or DIN (Fig. 2B) or for DIC concentrations in the epilimnion during sampling or experimental periods.

Epiphyton survey

Epiphyton grown on artificial substrates for 4 wk accumulated more dry mass, chl *a*, chl *b*, and fuco at sites with LGD than at C sites (Table 3). Both TP content (% dm) and accumulation (mass per unit area) were higher at sites with LGD compared to C sites (Table 3). On the contrary, the TN content of epiphyton did not differ between LGD and C sites. Epiphyton chl *a* and chl *b* accumulation varied significantly between sites and seasons, from 2.2 to 45.8 mg m^{-2} for chl *a* (Fig. 5) and from 0.2 to 15.1 m g m^{-2} for chl *b*. For each of the 3 pairs of sites, chl *a* and chl *b* accumulation was higher at LGD than at C sites by the end of summer (Student's *t*-tests, $p < 0.05$; Fig. 5).

Table 3. Results of linear mixed model (LMM) applied to data from the field sampling of epiphyton grown on artificial substrates exposed to sites with (LGD) or without (control = C) groundwater in Lake Stechlin, using raw data. $\chi 2$ and p are the results of an analysis of variance test applied to the linear mixed model with and without groundwater to evaluate its influence on the overall model. The difference between sites with and without groundwater discharge is the model output for the fixed effect flow, given as estimate ± SE. The mean and standard deviation (SD) of the data are given as a comparison. TP = total P, TN = total N.

Variable	Unit	*n*	χ^2	*p*	Difference LGD – C	Data mean ± SD
Dry mass	$g\ m^{-2}$	87	17.1	3.5×10^{-5}	4.5 ± 1.0	9.9 ± 6.3
Chlorophyll *a*	$mg\ m^{-2}$	86	23.6	1.2×10^{-6}	5.8 ±1.1	14.1 ± 8.1
Chlorophyll *b*	$mg\ m^{-2}$	86	29.8	4.7×10^{-8}	1.9 ± 0.32	3.4 ± 3.0
Fucoxanthin	$\mu g\ m^{-2}$	88	12.2	4.8×10^{-4}	0.61 ± 0.17	1.05 ± 1.0
TP accumulation	$mg\ m^{-2}$	84	12.4	4.2×10^{-4}	5.0 ± 1.4	14.8 ± 8.2
TN accumulation	$mg\ m^{-2}$	80	12.2	4.7×10^{-4}	55.1 ± 15.1	161 ± 83.2
C:P		77	0.08	0.8		475 ± 209
C:N		77	0	1		29.6 ± 2.8

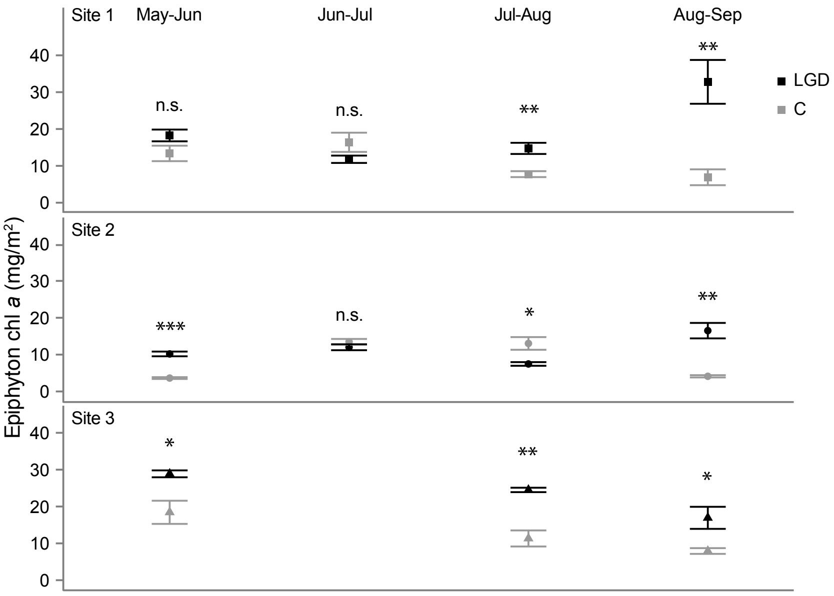

Figure 5. Mean (±SE) chlorophyll a (chl a) mass per unit area of epiphyton sampled from artificial substrates exposed in Lake Stechlin for 4 wk (during June–September 2013) at sites with (LGD) and without (C) groundwater discharge. n.s. = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (Student's *t*-test).

The molar C∶P ratios of epiphyton varied from 115 to 961 (Fig. 6A). Epiphyton had lower C∶P at LGD than at C sites (Table 3). A linear regression with sampling date showed a significant increase of C∶P during summer, with a higher regression quality and slope at C than at LGD sites ($r^2 = 0.69$, slope = 5.1; $r^2 = 0.44$, slope = 2.6, respectively). Both C and LGD sites surpassed C∶P = 369 in July, indicating P limitation (Kahlert 1998). At the same time, C∶P ratios became greater at C than at LGD sites. Epiphyton C∶N did not differ between C and LGD sites. These ratios remained >11, indicating N limitation in epiphyton (Kahlert 1998, Fig. 6B).

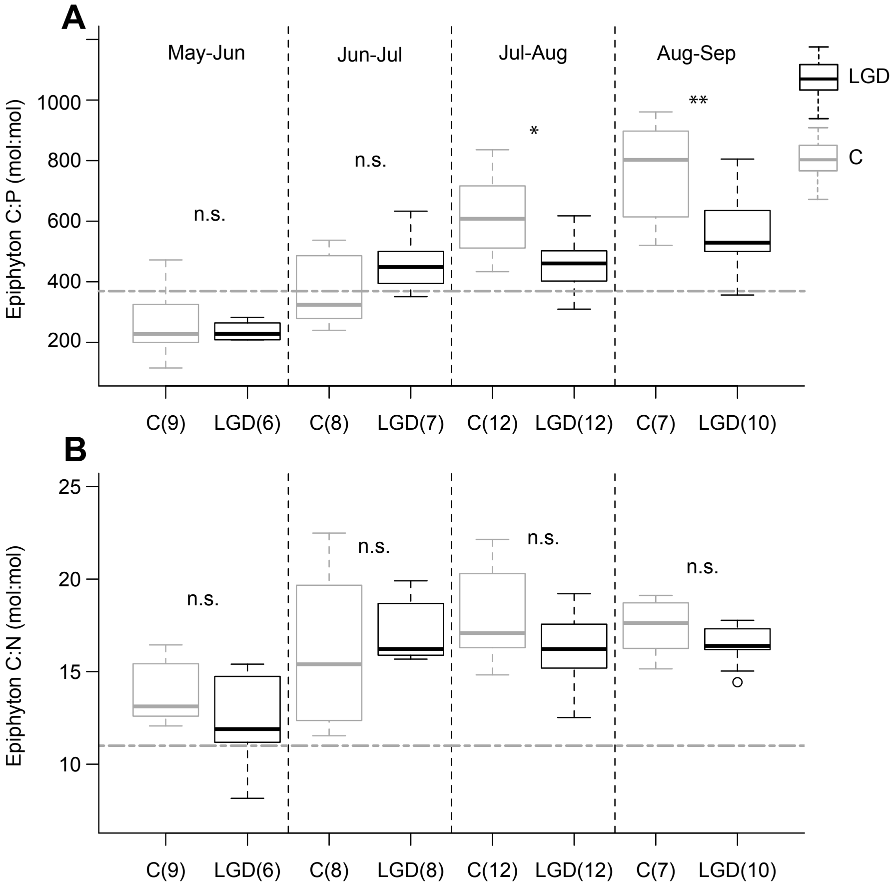

Figure 6. Box-and-whisker plots for molar C∶P (A) and C∶N (B) epiphyton grown on artificial substrates exposed in Lake Stechlin at control (C) and lacustrine groundwater discharge (LGD) sites during 4 experimental periods in summer 2013. Number of samples is indicated in brackets. Lines in boxes are medians, box ends are quartiles, and whiskers are 95% confidence intervals. Above the horizontal dashed line (C∶P = 369 and C∶N = 11), epiphyton is assumed to be P/N-limited (Kahlert 1998). n.s. = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$ (Student's *t*-test).

Epiphyton dry mass and TP content were negatively correlated at C and LGD sites (Spearman correlation, ρ = 20.59 and 20.58, respectively). Epiphyton P content did not differ between C and LGD sites (Mann–Whitney *U*-test, $p > 0.1$, Fig. 7), whereas TP accumulation was significantly greater at LGD than C sites (Welch's

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t-test, $p < 0.001$). Total C accumulation was greater at LGD than at C sites (Student's *t*-test, $p < 0.01$; LGD sites: 2.5 ± 1.2 g m^{-2}, C sites: 2.0 ± 1.0 g m^{-2}) and was significantly correlated with dry mass ($\rho = 0.97$, $p < 0.001$; Fig. 7) and ash mass ($\rho = 0.83$, $p < 0.001$).

Within 4 wk, epiphyton growth reduced the light availability for macrophytes by 44 ± 18% (with values ranging from 4 –76%) at locations without LGD, compared to 57 ± 16% (13–81%) at LGD sites. The difference between LGD and C sites was significant (Mann–Whitney *U*-test, $p < 0.001$).

Snail tracks visibly and significantly altered the epiphyton cover on the substrate at C1 in August. These samples produced strong outliers and were removed from stoichiometric tests, but not biomass and following calculations.

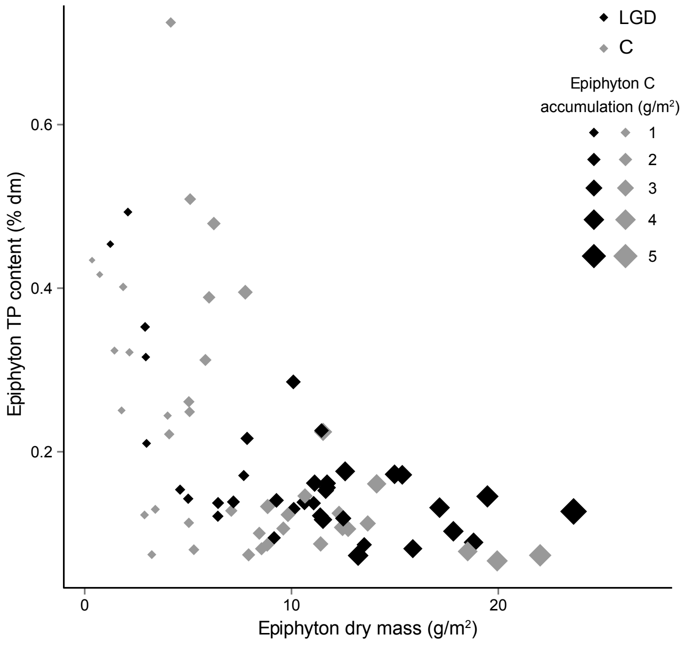

Figure 7. Scatterplot showing correlation between epiphyton dry mass and its total P (TP) content. Epiphyton was grown on artificial substrates exposed for 4 wk in Lake Stechlin in summer (May–September 2013) of 2013 at control (C) and lacustrine groundwater discharge (LGD) sites. The size of a point indicates epiphyton C accumulation.

Epiphyton experiment

After 4 wk of exposure, epiphyton dry mass and algae (chl *a*, chl *b*, and fuco) accumulation were significantly higher in open chambers with LGD than in closed chambers without LGD. LGD did not explain the differences in P accumulation or nutrient ratios in epiphyton (Table 4, Fig. 8). Epiphyton chl *a* contents in the experiment were similar to those obtained in the field survey (22.6 ± 25.1 mg m^{-2} chl *a* gathered from the experiment [closed and open chambers] and 14.1 ± 8.1 mg m^{-2} chl *a* from the survey [C and LGD sites], Mann–Whitney *U*-test, $p = 0.07$).

P concentrations in the leachate from the gravel of the chambers were greater at the end than at the beginning of the experiment (Mann–Whitney *U*-test, $p = 0.02$), but no difference was observed between open and closed chambers (Mann–Whitney *U*-test, $p = 0.68$).

Table 4. Results of a linear mixed model (LMM) applied to field experiments testing epiphyton development on artificial substrates exposed in chambers with (LGD) and without (control = C) groundwater. TP = total P, TN = total N. The application of the model follows the same method as described in Table 3.

Parameter	Unit	Transformation	*n*	χ^2	*p*	Difference LGD – C	Mean ± SD
Dry mass	g m^{-2}	log	101	6.6	0.01	0.12 ± 0.35	13.5 ± 13.4
Chlorophyll *a*	mg m^{-2}	log	91	9.5	0.002	0.26 ± 0.08	22.6 ± 25
Chlorophyll *b*	mg m^{-2}	log	90	7.1	0.008	0.23 ± 0.08	0.74 ± 0.88
Fucoxanthin	µg m^{-2}	log	89	14.9	< 0.001	0.25 ± 0.06	0.078 ± 0.056
TP accumulation	mg m^{-2}	log	99	2.1	0.14		12.4 ± 7.0
TN accumulation	mg m^{-2}	log	101	8.5	0.003	0.20 ± 0.07	0.22 ± 0.23
C:P		log	98	0.03	0.9		753 ± 737
C:N		log	98	0.1	0.7		18.6 ± 2.2

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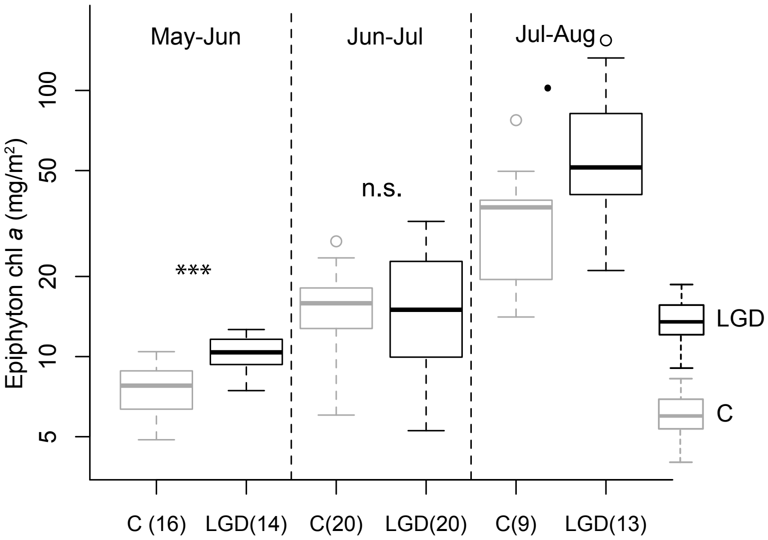

Figure 8. Box-and-whisker plots for log(chlorophyll a [chl a] mass per area) of epiphyton grown for 4 wk on artificial substrates exposed in experimental chambers without (control = C) and with lacustrine groundwater discharge (LGD) during 3 experiments in Lake Stechlin during summer 2014. Number of samples is indicated in brackets. Lines in boxes are medians, box ends are quartiles, whiskers are 95% confidence intervals, single points are outliers. n.s. = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (Student's *t*-test).

Discussion

Lacustrine groundwater discharge patterns and effect on P supply to the lake

Understanding groundwater-driven nutrient fluxes in Lake Stechlin required multiple and indirect monitoring methods because of diffuse seepage and low hydraulic gradients. Our data on stable-isotope signatures and EC in piezometers indicated the presence of LGD along the western, southern, and eastern shore, whereas groundwater recharge occurred in the northern bay. The borders between LGD and non-LGD zones may have varied because a modeling study indicated potential changes to subsurface catchments during wet or dry years (Holzbecher 2001). However, our study years (2012–2014) were not climatically extreme, and isotope measurements in sediment pore waters in 2013 validated our selection of LGD and C sites in 2012 (apart from C3, which proved to be a C site despite being in an otherwise LGD area).

In Lake Stechlin, lower pore-water SRP concentrations in deep than in shallow sediment layers indicated that long-distance transport from within the catchment may have a limited influence on sediment and water-column P. We assume that the littoral sediment P in Lake Stechlin is primarily of biogenic origin (e.g., leaves and wood from shoreline trees), which explains the high heterogeneity of pore-water P in upper layers. Higher pore-water SRP concentrations measured in summer occur as a result of temperature-controlled mineralization. LGD is assumed to transport some of this pore-water P into littoral waters. If LGD were strong, pore-water P concentrations would be influenced only marginally by near-surface mineralization processes because the groundwater would pass the near-surface zone rapidly. If advective fluxes were low compared to biogeochemical turnover rates, higher pore-water P concentrations might develop. In our field experiment, seepage water flowing through the P-poor artificial sediments of the experimental setup discharged with low P concentration, which confirms that the upper layers of sediments were the origin of P measured at LGD sites. This sediment P might become available to benthic primary producers through diffusion, but LGD is assumed to facilitate SRP availability through advective transport (Lewandowski et al. 2015). In this way, SRP from the sediments can reach epiphyton growing on surfaces situated above the sediments.

At LGD sites, all compounds dissolved in pore water may be transported analogously to P to the overlying water. However, NO_3^- and NO_2^- were lower in pore water than in lake water, indicating dilution resulting from LGD. The high concentrations of NH_4^+ in pore water at C sites result from local decomposition processes and may not have reached the water column. Higher DSi and DIC concentrations in pore waters compared to surface waters (Table 2) suggest a potential flux into the lake at LGD sites, as confirmed by higher DSi concentrations in the seepage bags of the *in situ* experiment. This flux of DIC and DSi probably originated from the groundwater because no concentration gradient was observed in shallow sediment layers.

Epiphyton is promoted at LGD sites

Our survey and *in situ* experiment revealed promotion of epiphyton by LGD in an oligomesotrophic lake. Enhanced epibenthic algal growth on the sediment surface by LGD also has been observed in a lake with similar seepage rates by Hagerthey and Kerfoot (1998). However, in our study, epiphyton grew on artificial substrates above the sediment surface mimicking submerged macrophytes. Thus, nutrients had to pass the benthic layer and travel through the water column to reach the epiphyton.

We think that the stimulation of epiphyton by LGD in our study was caused mainly by the additional supply of P. This conclusion is based on the following arguments. 1) C∶P measured in epiphyton sampled during the survey revealed less P limitation at LGD than at C sites, especially in late summer when P concentrations in the water column were low. 2) Epiphyton biomass was higher at LGD than at C sites at the end of summer when P concentrations in the water were low. 3) The effect of other variables, such as DIC, DSi, and N, on epiphyton was largely ruled out. DIC is assumed to be sufficiently available because Lake Stechlin is a hard-water lake and periphytic algae can use free CO_2 as well as HCO_3^-. In addition, wave action can increase CO_2 availability in shallow zones. DSi concentrations in the water column were higher than the half-saturation constants of Si intake by diatoms (Paasche 1973; Reynolds 2006). Epiphyton N content and C∶N ratio were not significantly different between LGD and C sites. However, epiphyton C∶N ratios revealed that N was, in addition to P, a limiting nutrient for epiphyton growth (Kahlert 1998) in Lake Stechlin. 4) Light availability (Qin et al. 2007; Vadeboncoeur et al. 2014), and wave exposure (Cattaneo 1990) may influence epiphyton growth, but we limited their influence via our survey design by pairing sites with similar wave and sun exposure. Epiphyton grazers, such as snails and chironomids (Hillebrand 2002; Mahdy et al. 2015), did not show higher abundance at C sites (data not shown).

Implications for pelagic nutrient concentrations and macrophytes

Epiphyton and, more generally, periphyton can store groundwater-borne nutrients temporarily during periods of rapid growth and, thereby, can limit nutrient availability to phytoplankton and hinder detection of nutrient import into the open water (Gaiser et al. 2004). In our study, P accumulation rates of epiphyton (2–35 mg P/m^2) were lower than those in more eutrophic systems (25–125 mg P/m^2; Jöbgen et al. 2004). Assuming uniform epiphyton biomass down to the maximum colonization depth of macrophytes (18.9 m in 2008; van de Weyer et al. 2009), we estimated that epiphyton and periphyton in Lake Stechlin may have stored P equivalent to 13% of the TP content of the overlying water column in summer 2013. However, only part of stored P is displaced to deeper sediment layers and stored for longer periods. The rest may become available again through grazing and decomposition. Benthic periphyton mats decrease advective transport of P by intercepting its diffusion from the sediment surface, leading to lower P flux from sediments to the water column than expected (Dodds 2003). Assuming a seepage rate of 13.1 L m^{-2} h^{-1} and SRP concentrations of 3 µg L^{-1}, summer epiphyton and periphyton could accumulate 56% of the P entering Lake Stechlin by LGD.

Epiphyton can contribute significantly to macrophyte shading (Jones and Sayer 2003; Roberts et al. 2003). In oligotrophic lakes, groundwater-borne nutrients are assumed to facilitate macrophyte growth (Lodge et al. 1989; Lillie and Barko 1990; Frandsen et al. 2012). However, additional epiphyton shading may counterbalance this effect (Périllon and Hilt 2016). Charophyte populations in shallow waters (where LGD usually occurs) have declined strongly since 1962 (van de Weyer et al. 2009). Moreover, our data show a significantly higher biomass and, thus, shading effect of epiphyton at LGD than at C sites. Higher epiphyton biomass also may contribute to a decrease in the resistance of shallow water charophytes to drag forces from waves (Schutten et al. 2004) and an increased susceptibility to herbivory (Hidding et al. 2016). Thus, epiphyton growth stimulation by LGD can have far reaching consequences for littoral submerged vegetation and their vital functions in lakes.

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References

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 51.

Belanger, T. V., D. F. Mikutel, and P. A. Churchill. 1985. Groundwater seepage nutrient loading in a Florida Lake. Water Res. **19**: 773–781.

Boström, B., and K. Pettersson. 1982. Different patterns of phosphorus release from lake sediments in laboratory experiments. Hydrobiologia **92**: 415–429.

Brothers, S., Y. Vadeboncoeur, and P. Sibley. 2016. Benthic algae compensate for phytoplankton losses in large aquatic ecosystems. Glob. Chang. Biol. **22**: 3865–3873.

Cattaneo, A. 1990. The effect of fetch on periphyton spatial variation. Hydrobiologia **206**: 1–10.

Dadheech, P. K., G. B. Selmeczy, G. Vasas, J. Padisák, W. Arp, K. Tapolczai, P. Casper, and L. Krienitz. 2014. Presence of potential toxin-producing cyanobacteria in an oligo-mesotrophic lake in Baltic lake district, Germany: An ecological, genetic and toxicological survey. Toxins (Basel). **6**: 2912–2931.

Dodds, W. K. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J. Phycol. **39**: 840–849.

Frandsen, M., B. Nilsson, P. Engesgaard, and O. Pedersen. 2012.

Groundwater seepage stimulates the growth of aquatic macrophytes. Freshw. Biol. **57**: 907–921.

Gaiser, E. E., L. J. Scinto, J. H. Richards, K. Jayachandran, D. L. Childers, J. C. Trexler, and R. D. Jones. 2004. Phosphorus in periphyton mats provides the best metric for detecting low-level P enrichment in an oligotrophic wetland. Water Res. **38**: 507–16.

Genkai-Kato, M., Y. Vadeboncoeur, L. Liboriussen, and E. Jeppesen. 2012. Benthic – planktonic coupling, regime shifts, and whole-lake primary production in shallow lakes. Ecology **93**: 619–631.

Giling, D. P., J. C. Nejstgaard, S. A. Berger, H.-P. Grossart, G. Kirillin, A. Penske, M. Lentz, P. Casper, J. Sareyka, and M. O. Gessner. 2016. Thermocline deepening boosts ecosystem metabolism: Evidence from a large-scale lake enclosure experiment simulating a summer storm. Glob. Chang. Biol. **23**: 1448–1462.

Hagerthey, S. E., and W. C. Kerfoot. 1998. Groundwater flow influences the biomass and nutrient ratios of epibenthic algae in a north temperate seepage lake. Limnol. Oceanogr. **43**: 1127–1242.

Hagerthey, S. E., and W. C. Kerfoot. 2005. Spatial variation in groundwater-related resource supply influences freshwater benthic algal assemblage composition. J. North Am. Benthol. Soc. **24**: 807–819.

Hansson, L.-A. 1992. Factors regulating periphytic algal biomass. Limnol. Oceanogr. **37**: 322–328.

Hesslein, R. H. 1976. An in situ sampler for close interval pore water studies. Limnol. Oceanogr. **21**: 912–914.

Hidding, B., E. S. Bakker, M. J. M. Hootsmans, and S. Hilt. 2016. Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. Oikos **125**: 1489–1495.

Hillebrand, H. 2002. Top-down versus bottom-up control of autotrophic biomass : a meta-analysis on experiments with periphyton. J. North Am. Benthol. Soc. **21**: 349–369.

Holman, I. P., M. J. Whelan, N. J. K. Howden, P. H. Bellamy, N. J. Willby, M. Rivas-Casado, and P. McConvey. 2008. Phosphorus in groundwater - an overlooked contributor to eutrophication? Hydrol. Process. **22**: 5121–5127.

Holzbecher, E. 2001. The dynamics of subsurface water divides - watersheds of Lake Stechlin and neighbouring lakes. Hydrol. Process. **15**: 2297–2304.

IAEA. 2009. Reference Sheet for VSMOW2 and SLAP2 international measurement standards. Ref. Sheet Int. Meas. Stand. 5 p.

Jöbgen, A., A. Palm, and M. Melkonian. 2004. Phosphorus removal from eutrophic lakes using periphyton on submerged artificial substrata. Hydrobiologia **528**: 123–142.

Jones, J. I., and C. D. Sayer. 2003. Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? Ecology **84**: 2155–2167.

Kahlert, M. 1998. C:N:P ratios of freshwater benthic algae. Arch. Hydrobiol. (Suppl) (Advanc. Limnol.) **51**: 105–114.

Kidmose, J., B. Nilsson, P. Engesgaard, M. Frandsen, S. Karan, F. Landkildehus, M. Søndergaard, and E. Jeppesen. 2013. Focused groundwater discharge of phosphorus to a eutrophic seepage lake (Lake Væng, Denmark): implications for lake ecological state and restoration. Hydrogeol. J. **21**: 1787–1802.

