

**Assessing potential impacts of multiple stressors on riverine
phytoplankton community by integrated models**

Dissertation

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Yueming Qu M. Sc.
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Erste Gutachterin: Prof. Dr. Nicola Fohrer

Zweiter Gutachter: Prof. Dr. Hans-Rudolf Bork

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Abstract

Rivers are among the most valuable resources on the earth, which closely related to human activities. They are particularly vulnerable to global changes because of highly complex interactions. Increasing pressures from both natural disturbance and anthropogenic activities challenge river ecosystem resilience. A better understanding of the ecological balancing of structure and functions under multiple stressors is thus critical for sustainable future as well as human wellbeing.

Phytoplankton are one of the important autotrophs in the food web of lowland rivers. They are sensitively reflecting multiple filters from spatial dispersals of species to the alteration of land-use pattern, flow regime and physicochemical condition. However, up to this thesis, the studies on how these factors overall affect the lotic phytoplankton community are still scarce. This study aims to examine river phytoplankton community structure in relation to combined abiotic gradients, and to consider the potential responses of this biomes under changing status.

As case study area the lowland catchment Treene (Schleswig-Holstein, Germany) is selected, since the eco-hydrological model Soil and Water Assessment Tool (SWAT) has already utilized in several studies before. It applies here in combination with various statistical models. The integrated model method is established for exploring the contributions of the spatial, local environmental, land-use and hydrological factors and their interactions on algal community pattern quantitatively. The main results of the thesis are as follows.

The investigations show high spatial and temporal variations of phytoplankton community at Treene catchment. Bacillariophyta, especially benthic diatoms, are dominant in most of the study area. Euglenophyta show higher existence percentage in the headwaters and exhibited a strong positive correlation to the share of agricultural land use as shown in a redundancy analysis (RDA). Cyanobacteria (specifically *Microcystis*) population are found with high abundance downstream of a lake, such situation will have a significant influence on the phytoplankton community composition. However, the influence from the lake vary along with seasons and limited within a relatively short distance after the lake. Both structural equation models (SEM) and RDA detect that higher phosphorous concentration directly causes the increase of *Microcystis* population, while the area of urban land-use indirectly affects their abundance during high flow period.

Variation partitioning and Mantel tests reveal that both species and traits composition more relate to hydrological and local physicochemical heterogeneity than to species dispersal, which confirm the suitability of lowland phytoplankton-based bioassessment in the study area. From SEM result, the skewness of 7 days discharge emerges as a key driver, and always indirectly effects on benthic diatoms during different hydrological periods. Due to the importance of flow regime in shaping pelagic algal community, it is necessary to include hydrological variables in long-term bio-monitoring campaigns and biodiversity conservation and restoration.

By the results of random forest (RF) model, the main stressors for local species richness are detected. The potential responses of phytoplankton species richness under altered water temperature and land-use scenarios are estimated by generalized linear mixed model (GLMM). RF illustrate that the shares of forest and pasture area have outstanding explanation to the variation of riverine phytoplankton species richness pattern. The results from the GLMM indicate that the interactions of land-use, water temperature and hydrological factors determine the species richness characteristics. The value of forest presence exerted stronger effects than pasture changes in antagonistic effects scenarios. The findings emphasize the significance of preservation of forest area in protecting the aquatic algal biodiversity for maintaining the river ecosystem functioning. There detects a one to two weeks' time lag of phytoplankton community variation when responding to hydrological alteration. It refers that the community structure and differences of composition are partly due to past events rather than contemporary factors.

The integrated modeling framework is a powerful approach for achieving progress in the interdisciplinary research in eco-hydrology and for a better understanding of the community structuring mechanism by separately analyzing multiple stressors at the watershed scale. Unfortunately, this study has not contained observation and detection of underwater lighting condition, grazing pressure and pesticides influences, which potentially have undeniable significant roles on shaping riverine phytoplankton community. It is valuable to involve them for the optimization of integrated models in the future also under consideration of socioeconomic aspects.

Zusammenfassung

Flüsse gehören zu den wertvollsten Ressourcen der Erde und stehen im engen Zusammenhang zu den menschlichen Aktivitäten. Aufgrund von hochkomplexen Wechselwirkungen sind sie besonders anfällig für globale Veränderungen. Zunehmende Belastungen durch natürliche Störungen und anthropogene Veränderungen beeinflussen die Widerstandsfähigkeit dieser Ökosysteme. Daher ist ein besseres Verständnis ihrer ökologischen Strukturen und Funktionen und der Auswirkungen von verschiedenen Einflussfaktoren auf die Ökosysteme von entscheidender Bedeutung für eine nachhaltige Entwicklung und für das Wohlergehen der Menschheit.

Phytoplankton gehört zu den wichtigsten Komponenten im Nahrungsnetz von Tieflandflüssen und reagiert sensitiv auf verschiedene Faktoren wie räumliche Ausbreitung von Arten, Veränderungen des Landnutzungsmusters und physikochemische Zustände. Bisher gibt es jedoch kaum Untersuchungen wie sich die genannten Faktoren auf die Gemeinschaft von lotischem Phytoplankton auswirken. Daher untersucht diese Doktorarbeit die Struktur von Phytoplankton in Bezug auf die genannten abiotischen Gradienten und die möglichen Reaktionen dieser Artengruppe bei Systemveränderungen.

Das Untersuchungsgebiet ist das Tieflandeinzugsgebiet der Treene in Schleswig-Holstein. In diesem Gebiet wurden bereits zahlreiche Studien mit dem ökohydrologischen Modell SWAT (Soil and Water Assessment Tool) durchgeführt. Das SWAT-Modell wird in dieser Arbeit in Kombination mit verschiedenen statistischen Modellen angewendet. Die integrierte Modellanwendung wird zur Quantifizierung des Einflusses von räumlichen, lokalen Umwelt-, Landnutzungs- und hydrologischen Faktoren und deren Wechselwirkungen auf Algengemeinschaften entwickelt.

Die Hauptergebnisse dieser Arbeit sind:

Es zeigen sich große räumliche und zeitliche Unterschiede in den Phytoplanktongemeinschaften im Treene-Einzugsgebiet. Bacillariophyta dominieren in den meisten Teilen des Einzugsgebietes. Dagegen treten Euglenophyta in einem höheren Anteil im Quellgebiet auf. Eine Redundanzanalyse (RDA) zeigt eine hohe positive Korrelation zum Anteil der Landwirtschaft. Cyanobakterien (insbesondere *Microcystis*) sind häufiger flussabwärts eines Sees zu finden, woraus eine signifikante Veränderung in der Zusammensetzung und Population von Phytoplankton resultiert. Allerdings variiert

der Einfluss des Sees zwischen den Jahreszeiten und ist räumlich auf eine relativ geringe Entfernung zum See konzentriert. Das Strukturgleichungsmodell (SEM) und die RDA zeigen, dass eine höhere Phosphorkonzentration zu einer Zunahme der *Microcystis*-Population führen kann. Dagegen beeinflusst der Anteil an urbanen Gebieten die Häufigkeiten während Hochwasserphasen.

Mit Variation-Partitioning und Manteltest zeigen einen stärkeren Zusammenhang der Zusammensetzung der Arten und ihren Ausprägungen mit den hydrologischen und lokalen physikochemischen Heterogenitäten als mit der räumlichen Ausbreitung. Damit lässt sich die Eignung von Phytoplankton zur biologischen Bewertung in Tieflandgebieten bestätigen. Die Ergebnisse des SEM zeigen, dass die Schiefe der 7-Tagesabflüsse ein wichtiger Faktor ist, der benthische Diatomeen in verschiedenen hydrologischen Phasen beeinflusst. Aufgrund der Bedeutung des Abflussregimes für die pelagischen Algengemeinschaften sollten hydrologische Variablen langfristig untersucht und in Analysen zum Erhalt und Wiederherstellung der biologischen Vielfalt berücksichtigt werden.

Die Ergebnisse eines Random Forest (RF) Modells werden zur Ableitung der Haupteinflussfaktoren für Artenreichtum genutzt. Mit Hilfe eines generalisierten linearen gemischten Modells (GLMM) wird der Artenreichtum an Phytoplankton in Szenarien mit veränderten Temperatur- und Landnutzungsbedingungen abgeschätzt. Das RF-Modell zeigt, dass die Anteile an Wald- und Weideflächen die Variation des Artenreichtums an Phytoplankton in Flüssen hervorragend erklären können. Die GLMM-Szenarien zeigen, dass das Zusammenwirken von Landnutzung, Wassertemperatur und hydrologischen Faktoren den Artenreichtum bestimmen. Die Auswirkungen von Waldflächen sind in den Szenarien stärker als Veränderungen in Weideflächen. Diese Ergebnisse zeigen, welche Bedeutung der Erhalt von Waldflächen für den Schutz der Artenvielfalt der Algen in Gewässer hat, um die Funktionalität von Flussökosystemen aufrechtzuerhalten. Der Einfluss von hydrologischen Änderungen auf die Phytoplanktongemeinschaften tritt ein bis zwei Wochen später auf. Hierbei soll darauf hingewiesen werden, dass Unterschiede in der Struktur von Phytoplankton vor allem auf Veränderungen in der Vergangenheit und weniger auf rezente Faktoren zurückzuführen sind.

Das hier präsentierte Modell-Framework ist eine leistungsfähige Methode, um Fortschritte in der interdisziplinären öko-hydrologischen Forschung zu erreichen und die Strukturmechanismen von Phytoplanktongemeinschaften besser zu verstehen. Hierbei konnte der Vorteil aufgezeigt werden, wenn die verschiedenen Einflussfaktoren auf der Einzugsgebietsskala getrennt betrachtet werden. Leider konnten in dieser Studie keine Beobachtungsdaten von Lichtverhältnissen unter Wasser sowie

von Weidedruck und Pestizideinfluss berücksichtigt werden, die möglicherweise eine wichtige Rolle bei der Ausbildung von Phytoplanktongemeinschaften darstellen. Es wäre wünschenswert, wenn diese Faktoren in zukünftigen Studien zur Optimierung von integrierten Modellen auch unter Hinzunahme von sozioökonomischen Aspekten berücksichtigt werden könnten.

摘要

集成模型评估多重压力下河流浮游植物群落的响应

河流是地球上最具价值的资源之一，自古以来人类逐水而居，河流与人类生活密切相关。随着人类活动干扰的日益增强，全球变化急剧威胁着河流生态系统的健康，维护和保持河流生态系统功能于人类福祉及构建可持续发展的未来至关重要。

生活在低地河流中的浮游植物群落是河流食物网中重要的自养微生物。它们敏锐地反映着从空间扩散到土地利用，以及水流情势、理化条件变化等多重环境压力的影响。然而到目前为止，关于河流浮游植物的研究仍十分有限，关于多重因素如何综合影响浮游植物群落仍存有许多疑问。据此，本论文旨在探究浮游植物群落结构与复合非生物因子间的关系，及其对环境变化的响应。

本文选择位于德国北部的典型低地流域 Treene 为研究区域，运用生态水文模型土壤和水分评估工具 (SWAT)，结合不同的统计方法 (包括方差分解，结构方程模型，机械学习等) 对浮游植物的响应进行模拟及定量分析。本研究主要结论如下：

调查显示，低地河流中的浮游植物群落结构具有显著的时空分布差异。在绝大部分研究区域，硅藻，尤其是底栖硅藻类群，优势显著。冗余分析显示，裸藻在河源区域所占比例较高，且与农业用地呈显著正相关。蓝藻，特别是微囊藻，在湖下游区域具有较高的丰度。在河湖串联系统中，与河流连通的湖泊显著的更改了下游河流藻类群落结构，其影响随季节变化强度不同。此外，结构方程模型以及冗余分析均证实较高的磷浓度将导致河流浮游植物组成中微囊藻的比例增加。此外，城镇占地面积比例的增加会间接的促进微囊藻生物量的增长。

水文情势与局地的环境属性是决定浮游植物群落的物种、性状组成，以及其 α 、 β 多样性特征的主要控制因素，而扩散作用在该研究中相对较弱。这一特征从另一方面证实了低地河流中运用浮游植物进行生物监测与生物评估的重要意义。流量偏度是一个值得的关注的水情度量因子，特别是周流量偏度变化率对浮游植物的群落构建具有因果指示性；并在不同的水文过程中，对底栖硅藻的结构特征始终具有间接的驱动作用。鉴于水文因子在浮游藻类群落构建中体现出的重要作用，建议将水文情势的调查纳入长期生态监测，并在生物多样性的保护与恢复工作中综合考量特征水文因子的变化特征。

运用集成模型评估环境变化对河流浮游植物物种丰富度的影响发现，土地利用特征与水温、水文因子共同决定物种丰富度特征。集水区中森林和牧草土地覆盖面积所占百分比对河流浮游

植物物种丰富度格局具有突出的解释率。研究结果显示森林面积对河流藻类生物多样性保持具有积极的作用，而牧草面积与藻类多样性指数呈负相关。在拮抗效应情景中，森林变化带来的影响比牧场变化的作用显著；组合情景表明，温度变化可调节土地利用对生物多样性的影响。研究发现浮游植物群落对水情变化的响应或有一至两周时滞性。河岸带森林分布与覆盖面积对水生生物多样性的维护具有重要意义。

研究提出集成模型框架为流域尺度下的生态-水文交叉学科研究。该研究有助于更好的理解多重压力下生物群落的形成机制，并可有效应用于其他流域的研究及有关的河流系统管理中。本研究虽未考虑水下光照条件，放牧压力和农药使用情况的影响，然这些因素对河流浮游植物的群落结构，及河流生态系统功能的影响不容忽视，值得纳入后续研究，并结合社会经济发展综合考虑。

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Chapter 1 General introduction

Rivers are the “arteries of our planet” (Hails et al., 2008). In transporting water and water components, they connect lakes, wetlands and terrestrial areas, serve as significant communication, transportation and navigation functions on the way to the ocean. However, the study of river ecosystem has still been relatively scarce and lagged way well behind marine, terrestrial and even lake ecosystems (Bernhardt et al., 2018). Rivers are complex mosaics of habitat types and environmental gradients, and characterized by high connectivity and spatial complexity (Tonkin et al., 2018a). River ecosystems are closely related to human activities. At the same time, they are threatened by a variety of pressures from both natural alterations and anthropogenic disturbances at different spatial and temporal scales (Vörösmarty et al., 2010). Humans are simultaneously producers as well as receptors of the effects by these multiple stressors. The maintenance and protection of river ecosystem health are thus critical to sustainable development as well as human wellbeing (Griggs et al., 2013; Poff et al., 2016).

1.1 Multiple stressors in river ecosystem

From historical time, rivers supplied critical benefits for human societies by providing drinking water, food from fisheries and irrigation, regulating biogeochemical balances, and enriching our aesthetic and cultural experience. The related fertile riparian soils and livable environment have attracted human settlements living next to the water. The common etymology of the words “river” and “rivalry” from the Latin *rivalis* (pertaining to streams or rivers) is evidence of the age-long conflict over shared resources between humans living on opposite banks of rivers (Arthington et al., 2006). The fact results in very few river systems on our planet that are still in pristine condition and have not been altered by human activities. It is no exaggeration to state that virtually all rivers on the Earth are subject to stressors. Global changes are influencing rivers including but not restricted to water flow interruptions, temperature increases, altered water residence time, changes in nutrients loads, increasing arrival of new chemicals, simplification of the physical structure of the systems and biodiversity losses (Tollefson, 2019). All of them affect the structure and functioning of the river ecosystem, and thereby their ecosystem services. The following key stressors are specially studied in this thesis.

1.1.1 Spatial factors

A taxa appearance must pass through nested filters from “history filter”, “dispersal filter” to “environmental filter” (Zobel, 1997). Filters are scaled structuring elements that influence the probability taxa with its specified functional traits being able to niche into the local community. Dispersal effect is an essentially natural process in selecting local biotic community from regional species pools (Fig. 1.1, modified from Hillebrand & Blenckner 2002). Spatial biogeographic patterns are increasingly studied in order to provide a better understanding of the ecology of living organisms (e.g. Soininen, 2007; Morlon et al., 2011) and to identify the processes involved in the maintenance or decline of biodiversity (Jackson et al., 2001; Tonkin et al., 2018a).

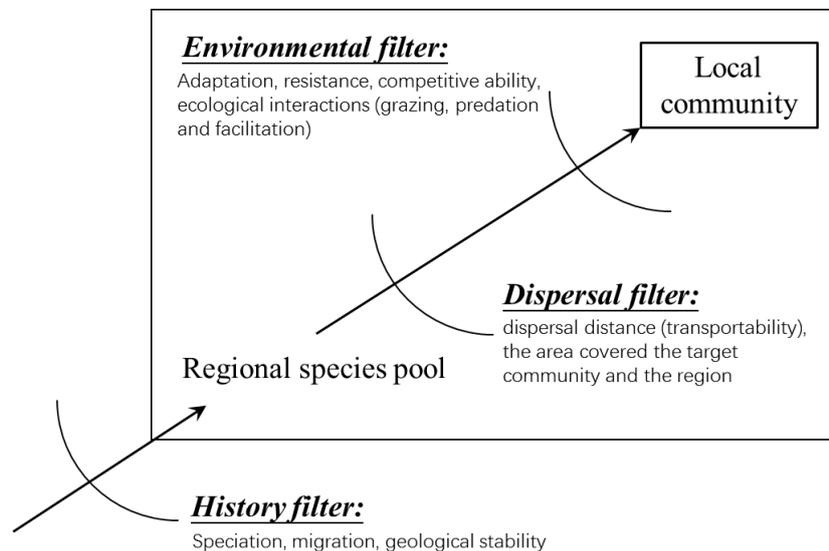


Fig. 1.1 A conceptual model visualizing the assembly of local communities through a series of nested filters (modified from Hillebrand & Blenckner 2002)

Linear-shaped sinks, dendritic network structure and single directional flow are special spatial characteristics of river. The unidirectionality flowing water gives a disproportionate effect on the biological spread (Fig 1.2, modified from Peterson et al., 2013), which in turn potentially changes the viability and intermediate coexistence of the aquatic biomes (Peterson et al., 2013). The dendritic network usually resulting in more frequent exchange is located in the mainstream, while higher isolation for the headstreams is observed (Brown and Swan, 2010). Spatial dispersal effects may mask the importance of environmental conditions affecting local communities, because very high or low dispersal rates may restrict species sorting, disassociating the otherwise strong relationship between

local communities and local environmental characteristics (Leibold et al., 2004; Winegardner et al., 2012). Riverine species with poor dispersal abilities, for example algae (Heino et al., 2010), against rapid short-term environmental changes are in jeopardy to be removed from a given river ecosystem, suffering from water level fluctuations, such as temporally dynamic flood and drought events (Alahuhta et al., 2018).

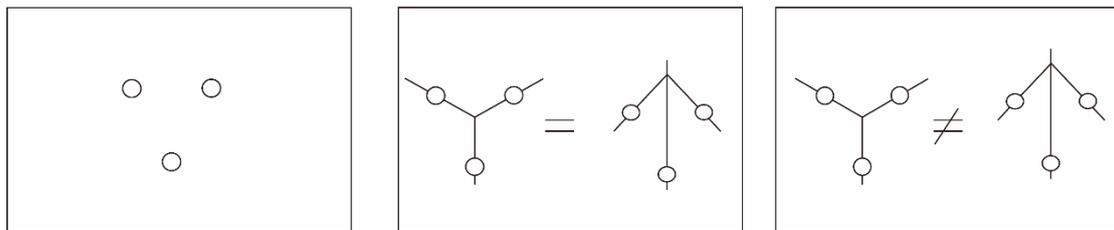


Fig. 1.2 The difference between two-dimensional landscape (left), topology network (middle) and unidirectional network (right) (modified from Peterson et al., 2013).

Under acknowledging rivers are organized into hierarchical dendritic networks, it emphasized the importance of dispersal dynamics regulating biodiversity in river ecosystem. Spatial processes are typically related to dispersal of species between sites. They are external processes that affect local community (Heino et al., 2015). Thus, spatial factors representing the dispersal effects should also be included in community analysis. The spatial variables express the spatial position of a site or distances between sites irrespective of local environmental conditions. They have been described by Principal Coordinates of Neighbor Matrices (PCNM) based on a Moran's Eigenvector Map (Borcard et al., 1992). They can illustrate spatial relations among sites at multiple scales, which is commonly used to describe species dispersal processes (Curry and Baird, 2015).

1.1.2 Flow regime

The river network is not static rather a fluvial ecological network; it changes with flowing water. The pivotal role of river's flow regime for sustaining biodiversity and ecological integrity receive fully awareness (Poff, 1997, 2018). Ecological processes in the river are governed by patterns of temporal variations in river flows, as hydrological conditions fundamentally govern the establishment, growth, reproduction, dispersal, and extinction of many freshwater organisms (Bunn and Arthington, 2002). Firstly, flow is a major determinant of physical habitat in streams, which in turn is a major determinant of biotic composition. Secondly, aquatic species have involved life history strategies primarily in direct

response to the natural flow regime. For example, the scale of spawning and recruitment redundancy for ecological resilience under overall habitat availability (Lytle and Poff, 2004).

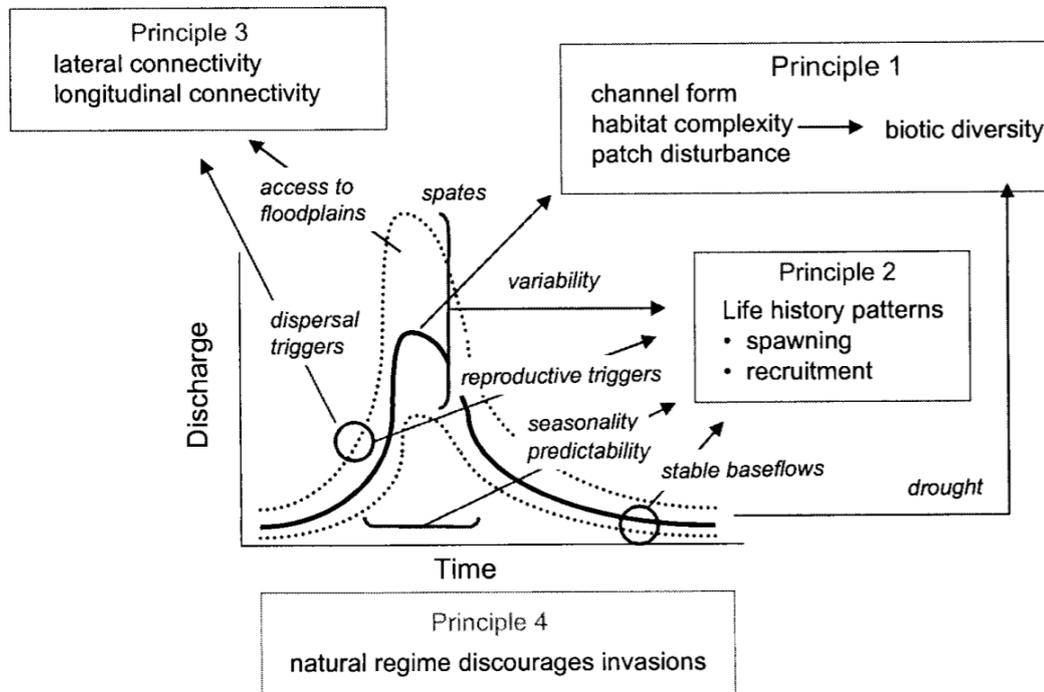


Fig. 1.3 Graphical representation of natural flow regime influence aquatic biodiversity over different aspects. (modified from Bunn & Arthington, 2002)

Thirdly, maintenance of natural patterns of longitudinal and lateral connectivity play indispensable role in ensuring the composition and population of many riverine species. As an instance, droughts and low flow events can trigger longitudinal dispersal of migratory aquatic organisms, and flooding events allow access to otherwise disconnected habitats. Finally, increasing flow alteration simplify aquatic biodiversity (Tonkin et al., 2018b) and more likely allow invasive exotic species adapted to modified habitat (Fig. 1.3).

The natural flow of a river varies on time scales of hours, days, seasons, years or longer. The characteristic pattern of river's flow quantity, timing and variability is the natural flow regime (Dunne and Leopold, 1978). Five critical components of the flow regime – magnitude, frequency, duration, timing and rate of change – directly and indirectly influence other regulators on ecological processes in river ecosystem (Poff et al., 1997, Kiesel et al., 2017 & 2019a). The magnitude of discharge is simply the amount of water moving through a fixed location per given unit time. The frequency of

occurrence refers to how often a flow given magnitude recurs over a specified time interval. The duration is the period of time associated with a particular flow event (e.g., a specific number of days by a ten-year flood, or when the flow exceeds some value). The timing of flow refers to the regularity with defined magnitude occurrence. The rate of change refers to how quickly flow changes from one magnitude to another (Poff and Ward, 1989; Puckridge et al., 1998; Walker et al., 1995). Alteration of flow thus has cascading effects on the ecological integrity of rivers (Fig 1.4, modified from Poff et al., 1997).

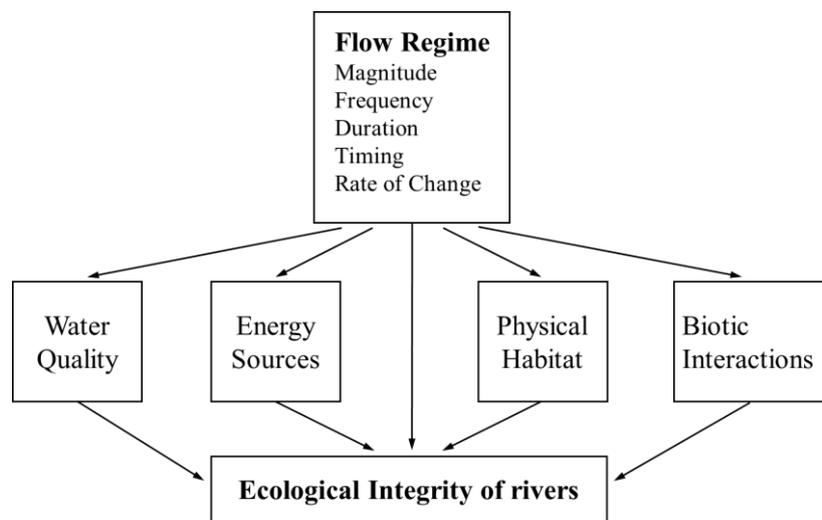


Fig. 1.4 The importance of flow regime in sustaining ecological integrity of streams. Flow regime influences streams through magnitude, frequency, duration, timing and rate of change both directly and indirectly (modified from Poff et al., 1997).

The flow regime has randomness, i.e., unexpected magnitude and timing of extreme condition, and regularity, i.e., predictability seasonal patterns (Kiesel et al., 2019a). Droughts and floods are natural disturbances of most running water ecosystem. However, the temporal dynamics will change due to climate change and the near-ubiquitous human control of river flows globally, which may have severe effects on riverine biological communities (Poff and Zimmerman, 2010; Rolls et al., 2018). Flow regime can be regarded as a direct factor affecting the community structure and function of aquatic organisms (Guse et al., 2015a). At the same time, flow regime can also be regarded as an indirect factor that affects the river ecological process by regulating water quality with dilution and changing the degree of connectivity (Kiesel et al., 2019b; Poff and Zimmerman, 2010). There is now a general agreement among scientists and many managers that in order to protect freshwater

biodiversity and maintain the river ecosystem services, we need to mimic components of natural flow variability and take into consideration of the flow events (Arthington, 2016; Lytle et al., 2017; Poff et al., 2016).

Relative interdisciplinary studies have promoted our understanding of the environmental flow guidelines and transferable hydro-ecological relations. Studies have already made innovation progress on benthic macroinvertebrate by SWAT-SDM (Soil and Water Assessing Tool – Species Distribution Model, Arnold et al., 1998; Elith and Leathwick, 2009) integrated modeling (Jähnig et al., 2012; Kuemmerlen et al., 2014). Application of the ELOHA (Ecological Limits of Hydrologic Alteration) framework identified a suite of hydrological variables that govern the structure of macrophytes and fish (Arthington et al., 2012; Kendy et al., 2009; Mackay et al., 2014). While the studies on riverine phytoplankton assemblages are still rarely reported. In this study, interdisciplinary analysis will be used to explore the quantitative and detail relationship between algae and their living hydrological conditions.

1.1.3 Land-use pattern

Rivers also have high dependency on their watershed. One major threat to rivers is the alteration of land-use patterns. Over the last 160 years, urban and agricultural land uses have changed markedly and to varying degrees across different European Ecoregions (Hurt et al., 2011). Changes in cropland and grassland occurred everywhere throughout Europe for multiple reasons, such as the cultivation of wetland regions (e.g., North Sea region along the Netherlands, German, and Danish coasts) (Ferreira et al., 2019). Together with the intensification of agricultural production, more stressors are at play, such as animal husbandry, and exponential increases in pesticide and fertilizer usage (dos Reis Oliveira et al., 2018; Wagner et al., 2016).

The impact of land-use change on water flow and riverine biota is difficult to estimate due to their complexity and interactive effects. The pristine forest land produces various habitats for various species, thus leads to high biodiversity and more healthy ecological status (Oeding et al., 2018; Wilkinson et al., 2018). On the contrary, agriculture intensification can impose a variety of stressors in streams, not only nutrient enrichment, chemical pollutants, but also augmented fine sediment inputs, increased frequency and intensity of disturbances via erosional events, lowered base flows and more

flashy flow patterns by water abstraction (Lange et al., 2014a). In addition, urban land aggravates the hydrological intensification effects by increasing peak discharges and reducing their durations (Davis et al., 2015). Land-use shifting processes summarize in the river reflecting various kinds of responses. For these reasons, land-use changes potentially cause severe biodiversity loss (Fig. 1.5), and play a critical role in mitigation of other stressors (Allan, 2004; Kremen and Merenlender, 2018).

It reported that increasing land-use intensity causes larger losses of plant and animal species richness in the arid lowlands than in humid submontane and montane zones (Peters et al., 2019). In a highly modified catchment of Australia, pasture grazing land had the poorest riverine health in the catchment followed by sugar cane and urban, while macadamia plantations and forest area were relatively healthy (Oeding et al., 2018). By simulations of land use change scenarios along a model cascade revealed that land use change leads to a stronger impact on nitrate concentrations, and further result in an increase as well as in a decrease in the abundances of macroinvertebrate species, depending on the species-specific sensitivity (Guse et al., 2015a).

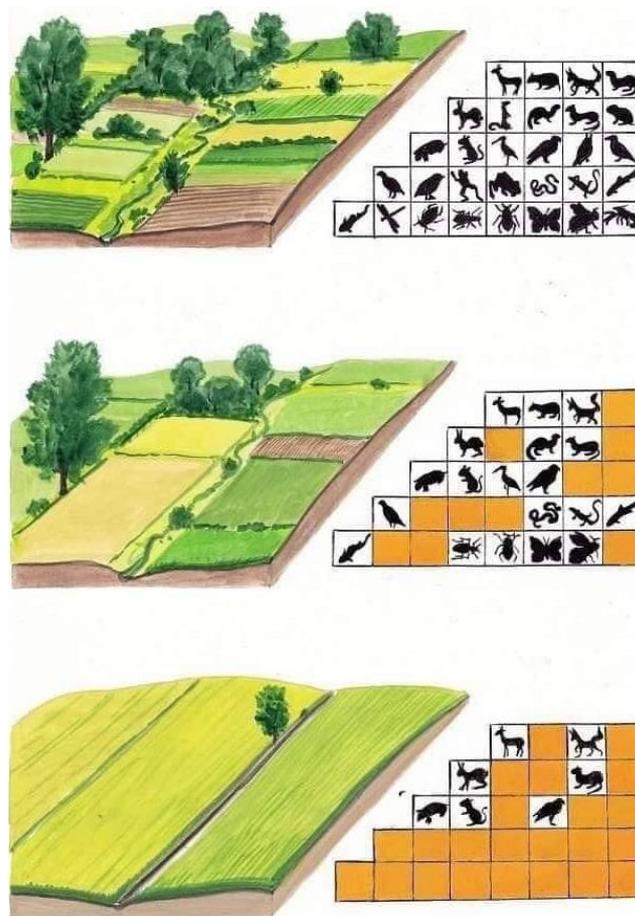


Fig. 1.5 Schematic diagram of the impact of industrial agriculture on biodiversity (modified from Potts et al., 2010).

1.1.4 Eutrophication

Eutrophication was formerly defined in reference to describe the natural aging process of lake ecosystem (Wetzel, 2001), however, now it has been used more often to refer to accelerated eutrophication of lakes, rivers, estuaries and marine waters, where the natural eutrophication process is advanced by human activities that add nutrients (Anderson et al., 2002). Eutrophication results in cascade problems in aquatic ecosystem. One dominant taxon can bloom in eutrophic waterbodies. The monotonous inedible species with poor nutritional value or unsuitable size for the consumers can disrupt the ecosystem functioning through food web. They cause oxygen depletion, and such anoxia-driven ‘vicious cycle’ enhances the bloom by efficiently recycling phosphorus from bottom sediment. The shifts result in increased costs of water treatment for public supply (Pretty et al., 2003) as well as the loss of biodiversity (Binzer et al., 2016).

Many organisms are competent bloom maker: Harmful red tide (e.g., *Cochlodinium polykrikoides* bloom) in coastal waters, invasive plants water hyacinth (*Eichhornia crassipes*), and cyanobacteria bloom (Anderson, 1997; Heisler et al., 2008; Mahamadi, 2011). Cyanobacteria is one of the most notorious ones, in particular, *Microcystis* as one of the most pervasive bloom-forming taxa (Harke et al., 2016). It can produce a diverse suite of potent and deadly cyanotoxins (Meriluoto et al., 2017), as well as other metabolites that result in taste and odor problems in drinking and recreational water supplies (Graham et al., 2010). *Microcystis* blooms can pose significant hazards on human and animal health, impair aquaculture, agricultural irrigation, lead to substantial economic damage (Carmichael and Boyer, 2016).

Natural catchment land-use changed into agriculture, industry or urban areas load excessive nitrogen (N) and phosphorus (P) to the rivers. Fertilizer application on land remains a major contributor to nonpoint nutrient pollution, and this source is still increasing at an alarming rate in many geographic regions (Michalak et al., 2013). Nutrient inputs from the catchment vary not only in quantity, but also in composition (the ratio of nitrogen and phosphorus, based on the form of fertilizer in use), and this has important implications for biotic community development (Huisman et al., 2018). Cyanobloom has not only been observed in lakes, but also reported increasing occurrence in rivers (Haakonsson et al., 2017; Lapointe et al., 2015; Philips et al., 2007; Yang et al., 2012). The linkage eutrophic lakes may

also serve as one possible reason. Introduced limnoplankton species, for instance *Microcystis*, can survive in the river regarding to the suitability of the instream environment (Otten et al., 2015). Application of pesticides in the related watershed may potentially stimulate the cyanobacteria bloom (Harris and Smith, 2016). Moreover, human activities can affect both water temperature and discharge in lotic ecosystems, favoring cyanobacterial growth (Haakonsson et al., 2017).