Kirillin, G., W. Phillip, C. Engelhardt, and G. Nützmann. 2013. Net groundwater inflow in an enclosed lake: From synoptic variations to climatic projections. Hydrol. Process. **27**: 347–359.

Köhler, J., J. Hachoł, and S. Hilt. 2010. Regulation of submersed macrophyte biomass in a temperate lowland river: interactions between shading by bank vegetation, epiphyton and water turbidity. Aquat. Bot. **92**: 129–136.

Lewandowski, J., K. Meinikmann, G. Nützmann, and D. O. Rosenberry. 2015. Groundwater - the disregarded component in lake water and nutrient budgets. 2. Effects of groundwater on nutrients. Hydrol. Process. **29**: 2922–2955.

Lillie, R. A., and J. W. Barko. 1990. Influence of sediment and groundwater on the distribution and biomass of *Myriophyllum spicatum* L. in Deveil’s Lake, Wisconsin. J. Freshw. Ecol. **5**: 417–426.

Lodge, D. M., D. P. Krabbenhoft, and R. G. Striegl. 1989. A positive relationship between groundwater velocity and submersed macrophyte biomass in Sparkling lake, Wisconsin. Limnol. Oceanogr. **34**: 235–239.

Mahdy, A., U. Scharfenberger, R. Adrian, and S. Hilt. 2015. Experimental comparison of periphyton removal by chironomid larvae and *Daphnia magna*. Inl. Waters **5**: 81–88.

McBride, M. S., and H. O. Pfannkuch. 1975. The distribution of seepage within lakebeds. J. Res. USGS **3**: 505–512.

Meinikmann, K., M. Hupfer, and J. Lewandowski. 2015. Phosphorus in groundwater discharge – A potential source for lake eutrophication. J. Hydrol. **524**: 214–226.

Meinikmann, K., G. Nützmann, and J. Lewandowski. 2014. Empirical quantification of lacustrine groundwater discharge - different methods and their limitations. In: Complex interfaces under change: Sea-river-groundwater-lakes. *Proceedings of symposia HP2 and HP3 held during the IAHS-IASPEI Assembly, Gothenburg, Sweden*. IAHS Publ. 85–90.

Murphy, J., and J. P. A. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta **27**: 31–36.

Neumeier, U., C. H. Lucas, and M. Collins. 2006. Erodibility and erosion patterns of mudflat sediments investigated using an annular flume. Aquat. Ecol. **40**: 543–554.

Paasche, E. 1973. Silicon and the ecology of marine plankton diatoms .

II . Silicate-uptake kinetics in five diatom species. Mar. Biol. **19**: 262–269.

Périllon, C., and S. Hilt. 2016. Groundwater influence differentially affects periphyton and macrophyte production in lakes. Hydrobiologia **778**: 91–103.

Qin, P., C. M. Mayer, K. L. Schulz, X. Ji, and M. E. Ritchie. 2007. Ecological stoichiometry in benthic food webs : Effect of light and nutrients on periphyton food quantity and quality in lakes. Limnol. Oceanogr. **52**: 1728–1734.

R Development Core Team, R. 2014. R: a language and environment for statistical computing. R Found. Stat. Comput. **1**: 409 pp.

Reynolds, C. S. 2006. The ecology of Phytoplankton, Cambridge University Press.

Rivett, M. O., P. A. Ellis, R. B. Greswell, R. S. Ward, R. S. Roche, M. G. Cleverly, C. Walker, D. Conran, P. J. Fitzgerald, T. Willcox, and J. Dowle. 2008. Cost-effective mini drive-point piezometers and multilevel samplers for monitoring the hyporheic zone. Q. J. Eng. Geol. Hydrogeol. **41**: 49–60.

Roberts, E., J. Kroker, S. Körner, and A. Nicklisch. 2003. The role of periphyton during the re-colonization of a shallow lake with submerged macrophytes. Hydrobiologia **506–509**: 525–530.

Roy, J. W., J. M. Robillard, S. B. Watson, and M. Hayashi. 2009. Non-intrusive characterization methods for wastewater-affected groundwater plumes discharging to an alpine lake. Environ. Monit. Assess. **149**: 201–11.

Schneider, S. C., M. Cara, T. E. Eriksen, B. Budzakoska Goreska, A. Imeri, L. Kupe, T. Lokoska, S. Patceva, S. Trajanovska, S. Trajanovski, M. Talevska, and E. Veljanoska Sarafiloska. 2014. Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low. Limnologica **44**: 90–97.

Schroeder, F., W. Traunspurger, K. Pettersson, and L. Peters. 2012.

Temporal changes in periphytic meiofauna in lakes of different trophic states. J. Limnol. **71**: 216–227.

Schutten, J., J. Dainty, and A. J. Davy. 2004. Wave-induced hydraulic forces on submerged aquatic plants in shallow lakes. Ann. Bot. **93**: 333–341.

Shaw, R. D., and E. E. Prepas. 1990. Groundwater-lake interactions: II. Nearshore seepage patterns and the contribution of ground water to lakes in central Alberta. J. Hydrol. **119**: 121–136.

Tomer, M. D., K. E. Schilling, C. A. Cambardella, P. Jacobson, and P. Drobney. 2010. Groundwater nutrient concentrations during prairie reconstruction on an Iowa landscape. Agric. Ecosyst. Environ. **139**: 206–213.

Vadeboncoeur, Y., S. P. Devlin, P. B. McIntyre, and M. J. Vander Zanden. 2014. Is there light after depth ? Distribution of periphyton chlorophyll and productivity in lake littoral zones. Freshw. Sci. **33**: 524–536.

Valiela, I., J. Costa, K. Foreman, J. M. Teal, B. Howes, and D. Aubrey. 1990. Transport of groundwater-borne nutrients from watershed and their effects on coastal waters. Biogeochemistry **10**: 177–197.

van de Weyer, K., J. Päzolt, P. Tigges, C. Raape, and S. Oldorff. 2009. Flächenbilanzierungen submerser Pflanzenbestände – dargestellt am Beispiel des Großen Stechlinsees (Brandenburg) im Zeitraum von 1962-2008. Naturschutz und Landschaftspfl. Brand. **18**: 137–142.

Zak, D., J. Gelbrecht, S. Zerbe, T. Shatwell, M. Barth, A. Cabezas, and P. Steffenhagen. 2014. How helophytes influence the phosphorus cycle in degraded inundated peat soils - Implications for fen restoration. Ecol. Eng. **66**: 82–90.

Zhang, X., Z. Liu, R. D. Gulati, and E. Jeppesen. 2012. The effect of benthic algae on phosphorus exchange between sediment and overlying water in shallow lakes: a microcosm study using ^{32}P as a tracer. Hydrobiologia **710**: 109–116.

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Chapter 3

Changes in submerged macrophyte colonization in shallow areas of an oligo-mesotrophic lake and the potential role of groundwater

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Abstract

Groundwater influx can significantly contribute to nutrient budgets of lakes and its influence is strongest in shallow littoral areas. In oligo- or mesotrophic systems, additional nutrient supply by groundwater influx may affect benthic primary producers and their interactions. Potential changes can be expected in community composition, biomass, stoichiometry and interactions between submerged macrophytes and epiphyton.

This study aimed at investigating whether enhanced epiphyton growth at sites with groundwater discharge may have contributed to a significant change in shallow littoral macrophyte abundance reported from oligo- mesotrophic Lake Stechlin during the last 50 years. In the 1960s, shallow littoral areas were dominated by small charophyte species such as *Chara aspera*, *C. filiformis* and *C. rudis*. Recent mappings indicated a strong decline of this shallow water charophyte community from 42 ha to 3 ha and a shift to the occurrence of macrophyte species typical of eutrophic lakes such as *Potamogeton perfoliatus*, *P. pectinatus* and *Myriophyllum spicatum*. We analyzed the nutrient content of macrophytes, and measured epiphyton growth at sites with different groundwater influence. Water column nutrient enrichment may have increased the abundance of eutrophic species, but this did not explain the decrease of charophytes. Our data suggest that enhanced epiphyton growth in shallow littoral areas with groundwater influx could impair the development of small charophytes by shading, increasing drag forces and the charophytes' sensitivity to herbivory.

Key words: seepage, eutrophication, charophytes, periphyton, nutrients, littoral

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Introduction

Submerged macrophytes have important functions in littoral zones of many lakes by influencing suspended solid retention, sediment oxygenation, and providing shelter or support for other primary producers and grazers (Carpenter and Lodge 1986). They have been suggested to stabilize clear-water conditions in both shallow (Scheffer et al. 1993) and deeper lakes (Hilt et al. 2010; Sachse et al. 2014). During the last century, higher nutrient loading to temperate lakes resulted in a decrease of charophytes (Blindow 1992; Baastrup-Spohr et al. 2013) and an increase of faster growing macrophyte species such as *Potamogeton pectinatus* (recently named *Stuckenia pectinata*), *Myriophyllum spicatum*, or *Ceratophyllum demersum* (Sand-Jensen et al. 2000). Eutrophication has also reduced the maximum colonization depth (Middelboe and Markager 1997), caused a shift to species with a shorter vegetation period (Sayer et al. 2010; Hilt et al. 2013) and ultimately led to a complete decline of submerged macrophytes (Sand-Jensen et al. 2000; Körner et al. 2002).

A major nutrient-promoted process impeding macrophytes is the development of phytoplankton and epiphyton competing for light. As macrophytes in deeper water are first affected by shading, maximum colonization depth of macrophytes are widely used as an indicator for lake water quality (Penning et al. 2008; Lyche-solheim et al. 2013; Søndergaard et al. 2013; Kolada et al. 2014). In the shallow littoral, however, macrophytes are supposed to be less affected by turbid water. Macrophytes therefore often find a refuge in shallow water of highly eutrophic lakes (Hilt et al. 2013). However, additional stress factors can affect macrophyte growth even in shallow waters. Macrophytes in the upper littoral may be influenced by water level fluctuations (Deegan et al. 2012), shading by shore vegetation (Köhler et al. 2010) and by epiphyton (periphyton growing on macrophytes, Phillips et al. 1978; Sand-Jensen and Søndergaard 1981; Tóth and Palmer 2016) and wave action (Chambers and Kalff

1987; Schutten et al. 2004). Shallow macrophytes may also be influenced by groundwater inflow (in the following termed lacustrine groundwater discharge, LGD), which predominantly takes place close to the shoreline (McBride and Pfannkuch 1975; Rosenberry et al. 2015).

LGD may constitute a significant component of the nutrient budget in nutrients-limited lakes (Lewandowski et al. 2015). Groundwater-borne nutrients may influence macrophyte biomass (Loeb and Hackley 1988; Lodge et al. 1989; Lillie and Barko 1990; Frandsen et al. 2012), and the stoichiometry (Sebestyen and Schneider 2004) and total phosphorus (TP) content of their tissue (Ommen et al. 2012). LGD, however, can also promote epiphyton growth (Hagerthey and Kerfoot 1998, 2005) which may increase shading and drag forces on macrophytes in shallow habitats (Périllon and Hilt 2016).

Here, we evaluate the changes in the abundance and species composition of shallow littoral macrophytes in a groundwater-fed oligo-mesotrophic hardwater lake and the potential role of LGD in this process. In a previous study, a potential impact of groundwater-mobilized nutrients on periphyton growth has been shown for this lake (Périllon et al. 2017). We hypothesize that this process may contribute to a change in macrophyte species composition towards a community with more species typical for eutrophic lakes and a decline of charophytes in shallow littoral areas. To test these hypotheses, we compared the macrophyte species composition and abundance in shallow areas in 1962, 2002, 2007, 2008 and 2014. In addition, macrophyte tissue nitrogen (N) and phosphorus (P) concentrations were measured in five macrophyte species at locations with and without LGD in 2014. Epiphyton development was monitored in summer 2014 on artificial substrates at four locations with or without LGD.

Material and methods

Lake Stechlin

Lake Stechlin is a temperate, monomictic hard-water lake in north-eastern Germany (Table 1), fed by groundwater and rainfall, with a stable water level since 1962. Short-term water level changes are controlled by climatic conditions such as wind and precipitations (Kirillin et al. 2013a).

In summer 2012, a piezometer campaign aimed at localizing areas with LGD using stable isotopes as indicators (Périllon et al. 2017). We generalized these data for the present study area (0–2 m deep) using the Voronoi polygons tool (QGIS 2.12.0) and selected the area situated between the shore and the 2 m depth line, using a bathymetric map (Fig. 1A). The areas located next to a piezometer with low $\delta^{18}O$ signature (between −10‰ and −6‰) were characterized as "LGD" and areas with higher $\delta^{18}O$ values (between −6‰ and −2‰) as "C" (control). The most eastern bay was excluded from the analysis due to its anthropogenic use as beach area (Fig. 1A). The main locations for LGD are in the southern, south-eastern and western littoral, while the outflow is concentrated in the northern littoral of the lake. All our sampling points were located in areas with stable groundwater flow direction, apart from the eastern control which could show inter-annual variation in flow direction, e.g. after wet years (Holzbecher 2001).

Table 1. Topographical, morphological, hydrological, and chemical parameters of Lake Stechlin (Krey, 1985; IGB, unpublished data).

Parameter	Mean ± sd
Drainage basin	12.6 km^2
Forested area in drainage basin	95 %
Maximum depth	69.5 m
Surface area	4.3 km^2
Volume	96.9×10^6 m^3
Mean depth	23.3 m
Effective fetch	2 000 m
Water retention time	>40 yrs
Water temperature[c]	19.1 ± 3.1 °C
Secchi transparency[a]	6.4 ± 1.7 m
Calcium[b]	49.6 ± 6.9 mg L^{-1}
Dissolved inorganic carbon[b]	20.6 ± 1.9 mg L^{-1}
NO_3^-nitrogen[b]	16 ± 24 µg L^{-1}
NH_4^+-nitrogen[b]	32 ± 30 µg L^{-1}
Total phosphorus[b]	11 ± 3 µg L^{-1}
Soluble reactive phosphorus[b]	2 ± 1 µg L^{-1}

[a] seasonal average, May-September, 2001-2010

[b] seasonal averages, May-September, 2000-2008, pooled samples, surface, 5m, 10m

[c] seasonal averages, May-September, 2014, pooled samples, surface, 5m, 10m

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Macrophyte mapping

Macrophyte surveys have been performed during the summers 1962 (Krausch 1964), 2002, 2007 (unpublished data of Landesumweltamt Brandenburg), 2008 (Van de Weyer et al. 2009), and 2014 (Van de Weyer et al. 2015, Fig. 1B). From 1962 we could only access the maps (Fig. 1B) and the list of species present in the whole lake (Table 2). In 2002, 2007, 2008 and 2014, macrophytes have been surveyed on 7 identical transects (straight lines that begin perpendicular to the shore). 13 further transects were surveyed in 2008 and 2014.

The mappings performed in 2008 and 2014 (20 transects) were most detailed. First, vegetation zones were mapped in June/July from a boat using an underwater camera and macrophyte were identified after sampling with a rake. Additionally, a diver followed the borders of specific populations of vegetation with a GPS buoy. Finally, divers mapped 20 transects to define more precisely macrophyte habitats and identify maximum colonization depths. Macrophyte species were determined following (Van de Weyer and Schmitt (2011) and the macrophyte zones were identified after Berg et al. (2004). For each vegetation zone, the coverage was estimated in the field using the decimal Londo scale (Londo 1976) and then translated into percentage of coverage, with values ranging between 0.1% (single macrophyte) to 97.5% (single species continuous cover).

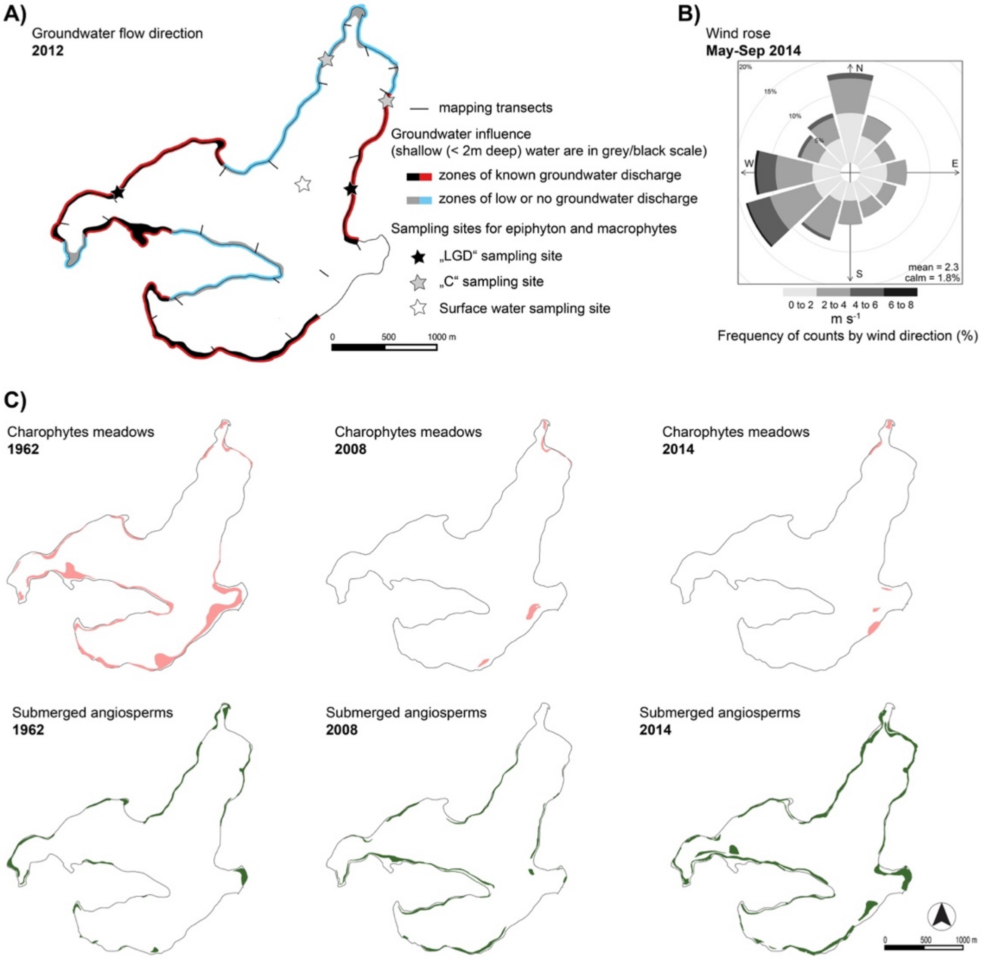

Figure 1. A) Areas of lacustrine groundwater discharge (LGD), mapped transects and macrophyte and epiphyton sampling points in Lake Stechlin (black stars for LGD sites, grey stars for control sites). B) Wind strength and directions, measured at the shore in summer 2014. C) Distribution of charophyte meadows and angiosperms in shallow littoral areas of Lake Stechlin in 1962, 2008 and 2014.

Macrophyte species were classified following the indicator values defined in Schaumburg et al. (2015) for the lake type TKg13 (carbonate-rich stratified water body of northern German lowlands with small watershed). “A” species are typical for pristine undisturbed conditions characteristic of this lake type, “B” species are more indifferent and “C” species indicate a deviation from reference

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conditions for this lake type (Schaumburg et al. 2004). The classification of charophytes (Kabus and Mauersberger 2011) and angiosperms (Ristow et al. 2006) in red list categories for Brandenburg, are presented in Table 2.

For data evaluation, we selected macrophyte data from the two first meters depth using QGIS. The indicator values were attributed following the species and the depth limits of vegetation zones: when the zone upper limit were shallower than 1m, the indicator values corresponding to 0–1 m (Schaumburg et al. 2015) were attributed to the macrophytes. Indicator values corresponding to 1–2 m were attributed to deeper zones.

First data analysis consisted of the comparison of the number of macrophytes species present at 0–2 m depth, in the 7 common transects studied in 2002, 2007, 2008 and 2014 (Fig. 2A). The number of macrophyte species typically growing in shallow littoral, are also represented for the year 1962 (Fig. 2A).

Further analysis required the calculation of coverage data within transects, using the data from 20 transects, in 2008 and 2014. The coverage of each macrophyte species were added for each indicator value and transects. The percentage of the littoral area covered by the vegetation zones were used as an adjustment value. Often species were observed as single plants, or only in few transects, therefore the obtained values averaged among transects and species, are low.

Epilimnion water quality

Data on surface water quality (Secchi depth, total phosphorus (TP) concentration) were gathered from regular monthly monitoring in the middle of Lake Stechlin (Kasprzak et al. 2017) from 1970 onwards. TP concentration were analysed according to DIN 38405. We selected data from the water surface (< 1 m depth), which were supposed to best represent conditions in the littoral zone, and the four years preceding the macrophyte surveys. The data were split in two periods:

summer (from May to September, when the lake is stratified) and winter. The 20–40 single data per period and parameter were presented as boxplot (Fig. 3). Previous analyses of water sampled at different littoral sites showed no difference to data of the lake centre (data not shown).

Macrophyte tissue nutrient concentration

We sampled submerged macrophytes in 40–70 cm water depth at four sites of Lake Stechlin in July and August 2014 (Figs. 1A, 4). At this depth, the macrophyte community only consisted of single plants. Five macrophyte species were present in sufficient quantity to be included: *Myriophyllum spicatum*, *Potamogeton lucens*, *P. perfoliatus*, *P. pectinatus* and *Najas marina*. We sampled selectively young tissue to reduce the possible effect of age on nitrogen (N) and phosphorus (P) contents. For *M. spicatum*, *P. lucens* and *P. perfoliatus* we selected new leaves, for *N. marina* and *P. pectinatus* we sampled the whole above-ground plant. For each macrophyte species, several leaves or plants were sampled per replicate, and we took 4 replicates per site and sampling date. The samples were gently cleaned from epiphyton and dried at 60 °C until the constant weight. The ground probes were analyzed with the ammonium molybdate spectrometric method for P. TC (total carbon) and TN (total nitrogen) tissue content were determined with a CHN elemental analyser (Vario EL, Germany).

Epiphyton biomass and shading

Epiphyton was sampled from transparent polypropylene sheets (IBICO, GBC, Chicago. Il, USA), that mimic macrophytes by being flexible and having a slightly rough surface. Strips (2 × 12 cm) were installed vertically in 50 cm water depth, fixed to the sediment with a metallic structure. The sampled surface extended from 5 cm above the sediment surface to around 30 cm from the water surface. The strips were installed parallel to the shore line, and in areas with limited shading from trees or macrophytes (Fig. 1A). After four weeks exposure, we sampled eight stripes at each location and transported them to the laboratory in a dark, humid and cooled box. Stripes were exposed to carbonated water to remove grazers, and epiphyton was scrubbed with a toothbrush into filtered (0.45µm cellulose acetate filters, Sartorius, Göttingen) lake water. The suspension obtained from two stripes was filtered on to a pre-weighed GF/F glass-fibre filter and dried for 12 h at 105 °C for obtaining epiphyton dry weight (dw). We obtained 4 replicates (each from two stripes), per site and per sampling campaign. Light attenuation by epiphyton was calculated following (Köhler et al. 2010) using the formula: light attenuation = (108 × epiphyton dw)/(9.2 + epiphyton dw). Carbon and N concentrations were obtained from the filters, and P concentrations from the suspension, following the same methods as described for the macrophytes (Fig. 5).

Statistics

To evaluate differences of epiphyton and macrophyte parameters between samples in LGD or in control (C) conditions, or between years, we applied Student's t-test for normally distributed data with equal variances, or for small samples sizes. When normality could not be attained, we applied the non-parametrical Mann-Whitney U test. All statistical analyses were performed with the software R (R Development Core Team 2014) and the data represented with ggplot2 (Wickham 2009).

Results

Macrophyte community composition and abundance

In 1962, low-growing charophyte meadows were abundant along two third of Lake Stechlin's littoral shore line and covered 42 ha (Fig.

1C). In 2008 and 2014, this area had shrunk to 3 ha, present in the northern bay and few stands further from the shore, next to the eastern bay (Fig. 1C). Submerged angiosperms were present along most of the lake shore, with a prevalence on the western shore and bay ends. They covered 17 ha in 1962, 13 ha in 2008 and 33 ha in 2014. Areas without submerged vegetation were only mapped in 2014, and covered 31 ha of the whole littoral.

Since 1962, species of all three indicator values (A, B and C) have been present in the lake, but the number of A species tended to decrease (Fig. 2A). Specifically, the A species *Chara aspera* and *C. rudis* have not been observed after 1962, and *C. intermedia* after 2002 (Table 2). *Najas marina ssp. intermedia* and *P. crispus* appeared as additional C species in 2002 and 2008, respectively. While healthy stands of the A specie *C. filiformis* were observed in 1962, in the last campaigns only isolated plants were reported.

The percentage of transect coverage per species have been calculated for 20 transects in 2008 and 2014, and show different patterns for A, B, C species (Fig. 2B). “A” species were less abundant in 2014 than in 2008 (Mann-Whitney *U* test, $p < 0.01$). There were no significant differences in the abundance between A and C species in 2008 (Mann- Whitney *U* tests, $p > 0.05$), while C species became more abundant than A species in 2014 (Mann-Whitney *U* tests, $p < 0.001$). The number of species per transect was extremely variable, and no significant differences between years were found. Also, we could not find any significant differences in macrophyte coverage between transects located in LGD sites and other transects.