Fig. 1.6 (A) Bloom of *Microcystis* in River Treene, Schleswig-Holstein State, Germany. (B and C) light microscope images of diverse colony morphologies of *Microcystis* (B, *M. aeruginosa*; C, *M. viridis*, respectively) in the River Treene.

1.2 Characteristics of riverine phytoplankton

Algae, together with higher plants, some bacteria and protists are important autotrophs in lotic food web (Allan and Castillo, 2007). They are primary producers who acquire energy from sunlight and materials from nonliving sources. Usually, phytoplankton are unable to maintain populations in fast-flowing streams, but can become abundant in slowly moving rivers and backwater zone. In lowland rivers, due to the low hydraulic gradients and strong dominance of muddy, sandy substrates, phytoplankton have an essential role in the fluvial food web, and they are the focus of this study (Fig 1.7).

Phytoplankton are increasingly being used as reliable environmental indicators in rivers globally (Wu et al., 2017), because they strongly respond to environmental changes. As for indicators of multiple stressors, impacts reflect on their abundance, biomass, biodiversity, and directly influence the provision of ecosystem services. The lowland riverine phytoplankton community serves as one of the important primary producers, which initiate and sustain the function of the whole ecosystem. At the

same time, they firstly and foremost receive and response to the natural and anthropogenic impacts. The estimation of the pattern of phytoplankton community traditionally includes their density, biomass, species composition, species richness and Shannon-Wiener diversity. Recently, more indexes of algae have evolved for better understanding their conditions, variations and monitoring rivers under changing environment. The indexes considered not only taxonomic diversity but also functional diversity, and across spatial scales from local community to regional metacommunity.

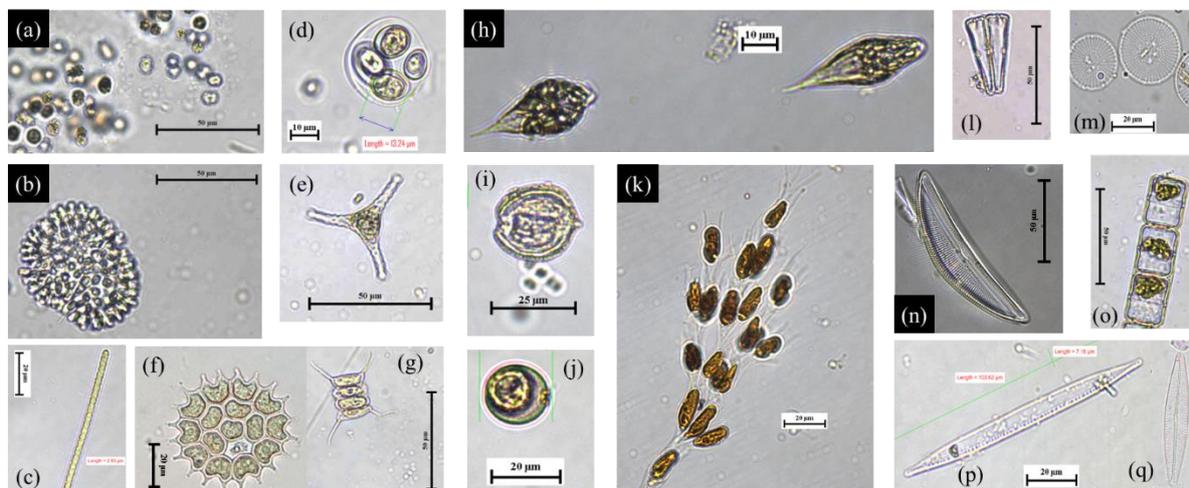


Fig. 1.7 Some typical phytoplankton species observed in the River Treene. (a) *Microcystis aeruginosa*; (b) *Woronichinia naegeliana*; (c) *Aphanizomenon flos-aquae*; (d) *Oocystis lacustris*; (e) *Staurostrum tetracerum*; (f) *Pediastrum boryanum*; (g) *Desmodesmus communis*; (h) *Euglena agilis*; (i) *Phacus alatus*; (j) *Trachelomonas volvocina*; (k) *Dinobryon sertularia*; (l) *Meridion circulare*; (m) *Stephanodiscus Hantzschii*; (n) *Amphora ovalis*; (o) *Melosira varians*; (p) *Nitzschia intermedia*; (q) *Navicula lanceolata*.

1.2.1 Phytoplankton functional groups

The phytoplankton functional group (PFG) classification method was introduced by Reynolds (2002), based on dominant or co-dominant algae species in a given environment, from their physiological, morphological and ecological attributes. This method summarized 37 assemblages (Table 1.1) according to their similar sensitivities and tolerance characteristics (Borics et al., 2007). This approach reduced large numbers of species by pooling them into several descriptive groups containing mutual adaptations, meanwhile not losing the information embedded in detailed species taxonomic lists (Padisák et al., 2009; Reynolds, 2006). Over the last few decades, PFG classification has been applied worldwide in different types of aquatic ecosystems from lakes (Crossetti et al., 2013; Tsai et al., 2014) to rivers (Abonyi, 2014; Abonyi et al., 2012). The traits-based phytoplankton functional groups are highly suggested as the robust bio-indicators for a better understanding of the

current ecological status (Padisák et al., 2005; Salmaso et al., 2015; Tapolczai et al., 2017).

Table 1.1 Descriptions and representatives of common phytoplankton functional groups which appeared in rivers, modified from Borics et al., 2007.

Codes	Described habitat from the reference	Representative species (genera) in the study area
A	Clear, well-mixed, base poor lakes	<i>Brachysira brebissonii</i> , <i>Cyclostephanos invistatus</i> , <i>Cyclostephanos dubius</i>
B	Vertically mixed, mesotrophic, small-medium lakes	<i>Stephanodiscus minutulus</i> , <i>Stephanodiscus neoastrea</i> , <i>Stephanodiscus parvus</i>
C	Well mixed, eutrophic small-medium lakes	<i>Asterionella formosa</i> , <i>Aulacoseira ambigua</i> , <i>Cyclotella meneghiniana</i>
D	Shallow, enriched turbid waters, including rivers	<i>Nitzschia</i> , <i>Ulnaria acus</i> , <i>Stephanodiscus hantzschii</i>
F	Clear epilimnia	<i>Kirchneriella obesa</i> , <i>Oocystis lacustris</i> , <i>Nephrocytium aghardianum</i> ,
G	Short, nutrient-rich water, small pools	<i>Eudorina elegans</i>
H1	Dinitrogen fixing Nostocleans	<i>Aphanizomenon flos-aquae</i> , <i>Aphanizomenon issatschenkoi</i>
J	Shallow, enriched lakes, ponds and rivers	<i>Scenedesmus</i> , <i>Desmodesmus</i> , <i>Crucigeniella</i> , <i>Pediastrum</i>
K	Short, nutrient-rich columns	<i>Aphanothece</i> , <i>Aphanocapsa</i>
Lo	Summer epilimnia in eutrophic lakes	<i>Woronichinia naegeliana</i> , <i>Merismopedia tenuissima</i> , <i>Peridiniopsis cunningtonii</i>
M	Small eutrophic lakes	<i>Microcystis</i>
N	Mesotrophic epilimnia	<i>Tabellaria flocculosa</i> , <i>Cosmarium granatum</i> , <i>Staurastrum tetracerum</i>
P	Eutrophic epilimnia	<i>Aulacoseira granulata</i> , <i>Closterium</i> , <i>Staurastrum chaetoceras</i>
S1	Turbid mixed layers	<i>Planktolyngbya linnetica</i> , <i>Planktothrix agardhii</i> , <i>Limnothrix redekei</i>
S2	Shallow, turbid mixed layers	<i>Spirulina</i>
TB	Highly lotic environments including rivers and rivulets	<i>Navicula</i> , <i>Achnanthes</i> , <i>Surirella</i>
W1	Small, organic ponds	<i>Euglena</i> , <i>Phacus Curvicauda</i> , <i>Lepocinclis acus</i>
W2	Shallow, mesotrophic, well mixed lakes	<i>Trachelomonas</i>
X1	Shallow well mixed layers in enriched condition	<i>Ankistrodesmus falcatus</i> , <i>Monoraphidium contortum</i>
X2	Mesotrophic, shallow well mixed layers	<i>Chroomonas acuta</i> , <i>Cryptomonas erosa</i> , <i>Cryptomonas ovata</i>
Y	Usually small well mixed enriched lakes	Large microflagellates (<i>Cryptomonas</i>)
Z	Clear, mixed layers	<i>Rhabdoderma lineare</i>

1.2.2 Algal traits

In addition to species composition, ecologists have recently started to investigate traits composition because it reflects the functional adaptation of organisms to the environment (Leonilde et al., 2017; Litchman and Klausmeier, 2008; Soininen et al., 2016). Usually traits are divided into two types: ecological traits (related to habitat preferences, like pH, oxygen and temperature tolerance, tolerance to hydrologic condition and organic pollution, etc.) and biological traits (e.g., life history, motility and nitrogen fixation behavioral and morphological characteristics, such as reproductive strategies, motility, cell size, life form; Table 1.2).

Table 1.2 Algae biological traits and their categories from morphological, physiological, behavioral and life-history, modified from Lange et al., 2015

	Functional traits	Categories
Morphology	(1) Cell size	pico (< 5 μm^3)
		nano (5-100 μm^3)
		micro (100-300 μm^3)
	(2) Life form	meso (300-600 μm^3)
		macro (600-1500 μm^3)
		very large (>1500 μm^3)
		Colonial
		filamentous
	(3) Guild	flagellate
		unicellular
		attachment_low
Physiology	(4) Nitrogen fixation	attachment_med
		attachment_high
		yes
Behavior	(5) Motility	no
		attached
		gliding
Life-history	(6) Reproductive technique	drift
		fission
	(7) Spore formation	fragmentation
		no forming spores
		zoospores
		akinetes
		oospores and zygospores

1.3 Motivations

1.3.1 Research gaps

River ecosystem is influenced by natural process and anthropogenic impacts at different spatial and temporal scales (Kiesel et al., 2019b). To shed light on the potential responses of aquatic community to changing environment, the characteristics of the catchment landscape and hydrological processes are important factors in the investigation. It has been aware that the alteration of natural flow regime dramatically affects river species (Poff, 1997). However, the current studies still have potential for improvements. Firstly, relative relationship studies only considered a few easy achievable variables like water level or river depth to represent the hydrological condition (Ji et al., 2017; Leland, 2003; Li et al., 2017). Secondly, the temporal scale of the parameters is misused. Contemporary or annual parameters maybe not appropriate enough to reflect the ecological responses at different life stage to hydrological events (Arroita et al., 2017; Kakouei et al., 2017), considering the time lags of their communities structuring process. Thirdly, the representative biological variables need to be analyzed more in detail. It is of relevance to analyze species appearance or density of course, however the specific responsible survival strategies or life history patterns may lead to better explanations (Jarić et al., 2019; Radinger et al., 2016). Several questions remain unclear: Which specific variables of flow alteration have a robust contribution to the community transformation? Do the changes have an impact on species, or more on a group of algae with similar functions, or actually act on particular biological traits? How the functional groups or biological traits composition respond to the flow alteration? Such issues raise my interests for further analyses and discussions in this thesis.

Regarding the land-use and land-cover characteristics, these factors can bring a series of potential impacts, such as water eutrophication, pesticide input, increased suspended solids, riparian vegetation changes, artificial changes in original river characteristics. Such multiple potential effects with complex interactions may bring unpredictable responses. Relevant studies on pesticides and nutrients under various land-use patterns have been conducted (Fohrer et al., 2014; Haas et al., 2016; Ulrich et al., 2018), while the direct and indirect cause-effect relationship of land-use condition and riverine phytoplankton community pattern has been scarcely explored. In addition, water body is also an important land-cover category for aquatic species. Especially, in a lentic-lotic continuum, lakes may act as purifying pollutants or adding new species to the downstream of the river.

To our knowledge up to now, there is quite limited research on the interplay of these stressors on riverine phytoplankton. Moreover, there are currently not enough research activities on the modulations from river network, alteration of flow regime, land-use pattern and instream physicochemical condition jointly. Furthermore, most of the existing river research dedicates on fishes, macrophytes, benthos, diatoms. Studies with a focus on phytoplankton are still lacking. However, the importance of phytoplankton in maintaining lowland river ecosystems cannot be ignored. The assessment of river algae should include cyanobacteria and other groups of algae, not just diatoms (Kelly, 2013; Lange et al., 2016).

1.3.2 Research questions

In summary, the fitness of riverine organisms seldom reflects pressures from a single source, and human pressures often alter more than one environmental factor. The theme of “multiple stressors” was intended to epitomize the challenges faced by rivers, since they are particularly vulnerable to external changes. A certain environmental stressor in river ecosystems rarely occurs alone. They often act jointly to produce complex interactions ranging from synergistic to antagonistic (Sabater et al., 2019a). In such situation, predicting the responses of rivers to changing environment is challenging and prospective because of the complexity of interactions among man-made drivers across natural hydrogeomorphic settings. Meanwhile, the projections based on environment alteration-ecological response relationship are important for stakeholders and decision makers. An interdisciplinary of hydrology and ecology urgently needs to be improved and promoted, by raising meaningful implications to propose suitable protection and management measures.

The major aim of this study is to reveal the responses of phytoplankton community patterns under multiple stressors. The main research questions of the research are:

- (1) *How high is the contribution of spatial factors for the riverine phytoplankton communities compared to local factors in the study area?*
- (2) *How does the flow regime act in structuring the algal taxonomic and functional compositions?*
- (3) *How strongly is the downstream riverine phytoplankton community influenced by the upstream eutrophic lentic waterbodies?*
- (4) *What are the causal relationships between multiple stressors and algal communities?*
- (5) *What are the possible responses of phytoplankton community to multiple stressors?*

1.4 Introduction of methods

In this thesis, the study river Treene located in Schleswig-Holstein state of Germany belonging to northern lowland region of Europe (Fig. 1.8). It is the largest tributary of the Eider River in a temperate climate zone influenced by marine climate, with mild temperature and high precipitation in winter. River substrate is mainly sand and gravel. The water flow is on average 3 m³/s for a catchment area of 481 km² (at catchment outlet Treia). The land cover is dominated by agriculture and pasture (together around 80%). Most of the wetlands are drained and the channels are anthropogenically modified. The northeast highland in the upstream part of the Treene catchment is characterized by slightly slopes and more fertile soils, allowing a cultivation of high value crops, such as wheat, barley and rapeseed (Eastern Hillands). The southwest part of the watershed is characterized by poorer sandy soils and low fertility with a higher percentage of pasture (Geest landscape). Several lakes are linked with the river in the catchment. The three largest ones are Sueden See (0.64 km²), Sankermarker See (0.56 km²) and Winderatter See (0.24 km²). The major tributaries are Bondenau, Kielstau, Bollingstedter Au, Jerrisbek, Juebek. The sub-catchment Kielstau has been appointed to an UNESCO Ecohydrological Demonstration (Fohrer and Schmalz, 2012). The Soltfeld and Treia gauging stations are part of the official gauging network of the Federal State Schleswig-Holstein. The eco-hydrological SWAT model has been well tested in the study area confirming the reliability for the further studies (Guse et al., 2014; 2015a; 2015b; Haas et al., 2016; 2017). Long-term spatial patterns of hydrological variables in a daily resolution was provided from the model results.

In order to have a better understanding of the current situation of this river and its relative watershed, long-term systematic monitoring and modelling was conducted for the lowland catchment Treene in North Germany. Studies involves various aspects of this watershed. Previous studies have covered the herbicides (Fohrer et al., 2014; Ulrich, 2010), nitrate (Guse et al., 2015b; Haas et al., 2017; Haas et al., 2016), hydrology (Guse et al., 2017; Guse et al., 2014; Kiesel et al., 2019a) and land-use (Dey, 2004; Haas et al., 2017) patterns, which regarding to abiotic part. There also biotic investigations for fish and macroinvertebrates (Guse et al., 2015a; Jähnig et al., 2012; Kail et al., 2015) with the application of ecohydrological modeling. The studies about phytoplankton carried out by Wu et al. (2018; 2014; 2012) has simulated the daily dynamic of chlorophyll *a* and developed a phytoplankton

index of biotic integrity (P-IBI) to assess effects of human disturbances in a sub-catchment of the study area.

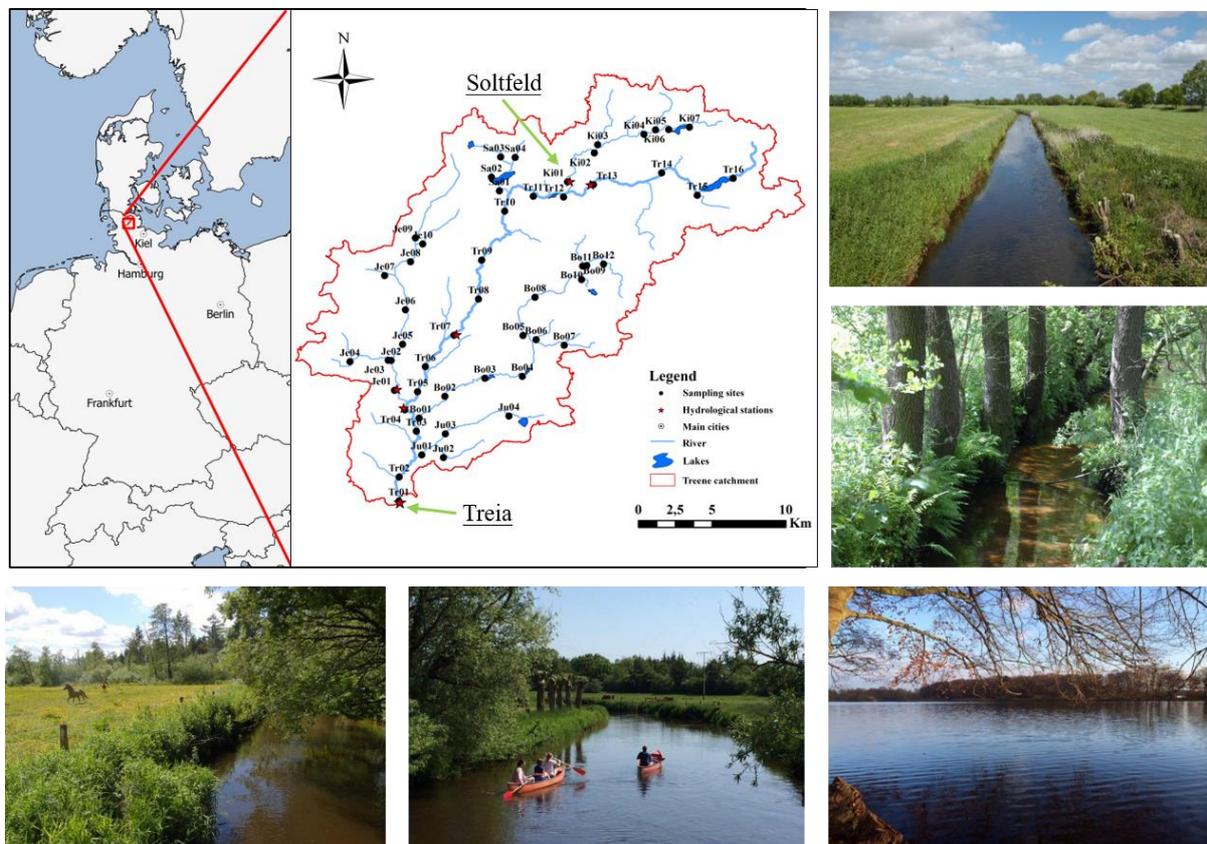


Fig. 1.8 The research area Treene catchment in northern Germany with various landscapes

Focusing on the overall research objective and the key research questions, studies of this thesis have combined with the background hydrological model (SWAT) to analyze the alteration of flow regime, using the investigations of river geomorphic condition and catchment land-use conditions from the government recording, and the field sampling in the study area. The combination of historical monitoring and simulated data with *in situ* monitoring data gives a comprehensive data processing, statistical analyzing and integrated model establishing. The phytoplankton community status has described from species, functional groups, functional traits, local and regional diversity, as various perspectives to handle their responses to coupling processes of river spatial factors, flow alteration, land use and eutrophication. With the help of redundancy analysis (RDA), variation partitioning analysis (VP), classification and regression tree (CART) model, structural equation model (SEM), random forest (RF) model, and general linear mixed model (GLMM) as statistical methods, the factors individual and interactive contributions on phytoplankton community are analyzed. This method route

(Fig. 1.9) makes a better understanding on the main influencing factors among multiple stressors, abiotic and biotic factors relationships, and the potential response of phytoplankton community structure on under different scenarios.

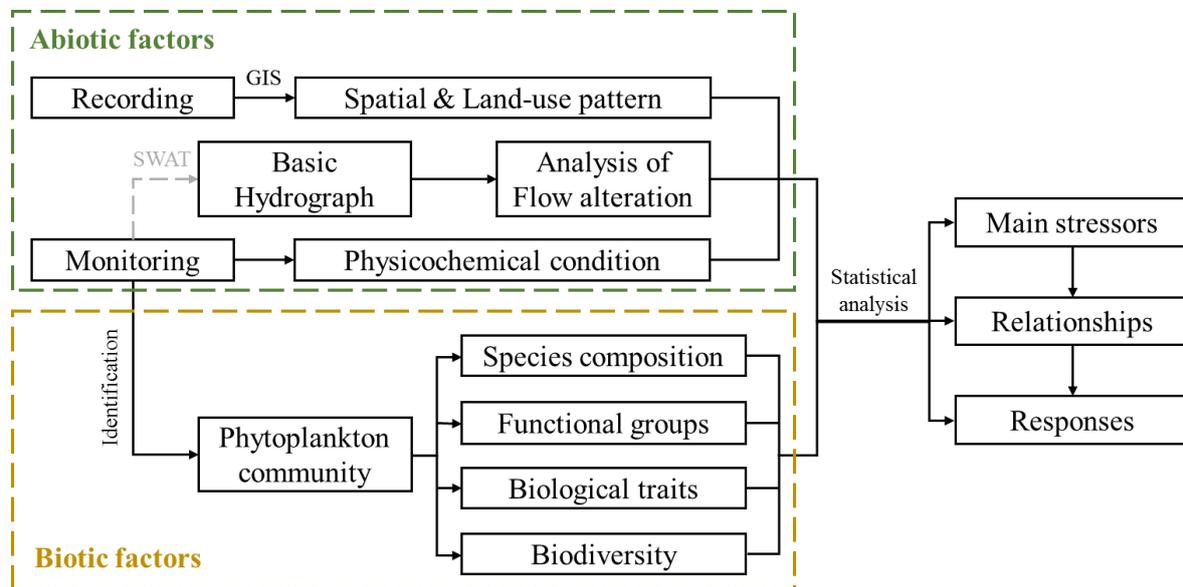


Fig 1.9 The method route of this thesis. More details of the methods description can be found from Chapter 2 to Chapter 6 in the methods part. The black line is conducted by the author. The grey dotted line is based on previous works.

Chapter 2 Riverine phytoplankton shifting along a lentic-lotic continuum under hydrological, physicochemical conditions and species dispersal

Yueming Qu^{1*}, Naicheng Wu^{1,2,3*}, Björn Guse^{1,4}, and Nicola Fohrer¹

¹ Department of Hydrology and Water Resources Management, Institute for Natural Resource Conservation, Kiel University, 24118 Kiel, Germany

² Aarhus Institute of Advanced Studies, Aarhus University, Høegh-Guldbergs Gade 6B, 8000 Aarhus C, Denmark

³ Department of Bioscience, Aarhus University, Ole Worms Allé 1, 8000 Aarhus C, Denmark

⁴ GFZ German Research Centre for Geosciences, Section Hydrology, Potsdam, Germany

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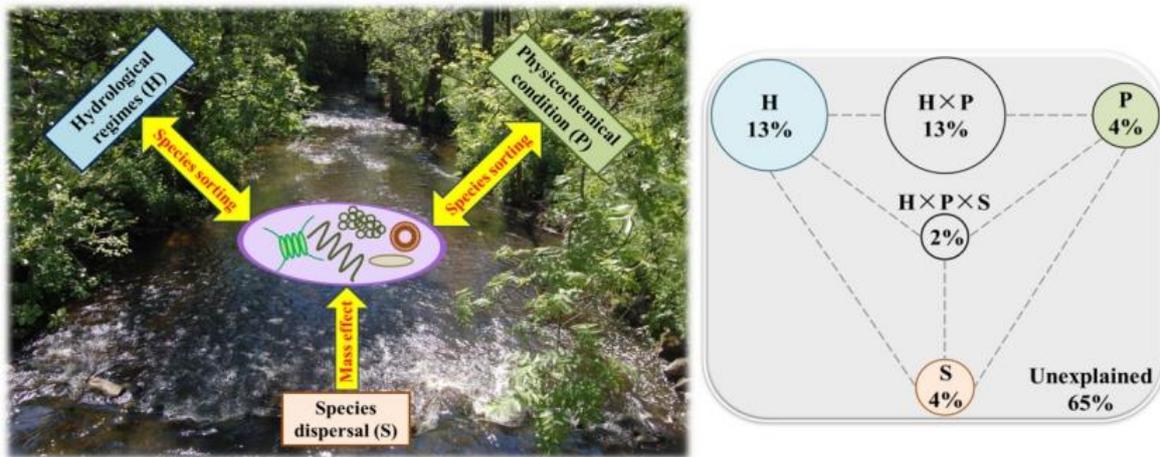
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Abstract

The importance of phytoplankton-based bio-assessment has been recently recognized in lowland rivers which are affected by multi-environmental factors. However, some basic questions remain unclear to date, such as: (i) spatial and temporal variations of phytoplankton, (ii) the impact of upstream lakes on downstream community, (iii) the main drivers for species composition or (iv) the regional biodiversity along a lentic-lotic continuum. To answer these questions, we collected and analyzed the fluvial phytoplankton communities along a lentic-lotic continuum from a German lowland catchment, where a well-established ecohydrological modelling predicted long-term discharges at each sampling site. Our results revealed very high spatial and temporal variations of phytoplankton community. The changes of a lake on downstream phytoplankton assemblages were significant, especially the nearest reach after the lake. However, these influences varied along with seasons and limited in a relatively short distance to the lake. Redundancy analysis and Mantel tests showed that phytoplankton composition and dissimilarities along the lentic-lotic continuum attributed more to local hydrological and physicochemical variables than species dispersal, which confirmed the suitability of lowland phytoplankton-based bioassessment. In addition, our findings highlighted the importance of flow regime in shaping phytoplankton community composition and regional beta diversities. This study emphasized the necessity to include the hydrological variables and their relationship with phytoplankton community in future bio-monitoring investigations.

Keywords: Riverine phytoplankton, Hydrological regimes, Physicochemical condition, Species dispersal, Lentic-lotic continuum, Lowland river

Graphical abstract



Highlights

- Phytoplankton community shows extremely high spatial and temporal variations.
- Lentic water body has significant effects on downstream phytoplankton community.
- Flow regimes impact algal local composition and regional beta diversities.
- Hydrological and physicochemical factors contribute more than dispersal in study area.

2.1 Introduction

Multiple stressors resulting from intensive anthropogenic activities are affecting the global water resources significantly (Hering et al., 2015). Generally, they include flow regime alteration, diffuse and point sources. For example, flow diversion due to dam construction disrupts the river's natural connectivity and impedes the cycling of organic matter, sediments and nutrients from upstream to downstream (Wu et al., 2012b). Flow conditions may determine the physical habitat conditions and directly or indirectly affect many other physicochemical variables which are key factors regulating ecological processes in aquatic ecosystems (Bhat et al., 2010). In addition, river eutrophication, which is often associated with diffuse (agriculture) and point source pollution (sewage), has exerted additional stressors for aquatic ecosystem (Hilton et al., 2006). This pollution leads to increased costs of water treatment for public supply (Pretty et al., 2003) as well as loss of biodiversity (Binzer et al., 2016). Since human-mediated disturbances and their effects become a pressing focus, it is essential to understand how the interaction between multiple stressors and their impacts on water quantity and quality issues as well as aquatic organisms, because these are fundamental for prioritizing global and regional conservation efforts and achieving sustainability of freshwater resources (Piggott et al., 2012). However, the combined effects of multiple, simultaneously operating stressors on a comprehensive set of aquatic organisms, particularly for algal communities are still to be studied (Lange et al., 2016).

Riverine phytoplankton are valuable bio-indicator for assessing water quality in aquatic systems since their irreplaceable role and sensitivity to multiple ecological stressors (EU, 2013; Pasztaleniec and Poniewozik, 2010; Wu et al., 2014; Zeng et al., 2017). In lowland rivers, phytoplankton instead of benthic algae serves as an important primary producer, due to the low hydraulic gradients and a strong dominance of muddy, sandy substrates (Wu et al., 2011; 2014). Although their important roles as bio-indicator were recognized recently (Stevenson et al., 2010; Thomas et al., 2016), the occurrence and distribution of phytoplankton in rivers are still unclear. It was believed that there was no true riverine plankton and the pelagic algae found in rivers which originate from either upstream lentic waterbodies or the periphyton (Hötzel and Croome, 1999). However, recent studies (Centis et al., 2010; Wu et al., 2011) argued that benthic diatom communities as the source of the riverine pelagic algae might be too simplistic and that the long retention time in lowland rivers allowed the reproduction of phytoplankton communities and development of substantial populations *in situ*. Various sources could be initial

reasons which lead to various algae species composition along the river (Gillett et al., 2016). The effects of upstream lake in the river system and the dynamics of the lentic-lotic linkage ecosystem attracts ecologists' increasing attentions (Arp and Baker, 2007; Jones, 2010; Ellis and Jones, 2013). Linkage lakes act as either sink or source for phytoplankton in river systems (Bridgeman et al., 2012; Miller and McKnight, 2015). A eutrophic lake in the upper stream may lead to serious problems in downstream river ecology. Lacustrine bloom-forming toxic Cyanobacteria (e.g. *Microcystis aeruginosa* Kützing) can be transferred to the river downstream which heavily impacted downstream river water quality (Jacoby and Kann, 2007; Yu et al., 2015). In this situation phytoplankton are definitely influenced by multiple factors, including hydrological regimes, physicochemical variables, and species dispersal during their floating processes.

One of the primary goals of ecological surveys is to describe the temporal and spatial distribution in relation to the abiotic factors. The relationships between aquatic organism and abiotic factors have been studied for a long time (Hering et al., 2006; Heathwaite, 2010; Mantyka - pringle et al., 2012). However, previous studies and monitoring investigations primarily focused on local physicochemical variables, especially on nutrients loading (Larson et al., 2007; Wang et al., 2016). The flow alteration and geographic characteristics also affected the structure and function of aquatic ecosystem (Lytle and Poff, 2004; Heino et al., 2015; Dong et al., 2016). Nevertheless, most of the publications about flow-ecology relationship preferred to prospect fish or invertebrate communities as targets (Stewart et al., 2014; Guse et al., 2015b; Kiesel et al., 2015) rather than focus on riverine phytoplankton community patterns.

In our research area, a lentic-lotic linkage lowland catchment, we are also facing this similar situation: very few studies investigating the relationships between algae community and abiotic factors. To find out the shaping factors, we examined fluvial phytoplankton community patterns from different levels of biological aspects, which were not only overall indexes, but also their spatiotemporal distribution in local and regional scales. In this study we ascertained following questions:

- (1) How large is the influence of upstream lake in the lentic-lotic linkage system among the spatiotemporal dynamics?
- (2) Which of hydrological regimes, physicochemical variables and species dispersal factors, is the key driving factor for shaping the lowland phytoplankton communities at local and regional scale?

2.2 Methods

2.2.1 Study area and field sampling

River Treene catchment (481 km² at the catchment outlet Treia) is part of the lowland area located in the State of Schleswig-Holstein in north Germany. Agriculture is the dominant land use in the study area (80%), and this sand-bed river is naturally heavily meandering with a small altitude gradient (elevations range from 2 to 80 m). The Bondenau, as one of its original tributaries, contains a lake (Sueden See, 0.64 km²) in the upstream. Our field samplings were conducted seasonally from December 2014 to September 2015, and 16 sites along the mainstream were visited each time. The sites were placed evenly under consideration of the lake and tributaries. The number count along the longitudinal axis of rivers from the upstream to outlet is T01-T16 (Fig. 2.1).

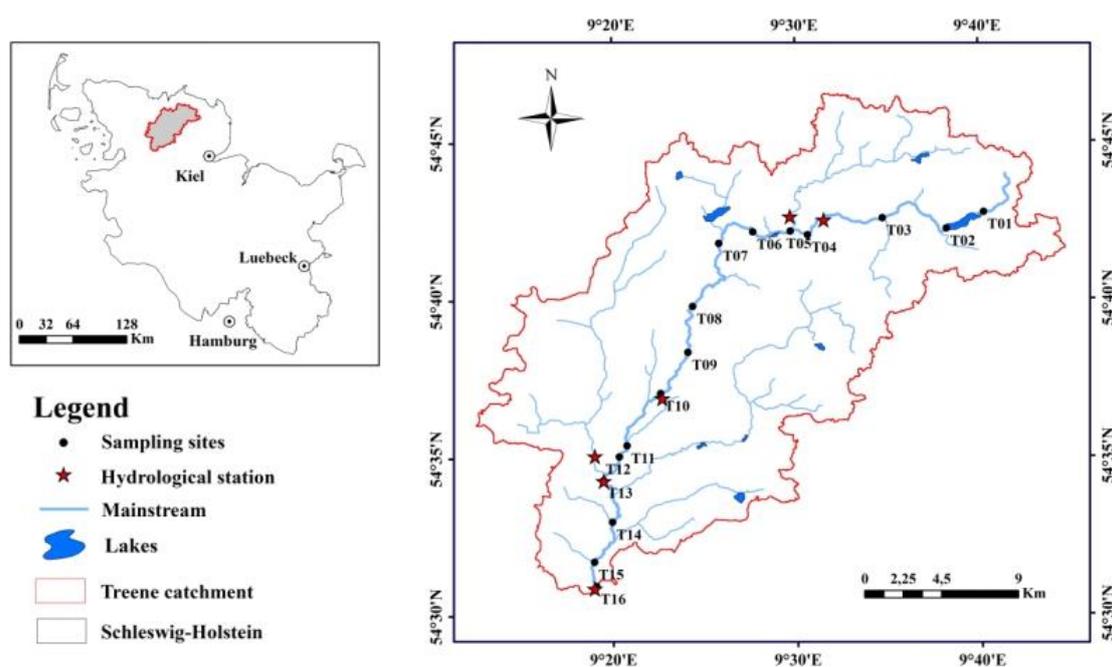


Fig. 2.1 The 6 hydrological stations and 16 sampling sites of Treene catchment (right) in the Schleswig-Holstein State (left) of Germany.