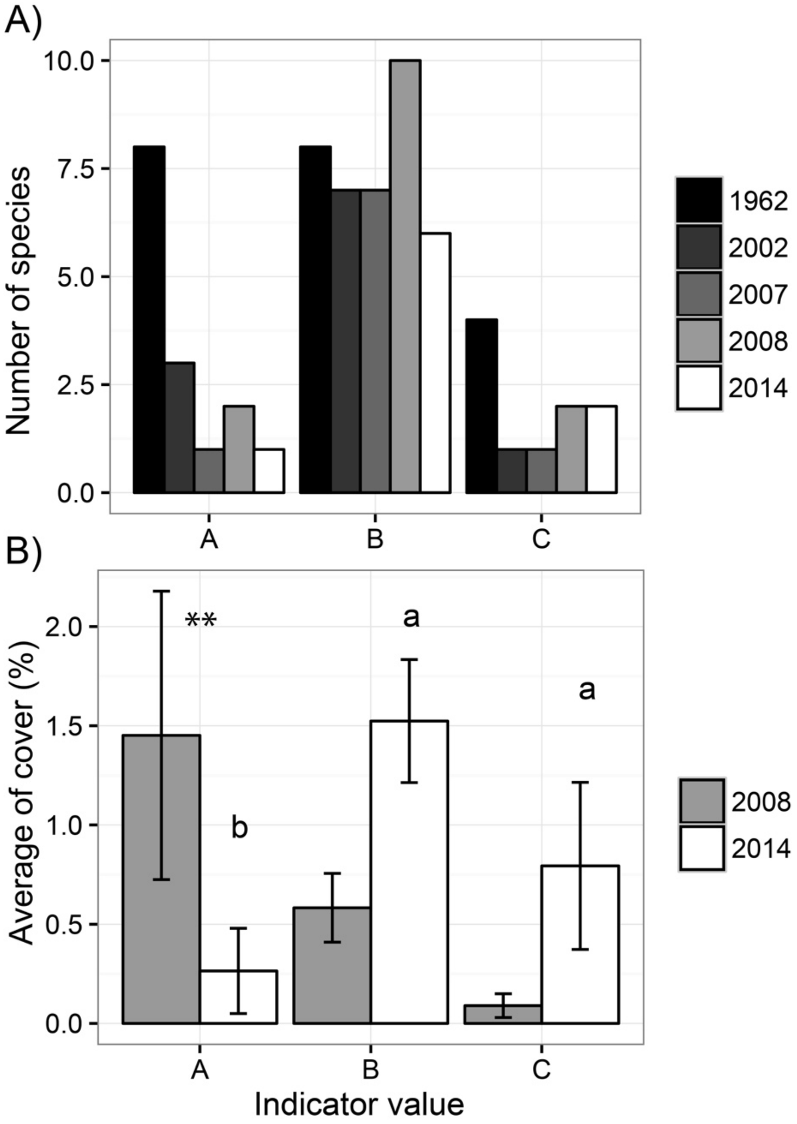

Figure 2. A) Number of macrophyte species with A, B and C indicator values present in shallow littoral areas (0–2 m depth) of Lake Stechlin during the survey in 1962 (whole lake), and in 2002, 2007, 2008 and 2014 (data from 7 identical transects). B) Average coverage (± standard error) of macrophyte species with A, B and C indicator values observed in 0–2 m depth of 20 transects surveyed in 2008 and 2014. The significant differences between years are indicated (Mann-Whitney *U* test, **: $p < 0.01$) and for each year, different letters are attributed to significantly different (Mann-Whitney *U* test, $p < 0.05$) coverages. The indicator values are attributed after (Schaumburg et al. (2015) for the “TKg13” lake type.

Table 2: Presence of macrophyte species in transects of the shallow littoral (0-2 m water depth) of Lake Stechlin in 2002, 2007, 2008 and 2014 (whole lake in 1962). Species group according to Schaumburg et al. (2015) at 0 to 1 m depth in lakes from the "TKg13" category. Taxa from group "A" are only abundant at undisturbed sites, "B" taxa are present in both disturbed and undisturbed sites, and "C" taxa only occur at disturbed sites. Red list categories for Brandenburg according to Kabus and Mauersberger (2011) for charophytes and Ristow et al. (2006) for angiosperms (1: critically endangered, 2: endangered, 3: vulnerable, "V": near threatened, *: least concern).

	Species group	Red list category	1962	2002	2007	2008	2014
Charophytes							
Chara aspera	A	2	x				
Chara contraria	B	V	x	x	x	x	
Chara filiformis	A	1	x	x		x	x
Chara globularis	B	*	x		x	x	x
Chara intermedia	A	3	x	x			
Chara rudis	A	2	x				
Chara tomentosa	A	3	x	x	x	x	x
Chara virgata	B	*	x	x		x	
Angiosperms							
Ceratophyllum demersum	C	*	x		x	x	x
Elodea canadensis	C	*	x				
Myriophyllum spicatum	B	V	x	x	x	x	x
Myriophyllum alterniflorum	B	2	x			x	
Najas marina ssp. intermedia	C	3		x	x	x	x
Nuphar lutea	B	*	x				x
Potamogeton crispus	C	*				x	x
Potamogeton filiformis	A	1	x			x	
Potamogeton gramineus	A	2	x				
Potamogeton lucens	B	3	x	x	x	x	x
Potamogeton natans	A	*	x				
Potamogeton pectinatus	B	*	x	x	x	x	x
Potamogeton perfoliatus	B	V		x	x	x	x
Potamogeton pusillus	C	3	x	x		x	x
Ranunculus circinatus	C	3	x			x	x

Surface water quality

Average TP concentrations of the surface water of Lake Stechlin were 12.0 ± 4.6 (sd) µg L^{-1} during the 16 observed years, with slightly higher values during the winter period (10.6 ± 3.3 µg L^{-1} in summer and 13.2 ± 5.3 µg L^{-1} in winter). Summer TP concentrations during the periods 2005–2008 and 2011–2014 were significantly higher than in the two previous time period (1970–1973 and 1999–2002, $p < 0.001$, Mann-Whitney *U* tests, Fig. 3). Winter TP concentrations in surface water of Lake Stechlin have increased from 1970–73 to 1999–2002 and to 2005–08 and did not significantly differ between the two last periods (Mann-Whitney *U* tests, $p < 0.001$, $p < 0.001$ and $p > 0.05$ respectively). Summer Secchi depths significantly decreased during the three last periods ($p < 0.001$, Mann- Whitney *U* tests), with means of 8.9 ± 1.3 m, 7.4 ± 1.3 m and 5.9 ± 1.4 m, respectively (Fig. 3).

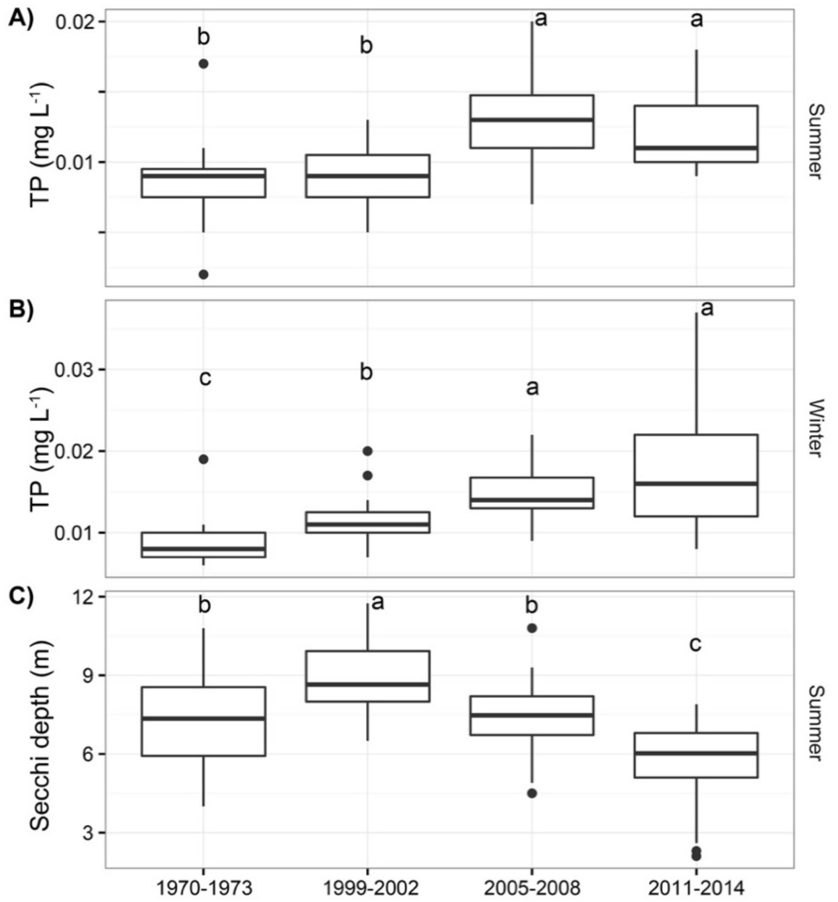

Figure 3. Characteristics of surface water during the 4 years preceding macrophyte mappings in Lake Stechlin. (A) Total phosphorus (TP) concentrations during the summer months: May to September, (B) TP concentrations during the winter months: October to April, (C) Secchi depth during the summer months. Box-and whisker plots boxes represent first, second and third quartiles, with upper and lower whiskers extending until respectively the highest and the lowest values that are within 1.5 x inter-quartile range. The small letters represent the rank of the datasets, after repeated Mann-Whitney *U* tests.

Macrophyte tissue nutrient concentrations

Macrophyte tissue P content ranged between 0.07% dw and 0.41% dw and the N content varied between 1.04% dw and 3.58% dw (Fig. 4). 89% of the P values were above 0.13% dw, and 98% of the N data were above 1.3% dw, the thresholds for P and N limitation suggested by Gerloff and Krombholz (1966).

Macrophyte tissue nutrient content mostly did not differ significantly between LGD and C sites (Student's *t*-tests, $p > 0.05$), apart from *N. marina spp. intermedia* and *P. lucens* ($p < 0.05$).

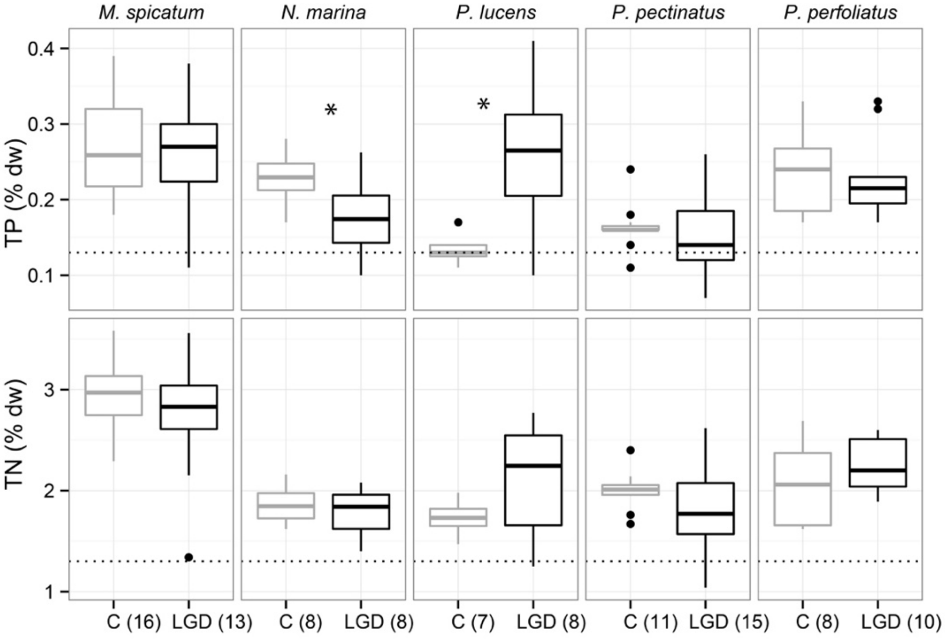

Figure 4. Nitrogen (N) and phosphorus (P) tissue concentrations in submerged macrophytes at locations with and without lacustrine groundwater discharge (LGD) in Lake Stechlin in 2014. The horizontal lines indicate the threshold of nutrient limitation (Gerloff and Krombholz 1966). Box-and whisker plots boxes represent first, second and third quartiles, with upper and lower whiskers extending until respectively the highest and the lowest values that are within 1.5 x inter-quartile range. An asterisk (*) is included if the result of the Student's t-test is significant ($p < 0.05$). The number of samples is indicated on the x-axis.

Epiphyton shading

Epiphyton dry weight varied between 0.97 g m^{-2} and 13.5 g m^{-2}, with averages of 7.4 g m^{-2} in LGD sites and 5.4 g m^{-2} in control sites. Light attenuation by epiphyton varied between 10.3% and 64.3% of incoming photosynthetically active radiation (PAR), and was significantly higher in LGD sites than in control sites (Student's *t*-test, $p < 0.05$, Fig. 5A). More P accumulated per area in epiphyton at LGD sites (12.4 ± 5.4 mg m^{-2}) compared to C sites (8.3 ± 3.9 mg m^{-2}) (Student's *t*-test, $p < 0.01$, Fig. 5D). Most of C:P ratio and all C:N ratio in epiphyton were above the thresholds for P or N limitation identified by Kahlert (1998).

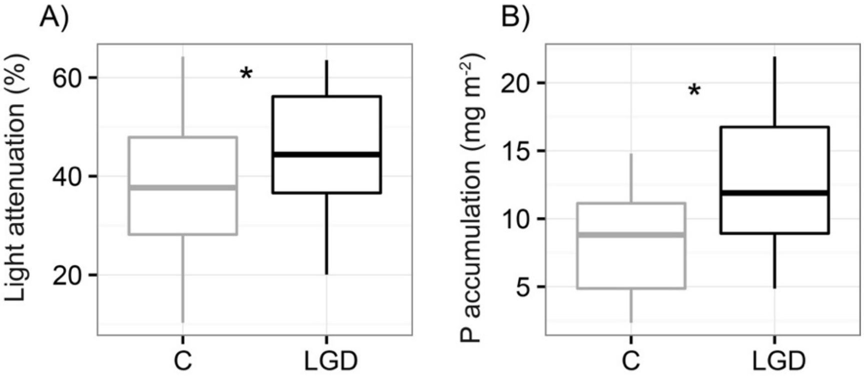

Figure 5. Light attenuation (A) and phosphorus accumulation (B) in the epiphyton at locations with (LGD) and without (C) lacustrine groundwater discharge in the littoral of Lake Stechlin in summer 2014. Box-and whisker plots boxes represent first, second and third quartiles, with upper and lower whiskers extending until respectively the highest and the lowest values that are within 1.5 x inter-quartile range. The results of Student's t-tests are included (* for $p < 0.05$).

Discussion

Shallow littoral macrophyte communities of Lake Stechlin showed strong changes in the last 50 years, both in species composition and abundance. Low-growing charophyte communities in shallow water have almost completely disappeared while macrophyte species typical of eutrophic conditions have become abundant, and more areas completely lack vegetation. Increasing summer TP concentrations and decreasing Secchi depth in surface water of Lake Stechlin indicate a process of slight eutrophication, especially during the last decade. This process may have promoted the occurrence and dispersal of angiosperm species typical for eutrophic conditions in the shallow littoral areas. A competition for space in the shallow littoral between low-growing charophytes and angiosperms seems unlikely as the vegetation is generally rather sparse. Our data on higher periphyton biomass at shallow areas without charophyte stands and with groundwater discharge and our previous detailed study on groundwater-mobilized nutrients potentially affecting periphyton growth (Périllon et al. 2017) indicate that groundwater may indirectly affect both macrophyte species composition and abundance in shallow littoral areas due to a stimulation of epiphyton growth shading the macrophytes, increasing the drag forces of waves, especially on small rootless charophytes and enhancing the sensitivity of macrophytes to disturbances by herbivor- ous and benthivorous cyprinids.

Macrophyte development in shallow littoral areas

In the shallow littoral areas of Lake Stechlin, we observed a strong decline of both presence and abundance of macrophyte species characteristic for the reference oligotrophic conditions in this lake type. The observed change in macrophyte abundance is especially visible between 2008 and 2014, however, this could be partly explained by inter-annual fluctuation, and a confirmation of this trend would need further data acquisition. The once abundant charophyte species *C. aspera* and *C. intermedia* had completely disappeared in 2008 and 2014, while *C. rudis* had declined in abundance. A decline of charophytes with increasing eutrophication has been described for the period since 1940 in several lakes in Southern Sweden (Blindow 1992) and in Denmark (Baastrup-Spohr et al. 2013). Both studies point to a disappearance of macrophytes in deep waters in alkaline lakes and consequently a prevalence of reduced light availability as a main reason for this historical decline. The situation in Lake Stechlin, and dozens of other lakes in the region (R. Mauersberger, pers. comm.) seems different.

Secchi disk transparencies and TP concentrations indicate a slight eutrophication trend, especially during the last decade. The reasons for this development are subject of current research (Kirillin et al. 2013b). The effect of decreasing light availability in the water column for shallow littoral habitats, however, was supposed to be of low relevance. Minimum light requirements for charophytes and submerged angiosperms are comparable (Sand-Jensen and Madsen 1991). In addition, Kovtun-Kante et al. (2014) have shown that shallow water *C. aspera* communities could adapt to reduced light availability. In contrast to light, the increasing P availability in the water column may have contributed to the decline of shallow water charophytes by promoting macrophyte species typical for eutrophic conditions. Richter and Gross (2013) reported that charophyte stands can negatively affect tallgrowing angiosperms under low P availability and high water clarity. This implies that taller angiosperms may outcompete low-growing charophytes at increasing TP concentrations. The tissue P- and N- contents of the five angiosperms abundant in Lake Stechlin were all well above the threshold levels indicating limiting conditions (Gerloff and Krombholz 1966), which suggests that nutrient availability in the sandy littoral was sufficient. P- and N- contents of M. spicatum were similar to the values obtained by Gross (2009) in the littoral of Lake Constance, at a time when charophytes increased and started replacing *Myriophyllum* during re-oligotrophicaton. However, angiosperm densities are still rather low and vegetation-free areas are abundant indicating that competition with angiosperms is at least not the only reason for the observed charophyte decline.

Potential influence of groundwater

A direct influence of groundwater discharge on angiosperm biomass or nutrient content could not be found in Lake Stechlin, contrary to other studies which showed a promotion of macrophytes by LGD (Lodge et al. 1989; Ommen et al. 2012; Frandsen et al. 2012). However, our data show an increased epiphyton growth at the LGD sites. A previous study indicated that sediment nutrients, and especially phosphorus, may have been mobilized by groundwater discharge (Périllon et al. 2017) and stimulated epiphyton growth.

The present epiphyton biomass production in Lake Stechlin was higher than 40 years ago. Scheffler (1981) applied the ^{14}C method and measured an epiphyton production of approximately 28 ± 7 mg C m^{-2} d^{-1} on artificial substrates exposed for 4 weeks at a sunny littoral site during May-September of 1971–74 while we measured 64.5 mg C m^{-2} d^{-1} at LGD sites and 51.6 mg C m^{-2} d^{-1} at control sites during the same season in 2014. The question, whether the observed development of epiphyton was significantly affected by changes in the groundwater discharge, P availability in the ground- water or in the littoral sediment, remains open. Since 2011, several wet summers caused increasing water levels in many lakes of the region after 30 years of droughts and falling lake levels (Kaiser et al. 2014). Changes in groundwater flow may have influenced nutrient fluxes and mobilization processes, but groundwater-induced nutrient fluxes were not studied in the past. However, one could speculate that P availability in the littoral may have increased due to a feedback with epi- and periphyton growth in the groundwater-influenced locations resulting in effects similar to those described as “nearshore P shunt” for areas with abundant dreissenids (Hecky et al. 2004).

This groundwater-promoted epiphyton growth in shallow littoral areas may have affected the low-growing charophyte species, which can currently only be found in littoral areas without LGD, in three ways. It may firstly have increased shading. In our study, epiphyton shading reached up to 64% reduction of incoming PAR and growth limitations have been observed for values between 26% PAR reduction for *P. pectinatus* (Vierssen and Hootsmans 1994) to 95% for charophytes (Middelboe and Markager 1997). Most of the littoral of Lake Stechlin is shaded by trees which together with epiphyton can significantly contribute to macrophyte biomass reductions (Köhler et al. 2010; Ali et al. 2011). Secondly, epiphyton may enhance sensitivity of macrophytes to wave drag forces (Schutten et al. 2004). Especially charophytes may be more sensitive to high fetch than other macrophytes, as observed by Schmieder and Lehmann (2004) in Lake Constance. This could explain the loss of charophytes at the wind-exposed northern sites. Finally, it has been shown in a modeling study and by a meta- analysis that epiphyton increases the susceptibility of macrophytes to herbivory (Hidding et al. 2016). An influence of herbivorous and benthivorous cyprinids on charophytes in Lake Stechlin has been suggested based on the increase of vegetation-free areas and typical sediment patterns indicating physical disturbance by benthivorous fish (Van de Weyer et al. 2015). Negative effects on charophytes have been shown in other lakes for a number of fish species such as common carp (Laguna et al. 2016), grass carp (Krupska et al. 2012), bream (Ten Winkel and Meulemans 1984) and rudd (Lake et al. 2002). Long-term exclosure experiments should be performed in the shallow littoral of Lake Stechlin to clarify the impact of herbivorous and benthivorous fish.

Conclusion

We conclude that there is a strong need for further research on the reasons of charophyte decline observed in the shallow littoral area of Lake Stechlin and other oligo-mesotrophic lakes in North-Eastern Germany. Their decline does not follow the typical pattern described for eutrophication of freshwater habitats with an initial loss of species in deeper water habitats. Groundwater-mobilized nutrients may play a role by promoting epiphyton growth leading to increased shading and sensitivity of wave action and herbivory, but further detailed studies need to clarify the origin of the nutrients and the impact of other factors such as disturbances by herbivorous and benthivorous cyprinids.

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References

Ali, M. M., S. A. Hassan, and A.-S. M. Shaheen. 2011. Impact of riparian trees shade on aquatic plant abundance in conservation islands. Acta Bot. Croat. **70**: 245–258.

Baastrup-Spohr, L., L. L. Iversen, J. Dahl-Nielsen, and K. Sand-Jensen. 2013. Seventy years of changes in the abundance of Danish charophytes. Freshw. Biol. **58**: 1682–1693.

Berg, C., J. Dengler, A. Abdank, and M. Isermann. 2004. Die Pflanzengesellschaften Meck- lenburg-Vorpommern und ihre

Gefährdung. 2 Bände., Weissdorn-Verlag.

Blindow, I. 1992. Decline of charophytes during eutrophication: comparison with angiosperms. Freshw. Biol. **28**: 9–14.

Carpenter, S. R., and D. M. Lodge. 1986. Effects of submersed macrophytes on ecosystem processes. Aquat. Bot. **26**: 341–370.

Chambers, P. A., and J. Kalff. 1987. Light and nutrients in the control of aquatic plant community structure. I. In situ experiments. J. Ecol. **75**: 611–619.

Deegan, B. M., S. D. White, and G. G. Ganf. 2012. Nutrients and water level fluctuations: a study of three aquatic plants. River Res. Appl. **28**: 359–368.

DIN 38405. Deutsche Einheitsverfahren (DEV) zur Wasser-, Abwasser- und Schlammuntersuchung. Anal. Stand. DIN 38405, part 21

Frandsen, M., B. Nilsson, P. Engesgaard, and O. Pedersen. 2012. Groundwater seepage stimulates the growth of aquatic macrophytes. Freshw. Biol. **57**: 907–921.

Gerloff, G. C., and P. H. Krombholz. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. **11**: 529–537.

Gross, E. M. 2009. Impact of reoligotrophication in Lake Constance on nutrient content and defensive phenolic compounds in Eurasian watermilfoil. Int. Assoc. Theor. Appl. Limnol. Vol 30, Pt 6, Proc. **30**: 897–899.

Hagerthey, S. E., and W. C. Kerfoot. 1998. Groundwater flow influences the biomass and nutrient ratios of epibenthic algae in a north temperate seepage lake. Limnol. Oceanogr. **43**: 1127–1242.

Hagerthey, S. E., and W. C. Kerfoot. 2005. Spatial variation in groundwater-related resource supply influences freshwater benthic algal assemblage composition. J. North Am. Benthol. Soc. **24**: 807–819.

Hecky, R. E., R. E. H. Smith, D. R. Barton, S. J. Guildford, W. D. Taylor, M. N. Charlton, and T. Howell. 2004. The nearshore phosphorus shunt: a consequence of ecosystem engineering by dreissenids in the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. **61**: 1285–1293.

Hidding, B., E. S. Bakker, M. J. M. Hootsmans, and S. Hilt. 2016. Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. Oikos **125**: 1489–1495.

Hilt, S., I. Henschke, J. Rücker, and B. Nixdorf. 2010. Can submerged macrophytes influence turbidity and trophic state in deep lakes? Suggestions from a case study. J. Environ. Qual. **39**: 725–33.

Hilt, S., J. Köhler, R. Adrian, M. T. Monaghan, and C. D. Sayer. 2013. Clear, crashing, turbid and back - long-term changes in macrophyte assemblages in a shallow lake. Freshw. Biol. **58**: 2027–2036.

Holzbecher, E. 2001. The dynamics of subsurface water divides - watersheds of Lake Stechlin and neighbouring lakes. Hydrol. Process. **15**: 2297–2304.

Kabus, T., and R. Mauersberger. 2011. Liste und Rote Liste der Armleuchteralgen (Characeae) des Landes Brandenburg. Natur und Landschaftpfl. Brand. **20**: 32pp.

Kahlert, M. 1998. C:N:P ratios of freshwater benthic algae. Arch. Hydrobiol. (Suppl) (Advanc. Limnol.) **51**: 105–114.

Kaiser, K., P. J. Koch, R. Mauersberger, P. Stüve, J. Dreibrodt, and O. Bens. 2014. Detection and attribution of lake-level dynamics in north-eastern central Europe in recent decades. Reg. Environ. Chang. **14**: 1587–1600.

Kasprzak, P., T. Shatwell, M. O.Gessner, T. Gonsiorczyk, G. Kirillin, G. Selmeczy, J. Padisák, and C. Engelhardt. 2017. Extreme weather event triggers cascade towards extreme turbidity in a clear-water lake. Ecosystems , doi:10.1007/s10021-017-0121-4

Kirillin, G., W. Phillip, C. Engelhardt, and G. Nützmann. 2013a. Net groundwater inflow in an enclosed lake: From synoptic variations to climatic projections. Hydrol. Process. **27**: 347–359.

Kirillin, G., T. Shatwell, and P. Kasprzak. 2013b. Consequences of thermal pollution from a nuclear plant on lake temperature and mixing regime. J. Hydrol. **496**: 47–56.

Köhler, J., J. Hachoł, and S. Hilt. 2010. Regulation of submersed macrophyte biomass in a temperate lowland river: interactions between shading by bank vegetation, epiphyton and water turbidity. Aquat. Bot. **92**: 129–136.