2.2.2 Physicochemical and biological measurements

At each site, water temperature (WT), pH, electric conductivity (EC), and dissolved oxygen (DO) of the surface water were measured *in situ* using Portable Meter (WTM Multi 340i and WTW Cond 330i, Germany). River width, water depth and flow velocity (FlowSens Single Axis Electromagnetic Flow Meter, Hydrometrie, Germany) were surveyed simultaneously. At the same time, water samples

were collected for nutrients analysis. They were partially filtered through GF/F glass microfiber filter (Whatmann 1825-047) to remove and collect the total suspended substances. Both filtered and unfiltered samples were kept frozen at -20°C until measurements. The concentrations of total phosphorus (TP), phosphate-phosphorus (PO_4^{3-}P), ammonium-nitrogen ($\text{NH}_4^+\text{-N}$), nitrate-nitrogen ($\text{NO}_3^-\text{-N}$), nitrite-nitrogen ($\text{NO}_2^-\text{-N}$), chloride (Cl^-) and sulphate (SO_4^{2-}) were measured according to the standard DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung) methods. DIN is the sum of nitrite ($\text{NO}_2^-\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$) and ammonia ($\text{NH}_4^+\text{-N}$). Nitrogen to phosphorus ratio (NPR) is the ratio of DIN and TP. Samples for phytoplankton analysis were collected using a plankton net with a mesh size of 20 μm . A known volume of water was filtered and fixed immediately by neutral lugol's solution. Algae samples were concentrated to 25 mL for further processing after natural sedimentation in the laboratory.

For the soft algae (non-diatom) identification, algae were counted with optical microscope (Nikon Eclipse E200-LED, Germany) at 400X magnifications in a Fuchs-Rosental chamber. The counting unit was individual (unicell) and at least 300 units were counted for each sample. Taxonomic identification of species was done according to the introductions of Hu (2006) and Burchardt (2014). To identify diatoms, permanent slides were prepared after oxidization (using 5 mL of 30% hydrogen peroxide, H_2O_2 , and 0.5 mL of 1 M hydrochloric acid, HCl), and then 0.1 mL of the diatom-ethanol mix was transferred on a 24mm X 24mm cover slip. A drop of Naphrax was used to mount the slides. Diatoms were identified with the optical microscope (Nikon Eclipse E200-LED, Germany) with 1000X magnification under oil immersion, based on the key books by Bey and Ector (2013), Hofmann (2011) and Bak (2012). Algae densities were expressed as cells/L.

2.2.3 Modeling and statistical analysis

2.2.3.1 Modeling discharge

A semi-distributed hydrological model is required for this analysis. The ecohydrological SWAT model (Soil and Water Assessment Tool, Arnold et al. 1998), which is discretized in subbasins, was selected. The SWAT model provides outputs of different hydrological variables for each subbasin in a daily resolution. In this model application, the model discretization for the Treene catchment resulted into 108 subbasins. Due to the semi-distributed model type, separate subbasins were also included for the major tributaries (Guse et al., 2014). This allows a consideration of the spatial heterogeneity.

To ensure accurate model results within the entire catchment, six hydrological stations were used in a multi-site calibration as shown in Fig. 2.1 (Guse et al., 2015a). The modeling period consisted of a calibration (2001 to 2005) and a validation period (2006 to 2016) for discharge. During the model validation, we only used five stations due to an early termination of the measurements at one hydrological station in 2014. The model performance was evaluated by using three performance measures (Nash-Sutcliffe Efficiency, Percent Bias, RSR (root mean square error divided by standard deviation)) (Guse et al. 2015a). The comparison of modeled and measured discharge yielded a reliable performance for the six hydrological stations. Thus, the results of this eco-hydrological model can be used for the consecutive analyses. Daily model results were extracted for subbasins which includes one or more a sampling point. For reason of consistency, each sampling point of this study was assigned to the model results from the closest outlet of a sub-basin outlet.

2.2.3.2 Data processing and statistical analysis

We classified the abiotic factors into three categories: hydrological regimes (H), physicochemical condition (P), and species dispersal (S). They composed as three explanatory gradients for biotic patterns.

Based on the modeled daily discharge time series from the SWAT model, we calculated 57 hydrologic indices describing different aspects of the flow regime (Olden and Poff, 2003), including the other two *in situ* parameters: depth and velocity, which constituted the hydrological regimes group (H). The 14 local physicochemical parameters measured from the sampling sites were composed as physicochemical condition group (P). For species dispersal group (S), except for the coordinates (X: Latitude, Y: Longitude), Moran's eigenvector maps were used to generate species dispersal variables representing geographical positions and dispersal across the rivers. This method is a powerful approach to detect spatial structures of varying scale in response data and more flexible than other eigenvector-based approaches for irregular sampling design (Tang et al., 2013a). In brief, this method proceeds as follows: i) a geographical distance matrix as Euclidean distance between each pair of sampling sites was calculated using the *earth.dist* function in the package *fossil* in R. ii) Principal Coordinates of Neighborhood Matrix (PCNM) analysis based on the geographical distance were used to compute species dispersal representing geographical positions through the *pcnm* function in R package *vegan*. The generated eigenvectors were considered as spatial variables (i.e., PCNMs), which could reflect

unmeasured broadscale variation in the modern environment or historical factors, e.g. natural dispersal-generated patterns demonstrating internal local-scale dispersal dynamics or regional-scale migration history (Svenning et al., 2009). PCNMs are ranked in descending order based on their eigenvalues and simultaneously coded in ascending order (starting from 1), and PCNMs with large eigenvalues and small code represent broad-scale spatial pattern, while the smaller eigenvalues with large code represent fine-scale patterns. PCNMs are commonly used to describe species dispersal processes (Curry and Baird, 2015). Usually, only PCNMs with positive eigenvalues are retained as spatial explanatory variables (Tang et al., 2013b). Among the 15 PCNMs generated, eigenvalues of PCNM component 1-10 were positive and thus 12 variables (including X, Y) were included in the species dispersal group (S).

For testing the differences of phytoplankton community composition and structure among the four seasons and 16 sites, multi-response permutation procedure (MRPP) was used (function: *mrpp*; package: *vegan*). The null hypothesis was that there was no difference among the groups in a Monte Carlo randomization procedure with 999 permutations. Classification and regression trees (CART) were used to find out the relative important abiotic factors to a single biological index (function: *rpart*; package: *party*).

To calculate the explanation of unique and interaction of abiotic gradients' effect on local phytoplankton community variations, a variation partitioning canonical redundancy analysis (partial RDA) was performed. For achieving a best performance, the phytoplankton abundance matrix was Hellinger transformed (Legendre and Gallagher, 2001; Legendre and Legendre, 2012). In the meanwhile, the three sets of abiotic variables were tested significance, when using all variables in the model of explaining variations of phytoplankton communities. Afterwards, a *forward selection* (Blanchet, 2008) was proceeded to choose a parsimonious subset of explanatory variables, and then modeled multivariate community structure (function: *cor*, *anova* — package: *stats*; function: *rda*, *varpart* — package: *vegan*; function: *forward.sel* — package: *adespatial*).

Shannon-Wiener Index based on algal density was used to assess the local phytoplankton community diversity (alpha diversity) (Shannon, 2001). Furthermore, Mantel test was applied to examine the phytoplankton communities' dissimilarity in regional scale (beta-diversity) along distance matrices (Mantel, 1967). The biological matrix was generated by Bray-Curtis similarity index based

on phytoplankton relative abundance data, since the index takes into account of the differences between species and emphasizes dominant species. Distance matrices were calculated for hydrological regimes (H), physicochemical condition (P), species dispersal (S), respectively by Euclidean distances approach (function: *mantel*, *mantel.partial*, *vegdist*; package: *adespatial*).

All analyses were performed with the R software (version 3.3.3, R Development Core Team, 2017).

2.3 Results

2.3.1 Environmental variations

During the sampling period, the main environmental parameters varied widely both seasonally and spatially. The annual water temperature in the study period is 10.8 °C. The results show that the average value of pH and EC increased from winter (December of 2014) to autumn (September of 2015), from 7.81 to 8.21, and 435.19 to 539.25 $\mu\text{s}/\text{cm}$, respectively. On the contrary, nitrogen compounds (ammonium-nitrogen, nitrate-nitrogen, DIN) decreased, and showed the smallest magnitude in autumn (0.05 mg/L, 8.70 mg/L and 8.76 mg/L, respectively). For the hydrological variables, mean flow of river discharge decreased from winter to autumn, and had a greater longitudinal variation in winter than in autumn in this study area. For instance, the intraday discharge (H01) varies from 0.31 to 18.3 m^3/s with a standard deviation (SD) of 5.68 in winter, while from 0.008 to 2.31 m^3/s with a SD of 0.77 in autumn. In addition, there are similar results from the frequency of flow events. We also found high flood pulse count (H45) had more days in winter and spring time. For example, high flood pulse count for 30 days (H45) have 8 days in December 2014, 13 days in March 2015, while zero day in June and September of 2015.

2.3.2 Temporal and longitudinal variation of phytoplankton community patterns

According to our four seasonal samplings in 16 sites from the River Treene, 334 algae species were identified belonging to seven families. The number of species assigned to Bacillariophyta, Cyanobacteria, and Chlorophyta were 217, 32 and 59 respectively. The dominant species switched along the longitudinal direction and across seasons. The site T01 is located at the most upstream part of Treene River and was mainly dominated by *Navicula lanceolata* Ehrenberg and *Melosira varians* C. Agardh. The site T02 is close to T01 (~1.7 km downstream) with a lake in between, the dominant

species shifted dramatically to *Microcystis*. At the sites afterwards (T03-T16), dominant species changed back to *Navicula lanceolata* Ehrenberg and *Melosira varians* C. Agardh. In addition, *Microcystis aeruginosa* Kützing remain in species composition, while their percentage decreased generally along the lentic-lotic continuum. The differences between T01 and the sites after the lake (e.g., T02-T16) can be explained by the lake in-between.

In different seasons, the species densities and diversities also showed a great variation (Fig. 2.2 & Fig. 2.3). The average algal densities in Dec. 2014, Mar. 2015, Jun. 2015, Sep. 2015 were 1.44 million cells/L, 0.72 million cells/L, 1.48 million cells/L and 7.74 million cells/L, respectively, while Shannon-Wiener Index were 2.43, 2.70, 2.38 and 2.34, respectively. Generally, species densities downstream of the lake increased significantly compared with T01, and afterwards showed a clear decline trend (from T02 to T16) (except for Dec., 2014), particularly for Mar. and Sep., 2015 (Fig. 2.2).

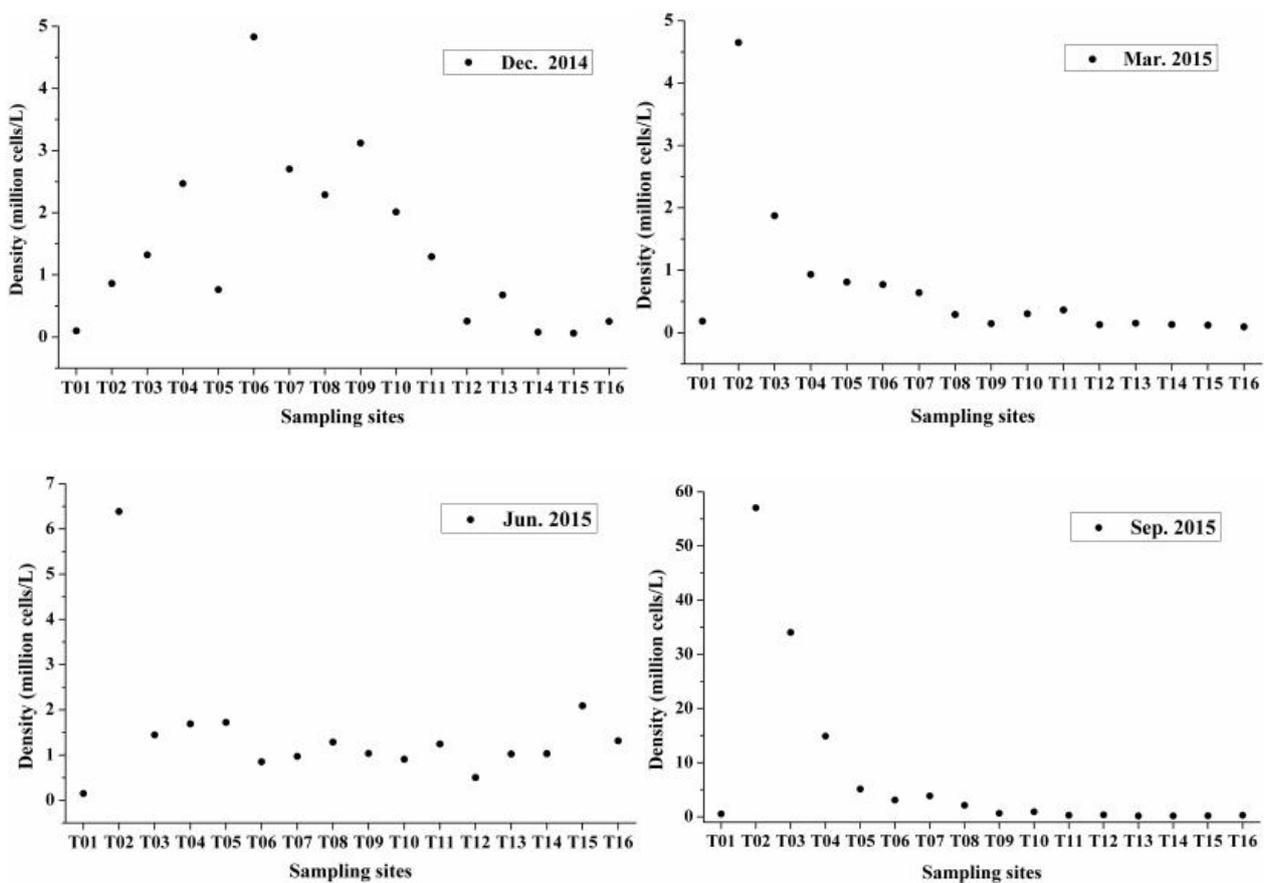


Fig. 2.2 Longitudinal variation of phytoplankton communities' density from up- (T01) to downstream site (T16) at different sampling seasons (Dec. 2014, Mar. 2015, Jun. 2015, Sep. 2015) in the Treene catchment.

Based on the Bray-Curtis similarities, we compared all the sites with T01, the site without lake impact, and we found that the similarities at T02 declined significantly and afterwards (T03-T15) stayed steady with high values without significant change. Furthermore, based on the MRPP results, which compared the community composition between T01 and the other 15 sites, in four times sampling, only T01 and T02 have significant dissimilarity ($A=0.1475$, $p=0.036$ based on MRPP), while the differences between T01 and other 14 sites (T03-T16) were nonsignificant respectively ($p>0.05$, based on MRPP). Thus, we could conclude that the impact of the lake at downstream sites was constrained to T02 and other sites were less affected. On the other hand, we also found that the phytoplankton community species composition and abundance in four seasons showed a significantly difference ($A=0.1519$, $p=0.001$ based on MRPP) demonstrating a significant temporal dynamic.

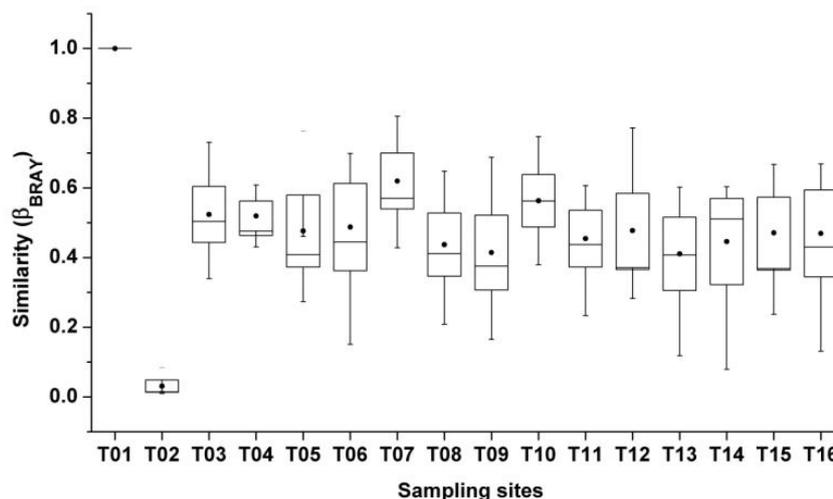


Fig. 2.3 Phytoplankton community similarity in the main stream of River Treene (the similarity calculation for all sites are compared with T01).

2.3.3 Driving factors for phytoplankton communities

2.3.3.1 Driving factors for phytoplankton density

With CART, the NPR was selected as the most important key factor for phytoplankton density during the one-year seasonal investigation, and the threshold value was 41.16 (Fig. 2.4). Samples were separated into two groups. The mean density was 1.10 million cells/L in Group 1 with $NPR \geq 41.16$, while, Group 2, with $NPR < 41.16$, were made up of larger density samples (the average value is 17.1 million cells/L) with a high portion of Cyanobacteria. On the other side, phytoplankton diversity gradients were classified into four groups by three potential abiotic factors: skewness of 30 days (H37), variability flows of 3 days (H11), and dissolved inorganic nitrogen (DIN). Their thresholds were 0.9,

0.006, 11.26, respectively (Fig. 2.5). Sites in Group 4 shared the characteristic of $H37 < 0.9$. Compared with the others, the Group 4 has the highest diversity, and samples came all from spring (campaign on March 2015). However, Group 1 with least diversity has been shaped by less nitrogen concentration and higher flow alteration.

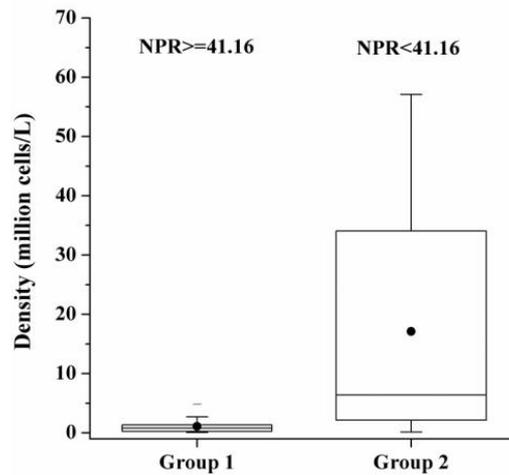


Fig. 2.4 Partition the variation of phytoplankton density by classification and regression tree (NPR represent for nitrogen to phosphorus ratio. NPR separate phytoplankton communities in two groups based on the magnification of density)

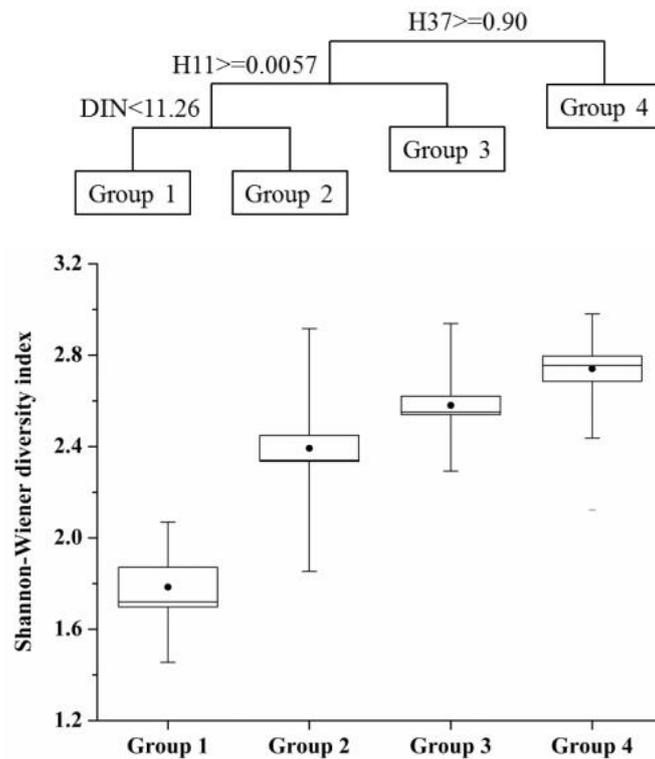


Fig. 2.5 Partition the variation of phytoplankton diversity by classification and regression tree ($H37$ represents skewness of 30 days, $H11$ represents variability flows of 3 days, DIN represents dissolved inorganic nitrogen. Shannon-Wiener diversity index were used for evaluating phytoplankton diversity variation)

2.3.3.2 The importance of explanatory variables on local phytoplankton community

When we consider the whole year, there are four hydrological (H) variables (i.e., mean flows of 30 days, skewness of 30 days, high flood pulse count for 30 days, and the rate of change in flow events from 30 days), and six physicochemical (P) variables (i.e., WT, Cond, PO_4^{3-}P , $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$) were selected by *forward selection*. The variance partitioning analysis (Fig. 2.6) showed that 12% of the total variation in the phytoplankton data could be explained by pure hydrological variables, which was much higher than that by pure physicochemical variables (4%). Whereas, the interaction between hydrologic indices and physicochemical property influenced the variation of phytoplankton and explained 13%, also stands a relatively important part. Species dispersal factors explained only 3%. Interaction of the three factors ($\text{H} \times \text{P} \times \text{S}$) was 2.2%, and a total of 65% variation was still left unexplained.

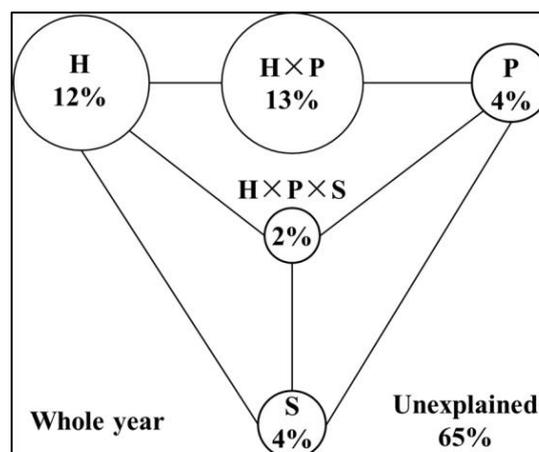


Fig. 2.6 Contribution of abiotic factors to local phytoplankton community (H represents hydrological variables, P represents physicochemical variables, S represents species dispersal).

2.3.3.3 Driving factors for regional phytoplankton communities

Mantel tests showed that the relative importance of multiple factors to phytoplankton dissimilarities (Bray-Curtis index) varied among seasons (Table 2.1). Based on the entire year data, phytoplankton dissimilarities increased positively with only physicochemical distance. Regarding to each season, the driving factors for the beta diversities (i.e., dissimilarities) were different (Table 2.1). For example, phytoplankton dissimilarities were decayed along hydrological and physicochemical distances together by the samples in Dec. 2014 and Mar. 2015. Based on the summer data (Jun. 2015), the effects of both hydrological distances and species dispersal distance on community dissimilarities were significant. In addition, the relationship between dissimilarity and hydrological distance were

stronger than that between dissimilarity and species dispersal distance. In September, we found three of them weakly correlated to local diversity changing.

Table 2.1 Results of Mantel tests for the correlation between phytoplankton community dissimilarities (Bray-Curtis index) and hydrological (H), physicochemical (P) and species dispersal (S) distances. Significance was expressed as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Index	H	P	S
Entire year	0.049	0.240***	0.030
Dec. 2014	0.281*	0.298*	-0.064
Mar. 2015	0.511***	0.423**	0.182
Jun. 2015	0.412**	0.218	0.256*
Sep. 2015	-0.017	0.050	0.119

2.4 Discussion

In this study, phytoplankton compositions and their metacommunities were shown to be excellent responders to both hydrological and physicochemical conditions. Moreover, the impact from species dispersal factors only delineated from geographic distinction, also slightly contributed to the biotic variations.

2.4.1 Hydrological factors

Hydrological variables have been identified as important factors in shaping riverine phytoplankton community in our study lowland river. The characteristics of flow alteration during a long period of time were detected as key hydrological driving factors for algal communities, specifically, monthly skewness and variability of the flows. In contrast to instant status, these monthly hydrological alterations influenced water temperature, oxygen level, light intensity as well as nutrient availability in water bodies (Richter et al., 1998, Mitrovic et al., 2011; Paerl et al., 2011), which reflecting the dynamic living condition of riverine phytoplankton community. A study in tropical streams detected that the combination of stream velocity and water depth plays a vital role in structuring diatom communities (Bere et al., 2016). Similarly, water depth was also found to be the most crucial factor for diatoms local species richness in subarctic streams, while the elevation significantly correlated with community dissimilarity (Teittinen et al., 2016). In Poyang Lake (China), Li et al. (2016) found water level as the main controlling factor that controlled the phytoplankton

seasonal variability, which actually reflected changes of water quality. Besides the hydrological factors themselves, there was more studies paid attention to combined impacts. For example, low flow attributed to increased irradiance and water temperature were conducive to sustaining cyanobacterial bloom over weeks (Ha et al., 1999). Additionally, low flow reduced velocities, and hence, higher water residence time and nutrients concentration enhanced potential toxic algal blooms and reduced dissolved oxygen levels (Whitehead, 2009). In contrast, riverine bloom was retarded by flow velocities above 0.03-0.05 m/s (Mitrovic et al., 2011). A model studied on benthic algae in New Zealand stream suggested that hydrologic regime and nutrient concentration interacted to shape patterns of biotic composition, and thus algal biomass was strongly dependent on nutrient supply when frequent floods decreased primary consumer biomass (Riseng et al., 2004). The results support that the hydrological indicators, separated (themselves) or combined with other environmental factors, stand as key influential factors, which similar to other studies in this research area. However, different biotic taxonomic assemblages reflect different preference to flow regimes.

Recently, Kiesel et al. (2017) improved hydrological model to explore benthic invertebrates' response, based on hydrological condition in the Treene catchment Guse et al. (2015b) predicted the impact of stressors on Treene River's biota, 27 organization for fishes focusing on hydraulic habitat and macroinvertebrates focusing more on water quality (nitrate). Hydrological indices describing various aspects of the flow regime can be selected to best match the particular ecological processes in local- or regional scale analyses. For example, duration high flow event correlated best with the abundance of individual benthic stream invertebrates (Kakouei et al., 2017). Predictability of flows (annual skewness of the flow) has been linked with mobility and colonizing ability of fish, while variability of pulse frequency with species richness (Puckridge et al., 1998). The timing of flow events impacts on spawning success, and high flows in spring would positively result in high red shiner density, due to their unique life-history strategies (Mims and Olden, 2012). Therefore, different flow alteration was associated with a variety of ecological responses (Poff and Zimmerman, 2010). A key implication of our findings for freshwater management is that long-term bio-monitoring campaigns should include hydrological variables.

2.4.2 Physicochemical factors

For ascertaining the influences from physicochemical factors, we found that NPR is a key driver for phytoplankton density (Fig. 2.2). This is a reasonable result, since the research catchment is located in a rural area, where agriculture stands as the main portion (80%) of land use pattern (Guse et al., 2015a). It has been reported that agriculture and point sources in this watershed were the major contributor for nitrate and ammonium, which has been noticed being the main factor affecting water quality in the stream water (Schmalz et al., 2015). With regard to phytoplankton densities, our samples were separated into two groups by CART. The samples with low NPR have high species densities and high percentage of Cyanobacteria. This is specifically the case for *Microcystis* which is known as a sign of nutrients enrichment in the water body. Along the longitudinal direction, dominant species always changed from lacustrine species (Cyanobacteria) to fluvial species (Bacillariophyta). This shift was in accordance with Yu et al. (2015) and Gillett et al. (2016) and illustrated the impact of lakes on downstream sites in different catchments. For example, Yu et al. (2016) observed in Tanglan River that the toxic *Microcystis* from Dianchi Lake dominated at the upper reaches, but replaced by Chlorococcales green algae and centric diatoms in the lower reach. Gillett et al. (2016) found that the headwater of Klamath River was dominated by planktonic blooming toxic Cyanobacteria resulting in generally low NPR. This condition promoted the development of N-fixing benthic diatoms with cyanobacterial endosymbionts from family Epithemiaceae. This trend followed a wide acknowledged pattern from previous studies in lakes with high NPR in oligotrophic status, while low NPR in eutrophic status (Downing and McCauley, 1992; Xu et al., 2010). Studies also found that low NPR was corresponded to *Microcystis* bloom (Smith, 1983; Xie et al., 2003; Orihel et al., 2015).

Both total density and cyanobacterial density decreased gradually in September from sites T02 to T05 (with a distance of 6 km). As a typical lacustrine species, *Microcystis spp.* was coming from the lake in the upstream. Despite the contribution of decreasing concentration of nitrogen, the concentration of phosphorus in those sites was relatively higher than the others, which also implied the possibility to high phytoplankton density. These results were similar to Wu et al. (2014), which were not surprising since major nutrients, such as nitrogen and phosphorus, were primary elements for algae growing. Increased nutrient concentrations can potentially stimulate algal growth and hence enhance gross primary production and ecosystem respiration in aquatic ecosystems (Ye et al., 2016). On the other hand, nutrient remain low concentration may due to rapid uptake by algae as well (Jirsa

et al., 2013). Similar to the lake-river system, reservoir-river systems are also facing a dominance of toxic algae, which exported from the reservoir. *Microcystis* cells can withstand passage through hydroelectric installations and transport over distance on the downstream (Otten et al., 2015). Studies in a dammed lowland river in Poland also observed summer-autumn dominance of toxic Cyanobacteria. They concluded that higher rate of flushing induced a more rapid species dispersal, while low water level led to an increase in phytoplankton population (Grabowska and Mazur-Marzec, 2016). In addition, among the local variables, water temperature, conductivity as well as nutrients were selected in RDA as significantly correlated to phytoplankton composition, which was in agreement with an earlier report within this study area (Wu et al., 2011). Our results from Mantel tests were consistent with Soinenen et al. (2016), and suggested that local environment was highly important for phytoplankton at larger (regional) scale as well. These findings further emphasized the suitability of lowland phytoplankton as bio-indicator for local habitat changes since they were more affected by local environment rather than spatial effects as indicated by species dispersal variables (Table 2.1). Nevertheless, as for phytoplankton based bio-monitoring and bio-assessment, the impacts of upstream lake or reservoir should be paid more attentions, which also remains a need to identify in the future studies how far the lake might affect the downstream phytoplankton community in different seasons.

2.4.3 Species dispersal

Species dispersal has a weak relationship with phytoplankton in both RDA analyses and Mantel tests in our study area, considering of the whole year. This was inconsistent with the research of Dong et al. (2016) in high mountain stream, which concluded that directional processes and dispersal had prevailing effect on algae metacommunity structuring rather than local physicochemical factors. However, the Treene watershed is characterized by lower hydraulic gradients (Kiesel et al., 2010; Pfannerstill et al., 2014), low altitude gradient (elevations range from 2 to 80 m), and relatively smaller geographic location scale (with a size of 481 km²). Besides, as a typical rural area, agricultural streams reduce retention time due to the alteration of stream channels, which result in a low percentage of nutrient removal. Nevertheless, in a large-scale river basin, the phytoplankton composition and functional groups can only be significantly explained by environment, not dispersal processes (Huszar et al., 2015). On the other hand, species dispersal mechanisms imposed by the dendritic structure stream networks can be evident at very small spatial extents (Göthe et al., 2013). The River Treene has

several tributaries in the catchment. The tributaries stand with different landscape and scale, which lead to distinctive species composition and abundance as well. In this study, we focused only on the mainstream of the river, while following investigation will progress the species dispersal mechanisms by the whole watershed scale.

The unexplained variation of phytoplankton community remained still very high (65%, Fig. 2.6). Despite the three factors discussed above, some quite vital elements are not included here due to some limitations. For example, light availability (Kirk, 1994), dissolved reactive silica (Tavernini et al., 2011) as well as grazing from both planktonic grazer (Kang et al., 2015) and benthic invertebrate filter-feeder (Rossetti et al., 2009). The contribution of those components varies along the river continuum and through the year. In many cases, the impacts of one are dependent on another. Water discharge itself may produce changes on the physicochemical condition, and thus affecting phytoplankton communities (Descy, 1993). An observation illustrated that land use and lake use also potential drivers of phytoplankton biomass dynamics (Borics et al., 2013). Therefore, either directly or indirectly factors, there are far more potential contributors related to the riverine algal assemblages worth much concern.

2.5 Conclusion

In this study, the impact of hydrological regimes, physicochemical condition and species dispersal on riverine phytoplankton along a lentic-lotic continuum was analyzed. The main outcomes are:

- (1) Phytoplankton community in a lentic-lotic continuum catchment showed very high spatial and temporal variations.
- (2) The impacts of upper lake on downstream phytoplankton assemblages were significant, especially for the first site (T02) after the lake. However, these effects varied along with seasons and remained only to a relatively short distance in our research area.
- (3) RDA and Mantel tests showed that phytoplankton composition and dissimilarities along the lentic-lotic continuum were shaped more by local hydrological and physicochemical variables than species dispersal factors, which confirmed the suitability of lowland phytoplankton-based bioassessment. However, upstream lake impacts should be taken into consideration in future biomonitoring campaigns.

(4) The flow regime has been proved as a key driver for local phytoplankton community patterns and regional beta diversity, although its relative importance showed seasonal variations. Further examination on the flow alteration in a finer resolution will gain a deeper understanding of the roles of hydrological condition in structuring phytoplankton communities.

Chapter 3 Effects of land use pattern and physicochemical conditions on phytoplankton communities in a German lowland catchment

Yueming Qu^{a*}, Naicheng Wu^{a, b, c*}, Kristė Makarevičiūtė^d, Björn Guse^{a, e}, and Nicola Fohrer^a

^a Department of Hydrology and Water Resources Management, Kiel University, Olshausenstr. 75, Kiel 24118, Germany

^b Aarhus Institute of Advanced Studies, Aarhus University, Høegh-GuldbergsGade 6B, 8000 Aarhus C, Denmark

^c Department of Bioscience, Aarhus University, Ole Worms Allé 1, 8000 Aarhus C, Denmark

^d Helmholtz Centre for Ocean Research Kiel (GEOMAR), DüsternbrookerWeg 20, 24105 Kiel, Germany

^e GFZ German Research Centre for Geosciences, Section Hydrology, Potsdam, Germany

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Abstract

To understand the lowland riverine phytoplankton community (of species and functional groups) and to investigate the effects of abiotic factors on phytoplankton variance during high flow season, 59 sites of the Treene catchment, northern Germany, were visited in December 2014. We observed 327 species belonging to 6 taxonomic groups and 21 phytoplankton functional groups. Bacillariophyta, especially benthic pennales (corresponding to functional groups TB), were dominant in most of the study area. Cyanobacteria (specifically indicated functional groups M and S1) which are typically found in lake environments were also found in the main stream of the River Treene. Furthermore, Euglenophyta (represented by functional groups W1 and W2) showed a higher percentage in the headwaters. Redundancy analysis indicated that the land use pattern and physicochemical condition co-contributed to phytoplankton communities' variance. The total explained variance (68.4%) of phytoplankton data was partitioned into 3 parts: purely physicochemical factors contributed to 24.1% of the explanation, followed by land use characteristics (15.6%), and their shared effects (28.7%). Functional groups W1 and W2 exhibited a strong positive correlation to the percentage of agricultural land use. The percentage of urban land use and phosphate concentration in the stream were interrelated with functional groups Lo and M, which indicated mesotrophic to eutrophic water conditions. The unexplained variance (31.6%) might result from hydrological regimes, which will be taken into account in subsequent studies.

Key words: lowland riverine phytoplankton, land use pattern, physicochemical conditions, redundancy analysis

3.1. Introduction

Freshwater ecosystems, particularly rivers, are under severe pressure from multiple stressors, including habitat alteration, nutrient enrichment and diffuse or point source impacts (Kristensen, 2012). Unlike lakes, lotic ecosystems have unidirectional water movement along a slope and fast renewal rates (Wetzel, 2001). However, compared to mountain streams with steep valleys and rushing flow (Dong et al., 2016), lowland rivers have relatively longer residence times and intensive land use (Stoate et al., 2001). Lowland rivers are strongly influenced by the geology, vegetation and human activities (Poff et al., 1997). Therefore, aquatic environments in lowland areas do not only depend on processes within the water body, but very much on land use as a cause of pollution, erosion, water quantity and related problems (Hoorman et al., 2008), indicating that an analysis on the catchment scale would be highly beneficial.

Fluvial algae are important primary producers in lowland riverine ecosystems. In addition, they have a quicker response than other biota to abiotic factors (Kim et al., 2015). Potamoplankton is a sensitive bio-indicator in aquatic ecosystems as changes in the physical, chemical and biological environment are rapidly manifested in their composition and biomass (Wu et al., 2011). Nutrient enrichment from diffuse and point sources, coupled with low flow conditions, is believed to cause ecosystem degradation through eutrophication and a shift in the composition of algal communities in UK lowland rivers (Lázár et al., 2016). Dahm et al. (Dahm et al., 2013) found a strong response of diatoms to hydromorphological conditions in lowland rivers, mainly driven by flow conditions and shading on diatom communities in lowland rivers. River ecosystems and their algal communities are shaped by a wide variety of processes and conditions which render them very heterogeneous. In these turbid, turbulent and deep systems, phytoplankton is characterized by assemblages with low density and biomass, while and high species richness due to the occurrence of many sporadic species (Devercelli and O'Farrell, 2013). Phytoplankton is used as a monitoring tool to determine water quality and to help to understand the characteristics and variations in aquatic ecosystems (Crossetti and Bicudo, 2008).

The phytoplankton functional group (PFG) classification method was introduced by Reynolds et al. (Reynolds et al., 2002), based on dominant or co-dominant algae species in a given environment,

from their physiological, morphological and ecological attributes. This method summarized 37 assemblages according to their similar sensitivities and tolerance characteristics (Borics et al. 2007). This approach reduced large numbers of species by pooling them into several descriptive groups containing mutual adaptations, without losing the information embedded in detailed species taxonomic lists (Wang et al. 2011, Wu et al. 2017). Over the last few decades, PFG classification has been applied worldwide in different types of aquatic ecosystems from lakes (Crossetti et al. 2013, Tsai et al. 2014) to rivers (Abonyi et al., 2012; Abonyi et al., 2014).

Previous studies demonstrated that phytoplankton abundance was influenced by numerous factors, including resource availability (Reynolds 2006; Jeppesen et al. 2005), catchment land use (Crosbie and Chow-Fraser, 1999), hydrological conditions (Schindler, 2006), and grazing pressure (Mazumder, 1994; Obertegger et al., 2007). These factors might individually influence phytoplankton in independent ways. At the same time, all variables are potentially correlated with each other (Bowes et al., 2016; Waylett et al., 2013). However, to our knowledge, few studies explicitly quantified the relationship between land use pattern and lowland riverine phytoplankton communities. Moreover, limited studies simultaneously assessed the relative importance of river physicochemical parameters and watershed land use patterns as drivers of algal conditions. In addition, we also noticed that plenty of studies (Ke et al. 2008, Moorhouse et al. 2018) had focused on growing season, while the situation in winter time was still lack of study. However, winter time is the high flow period of the River Treene (Guse et al. 2015a). In former studies (Qu et al., 2018; Wu et al., 2018), we detected that hydrological regime is an important influential factor for phytoplankton community. The specific research on the wet period of time would thus have unique importance. Therefore, we investigated the spatial distribution of potamoplankton communities during the high flow hydrological period in a lowland river of northern Germany. Based on the method of PFG, we examined the relationship between abiotic factors and the lowland riverine phytoplankton communities. Furthermore, we analyzed the contributions of distinct abiotic factors to phytoplankton communities' variance. As one part of series study, it can serve as a reference to supplement previous researches (Wu et al. 2011; 2012) and to support future studies.

3.2 Methods

3.2.1 Study area and sampling

The lowland catchment Treene is located in northern Germany, in the state of Schleswig-Holstein (Fig. 3.1). It is a major tributary of the Eider River, with a basin area of 481 km² (Guse et al., 2015). The Treene catchment is in a temperate climate zone, influenced by marine climate, with mild temperatures and high precipitation in winter. Sandy, loamy and peat soils are characteristic for the basin. The maximum height difference is 76 m.

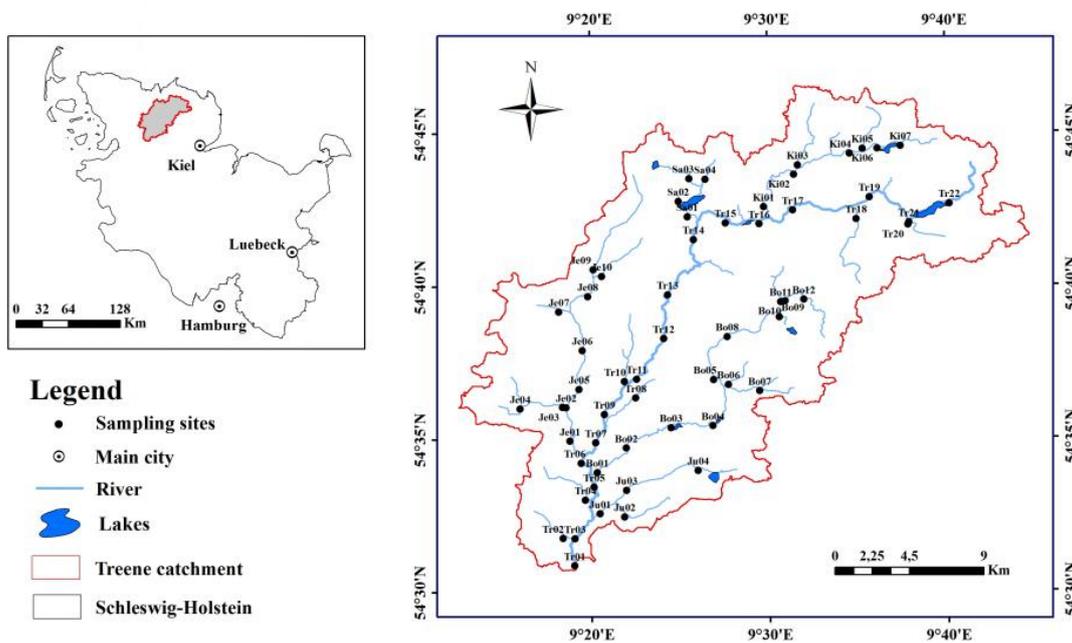


Fig. 3.1 The location of sampling sites of the Treene catchment in Schleswig-Holstein state of Germany. Bo represents for Bollingstedter Au, Je for Jerrisbek, Ju for Juebek, Ki for Kielstau, Sa for Sankermark See, and Tr for mainstream of Treene. The numbers increased along the longitudinal axis of rivers from the outlet to upstream.

The watershed is in a rural area with low human population densities. Around 5% of the area is covered by urban areas (Statistikamt Nord 2013). Agricultural land use dominates the landscape in the Treene catchment. Around 50% of the area is used for agriculture and around 30% for winter pastures. The rivers Bondenau and Kielstau are the sources of Treene. The major tributaries of the river Treene up to station Treia are Bollingstedter Au, Jerrisbek, and Juebek. There are several lakes (top three from size: Sueden See: 0.64 km², Sankermarker See: 0.56 km², Winderatter See: 0.24 km²) in the catchment, mainly located upstream of the river. The northeast Hillands in the upstream part of the Treene catchment are characterized by slight slopes and more fertile soils, allowing a cultivation of high value crops, such as wheat, barely or rape. However, the southwest part of the watershed (Jerrisbek and Juebek and Middle Treene catchments) is characterized by poorer sandy soils and low fertility, with a

higher percentage of grassland (Guse et al. 2015b). During field surveys 59 sampling sites were visited in the mainstream and its tributaries in December, 2014. Abbreviations were used for the sites based upon the subbasins they contributed to. Therefore, Bo stood for Bollingstedter Au, Je for Jerrisbek, Ju for Juebek, Ki for Kielstau, Sa for Sankermark See, and Tr for mainstream of Treene. Lower numbers were assigned to sites closer to the outlet, and higher numbers to more upstream sites (Fig. 3.1).

3.2.2 Measurements of abiotic and biotic variables

Abiotic variables included basic water physicochemical parameters. Water temperature (WT), pH, electric conductivity (EC), and dissolved oxygen (DO) of the surface water were measured in situ using Portable Meter (WTW Multi 340i and WTW Cond 330i, Germany). Simultaneously, river width, depth and velocity were surveyed at the sampling points (velocity – using FlowSens Single Axis Electromagnetic Flow Meter, Hydrometrie, Germany). The water samples for nutrients analysis were stored in pre-cleaned plastic bottles (1 L each) from each sampling sites. They were partially filtered through GF/F glass microfiber filter with a pore size of 0,7 μm (Whatmann 1825-047) to isolated the total suspended solids. The filtered and unfiltered samples were both stored under -20°C until measurement. The concentrations of total phosphorus (TP), phosphate-phosphorus ($\text{PO}_4\text{-P}$), ammonium-nitrogen ($\text{NH}_4\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$), chloride (Cl) and sulphate (SO_4) were measured according to the standard methods of DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). The summary of nitrite-nitrogen ($\text{NO}_2\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$) and ammonia-nitrogen ($\text{NH}_4\text{-N}$) was conducted as total dissolved inorganic nitrogen (DIN). Total suspended particulates (TSP) were measured according to Standard Operating Procedure for Total Suspended Solid Analysis (U.S.GeologicalSurvey, 1998). Inorganic carbon (IC), dissolved total carbon (DTC) and dissolved organic carbon (DOC) were measured with a DIMA-TOC-100 total organic carbon analyzer, using the infrared spectroscopy method by DIMA-TOC-100 total organic carbon analyzer (Dimatec Analysentechnik GmbH, Germany).

The algal samples were collected using a phytoplankton net with a mesh size of 20 μm . A known volume of water (10-30 liters, depending on site) was filtered and fixed immediately by neutral lugol's solution. The algal classification included two parts. Firstly, the soft algae (non-diatom) identification were counted using an optical microscope (Nikon Eclipse E200-LED, Germany) at $\times 400$ magnifications in a Fuchs-Rosenthal chamber. The counting unit was individual (unicell) and at least 300 units were counted in each sample. Taxonomic identification of species was conducted according to previous publications (Hu and Wei, 2006) and (Burchardt, 2014). Secondly, for the diatoms identification, permanent slides were prepared after oxidization using 5 mL of 30% hydrogen peroxide

(H₂O₂), and 0.5 mL of 1 mol/L hydrochloric acid (HCl). Then, 0.1 mL of the diatom-ethanol mix was transferred on a 24 × 24 mm cover slip, and was left to dry. A drop of Naphrax was used to mount the slides. Diatoms were identified with the optical microscope (Nikon Eclipse E200-LED, Germany) at × 1000 under oil immersion, using the key books by (Bey and Ector, 2013), (Hofmann et al., 2011) and (Bağ et al., 2012). Algal biomass was calculated using the approximation of cell morphology to regular geometric shapes, assuming the fresh weight unit as expressed in mass, where 1 mm³/L = 1 mg/L (Huang et al., 2000; Wetzel, 2001). Based on the criteria proposed by Reynold et al. (Reynolds et al., 2002), species contributing more than 5% to the total biomass were grouped into functional groups, and the phytoplankton functional classification was conducted according to Reynold et al. (2002), Borics et al. (2007) and Padisák et al. (Padisák et al., 2009).

3.2.3 Data analysis

Land use data were obtained from Schleswig-Holstein State Bureau of Surveying and Geo-information (LVERMGEO-SH, 2012). Land use analysis was performed via ArcGIS processing. Watershed area upstream from each sampling site was determined and land use within this area was considered to be the land use affecting the sampling site. Eleven land use types were classified: agricultural land-generic (AGRL), deciduous forest (FRSD), evergreen forest (FRST), rangeland (RNGE), industrial (UIDU), residential-low density (URLD), residential-medium density (URMD), water (WATR), wetland (WETL), winter pasture (WPAS).

Preliminary analysis was conducted using Detrended Correspondence Analysis in order to select linear or unimodal methods. Since the resulting gradient length of functional groups was between 3-4 standard deviations, both Canonical Correspondence analysis (CCA) and Redundancy Analysis (RDA) were considered acceptable. We found RDA performed better in selecting the variable for describing the PFG and sample distribution in Canoco 5.0 software. Monte Carlo simulations with 999 permutations were used to identify the significance of the abiotic variables in explaining the PFG data in RDA.

3.3 Results

3.3.1 Variations of abiotic factors

The distribution patterns of nutrition were spatially heterogeneous during the study period. The average values of PO₄-P and NH₄-N concentrations were much higher in Kielstau than in other subcatchments (Fig. 3.2), while the PO₄-P concentrations in Bollingstedter Au and Jerrisbek were lower than the mean value of the whole catchment. Except for the tributary Kielstau, the NH₄-N

concentrations in other subcatchments were less distinctive. There was no significant difference in SO_4 concentration between all the subcatchments in the catchment, whereas the subcatchment Jerrisbek has a slightly higher value compared to the other subcatchments. The maximum value of DIN (6.79 mg/L) was found in the samples from Sankelmarker See. Samples from the outlet of the lake tended to exhibit higher DIN values.

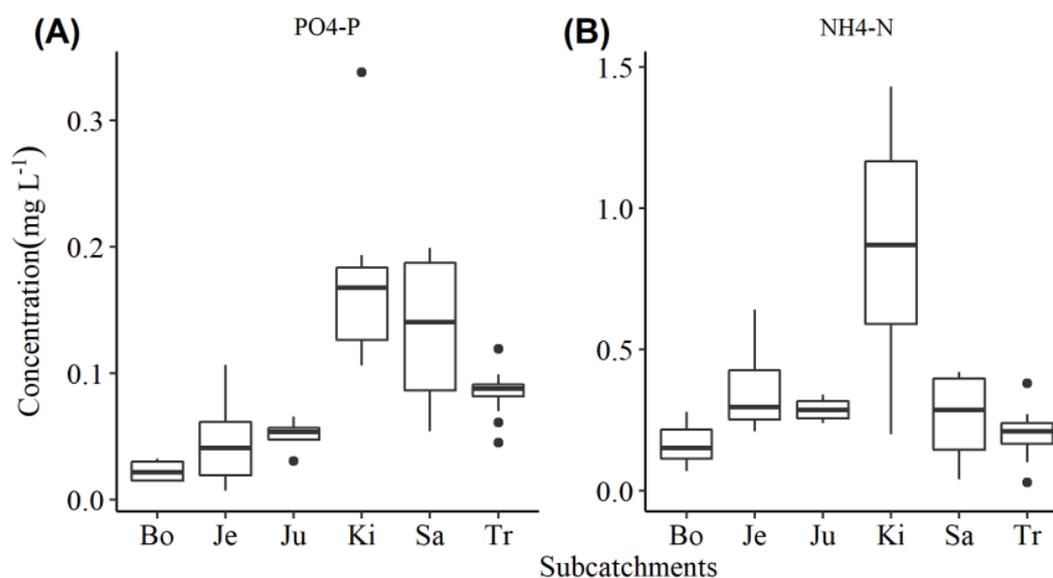


Fig. 3.2. Nutrients comparison across subcatchments of the River Treene (A) phosphate-phosphorous ($\text{PO}_4\text{-P}$), (B) ammonia-nitrogen ($\text{NH}_4\text{-N}$). Subcatchments: Bo-Bollingstedter Au, Je-Jerrisbek, Ju-Juebek, Ki-Kielstau, Sa-Sankelmarker See, Tr-main stream of Treene.

3.3.2 Characteristics of phytoplankton communities

In this study, 327 taxa of phytoplankton species were identified in the main river of Treene and their tributaries, distributed in 6 taxonomic categories, i.e., Bacillariophyta (239), Chlorophyta (42), Cyanophyta (30), Euglenophyta (10), Cryptophyta (4) and Dinophyta (2). The dominant group Bacillariophyta contributed to more than half (56.42%) of the total algal biomass. With a high spatial heterogeneity, the described species belonged to 21 functional groups of phytoplankton (Table 3.1). The group TB, mainly represented by benthic pennales, was the most common PFG in the whole catchment (Fig. 3.3). It was the dominant group for most of the sites, including the tributaries Jerrisbek and Bollingstedter Au and sites located in downstream of the main river of Treene. Group D, described as sensitive to nutrient depletion, were found in 97% of the sites. However, they contributed low biomass in all sites. PFG S1 were also rather common, occurring in 70-80% of the sites. The group S1, including representative free-floating filament cyanobacteria, was found in 41 out of 59 sites, whereas they were not observed in the entire tributary of Jerrisbek. Groups B, J and P, indicating a more stagnant

environment, appeared in more than half of the sites. Group J, which is associated with nutrient-enriched conditions, contributed a relatively high biomass in this study, especially in the Kielstau tributary. Group M, which was mainly represented by *Microcystis aeruginosa*, an algal species able to produce the toxin microcystin, occurred in 41% of the sampling sites. In addition, this group was characterized by relatively high biomass in several sites of the main river of Treene and the site Ki04 in the tributary Kielstau.

Table 3.1 Phytoplankton functional groups list of the river Treene, with their descriptions and representatives modified from Reynolds et al. (2002), Borics et al. (2007) and Padisák et al. (2009)

Codes	Described habitat from the reference	Representative species (genera) in the study area
A	Clear, well-mixed, base poor lakes	<i>Brachysira brebissonii</i> , <i>Cyclostephanos invistatus</i> , <i>Cyclostephanos dubius</i>
B	Vertically mixed, mesotrophic, small-medium lakes	<i>Stephanodiscus minutulus</i> , <i>Stephanodiscus neoastrea</i> , <i>Stephanodiscus parvus</i>
C	Well mixed, eutrophic small-medium lakes	<i>Asterionella formosa</i> , <i>Aulacoseira ambigua</i> , <i>Cyclotella meneghiniana</i>
D	Shallow, enriched turbid waters, including rivers	<i>Nitzschia</i> , <i>Ulnaria acus</i> , <i>Stephanodiscus hantzschii</i>
F	Clear epilimnia	<i>Kirchneriella obesa</i> , <i>Oocystis lacustris</i> , <i>Nephroclytium aghardianum</i> ,
G	Short, nutrient-rich water, small pools among macrophytes	<i>Eudorina elegans</i>
H1	Dinitrogen fixing Nostocleans	<i>Aphanizomenon flos-aquae</i> , <i>Aphanizomenon issatschenkoi</i>
J	Shallow, enriched lakes, ponds and rivers	<i>Scenedesmus</i> , <i>Desmodesmus</i> , <i>Crucigeniella</i> , <i>Pediastrum</i>
K	Short, nutrient-rich columns	<i>Aphanothece</i> , <i>Aphanocapsa</i>
Lo	Summer epilimnia in eutrophic lakes	<i>Woronichinia naegeliana</i> , <i>Merismopedia tenuissima</i> , <i>Peridiniopsis cunningtonii</i>
M	Small eutrophic lakes	<i>Microcystis</i>
N	Mesotrophic epilimnia	<i>Tabellaria flocculosa</i> , <i>Cosmarium granatum</i> , <i>Staurastrum tetracerum</i>
P	Eutrophic epilimnia	<i>Aulacoseira granulata</i> , <i>Closterium</i> , <i>Staurastrum chaetoceras</i>
S1	Turbid mixed layers	<i>Planktolyngbya linnetica</i> , <i>Planktothrix agardhii</i> , <i>Limnothrix redekei</i>
S2	Shallow, turbid mixed layers	<i>Spirulina</i>
TB	Highly lotic environments including rivers and rivulets	<i>Navicula</i> , <i>Achnanthes</i> , <i>Surirella</i>
W1	Small, organic ponds	<i>Euglena</i> , <i>Phacus Curvicauda</i> , <i>Lepocinclis acus</i>
W2	Shallow, mesotrophic, well mixed lakes	<i>Trachelomonas</i>
X1	Shallow well mixed layers in enriched condition	<i>Ankistrodesmus falcatus</i> , <i>Monoraphidium contortum</i>
X2	Mesotrophic, shallow well mixed layers	<i>Chroomonas acuta</i> , <i>Cryptomonas erosa</i> , <i>Cryptomonas ovata</i>
Z	Clear, mixed layers	<i>Rhabdoderma lineare</i>

As shown in the map of PFG distribution in the Treene catchment (Fig. 3.3), there was a high spatial heterogeneity in PFG spatial distribution. The composition of phytoplankton assemblages in

the different subcatchments of the river Treene was therefore investigated. High variation of PFG's biomass was found among the subcatchments. The tributaries Jerrisbek, Bollingstedter Au and Juebek were very similar in their PFG composition and mainly characterised by diatoms from the group TB. The domination of group TB was the most pronounced in Jerrisbek. In Juebek and in Bollingstedter Au group J and group C, respectively, were subdominant. The most diverse subcatchment was Sankermarker See. PFG composition was likely influenced by the presence of lakes and tributaries. The common FG in the Sankermarker See, including S1, W2, N, B, were characteristic for lentic water bodies. In the upstream tributary Kielstau Group, J was dominant, indicating enriched conditions of nutrients.

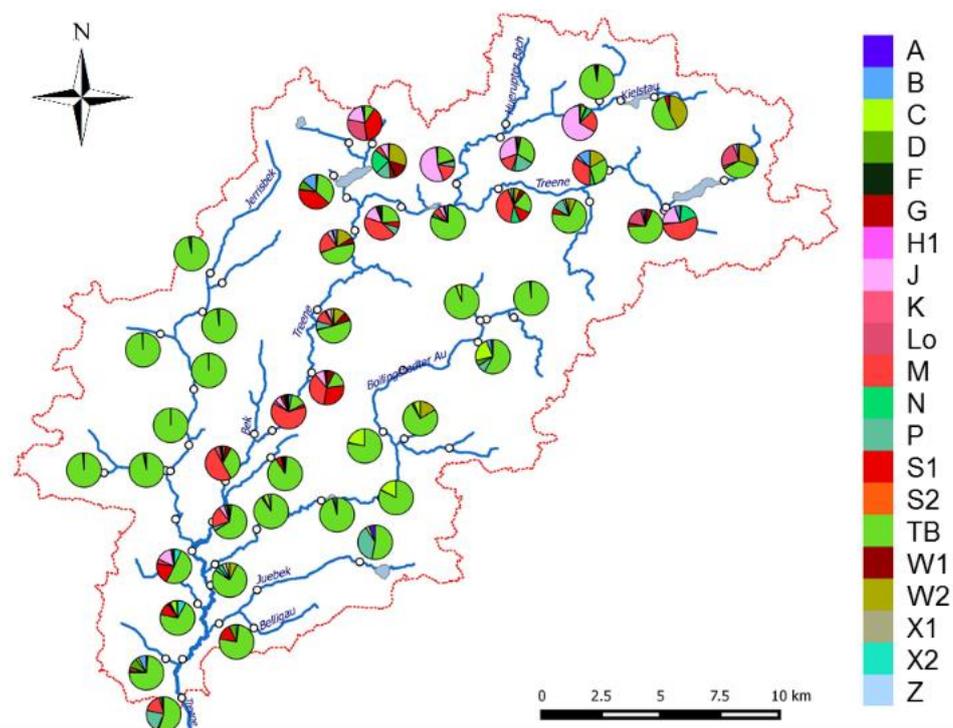


Fig. 3.3 The spatial distribution of phytoplankton functional group composition of Treene catchment. The codes are as in Table 3.1.

3.2.3 Relationship between phytoplankton and abiotic factors

The result of Detrended Correspondence Analysis based on functional groups showed that the maximum of gradient lengths was 3.32 (between 3 and 4), indicating that both linear and unimodal ordination method could be chosen to analyze the relationships between the biotic and abiotic variables (Lepš and Šmilauer, 2003). RDA was selected due to a higher explanation of the variation of PFG (68.37%). The test for significance of all canonical axes by Monte Carlo simulation showed that all canonical axes were significant ($F = 3.4$, $p = 0.002$, 999 permutations under reduced model). There

were 8 abiotic variables screened by forward selection of RDA: DOC, PO4-P, TSP, SO4, WATR, WPAS, AGRL, and URMD. Among them, 4 significant abiotic factors were: WATR ($r^2 = 0.32$, $p < 0.001$), URMD ($r^2 = 0.32$, $p < 0.001$), WPAS ($r^2 = 0.29$, $p < 0.001$), and SO4 ($r^2 = 0.28$, $p < 0.001$). The relationships between the abiotic factors were further assessed by correlation analysis.

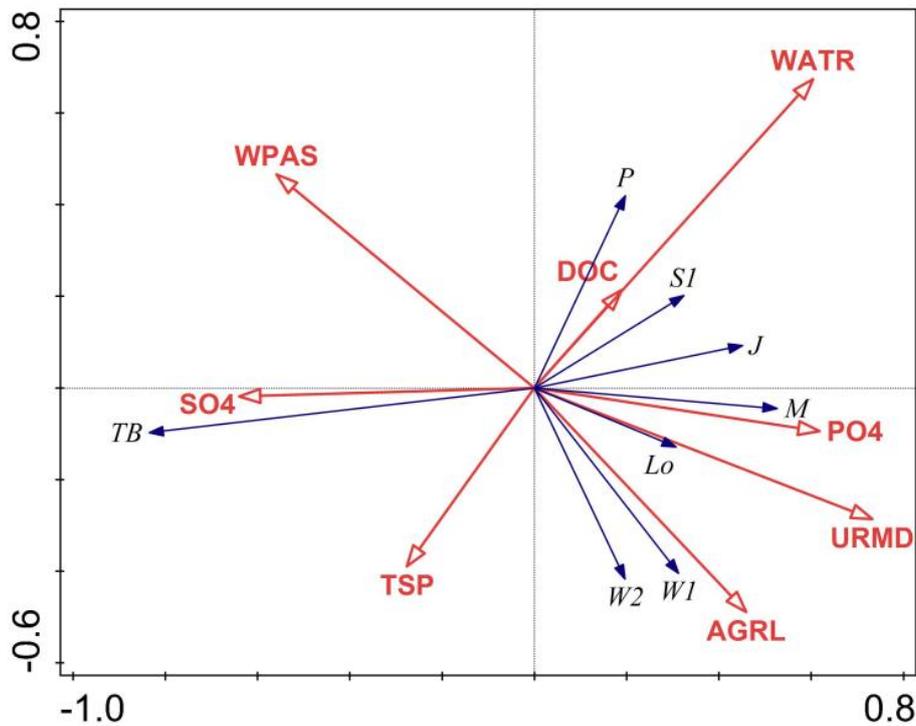


Fig. 3.4 Canonical correspondence triplets for functional groups and environmental variables. Functional groups are represented in blue arrows, and red arrows show abiotic variables (only variables having significant influence are shown). Physicochemical codes: DOC – dissolved organic carbon, PO4 - phosphate-phosphorous (PO4-P), TSP- total suspended particulates, SO4 – sulphate; Land use coden: WATR-water bodies, WPAS – winter pastures, AGRL – agricultural land use, URMD – urban areas.

Fig. 3.4 demonstrated the ordination diagram of the major functional groups (blue arrows) and the most important abiotic factors (red arrows) of the River Treene based upon the results of RDA. The first axis of RDA was mainly correlated with PO4-P, URMD, WATR, and SO4. The second axis was mainly positively correlated with DOC, TSP, and WPAS. Correlation analysis together with RDA revealed that WPAS had a significant negative correlation with AGRL ($r^2 = -0.97$, $p < 0.001$) and URMD ($r^2 = -0.42$, $p < 0.001$) land cover. PO4-P was positively correlated with URMD ($r^2 = 0.35$, $p < 0.01$). SO4 had a significant positive effect on functional group TB. Functional groups of W1 and W2 exhibited a strong positive correlation to the percentage of AGRL. Fig. 3.4 also showed that URMD and PO4-P in the stream were interrelated with functional groups M and Lo.

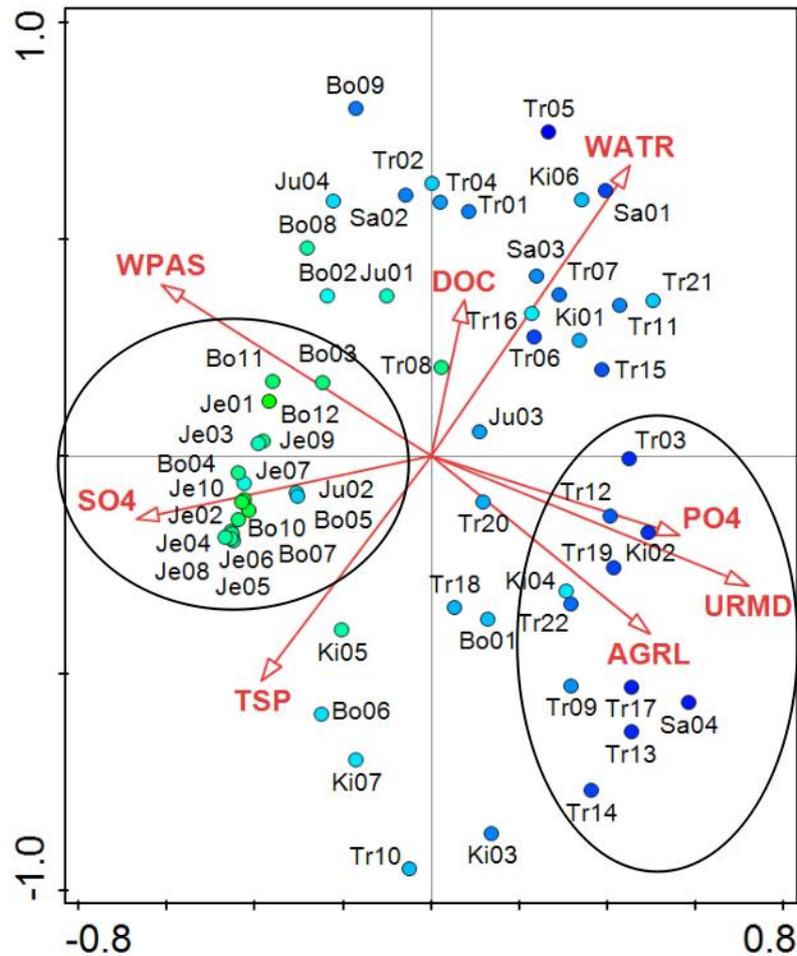


Fig. 3.5 RDA ordination diagram of the sampling sites and environmental variables. Sites are represented as dots with color gradient corresponding to sample's functional group diversity value (using Shannon - Wiener index, from green to blue, representing the value from low to high, 0.177 to 1.952, respectively). Arrows show abiotic variables (only variables having significant influence are shown). Physicochemical codes: DOC – dissolved organic carbon, PO4 - phosphate-phosphorous (PO4-P), TSP - total suspended particulates, SO4 – sulphate; Land use coden: WATR - water bodies, WPAS – winter pastures, AGRL – agricultural land use, URMD – urban areas.

The spatial variation of phytoplankton functional groups in Treene catchment was well described by the distributions of the samples in the RDA ordination diagram (Fig. 3.5). The samples in tributary Jerrisbek and Bollingstedter Au with lower biodiversity were in the negative side of the first RDA axis. It revealed that the location of functional group TB showed best performance in these tributaries, most of which showed higher sulphate concentration and higher percentage of winter pastures in their hydrological catchment area. The RDA ordination diagram demonstrated that sulphate and winter pasture land cover had a positive correlation with the sampling sites dominated by TB. The sampling sites on the positive side of the RDA axis, which included the tributary of Sankermarker See, Kielstau, and mainstream of River Treene, exhibited a higher biodiversity. These sites were associated with higher phosphate-phosphorous concentrations, and in land cover pattern with higher percentage of agriculture, urban areas or water bodies. Furthermore, the variation partitioning based on RDA

indicated a co-contribution of the land use characteristics and physicochemical factors to phytoplankton communities' variance. The total explained variance (68.4%) of phytoplankton data was partitioned into 3 parts: physicochemical factors contributed 24.1%, followed by land use characteristics (15.6%) and their shared effects (28.7%). The unexplained variance, residuals, counted for approximately 31.6% (Fig. 3.6).

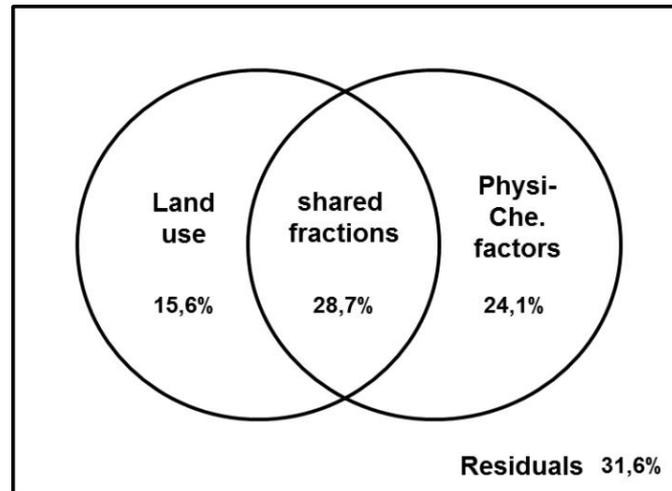


Fig 3.6 Contribution of abiotic factors to phytoplankton communities (Pure land use characteristics (Land use) contributed 15.6% of the phytoplankton variation, pure physicochemical factors (Physi-Che. factors) 24.1%, their shared effects explained 28.7%, and about 31.6% unexplained variance.

3.3 Discussion

The Treene catchment is a mesoscale sand-bed river in the northern German lowlands and agriculture is the dominant land use type. The highest percentage of arable land is in the eastern parts, while the percentage of winter pasture increases from east to west. The distribution of phytoplankton communities also varied from northeast to southwest, from more diverse composition to one dominant functional group.

Functional group TB, representing benthic diatoms, was the most represented group throughout the whole investigated area: it was observed in 54% of the sites and contributed more than 50% of the total phytoplankton biomass. It was particularly dominant in the tributaries of Jerrisbek. TB was previously found in highly lotic environments including rivers and riverlets (Borics et al. 2007, Padisak et al. 2009, Abonyi et al. 2014, Borics et al. 2014). The tributary Jerrisbek, located in the southwest of the catchment with a flatter Geest landscape, was characterized by poor sandy soils and low fertility. In the present study, we also observed a much lower value of both biomass and nutrient concentrations in this area. Thus, riverine phytoplankton with a high proportion of TB may indicate oligotrophic conditions of the river (Borics et al., 2007). Although TB was rare to be a dominant species, it might

be explained by the season in which sampling was conducted. One-time investigation in a winter time was performed in this study. Usually, the largest water flow occurs in the winter season in the Treene catchment (Guse et al., 2015), and during high-discharge period, tycho planktonic diatoms, such as TB, become dominant (Mihaljević et al., 2014). Accordingly, Katz et al. (Katz et al., 2015) reported the phenomenon of a “Melosia year”, which is an under-ice diatom bloom. It emphasized that winter primary biota might play an important role in aquatic metabolism and trophic dynamics. Certain hydrological conditions might lead to typical phytoplankton community scenarios. Diatoms, tolerating water column mixing, were reported to be a significant portion of the total phytoplankton biomass in floodplain landscapes (Stević et al., 2013). Due to the dilution and flushing effects, their biomass was low even when dominant.