Kolada, A., N. Willby, B. Dudley, P. Nõges, M. Søndergaard, S. Hellsten, M. Mjelde, E. Penning, G. Van Geest, V. Bertrin, F. Ecke, H. Mäemets, and K. Karus. 2014. The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. Ecol. Indic. **45**: 407–415.

Körner, S., E. S. G. Schreiber, and N. Walz. 2002. Herbivory on submerged macrophytes -- who's to blame? Verhandlungen der Int. Vereinigung für Theor. und Angewante Limnol. **28**: 967–970.

Kovtun-Kante, A., K. Torn, and J. Kotta. 2014. In situ production of charophyte communities under reduced light conditions in a brackish-water ecosystem. Est. J. Ecol. **63**: 28–38.

Krausch, H. D. 1964. Die Pflanzengesellschaften des Stechlinsee-Gebietes I. Die Gesellschaften des offenen Wassers. Limnologica **2**: 145–203.

Krey, L. 1985. The lakes of the Lake Stechlin area: aspects of their morphometry, p. 29–40. *In* Lake Stechlin. Dr. W. Junk Publishers.

Krupska, J., M. Pełechaty, A. Pukacz, and P. Ossowski. 2012. Effects of grass carp introduction on macrophyte communities in a shallow lake. Oceanol. Hydrobiol. Stud. **41**: 35–40.

Laguna, C., J. J. López-Perea, J. Viñuela, M. Florín, J. Feliu, Á. Chicote, S. Cirujano, and R. Mateo. 2016. Effects of invasive fish and quality of water and sediment on macrophytes biomass, and their consequences for the waterbird community of a Mediterranean floodplain. Sci. Total Environ. **551**–**552**: 513–521.

Lake, M. D., B. J. Hicks, R. D. S. Wells, and T. M. Dugdale. 2002. Consumption of submerged aquatic macrophytes by rudd (*Scardinius erythrophthalmus* L.) in New Zealand. Hydrobiologia **470**: 13–22.

Lewandowski, J., K. Meinikmann, G. Nützmann, and D. O. Rosenberry. 2015. Groundwater - the disregarded component in lake water and nutrient budgets. 2. Effects of groundwater on nutrients. Hydrol. Process. **29**: 2922–2955.

Lillie, R. A., and J. W. Barko. 1990. Influence of sediment and groundwater on the distribution and biomass of *Myriophyllum spicatum* L. in Deveil's Lake, Wisconsin. J. Freshw. Ecol. **5**: 417–426.

Lodge, D. M., D. P. Krabbenhoft, and R. G. Striegl. 1989. A positive relationship between groundwater velocity and submersed macrophyte biomass in Sparkling lake, Wisconsin. Limnol. Oceanogr. **34**: 235–239.

Loeb, S. L., and S. H. Hackley. 1988. The distribution of submerged macrophytes in Lake Tahoe, California and Nevada, and the possible influence of groundwater seepage. Verhandlungen der Int. Vereinigung für Theor. und Angewante Limnol. **23**: 1927–1933.

Londo, G. 1976. The decimal scale for releves of permanent quadrats. Vegetatio **33**: 61–64.

Lyche-solheim, A., C. K. Feld, S. Birk, G. Phillips, L. Carvalho, G. Morabito, U. Mischke, N. Willby, M. Søndergaard, S. Hellsten, A. Kolada, M. Mjelde, J. Böhmer, O. Miler, M. T. Pusch, C. Argillier, E. Jeppesen, T. L. Lauridsen, and S. Poikane. 2013. Ecological status assessment of European lakes : a comparison of metrics for phytoplankton , macrophytes , benthic invertebrates and fish. Hydrobiologia **704**: 57–74.

McBride, M. S., and H. O. Pfannkuch. 1975. The distribution of seepage within lakebeds. J. Res. USGS **3**: 505–512.

Middelboe, A. L., and S. Markager. 1997. Depth limits and minimum light requirements of freshwater macrophytes. Freshw. Biol. **37**: 553–568.

Ommen, D. A. O., J. Kidmose, S. Karan, M. R. Flindt, P. Engesgaard, B. Nilsson, and F. Ø. Andersen. 2012. Importance of groundwater and macrophytes for the nutrient balance at oligotrophic Lake Hampen, Denmark. Ecohydrology **5**: 286–296.

Penning, W. E., M. Mjelde, B. Dudley, S. Hellsten, J. Hanganu, A. Kolada, M. van den Berg, S. Poikane, G. Phillips, N. Willby, and F. Ecke. 2008. Classifying aquatic macrophytes as indicators of eutrophication in European lakes. Aquat. Ecol. **42**: 237–251.

Périllon, C., and S. Hilt. 2016. Groundwater influence differentially affects periphyton and macrophyte production in lakes. Hydrobiologia **778**: 91–103.

Périllon, C., F. Pöschke, J. Lewandowski, M. Hupfer, and S. Hilt. 2017. Stimulation of epiphyton growth by lacustrine groundwater discharge to an oligo-mesotrophic. Freshw. Sci. **36**, doi:10.1086/692832.

Phillips, G. L., D. Eminson, and B. Moss. 1978. A mechanism to account for macrophytes decline in progressively eutrophicated freshwaters. Aquat. Bot. **4**: 103–126.

R Development Core Team, R. 2014. R: a language and environment for statistical computing. R Found. Stat. Comput. **1**: 409 pp.

Richter, D., and E. M. Gross. 2013. *Chara* can outcompete *Myriophyllum* under low phosphorus supply. Aquat. Sci. **75**: 457–467.

Ristow, M., A. Herrmann, H. Illig, H.-C. Klage, G. Klemm, V. Kummer, B. Machatzi, S. Rätzel, R. Schwarz, and F. Zimmermann. 2006. Liste und Rote Liste der etablierten Gefäßpflanzen Brandenburgs.

Naturschutz und Landschaftspfl. Brand. Bg. **15**: 163.

Rosenberry, D. O., J. Lewandowski, K. Meinikmann, and G. Nützmann. 2015. Groundwater - the disregarded component in lake water and nutrient budgets. Part 1: effects of groundwater on hydrology. Hydrol. Process. **29**: 2895–2921.

Sachse, R., T. Petzoldt, M. Blumstock, S. Moreira, J. Rücker, J. H. Janse, W. M. Mooij, S. Hilt, and P. Marlene. 2014. Extending one-dimensional models for deep lakes to simulate the impact of submerged macrophytes on water quality. Environ. Model. Softw. **61**: 410–423.

Sand-Jensen, K., and T. V. Madsen. 1991. Minimum light requirements of submerged freshwater macrophytes in laboratory growth experiments. J. Ecol. **79**: 749–764.

Sand-Jensen, K., T. Riis, O. Vestergaard, and S. E. Larsen. 2000. Macrophyte decline in Danish lakes and streams over the past 100 years. J. Ecol. **88**: 1030–1040.

Sand-Jensen, K., and M. Søndergaard. 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Rev. der gesamten Hydrobiol. **66**: 529–552.

Sayer, C. D., T. A. Davidson, and J. I. Jones. 2010. Seasonal dynamics of macrophytes and phytoplankton in shallow lakes: A eutrophication-driven pathway from plants to plankton? Freshw. Biol. **55**: 500–513.

Schaumburg, J., C. Schranz, G. Hofmann, D. Stelzer, S. Schneider, and U. Schmedtje. 2004. Macrophytes and phytobenthos as indicators of ecological status in German lakes—a contribution to the implementation of the Water Framework Directive. Limnologica **34**: 302–314.

Schaumburg, J., C. Schranz, D. Stelzer, and A. Vogel. 2015. Verfahrensanleitung für die ökologische Bewertung von Seen zur

Umsetzung der EU- Wasserrahmenrichtlinie : Makrophyten und Phytobenthos Phylib.

Scheffer, M., S. Hosper, M. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative equilibria in shalow lakes. Trends Ecol. Evol. **8**: 275–279.

Scheffler, W. 1981. Quantitative Erfassung des Periphytons im Stechlinsee. Limnologica **13**: 101–122.

Schmieder, K., and A. Lehmann. 2004. A spatio-temporal framework for efficient inventories of natural resources: a case study with submersed macrophytes. J. Veg. Sci. **15**: 807–816.

Schutten, J., J. Dainty, and A. J. Davy. 2004. Wave-induced hydraulic forces on submerged aquatic plants in shallow lakes. Ann. Bot. **93**: 333–341.

Sebestyen, S. D., and R. L. Schneider. 2004. Seepage patterns, pore water, and aquatic plants: hydrological and biogeochemical relationships in lakes. Biogeochemistry **68**: 383–409.

Søndergaard, M., G. Phillips, S. Hellsten, A. Kolada, F. Ecke, H. Mäemets, M. Mjelde, M. M. Azzella, and A. Oggioni. 2013. Maximum growing depth of submerged macrophytes in European lakes. Hydrobiologia **704**: 165–177.

Team, Q. D. 2015. QGIS Geographic Information System.

Tóth, V. R., and S. C. J. Palmer. 2016. Acclimation of *Potamogeton perfoliatus* L. to periphyton accumulation-induced spectral changes in irradiance. Hydrobiologia **766**: 293–304.

Vierssen, W. Van, and M. J. M. Hootsmans. 1994. Photoperiodic effects on photosynthesis and tuber production in Potamogeton pectinatus L., p. 118–134. *In* W. Van Vierssen, M.J.M. Hootsmans, and J.E.Vermaat [eds.], Lake Veluwe, a macrophyte-dominated system under eutrophication stress. Kluwer Academic Publishers.

Van de Weyer, K., S. Meis, and V. Krautkrämer. 2015. Die Makrophyten des Großes Stechlinsees, des Wummsees und des Wittwensees. Fachbeiträge des LUGV, H. 145

Van de Weyer, K., J. Päzolt, P. Tigges, C. Raape, and S. Oldorff. 2009. Flächenbilanzierungen submerser Pflanzenbestände – dargestellt am Beispiel des Großen Stechlinsees (Brandenburg) im Zeitraum von 1962-2008. Naturschutz und Landschaftspfl. Brand. **18**: 1–6.

Van de Weyer, K., and C. Schmitt. 2011. Bestimmungsschlüssel für die aquatischen Makrophyten (Gefäßpflanzen, Armleuchteralgen und Moose) in Deutschland: Band 1: Bestimmungsschlüssel. Fachbeiträge des LUGV Brand. **119**: 164pp.

Wickham, H. 2009. ggplot2: Elegant graphics for data analysis., Springer-Verlag.

Ten Winkel, E. H., and J. T. Meulemans. 1984. Effects of fish upon submerged vegetation. Hydrobiol. Bull. **18**: 157–158.

Chapter 4

Groundwater discharge gives periphyton a competitive advantage over macrophytes

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Abstract

Lacustrine groundwater discharge (LGD) can significantly contribute to nutrient budgets of lakes. It mainly occurs in shallow littoral zones, and thus potentially affects macrophytes and periphyton. These benthic primary producers asymmetrically compete for resources, with rooted macrophytes and periphyton being superior for either sediment nutrients or light, respectively. We hypothesized that LGD can advectively transport sediment phosphorus (P) into the water, thereby supporting periphyton growth. Above a threshold level of sediment P concentrations, negative effects of periphyton shading should exceed positive effects of sediment P on macrophytes. To test these hypotheses, we performed laboratory experiments comparing the growth of macrophytes (*Potamogeton pectinatus*) and periphyton at four different sediment P concentrations without and with LGD.

Our data show advective transport of sediment P by LGD and an increased periphyton biomass with rising sediment P concentration in LGD treatments but not in controls. These results experimentally confirm earlier field studies on the positive influence of LGD on periphyton biomass. The hypothesized negative effect of this process on macrophytes was shown by a negative correlation between macrophyte growth rates and periphyton shading. This only occurred in treatments with LGD and when experiments were performed with young plants. Macrophytes died when shading levels exceeded 75% of incident light.

We conclude that LGD can significantly affect the competition between benthic primary producers at the expense of macrophytes. This process is probably most relevant in oligotrophic water bodies or during periods of P deficiency in the open water, when advective transport of sediment P by LGD can release periphyton from nutrient limitation. Global change effects on LGD can thus translate into changes in composition and productivity of benthic habitats.

Key words: asymmetric competition, periphyton, macrophytes, groundwater

Introduction

Eutrophication is one of the most dominant stress factors to lakes (Hasler 1947; Smith 2003). Direct runoff from sewer systems and agricultural catchments are supposed to be the main nutrient sources (Arle et al. 2017). However, nutrient input by lacustrine groundwater discharge (LGD) is often significant in lakes, and its contribution to lake eutrophication has often been overlooked (Lewandowski et al. 2015; Périllon and Hilt 2016).

LGD may increase the availability of limiting nutrients for primary producers in lakes through nutrient transport from the catchment into the lake (Frandsen et al. 2012; Lewandowski et al. 2015). It may also mobilize nutrients from the upper sediment layers, which usually have higher concentrations than the lake water (Hagerthey and Kerfoot 1998; Périllon et al. 2017). Additional positive influences of LGD on benthic primary producers include flushing of phytotoxins from sediments and reducing seasonal temperature variation in the littoral (Lodge et al. 1989).

Groundwater influx to lakes usually is greatest in the shallow littoral and exponentially decreases with distance offshore (McBride and Pfannkuch 1975; Shaw and Prepas 1990). Benthic primary producers growing in these areas are thus supposed to be particularly influenced by nutrient influx through LGD, which has been shown separately for periphyton (Hurley et al. 1985; Hagerthey and Kerfoot

1998; Hunt et al. 2006; Périllon et al. 2017) and macrophytes (Lodge et al. 1989; Lillie and Barko 1990; Frandsen et al. 2012).

However, periphyton and macrophytes are competitors, both for nutrients and for light. While both can directly take up nutrients from the water column, rooted macrophyte species have a competitive advantage due to their access to nutrients in the sediment (Barko and Smart 1980; Jaynes and Carpenter 1986). In contrast, periphyton is the superior competitor for light and can intercept up to 98% of incoming light (Tóth 2013; Tóth and Palmer 2016) with detrimental effects on submerged macrophyte growth (e.g., Sand-Jensen and Søndergaard 1981; Roberts et al. 2003; Klančnik et al. 2014). It can also increase the sensitivity of submerged macrophytes to herbivory (Hidding et al. 2016). Consequently, Périllon and Hilt (2016) hypothesized that both, macrophytes and periphyton, are only promoted by nutrients from LGD up to a certain threshold above which periphyton shading limits growth of submerged macrophytes.

To test this hypothesis, we conducted laboratory experiments measuring the growth of periphyton on artificial substrata and of submerged macrophytes rooted in sediment containing different levels of phosphorus (P) concentrations crossed with and without simulated LGD. We specifically expected that

1) periphyton is promoted by LGD through a mobilization of sediment-bound P, while macrophytes are promoted by increasing P concentrations in sediments, independent of LGD (Fig 1), and

2) periphyton shading impedes macrophyte growth in treatments with LGD above a threshold level of P concentrations in the sediment (Fig 1).

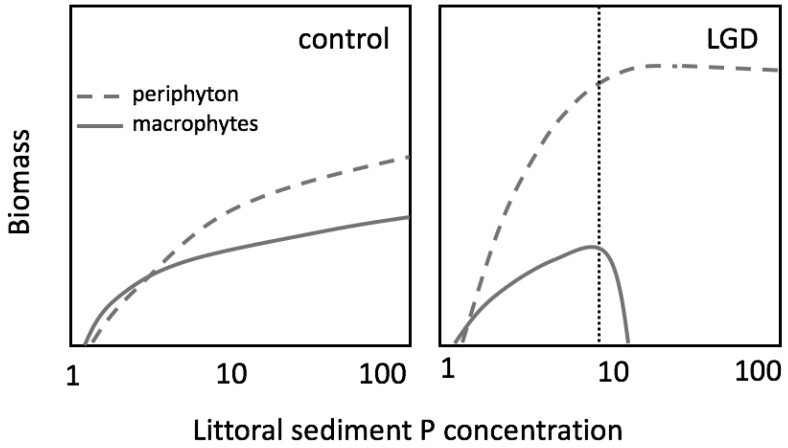

Fig 1: Conceptual model on the response of rooted submerged macrophytes and periphyton biomass to increasing littoral sediment phosphorus (P) concentrations without (control) and with lacustrine groundwater discharge (LGD)

Material and methods

Experimental setup

We tested the effect of LGD on macrophyte-periphyton interactions in two four-week laboratory experiments each consisting of a control (Co) and a flow-through (FT) treatment simulating LGD. Initially, plastic strips with pre-grown periphyton and macrophytes (*Potamogeton pectinatus*) growing in artificial sediments were added to aquaria containing nutrient solution. Experiment (exp.) 1 was performed using tubers (starch-rich overwintering organs of *P. pectinatus*) and periphyton of early spring, while young rooted macrophytes and periphyton of early summer were used in exp. 2 to simulate different field situations (Fig 2, Table 1).

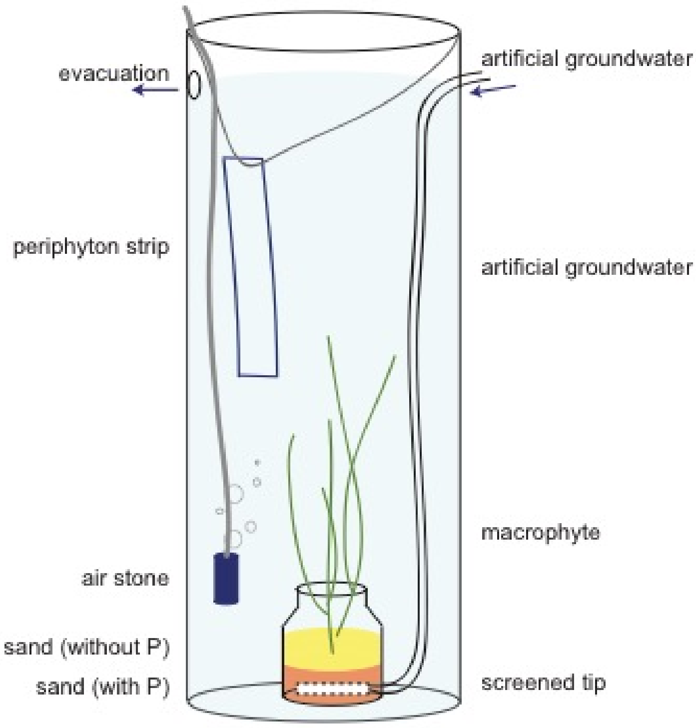

Fig 2: Experimental system for testing the effect of circulation of artificial groundwater on the growth of macrophytes (*Potamogeton pectinatus*) and periphyton grown on artifical strips. Systems without circulation were built without screened tip, artificial groundwater tube and evacuation hole.

Table 1: Comparison of experimental setup and water parameters at the end of both experiments. Statistical tests for significant differences were performed using Mann-Whitney *U*-tests (u), Welch's *t*-test (w) or Student's *t*-test (t) and results are indicated as "ns" ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.001$).

Experiment	Exp. 1			Exp. 2			Difference between exp. 1&2
Treatment	Co	FT	Sign.	Co	FT	Sign.	
Origin of periphyton/ date of harvest (after 2 weeks exposure)	Lake Stechlin, 12. March 2015			Lake Stechlin, 9. June 2015			
Macrophyte material	Tubers			Young plants			
Origin of macrophytes, date of harvest	Lake Müggelsee, 11. March 2015			Lake Stechlin, 9. June 2015			
Periphyton/macrophyte growing time	26/29 days			30/32 days			
pH mean ± sd (n)	8.3 ± 0.05 (16)	8.2 ± 0.08 (16)	*, u	8.46 ± 0.05 (5)	8.43 ± 0.05 (4)	ns, t	**, u
O_2 (mg L^{-1})	9.01 ± 0.03 (16)	9.03 ± 0.06 (16)	ns, u	8.46 ± 0.05 (5)	8.50 ± 0.08 (4)	ns, t	**, u
T (°C)	22.2 ± 0.1 (16)	22.2 ± 0.2 (16)	ns, u	24.2 ± 0.2 (5)	24.1 ± 0.3 (5)	ns, t	**, u
NO_3^- (mg L^{-1})	6.9 ± 0.2 (4)	7.0 ± 0.3 (4)	ns, t	6.44 ± 1.06 (4)	6.43 ± 0.25 (4)	ns, t	ns, w
DSi (mg L^{-1})	8.2 ± 0.8 (4)	6.3 ± 3.1 (4)	ns, t	11.4 ± 0.6 (4)	9.3 ± 2.1 (4)	ns, t	*, w
DIC	n.d.	n.d.	n.d.	22.3 ± 0.5 (4)	21.2 ± 0.1 (4)	ns, t	n.d.

P concentrations of sediments were adjusted to four different levels: 0, 0.008, 0.08 and 0.8 mg P g^{-1} sand, with four replicates each (Fig. 3). For each experiment, 32 glass bottles were filled with 2 L of nutrient solution without P (Nicklisch 1999). A small glass bottle (50 mL) was put at the bottom of each aquarium (Fig 2), containing 20 mL of pure sea sand (Roth, Germany) mixed with tricalcium phosphate (Roth, Germany, infrared spectrometry revealed the existence of hydroxyl- and carbonate groups, the product being mostly composed of hydroxyl-apatite). 20 mL pure sea sand was put on top of this layer to prevent diffusion of P into the overlying water (Wüstenberg et al. 2011).

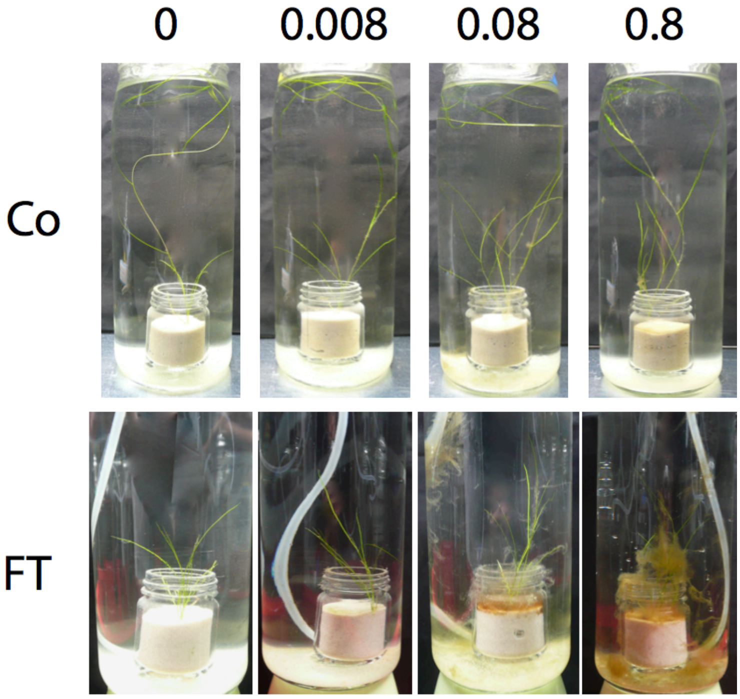

Fig 3: *Potamogeton pectinatus* plants and periphyton development in treatments without (Co) and with (FT) simulated lacustrine groundwater discharge in the exp. 1, after three weeks of exposure. Air stones are removed for the picture. Numbers in the upper row represent concentrations of P in the sediment at the installation (mg P g^{-1} sand).

Air was bubbled into the aquaria through an air stone providing oxygen and carbon dioxide, and preventing stratification of the water (Fig 2). Fluorescent lamps (Biolux ® L 18W/965 from OSRAM) provided light from the top in a 12:12h period, with an intensity ranging from 100 to 180 µmol photon m^{-2} s^{-1} at the top of the aquaria, 60 to 100 µmol photon m^{-2} s^{-1} at the bottom. The room temperature was fixed at 20°C, resulting in a water temperature between 20 and 25°C (at the end of the light period) during the experiments (Table 1). To prevent gradients in light and temperature between treatments and replicates, the location of the aquaria was changed each week.

In flow-through (FT) treatments, nutrient solution was pumped with a peristaltic pump at a rate of 8 mL h^{-1} into the bottom layer of the sand through a screened tip (Fig 2). In control (Co) treatments, nutrient solution was only added weekly to compensate for evaporation losses.

Periphyton

Pre-grown periphyton originated from Lake Stechlin, an oligo-mesotrophic lake in North-eastern Germany (Périllon et al. 2017). Plastic strips (GBC, polypropylene, 2 x 15cm) were exposed in the littoral in late winter (exp. 1) and late spring (exp. 2) for two weeks (Table 1). Strips were transported separately in closed tubes and hung vertically into the aquaria (one each) (Fig 2).

Periphyton dry weight (dw), total P concentration, carbon (C) and nitrogen (N) concentration and pigment content were analyzed as described in Köhler et al. (2010) at the start (4 strips) and the end of each experiment after scraping it from the strips with a toothbrush and rinsing with nutrient solution. Shading (light attenuation (A), as % of PAR, photosynthetic active radiation) was calculated from periphyton dw using the hyperbolic curve fit found by Köhler et al. (2010): $A = 108 \times dw / (9.2+dw)$. Chlorophyll-*a* (chl-*a*) content of the dw was used as proxy for total algae content, while the share of chlorophyll-*b* (chl-*b*), fucoxanthin (fuco) and myxoxanthophyll (myxo) in total chl-*a* was used to indicate the share of green algae, diatoms and cyanobacteria, respectively.

Macrophytes

The tested macrophyte species, sago pondweed (*Potamogeton pectinatus* L.*,* also known as *Stuckenia pectinata*) has a nearly worldwide distribution (Kantrud 1990) and is one of the most common macrophyte species in temperate lakes (Hilt and Gross 2008). It forms tubers (starch-rich overwintering organs), which were used in exp. 1 (Table 1). After size and wet weight measurement, one tuber (12-23 mm) was buried between the two different sand layers (Fig 2) in each bottle. For exp. 2, young plants containing 2 to 3 leaves of max. 4.2 cm length, roots and no tubers, were harvested from the oligo-mesotrophic Lake Stechlin in June 2015 (Table 1). On the same day, they were photographed on a flat surface to determine their size and planted, one per bottle. The size and dw (60 °C to weight constancy) of an additional 12 tubers and 15 plants were determined to arrive at a size-dw-ratio needed to calculate the initial dw_{start} of the macrophytes in the bottles.

During the experiment, leave number and plant height were registered every second day (Fig S1). At the end of each experiment, macrophytes were sampled and scanned (exp. 1) or photographed (exp. 2). Their leave surface, length of leaves and roots, maximum leave and root length, and internode length were measured using the software ImageJ (Schneider et al. 2012). Afterwards, they were dried at 60°C until weight constancy, and their final dw_{end} was measured. The dw_{start} was calculated from tuber weight (exp. 1) or from correlations between surface area and dw at the start (exp. 2). Relative growth rate (RGR) of macrophytes was calculated as

$$RGR=(\ln(dw_{end})-\ln(dw_{start}))/(t_{end}-t_{start}) \qquad \text{(equ. 1)}$$

Water and sediment analysis

At the end of the experiment, oxygen concentration, pH and temperature were measured in each aquarium, both next to water surface and next to sediment surface (exp. 1), or in one aquarium per treatment (exp. 2).

After periphyton sampling, 60 mL of water were sampled in the center of each aquarium, and analysed for soluble reactive phosphorus (SRP, all replicates, molybdenum-blue method), dissolved silica (DSi, one per treatment, continuous flow analysis photometry, Fig S2), total phosphorus (TP, one per treatment, molybdenum-blue method), dissolved inorganic carbon (DIC, one per treatment, nondispersive infrared after combustion), nitrate, nitrite and ammonium (NO_3^-, NO_2^- and NH_4^+, one per treatment, continuous flow analysis photometry). In exp. 1, SRP was also measured each week in one replicate per treatment, and in each flow-through aquarium with highest sediment P concentration (Fig S3). In exp. 2, additional SRP measurements were only done in two aquaria per treatment at the end of the first week.

At the end of exp. 1, both layers of sediment were sampled separately, after removing the top centimeter of sediment. The sediment was air-dried, and TP was analyzed after digestion by sulfate and hydrogen peroxide, using the molybdenum-blue spectrometric method. Six sand samples of exp. 2 were analyzed for TP to verify the similarity between both experiments.

Statistical analyses

Water temperature, pH, oxygen and water chemical parameters (apart from P) were compared between treatments (Co and FT) using the non-parametric Mann-Whitney *U*-test or Welch's *t*-test for normally distributed data without equal variance (Table 1), or Student *t*-test. Paired tests were used to compare beginning and end growth parameters of macrophytes in exp. 2.

Two-way analysis of variance (ANOVA) with groundwater influence (df = 1) and sediment P concentration (df = 3) as fixed factors was applied to compare water P concentrations and several parameters of periphyton and macrophytes between treatments. The influence of sediment P concentrations on periphyton and macrophyte growth was tested separately for Co and FT treatments using Tukey's honest significant difference (HSD) test.

Statistical analyses were performed using the software R (R Development Core Team 2014).

Results

Periphyton

In both experiments, periphyton had a higher dw at the end than at the beginning of the experiment (0.29 ± 0.11 g m^{-2} at the beginning of exp. 1 and 1.71 ± 0.38 g m^{-2} in exp. 2), and was significantly higher in FT treatments than in controls (Figs 3-5, Table 2). Periphyton dw significantly increased with increasing sediment P concentration in FT treatments, while that was not the case in controls (Figs 4, 5, Table 2). Overall, periphyton biomass was significantly higher in exp. 2 than in exp. 1 (Mann-Whitney *U*-test, $p < 0.001$), on average about 10 times (Fig 4, 5, Table 2).

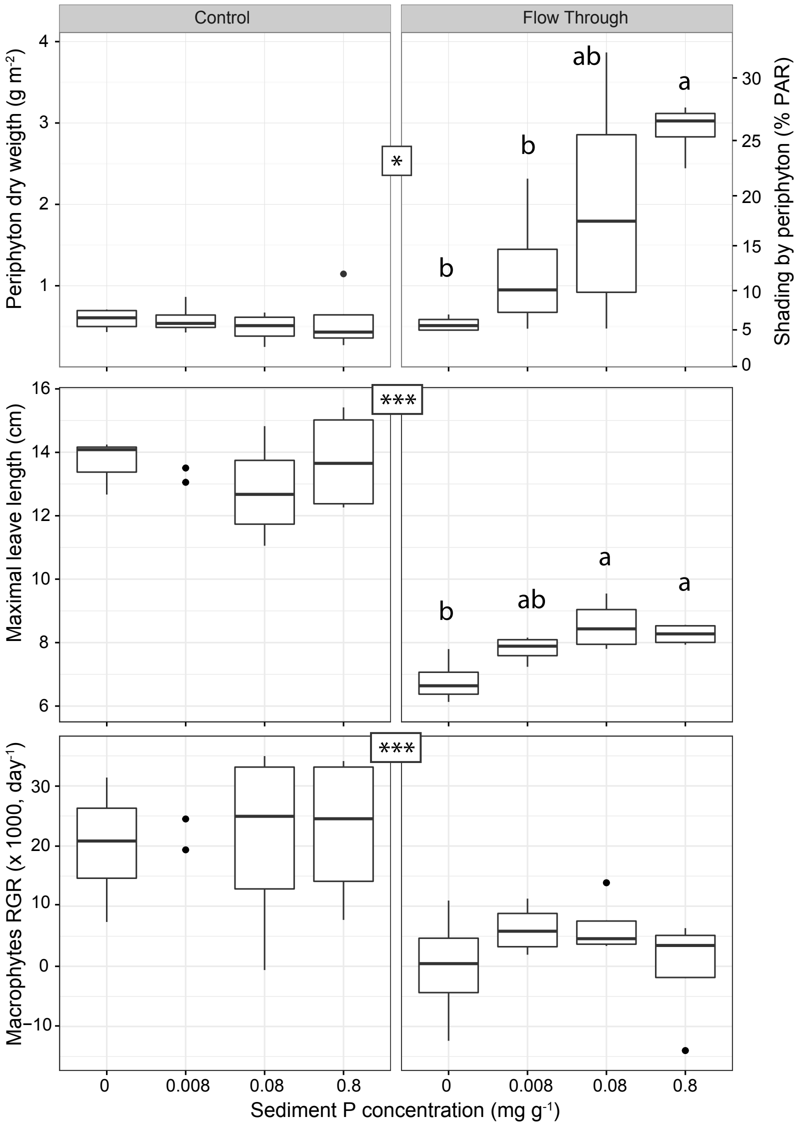

Fig 4: Periphyton biomass, shading (calculated after Köhler et al. (2010)), macrophyte leave length and relative growth rate (RGR) in exp. 1. Box-and-whisker plots represent median, first and third quartiles as limits of the box, and the highest and lowest data as whiskers of the plot, as long as they are not identified as outliers. Results are represented as single points when more than one replicate was lost. Significant differences between control and groundwater treatments are indicated with asterisks (* = $p < 0.05$, *** = $p < 0.001$). Letters indicate significant differences between sediment P concentration treatments in the flow-through treatment simulating LGD (Tukey's HSD test).

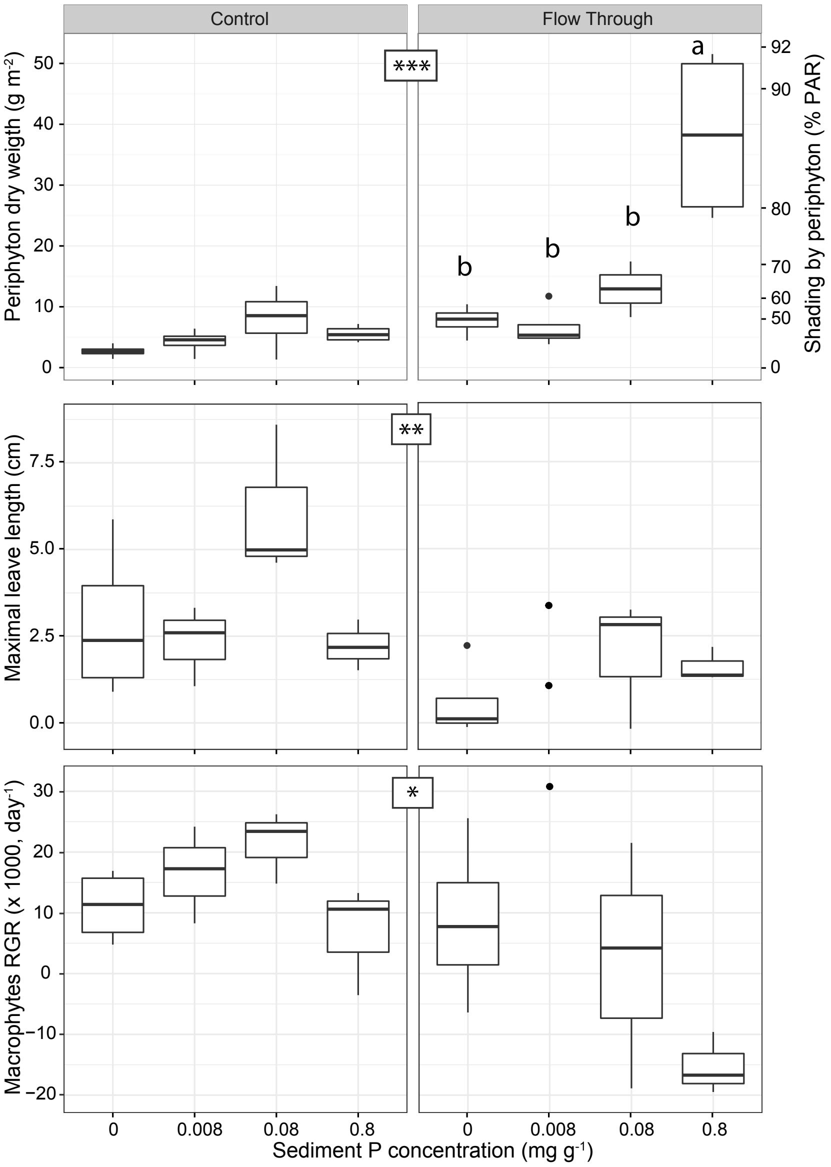

Fig 5: Periphyton biomass, shading (calculated after Köhler et al. (2010)), macrophyte leave length and relative growth rate (RGR) in experiment 2. Box-and-whisker plots represent median, first and third quartiles as limits of the box, and the highest and lowest data as whiskers of the plot, as long as they are not identified as outliers. Results are represented as single points when more than one replicate was lost. Significant differences between control and groundwater treatments are indicated with asterisks (* = $p < 0.05$, *** = $p < 0.001$). Letters indicate significant differences between sediment-P concentration treatments in the flow-through treatment simulating LGD (Tukey's HSD test).

In exp. 1, periphyton accumulated 0.3 to 9.2 mg P m^{-2}, which represented 0.07 % to 1.3 % of its dry weight (Table 2, Table S1). In exp. 2, periphyton accumulated 0.8 to 19.3 mg P m^{-2}, which represented 0.02 % to 0.4 % of its dry weight (Table 2). In both experiments, P accumulation in periphyton was higher in FT treatments than in controls and significantly increased with increasing sediment P concentration (Table 2). On the contrary, there was no influence of LGD on the P content in the periphyton (% dw). C:P ratios were above values indicating P limitation (369, Kahlert 1998), apart from exp. 1, with FT and highest sediment P concentrations. In both experiments, C:P ratio of the introduced periphyton was under C:P=369. Periphyton C:N ratios were around or above 11 in exp. 1 and 2, respectively.

In exp. 1, pigment analyses revealed the presence of chl-*a* and fuco in all samples, and chl-*b* was present in few samples. The pigments chl-*a*, chl-*b*, mixo and fuco were detected in most samples of exp. 2. Chl-*a* concentrations and the share of chl-*a* in the total periphyton biomass were higher in FT treatments than in controls (Table 2, Table S1). In Co, these parameters did not depend on sediment P concentrations, whereas in FT treatments, pigment concentrations increased with sediment P concentrations. The share of chl-*b* in total periphyton chl-*a* in exp. 1, and myxo in chl-*a* in exp. 2 decreased with increasing sediment P concentrations in FT treatments. In both experiments, the share of fuco in chl-*a* increased with increasing sediment P concentration in FT treatments (Table 2).

Table 2: Results of two-way ANOVA applied on periphyton growth parameters (total phosphorus (TP), chlorophyll-*a* (chl-*a*), chlorophyll-*b* (chl-*b*), fucoxanthin (fuco), myxoxanthophyll (myxo), carbon (C) to phosphorus (P) ratio) of exp. 1 (spring) and exp. 2 (summer). Results of two-way ANOVA are given as *p* probability, for flow (FT: Flow-through or Co: control), sediment P concentration (in mg P/g sediment) or for the interaction (Flow x P conc). Letters indicate results of Tukeys HSD posthoc tests.

Exp.	Parameter	Trans-formation	N	Flow x P conc	Flow	Ranking flow	Circulation P concentration	Co 0	Co 0.008	Co 0.08	Co 0.8	FT 0	FT 0.008	FT 0.08	FT 0.8
1	dw [g m^{-2}]	log(x+0.01)	31	$p < 0.001$	$p < 0.001$	Co < FT	$p = 0.02$					c	bc	ab	a
	TP [mg m^{-2}]	none	31	$p < 0.001$	$p < 0.001$	Co < FT	$p < 0.001$					b	b	b	a
	TP [% dw]	none	31	$p < 0.001$	$p > 0.1$		$p = 0.04$	a	ab	a	b	ab	b	b	a
	C:P ratio (molar)	none	31	$p < 0.001$	$p > 0.1$		$p = 0.02$	b	ab	b	a	ab	a	bc	c
	chl-*a* [mg m^{-2}]	log(x+0.01)	29	$p = 0.005$	$p < 0.001$	Co < FT	$p = 0.008$					b	b	ab	a
	chl-*a* [%dw]	log(x+0.01)	31	$p = 0.02$	$p = 0.08$		$p = 0.004$					b	b	ab	a
	fuco [%chl-*a*]	none	30	$p = 0.006$	$p = 0.02$	Co < FT	$p = 0.03$	a	b	ab	ab	b	ab	ab	a
2	dw [g m^{-2}]	log(x+0.01)	30	$p = 0.004$	$p < 0.001$	Co < FT	$p < 0.001$					b	b	b	a
	TP [mg m^{-2}]	none	32	$p < 0.001$	$p < 0.001$	Co < FT	$p < 0.001$					b	b	b	a
	TP [% dw]	none	31	$p > 0.1$	$p > 0.1$		$p = 0.05$								
	C:P ratio	none	32	$p > 0.1$	$p > 0.1$		$p = 0.03$								
	chl-*a* [mg m^{-2}]	log(x+0.01)	32	$p > 0.1$	$p < 0.001$	Co < FT	$p < 0.001$					b	b	b	a
	chl-*a* [%dw]	none	31	$p = 0.01$	$p < 0.001$	Co < FT	$p < 0.001$	ab	ab	b	a	b	b	b	a
	chl-*b* [%chl-*a*]	none	31	$p > 0.1$	$p > 0.1$		$p = 0.04$					ab	ab	a	b
	fuco [%chl-*a*]	none	31	$p = 0.06$	$p = 0.07$		$p < 0.001$	ab	b	b	a	b	b	b	a
	myxo [%chl-*a*]	none	32	$p = 0.008$	$p = 0.005$	Co < FT	$p < 0.001$					a	a	a	b

Macrophytes

At the start of both experiments, tuber weight (exp. 1) and plant leave number and length, root number and length and dw (exp. 2) were not significantly different between treatments (two-way ANOVA, $p > 0.1$).

In exp. 1, final macrophyte leaves length, internode length and root numbers were significantly higher in Co than in FT treatments (Fig 4, Table 3). On the contrary, roots were longer in FT than in Co treatments (Table 3, Table S2). Internode length was lowest in the treatment without P and highest in the second highest concentration, in FT and Co treatments (Table 3). Leaves were significantly longer in the FT treatments with the two highest P concentrations. Macrophyte RGR were significantly higher in Co than in FT treatments (Fig 4), but did not significantly differ between levels of sediment P concentrations (Table 3). This difference was observed especially between the 10th and 20th day after planting, and concerns both leaves length and leaves number (Fig S1).

In exp. 2, macrophyte leaves and internodes grew during the experimental period (both paired Mann-Whitney *U*-test between begin and end, $p < 0.001$), but no significant change was measured in roots and rhizome length (both Student's *t*-test, $p > 0.05$). Macrophytes grew far less than in exp. 1 (Fig 5, Table S2), and especially the ratio between internode and leave growth was lower in exp. 2 than in exp. 1 (Mann-Whitney *U*-test, $p < 0.001$). No difference between Co and FT treatments were observed in plant height and visible leaves number, through the experimental period (Fig S3), but higher leaves length and RGR were measured in macrophytes growing in Co than in FT treatments (Table 3, Table S3). Sediment P concentrations had no significant influence on any of the macrophyte growth parameters (Table 3).

Table 3: Results of two-way ANOVA applied to different macrophyte parameters of the exp. 1 (in spring) and exp. 2 (in summer). The parameters are related to leaves, internodes, above-sediment parts (“L+I”), roots and whole plant. Two-way ANOVA results are given as *p* probability, for flow (FT: Flow-through or Co: control), sediment P concentration (in mg P/g sediment) or for the interaction (Flow x P conc). Letters indicate results of Tukey’s HSD posthoc tests.

Exp.	Parameter	Unit	Trans-formation	N	Flow x P conc	Flow	Ranking flow	Circulation P concentration	Co 0	Co 0.008	Co 0.08	Co 0.8	FT 0	FT 0.008	FT 0.08	FT 0.8
1	Leaves	Number	none	31	$p > 0.1$	$p > 0.1$		$p > 0.1$								
	Leaves	Maximal length [cm]	none	29	$p > 0.1$	$p < 0.001$	Co > FT	$p = 0.05$					b	ab	a	a
	Internodes	Added length [cm]	none	27	$p < 0.001$	$p < 0.001$	Co > FT	$p < 0.001$	b	b	a	b	b	ab	a	ab
	L+I	Surface [mm^2]	log	28	$p > 0.1$	$p < 0.001$	Co > FT	$p > 0.1$								
	Roots	Number	none	29	$p > 0.1$	$p < 0.001$	Co > FT	$p > 0.1$								
	Roots	Maximal length [cm]	log	31	$p > 0.1$	$p = 0.003$	Co < FT	$p > 0.1$								
	Plant	RGR [day^{-1}]	none	31	$p > 0.1$	$p < 0.001$	Co > FT	$p > 0.1$								
2	Leaves	Number	none	24	$p > 0.1$	$p > 0.1$		$p > 0.1$								
	Leaves	Maximal length [cm]	none	25	$p > 0.1$	$p = 0.008$	Co > FT	$p = 0.07$								
	Internodes	Added length [cm]	log	24	$p > 0.1$	$p > 0.1$		$p > 0.1$								
	L+I	Surface [mm^2]	log	26	$p > 0.1$	$p > 0.1$		$p > 0.1$								
	Plant	RGR [day^{-1}]	none	25	$p < 0.05$	$p < 0.05$	Co > FT	$p = 0.09$								

Macrophyte-periphyton interaction

Macrophyte RGR and periphyton shading were only negatively correlated in exp. 2, and here only in the FT treatments (Fig 6).

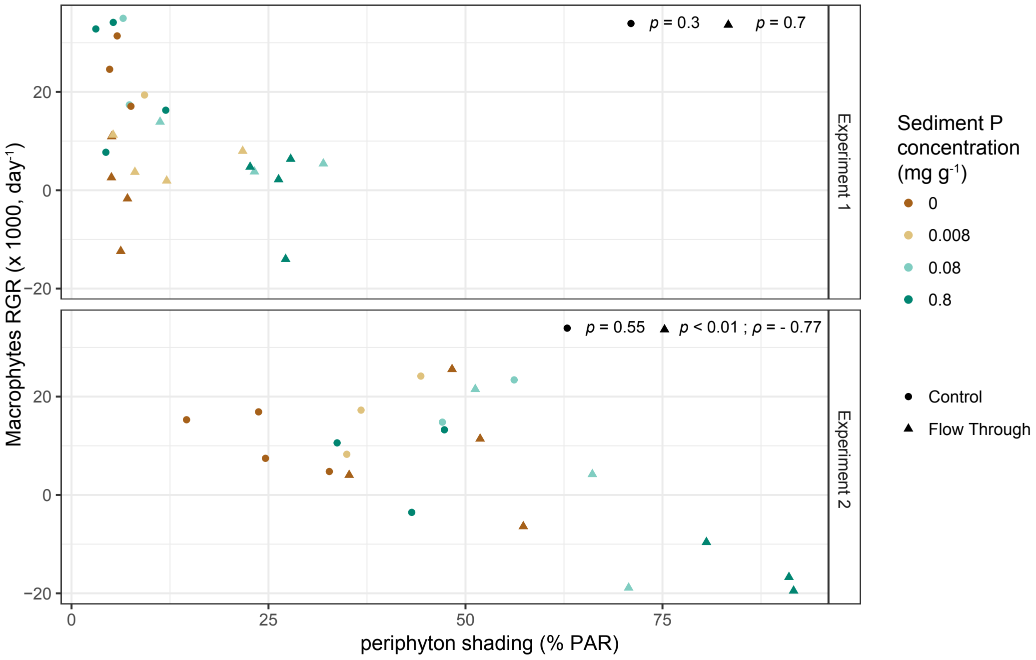

Fig 6: Correlations between periphyton shading (% of incoming light) and macrophyte relative growth rate (RGR) in exp. 1 (*Potamogeton pectinatus* grown from tubers, early spring periphyton) and exp. 2 (*P. pectinatus* young plants, early summer periphyton) for control and flow-through samples with different sediment phosphorus (P) concentrations. The result of Pearson correlation are presented separately for each experiment and LGD treatment.

Water quality

Most water parameters were not different between both experiments and between Co and FT treatments (Table 1). Ammonium, nitrite, SRP and TP concentrations were below detection limits. Contrary to trials without macrophytes and periphyton, SRP concentrations measured in the water column remained below or close to detection limit throughout both experiments, especially in the Co treatments and in exp. 2 (Fig S3).

Final sediment P concentrations remained close to the initial conditions (0.006 ± 0.001 mg g^{-1}, 0.01 ± 0.005 mg g^{-1}, 0.08 ± 0.02mg g^{-1}, 0.75 ± 0.08 mg g^{-1}) without differences between treatments (Student's *t*-tests, $p > 0.05$).

Discussion

Our results clearly show that P transported from sediments into the water column by flow-through conditions simulating LGD strongly promoted the development of periphyton biomass. No clear threshold of sediment P concentrations could be detected above which the negative effects of periphyton on macrophytes prevail. However, our hypothesis was still confirmed as macrophyte growth rates and periphyton shading were only negatively correlated in treatments with LGD. LGD can thus significantly affect the competition between benthic primary producers at the expense of macrophytes, at least under P-deficient conditions in the open water when LGD increases availability of sediment P for periphyton.

Our experiment confirmed an advective transport of P from sediments to the water column resulting in higher periphyton growth. Similar results have been reported from a field study showing higher periphyton biomass at sites with LGD as compared to control sites in an oligo-mesotrophic lake (Périllon et al. 2017). Pre-experiment trials without macrophytes and periphyton showed that P concentrations in the water column of FT treatments increased with rising sediment P concentration. In contrast, P concentrations in control treatments remained below detection limits due to the low solubility of hydroxyapatite (0.02 g / 100 g) and the additional layer of pure sand, preventing P diffusion into the water column.

Periphyton biomass responded positively to additional P availability in the water column in both experiments. Both total periphyton biomass as well as its chl-*a* content were promoted by sediment P in FT treatments. Due to the exclusion of interfering processes such as grazing and sediment heterogeneity, the effect of advective P transport by LGD was more evident in our laboratory experiments that in field studies (Hagerthey and Kerfoot 1998; Périllon et al. 2017).

The periphyton community composition with a dominance of diatoms in exp. 1, and a coexistence of green algae and diatoms in exp. 2, resembled common periphyton community developments in temperate lakes in spring and summer, respectively (Liboriussen 2003). Additional P availability promoted both communities.

LGD affects macrophyte growth from tubers

Our study revealed an unexpected influence of FT conditions on *P. pectinatus* when grown from tubers as in exp. 1. Here, macrophytes grown in controls showed a strong shoot elongation while those in FT treatments were shorter and had less numerous but longer roots. Longer roots have already been observed for macrophytes growing at LGD locations as compared to others without the influence of LGD in a field study by Ommen et al. (2012). The longer shoots in control treatments of exp. 1, which were independent of sediment P concentrations, might have been the result of lacking physical disturbance by LGD. However, field studies reported the opposite: above-ground parts of *P. pectinatus* grown from tubers were higher at sites exposed to waves (Kautsky 1987; Idestam-Almquist and Kautsky 1995). In our experiment, however, air bubbling caused high turbulence, which was present in both treatments and supposed to exceed physical effects of groundwater flow. More likely, FT conditions may have influenced oxygen availability in the sediment. Strong internode elongation has been observed in *P. pectinatus* plants grown from tubers under anaerobic conditions (Summers and Jackson

1994; Dixon et al. 2006). While in our experiments, the water column was always oxic (Table 1), we assume that sediments in controls were more anoxic than those with a through-flow of nutrient solution. This might have caused stronger shoot elongation in controls of exp. 1, when plants growing from tubers were exposed to anoxic sediment. Groundwater entering lakes is usually relatively poor in oxygen and might thus not have a comparable effect.

Effect of sediment P on macrophytes partially confirmed

We expected a positive effect of increasing sediment P concentrations on macrophyte growth in the control treatments and in FT treatments with lower periphyton shading. Other studies had shown a positive influence of sediment nutrients on the growth of above-ground parts of e.g. *M. spicatum* and *P. crispus* (Cao et al. 2012; Xie et al. 2013a, b). However, we could only confirm such effects for maximum leaf lengths in FT treatments of exp. 1. In controls, the assumed effect of sediment anoxia on shoot elongation (see previous paragraph) might have exceeded any sediment P effects and P reserves in tubers (Hodgson 1966) might have been sufficient to support this elongation. In exp. 2, periphyton biomass and thus shading was generally higher in both control and FT treatments than in exp. 1 and young plants without access to energy stored in tubers may have suffered more from this shading than plants grown from tubers (Fig 6).