In the River Treene, biomass proportion differed between different sampling sites. A relatively high phytoplankton biomass was observed in the upstream tributary Kielstau, where group J was dominant and groups M, TB were co-dominant. The groups M and J showed a higher percentage upstream of Treene. The species in group J mainly belonged to Chlorococcales, including typical species such as *Pediastrum* spp., *Scenedesmus* spp. and *Coelastrum* spp.. During the study period, *Pediastrum boryanum* was abundant. Algae of this group preferably grow in shallow, mixed and highly enriched habitats, including low-gradient rivers (Borics et al. 2007). From the RDA ordination diagram, Groups M and J were located in close proximity, indicating similar environmental preferences. Meanwhile, as previously stated, group M tended to abound in eutrophic to hypertrophic circumstances in small- to medium-sized water bodies (Reynolds et al. 2002; Padisák et al. 2009). Therefore, M and J might co-occur in nutrient-rich waterbodies. All the *Microcystis* species belong to M functional group. In the present study, three important species were observed to belong to group M: *Microcystis aeruginosa*, *Microcystis viridis*, and *Microcystis wesenbergii*. Various reasons could promote the dominance of *Microcystis*, including resource competition, low light tolerance, superior cellular nutrient storage, and ammonium nitrogen exploitation, etc. (Wang et al., 2011; Yoshida et al., 2008). Regarding to the investigated river, this specificity could be explained by the RDA ordination graph, which showed a positive correlation with high values of phosphate, urban and agricultural areas and a negative correlation with the percentage of winter pasture land use. As reported in previous studies, the northeast area had a higher percentage of agricultural land use, which led to allochthonous nutrient input from surrounding streams or drainages. Nutrient loads were mainly caused by manure, fertilizer, and pesticide application in agricultural areas (Guse et al. 2015b, Ulrich et al. 2018). In watersheds dominated by agriculture, drainage tiles and open ditches enhance the subsurface movement of N, transporting around 50% of N from agricultural fields into nearby waterbodies (Hayes et al., 2015).

The sites in upstream of Treene and Sankmarker See were characterized by a heterogeneous phytoplankton community composition. At the headstream (Tr22), PFG composition was rather even, dominated by TB, W2 and Lo. However, only in one site (Tr21) downstream, the phytoplankton community composition was considerably different, with N, J and M functional groups, indicating stagnant, enriched conditions, dominating. The river goes through a lake (Sueden See) between 2 sampling points, which likely greatly affected the phytoplankton composition. A lake between Tr16 and Tr15 could have influenced the decline of benthic diatoms. Total biomass increased, and the groups M and J, characteristic for eutrophic lakes, were favored. In addition, several new PFG (re)appeared, e.g. W1 and W2 which contributed to the high biomass. An upstream eutrophic lake might supply a large amount of dominant lacustrine algae to the river downstream (Yu et al. 2015, Yu et al. 2017). The influence of lakes on downstream phytoplankton communities should be addressed (Qu et al., 2018).

In the main stream, the total biomass increased going downstream, reaching a peak at site Tr14. Around site Tr14, the medium-sized city Tarp and a wastewater plant of size class 3 (LAND-SH, 2006) may have contributed to the high biomass. The analysis of a nutrient enriched lowland river in the Salado (Argentina) identified 3 scenarios regarding the local hydrological conditions. In high water periods, a longitudinal discontinuity with lower phytoplankton concentrations downstream could be attributed to dilution and enhancement of water velocity (Devercelli and O'Farrell, 2013). In the present study, downstream from Tr14, both the total biomass and TB biomass continually decreased. Downstream of the river Treene (Tr07 - Tr01), the total biomass was very low, with benthic diatoms accounting for approximately half of the total biomass. This decrease in biomass might be explained by dilution from the inflow of the Bollingstedter Au, Jerrisbek and other smaller tributaries, where the algal biomass was very low.

Lotic ecosystem health is associated with stream morphology, land use pattern, and nutrient levels (Choi et al., 2015). It has long been recognized that the ecological status of aquatic systems is related to the terrestrial environment of the basin. An expansion of intensive agriculture might enhance the growth of phytoplankton, especially in the upper reaches of the river (Bussi et al., 2016). Changes in phytoplankton diversity were probably due to a combination of changes in land use, resulting in alterations in nutrient ratios and concentrations (Van Chu et al., 2014). Choi et al. (2015) concluded that physical parameters such as stream morphology and land use patterns can influence nutrient conditions and these factors directly and indirectly determine the levels of primary production and trophic composition. In contrast, other studies demonstrated that land use had no significant impact on lowland phytoplankton productivity (Borics et al., 2013). In the present study, we analyzed the

contribution from land use characteristics and physiochemical factors to phytoplankton communities' variances. These results suggested that physicochemical factors had a higher potential to explain the phenomenon than land use characteristics. However, they had overlapping parts, and a certain portion of unexplained factors could also be noted. These results reveal the interaction between land use pattern and nutrient levels, while the unexplained variance may result from hydrologic and hydraulic factors (Qu et al. 2018, Wu et al. 2018). In addition, understanding the causal effects of land use on riverine phytoplankton across different hydrologic periods (e.g. dry and wet) are highly needed in the future studies, as this is a critical prerequisite for robust bio-assessment and water resources management.

3.4 Conclusion

In this study, the spatial patterns of potamoplankton communities were analyzed on a functional level in contrast to the classical taxonomic approach. Abiotic factors from land use scenarios of the watershed to physicochemical conditions in the waterbody were analyzed in relation to the spatial distribution of phytoplankton. The main outcomes are:

1. Diatoms, specifically functional group TB, were dominant during the study period.
2. Abiotic factors interact causing phytoplankton variance: Functional groups of W1, W2 showed a close correlation to the percentage of agricultural land use. The percentages of urban land use and phosphate concentrations in the stream were interrelated with functional groups of M and J, which indicate mesotrophic to eutrophic water conditions.
3. The study has left unexplained variance of phytoplankton composition, indicating that the hydrologic and hydraulic characteristics of the river basin are also important features.

Chapter 4 Riverine phytoplankton functional groups response to multiple stressors variously depending on hydrological periods

Yueming Qu^{1*}, Naicheng Wu^{1,2,3*}, Björn Guse^{1,4}, Kristė Makarevičiūtė⁵, Xiuming Sun¹, Nicola Fohrer¹

¹ Department of Hydrology and Water Resources Management, Institute for Natural Resource Conservation, Kiel University, Olshausenstr. 75, 24118 Kiel, Germany

² Aarhus Institute of Advanced Studies, Aarhus University, Høegh-Guldbergs Gade 6B, 8000 Aarhus C, Denmark

³ Department of Bioscience, Aarhus University, Ole Worms Allé 1, 8000 Aarhus C, Denmark

⁴ GFZ German Research Centre for Geosciences, Section Hydrology, Potsdam, Germany

⁵ GEOMAR Helmholtz Centre for Ocean Research Kiel, DüsternbrookerWeg 20, 24105 Kiel, Germany

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Abstract

Rivers and related freshwater ecosystems are facing increasing natural disturbance and anthropogenic stressors. Understanding the key ecological processes that govern the riverine biota in aquatic ecosystems under multiple pressures has crucial importance. However, there is still insufficient knowledge in quantifying of stressors interactions. Moreover, the understanding of the responses of riverine phytoplankton to multiple stressors is still scarce from catchment aspect. As an interdisciplinary study, the catchment hydrological processes were linked to ecological responses in this study, and we chose phytoplankton functional groups (PFGs) instead of taxonomic classifications of algae to examine their responses to land-use pattern (L), hydrological regime (H), and physicochemical condition (P) across two contrasting hydrological periods (dry, wet). The traits-based phytoplankton functional groups are highly suggested as robust bio-indicators for better understanding the current ecological status. The hydrological regime was described by a matrix index of hydrological alteration based on the outputs of a well-established ecohydrological model (SWAT). The results from variation partitioning analysis showed that P and H dominate during the dry period and P in high flows. Structural equation models (SEM) showed that the skewness of 7 days discharge emerged as a key driver of H, and had always an indirect effect on functional group **TB** (benthic diatoms) during both hydrological periods. The functional group **M** (mainly composed by *Microcystis*) has directly related to phosphorous in both periods, while indirectly to L of urban area in high flow period, and water bodies in low flow period. This study emphasized that climate change and anthropogenic activities such as altering flow regime and land-use pattern affect directly or indirectly riverine phytoplankton via physicochemical conditions. In addition, our findings highlighted that biomonitoring activities require detailed investigation in different hydrological periods. SEM is recommended for improved understanding of phytoplankton responses to the changing environment, and for future studies to fulfill the increasing demand for sustainable watershed management regarding aquatic biota.

Keywords: Phytoplankton functional groups, Land-use pattern, Hydrological regime, Physicochemical condition, Structural equation model

4.1 Introduction

Riverine phytoplankton is one of the vital primary producers in the river ecosystem, and acts as robust bio-indicator responding to multiple stressors (Hilton et al., 2006). It is widely used for bio-assessment of freshwater ecosystems in recent years (EC, 2000). Benthic diatoms are one of significant elements of riverine phytoplankton recruitment, especially in the fine substrate rivers (Bolgovics et al., 2017). Short water residence time lead to benthic diatoms dominance (Reynolds, 1994; Wang et al., 2018), while dynamic flow velocity results in shifts of the share of benthic diatoms in the phytoplankton community (Wu et al., 2007). Moreover, flow alteration influences riverine phytoplankton assemblages by altering nutrient delivery, light availability, dewatering of habitats or severe vertical mixing (Reynolds, 2006). Despite diatom dominance, several studies demonstrate that running water can also suffer the negative consequences of cyanobacterial blooms, resulting from anthropogenic eutrophication and changing environment (Bowling et al., 2016; Stanković et al., 2012). Intensive agriculture and urbanization strongly affect freshwater biodiversity through flow modification and nutrient over-enrichment by fertilizer, pesticides, and sewage fluxes (Bussi et al., 2016; Harris and Smith, 2016; Katsiapi et al., 2012). Changing climate conditions, specifically global warming and altered rainfall patterns, play additional interactive roles in modulating cyanobacterial blooms frequency, intensity and geographic distribution in a long-term aspect (Paerl, 2017; Paerl et al., 2011). Therefore, multiple stressors including natural disturbances (drought and floods) and anthropogenic stressors (human-induced water pollution, eutrophication) are affecting riverine phytoplankton directly or indirectly. Understanding the key ecological processes that govern riverine phytoplankton community in aquatic ecosystems under multiple pressures has crucial importance. It is a fundamental prerequisite for robust bio-assessment, as well as sustainable watershed management. However, investigations and analysis of stressors interactions were much insufficient. Studies about the response of riverine phytoplankton to multiple stressors from catchment scale are still scarce.

Interactions of multiple stressors determine the occurrence and survival of algae. In turn, phytoplankton response to stressors by associated functions of tolerance, preference, and sensitivities of distinct traits (Kruk et al., 2017; Litchman and Klausmeier, 2008). In this study, we chose phytoplankton functional groups (PFGs) instead of taxonomic classifications of phytoplankton dynamics to investigate their response to multiple stressors in a better way: land-use pattern (L), hydrological regime (H) and physicochemical condition (P) (see Fig. 4.1, a schematic diagram developing a conceptual framework). PFGs concept suggested aggregating species with similar features into a few functional groups. Each group was described according to the physiological,

morphological and ecological traits, as well as common environmental sensitivities and tolerances (Borics et al., 2007; Reynolds et al., 2002). For elaboration of the phytoplankton-based quality assessment, Borics et al. (2007) replenished and evaluated PFGs in rivers, considering four parts: trophic state, turbulence character, time sufficient for development of the given assemblage and risk. Code **TB** (composed by benthic diatoms) and code **M** (mainly composed by limnophilic *Microcystis* spp.) represent two highly different functional groups of riverine phytoplankton. **TB** is assigned to mesotrophic, highly lotic preference benthic species with low risk of harm, while **M** assigned to hypertrophic nutrient status, lentic preference, climax assemblages with potential toxicity. Mischke et al. (2011) also selected the proportions of Cyanobacteria and Pennales (as typical benthic diatom) to assess the trophic status of German Rivers. They concluded an unhealthy status with a high proportion of Cyanobacteria, while they detected a healthy status with a high percentage of Pennales. We were working with a lentic-lotic continuum watershed in a rural area under natural hydrology disturbance and human-induced stressors. Based on the previous investigation of this catchment, we found nutrients, especially the nitrate loads, were highly related with agriculture activities in the Treene basin (Haas et al., 2016). **TB** were dominant in most of the study area, while their share varied in time and space (Qu et al., 2018b). The temporary intensive occurrence of *Microcystis* in dry season received high concerns from the local stakeholders (Qu et al., 2018a).

Therefore, we are especially interested in these two functional groups **TB** and **M**, in addition to the phytoplankton community characteristics. We hypothesized that: 1) High flow condition promote to **TB** domination over the others; 2) High share of agriculture land-use rise ascendancy of **M** in the community. Our study aims to disentangle the causal-effects relationship of multiple stressors and riverine phytoplankton community (indicated by PFGs) from wet and dry seasons. The answers would give novel insights into the complex pathways of joint impact of natural and anthropogenic descriptors on riverine phytoplankton community in lowland rivers across contrasting hydrological periods.

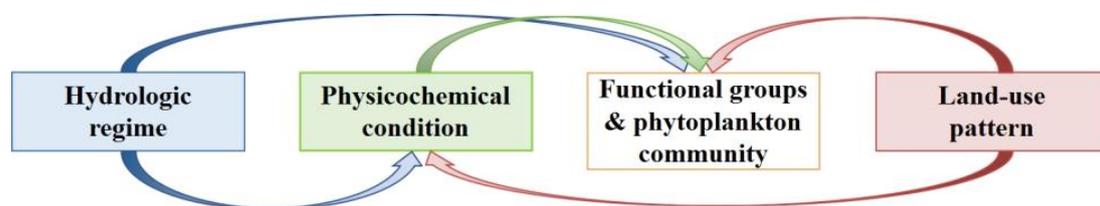


Fig. 4.1 Schematic diagram showing the major factors (e.g., hydrological regime, physicochemical condition, and land-use pattern), which govern the pattern of phytoplankton functional groups and community structures. Those controls include state factors, interactive controls, direct controls and indirect controls, which eventually causes the difference of phytoplankton communities.

4.2 Materials and methods

4.2.1 Study area and sampling sites

This study was carried out in Treene River, a lowland watershed located in the northern Germany. As a lowland catchment, the maximum elevation of Treene basin is 76 m, with watershed area of 517 km². The Treene catchment is in a temperate climate zone, which is influenced by marine climate. Meanwhile it has a typical seasonal pattern of discharge, with the highest water flow in winter and the lowest in summer and autumn (Guse et al., 2015a). Field surveys were carried out in two hydrological periods (a wet period in December 2014 and a dry period in September 2015) on 59 sampling sites which covered mainstream and tributaries of the catchment. The abbreviation of the sites names are according to each sub-basin where they are located in: Bo for Bollingstedter Au, Je for Jerrisbek, Ju for Juebek, Ki for Kielstau, Sa for Sankermark See, and Tr for the mainstream of Treene. The numbers count along the longitudinal axis of rivers from the outlet to upstream. The sampling points in close distance of lakes are not located in the lake, but rather are situated systematically downstream following the lakes (Fig. 4.2).

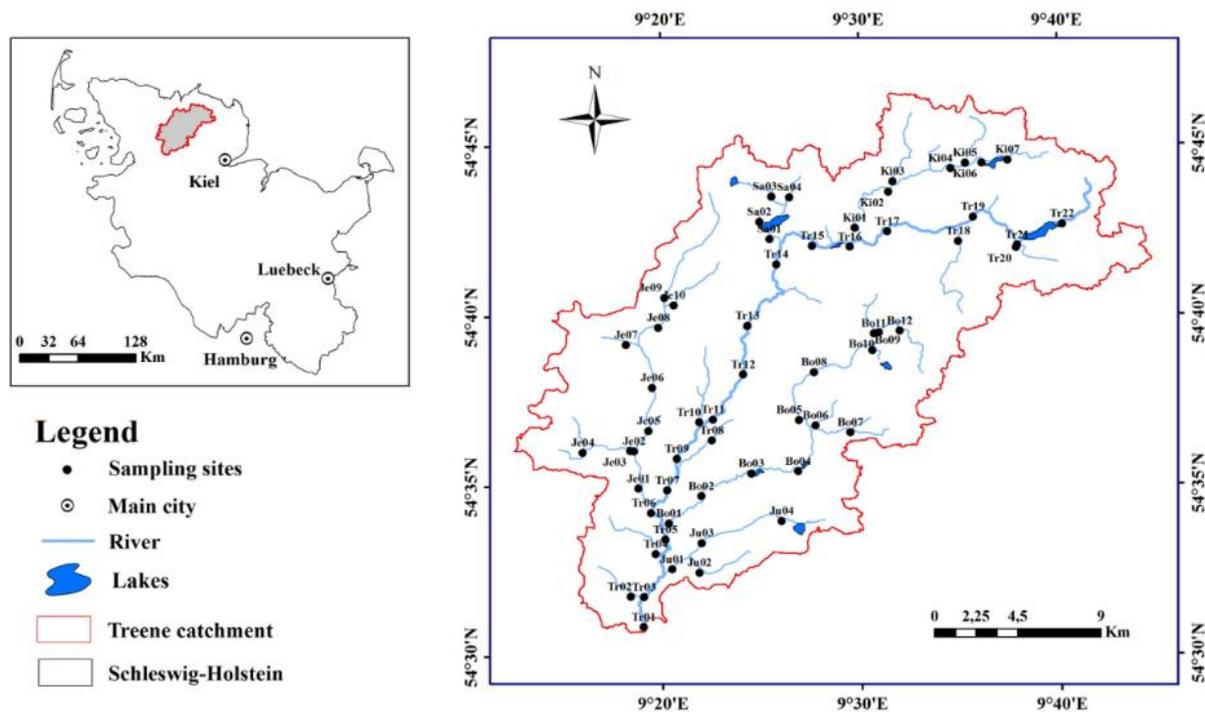


Fig. 4.2 The location of the Treene catchment and 59 sampling points in Schleswig-Holstein state of Germany.

4.2.2 Land-cover pattern analysis (L)

The catchment landscape is dominated by agricultural land-use. Around 50% of the area is covered by arable land, and around 30% by winter pasture. Soils in the north of the catchment (natural region of Angeln) are nutrient-rich and have a pH value ranging 6 to 7, while the pH of soils in the south-east of the catchment (Geest) varies from 4 to 5.5 (Tavares, 2006). Therefore, in the Angeln natural region, corresponding to Kielstau and Treene upstream catchments, agricultural fields are more common, meanwhile, in the southern Geest region (e.g., Jerrisbek and Juebek catchments) pastures constitute to a bigger part. The Treene catchment can be characterized by a slightly undulating landscape, with various depressions and lakes (Kiesel et al., 2010). Land-use data was provided by the Schleswig-Holstein State Bureau of Surveying and Geo-information (LVERMGEO-SH, 2012). The land-use analysis performed via ArcGIS software (Version 10.0, ESRI, US) processing (Fig. A. 1). Eight land-use types were classified: agricultural land-generic (AGRL), forests (FRST), rangeland (RNGE), industrial area (UIDU), urban areas (URMD), water bodies (WATR), wetland (WETL) and winter pasture (WPAS). The upstream watershed area from each sampling site was accumulated, and the land-use within this area was considered as the land-use pattern affecting the sampling site. In this case, the sites located at downstream and near to the lakes or cities presented a higher percentage of water bodies or urban area. For example, the sites located on the upstream region of a city has none percentage of urban area land-use, while the downstream sites have decreasing percentages along the river flow.

4.2.3 Hydrological regime analysis (H)

Except for water depth and velocity which were measured *in situ* at the sampling points (velocity – using FlowSens Single Axis Electromagnetic Flow Meter, Hydrometrie, Germany), the hydrological regime was mainly described by the indicators of hydrological alteration (IHA) (Olden and Poff, 2003). The IHA, illustrating the hydrographic signatures from the duration, frequency, timing magnitude and rate of flow events, are ecological relevant and can be calculated based on daily discharge data and are also used in hydrology (Kiesel et al., 2017; Pool et al., 2017). The daily discharge time series in this study based on the output of the ecohydrological SWAT model (Soil and Water Assessing Tool). In this model application, the model discretization for the Treene catchment resulted into 108 sub-basins. In a multi-site calibration, six hydrological stations which distributed in the catchment were used to consider the spatial heterogeneity in reproducing discharge. The modeling period subdivided into a calibration (2001 to 2005) and a validation period (2006 to 2016). To evaluate the model performance, we used three well-known performance measures: Nash-Sutcliffe Efficiency, Percent Bias and RSR

(root mean square error divided by standard deviation) (see Guse et al., 2015b for details). Daily modeled discharge values were used for subbasins in which at least one sampling point located. At this moment, the sampling points were related to the model results from the closest outlet of a sub-basin outlet. We used 57 hydrological indices from IHA describing the traits of hydrological regime. The final hydrological matrix included: magnitude, frequency, rate of flow events and *in situ* measurement.

4.2.4 Physicochemical factors analysis (P)

Physicochemical factors collection included a field *in situ* measurements and laboratory measurement. Water temperature (WT), pH, electric conductivity (EC) and dissolved oxygen (DO) of the surface water were measured *in situ* using Portable Meter (WTM Multi 340i and WTW Cond 330i, Germany) at each sampling site. Simultaneously, two water samples were stored in pre-cleaned plastic bottles (500 ml each) for nutrient analysis in the laboratory. They were partially filtered through GF/F glass microfiber filter (Whatmann 1825-047) for collecting the total suspended substances. Both filtered and unfiltered samples were kept frozen at -20 °C until measurement. Concentrations of total phosphorus (TP), phosphate-phosphorus ($\text{PO}_4^{3-}\text{-P}$), ammonium-nitrogen ($\text{NH}_4^+\text{-N}$), nitrate-nitrogen ($\text{NO}_3^-\text{-N}$), nitrite-nitrogen ($\text{NO}_2^-\text{-N}$), chloride (Cl^-) and sulfate (SO_4^{2-}) were measured using the standard methods of DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). Dissolved inorganic nitrogen (DIN) was defined as the summation of nitrite-nitrogen ($\text{NO}_2^-\text{-N}$), nitrate-nitrogen ($\text{NO}_3^-\text{-N}$) and ammonium-nitrogen ($\text{NH}_4^+\text{-N}$), and the nitrogen to phosphorus ratio (NPR) is the ratio between DIN and TP. In this study, we chose DIN:TP ratio instead of the TN:TP ratio, since DIN:TP can better discriminate the N and P limitation of phytoplankton (Bergström, 2010). Total suspended solids (TSS) were measured according to Standard Operating Procedure for Total Suspended Solid Analysis (Federation and Association, 2005).

4.2.5 Phytoplankton collecting and processing

Phytoplankton samples were quantitatively analyzed with a known concentration from the subsurface (5 - 40 cm) water of the river by plankton net. Identification included two steps. First, soft algae were identified in a Fuchs-Rosenthal chamber with an optical microscope (Nikon Eclipse E200-LED, Germany) at $\times 400$ magnification, after employing the sedimentation method for the samples (Sabater et al., 2008). Taxonomic identification was based on the references of Hu and Wei (2006) and Burchardt (2014). Second, for further determination of the diatom species in the samples, permanent slides were prepared after oxidization (using 5ml of 30% hydrogen peroxide, H_2O_2 , and 0.5ml of 1mol/l hydrochloric acid, HCl), and then 0.1 ml of the diatom-ethanol mix was transferred on a 24 \times 24 mm coverslip. Diatoms were identified with the optical microscope (Nikon Eclipse E200-LED,

Germany) at $\times 1000$ under oil immersion, based on the key books by Bey (2013), Hofmann et al. (2011) and Bak et al. (2012). Algal biomass was calculated using the approximation of cell morphology to regular geometric shapes, assuming the fresh weight unit as expressed in mass, where $1 \text{ mm}^3/\text{L} = 1 \text{ mg/L}$ (Huang, 2000; Wetzel and Likens, 2013). We then calculated the relative biomass of every species as their abundance. Based on the criteria proposed by Reynold (2002), species that contributed more than 5% of the total biomass were sorted into functional groups, and the phytoplankton functional classification was done according to Reynolds et al. (2002), Borics et al. (2007) and Padisák et al. (2009). Species not mentioned in the references were assigned to a group according to their morphological and ecological characteristics and the environmental conditions prevailing during their greatest occurrence (Devercelli, 2006). In this study, we specially focused on the functional group **M** (species mainly from genus: *Microcystis* consisted by *Microcystis aeruginosa*, *M. wesenbergii*, *M. viridis*) and **TB** (mainly composed of benthic Pennales, typically genus such as: *Navicula*, *Nitzschia*, *Gomphonama*, *Fragilaria*). From the references, this two group of algae have different strategies, preference of living, and they present two characteristic conditions: highly lotic river sections with **TB** dominance, while **M** dominance in eutrophicated lentic habitats (Borics et al., 2007).

4.2.6 Statistical analysis

For achieving the best performance, the phytoplankton functional groups abundance matrix was Hellinger transformed (Legendre and Gallagher, 2001; Legendre and Legendre, 2012). Firstly, the analysis of PERMANOVA was conducted for testing the difference of PFG composition (function: *adonis* of the R-package: *vegan*). In the meanwhile, the three sets of abiotic variables (i.e., L, H and P) collinearity was tested when using all variables in the model of explaining variations of phytoplankton communities (function: *cor* of the R-package: *stats*). Afterwards, a forward selection (Blanchet et al., 2008) was carried out to choose a parsimonious subset of explanatory variables (function: *forward.sel* of the R-package: *adespatial*), and then the multivariate community structure was modeled under variation partitioning analysis (Borcard et al., 1992) (function: *rda*, *varpart* of the R-package: *vegan*). We then constructed structural equation model (SEM) (Westland, 2016) for quantitative evaluation of the relationship between human and natural stressors on PFG composition, as well as the potential effect of land-use and hydrology on in-stream nutrients. We used reduced-multidimensional data rather than original PFG matrices in the analysis since SEM can only handle one-dimensional variables. Nonmetric multidimensional scaling (NMDS) ordination was applied to produce reduced-dimensional data of PFG composition, measured as the first NMDS axis scores for each site (i.e., NMDS1) to represent the main condition of the whole phytoplankton community composition (function: *metaMDS* of the R-package: *vegan*). To achieve the SEM, we first proposed the conceptual model for the three

bio-indicators (PFG: **TB**, **M** and NMDS1). The conceptual model included all possible pathways between the response variables and their key abiotic variables, which identified by general linear model (function: *glm* and *step* of the R-package: *stats*). In addition to the direct pathways, we also checked the indirect ones to see if variables exerted further effects via mediation variables. From the initial model, we then specified the pathways. All non-significant paths were eliminated stepwise until all remaining paths were significant related to the response variables. Standardized path coefficients were calculated for each pathway for a better comparable in the SEM. The overall final model fit was evaluated with root mean square error of approximation (RMSEA), and the comparative fit index (CFI). RMSEA approach to 0, and CFI close to 1.0 indicate a good fit of the model (Grace, 2006). In this study, SEM was constructed by *sem* in the R-package: *lavaan*.

All analyses were conducted with the R software (version 3.5.1, R Development Core Team, 2018).

4.3. Results

4.3.1 Description of watershed hydrological regime and physicochemical condition

From the model outputs, the indicators of hydrological alteration varied across the two studied periods. Water flow has a higher magnitude and fluctuation in the high flow (December) than in the low flow period (September) (e.g., at the outlet of the catchment Tr01, Fig. A. 2). Some other typical hydrological indices, for instance, H20 which represents the skewness of seven days of discharge, has an opposite spatial trend during these two periods (Fig. A. 3). The index H20 ranges from -0.775 to 2.439. Most values of H20 were positive with a range of 0.043 to 2.439. They were negative skewed only in the upstream of the main river (specifically sites: Tr16 - Tr22) in high flow period, and tributary Jerrisbek during dry season. We observed a relatively high value in the tributary Jerrisbek during the wet season (with an average value of 2.038). The key hydrological indicators were summarized after pre-selection excluding the ones with significant multi-collinearity (Table 4.1).

The local physicochemical variables measured *in situ* varied considerably among different hydrological periods and sub-catchments (Fig. A. 4). Firstly, the difference of water temperature (WT) in two seasons was remarkable, and mean values in December 2014 and September 2015 were 5.69 °C and 14.24 °C, respectively (Table 4.1). The value of pH followed a similar pattern in different sub-basins, while mean value in September was slightly higher than in December ($8.084 > 7.487$, Table 4.1). The concentrations of ammonium-nitrogen ($\text{NH}_4^+\text{-N}$) showed a higher concentration in December compared to September (average: $0.307 \text{ mg/L} > 0.158 \text{ mg/L}$, Table 4.1). Conversely, nitrate-nitrogen

(NO₃⁻-N) concentration showed lower in December than in September (average: 3.551 mg/L < 9.243 mg/L, Table 4.1). Dissolved inorganic nitrogen (DIN) followed a similar pattern as nitrate-nitrogen. We observed a decreasing trend of phosphate-phosphorus (PO₄³⁻-P) concentration while an increasing trend of nitrogen to phosphorous ratio (NPR) along the mainstream of Treene (Tr) in September 2015. Except for the sub-basin Juebek, the average value and fluctuation of NPR in September was higher than in December (Fig. A. 4). The concentrations of NH₄⁺-N and PO₄³⁻-P in sub-basin Kielstau were always significantly higher than other sub-basins at both investigation periods. Generally, the nutrient contents such as NO₃⁻-N, DIN and NPR in wet season were lower than in dry season (Table 4.1).

Table 4.1 Summary of important hydrological (H), and physicochemical (P) variables with their codes and descriptions in this study. Variables with significant multicollinearity (spearman correlation coefficient greater or equal to 0.75) are excluded from the table.

Variables						
Code	Unit	Description	High flow		Low flow	
			Mean	SD	Mean	SD
H Hydrological parameter						
H01	m ³ /s	Discharge at the sample day	2.274	4.38	0.299	0.52
H20	-	Skewness of 7 days' discharge	0.955	0.686	0.814	0.696
H36	-	Skewness of 30 days' discharge	1.143	0.636	1.428	0.435
H41	days	Low flood pulse counts 30 days	13.373	10.257	22.373	3.737
H42	days	High flood pulse counts 3 days	1.492	1.467	0	0
H55	-	Rate of change in 7 days	-0.038	0.089	-0.017	0.032
P Physicochemical parameter						
WT	°C	Water temperature	5.69	1.581	14.237	1.619
pH	-	pH	7.487	0.474	8.084	0.407
NH4	mg/L	Ammonium-nitrogen (NH ₄ ⁺ -N)	0.307	0.276	0.158	0.266
NO3	mg/L	Nitrate-nitrogen (NO ₃ ⁻ -N)	3.551	1.772	9.243	6.784
NO2	mg/L	Nitrite-nitrogen (NO ₂ ⁻ -N)	0.02	0.019	0.054	0.119
TP	mg/L	Total phosphorus	0.225	0.116	0.211	0.209
PO4	mg/L	Phosphate-phosphorus (PO ₄ ³⁻ -P)	0.077	0.058	0.072	0.109
NPR	-	Nitrogen to phosphorus ratio	21.002	14.926	79.739	67.715
SO4	mg/L	Sulfate (SO ₄ ²⁻)	31.819	10.923	44.087	14.572

4.3.2 Variation of phytoplankton assemblages

We observed 396 algal taxa from the 118 samples and they were classified into 21 phytoplankton functional groups (PFGs). Among them, there were 16 groups in December 2014, while 19 groups in September 2015. The PERMANOVA analysis showed a significant dissimilarity of phytoplankton functional groups composition both temporal (high water flow period and low water flow period) and spatial (sub-basins). We also found that biomass increased significantly from high flow to low flow period in the sub-basins of Tr, Sa and Ju, while fewer changes occurred in the sub-basins of Bo, Je and Ki (Fig. 3). In both hydrological periods, functional group **TB** constituted a high portion in sub-basins:

Bo (87% - Dec. 2014, 68% - Sep. 2015) and Je (98% - Dec.2014, 64% - Sep. 2015). In contrast, the functional group **M** percentage increased in the sub-basin of Tr, Ki, Sa and Ju from December to September.

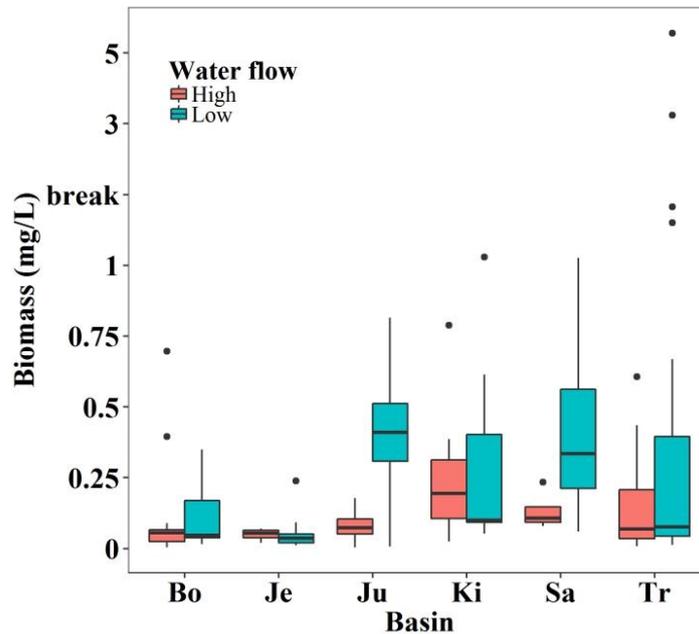


Fig. 4.3 Average biomass in different basins (Bo represents for sub-basin Bollingstedter Au, Je for Jerrisbek, Ju for Juebek, Ki for Kielstau, Sa for Sankermark See and Tr for mainstream of Treene)

4.3.3 Effect of abiotic factors on phytoplankton functional groups

4.3.3.1. Relationship described by variation partitioning

In wet season (Dec. 2014), there were 3 L, 5 H and 5 P variables selected by a forward selection. All the groups showed significant relationships with PFGs (by *anova* function in R, $p < 0.001$). According to variation partitioning analysis, the three sets could explain 44% of the variation in PFGs (Fig. 4). The variations purely explained by H, L and P were 4%, 2% and 6%, respectively, while the shared fraction of the three variables was 14%. In general, the joint contribution by H and P ($H \times P$, 9%) was higher than those by $H \times L$ (2%) and $P \times L$ (7%). In the dry season (Sep. 2015), there were 2 L, 1 H and 6 P variables selected by a forward selection. The PFGs variation was mostly related to the effect of P (their pure effect explained 10% of the variation), whereas the pure effect of H was the least (only accounted for 0.4%). The variation partitioning also showed that both wet and dry periods had similar explanation fraction of land cover (Dec. 2014: 2%; Sep. 2015: 2%).