Negative net effect of LGD on macrophyte growth due to periphyton shading

Our study revealed a negative correlation between periphyton shading and macrophyte RGR rates when simulated LGD promoted periphyton growth and *P. pectinatus* grew from young plants without tubers. In general, such negative correlations between macrophyte and periphyton biomass are known, and mainly attributed to shading effects of epiphyton on macrophytes (Sand-Jensen 1977; Jones et al. 2002; Grutters et al. 2017). In turn, epiphyton growth might be impaired at higher macrophyte biomass by allelopathic activities, however, *P. pectinatus* is recognized as a species with low allelopathic activity (Hilt and Gross 2008).

The response of macrophytes to periphyton shading differs (Xie et al. 2013b) suggesting that there is no universal correlation between macrophyte and periphyton growth. However, a promotion of epiphyton biomass by LGD is expected to negatively affect macrophytes above certain, probably species-specific, threshold levels in shading. In our experiments, young *P. pectinatus* plants could not cope with shading levels above 75% of incident light (with light conditions similar to sunny summer day in the region), corresponding to periphyton biomass of 21 g dw m^{-2}.

Conclusions

We conclude that LGD differentially affects periphyton and macrophytes at the expense of the latter. LGD generally supports periphyton growth by increased P availability in the water column, and this effect becomes more pronounced with increasing sediment P concentrations. Macrophytes can also gain from increased sediment P concentrations, but only at low periphyton shading. Although a clear threshold in sediment P concentrations could not be detected, young *P. pectinatus* plants were negatively affected at periphyton shading levels above 75% of incident light. LGD may thus be a relevant factor for submerged macrophyte abundance. Present and future global climate change is assumed to potentially affect LGD (Green et al. 2011; Kirillin et al. 2013; Taylor et al. 2013). Our study indicates that such changes can translate into significant changes in composition and productivity of benthic primary producers with potential consequences for whole lake water quality (Scheffer et al. 1993) and productivity (Vadeboncoeur et al. 2003).

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References

Arle, J., K. Blondzik, U. Claussen, A. Duffek, S. Grimm, F. Hilliges, B. Kirschbaum, I. Kirst, D. Koch, J. Koschorreck, P. Lepom, W. Leujak, V. Mohaupt, S. Naumann, U. Pirntke, J. Rechenberg, P. Schilling, A. Ullrich, J. Wellmitz, S. Werner, and R. Wolter. 2017. Gewässer in Deutschland : - Zustand und Bewertung.

Barko, J. W., and R. M. Smart. 1980. Mobilization of sediment phosphorus by submersed freshwater macrophytes. Freshw. Biol. **10**: 229–238.

Cao, J., Y. Wang, and Z. Zhu. 2012. Growth response of the submerged macrophyte Myriophyllum spicatum to sediment nutrient levels and water-level fluctuations. Aquat. Biol. **17**: 295–303.

Dixon, M. H., S. A. Hill, M. B. Jackson, R. G. Ratcliffe, and L. J. Sweetlove. 2006. Physiological and metabolic adaptations of *Potamogeton pectinatus* L. tubers support rapid elongation of stem tissue in the absence of oxygen. Plant Cell Physiol. **47**: 128–140.

Frandsen, M., B. Nilsson, P. Engesgaard, and O. Pedersen. 2012.

Groundwater seepage stimulates the growth of aquatic macrophytes. Freshw. Biol. **57**: 907–921.

Green, T. R., M. Taniguchi, H. Kooi, J. J. Gurdak, D. M. Allen, K. M. Hiscock, H. Treidel, and A. Aureli. 2011. Beneath the surface of global change: Impacts of climate change on groundwater. J. Hydrol. **405**: 532–560.

Grutters, B. M. C., E. M. Gross, E. van Donk, and E. S. Bakker. 2017. Periphyton density is similar on native and non-native plant species. Freshw. Biol. 906–915.

Hagerthey, S. E., and W. C. Kerfoot. 1998. Groundwater flow influences the biomass and nutrient ratios of epibenthic algae in a north temperate seepage lake. Limnol. Oceanogr. **43**: 1127–1242.

Hasler, A. D. 1947. Eutrophication of lakes by domestic drainage. Ecology **28**: 383–395.

Hidding, B., E. S. Bakker, M. J. M. Hootsmans, and S. Hilt. 2016. Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. Oikos **125**: 1489–1495.

Hilt, S., and E. M. Gross. 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? Basic Appl. Ecol. **9**: 422–432.

Hodgson, R. H. 1966. Growth and carbohydrate status of sago pondweed. Weed Sci. Soc. Am. **14**: 263–268.

Hunt, R. J., M. Strand, and J. F. Walker. 2006. Measuring groundwater–surface water interaction and its effect on wetland stream benthic productivity, Trout Lake watershed, northern Wisconsin, USA. J. Hydrol. **320**: 370–384.

Hurley, J. P., D. E. Armstrong, G. J. Kenoyer, and C. J. Bowser. 1985. Ground Water as a silica source for diatom production in a precipitation-dominated lake. Science (80-.). **227**: 1576–1578.

Idestam-Almquist, J., and L. Kautsky. 1995. Plastic responses in morphology of *Potamogeton pectinatus* L. to sediment and above-sediment conditions at two sites in the northern Baltic proper. Aquat. Bot. **52**: 205–216.

Jaynes, M. ., and R. Carpenter. 1986. Effects of vascular and nonvascular macrophytes on sediment redox and solute dynamics. Ecology **67**: 875–882.

Jones, J. I., J. O. Young, J. W. Eaton, and B. Moss. 2002. The influence of nutrient loading, dissolved inorganic carbon and higher trophic levels on the interaction between submerged plants and periphyton. J. Ecol. **90**: 12–24.

Kahlert, M. 1998. C:N:P ratios of freshwater benthic algae. Arch. Hydrobiol. (Suppl) (Advanc. Limnol.) **51**: 105–114.

Kantrud, H. A. 1990. Sago pondweed (Potamogeton pectinatus L.): a literature review. 1–98.

Kautsky, L. 1987. Life-cycles of three populations of *Potamogeton pectinatus* L. at different degrees of wave exposure in the Askö area, northern baltic proper. Aquat. Bot. **27**: 177–186.

Kirillin, G., W. Phillip, C. Engelhardt, and G. Nützmann. 2013. Net groundwater inflow in an enclosed lake: From synoptic variations to climatic projections. Hydrol. Process. **27**: 347–359.

Klančnik, K., D. Gradinjan, and A. Gaberščik. 2014. Epiphyton alters the quantity and quality of radiation captured by leaves in submerged macrophytes. Aquat. Bot. **120**: 229–235.

Köhler, J., J. Hachoł, and S. Hilt. 2010. Regulation of submersed macrophyte biomass in a temperate lowland river: interactions between shading by bank vegetation, epiphyton and water turbidity. Aquat. Bot. **92**: 129–136.

Lewandowski, J., K. Meinikmann, G. Nützmann, and D. O. Rosenberry. 2015. Groundwater - the disregarded component in lake water and nutrient budgets. 2. Effects of groundwater on nutrients. Hydrol. Process. **29**: 2922–2955.

Liboriussen, L. 2003. Production, regulation and ecophysiology of periphyton in shallow freshwater lakes. University of Aarhus.

Lillie, R. A., and J. W. Barko. 1990. Influence of sediment and groundwater on the distribution and biomass of *Myriophyllum spicatum* L. in Deveil's Lake, Wisconsin. J. Freshw. Ecol. **5**: 417–426.

Lodge, D. M., D. P. Krabbenhoft, and R. G. Striegl. 1989. A positive relationship between groundwater velocity and submersed macrophyte biomass in Sparkling lake, Wisconsin. Limnol. Oceanogr. **34**: 235–239.

McBride, M. S., and H. O. Pfannkuch. 1975. The distribution of seepage within lakebeds. J. Res. USGS **3**: 505–512.

Nicklisch, A. 1999. Competition between the cyanobacterium *Limnothrix redekei* and some spring species of diatoms under P-limitation. Int. Rev. Hydrobiol. **84**: 233–241.

Ommen, D. A. O., J. Kidmose, S. Karan, M. R. Flindt, P. Engesgaard, B. Nilsson, and F. Ø. Andersen. 2012. Importance of groundwater and macrophytes for the nutrient balance at oligotrophic Lake Hampen, Denmark. Ecohydrology **5**: 286–296.

Périllon, C., and S. Hilt. 2016. Groundwater influence differentially affects periphyton and macrophyte production in lakes. Hydrobiologia **778**: 91–103.

Périllon, C., F. Pöschke, J. Lewandowski, M. Hupfer, and S. Hilt. 2017. Stimulation of epiphyton growth by lacustrine groundwater discharge to an oligo-mesotrophic. Freshw. Sci. **36**, doi:10.1086/692832.

R Development Core Team, R. 2014. R: a language and environment for statistical computing. R Found. Stat. Comput. **1**: 409 pp.

Roberts, E., J. Kroker, S. Körner, and A. Nicklisch. 2003. The role of periphyton during the re-colonization of a shallow lake with submerged macrophytes. Hydrobiologia **506–509**: 525–530.

Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. Aquat. Bot. **3**: 55–63.

Sand-Jensen, K., and M. Søndergaard. 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Rev. der gesamten Hydrobiol. **66**: 529–552.

Scheffer, M., S. Hosper, M. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative equilibria in shalow lakes. Trends Ecol. Evol. **8**: 275–279.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods **9**: 671–675.

Shaw, R. D., and E. E. Prepas. 1990. Groundwater-lake interactions: II. Nearshore seepage patterns and the contribution of ground water to lakes in central Alberta. J. Hydrol. **119**: 121–136.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems: A Global Problem. Environ. Sci. Pollut. Res. **10**: 126–139.

Summers, J. E., and M. B. Jackson. 1994. Anaerobic conditions strongly promote extension by stems of overwintering tubers of *Potamogeton pectinatus* L . J. Exp. Bot. **45**: 1309–1318.

Taylor, R. G., B. Scanlon, P. Doll, M. Rodell, R. van Beek, Y. Wada, L. Longuevergne, M. Leblanc, J. S. Famiglietti, M. Edmunds, L. Konikow, T. R. Green, J. Chen, M. Taniguchi, M. F. P. Bierkens, A. MacDonald, Y. Fan, R. M. Maxwell, Y. Yechieli, J. J. Gurdak, D. M. Allen, M. Shamsudduha, K. Hiscock, P. J.-F. Yeh, I. Holman, and H. Treidel. 2013. Ground water and climate change. Nat. Clim. Chang. **3**: 322–329.

Tóth, V. R. 2013. The effect of periphyton on the light environment and production of Potamogeton perfoliatus L. in the mesotrophic basin of Lake Balaton. Aquat. Sci. **75**: 523–534.

Tóth, V. R., and S. C. J. Palmer. 2016. Acclimation of *Potamogeton* *perfoliatus* L. to periphyton accumulation-induced spectral changes in irradiance. Hydrobiologia **766**: 293–304.

Vadeboncoeur, Y., E. Jeppesen, M. J. Vander Zanden, H.-H. Schierup, K. Christoffersen, and D. M. Lodge. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnol. Oceanogr. **48**: 1408–1418.

Wüstenberg, A., Y. Pörs, and R. Ehwald. 2011. Culturing of stoneworts and submersed angiosperms with phosphate uptake exclusively from an artificial sediment. Freshw. Biol. **56**: 1531–1539.

Xie, D., D. Yu, W. H. You, and L. G. Wang. 2013a. Morphological and physiological responses to sediment nutrients in the submerged macrophyte *Myriophyllum spicatum*. Wetlands **33**: 1095–1102.

Xie, D., D. Yu, W. You, and L. Wang. 2013b. Algae mediate submerged macrophyte response to nutrient and dissolved inorganic carbon loading : A mesocosm study on different species. Chemosphere **93**: 1301–1308.

Supplementary

Table 1. Average and standard deviation of periphyton growth parameters (supplementary to Table 2 in manuscript), for flow-through (F-T) and control (C) set-ups, and 4 different Sediment-P concentrations (in mg g^{-1}). The parameters are total phosphorus (TP), chlorophyll-*a* (chl-*a*), chlorophyll-*b* (chl-*b*), fucoxanthin (fuco), myxoxanthophyll (myxo), and molar nutrient ratios.

Exp	Circulation	Co	Co	Co	Co	FT	FT	FT	FT
	P concentration	0	0.008	0.08	0.8	0	0.008	0.08	0.8
1	dw [g m^{-2}]	0.58±0.13	0.59±0.19	0.48±0.19	0.57±0.39	0.53±0.09	1.17±0.81	1.98±1.51	2.92±0.33
	TP [mg m^{-2}]	1.22±0.46	0.90±0.21	1.04±0.45	0.63±0.45	0.86±0.13	1.14±0.92	3.05±0.88	7.94±1.40
	TP [% dw]	0.11±0.02	0.16±0.05	0.20±0.03	0.21±0.02	0.09±0.02	0.14±0.07	0.17±0.05	0.28±0.07
	C:P ratio	386±28	515±177	376±61	688±171	490±117	651±118	395±88	203±60
	C:N ratio	11.1±1.1	11.8±0.9	11.9±0.7	11.4±1.1	12.1±0.8	10.6±0.9	10.0±0.9	7.9±0.8
	chl-*a* [mg m^{-2}]	1.41±0.44	0.87±0.35	1.42±1.20	1.10±0.54	1.15±0.65	2.92±3.73	6.33±6.81	22.49±8.60
	chl-*a* [%dw]	0.23±0.11	0.14±0.03	0.28±0.15	0.21±0.04	0.21±0.09	0.18±0.13	0.26±0.11	0.80 ±0.38
	fuco [%chl-*a*]	31.5±6.6	26.4±4.1	31.4±3.6	30.4±0.9	27.5±2.7	32.1±5.4	35.5±3.3	38.0±4.2
2	dw [g m^{-2}]	2.69±1.05	4.24±2.07	7.96±5.12	5.54±1.37	7.70±0.38	6.5±63.52	12.91±3.94	38.15±14.29
	TP [mg m^{-2}]	1.76±0.82	2.30±1.03	2.71±1.85	2.80±0.68	3.04±0.94	3.19±1.44	4.25±1.26	16.8±1.85
	TP [% dw]	0.04±0.02	0.05±0.01	0.06±0.00	0.05±0.01	0.03±0.01	0.04±0.00	0.05±0.02	0.05±0.02
	C:P ratio	1222±324	1305±135	1975±651	1355±138	1795±79	1618±715	2225±423	1402±558
	C:N ratio	13.9±1.2	14.5±0.5	14.3±2.9	14.6±1.0	13.9±0.3	13.9±0.8	14.2±0.7	14.6±0.9
	chl-*a* [mg m^{-2}]	2.82±1.47	3.83±2.05	6.40±5.33	8.01±4.66	10.61±4.68	8.35±6.01	19.49±7.95	91.92±41.37
	chl-*a* [%dw]	0.10±0.02	0.09±0.01	0.08±0.02	0.14±0.05	0.13±0.02	0.12±0.03	0.15±0.04	0.24±0.04
	chl-*b* [%chl-*a*]	3.72±3.29	6.88±3.26	5.94±4.05	4.54±1.27	5.54±1.39	4.17±1.59	6.72±1.26	3.66±1.52
	fuco [%chl-*a*]	19.4±2.8	16.7±2.4	16.8±4.0	21.6±1.3	18.5±1.8	19.1±2.9	14.9±1.2	26.08±2.1
	myxo [%chl-*a*]	0.77±0.58	1.69±0.42	1.60±0.50	1.24±0.35	1.86±0.14	2.03±0.47	2.20±0.18	0.89±0.09

Table 2. Average and standard deviation of macrophytes parameters (supplementary to Table 3 in manuscript), for flow-through (F-T) and control (C) set-ups, and 4 different Sediment-P concentrations (in mg g^{-1}).

Exp		Circulation	Co	Co	Co	Co	FT	FT	FT	FT
		P concentration	0	0.008	0.08	0.8	0	0.008	0.08	0.8
1	Leaves	number	12.00±4.69	9.00±5.29	15.25±4.99	13.00±2.71	11.75±2.22	12.25±1.26	13.00±1.83	11.75±3.77
	Leaves	Maximal length [cm]	12.3±2.6	11.8±2.6	12.8±1.6	13.7±1.6	6.8±0.7	7.8±0.4	8.6±0.8	8.3±0.3
	Internodes	Added length [cm]	27.6±16.2	21.2±16.0	36.0±19.1	32.0±4.1	6.4±11.7	11.7±1.5	14.3±3.0	12.6±4.1
	L+I	Surface [mm^2]	7.82±4.61	5.83±4.28	10.37±4.84	9.09±1.77	2.62±0.90	3.56±1.14	4.06±0.95	3.83±1.38
	Roots	Number	8.50±3.32	6.00±3.61	6.50±1.00	7.50±3.32	4.00±2.58	3.50±0.58	4.00±0.82	3.25±0.96
	Roots	Maximal length [cm]	4.43±1.29	4.20±1.72	5.34±1.99	5.86±0.28	6.21±2.39	7.38±3.00	8.35±2.23	7.86±2.44
	Plant	RGR [x1000, day^{-1}]	20.1±10.3	21.9±3.6	28.3±9.5	22.7±12.9	6.8±5.9	6.2±4.2	6.6±4.9	4.4±2.1
2	Leaves	Number	2.0±1.15	4.67±2.08	2.75±1.71	2.00±0	2.50±1.73	5.00±5.66	3.50±0.71	2.50±0.71
	Leaves	Maximal length [cm]	2.88±2.25	2.33±1.15	6.07±2.20	2.23±0.73	0.82±1.22	2.22±1.63	3.04±0.30	1.62±0.49
	Internodes	Added length [cm]	0.82±0.51	2.14±1.33	1.81±1.78	0.85±0.63	0.32±0.23	2.96±4.09	1.25±0.07	0.39±0.40
	L+I	Surface [mm^2]	0.53±0.36	1.24±0.10	1.46±1.45	0.40±0.10	0.45±0.22	1.14±1.23	0.69±0.41	0.56±0.39
	Plant	RGR [x1000, day^{-1}]	11.1±5.9	16.6±8.0	6.2±30.9	6.8±9.0	8.7±13.4	12.9±25.3	2.3±20.3	-15.3±5.1

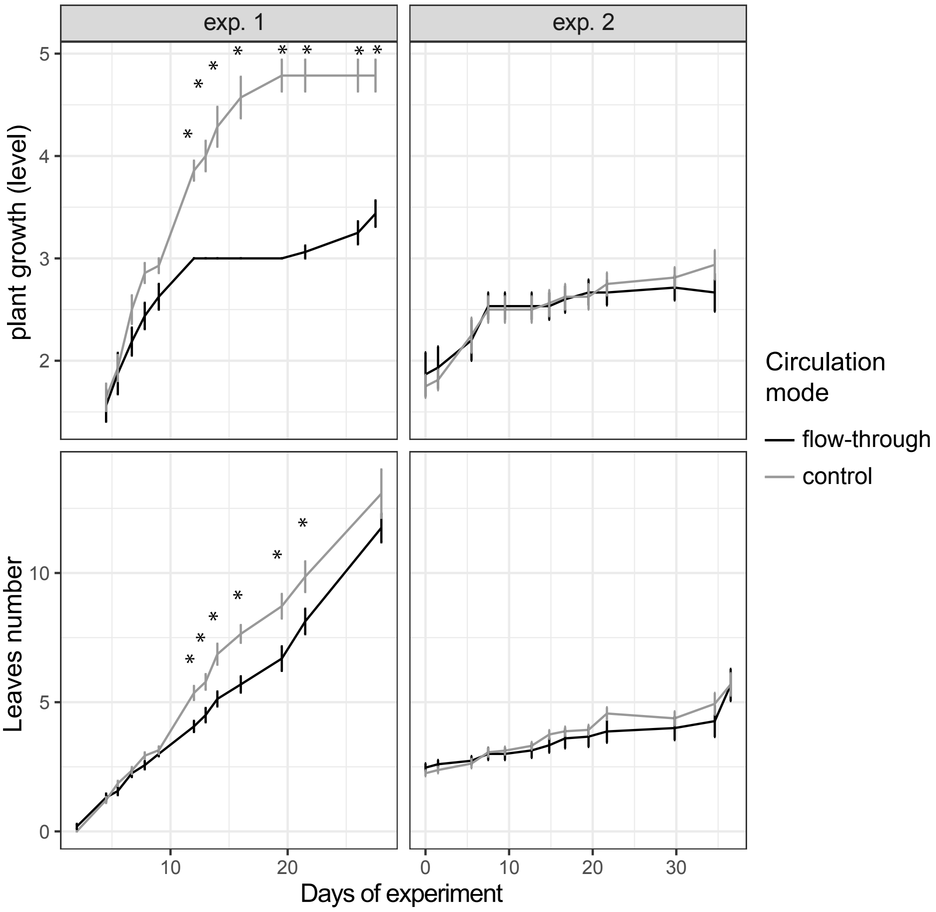


Figure 1. Macrophytes height (top) and leaves number (bottom) during the experimentation time, as mean and standard errors of the visual observation. A star is added when the difference between Co and FT groups were significant ($p < 0.05$, Mann-Whitney U tests and Student's t-tests). The plant height were measured in relation to objects present in the bottles. More precise measurements were not possible because of optical distortions due to water and glass.

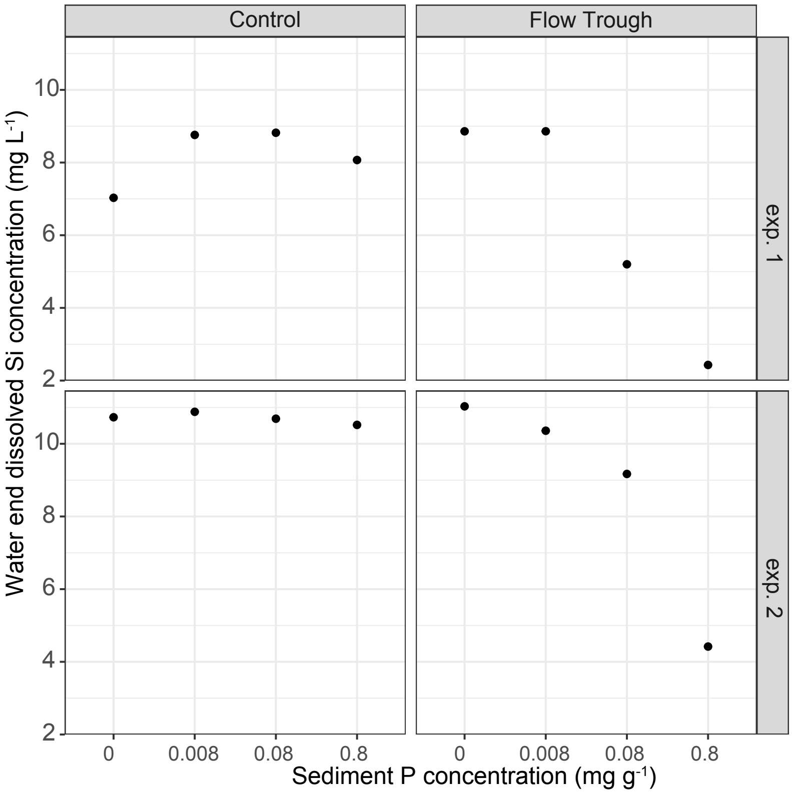


Figure 2. Silica concentrations in water column measured at the end of the experiment

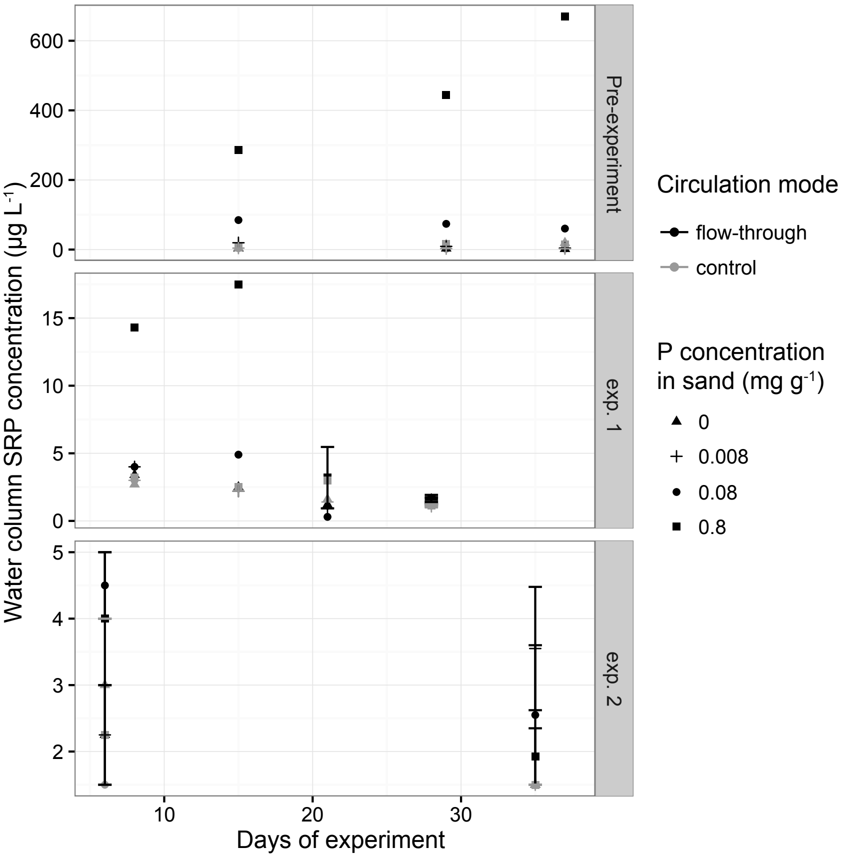


Figure 3. SRP measured the water column in both experiment and a previous experiment (without replicates) without macrophyte and periphyton. Means and single data are presented as points, standard errors are present if several replicates have been sampled and measured. SRP concentration (in µg/L) are represented with different scales for different experiments.

Discussion

Synthesis of study results

Detection of LGD and sediment nutrient mobilization

Studying the effect of LGD on benthic primary producers required the monitoring of ground- and porewater. The aims were to confirm the existence and the direction of groundwater fluxes in each samples site and to identify the origin of nutrients that were made available to benthic primary producers via LGD. Multiple methods were applied in Lake Stechlin to overcome the complexity of groundwater-surface water interactions (see introduction).