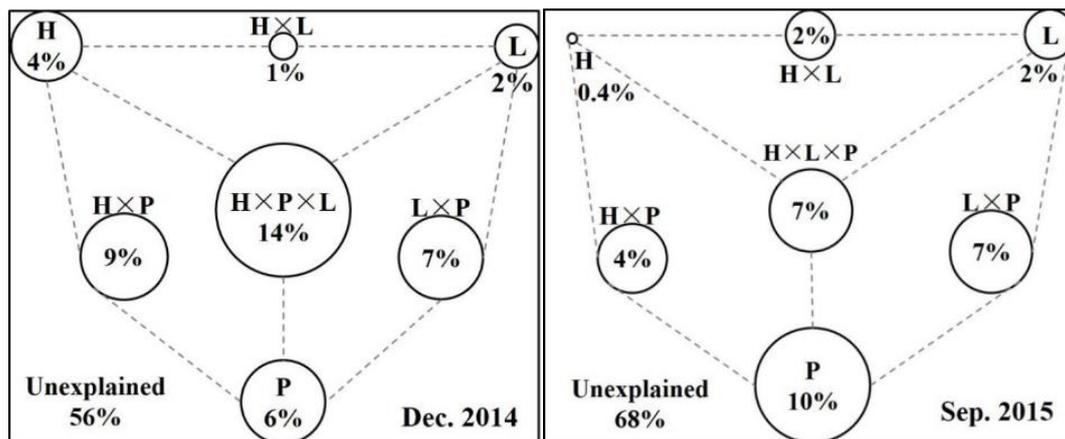


Fig. 4.4 Contributions of the hydrological (H), land-cover (L) and physicochemical (P) variables to the variances in phytoplankton functional groups (PFGs). Each diagram represents a given biological variation partitioned into the pure effects of H, L and P (i.e. when removing the variations caused by other two factors), interaction between any two variables (H×L, L×P, H×P), interaction of all three factors (H×L×P) and unexplained variation (total variation = 100). The analysis includes two scenarios: left: illustrate the situation in December of 2014 (n=59), right: show the results in September of 2015 (n=59).

4.3.3.2 Relationships described by structural equation model

From the general linear model results, we got a general idea about the effects of the multiple stressors. For further investigation of the causal relationship between the abiotic and biotic variables, we fitted structural equation models (SEMs) to infer the direct and indirect effects of specific abiotic variables on bio-indicators (indicated by NMDS1 and phytoplankton functional groups **M**, **TB**) (Fig. 4.5).

In high flow period (Dec. 2014), SEMs indicated that group **M** was directly governed by $\text{PO}_4^{3-}\text{-P}$ and TP ($\beta=0.276, 0.378$, respectively, standardized coefficient), while indirectly affected by URMD via $\text{PO}_4^{3-}\text{-P}$ and TP ($\beta=0.558, -0.448$, respectively, standardized coefficient). The hydrological variable H20 (skewness of 7 days' discharge) was mediated through sulfate (SO_4^{2-}) effect on **TB**. The SEM explained 72.8% of the variation of NMDS1. The explanations come from H36 (skewness of 30 days' discharge), $\text{PO}_4^{3-}\text{-P}$, TP, SO_4^{2-} directly, and URMD indirectly via H20.

During the low flow period (Sep. 2015), the strongest relationship ($\beta=0.525$, standardized coefficient) was observed in the SEM analysis between functional group **TB** and dissolved oxygen (DO). H20 exerted an indirect effect on **TB** via DO ($\beta=-0.465$, standardized coefficient). Area covered by water (WATR) appeared as the key variable for the group **M**, directly ($\beta=0.503$, standardized coefficient) and indirectly through NPR ($\beta=-0.318$, standardized coefficient). The SEM explained 31% of the community variation (represented by NMDS1) was negatively affected by DO, and positively

affected by WT and WATR.

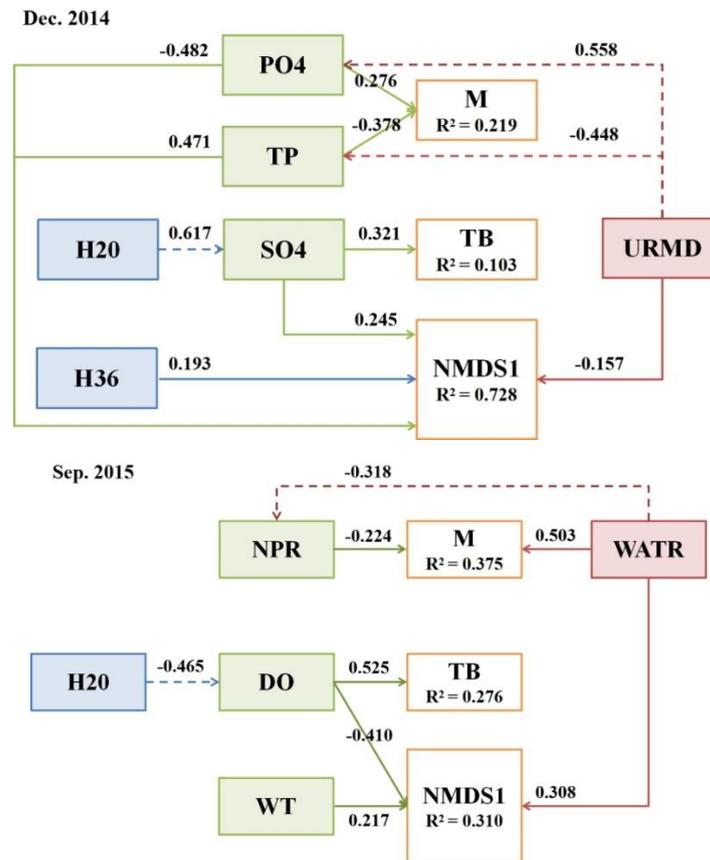


Fig. 4.5 Structural equation models embody the causal relationships between hydrological regime (H), land-use pattern (L), physicochemical condition (P) and phytoplankton bio-indicators. Solid arrows represent direct paths ($p < 0.05$), and dashed arrows represent indirect paths. The values corresponding to the path coefficients have standardized effect size. The models are evaluated using R^2 . The hydrological parameters are in blue squares, physicochemical parameters in green squares, land-use parameters in red squares, and the orange squares represent phytoplankton functional groups (**M**, **TB**) and community index (NMDS1). The analysis includes two scenarios: the upper one illustrates the situation in December of 2014 ($n=59$), the lower one shows the results on September of 2015 ($n=59$). The environmental variables included: H20: skewness of 7 days' discharge (including the sampling day); H36: skewness of 30 days' discharge (including the sampling day); WATR: area covered by water; URMD: urban area with residential-medium density; PO4: phosphate- phosphorus; TP: total phosphorus; SO4: sulfate; NPR: nitrogen to phosphorus ratio; DO: dissolved oxygen; WT: water temperature.

4.4 Discussion

4.4.1 Response of TB to multiple stressors

We observed a higher contribution of hydrological regime during high water period (Fig. 4.4). The result demonstrated the importance of flow regime in shaping phytoplankton community population and composition, which was also supported by previous results in the study region (Qu et al., 2018a). Among the hydrological variables, skewness of discharge in 7 days (H20) emerged as the key factor in shaping the pattern of **TB** both in wet and dry periods of the study (Fig. 4.5). The index

H₂O provided two opposite trends across different hydrological periods. One river could present two types of hydrograph on skewness depending on the hydrological conditions, such as in the river Piquiri, South Brazil, where small (large) floods present positive (negative) skewness (Fleischmann et al., 2016). The maximum value of skewness of flow in the studied catchment was observed in the tributary Jerrisbek during the wet season. As we know, the Jerrisbek tributary is in the flatter hill region. Additionally, the region had a higher share of forest and pasture, which turned to interfluvial wetland during flooding, which leads to low flood wave attenuation (Junk et al., 2011). A larger skewness implied higher discharge and runoff variability (Poff and Zimmerman, 2010).

Higher percentage of **TB** was observed in the tributary of Jerrisbek compared to other areas of the river. The PFG **TB** as typical potamal phytoplankton better dominated in high flow condition of small rivers (Stanković et al., 2012). The results support the hypothesis that increased water discharge can trigger higher share of **TB** in the community, due to their relatively high tolerance to flushing and turbulence (Borics et al., 2007). Phytoplankton communities were co-dominated by planktic and benthic algae. The proportion of silicified benthic diatoms increased when water residence time decreased (Beaver et al., 2013). Consistent with this, Wang et al. (2018) demonstrated that the community composition of benthic diatoms in river plankton was not randomly distributed. Additionally, it could be related to algal hydrological constraints based on their morphological and functional traits aspects in a meaningful way. Likewise, B-Béres et al. (2016) also emphasized that the trait of guild was a sensitive indicator for the environmental conditions in the lowland rivers and streams. Furthermore, we also observed a significant positive relationship between sulfate and benthic diatoms. The results revealed the possible importance of sulfate for diatoms growth. Based on a controlling experiment, sulfate is likely to be a promotion factor, which would be a benefit to the photosynthetic characteristics of benthic diatoms (Lengyel et al., 2015).

4.4.2 Response of **M** to multiple stressors

During high flow period, domestic area (URMD) is selected as an indirectly structuring factor to **M** via essential nutrient phosphorus (Droop, 1974), and the soluble phosphate was positive related with **M** (Fig. 4.4). It was surprisingly against with our hypotheses that the key factor would be the major land-use of agriculture. However, the results are consistent with other previous findings. In the Taizi River basin (China), they reported the pollution sources from built-up land areas were existing in both rainy and dry seasons as a point source, where the population is dense and industrial activities are intensive (Bu et al., 2014). It was reported that the urbanized land-use had better production of water quality than agricultural land-use, indicating that urbanized land-use is the primary contributor to degraded water quality rather than agricultural land-use (Baker, 2003; Schoonover and Lockaby,

2006). Additionally, it was reported a continuous high supplement of nitrogen, while controlled phosphorus in the rural area (Mischke et al., 2011). The condition of phosphorous showed as an important nutrient factor, especially for the growth of **M**.

During the low flow period, the area covered by water body (WATR) appeared as another key factor for controlling the functional group **M** with a direct positive relationship (Fig. 4.5). The result indicated that the lake act as a source of *Microcystis* rather than a sink of purification in the catchment. The biomass of the percentage of **M** was the highest on the sites after lakes. On the contrary, Lake Durowskie (west Poland), which is also located in a farmland-dominant catchment, has suffered inflow with severe cyanobacteria blooms by the linked Struga Gołaniecka River. However, the outflow water quality recovered by three restoration methods in the lake (Gołdyn et al., 2014; Kowalczevska-Madura et al., 2018). As a typical planktic group, **M** has been regarded as a group with the preference of small, eutrophic lacustrine habitat (Borics et al., 2007), while sensitivities to flushing and low light (Reynolds et al., 2002). In lentic area, high phytoplankton bio-volume may dominate by bloom-forming cyanobacteria occurring due to high water residential time (Rangel et al., 2016). The upstream dam resulted in lake type eutrophic, epilimnetic *Microcystis* dominance, while replaced by benthic diatoms in the middle sections of the River Loire (France) in late summer (Abonyi et al., 2012).

Moreover, we also observed that the ratio of nitrogen to phosphorous was directly negative related to group **M** during the dry season (Fig. 4.5). On the one hand, the result showed that the content of phosphorous acted as the shaping factor for *Microcystis* development (Schindler et al., 2016). On the other hand, the negative relationship between NPR and **M** consistent with the previous studies that cyanobacteria bloom was favored by low NPR (Orihel et al., 2015; Smith, 1983).

Finally, we are specially facing a high biomass of **M** on the low flow period, when local farmers fertilized crops and used pesticides more extensively in the study region (Ulrich et al., 2018). Cyanobacteria had been noticed by higher tolerance to herbicides than other phytoplankton taxa (Bérard et al., 1999), particularly under status of enhanced nutrient supply (Harris and Smith, 2016), indicating pesticides might potentially stimulated the dominance of **M** during low flow period.

4.4.3 Response of phytoplankton community to multiple stressors

During the high flow period, the phytoplankton community pattern had a higher influence from hydrological indexes compare to the dry period (Fig. 4.5). The hydrological regime mainly contributed to the low biomass and high similarity of the phytoplankton composition during the high flow period, due to the high connectivity and extensive dispersal stochasticity (Rodrigues et al., 2018). On the contrary, there was a relative high level of total biomass during the dry season (Fig. 4.3), and a high

share of contribution from physicochemical condition in structuring the phytoplankton community (Fig. 4.4). Firstly, it can be expected a light limitation in winter months in the Northern Hemisphere. In addition, we could infer even higher light availability in the dry season due to the low water level (Nõges et al., 2016). Moreover, water temperature had a positive contribution to the phytoplankton population development in September (Fig. 4.5). This phase of the year had more suitable temperature for algae growth compared to December. Warm autumn water temperatures combined with anthropogenic eutrophication attributed to the cause of riverine algal blooms during drought period (Bowling et al., 2016).

Overall, our findings suggest that the impacts of flow regime, land-use pattern, physicochemical condition and their potential interactions on riverine PFGs (**TB**, **M** and the community) varied greatly across hydrological periods. However, traditional biomonitoring campaigns and management practices, which focused on improving local abiotic variables to increase local biodiversity, were often based on one-time sampling data and fairly ignored the potential bias resulting from temporal variations, particularly different hydrological periods (Stubington et al., 2017b). The designation was probably one of the reasons that have resulted into many problems and delays in implementation of recent international water framework directive policies such as EU WFD (Voulvoulis et al., 2017). We therefore advocate that sampling programs and analyses that target future environmental policies should take different hydrological periods into account (Stubington et al., 2017a). Furthermore, studies address that detailed causal relationships between changing environment and aquatic organisms could significantly improve our basic understanding of ecological responses to multiple stressors. Sustainable watershed management requires holistic approaches that assess collective biotic and abiotic data to better predict the impacts of anthropogenic activities and climate change on aquatic ecosystems (Dudgeon et al., 2006).

4.5 Conclusion

In this study, the phytoplankton biomass and functional groups composition varied spatiotemporally during two contrasting seasonal hydrological periods. Their responses to physicochemical conditions, hydrological regime and land-use pattern were different during different hydrological periods. we detect that:

(1) The hydrological regime contributed more during the high flow period in structuring the phytoplankton community. The skewness of 7 days discharge emerged as a key driver of hydrological regime, and it always had an indirect effect on functional group **TB** during two hydrological periods.

(2) Anthropogenic stressor by urban land-use acted as a critical driver for functional group **M** especially during high flow period. The group **M** was directly related to phosphorous relevant indicators indicating its sensitivity to the concentration of phosphorous. The area with a higher share of water bodies was also a key causing factor during the low flow period, guide the lacustrine zone as its origin.

Our results provide evidence that, for a more comprehensive and accurate understanding of the aquatic status, we should also take consideration of different hydrological periods. Secondly, we recommend functional groups as effective indicators of phytoplankton dynamics to simplify the pattern and seize the key issue to achieve a better knowledge of the relationship between phytoplankton and multiple environmental stressors. Climate change and anthropogenic activities, for instance altering flow regime and land-use pattern, act as important ecological filters of riverine phytoplankton community via physicochemical conditions. Further studies across lake-river continuums are needed to enhance our understanding toward incorporating hydrological regime, land-use pattern, as well as physicochemical conditions into investigation designs, which would enable us to keep the pace with increasing demand for sustainable watershed management. Structure equation model disentangle the contribution of multiple stressors. It would be an outperforming method to generalize results, identify common patterns, and predict response of phytoplankton to environmental changes in future studies.

Chapter 5 Hydrological and environmental variables outperform spatial factors in structuring species, trait composition and beta diversity of pelagic algae

Naicheng Wu^{1,2,3*}, Yueming Qu¹, Björn Guse¹, Kristė Makarevičiūtė⁴, Szewing To¹, Tenna Riis³ and Nicola Fohrer¹

¹Department of Hydrology and Water Resources Management, Institute for Natural Resource Conservation, Kiel University, 24118 Kiel, Germany

²Aarhus Institute of Advanced Studies, Aarhus University, Høegh-Guldbergs Gade 6B, 8000 Aarhus C, Denmark

³Department of Bioscience, Aarhus University, Ole Worms Allé 1, 8000 Aarhus C, Denmark

⁴Helmholtz Centre for Ocean Research Kiel (GEOMAR), DüsternbrookerWeg 20, 24105 Kiel, Germany

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Abstract

Aim There has been increasing interest in algae based bio-assessment, particularly trait-based approaches are increasingly suggested. However, the main drivers, particularly the contribution of hydrological variables, of species composition, trait composition and beta diversity of algae communities are less studied. The aim of this study was to link species and trait composition to multiple factors (i.e., hydrological variables, local environmental variables and spatial factors), and to determine their relative roles in shaping species composition, trait composition and beta diversities of pelagic algae communities.

Location Treene catchment, Germany.

Methods Pelagic algae were collected from a German lowland catchment, where a well proven ecohydrological modelling enabled to predict long-term discharges at each sampling site. We related algal species, trait composition and beta diversities to hydrological, local environmental and spatial variables. Canonical redundancy analysis with variation partitioning was used to study species and trait composition in relation to multiple factors, while Mantel tests was conducted to examine the relationships between beta diversities with the multiple factors.

Results Both trait and species composition showed significant correlations with hydrological, environmental and spatial variables, and variation partitioning revealed that the hydrological and local environmental variables outperformed spatial variables. Mantel tests showed that both species and trait-based beta diversities were mostly related to hydrological and environmental heterogeneity with hydrological contributing more than environmental variables, while purely spatial impact was less important.

Main conclusions Our findings revealed the relative importance of hydrological variables in shaping pelagic algae community and their spatial patterns of beta diversities, emphasizing the need to include hydrological variables in long-term bio-monitoring campaigns and biodiversity conservation or restoration. A key implication for biodiversity conservation was that maintaining the instream flow regime and keeping various habitats among rivers are of vital importance. However, further investigations at multi-spatial and temporal scales are greatly needed.

Keywords: Beta diversity, functional traits, lowland river, multiple stressors, pelagic algae, species composition, ecohydrological modelling

5.1 Introduction

Although rivers cover only 0,8% of the landmasses on the earth, they contain more than 6% of global species and are thus invaluable for biodiversity and ecosystem services (Altermatt *et al.*, 2013). They also act as conveyor belts of biodiversity information by dictating dispersal pathways (Deiner *et al.*, 2016) and thus river ecosystems are a biodiversity hotspot. With arising from human-mediated fast global change, water quality was degraded and the use of aquatic organisms in bio-assessments became popular in last decades. Studying the river organisms in relation to abiotic factors, and identifying spatial patterns of biodiversity as well as their driving mechanisms have become a major trend of community ecology as basis for prioritizing global and regional conservation efforts (Myers *et al.*, 2000; Wang *et al.*, 2016). As the major primary producer, algae are increasingly being used as reliable environmental indicators in streams and rivers globally, especially in the context of recent international water framework directive policies such as EU WFD (Hering *et al.*, 2006; Lange *et al.*, 2016; Wu *et al.*, 2017) because they strongly respond to environmental changes (Stevenson *et al.*, 2010; Larras *et al.*, 2017).

The relationships between river algae and abiotic factors have been studied with a long history. Nevertheless, previous studies and biomonitoring campaigns focused mostly on local environmental variables such as nutrients (Kelly & Whitton, 1995; Lange *et al.*, 2011), pH, temperature (Wu *et al.*, 2011; Çelekli *et al.*, 2014) and recently also spatial factors (Heino & MykrÄ, 2008; Tang *et al.*, 2013b; Rezende *et al.*, 2014; Wu *et al.*, 2014). By comparison, little attention has been paid to hydrological factors such as flow regime, although many studies have shown that riverine algal communities are linked to flow velocity and discharge (Jowett & Biggs, 1997; Biggs *et al.*, 1999; Riseng *et al.*, 2004; Munn *et al.*, 2010; Wu *et al.*, 2010) and catchment wetness (Wu *et al.*, 2016). Yet a profound understanding on the interaction of hydrological variables and river organisms, specifically algae, is still missing.

In addition to species composition, ecologists have recently started investigating trait composition too because it reflects the functional adaption of organisms to its environment (McGill *et al.*, 2006; Soininen *et al.*, 2016). Usually traits are divided into two types: ecological traits (related to habitat preferences, like pH, oxygen and temperature tolerance, tolerance to organic pollution, etc.) and biological traits (e.g., life history, physiological, behavioral and morphological characteristics, such as reproductive strategies, motility, cell size, life form, etc.). In comparison with traditional taxonomic indices, biological traits show greater consistency in their responses across temporal and spatial scales (Menezes *et al.*, 2010; Soininen *et al.*, 2016) and furthermore give important insights into the

mechanisms driving the community and ecosystem processes along the gradients of influential factors (Litchman & Klausmeier, 2008). Traits can furthermore serve to disentangle multiple interacting influential factors (Lange *et al.*, 2014; Baattrup-Pedersen *et al.*, 2016). Traits have been used for different purposes in terrestrial plants (Grime, 1979; Tilman, 1980) and macroinvertebrate (Menezes *et al.*, 2010), but only very recently been considered for freshwater algae (McGill *et al.*, 2006; Lange *et al.*, 2016; Tapolczai *et al.*, 2016), particularly in phytoplankton studies (Reynolds *et al.*, 2002; Padisák *et al.*, 2009; Colina *et al.*, 2016; Thomas *et al.*, 2016). Recent studies have shown the advantages of applying traits for biomonitoring of freshwater ecosystems and for biodiversity conservation (McGill *et al.*, 2006; Litchman & Klausmeier, 2008; Menezes *et al.*, 2010; Lange *et al.*, 2011; Di Battista *et al.*, 2016; Soininen *et al.*, 2016). For instance, Soininen *et al.* (2016) concluded from a large-scale study that trait distributions are driven primarily by the local environmental and less dependent on the spatial location, which makes them better suited for researches on global environmental change. However, the comparisons between species and trait composition in relation to abiotic factors at catchment scale, in particular multiple stressors, are still poorly documented.

Rivers are widely affected by a mixture of stressors caused by anthropogenic activities (Hering *et al.*, 2015). Generally, they include flow regime alteration, diffuse and point sources. For example, flow diversion due to dam construction can disrupt the river's natural connectivity and impede the cycling of organic matter, sediments and nutrients from up- to downstream (Wu *et al.*, 2012b). In addition, global land use and climate changes have addressed additional stressors for rivers. The patterns of species composition in biological communities are governed by both local and spatial processes (Curry & Baird, 2015). Dispersal limitation creates spatial structure in assemblage composition because the probability of successful movement between locations is negatively related to the geographic distance between them. Spatial variables such as altitude or geographic location can play important and confounding roles determining the presence, absence and abundance of the algal species and consequently influence the algae-based bio-assessment (Wu *et al.*, 2014). One previous view of algae distributions was that they were ubiquitous and could disperse everywhere due to the immense population sizes, especially over a long time period (Fenchel & Finlay, 2004). If this theory was right, the similar algae species should be found at all places with similar environmental conditions, which was usually not the case leading to a large portion of variation explained by spatial factors (Soininen *et al.*, 2004; Smucker & Vis, 2011). Thus, it is difficult to determine whether an absent species is due to the unallowable environmental conditions or it has not dispersed to that location. Studying spatial geographic influences on algal composition is therefore a fundamental step in describing ecological patterns, making bio-monitoring more robust, which ultimately achieves

sustainable management (Smucker & Vis, 2011). Nevertheless, the current biomonitoring using algae often focuses on the local environmental conditions with seldom regarding to processes operating at larger spatial scales.

The purpose of this study is to assess the influence of different factors (e.g., hydrological variables, local environmental variables and spatial factors) on shaping species composition, trait composition and beta diversities of riverine pelagic algae communities in a German lowland catchment (Fig. 5.1). We had two main questions for this research: i) how much do hydrological variables contribute to variations of species and trait compositions compare to local environmental and spatial variables? ii) what are the major drivers of the species, trait composition and beta diversities of pelagic algae communities? The hypotheses were that 1) hydrological, environmental and spatial variables interacted to determine species composition, trait composition and beta diversity of pelagic algae, 2) hydrological variables would be a key driver of species composition, trait composition and beta diversity, 3) trait distributions are less dependent on historic (i.e. spatial) than species composition, and are driven primarily by the local environment.

5.2 Methods

5.2.1 Description of the study area

The Treene catchment with a basin area of 481 km² is located in Northern Germany as a part of a lowland area (Fig. 5.1). Sandy, loamy and peat soils are characteristic for this area. Land use is dominated by agriculture and pasture. Around 50% of the area is covered by arable land and ~ 30% by winter pasture (Guse *et al.*, 2015a). The major tributaries are Bondenau (Bo), Kielstau (Ki), Bollingstedter Au (Bo), Jerrisbek (Je), Juebek (Ju) and Sankermark See (Sa). It is in a temperate climate zone, influenced by marine climate, with mild temperature and high precipitation in winter and the maximum elevation gradient is 76 m. There are several lakes (top three from size: Sueden See: 0.64 km², Sankermarker See: 0.56 km², Winderatter See: 0.24 km²) in the catchment, mainly located in the upstream areas of the river. As a nested sub-catchment of the Treene, the Kielstau catchment (50 km²) (Fig. 5.1) has been appointed to an UNESCO Ecohydrological Demonstration Site in the year 2010 (Fohrer & Schmalz, 2012). The Soltfeld gauging station (at the outlet of the Kielstau catchment), and Treia gauging station (at the outlet of Treene catchment) are a part of the official gauging network of the Federal State Schleswig-Holstein (Fig. 5.1A). In addition, four more spatially distributed hydrological stations with continuous daily discharge time series were used for this study as shown in Fig. 5.1B. The Treene catchment was selected because of the reliability of the well proven hydrologic SWAT model (Guse *et al.*, 2015b; Guse *et al.*, 2015a), which enables to simulate long-term discharges

at different sampling sites. These conditions, which are rare in previous studies, are ideal to apply and test our hypotheses.

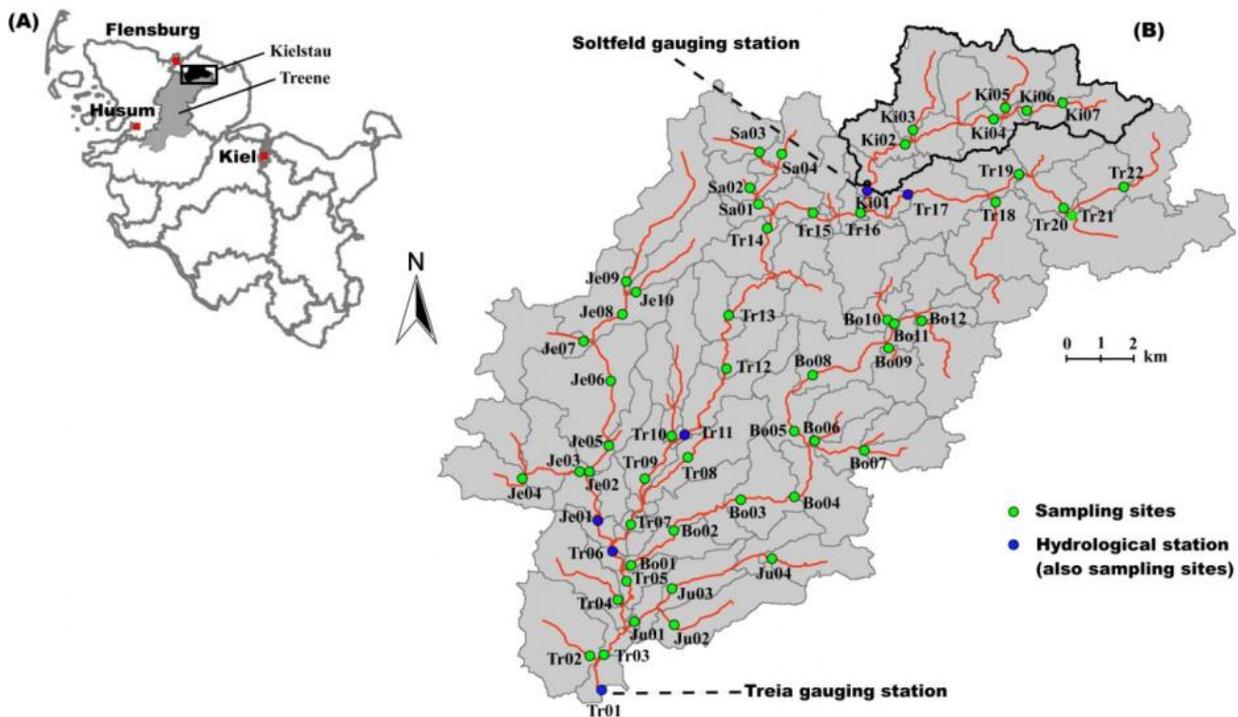


Fig. 5.1 The location of 6 hydrological stations and sampling sites of the Treene catchment (B) in Schleswig-Holstein state (A) of Germany. Sub-basins of Treene as derived by the ecohydrological model SWAT are shown too.

5.2.2 Sampling methods and primary procedures

Field surveys were carried out in the main stream and its tributaries in December, 2014. We visited 59 sampling sites and abbreviated the sites according to each sub-basin they were located in. Pelagic algae were collected using plankton net with a mesh size of 20 μm . A known volume of water (10-30 liters, depending on site) was filtered and fixed immediately by neutral lugol's solution. Algae samples were concentrated to 25 mL for further processing after natural sedimentation in the laboratory. Simultaneously, at each sampling point, water temperature (WT), pH, electric conductivity (EC), and dissolved oxygen (DO) of the surface water were measured *in situ* using Portable Meter (WTM Multi 340i and WTW Cond 330i, Germany). Besides, river width, depth and velocity were surveyed at the sampling points (velocity – using FlowSens Single Axis Electromagnetic Flow Meter, Hydrometrie, Germany).

Concurrently, water samples were taken in two pre-cleaned plastic bottles (500 ml each) for water chemistry measurement in the laboratory. In the lab, water samples were partially filtrated through GF/F glass microfiber filter (Whatmann 1825-047) for measurements of phosphate-phosphorus (PO_4 -

P), ammonium-nitrogen (NH₄-N), nitrate-nitrogen (NO₃-N), nitrite-nitrogen (NO₂-N), chloride (Cl⁻) and sulphate (SO₄²⁻) according to the standard methods DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). DIN is the sum of nitrite (NO₂-N), nitrate (NO₃-N) and ammonia (NH₄-N). The concentrations of total phosphorus (TP) were measured with unfiltered water samples. Total suspended solids (TSS) were measured according to Standard Operating Procedure for Total Suspended Solid Analysis (US Environmental Protection Agency, 1997). Inorganic carbon (IC), dissolved total carbon (DTC) and dissolved organic carbon (DOC) were measured with a DIMA-TOC-100 total organic carbon analyzer, according to infrared spectroscopy method (Dimatec Analysentechnik GmbH, Germany).

5.2.3 Algae preparation and identification

For the soft algae (non-diatom) identification, algae were counted with optical microscope (Nikon Eclipse E200-LED, Germany) at ×400 magnifications in a Fuchs-Rosental chamber. The counting unit was individual (unicell) and at least 300 units were counted for each sample. Taxonomic identification of species was done according to Hu and Wei (Hu & Wei, 2006) and Burchardt (Burchardt, 2014).

To identify diatoms, permanent diatom slides were prepared after oxidizing the organic material by Hydrogen Peroxide Method (30% H₂O₂ solution) and mounted in Naphrax (Northern Biological supplies Ltd., UK, R1=1.74) following Biggs and Kilroy (2000). A minimum of 300 valves were counted for each sample using a Zeiss Axioskop microscope at 1000× under oil immersion. Diatoms were identified to the lowest taxonomic level possible (mainly species level) according to following key books (Simonsen, 1987; Round *et al.*, 1990; Lange-Bertalot, 2000a, 2000b, 2005, 2007; Bey & Ector, 2013), Hofmann (2011) and Bak (2012). Algae densities were expressed as cells/L.

5.2.4 Biotic datasets

We used both traditional taxonomic composition and a functional perspective based on species traits composition.

- 1) Species composition (Sp): inclusion of all observed 327 algal species with their relative abundances.
- 2) Trait composition (Tr): we assigned 327 algal species to different functional traits: cell sizes (pico, nano, micro, meso, macro and large), guilds (low profile, high profile, motile and planktonic guild), life form (colonial, filamentous, flagellate and unicellular), eco-morphology (combination between cell sizes and guilds + life form), nitrogen fixation species, reproductive strategies (fission and fragmentation) and spore formation (no spore, akinetes, oospores and zygospores). Traits with medians

of 0 were eliminated because they would prevent the further statistical analyses and thus 44 traits were retained for final Tr dataset.

3) Beta diversities (β): to calculate the pairwise dissimilarities, we used the Bray-Curtis similarity index on Sp and Tr separately (i.e., $Sp\beta_{BRAY}$, $Tr\beta_{BRAY}$), since this index takes into account differences in abundances and emphasizes dominant species/trait (Magurran, 2004). Similarly, we also employed Jaccard similarity index on Sp and Tr, respectively (i.e., $Sp\beta_{JACC}$ and $Tr\beta_{JACC}$).

5.2.5 Abiotic datasets

Three abiotic datasets were formed.

(1) Hydrological variables (Hv): except for *in situ* measured width, water depth and flow velocity at the sampling point, long-term flow discharges (2010-2016) of each sampling site were simulated by the ecohydrological SWAT model (Soil and Water Assessment Tool) (Arnold *et al.*, 1998). The SWAT model is a semi-distributed model which provides daily outputs of a large set of hydrological variables for each subbasin. In this case study, the Treene catchment was subdivided into 108 subbasins (Fig. 5.1), which also covered the tributaries of the Treene (Guse *et al.*, 2014). Thus, the spatially distributed model results consider the spatial heterogeneity in the catchment. To obtain reliable spatially distributed model results, a multi-site calibration approach was selected. Thus, six hydrological stations were included in the calibration procedure with the aim to obtain good model results for all stations (Guse *et al.*, 2015a). We used a calibration period from 2001 to 2005 and validate the model from 2006 to 2016 for discharge. Since the measurements at one hydrological station ended in 2014, only five stations were used in the model validation. To assess the model performance, three typical performance measures namely Nash-Sutcliffe Efficiency (NSE), Percent Bias (PBIAS) and the root mean square error deviation (RSR) were used. The modelled discharge shows a good matching with the measured data, in particular under consideration of the multi-site approach with joint model performance estimation for six hydrological stations (for details see Guse *et al.* 2015a). Based on the well performing model, daily model results for the investigation period of this study were provided for all sub-basins with sampling points. In order to obtain reliable results, always the next sub-basin outlet was used for each sampling point under consideration of the river network. Then we calculated the different hydrological indices according to Olden and Poff (Olden & Poff, 2003), which mainly included magnitude of flow events, frequency of flow events, rate of change in flow events and *in situ* measurement. Finally, 11 hydrological variables were selected after excluding the ones with significant multicollinearity (Table 5.1).