In chapter 2, the results of littoral zone piezometer samplings, 6-chamber peepers ("6K-peepers"), and seepage measurements are presented. In addition, 1-chamber (>60 mL) peepers ("1K-peepers") were installed within the experimental chambers used in the field experiment (chapter 2, Fig. 5). The results of these measurements [supplement S1] confirmed the conclusions which were derived from other independent methods, and will be included in the discussion.

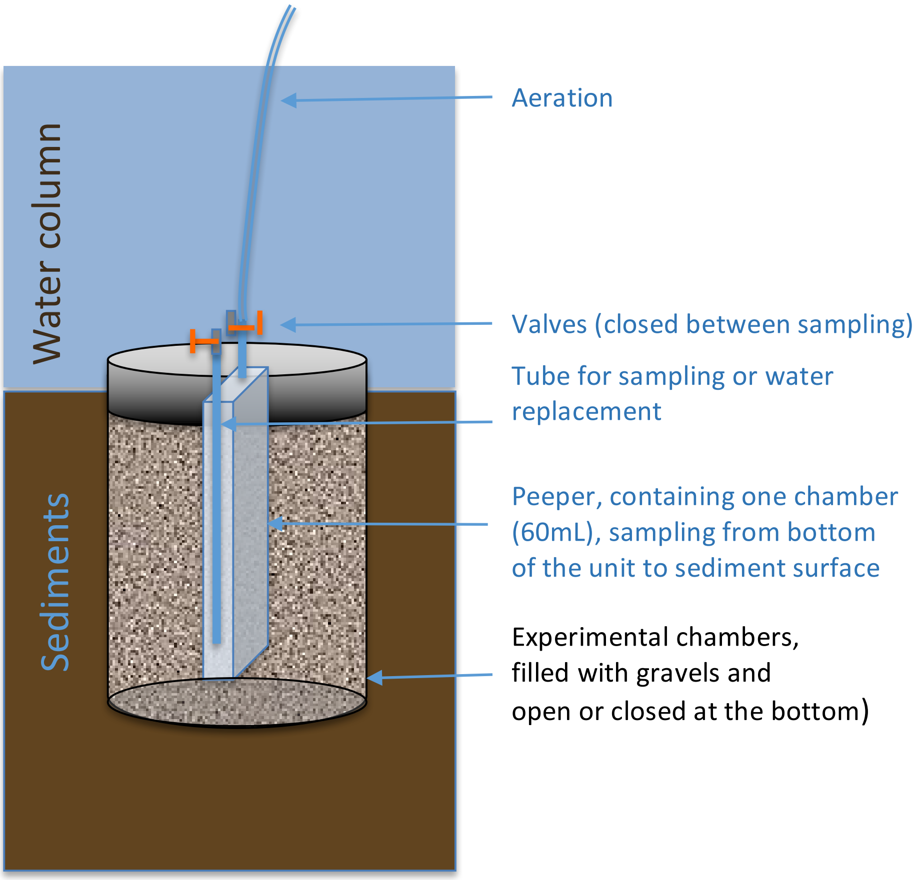


Figure 5. Experimental setup and sampling of a dialyze pore water sampler ("1K-peeper") used in the field experiment in Lake Stechlin. The method was adapted from Hesslein (1976) to permit repeated field sampling.

Groundwater featured a lower ratio of heavy stable water isotopes (^{2}H and ^{18}O) relative to lake water. This allowed the use of water stable isotope signatures to trace its origin. Based on those measurements, the inflow of groundwater into the lake could be confirmed at different survey sites [chapter 2].

Groundwater also contained higher concentrations of anions and cations than lake water. Consequently, EC measurements were used as a second independent tracer to identify LGD [chapter 2]. EC measurements were generally used as a decision-making tool during field experiments, as it provided a real-time, in-field indication of the origin of the water being sampled in seepage bags and 1K-peepers. However, sediment water samples occasionally provided higher EC values than lake water samples, even when the sediments were known not to be not influenced by groundwater. This would have been due to internal sediment processes. Stable isotope ratios were therefore prioritized over EC measurements to provide the most reliable information on water flow direction.

This study revealed that P entering Lake Stechlin, and likely many other lakes within catchments featuring low anthropogenic influences, was mobilized by LGD directly from the surface sediments, rather than being transported from the broader watershed area into the lake. This finding was confirmed by low P concentrations measured in relatively deep pore water (piezometers) as well as measurements of seepage through artificial sediments low in P [chapter 2]. Furthermore, 6K-peepers chamber measurements [chapter 2] revealed high SRP concentrations only in the upper 6 cm of the sediments, and extremely low SRP concentrations in the artificial sediments of *in situ* experimental chambers [1K-peepers, S1]. This indicated that P was mobilized from the surface sediments as the groundwater flowed through them. This situation was reproduced in laboratory experiments using artificial sediments enriched with P, and a flow-through setup where P was absent from water column and artificial groundwater [chapter 4].

In contrast to P, nitrogen (N) was not mobilized by groundwater. This was evidenced by a piezometer survey [chapter 2], and confirmed by the presence of low dissolved inorganic N concentrations in the experimental chambers' seepage [chapter 2] as well as 1K-peepers [S1].

In contrast to P and N, groundwater was a source of DIC and DSi, as both parameters were measured in elevated concentrations in piezometers, 6K-peeper sediment chambers, experimental seepage, and 1K-peepers [S1]. The absence of a gradient within 6K-peeper sediment chambers and the high concentration measured in both experimental seepage bags and 1K-peepers together indicate that the sediments had a negligible effect on DIC and DSi fluxes into the lake at LGD sites.

In addition to inorganic nutrients, groundwater also transports organic compounds which can be utilized by the non-algal constituents of periphyton, such as fungi (Fitter and Hillebrand 2009). Scinto and Reddy (2003) measured a comparable consumption of organic P and inorganic P in periphyton, and explained this finding by citing the ability of periphyton to utilize organic P through the production of phosphatase enzymes. Fungal degradation products can in turn serve as a nutrient source for the algae present in periphyton (Cross et al. 2005). Such processes may have additionally affected the facilitation of periphyton growth by LGD, but were beyond the scope of this study.

Finally, both pore water and groundwater monitoring are essential to evaluate the nutrient availability for benthic primary producers. For instance, in Lake Stechlin, P contained in littoral sediments was conveyed to the water column by groundwater fluxes.

Groundwater promotes epiphyton

In contrast to existing studies showing a positive impact of LGD on periphyton growing on the sediment surface (Hagerthey and Kerfoot 1998), this study for the first time revealed an effect of LGD on periphyton growing above the sediment surface (e.g., epiphyton growth on submerged macrophytes). These results are based on field surveys carried out in two subsequent years [chapters 2 & 3], a controlled field experiment [chapter 2] at Lake Stechlin, and controlled laboratory experiments [chapter 4]. In the presence of LGD, epiphyton accumulated more dry weight and chlorophyll-*a* compared to control conditions without LGD. All algal groups present in the epiphyton, including diatoms, cyanobacteria, and green algae, were promoted by LGD, confirming our hypothesis that LGD would facilitation periphyton production.

For the epiphyton of Lake Stechlin, as for most benthic autotrophs in lakes (Elser et al. 2007), P was determined to be the limiting nutrient from both field and laboratory surveys and experiments. SRP concentrations in the water column were consistently close to or below the detection limit of 3µg L^{-1}. The molar C:P ratio of epiphyton sampled *in situ* and grown in laboratory experiments was generally high, and often larger than the threshold value of 369, indicating that P limited growth (Kahlert, 1998).

In Lake Stechlin, P-depleted conditions in the epilimnion developed at the end of summer [chapter 2], as is typical for stratified oligotrophic lakes (e.g. Vrba et al. 1993). Interestingly, when comparing sites with and without LGD during this P-depleted period, epiphyton C:P ratios were significantly higher at sites with LGD, indicating a lower P limitation for epiphyton growing at groundwater seepage sites. This finding indicates that epiphyton communities benefit from the mobilization of sediment P through groundwater seepage, especially when the supply of water column P does not meet their needs. However, there are other nutrients which may potentially limit periphyton development in natural systems, including nitrogen, inorganic C, silica (Si), and various microelements.

Epiphyton in Lake Stechlin was potentially N-limited during the summer, as indicated by its low C:N ratios [chapters 2 & 3]. However, in contrast to P, LGD would not have affected this limitation due to the low mobilization rates of N through groundwater [chapter 2]. Groundwater seepage therefore did not influence epiphyton N supply in this study. Moreover, during 2013 and 2014 in Lake Stechlin, epiphyton contained N-fixing cyanobacteria which were especially abundant towards the end of the summer [data not shown].

Periphytic diatoms in Lake Stechlin were not believed to be Si-limited, based on their uptake kinetics reported in the literature [chapter 2], and relatively high dissolved Si concentrations in the water. Concentrations reached 0.23 ± 0.12 mg L^{-1} [chapter 2] in the summertime, which is higher than what is usually found in oligotrophic (Hurley et al. 1985) and more productive lakes (e.g. Shatwell et al. 2008). This assumption was confirmed by the fact that diatoms did not respond more positively to Si-rich groundwater than other algal groups (linear mixed model applied on the ratio fucoxanthin/chlorophyll-a: $p > 0.05$, data not shown).

The direct effect of groundwater on macrophytes

Based on previous studies from Lodge et al. (1989); Ommen et al. (2012) and Frandsen et al. (2012), we expected to observe a direct influence of LGD on macrophyte distribution, density, and growth form in Lake Stechlin. Surprisingly, transect data from macrophyte mapping campaigns (used as a proxy for macrophyte cover) showed no significant correlation between macrophyte species distribution and groundwater discharge areas in Lake Stechlin [S2]. The high variability of macrophytes cover may have hidden the potential influence of groundwater. What is more, due to patchiness and low growth densities of macrophytes in the littoral areas of Lake Stechlin, macrophyte biomass determination was not possible.

In the lab experiment, macrophyte growth form was affected by simulated LGD. Macrophytes growing from turions were far smaller in when exposed to simulated LGD compared to controls. This was probably the result of different redox conditions in the sediments, as simulated LGD may have transported oxygen into the sediments. This is, however, not the case under field conditions, as groundwater is often poor in oxygen. Thus, the experiments could not show that LGD directly affects macrophyte development.

In addition, nutrient content in macrophyte tissues from field samples did not verify the hypothesis that LGD affects the nutrient availability of macrophytes. Other studies showed no consistent results regarding the response of plant nutrient content to different nutrient concentrations in the water columns and sediments. Whereas Atkinson and Smith (1983) and Robach et al. (1995) found a strong relationship between plant nutrient content and environment nutrient concentrations, Demars and Edwards (2007) reported a weak response of macrophytes to nutrient content in the sediments. In fact, several studies pointed out that each species must be considered separately (Demars and Edwards 2007; Xie et al. 2013; Li et al. 2015). In Lake Stechlin, sediments apparently contained sufficient nutrients to meet the macrophytes' nutrient requirements [chapter 3]. Consequently, LGD and the concomitant release of nutrients did not directly impact macrophytes in shallow littoral areas.

An additional approach to trace the influence of LGD on macrophytes in Lake Stechlin has been used by analyzing the isotopic signature of hydrogen in lipids found in several different macrophyte species sampled from LGD and control sites (Aichner et al. in revision). The origin of water used for the biosynthesis of n-alcanes can be identified by the hydrogen isotopic composition in these compounds. However, no significant differences could be found between plants at LGD vs. control sites. This may be due to the low LGD flux, and thus a prevalence of the lake water signal in all plants (Aichner et al. in revision).

Influence of groundwater on macrophyte-periphyton interactions

The field studies in Lake Stechlin presented in this thesis revealed the existence of indirect effects of LGD on macrophytes, especially in shallow charophyte meadows [chapter 3]. A direct identification of these effects has been investigated using a field experiment [chapter

2] which involved both epiphyton and macrophytes (*Potamogeton pectinatus*, data not shown). However, herbivory made macrophyte growth measurements impossible.

To overcome these issues, a series of laboratory experiments with and without simulated groundwater seepage was set up to study macrophyte-periphyton interactions. Those experiments showed that macrophytes are indirectly affected by LGD, as macrophytes growth was impaired by epiphyton shading, which was promoted by LGD [chapter 4].

Shading by epiphyton was considered the most significant interaction leading to macrophyte decrease [chapter 4]. Epiphyton has long been known for its shading effect on macrophytes (Phillips et al. 1978; Sand-Jensen and Søndergaard 1981; Tóth and Palmer 2016). In the laboratory experiments, a negative effect of epiphyton on macrophytes was observed beginning at 75% shading, corresponding to an epiphyton density of 21 g dry weight m^{-2} [chapter 4].

Epiphyton shading may also increase the susceptibility of macrophytes for grazing pressure (Hidding et al. 2016), but this was not monitored in the present studies. Possible epiphyton-macrophytes interactions which involve the release of substances by macrophytes (nutrients or allelopathic substances) were not measured, as periphyton was sampled on artificial substrata.

Validation of the conceptual model

A conceptual model was proposed, based on the potential effects of groundwater-mediated nutrient loading on benthic primary producers and their interactions [chapter 1]. Extending this model to higher nutrient loading, three different conditions may be expected: (1) with low groundwater nutrient loading (under oligotrophic conditions), both macrophyte and periphyton production rates respond positively to any additional nutrient supply facilitated by groundwater fluxes; (2) at intermediate nutrient loading rates (approaching mesotrophic conditions), periphyton development impairs macrophytes by shading; (3) at high nutrient loading rates (producing eutrophic conditions), both macrophytes and periphyton are negatively affected by phytoplankton, which limit light availability in the benthic environment (Fig. 6).

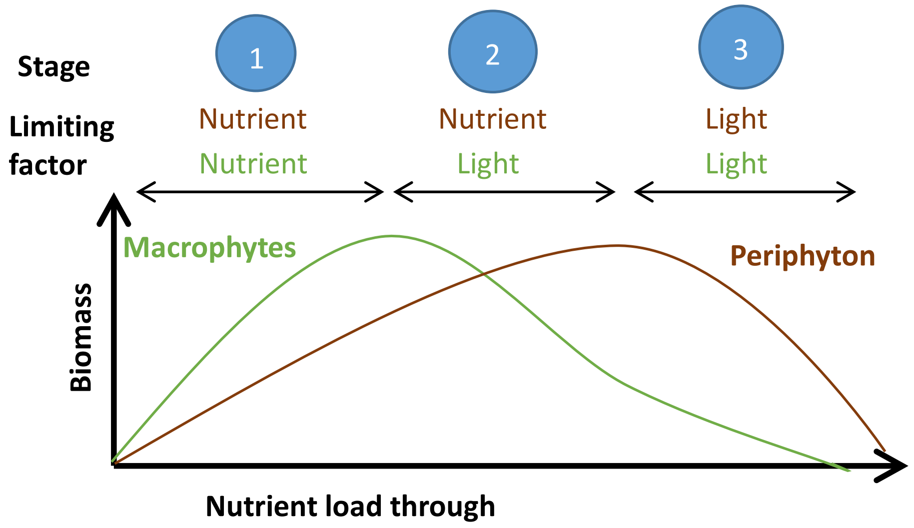


Figure 6. Extrapolation of the concept presented in [chapter 1], including turbid state

The field survey, field experiment, and lab experiment presented in this thesis confirmed the predicted response of periphyton to groundwater nutrient loading, represented above as stages 1 and 2. Indeed, periphyton was always promoted by groundwater-mediated nutrient loading, while few or no algae were present in the water column.

For macrophytes, the model's predictions could partly be verified in the laboratory experiment. Macrophyte growth was limited by artificial LGD-stimulated summer periphyton development, confirming the response of macrophytes to nutrient loading in stage 2

(Fig. 6). The positive effects of groundwater-mediated nutrient loading on macrophytes predicted for stage 1 (Fig. 6), however, were not observed [chapter 4].

The threshold at which macrophytes switch from nutrient to light limitation could not be defined for P loads, or for sediment P content, but only by periphyton biomass. Under experimental light conditions (reflecting the natural conditions of a German sunny day; Köhler et al. 2010), light-limitation of the studied macrophyte species (*P. pectinatus*) was reached when periphyton biomass surpassed 21 g dw m^{-2}.

In Lake Stechlin, periphyton biomasses above 21 g dw m^{-2} have often been measured, especially in LGD areas. The prevalence of negative effects of groundwater-mediated nutrient loading on macrophytes via periphyton shading (stage 2) thus seems possible in Lake Stechlin, particularly at sites that are additionally shaded by trees (see Köhler et al. 2010).

Implications for water quality and lake ecology

The importance of the water-sediment and shore-lake interfaces

The water-sediment boundary plays a crucial role in understanding groundwater-surface water interactions (Frape and Patterson 1981). In the littoral zone of Lake Stechlin, the presence of P-rich sediments supports the P supply of benthic primary producers [chapters 2-4]. Lacustrine groundwater discharge in those areas was shown to enhance the release of nutrients from the sediments, thereby differentially affecting the development of macrophytes and periphyton.

Since the lake shore is directly influenced by terrestrial inputs, such as leaf litter, woody branches, and surface runoff, littoral sediment quality and nutrient content can be highly heterogeneous, both spatially and temporally. This heterogeneity affects the availability of nutrients and alters seepage patterns, which eventually impact the development of primary producers in those shallow areas. To date, however, few studies have focused on the flow of nutrients from shorelines into lake systems, focusing on shallow littoral areas. Further research is thus required to improve our understanding of the processes in this habitat, and how they may influence the composition and structural function of benthic primary producers.

Role of periphyton for water quality

Periphyton can stabilize clear water states in lakes because it immobilizes P released from the sediments, thus hindering phytoplankton development (Genkai-Kato et al. 2012). This P fixation by periphyton is especially efficient when the P load via groundwater seepage increases, as periphyton is able to store excess P (chapter 2, Stevenson and Stoermer 1982). This P storage ability has been proposed as a potential tool in lake restoration efforts: artificial substrates could be installed in the water column, and removed after substantial periphyton development (Jöbgen et al. 2004). From another angle, the immobilization of SRP by periphyton from the water column potentially leads to an underestimation of eutrophication processes in lakes [chapters 2 & 4] when only water column parameters are being considered. It is therefore important to consider full periphyton communities with quantitative biomass assessments, and not only benthic diatom species (Szabó et al. 2017), when studying trophic changes in lakes. This is particularly important when considering lakes whose P input may be governed by LGD.

Periphyton may also influence P burial in the sediments. Photosynthetically-active periphyton (when autotrophic, during the day) may lead to sediment oxygenation, and thus facilitate P fixation in the uppermost sediment layer (Carlton and Wetzel 1988; Zhang et al. 2012). Moreover, periphyton may increase the pH at the sediment surface, enhancing calcium phosphate precipitation (Dodds 2003). On the other hand, periphyton may have the opposite effect on P mobility. Thick periphyton mats covering the sediment surface may be heterotrophic, favoring P mobilization from the sediments. However, these periphyton-sediment interactions may be less important where groundwater seepage occurs, because the vertical groundwater flux would override such processes and govern the redox and pH conditions at the sediment surface at a larger scale. Most importantly, it may be less probable that groundwater seepage takes place at sites where a continuous and compact periphyton mat exists, since such mats considerably lower hydraulic conductivity.

Calcite precipitation, a groundwater-promoted process

Calcite precipitation takes place in water bodies rich in Ca^{2+} and CO_3^{2-} ions, such as Lake Stechlin (Fuchs et al. 2016). Calcium and carbonate ions co-precipitate with other ions, such as phosphate, when the pH rises because of photosynthetic activity. Because the ions involved originate from the subsurface watershed, calcite precipitation may be considered as a groundwater-induced phenomenon (Holzbecher and Nützmann 2000) in Lake Stechlin.

Calcite precipitation displaces a significant amount of P from water column to the sediments, therefore promoting benthic over planktonic P uptake. In Lake Stechlin, this process accounts for 60 kg P km^{-2} yr^{-1}(average, Holzbecher and Nützmann 2000), which is in the same order of magnitude compared to P fixation by periphyton (81 ± 45 kg-P km^{-2} yr^{-1} (average ± standard deviation) in the summer of 2013 [chapter 2].

Calcite precipitation can also be induced locally through benthic primary producers. Macrophytes may induce calcite precipitation by increasing the pH at the surface of photosynthetically-active tissues. This calcite encrustation constitutes an opaque layer which increases the light limitation of macrophytes (Hutchinson 1975). Also periphyton frequently contains calcite as one of its abiotic constituents (Hagerthey et al. 2011). Calcite precipitation affects macrophyte-periphyton interaction, because the encrustation on macrophytes may support colonization by epiphytic organisms, and calcite contained in the periphyton matrix enhances macrophyte shading. Finally, as P is co-precipitated with calcite, the formation of calcite encrustation on macrophytes and within periphyton mats increases P storage in the sediments (Kufel and Kufel 2002; Dodds 2003).

Feedback between macrophytes and lake transparency

The key role of submerged macrophytes for stabilization of clear water states has been theorized for shallow lakes (Scheffer et al. 1993) and confirmed for deep lakes (Hilt et al. 2010; Sachse et al. 2014). This stabilization is facilitated by feedbacks between macrophytes and lake transparency, involving mechanisms such as sediment P fixation, nutrient uptake, and the influence of grazers communities (Scheffer et al. 1993). The switch between a macrophyte rich clear water state and a vegetation free turbid state is often abrupt, although Sachse et al. (2014) suggested that this change could be more gradual in deep lakes. The transition between these two different primary producers dominated states is induced by perturbations, such as changes in the external nutrient load (e.g. Hilt et al. 2013), water level fluctuations (Blindow et al. 1993), or modification of the trophic cascade (Jones and Sayer 2003).

The results of the present study imply that high periphyton development, by hindering macrophytes growth, may compromise the beneficial effect of macrophytes on the lake's ecosystem [chapter 4]. Consequently, as groundwater promotes periphyton, groundwaterborn nutrients may trigger the transition from a clear to a turbid water state. However, the transition from one state to the other would be delayed at first, because periphyton only harms macrophytes when its biomass exceeds a given threshold [chapter 4].

Shallow and deep submerged macrophytes seem to react differently to trophic changes. For example, during re-oligotrophication of Lake Müggelsee, Berlin, the littoral was quickly recolonised by macrophytes, whereas the maximum colonization depth increased only slowly (Hilt et al. 2013). As a matter of fact, macrophytes at deep areas are shaded by both periphyton and phytoplankton, whereas the influence of water column turbidity on macrophytes may be less pronounced in the shallow littoral. In case of Lake Stechlin, macrophytes in the shallow areas declined much faster than in the deeper parts [chapter 3]. Hence, periphyton appears to play a significant role in this change, while an increase in overall water column turbidity does not occur. The case of Lake Stechlin underlines that both maximum colonization depth of macrophytes and species coverage should be considered for evaluating the ecological status of lakes (Schaumburg et al. 2004). Our observations imply that the state of shallow macrophyte stands and the overgrowth by epiphyton may be used as an early indicator for changes in lake trophy.

Potential effects of groundwater on charophyte meadows in shallow littoral zones

Charophytes meadows are sensible macrophyte communities that are characteristic for environments with low nutrient concentration (Richter and Gross 2013), and are therefore often used as an indicator for lake trophy (Schaumburg et al. 2014). In Lake Stechlin, and in numerous other oligo-mesotrophic calcareous lakes in north-east Germany, the extent of the shallow charophyte meadows, has dramatically declined during the past 25 years (R. Mauersberger, pers.

comm.). The results of the present study indicate that LGD may play a role in the decline of charophyte meadows by promoting periphyton growth, which simultaneously affects charophytes through shading and by increasing their susceptibility to herbivors and wave action [chapter 3].

Alterations in LGD and in the accompanied diffuse nutrient inputs due to changing groundwater levels and human activities in the catchment could be contributing causes to the observed disappearance of charophyte meadows in this region. Indeed, changes in the amount and direction of groundwater flow as well as the nutrients released with it, may result from changes in climate (Green et al. 2011). Monitoring and modeling approaches of Lake Stechlin's watershed have shown that dry years may lead to higher groundwater flow rates from the neighboring eutrophic Lake Dagow (Holzbecher 2001). This change in groundwater flow in the catchment of Lake Stechlin is likely to occur again in the future according to climate change forecasts (Kirillin et al. 2013). Therefore, a further decline of charophytes in Lake Stechlin, but also in other nutrient-scarce seepage lakes can be expected.

Conclusion and outlook

Interactions between groundwater seepage and benthic primary producers in the shallow littoral zones of lakes are highly complex, but of potential significance for the entire lake ecosystem. These ecohydrological and ecological processes are strongly linked, but were traditionally considered separately. An improved understanding of the effects of changing environmental conditions on lake ecosystems, however, requires interdisciplinary approaches.

In the present studies, it has been demonstrated for the first time that periphyton, and epiphyton in particular, can be promoted by groundwater-mediated nutrient fluxes if nutrient concentrations in the lake water column are low. This process has been shown to have potentially positive and negative effects on lake water quality. On one hand, macrophytes suffer from epiphyton shading above certain epiphyton biomasses, and are thus indirectly negatively affected by lacustrine groundwater discharge (LGD), thus compromising their role in stabilizing clear-water conditions in lakes. On the other hand, below this threshold level, periphyton can store significant amounts of nutrients, and thus increase the resistance of lentic ecosystems to the effects of additional nutrient loading facilitated by LGD. Future experimental and field studies should be expanded to examine the effects of other substances potentially transported or mediated by LGD, including the effects of dissolved organic and inorganic carbon on benthic primary producers and their interactions.

The findings of the present studies can be generalized to systems with similar hydrological and ecological characteristics (oligo- to mesotrophic temperate hard water lakes with rather moderate LGD fluxes). Overall, the effects of groundwater-mediated nutrient fluxes on benthic primary producers are expected to be stronger in lakes and at locations with elevated LGD. Changes in future local weather, climate, and land use may have far-reaching consequences on groundwater fluxes, groundwater nutrient concentrations, and LGD. Predictions of the consequences of these changes for lake ecosystems (i.e. by ecosystem modeling) should consider the direct effects of LGD on benthic primary producers as well as direct and indirect cascading effects on lake water quality.

References

Aichner, B., S. Hilt, C. Périllon, M. Gillefalk, and D. Sachse. Biosynthetic hydrogen isotopic fractionation factors during lipid synthesis in submerged aquatic macrophytes: Effect of groundwater discharge and salinity. In revision, Org. Geochem.

Ali, M. M., S. A. Hassan, and A.-S. M. Shaheen. 2011. Impact of riparian trees shade on aquatic plant abundance in conservation islands. Acta Bot. Croat. **70**: 245–258.

Atkinson, M. J., and S. V. Smith. 1983. C : N : P ratios of benthic marine. Limnol. Oceanogr. **28**: 568–574.

Bakker, E. S., K. A. Wood, J. F. Pagès, G. F. C. Veen, M. J. A. Christianen, L. Santamaría, B. A. Nolet, and S. Hilt. 2016. Herbivory on freshwater and marine macrophytes: a review and perspective. Aquat. Bot. **135**: 15–36.

Barko, J. W., and R. M. Smart. 1980. Mobilization of sediment phosphorus by submersed freshwater macrophytes. Freshw. Biol. **10**: 229–238.

Belanger, T. V., D. F. Mikutel, and P. A. Churchill. 1985. Groundwater seepage nutrient loading in a Florida Lake. Water Res. **19**: 773–781.

Blindow, I., G. Andersson, A. Hargeby, and S. Johansson. 1993. Long-term pattern of alternative stable states in two shallow eutrophic lakes. Freshw. Biol. **30**: 159–167.

Burkholder, J. M., and R. G. Wetzel. 1989. Microbial colonization on natural and artificial macrophytes in a phosphorus-limited, hardwater lake. J. Phycol. **25**: 55–65.

Carlton, R. G., and R. G. Wetzel. 1988. Phosphorus flux from lake sediments : Effect of epipelic algal oxygen production. Limnol. Oceanogr. **33**: 562–570.

Carpenter, S. R., and D. M. Lodge. 1986. Effects of submersed macrophytes on ecosystem processes. Aquat. Bot. 341–370.

Cattaneo, A. 1990. The effect of fetch on periphyton spatial variation. Hydrobiologia **206**: 1–10.

Chappuis, E., E. Gacia, and E. Ballesteros. 2014. Environmental factors explaining the distribution and diversity of vascular aquatic macrophytes in a highly heterogeneous Mediterranean region. Aquat. Bot. **113**: 72–82.

Cross, W. F., J. P. Benstead, P. C. Frost, and S. A. Thomas. 2005. Ecological stoichiometry in freshwater benthic systems: Recent progress and perspectives. Freshw. Biol. **50**: 1895–1912.

Demars, B. O. L., and A. C. Edwards. 2007. Tissue nutrient concentrations in freshwater aquatic macrophytes: High inter-taxon differences and low phenotypic response to nutrient supply. Freshw. Biol. **52**: 2073–2086.

Dodds, W., and B. J. F. Biggs. 2002. Water velocity attenuation by stream periphyton and macrophytes in relation to growth form and architecture. J. North Am. Benthol. Soc. **21**: 2–15.

Dodds, W. K. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J. Phycol. **39**: 840–849.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. **10**: 1135–1142.

Fitter, A., and H. Hillebrand. 2009. Microbial food web structure affects bottom-up effects and elemental stoichiometry in periphyton assemblages. Limnol. Oceanogr. **54**: 2183–2200.

Frandsen, M., B. Nilsson, P. Engesgaard, and O. Pedersen. 2012. Groundwater seepage stimulates the growth of aquatic macrophytes. Freshw. Biol. **57**: 907–921.

Frape, S. K., and R. J. Patterson. 1981. Chemistry of interstitial water and bottom sediments as indicators of seepage patterns in Perch Lake, Chalk River, Ontario. Limnol. Oceanogr. **26**: 500–517.

Frost, P. C., H. Hillebrand, and M. Kahlert. 2005. Low algal carbon content and its effect on the C : P stoichiometry of periphyton. Freshw. Biol. **50**: 1800–1807.

Fuchs, A., G. B. Selmeczy, P. Kasprzak, J. Padisák, and P. Casper. 2016. Coincidence of sedimentation peaks with diatom blooms, wind, and calcite precipitation measured in high resolution by a multi-trap. Hydrobiologia **763**: 329–344.

Gasith, A., and A. D. Hasler. 1976. Airborne litterfall as a source of organic matter in lakes. Limnol. Oceanogr. **21**: 253–258.

Genkai-Kato, M., Y. Vadeboncoeur, L. Liboriussen, and E. Jeppesen. 2012. Benthic – planktonic coupling, regime shifts, and whole-lake primary production in shallow lakes. Ecology **93**: 619–631.

Green, T. R., M. Taniguchi, H. Kooi, J. J. Gurdak, D. M. Allen, K. M. Hiscock, H. Treidel, and A. Aureli. 2011. Beneath the surface of global change: Impacts of climate change on groundwater. J. Hydrol. **405**: 532–560.

Hagerthey, S. E., B. J. Bellinger, K. Wheeler, M. Gantar, and E. Gaiser. 2011. Everglades periphyton: A biogeochemical perspective. Crit. Rev. Environ. Sci. Technol. **41**: 309–343.

Hagerthey, S. E., and W. C. Kerfoot. 1998. Groundwater flow influences the biomass and nutrient ratios of epibenthic algae in a north temperate seepage lake. Limnol. Oceanogr. **43**: 1127–1242.

Hansson, L.-A. 1992. Factors regulating periphytic algal biomass. Limnol. Oceanogr. **37**: 322–328.

Hayashi, M., and D. O. Rosenberry. 2002. Effect of ground water exchange on the hydrology and ecology of surface water. Ground Water **40**: 309–316.

Hecky, R. E., and R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. J. North Am. Benthol. Soc. **14**: 631–653.

Heinen, E. A., and J. McManus. 2004. Carbon and nutrient cycling at the sediment-water boundary in western lake superior. J. Great Lakes Res. **30**: 113–132.

Hesslein, R. H. 1976. An in situ sampler for close interval pore water studies. Limnol. Oceanogr. **21**: 912–914.

Hickey, M., and C. King. 2000. The Cambridge illustrated glossary of botanical terms, Cambridge University Press.

Hidding, B., E. S. Bakker, M. J. M. Hootsmans, and S. Hilt. 2016. Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. Oikos **125**: 1489–1495.

Hilt, S., and E. M. Gross. 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? Basic Appl. Ecol. **9**: 422–432.

Hilt, S., I. Henschke, J. Rücker, and B. Nixdorf. 2010. Can submerged macrophytes influence turbidity and trophic state in deep lakes? Suggestions from a case study. J. Environ. Qual. **39**: 725–33.

Hilt, S., J. Köhler, R. Adrian, M. T. Monaghan, and C. D. Sayer. 2013. Clear, crashing, turbid and back - long-term changes in macrophyte assemblages in a shallow lake. Freshw. Biol. **58**: 2027–2036.

Holman, I. P., M. J. Whelan, N. J. K. Howden, P. H. Bellamy, N. J. Willby, M. Rivas-Casado, and P. McConvey. 2008. Phosphorus in groundwater - an overlooked contributor to eutrophication? Hydrol. Process. **22**: 5121–5127.

Hölting, B., and W. G. Coldewey. 2008. Hydrogeologie: Einführung in der Allgemeine und Angewandte Hydrogeologie, Spektrum Akademischer Verlag.

Holzbecher, E. 2001. The dynamics of subsurface water divides - watersheds of Lake Stechlin and neighbouring lakes. Hydrol. Process. **15**: 2297–2304.

Holzbecher, E., and G. Nützmann. 2000. Influence of the subsurface watershed on eutrophication - Lake Stechlin case study. Ecol. Eng. **16**: 31–38.

Hurley, J. P., D. E. Armstrong, G. J. Kenoyer, and C. J. Bowser. 1985. Ground Water as a silica source for diatom production in a precipitation-dominated lake. Science (80-.). **227**: 1576–1578.

Hutchinson, G. E. 1975. A Treatise on Limnology. Volume III, Limnologic. John Wiley and Sons.

Idestam-Almquist, J., and L. Kautsky. 1995. Plastic responses in morphology of *Potamogeton pectinatus* L. to sediment and above-sediment conditions at two sites in the northern Baltic proper. Aquat. Bot. **52**: 205–216.

Jacobsen, L., and M. R. Perrow. 1998. Predation risk from piscivorous fish influencing the diel use of macrophytes by planktivorous fish in experimental ponds. Ecol. Freshw. Fish **7**: 78–86.

Jäger, C. G., and S. Diehl. 2014. Resource competition across habitat boundaries : asymmetric interactions between benthic and pelagic producers. Ecol. Monogr. **84**: 287–302.

Jaynes, M. ., and R. Carpenter. 1986. Effects of vascular and nonvascular macrophytes on sediment redox and solute dynamics. Ecology **67**: 875–882.

Jöbgen, A., A. Palm, and M. Melkonian. 2004. Phosphorus removal from eutrophic lakes using periphyton on submerged artificial substrata. Hydrobiologia **528**: 123–142.

Jones, J. I., J. W. Eaton, and K. Hardwick. 2000. The influence of periphyton on boundary layer conditions: A pH microelectrode investigation. Aquat. Bot. **67**: 191–206.

Jones, J. I., and C. D. Sayer. 2003. Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? Ecology **84**: 2155–2167.

Kahlert, M. 1998. C:N:P ratios of freshwater benthic algae. Arch. Hydrobiol. (Suppl) (Advanc. Limnol.) **51**: 105–114.

Kahlert, M., A. T. Hasselrot, H. Hillebrand, and K. Pettersson. 2002. Spatial and temporal variation in the biomass and nutrient status of epilithic algae in Lake Erken , Sweden. Freshw. Biol. **47**: 1191–1215.

Karan, S., J. Kidmose, P. Engesgaard, B. Nilsson, M. Frandsen, D. a O. Ommen, M. R. Flindt, F. O. Andersen, and O. Pedersen. 2014. Role of a groundwater-lake interface in controlling seepage of water and nitrate. J. Hydrol. **517**: 791–802.

Kautsky, L. 1987. Life-cycles of three populations of Potamogeton pectinatus L. at different degrees of wave exposure in the Askö area, northern baltic proper. Aquat. Bot. **27**: 177–186.

Kirillin, G., W. Phillip, C. Engelhardt, and G. Nützmann. 2013. Net groundwater inflow in an enclosed lake: From synoptic variations to climatic projections. Hydrol. Process. **27**: 347–359.

Köhler, J., J. Hachoł, and S. Hilt. 2010. Regulation of submersed macrophyte biomass in a temperate lowland river: interactions between shading by bank vegetation, epiphyton and water turbidity. Aquat. Bot. **92**: 129–136.

Kufel, L., and I. Kufel. 2002. *Chara* beds acting as nutrient sinks in shallow lakes—a review. Aquat. Bot. **72**: 249–260.

Laskov, C., C. Herzog, J. Lewandowski, and M. Hupfer. 2007. Miniaturized photometrical methods for the rapid analysis of phosphate , ammonium , ferrous iron , and sulfate in pore water of freshwater sediments. Limnol. Oceanogr. methods **4**: 63–71.

Lee, R., J. A. Cherry, and J. F. Pickens. 1980. Groundwater transport of a salt tracer through a sandy lakebed. Limnol. Oceanogr. **25**: 45–61.

Lewandowski, J., K. Meinikmann, G. Nützmann, and D. O. Rosenberry. 2015. Groundwater - the disregarded component in lake water and nutrient budgets. 2. Effects of groundwater on nutrients. Hydrol. Process. **29**: 2922–2955.

Li, W., T. Cao, L. Ni, G. Zhu, X. Zhang, H. Fu, X. Song, and P. Xie. 2015. Size-dependent C, N and P stoichiometry of three submersed macrophytes along water depth gradients. Environ. Earth Sci. **74**: 3733–3738.

Liboriussen, L., and E. Jeppesen. 2003. Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. Freshw. Biol. **48**: 418–431.

Liboriussen, L., and E. Jeppesen. 2006. Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations. Freshw. Biol. **51**: 95–109.

Lodge, D. M., D. P. Krabbenhoft, and R. G. Striegl. 1989. A positive relationship between groundwater velocity and submersed macrophyte biomass in Sparkling lake, Wisconsin. Limnol.

Oceanogr. **34**: 235–239.

Madsen, J. D., P. a Chambers, W. F. James, E. W. Koch, and D. F. Westlake. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia **444**: 71–84.

McBride, M. S., and H. O. Pfannkuch. 1975. The distribution of seepage within lakebeds. J. Res. USGS **3**: 505–512.

Meinikmann, K., M. Hupfer, and J. Lewandowski. 2015. Phosphorus in groundwater discharge – A potential source for lake eutrophication. J. Hydrol. **524**: 214–226.

Moss, B., S. Kosten, M. Meerhoff, R. W. Battarbee, E. Jeppesen, N. Mazzeo, K. Havens, G. Lacerot, Z. Liu, L. De Meester, H. Paerl, and M. Scheffler. 2011. Allied attack: climate change and eutrophication. Inl. Waters **1**: 101–105.

Neumeier, U., C. H. Lucas, and M. Collins. 2006. Erodibility and erosion patterns of mudflat sediments investigated using an annular flume. Aquat. Ecol. **40**: 543–554.

Ommen, D. A. O., J. Kidmose, S. Karan, M. R. Flindt, P. Engesgaard, B. Nilsson, and F. Ø. Andersen. 2012. Importance of groundwater and macrophytes for the nutrient balance at oligotrophic Lake Hampen, Denmark. Ecohydrology **5**: 286–296.

Phillips, G. L., D. Eminson, and B. Moss. 1978. A mechanism to account for macrophytes decline in progressively eutrophicated freshwaters. Aquat. Bot. **4**: 103–126.

Phillips, G., N. Willby, and B. Moss. 2016. Submerged macrophyte decline in shallow lakes : What have we learnt in the last forty years ? Aquat. Bot. **135**: 37–45.

Richter, D., and E. M. Gross. 2013. *Chara* can outcompete *Myriophyllum* under low phosphorus supply. Aquat. Sci. **75**: 457–467.

Robach, F., I. Hajnsek, I. Eglin, and M. Trémolières. 1995. Phosphorus sources for aquatic macrophytes in running waters : water or sediment ? Acta Bot. Gall. **142**: 719–731.

Rösel, S., A. Rychła, C. Wurzbacher, and H.-P. Grossart. 2012. Effects of pollen leaching and microbial degradation on organic carbon and nutrient availability in lake water. Aquat. Sci. **74**: 87–99.

Rosenberry, D. O., J. Lewandowski, K. Meinikmann, and G. Nützmann. 2015. Groundwater - the disregarded component in lake water and nutrient budgets. Part 1: effects of groundwater on hydrology. Hydrol. Process. **29**: 2895–2921.

Sachse, R., T. Petzoldt, M. Blumstock, S. Moreira, J. Rücker, J. H. Janse, W. M. Mooij, S. Hilt, and P. Marlene. 2014. Extending one-dimensional models for deep lakes to simulate the impact of submerged macrophytes on water quality. Environ. Model. Softw. **61**: 410–423.

Salant, N. L. 2011. “ Sticky business ” : The influence of streambed periphyton on particle deposition and in filtration. Geomorphology **126**: 350–363.

Sand-Jensen, K., and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. **41**: 137–175.

Sand-Jensen, K., and M. Søndergaard. 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Rev. der gesamten Hydrobiol. **66**: 529–552.

Schafran, G. C., and C. T. Driscoll. 1990. Porewater acid/base chemistry in near-shore regions of an acidic lake - The influence of groundwater inputs. Biogeochemistry **11**: 131–150.

Schaumburg, J., C. Schranz, G. Hofmann, D. Stelzer, S. Schneider, and U. Schmedtje. 2004. Macrophytes and phytobenthos as indicators of ecological status in German lakes—a contribution to the implementation of the Water Framework Directive. Limnologica **34**: 302–314.

Schaumburg, J., C. Schranz, and D. Stelzer. 2014. Bewertung von Seen mit Makrophyten & Phytobenthos gemäß EG-WRRL- Anpassung des Verfahrens für natürliche und künstliche Gewässer sowie Unterstützung der Interkalibrierung. Bayerisches Landesamt für Umwelt. Endbericht im Auftrag der LAWA 161 S.

Scheffer, M., S. Hosper, M. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative equilibria in shalow lakes. Trends Ecol. Evol. **8**: 275–279.

Schroeder, F., W. Traunspurger, K. Pettersson, and L. Peters. 2012. Temporal changes in periphytic meiofauna in lakes of different trophic states. J. Limnol. **71**: 216–227.

Schutten, J., J. Dainty, and A. J. Davy. 2004. Wave-induced hydraulic forces on submerged aquatic plants in shallow lakes. Ann. Bot. **93**: 333–341.

Scinto, L. J., and K. R. Reddy. 2003. Biotic and abiotic uptake of phosphorus by periphyton in a subtropical freshwater wetland. Aquat. Bot. **77**: 203–222.

Sebestyen, S. D., and R. L. Schneider. 2004. Seepage patterns, pore water, and aquatic plants: hydrological and biogeochemical relationships in lakes. Biogeochemistry **68**: 383–409.

Shatwell, T., J. Köhler, and A. Nicklisch. 2008. Warming promotes cold-adapted phytoplankton in temperate lakes and opens a loophole for Oscillatoriales in spring. Glob. Chang. Biol. **14**: 2194–2200.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems: A global problem. Environ. Sci. Pollut. Res. **10**: 126–139.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science : where do we go from here ? Trends Ecol. Evol. **24**: 201–207.

Spears, B. M., L. Carvalho, R. Perkins, and D. M. Paterson. 2008. Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. Water Res. **42**: 977–86.

Stevenson, R. J., and E. F. Stoermer. 1982. Luxury consumption of phosphorus by benthic algae. Bioscience **32**: 682–683.

Strand, J. A., and S. E. B. Weisner. 1996. Wave exposure related growth of epiphyton : implications for the distribution of submerged macrophytes in eutrophic lakes. Hydrobiologia **325**: 113–119.

Szabó, B., J. Padisak, G. B. Selmeczy, L. Krienitz, P. Casper, and C. Stenger-Kovacs. 2017. Spatial and temporal patterns of benthic diatom flora in Lake Stechlin , Germany. Turk. J. Botany **41**: 211–222.

Timms, R. M., and B. Moss. 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. Limnol. Oceanogr. **29**: 472–486.

Torn, K., A. Kovtun-Kante, K. Herkül, G. Martin, and H. Mäemets. 2015. Distribution and predictive occurrence model of charophytes in Estonian waters. Aquat. Bot. **120**: 142–149.

Tóth, V. R., and S. C. J. Palmer. 2016. Acclimation of *Potamogeton perfoliatus* L. to periphyton accumulation-induced spectral changes in irradiance. Hydrobiologia **766**: 293–304.

Tugend, K. I., M. S. Allen, and M. W. Binford. 2004. Potential use of remote sensing to assess effects of wave action on plant re-establishment. J. Aquat. Plant Manag. **42**: 54–60.

Vadeboncoeur, Y., S. P. Devlin, P. B. McIntyre, and M. J. Vander Zanden. 2014. Is there light after depth ? Distribution of periphyton chlorophyll and productivity in lake littoral zones. Freshw. Sci. **33**: 524–536.

Vadeboncoeur, Y., E. Jeppesen, M. J. Vander Zanden, H.-H. Schierup, K. Christoffersen, and D. M. Lodge. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnol. Oceanogr. **48**: 1408–1418.

Vadeboncoeur, Y., and A. D. Steinman. 2002. Periphyton function in lake ecosystems. Sci. World J. **2**: 1449–1468.

Vesterinen, J., J. Syväranta, S. P. Devlin, and R. I. Jones. 2016. Periphyton support for littoral secondary production in a highly humic boreal lake. Freshw. Sci. **35**: 1235–1247.

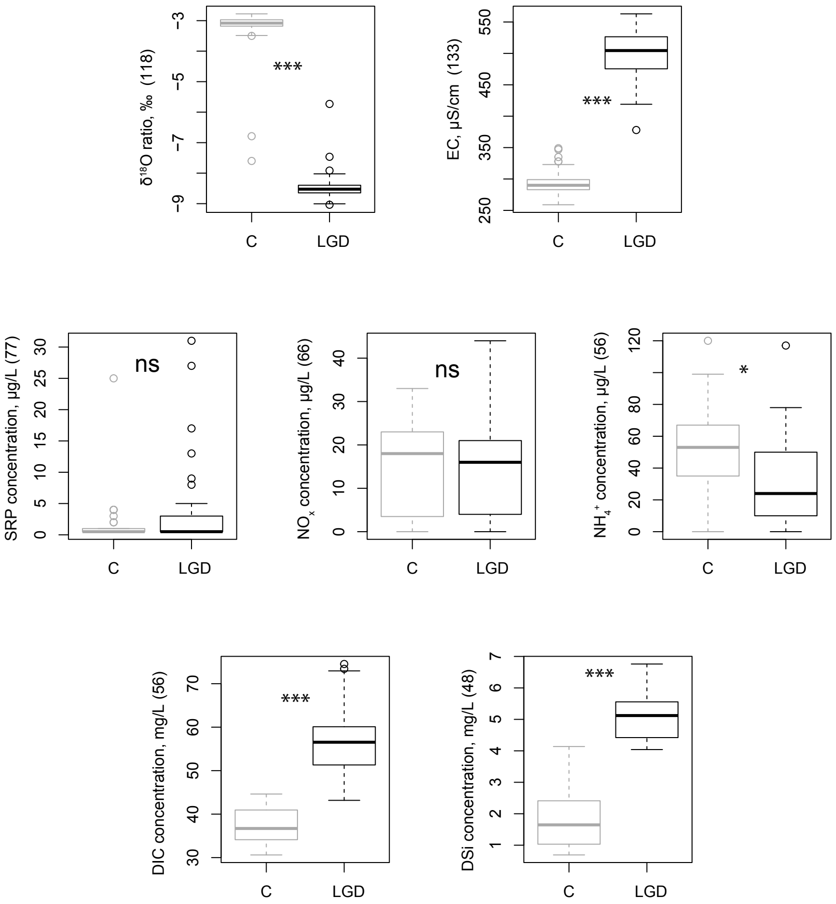
Vrba, J., J. Komárkova, and V. Vyhnálek. 1993. Enhanced activity of alkaline phosphatases - phytoplankton response to epilimnetic phosphorus depletion. Water Sci. Technol. **28**: 15 LP-24.

Wetzel, R. G. 1983. Opening remarks, *In* R.G. Wetzel [ed.], Periphyton of Freshwater Ecosystems. Dr W. Junk Publishers.

Xie, D., D. Yu, W. You, and L. Wang. 2013. Algae mediate submerged macrophyte response to nutrient and dissolved inorganic carbon loading : A mesocosm study on different species. Chemosphere **93**: 1301–1308.

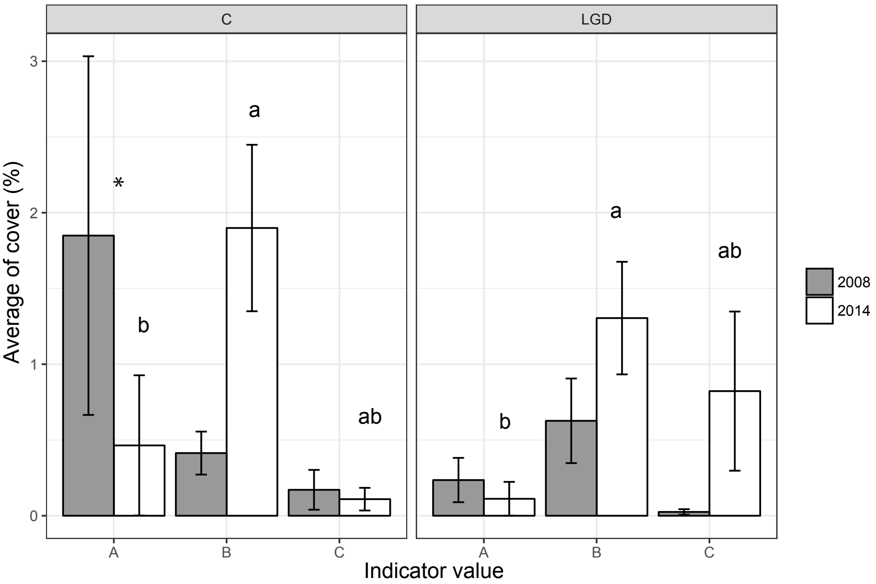
Zhang, X., Z. Liu, R. D. Gulati, and E. Jeppesen. 2012. The effect of benthic algae on phosphorus exchange between sediment and overlying water in shallow lakes: a microcosm study using ^{32}P as a tracer. Hydrobiologia **710**: 109–116.

Supplement 1



Supplement 1: Nutrients content of pore water sampled in the experimental units without (C, grey) or with (LGD, black) groundwater inflow, sampled with passive samplers (1K-peepers). The total number of samples are indicated, with maximum 5 sample per treatment and per campaign. The result of Student's t-test, Mann-Whitney U test and Welch's t-test appear as significance level ("ns" p>0.05, *: p<0.05, ***: p<0.001). This supplement is referred as [S1] in the dissertation.

Supplement 2



Supplement 2: Average coverage (±standard error) of macrophyte species with A, B, and C indicator values observed at 0–2m depths of 20 transects surveyed in 2008 and 2014. Data are represented separately for transects which were either influenced or uninfluenced by groundwater.

The indicator values are attributed following Schaumburg et al. (2015) for the "TKg13" lake type.

The significant differences between years are indicated (Mann-Whitney *U* test, *: $p < 0.05$) and for each year and groundwater influence, different letters are attributed to significantly different coverages (Mann-Whitney *U* test, $p < 0.05$). For each year and indicator value, no significant difference was observed between groundwater influences.

This supplement is referred as [S2] in the dissertation.

Declaration

I hereby declare that this thesis and the work presented in it is entirely my own, except where otherwise indicated. I have only used the documented utilities and references.

I certify that this work has not been submitted to any other institution of higher education.

Potsdam, 19.06.2017 ……………………………………

Cécile Périllon