Table 5.1 Summary of hydrological (Hv), environmental (Ev) and spatial (SV) variables with their codes and descriptions in this study. Variables indicating significant multicollinearity (with variance inflation factor >10 and Spearman correlation coefficient >=0.75) are excluded. For spatial variables, only the variables after forward selection are shown here (see also Table 5.2).

Variables					
Code	Unit	Description	Mean	Min	Max
Hv		Hydrological variables			
Hv01	m ³ /s	Discharge at the sample day	2.27	0.01	18.30
Hv12	-	Skewness of 3 days' ahead discharge (including the sampling day)	0.32	-1.73	1.73
Hv13	-	Skewness of 3 days' ahead discharge (excluding the sampling day)	0.90	-1.69	1.73
Hv20	-	Skewness of 7 days' ahead discharge (including the sampling day)	0.95	-0.22	2.44
Hv21	-	Skewness of 7 days' ahead discharge (excluding the sampling day)	0.84	-1.58	2.64
Hv36	-	Skewness of 30 days' ahead discharge (including the sampling day)	1.14	-0.31	3.28
Hv40	D	Low flood pulse count in the past 14 days	4.71	0.00	14.00
Hv45	D	High flood pulse count in the past 30 days	4.31	0.00	12.00
Hv54	-	Rate of change (i.e. slope) in the last 3 days before the sampling day	0.18	-0.01	1.50
Hv55	-	Rate of change (i.e. slope) in the last 7 days before the sampling day	-0.04	-0.31	0.09
VELO	m/s	Flow velocity at the sampling point	0.98	0.00	10.24
Ev		Environmental variables			
WT	°C	Water temperature	5.69	0.20	8.40
PH	-	pH	7.49	6.74	9.73
DO	mg/L	Dissolved oxygen	9.49	4.61	12.30
TP	mg/L	Total phosphorus	0.22	0.06	0.63
PO4	mg/L	Orthophosphate-phosphorus (PO ₄ -P)	0.08	0.01	0.34
NH4	mg/L	Ammonium-nitrogen (NH ₄ -N)	0.31	0.03	1.43
NO3	mg/L	Nitrate-nitrogen (NO ₃ -N)	3.55	1.03	8.43
NO2	mg/L	Nitrite-nitrogen (NO ₂ -N)	0.02	0.00	0.05
CL	mg/L	Chloride (Cl ⁻)	24.92	14.20	41.70
SO4	mg/L	Sulphate (SO ₄ ²⁻)	31.82	12.90	73.10
TSP	mg/L	Total suspended particulates	12.08	2.60	46.28
DTC	mg/L	Dissolved total carbon	41.59	25.60	70.40
DOC	mg/L	Dissolved organic carbon	10.45	-0.15	29.50
AGRL	%	Agricultural Land-Generic (%)	51.83	15.04	79.65
FRSD	%	Deciduous forest (%)	2.23	0.01	9.89
FRSE	%	Evergreen forest (%)	1.02	0.02	9.02
FRST	%	Forests mixed (%)	2.46	0.00	13.47
FR	%	Forest in total (%)	5.71	0.86	15.13
RNGE	%	Rangeland (%)	0.70	0.00	4.33
UIDU	%	Industrial (%)	4.20	2.98	8.41
URLD	-	Residential-Low Density	0.43	0.00	3.98

UR	-	Residential in total	5.65	1.75	12.26
WATR	%	Water (%)	1.71	0.62	5.42
WETL	%	Wetlands (%)	1.01	0.00	7.19
WPAS	%	Winter pasture (%)	29.18	7.22	70.97
Sv		Spatial variables			
X	°N	Latitude	54.64	54.51	54.74
Y	°E	Longitude	9.43	9.27	9.67
PCNM1	-	Principal coordinates of neighborhood matrix1	0.00	-0.14	0.23
PCNM3	-	Principal coordinates of neighborhood matrix3	0.00	-0.29	0.24
PCNM6	-	Principal coordinates of neighborhood matrix6	0.00	-0.34	0.26
PCNM7	-	Principal coordinates of neighborhood matrix7	0.00	-0.31	0.28
PCNM10	-	Principal coordinates of neighborhood matrix10	0.00	-0.33	0.26
PCNM11	-	Principal coordinates of neighborhood matrix11	0.00	-0.24	0.55

(2) Environmental variables (Ev): Ev includes *in situ* and lab measured physicochemical variables (see above). Furthermore, land use data was obtained from Schleswig-Holstein State Bureau of Surveying and Geo-information (LVERMGEO-SH, 2012). Land use analysis was performed via GIS processing. Watershed area upstream from each sampling site was determined and land use within this area was considered as the land use affecting the sampling site. A total of 25 variables were retained after excluding the ones with significant multicollinearity (Table 5.1).

3) Spatial variables (Sv): except for the coordinates (X: Latitude, Y: Longitude), Moran's eigenvector maps were used to generate spatial variables representing geographical positions and dispersal across the rivers. This method is a powerful approach able to detect spatial structures of varying scale in response data and more flexible than other eigenvector-based approaches for irregular sampling design (Tang *et al.*, 2013a), as the case in our study. In brief, this method proceeds as follows: i) a geographical distance matrix as Euclidean distance between each pair of sampling sites calculated using the *earth.dist* function in the package *fossil* in R (Version 3.3.2). ii) Principal Coordinates of Neighborhood Matrix (PCNM) analysis based on the geographical distance were used to compute spatial variables (i.e. historic factors) representing geographical positions through the *pcnm* function in R package *vegan* (Version 2.4-2). The generated eigenvectors were considered as spatial variables (i.e., PCNMs), which could reflect unmeasured broadscale variation in the modern environment or historical factors, e.g. natural dispersal-generated patterns demonstrating internal local-scale dispersal dynamics or regional-scale migration history (Svenning *et al.*, 2009). PCNMs are ranked in descending order based on their eigenvalues and simultaneously coded in ascending order (starting from 1), and PCNMs with large eigenvalues and small code represent broad-scale spatial pattern, while the smaller eigenvalues with large code representing fine-scale patterns. PCNMs are commonly used to describe species dispersal processes (Curry & Baird, 2015). Usually, only PCNMs with positive eigenvalues

are retained as spatial explanatory variables (Tang *et al.*, 2013b). Among the 58 PCNMs generated, eigenvalues of PCNM components 1-37 were positive and thus 39 variables (including X, Y) were used in the following analyses (Table 5.1).

5.2.6 Data analyses

All analyses were performed with the R software (version 3.3.2, R Development Core Team 2016).

To explore the potential impacts of hydrological variables on trait and species compositions (*question i*), the following preliminary data analyses were conducted. Firstly, trait and species composition with relative abundance (0-100%) was Hellinger transformed (using function *decostand* in R package *vegan*), respectively, in order to reduce the weight of abundant species/trait while preserves Euclidean distances between samples in the multidimensional space. Secondly, the variables in abiotic datasets (Hv, Ev and Sv) with significant multicollinearity (with variance inflation factor >10 and Spearman's rank correlation coefficient $|r| \geq 0.75$) were excluded (details see also above). A preliminary detrended correspondence analysis (DCA, using function *decorana* in R package *vegan*) on the Hellinger transformed trait and species data produced a longest gradient length of 2.03 and 4.82 along the first axis, suggesting that redundancy analysis (RDA) and canonical correspondence analysis (CCA) were appropriate for Tr and Sp, respectively (Lepš & Šmilauer, 2003). We performed RDA using the *rda* function and CCA using *cca* function and tested the significance using the *anova* function. Only if it was significant, a forward selection could be proceeded to get a parsimonious model with two stopping criteria: significance level and the adjusted coefficient of determination (AdjR^2) of the global model (Blanchet *et al.*, 2008). Forward selection was performed by the *forward.sel* function in R package *packfor*. The selected variables were then used as explanatory variables for the following variation partitioning analysis by using *varpart* function R package *vegan* (Version 2.4-2).

Next, we ran Mantel tests in order to examine the changes in trait and species composition along hydrological, environmental and spatial gradients (*question ii*). The Mantel test has been utilized as a distance-based approach to study community beta diversities in relation to distance matrices (Wang *et al.*, 2012; Teittinen *et al.*, 2016). The significance of this distance-decay relationship, which measures how dissimilarity decays with increasing distance between pairwise sites, was determined using Mantel test with 9999 permutations. In brief, the Mantel statistic r (range -1 to 1) is a correlation between two dissimilarities or distance matrices. We first constructed dissimilarity matrices for biotic data (i.e., beta diversities, $\text{Sp}\beta_{\text{BRAY}}$, $\text{Tr}\beta_{\text{BRAY}}$, $\text{Sp}\beta_{\text{JACC}}$ and $\text{Tr}\beta_{\text{JACC}}$, for details see above) and Euclidean distances separately for the hydrological, environmental and spatial variables (i.e., Hvdis ,

Evdis, Svdis). In addition to simple Mantel tests using two matrices, we used partial Mantel tests to tease apart the pure effects of hydrological, environmental and spatial variables on biotic matrices, and the significance was assessed using 9999 permutations, as described above. Mantel and partial Mantel tests were run using functions *mantel* and *mantel.partial*, respectively, in R package *vegan* (Version 2.4-2).

5.3 Results

5.3.1 Variability of abiotic factors

During the sampling period, river reaches of the study area (Fig. 5.1) varied widely in water quality and habitat characteristics and the main abiotic variables are summarized in Table 5.1. For example, water temperature (WT) ranged from 0.20 to 8.40 °C (mean: 5.69 °C), pH ranged from 6.74 to 9.73 (mean: 7.49), total phosphorus (TP) averaged 0.22 mg/L (0.06 to 0.63 mg/L), and ammonium-nitrogen (NH₄-N) ranged from 0.03 to 1.43 mg/L (mean: 0.31 mg/L), while total suspended particulates (TSP) averaged 12.08 mg/L (2.60-46.28 mg/L). Land use in the catchment was mainly open canopy and dominated by high agricultural land (%) of 51.83% (15.04-79.65%), while forest cover was low (mean coverage was 5.71% ranging from 0.86 to 15.13%). Due to a heavy rainfall event during the sampling period, hydrological variables varied greatly among the sampling sites. For instance, flow velocity (VELO) ranged from 0 to 10.24 m/s with an average of 0.98 m/s, discharge (Hv01) ranged from 0.01 to 18.30 m³/s with a mean of 2.27 m³/s, while skewness of flows (Hv12, Hv13, Hv20, Hv21 and Hv36), low flood pulse count (Hv40), high flood pulse count (Hv45) and change rates of flows (Hv54, Hv55) also showed large ranges (for details see Table 5.1). In addition, the spatial variables showed a small variation with latitude ranging from 54.51 to 54.74° N and longitude from 9.27 to 9.67° E, which was due to the relatively small catchment of Treene (481 km²).

5.3.2 Drivers of traits and species composition

In the RDA analysis for trait composition (Tr), hydrological (Hv), environmental (Ev) and spatial variables (Sv) all showed significant relationships with trait composition (by *anova* function in R package *vegan*, Table 5.2). Five Hv, 9 Ev and 7 Sv variables were selected by forward selection. According to variation partitioning analysis, the three sets could explain 57.0% variation of trait composition (Fig. 5.2A). The pure effects of Hv (3.7%) and Ev (6.0%) accounted for larger parts than the pure effect of Sv (1.5%), while the joint effect of Hv, Ev and Sv was the largest with 22.2%. In general, the joint contribution by Hv and Ev (Hv*Ev) (Tr: 15.4%, Sp: 9.5%) was higher than those by Hv*Sv (Tr: 2.7%, Sp: 2.0%) and Ev*Sv (Tr: 5.5%, Sp: 3.7%) (Fig. 5.2). The unexplained fraction of

trait composition (43.0%) was lower than for species composition (62.5%) (Fig. 5.2). However, the variation partitioning (Fig. 5.2) showed that both trait and species composition were less dependent on spatial factors, rejecting our third hypothesis.

Table 5.2 Results of forward selection of hydrological variables (Hv), environmental variables (Ev) and spatial variables (Sv) for trait (Tr) and species (Sp) composition respectively. The selected variables are in the order in which they were selected in the forward selection procedure. AdjR²Cum (cumulative adjusted R-square), F and p values are shown. All selected variables show no significant multicollinearity (with variance inflation factor VIF <10, by *vif.cca* function in R package *vegan*). Codes of variables are as in Table 5.1. Significance was expressed as * p < 0.05, ** p < 0.01, *** p < 0.001 (by *anova* function in R package *vegan*).

Trait composition (Tr)				Species composition (Sp)			
Variables	AdjR ² Cum	F	p	Variables	AdjR ² Cum	F	P
Hv***				Hv***			
Hv21	0.23	17.88	0.001	Hv21	0.15	11.29	0.001
Hv40	0.35	12.20	0.001	Hv40	0.22	6.36	0.001
Hv55	0.39	4.53	0.004	Hv55	0.25	2.83	0.008
Hv45	0.42	3.93	0.011	Hv45	0.28	3.35	0.003
Hv36	0.44	2.75	0.031	Hv36	0.29	2.07	0.036
Ev***				Ev***			
PO4	0.14	10.17	0.001	SO4	0.06	4.45	0.001
TP	0.33	16.93	0.001	UR	0.10	3.55	0.001
SO4	0.36	3.93	0.003	WPAS	0.13	2.98	0.001
PH	0.39	3.46	0.004	WATR	0.15	2.44	0.005
WT	0.41	3.39	0.005	PO4	0.17	2.33	0.008
DTC	0.44	3.25	0.011	TP	0.22	4.53	0.001
WPAS	0.46	3.76	0.003	PH	0.24	2.51	0.004
FRST	0.48	2.35	0.040	DTC	0.26	2.37	0.003
NO2	0.49	2.29	0.043	WT	0.28	2.08	0.009
				NH4	0.29	1.83	0.024
				FRST	0.30	1.66	0.037
Sv***				Sv*			
PCNM6	0.09	6.84	0.001	PCNM6	0.05	4.02	0.001
PCNM7	0.17	6.56	0.001	PCNM7	0.08	2.86	0.004
PCNM3	0.22	4.52	0.003	PCNM3	0.10	2.59	0.016
PCNM10	0.25	3.29	0.020	PCNM10	0.12	2.24	0.008
X	0.28	2.74	0.036	X	0.14	2.26	0.010
PCNM11	0.30	2.54	0.039	PCNM1	0.17	2.78	0.002
Y	0.32	2.79	0.023				

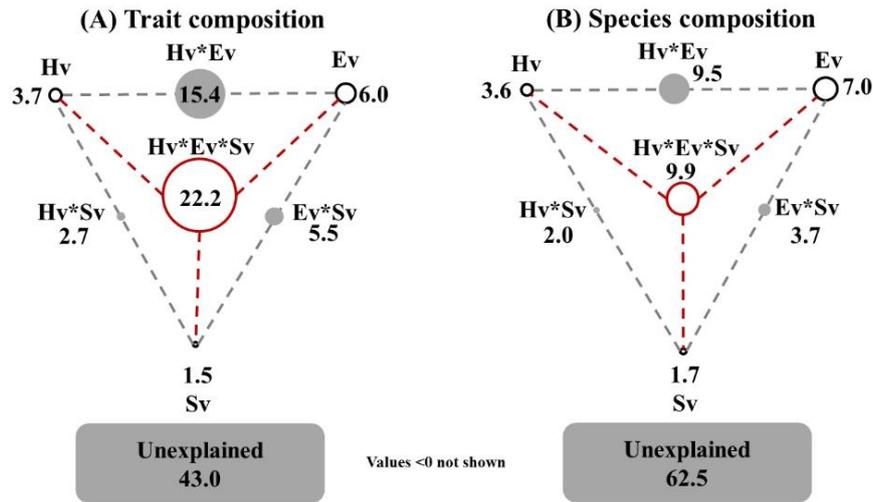


Fig. 5.2 Contributions of the hydrological (Hv), environmental (Ev) and spatial variables (Sv) to the variances in trait (A) and species composition (B). Each diagram represents a given biological variation partitioned into the pure effects of Hv, Ev and Sv (i.e. when removing the variations caused by other two factors), interaction between any two variables (Hv*Ev, Hv*Sv, Ev*Sv), interaction of all three factors (indicated by red circle), and unexplained variation (total variation = 100). The geometric areas of circles were proportional to the respective percentages of explained variation. More details on the selected variables are shown in Table 5.2. Similarly, in the CCA analysis for species composition (Sp), Hv, Ev and Sv all showed significant relationships with species composition (by *anova* function in R package *vegan*) and 5 Hv, 11 Ev and 6 Sv variables were selected by forward selection (Table 5.2). Variation partitioning indicated that the three sets explained only 37.5% variation of species composition. The variation purely explained by Hv, Ev and Sv were 3.6%, 7.0% and 1.7%, respectively, while the shared fraction was 9.9% (Fig. 5.2B).

5.3.3 Main drivers of traits and species-based beta diversities

Mantel testes showed that trait dissimilarities (i.e., beta diversities) based on both Bray-Curtis and Jaccard indices ($Tr\beta_{BRAY}$ and $Tr\beta_{JACC}$) increased significantly with hydrological (Hvdis), environmental (Evdis) and spatial distances (Svdis) (Fig. 5.3, Table 5.3). The relationships between trait dissimilarities ($Tr\beta_{BRAY}$ and $Tr\beta_{JACC}$) and hydrological distances (Hvdis) were consistently stronger than the relationships with environmental distances (Evdis), while the weakest relationships were with spatial distances (Svdis) (Fig. 5.3). Based on partial Mantel tests, the pure effects of hydrological and environmental distances on trait dissimilarities were significant using both indices, whereas the pure effect of spatial distance was nonsignificant using the both indices (Table 5.3).

Table 5.3 Results of Mantel and partial Mantel test for the correlation between β diversities for traits (Tr) and species (Sp) (Bray-Curtis and Jaccard: $Tr\beta_{BRAY}$, $Tr\beta_{JACC}$, $Sp\beta_{BRAY}$ and $Sp\beta_{JACC}$) and hydrological (Hvdis), environmental (Evdis) and spatial Euclidean distances (Svdis). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. † the pure effect while controlling for the other two distances.

Index	Hvdis	Evdis	Svdis	Hvdis [†]	Evdis [†]	Svdis [†]
$Tr\beta_{BRAY}$	0.287***	0.179***	0.060*	0.311**	0.218**	0.016
$Tr\beta_{JACC}$	0.301***	0.186***	0.069**	0.327**	0.228**	0.023
$Sp\beta_{BRAY}$	0.218***	0.188***	0.032	0.242**	0.216**	-0.013
$Sp\beta_{JACC}$	0.224***	0.179***	0.039	0.247**	0.207**	-0.004

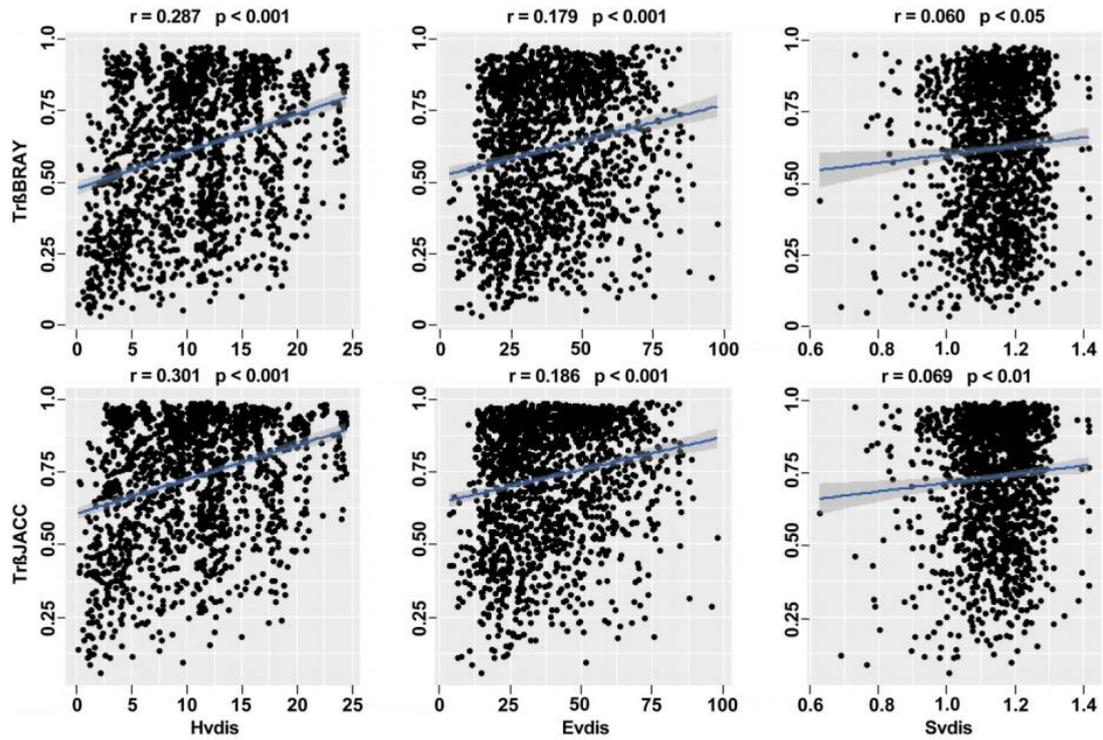


Fig. 5.3 Relationship between trait dissimilarities (Bray-Curtis and Jaccard: $Tr\beta_{BRAY}$ and $Tr\beta_{JACC}$) and hydrological (Hvdis), environmental (Evdiss) and spatial Euclidean distances (Svdis). The relationships were statistically significant according to the Mantel test (9999 permutations, $p < 0.05$, see Table 5.3). Regression lines based on linear models are shown by solid blue lines and grey areas around the blue lines indicate 97.5% confidence bands.

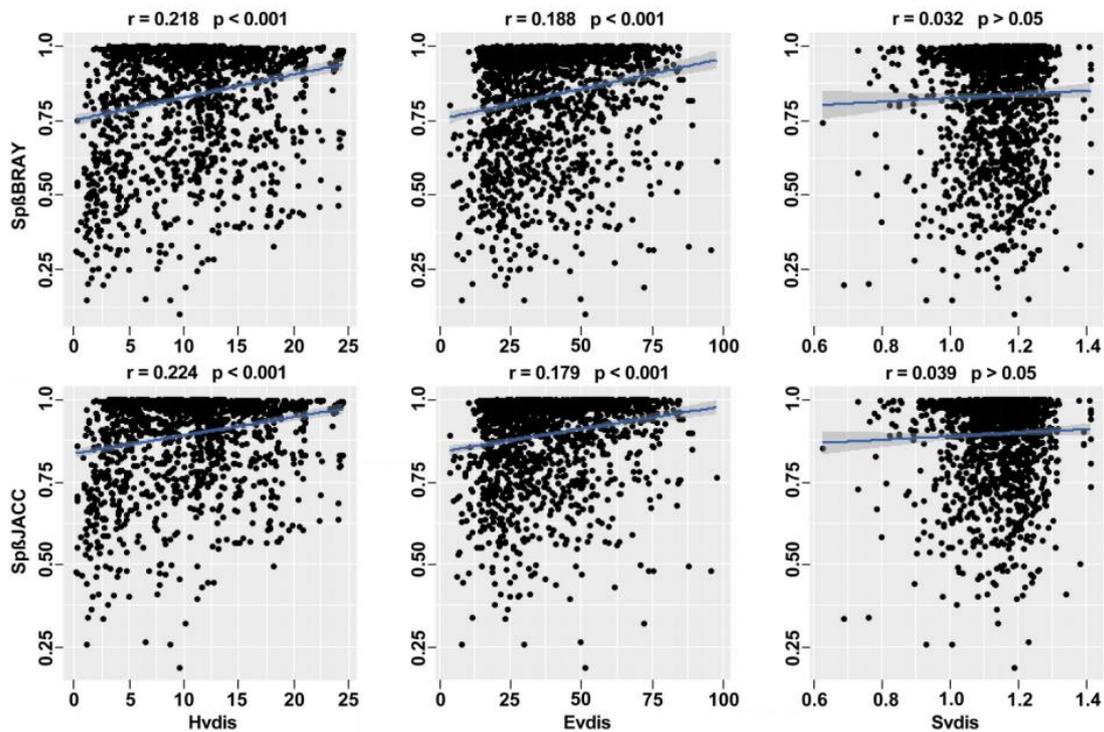


Fig. 5.4 Relationship between species dissimilarities (Bray-Curtis and Jaccard: $Sp\beta_{BRAY}$ and $Sp\beta_{JACC}$) and hydrological (Hvdis), environmental (Evdiss) and spatial Euclidean distances (Svdis). The relationships were statistically significant according to the Mantel test (9999 permutations, $p < 0.05$, see Table 5.3). Regression lines based on linear models are shown by solid blue lines and grey areas around the blue lines indicate 97.5% confidence bands.

As for species dissimilarities based on both Bray-Curtis and Jaccard indices ($Sp\beta_{BRAY}$ and $Sp\beta_{JACC}$), similar results were found (Fig. 5.4, Table 5.3). The pairwise species compositional dissimilarities ($Sp\beta_{BRAY}$ and $Sp\beta_{JACC}$) significantly increased with the corresponding changes in hydrological (Hvdis) and environmental distances (Evdiss). Further, the relationships between species dissimilarities ($Tr\beta_{BRAY}$ and $Tr\beta_{JACC}$) and hydrological distances (Hvdis) were consistently stronger than the relationships with environmental distances (Evdiss) (Table 5.3). In contrast, there was no significant spatial distance-decay for the both indices ($p>0.05$) (Fig. 5.4). According to partial Mantel tests, the pure effect of spatial distance was nonsignificant, while the pure effects of hydrological and environmental distances on species dissimilarities were significant using both indices (Table 5.3).

5.4 Discussion

One of the long-standing tasks in ecology is to explore the factors controlling the abundance and distribution patterns of aquatic organisms and the causes underlying these patterns. Although the relationship between algae community and abiotic factors (e.g., resources, disturbances, grazers, etc.) has been intensively investigated, the relative roles of different factors to algal variations remain controversial (Wu *et al.*, 2011). For example, some studies found that the geographical topography (e.g., altitude, latitude, longitude) and climate were the dominant factors regulating algae variation (Tang *et al.*, 2013b; Bae *et al.*, 2014; Wu *et al.*, 2014). In contrast, local environmental variables (e.g., substrate composition, sediments, nutrients, oxygen contents, bio-interaction) were often considered to be the main regulating factors (Bae *et al.*, 2014; Bussi *et al.*, 2016). Besides, previous studies have rarely taken hydrological variables into consideration. This might be due to the fact that acquisition of accurate hydrological variables needs long-term discharge data at different sampling sites, which is often time-consuming (e.g., the measurement of discharge). Obtaining data from field hydrological stations is an alternative way but it is normally impossible for every sampling site because of the limited numbers of hydrological stations, for instance only 6 stations in our catchment with 59 sampling sites (Fig. 5.1). At this situation, a well-proven hydrological modelling would be a good choice since it enables to predict long-term discharge variations at different sampling sites, as the case in our study area.

In this study we used hydrological modelling to obtain hydrological data for 59 sites and as expected, found that the hydrological variables, e.g., skewness of flow (Hv21, Hv36), flood pulse count (Hv40, Hv45), change rate of flow (Hv55), were the most important factors affecting both trait and species composition. Hydrological conditions are general factors that determine the physical habitat conditions and affect (directly or indirectly) many other environmental variables that are key factors

in pelagic algae community development, such as nutrient delivery, sediment transportation, residence time, disturbance intensity, temperature, light availability, and dissolved oxygen. That was also the reason why hydrological variables showed a higher shared effect with local environmental variables (Fig. 5.2). With the large number of available hydrologic metrics in use today, flow variabilities such as the magnitude, frequency, duration, timing, and rate of change of flows were the most important factors regulating ecological processes in aquatic ecosystems (Bhat *et al.*, 2010). A previous study on the relations among 83 hydrologic metrics and changes in algal communities of United States was consistent with our study and demonstrated that up to 73% of variance of specific algal community metrics could be explained by hydrological variables (Steuer *et al.*, 2010). Moreover, a recent study (Wu *et al.*, 2016), which was in line with our finding, also found that hydrological conditions were the highest contributor to the temporal variations of pelagic algae communities. Skewness of flows was found to be one of the most consistently dominant indices across all stream types and may be a particularly important measure of flow condition for certain riverine taxa (Olden & Poff, 2003), e.g., annual skewness of the flow has been linked to fish mobility and colonizing ability (Puckridge *et al.*, 1998). High flow event frequency (e.g., flood pulse count, change rate of flow), which was found to be transferable across stream type, was the most ecologically relevant hydrologic condition metrics. Previous studies with the aim of characterizing the response of phytoplankton to high flow events have indicated the importance of flow events in driving the patterns of phytoplankton distribution (Cook *et al.*, 2010; Saeck *et al.*, 2013). However, how does individual hydrological variable affect the pelagic algae composition and diversity was still less investigated so far and a possible reason was that few studies have the necessary temporal and spatial resolution to fully characterize these effects. This also remains a need to identify their individual or joint impacts and associated mechanisms in the future studies by means of intensive field campaigns or indoor mesocosms. Furthermore, prompted by the importance of hydrological variables to algal communities, we therefore advocate that planning for long-term monitoring and biodiversity conservation or restoration should include hydrological variables.

Besides, interdisciplinary collaboration between ecology and hydrology warrants further attention since they can advance our knowledge in understanding the aquatic organisms in relation to abiotic factors particularly the hydrologic conditions. This is in line with combining measurement campaigns with coupled abiotic-biotic modeling with the aim to improve the abundance/occurrence of biota and their eco-hydrologic drivers. As shown in this study, spatially-distributed hydrological model studies allow an identification of hydrological situation which can be used to describe the abundance and occurrence of biota. The results of hydrological models can be used both as input for ecological habitat

models (Guse *et al.*, 2015b), to describe the habitat of different biota (Kiesel *et al.*, 2009) and for consecutive data analysis based on the model results as shown here and also in recent studies (Kiesel *et al.*, 2017).

Our results also demonstrated that the spatial factors were less important than local hydrological and environmental variables for both trait and species composition (Fig. 5.2). For lowland rivers, the question about where do riverine pelagic algae come from is an important issue and has long been debated since it directly determines the suitability of pelagic algae based bio-assessment, which become more and more popular at lowland catchments (Wu *et al.*, 2012a). Historically, it was believed there was no true riverine plankton and the pelagic algae found in rivers were believed to come from either upstream lentic waterbodies or the benthos (Hötzel & Croome, 1999). Obviously, if this view was right, the riverine pelagic algae were not suitable as a bio-indicator because they were flushed or drifted and not the adaptation to the local environmental habitats. As a consequence, riverine pelagic algae are less used for bio-monitoring than other communities, such as periphyton and benthic invertebrates. However, recent researches (Centis *et al.*, 2010; Wu *et al.*, 2011) have argued that benthic diatom communities are the source of the riverine pelagic algae may be too simplistic and they believed that planktonic algal species do reproduce within rivers and many species develop substantial populations *in situ*. Disentangling the relative roles of local and spatial variables on spatial pattern of the community is a promising way to understand the source of pelagic algae communities. Based on the metacommunity theory (Heino *et al.*, 2015), the observed community at a certain point are shaped by two broad categories of effects - local and regional (i.e. spatial) effects. Local effects are largely due to environmental constraints or species interactions, while spatial effects are driven by the flux of organisms from the regional species pool (Brown & Swan, 2010). Our results in this study showed that the pelagic community in Treene catchment was more affected by local effects (e.g., local hydrological and environmental variables) than spatial effects as indicated by spatial variables (Fig. 5.2). These findings supported the recent researches, and further emphasized the suitability of lowland pelagic algae as bio-indicator for local habitat changes. Nevertheless, factors such as interaction between organisms (niche competition), dispersal ability, species evolution, which were not considered in this study, may have reduced the explainable variations. Furthermore, the relative importance of different factors may vary among different regions and might depend on the spatial extent of the study area.

Another interesting finding showed that trait and species composition were both less dependent on spatial factors (Fig. 5.2), which contradicted our third hypothesis. As an alternative to species-based approaches, use of trait-based approaches in biomonitoring has been advocated in recent years, in particular because of the demand of mechanistic understanding of biological responses (Baattrup-

Pedersen *et al.*, 2017). Based on previous studies (Passy, 2007; B-Béres *et al.*, 2016; Lange *et al.*, 2016; Soininen *et al.*, 2016), trait composition would track local environment gradients better than species composition and was less dependent on historic (i.e. spatial) factors, making them better suitable for research on global environmental change (Soininen *et al.*, 2016). Nevertheless, our finding was rather unexpected compared to a recent similar study (Soininen *et al.*, 2016). These differences between findings may be related to the spatial scale of studied areas. In comparison to a single catchment (this study), the previous research compared the trait and species composition at a global scale (Soininen *et al.*, 2016). Generally, for species distribution, the importance of spatial effects increased with geographical distance as dispersal limitation, and at large scales, spatial effects might outperform local environmental effects (Heino *et al.*, 2010; Wu *et al.*, 2014). Therefore, further comparisons between trait and species composition in relation to different factors at multi-spatial scales are greatly needed.

Mantel tests suggested that the importance accounting for the among-site differences in species and traits-based beta diversities was: hydrological variables > environmental filtering, without effects of historic (spatial) factors. Identifying mechanisms underlying the spatial patterns of biodiversity is another important task in community ecology, since these are fundamental to the appropriate biodiversity conservation and restoration (Myers *et al.*, 2000; Wang *et al.*, 2016). Focusing on pelagic algae in a catchment with short geographical distances and incorporating multiple factors enabled the disentanglement of pure hydrological, environmental and spatial gradients in our study. Our results revealed a clear distance-decay of community dissimilarity with increasing hydrological and environmental distances (Fig. 5.3, Fig. 5.4, Table 5.3). However, the relative roles of different distance matrices showed considerable variability, for instance, the importance of hydrological distance was consistently stronger than environmental distance, while importance of spatial distance was the lowest (or even nonsignificant). A key implication of our findings for biodiversity conservation is that maintaining the instream flow regime and keeping various habitats among rivers are of vital importance.

In conclusion, the present study has revealed the clear major role of flow regime (indicated by hydrological variables) in structuring riverine algae communities and beta diversity patterns, which has outperformed with local environmental variables and spatial factors. Our findings further emphasize the fundamental importance of considering hydrological variables, particularly when planning for long-term monitoring and biodiversity conservation or restoration. Although both trait and species composition showed significant correlations with hydrological, environmental and spatial variables, respectively, higher variation of trait composition (57.0%) than species composition (37.5%) was caught by these factors. This emphasizes the merit of applying traits for biomonitoring and

management of freshwater ecosystems. As our sampling covered only one catchment, we admit that the generality of these findings will be assessed later by other investigations in different systems. We also advocate that researchers should consider multi-spatial and temporal scales explicitly in studies of biodiversity conservation, as pattern may change with study scales (Soininen *et al.*, 2007; Li *et al.*, 2012; Tang *et al.*, 2016).

Chapter 6 Quantifying projected effects of global change on riverine phytoplankton species richness

Yueming Qu^{1*}, Naicheng Wu^{1,2*}, Björn Guse^{1,3}, Nicola Fohrer¹

¹ Department of Hydrology and Water Resources Management, Institute for Natural Resource Conservation, Kiel University, Olshausenstr. 75, 24118 Kiel, Germany

² Department of Health and Environmental Sciences, Xi'an Jiaotong-Liverpool University, Chongwen Road 8, 215123 Suzhou, China

³ GFZ German Research Centre for Geosciences, Section Hydrology, Potsdam, Germany

* Corresponding authors: yqu@hydrology.uni-kiel.de (Y. Qu), nwu@hydrology.uni-kiel.de (N. Wu)

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Abstract

1. Global change accelerates biodiversity loss, especially in vulnerable freshwater ecosystems exposed to multiple stressors. The trade-off between biodiversity conservation and ecosystem services is a key challenge. It is valuable to understand how multiple stressors translate into loss of diversity and to evaluate the potential risks of future land-use and temperature changes.
2. In this study, we aim to analysis the possible responses of riverine phytoplankton biodiversity to multiple stressors by a mixed-model approach. Firstly, a random forest model was developed for ranking the stressors' importance, and then a generalized linear mixed model for quantifying and projecting the potential response of phytoplankton biodiversity under the main stressors' interactive impacts was established. We focused on the response of species richness. Multi-metric environmental filters included water flow regime, watershed land-cover pattern, spatial factors and local physicochemical conditions.
3. The results illustrated that the interactions of land-use, water temperature and hydrological factors determine the species richness characteristics. The catchment land-use factors, more precisely the share of forest and pasture area, have outstanding explanation to the variation of riverine phytoplankton species richness. Forest share had a positive effect while pasture affected species richness in a negative way. The value of forest presence exerted stronger effects than pasture changes in antagonistic effects scenarios.
4. Besides, we found that the weekly and biweekly alteration of hydrological indices are good indicators of the phytoplankton biodiversity patterns. Water temperature showed a negative correlation with species richness indicating that the phytoplankton biodiversity decreases with increasing temperature. Under interactive scenarios, the additional temperature changes modulate the effects of land-use on biodiversity.
5. *Synthesis and applications.* Our findings highlighted that forest land-cover along the river had a significant positive contribution to phytoplankton diversity. This emphasizes the significance of preservation of forest area in protecting the aquatic algal biodiversity for maintaining the river ecosystem functioning. Our results also enlightened time lag for the phytoplankton community to rebalancing their structure or resilience the functioning, which should be taken into consideration for future studies and management.

Keywords: Multiple stressors, Riverine phytoplankton, Species richness, Random forest, Generalized linear mixed model

6.1 Introduction

Environmental change accelerates the loss of biodiversity and threatens vulnerable freshwater ecosystems (Maxwell et al., 2016; Alahuhta et al., 2018). Freshwater ecosystems are among the most imperiled on earth, with rivers being particularly susceptible to global change due to several factors, for example, simplification of the habitat, altered water residence times, changes in nutrient loads and increasing arrival of new chemicals (Jackson et al., 2016; Ormerod et al., 2010). Compared with marine, land and even lake ecosystems, studies on river ecosystems are still relatively scarce. However, rivers are important linkage among all other different ecosystems, and are closely related to human activities in the watershed (Tang et al., 2017). The maintenance and protection of river ecosystem health is thus critical to human health and social development.

River ecosystems are synergistically influenced by multiple stressors including natural factors (i.e. dispersal, slope, altitude etc.) and anthropogenic factors (i.e. climate change, land-cover change, eutrophication etc.). First of all, the river network structure plays an important role in structuring the aquatic biotic community which is passively diffuse with water flow (Heino et al., 2015). For example, the single directionality of rivers can give a disproportionate effect on biological spread, which in turn potentially changes the viability and intermediate coexistence of populations (Heino et al. 2009; Woodward et al. 2011). Secondly, ecological processes in the river are highly correlated with flow regime (Rolls et al., 2018). The flow regime has randomness, i.e. unexpected magnitude and timing of extreme events, and regularity, i.e. typical seasonal patterns (Kiesel et al., 2019a). Flow regime can be regarded as a direct factor affecting the community structure and function of aquatic organisms (Guse et al., 2015b; Wu et al., 2019). At the same time, flow regime can also be considered as an indirect factor that affects the ecological processes in rivers by regulating water quality and changing the degree of connectivity (Kiesel et al., 2019b; Poff and Zimmerman, 2010). Moreover, land coverage also acts as a crucial factor for biodiversity (Allan, 2004; Kremen and Merenlender, 2018). Land cover change influences rivers by affecting runoff rate and the input rate of sediment, chemical pollutants, wood debris and so on (dos Reis Oliveira et al., 2018; Wagner and Waske, 2016). Land-use change from wetland to rangeland can generally increase peak discharge volume and reduce the peak discharge duration, which increases hydrological intensification (Davis et al., 2015), while forest land produces various habitat conditions for various species which leads to high biodiversity and healthier status (Oeding et al., 2018; Wilkinson et al., 2018). The impact of land-use change on water flow and riverine biota is difficult to estimate due to their complexity and interactive effects. It is undeniable that the prediction of the impact of multiple stressors on the dynamics of communities is an arduous task

(Hering et al., 2015; Vaughn, 2010). However, the answers for how stressors interact and how they translate into losses of diversity are limited, and further studies are still needed.

To mitigate the impact of environmental changes, ensure sustainable ecosystems services and alleviate further declines of freshwater biodiversity and develop adaptive strategies. It is crucial to understand how the environment interacts with biodiversity patterns in microorganisms (Millennium Ecosystem Assessment, 2005). This is particularly true for phytoplankton communities, where shifts in communities' composition (e.g. leading to species pools dominated by harmful cyanobacteria) may have profound effects on aquatic ecosystem functioning and on the quality of aquatic resources (Jackson et al., 2016). As the significant primary producer at the basis of the food web, the riverine phytoplankton are the foundation for the whole ecosystem status and functioning. Moreover, the phytoplankton community is a dynamic and open structure that is especially impacted by external drivers (Wu et al., 2017). In this study we specifically focus on their species richness response, because species richness is a key characteristic affecting ecosystem functioning (Cardinale et al., 2006; Soliveres et al., 2016). It does not only reflect the complex relationship between biology and the environment, but also reflects the richness of biological resources. Therefore, we are trying to develop a novel interdisciplinary modelling approach to evaluate the impacts of potential global changes (land-use and temperature changes) on riverine phytoplankton species richness. We aim to: (I) rank the stressors' importance, (II) quantify the respondent-stressors relationship, (III) estimate the potential responses of phytoplankton species richness under different scenarios.

6.2 Materials and methods

6.2.1 study area

The data for this study was taken from field investigations and a modeled data base from a long-time monitoring study (Guse et al., 2015b). Our study river, Treene, is located in the Schleswig-Holstein state of Germany, belonging to the northern lowland region of Europe. It is in a temperate climate zone, which is influenced by marine climate, and its watershed covers an area of 481 km² at the outlet Treia. Field surveys were carried out included representative samples from all 4 seasons (December of 2014, March of 2015, June of 2015 and September of 2015, respectively) on 53 sampling sites which covered the mainstream and major tributaries of the catchment (Fig. 6.1), resulting in a total of 212 samples. At each sampling site, we conducted four parts of investigation: phytoplankton species composition, physicochemical condition, hydrological regime, land-use pattern and spatial variables.

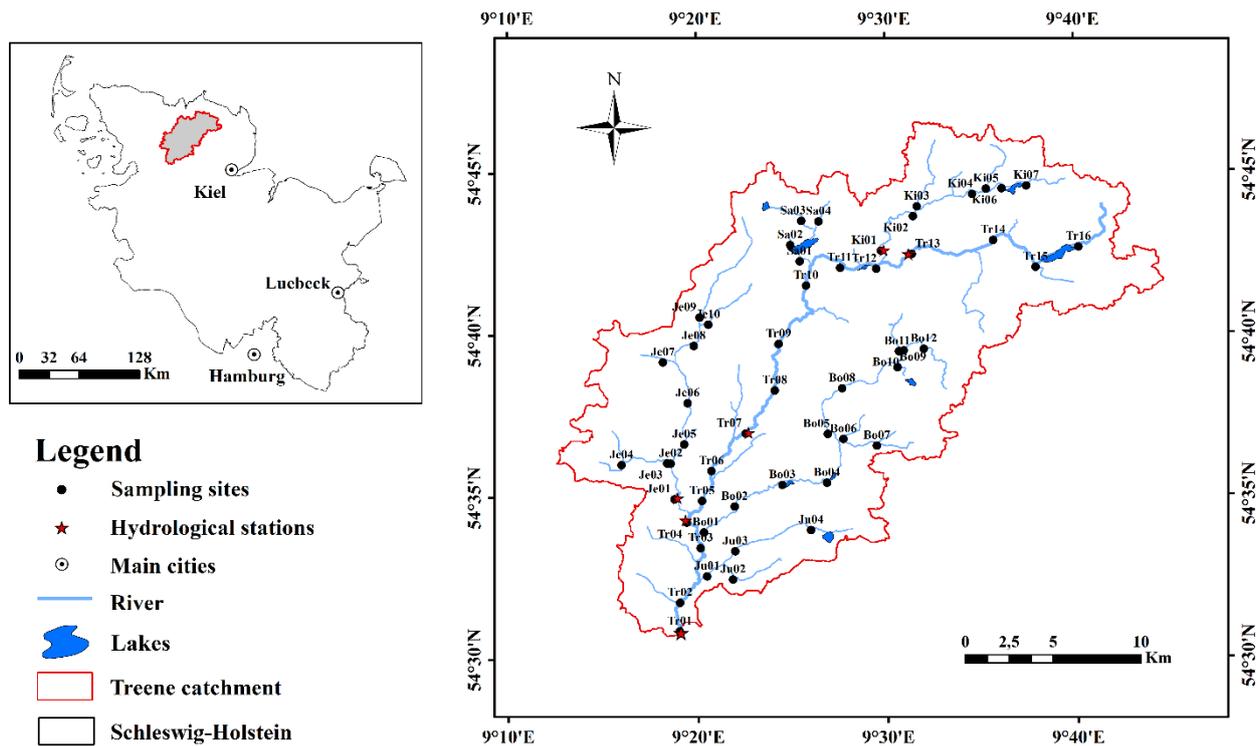


Fig. 6.1 The location of the Treene catchment up to Treia, 53 sampling points and 6 hydrological stations in Schleswig - Holstein state of Germany. The abbreviated sites named according to each sub-basin where they are located: Bo for Bollingstedter Au, Je for Jerrisbek, Ju for Juebek, Ki for Kielstau, Sa for Sankermark See, and Tr for the mainstream of Treene. The numbers count along the longitudinal axis of rivers from the outlet to upstream. The sampling points in close distance of lakes are not located in the lake but situated systematically downstream following the lakes.

6.2.2 Phytoplankton

At each sampling site, phytoplankton samples were quantitatively collected with a known concentration from the surface (0 m to 0.5m depth) of the river. Algal species were identified by two different magnifications under an optical microscope (Nikon Eclipse E200-LED, Germany): first at $\times 400$ magnification for classification of the soft algae, second at $\times 1000$ under oil immersion for determination of the diatom species. Permanent diatom slides were prepared after acid digestion for the second step. The counting unit was individual (unicell) and at least 300 units were counted for each sample. All samples were counted in species identification for calculating the species richness. Taxonomic identification was done according to Hu and Wei (2006), Burchardt (2014), Bey (2013), Hofmann (2011) and Bak (2012).

6.2.3 Spatial variables

Spatial variables were described by Principal Coordinates of Neighbour Matrices (PCNM) based on a Moran's Eigenvector Map (Borcard et al., 1992). The PCNM variables effectively model spatial structures among sites, they can illustrate spatial relations among sites at multiple scales, which is

commonly used to describe species dispersal processes (Curry and Baird, 2015). The spatial variables with small code (e.g. PCNM1) indicate broad-scale spatial pattern, while fine-scale pattern with larger code. The spatial variables were computed using the function 'pcnm' of the R package *vegan* (version 2.5-2) in the R base version 3.5.1 (R Core Team, 2018). There are 32 eigenvalues of PCNM component which were included in the spatial variables.

6.2.4 Physicochemical condition

The physicochemical condition is characterized by 14 parameters. Water temperature (WT), pH, electric conductivity (EC), and dissolved oxygen (DO) of the surface water were measured in situ with Portable Meter (WTM Multi 340i and WTW Cond 330i, Germany), while water depth was measured with a measuring tape and flow velocity using a digital water velocity meter (FlowSens Single Axis Electromagnetic Flow Meter, Hydrometrie, Germany). Simultaneously, two water samples (500 ml each) were collected in the field for analyzing the nutrients. Part of them were filtered immediately through GF/F glass microfiber filter (Whatmann 1825-047) when reaching the lab. Both filtered and unfiltered samples were kept frozen at -20°C until measurement. The concentration of total phosphorus (TP) was measured using the unfiltered water samples, and the remaining parameters including orthophosphate (PO₄-P), ammonium-nitrogen (NH₄-N), nitrate-nitrogen (NO₃-N), nitrite-nitrogen (NO₂-N), chloride (Cl) and sulphate (SO₄) were measured using the filtered samples according to the standard methods of DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). DIN is the sum of NH₄-N, NO₃-N and NO₂-N. Nitrogen to phosphorus ratio (NPR) is the ratio of DIN and TP. Total suspended solids (TSS) were measured according to Standard Operating Procedure for Total Suspended Solid Analysis (U.S.GeologicalSurvey, 1998).

6.2.5 Flow regime

An ecohydrological model Soil and Water Assessment Tool (SWAT) (Arnold and Allan, 1999) was implemented for the study catchment to model site-specific hydrological stressors of the phytoplankton community. In the SWAT model, the water balance is resolved and the most relevant hydrological processes are calculated. The spatially distributed SWAT model provides model results for several variables for each sub-basin. Thus, spatial patterns of different hydrological variables can be derived in a daily resolution. The SWAT model was already applied worldwide and has been well established in our study area in long-term daily resolution (Guse et al., 2015a; Haas et al., 2016). Our modeling period was sub-divided into a calibration period (2001 to 2005) and a validation period (2006 to 2016) based on the hydrological stations in the Treene catchment (Fig. 1), The model performance has been evaluated by Nash-Sutcliffe Efficiency, Percent Bias and RSR (root mean square error divided

by standard deviation) (Guse et al., 2015b). Discharge values of the sampling point were related to the SWAT model result from the closest outlet of a sub-basin. Based on the daily modeled discharge, we then calculated 86 ecological relevant hydrological alteration indices (Appendix Table A. 1) for further analyses, which included the flow event of magnitude, frequency, rate of flow events (Kiesel et al., 2017; Olden and Poff, 2003).

6.2.6 Land-use pattern

Land-use data of our study area was downloaded from the Schleswig-Holstein State Bureau of Surveying and Geo-information (LVERMGEO-SH, 2012). Eleven land-use types were classified: agricultural land-generic (AGRL), deciduous forest (FRSD), evergreen forest (FRST), evergreen forest (FRSE), rangeland (RNGE), industrial (UIDU), residential-low density (URLD), residential-medium density (URMD), water (WATR), wetland (WETL), winter pasture (WPAS). ArcGIS software (Version 10.0, ESRI, US) was used to process the area of each sampling site by land-use category. The land-use area is accumulative along the longitudinal river continuum. The upstream watershed was determined for each site, and the land-use areas were converted to proportions for following analyses.

6.2.7 Statistical methods

To explore the relationships between environmental stressors and species richness, we conducted statistical analysis methods work flow as follows: firstly, multi-stressors (physicochemical, hydrological, land-use and spatial variables) were pre-selected excluding the ones with significant multi-collinearity (with variance inflation factor >10 (O'brien, 2007) and Spearman's rank correlation coefficient $|r| \geq 0.75$). All abiotic variables were tested for collinearity by function 'cor' in R package of stats. Secondly, we chose random forest (RF) model to rank and identify multiple stressors hierarchy, and raised the robust influencing variables to our target bio-indicator (i.e. species richness). In this study, we ran a RF using function 'randomForest' from the R package randomForest. To rank the variables importance, we used the function 'gg_vimp' in the package ggRandomForests. Later, we analyzed the response pattern of species richness to select the robust predictors from the RF models. Partial dependence plots were generated for showing the response against the robust predictors (function: partialPlot, package: randomForest). Thirdly, we applied generalized linear mixed model (GLMM) to setup the best described model of species richness pattern by a set of predictors from the result of RF model. To avoid spatial auto-correlation in the data-set, the sampling sites were set as (spatial) random effect. The main stressors on phytoplankton species richness were included in the algorithms of the model (function: glmer, package: lme4). Finally, three key stressors (land-use parameters: FRSD, WPAS, and climate parameter: WT) in this GLMM model were increased and

decreased individually and interactively under different assumed scenarios (Table 6.1). Based on the established model (from GLMM), the dynamical changes of the response of the phytoplankton species richness were defined by the model output. All the statistical analyses were performed with the R software (version 3.5.1, R Development Core Team 2018).

Table 6.1 Land-use and climate change scenarios implemented into generalized linear mixed model

Groups	Scenarios	Descriptions
S_1	Forest land increase	FRSD + 5%
S_2	Forest land decrease	FRSD - 5%
S_3	Pasture land increase	WPAS + 5%
S_4	Pasture land decrease	WPAS - 5%
S_5	Temperature increase	WT + 5°C
S_6	Temperature decrease	WT - 5°C
S_7	5% of WPAS turn to FRSD	FRSD + 5% & WPAS - 5%
S_8	5% of FRSD turn to WPAS	FRSD - 5% & WPAS + 5%
S_9	S_7 with temperature increase	WT + 5°C & FRSD + 5% & WPAS - 5%
S_10	S_8 with temperature increase	WT + 5°C & FRSD - 5% & WPAS + 5%
S_11	S_7 with temperature decrease	WT - 5°C & FRSD + 5% & WPAS - 5%
S_12	S_8 with temperature decrease	WT - 5°C & FRSD - 5% & WPAS + 5%

6.3 Results

6.3.1 Ranking the environmental factors importance

To identify the main factors that influence the phytoplankton variations, RF models were calibrated based on the 212 samples with 143 environmental factors and one target variable: phytoplankton species richness. The performance criteria were tested automatically by the RF OOB procedure, which is similar to cross-validation. An improved importance analysis using conditional variable importance measure was applied to the models (Fig. 6.2). The higher the factor scores are, the higher is its impact/relevance. The top four stressors affecting the phytoplankton species richness were: the share of deciduous forest land-use area (FRSD), pasture (WPAS), skewness of 14 days flow (Hv28) and low flow pulse count for 14 days (Hv40). The result illustrated that the percentage area of forest was the most influential variable (with an importance share of 33.20%), followed by the share of pasture (27.98%).

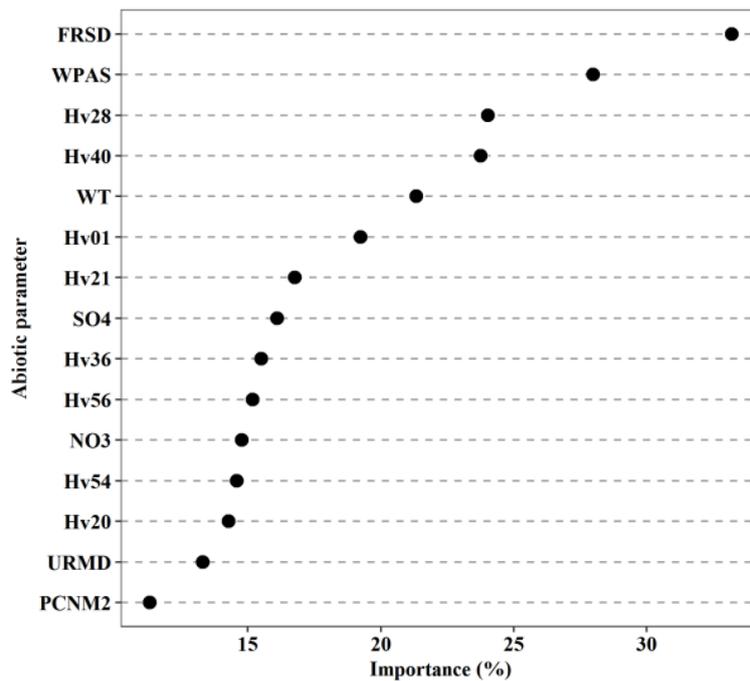


Fig 6.2 Dot plot ordered by parameters' importance in a Random Forest model. Large numbers of the variable importance indicate a high predictive capacity of a variable, the top 15 predictors (FRSD - forest, WPAS - pasture, Hv28 - skewness of 14 days flow, Hv40 - low flow pulse count for 14 days, WT - water temperature, Hv01 - daily discharge, Hv21 - skewness of 7 days flow (within the sampling day), SO4 - sulfate, Hv36 - skewness of 30 days flows, Hv56 - rate of change 14 days, NO3 - nitrate, Hv54 - rate of change 3 days, Hv20 - skewness of 7 days flow (without the sampling day), URMD - urban area, PCNM2 - principal coordinates of neighbor matrices in large-scale spatial relations among sites) are showing in the figure.

The partial dependency maps (Fig. 6.3) illustrated the partial dependency influence by four major drivers: land-use factors - the share of forest and pasture, hydrological factor - skewness of 14 days flow and water temperature, individually (also see species richness responses to skewness of 7 days flow, low flow pulse count for 14 days with less variation of species richness in Appendix Fig. A. 1). A general increase of the species richness with the percentage of forest, could be observed from the model output. For pasture, this relationship remained until a substantial decrease of the species richness when the percentage of pasture exceeded approximately 20%. The share of pasture land-use area (with average percentage of 28.48%) is much higher than the area of forest (average percentage of 5.38%) in Treene catchment. Forest area is plaque distribution in the catchment, with scarce valuable existence. Skewness of 14 days flow (Hv28) ranges from -1 to 4 in general and the values are concentrated around 1. The value of Hv28 changes from less than 1 to higher than 1 lead to species loss. The species richness decreases with increasing water temperature. Relative changes intensify from 5 to 10 °C.

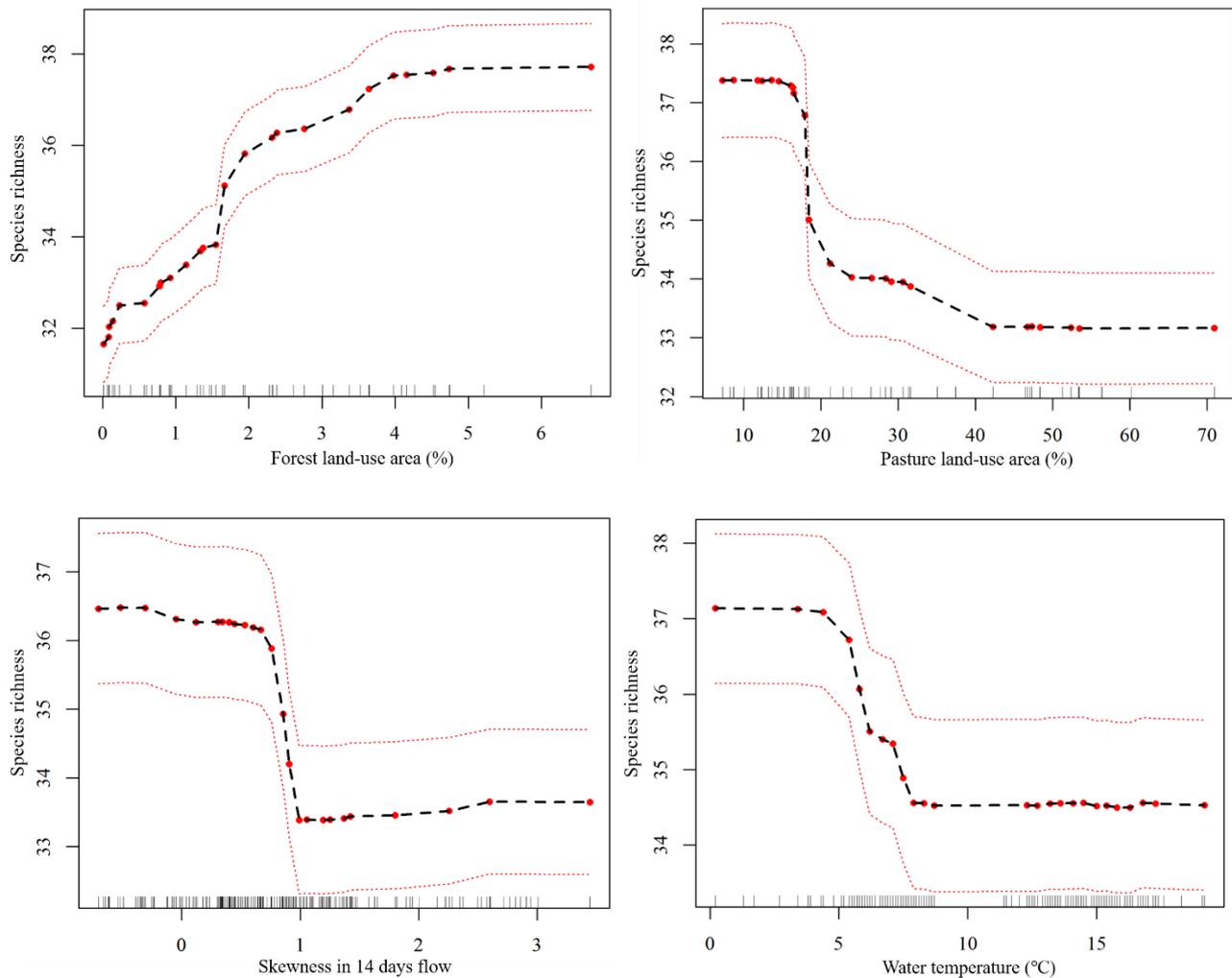


Fig 6.3. Partial dependence plots showing the response of species richness against the top predictors: forest land-use area (%), pasture land-use area (%), skewness in 14 days flow and water temperature (°C).

6.3.2 Responses of species richness to multiple stressors

The results above presented the rank of stressors for the phytoplankton species richness, and the share of forest and pasture (%) proved as the most important drivers for phytoplankton species richness in the study area. Based on the rank of stressors' importance, we firstly fitted the best predictable biodiversity-environment relationship by the generalized linear mixed model. The sampling sites information was regarded as a random effect, six stressors (WT, Hv21, Hv28, Hv40, FRSD and WPAS) were selected in this model, fulfilling the highest goodness of fit ($R^2=0.66$). Despite the high relationship with the share of forest area, the findings supported that the species richness was temperature dependent, and the flow regime of weekly period of time (e.g., hydrological indices: Hv21, Hv28 and Hv40, describe the flow alteration within one to two weeks) was better reflected in the algal biodiversity (Table 6.2).

Table 6.2 Results of fixed effects from the generalized linear mixed model (GLMM) fit by maximum likelihood for species richness. The random effects accounted by the sites, with a variance of 33.12, standard deviation of 5.75. In this table, Std. Error is the standard deviation of the sampling distribution of the estimate of the coefficient under the standard regression assumptions. The z value is the Wald statistic for testing the hypothesis that the corresponding parameter (regression coefficient) is zero. $P(>|z|)$ is the tail area in a 2-tail test, i.e. the test within a 2-sided outer hypothesis (Bolker et al., 2009).

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	46.705	3.088	15.124	<2e-16
WT	-0.746	0.100	-7.476	7.69e-14
Hv21	-1.887	0.572	-3.299	0.00097
Hv28	-2.409	0.566	-4.259	2.05e-5
Hv40	-0.412	0.084	-4.896	9.78e-07
FRSD	3.083	0.630	4.893	9.92e-07
WPAS	-0.179	0.064	-2.801	0.00510

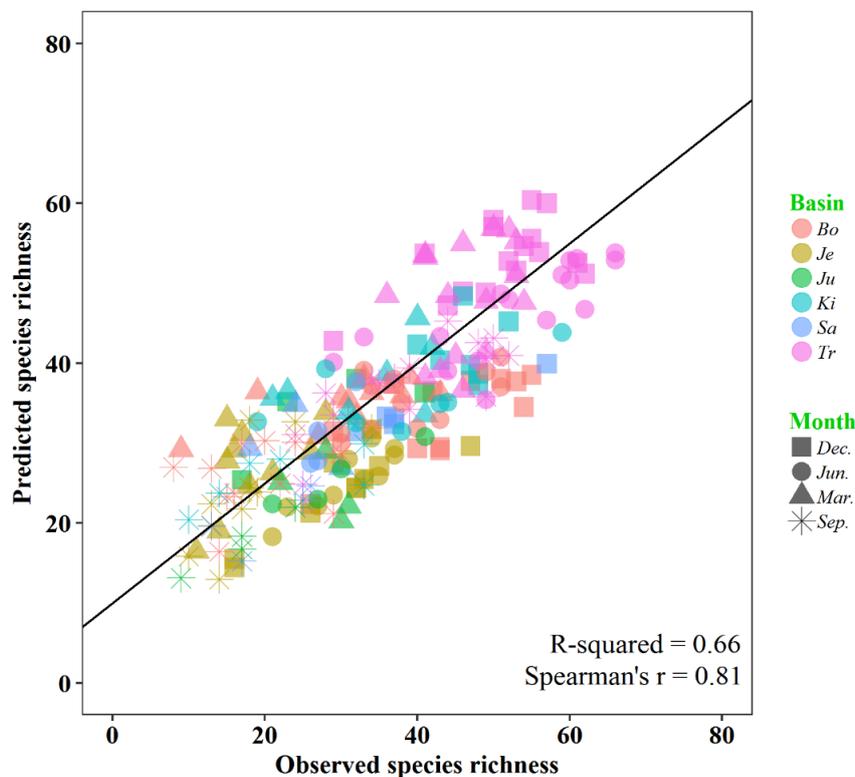


Fig 6.4 Observed and predicted variation of species richness by generalized linear mixed model. Basin: Bo represent for Bollingstedter Au, Je for Jerrisbek, Ju for Juebek, Ki for Kielstau, Sa for Sankermark See, and Tr for the mainstream of Treene; Month: Dec. for December, Jun. for June, Mar. for March, Sep. for September. The black line is the regression line of the model output.

Simulated species richness was calculated by the GLMM. A visual comparison of species predictions and observed data is presented in the Fig. 6.4, with a linearity of r square equals 0.66 was observed, which indicated a good prediction of the model. This figure also showed the spatial and temporal distribution of the phytoplankton species richness. The result evidently indicated that

phytoplankton species richness in the mainstream of the river (with pink color) was higher than in the tributaries. Seasonal variations can also be distinct from the graph where the lowest species richness appeared in September (expressed by star).

Table 6.3 Land-use and climate change scenarios into model and the projected changes of phytoplankton species richness (SR) response

Groups	Scenarios	Descriptions	Average value of SR	Rate of change (%)
TRUE			35.10	0
S_1	Forest land increase	FRSD + 5%	50.33	+43.39
S_2	Forest land decrease	FRSD - 5%	28.72	-18.18
S_3	Pasture land increase	WPAS + 5%	34.02	-3.08
S_4	Pasture land decrease	WPAS - 5%	35.81	+2.02
S_5	Temperature increase	WT + 5°C	31.19	-11.15
S_6	Temperature decrease	WT - 5°C	38.65	+10.10
S_7	5% of WPAS turn to FRSD	FRSD + 5% & WPAS - 5%	51.23	+45.95
S_8	5% of FRSD turn to WPAS	FRSD - 5% & WPAS + 5%	27.82	-20.73
S_9	S_7 with temperature increase	WT + 5°C & FRSD + 5% & WPAS - 5%	47.50	+35.32
S_10	S_8 with temperature increase	WT + 5°C & FRSD - 5% & WPAS + 5%	24.09	-31.35
S_11	S_7 with temperature decrease	WT - 5°C & FRSD + 5% & WPAS - 5%	54.96	+56.58
S_12	S_8 with temperature decrease	WT - 5°C & FRSD - 5% & WPAS + 5%	31.55	-10.10

To better understand the potential responses of phytoplankton, we assumed eight scenarios to quantify and test (forecast) the response of species richness by the RF-informed GLMM model. To reduce complexity, the land-use and climate change scenarios mainly focused on the changes of the top three stressors: forest, pasture shares and water temperature. The two land-use factors: forest and pasture influence on species richness were opposite in the RF results (Table 6.1). Similarly, the percentage of forest area had a positive effect on species richness, while the pasture showed a negative effect (scenarios: S_1, S_2, S_3 and S_4). Climate change indicated by water temperature changes have negative effects on phytoplankton species richness (scenarios: S_5, S_6). The value of forest presence exerted stronger effects than pasture changes in antagonistic effects scenarios (Table 6.3). When superimposed on land-use changes scenarios (S_9, S_10, S_11 and S_12), the combined temperature-land-use scenarios always led to fewer negative but larger positive additive effects (Table 6.3). The results indicated that temperature changes modulate the effects of land-use on biodiversity.

6.4 Discussion

6.4.1 Individual impacts

In recent years, biodiversity loss has caused widespread concern. Across every continent and in various habitats, we try to investigate and understand the causes and mechanisms for balancing human needs of ecosystem services and the maintenance of biodiversity. In river ecosystems, considering river characteristics, we attempted to model and quantify the effects of multiple stressors on phytoplankton species richness. From the models results, spatial factors were not the most important drivers in structuring the species richness. This was likely due to the relatively small with low variation of altitude across our target catchment (Heino et al., 2010). Moreover, our main objective was to identify the factors influencing local biodiversity, while spatial factors might explain more to the region species richness pattern (Wu et al., 2018).

Water temperature emerged as an important abiotic factor contributing 21.34% share of importance to the variation of phytoplankton species richness. It was selected in the final predicted model with a negative effect. Studies have reported that an increase in temperature can stimulate primary producers' population (Jackson et al., 2016). However, the algal biodiversity (i.e., species richness) would not increase with raising temperature. Global warming was considered likely to cause a profound loss in biodiversity by indirectly affecting competitive interactions among phytoplankton taxa (Urrutia-Cordero et al., 2017). A higher population of algae community dominated by a few species may lead to reductions or losses of ecosystem functioning, when considering the food web situation. By contrast, a higher diversity of biomes commonly had higher biomass and productivity from a wide range of taxa and ecosystems aspect (Duffy et al., 2017).

Hydrological indices, describing the flow regime of the watershed, contributed to several important stressors which effected the phytoplankton biodiversity pattern (Fig. 6.2). Usually, hydrological indices with long period of time, such as seasonal and annual, will lead to a better understanding and prediction for the hydrological process (Kiesel et al., 2019b). Hydrological variables measured over weekly and biweekly period of time are overlooked. However, our results showed that the most critical hydrological indices (Hv21–skewness of 7 days flow, Hv28–skewness of 14 days flow and Hv40–low flow pulse count for 14 days) which described flow alteration over a shorter period of time (from one to two weeks) were better indicators of phytoplankton species richness (Fig. 6.2 and Fig. 6.3). We assume that this can be explained by the short life cycle of phytoplankton on the one hand (Lehtinen et al., 2017). On the other hand, it enlightened the time lags for the phytoplankton community to rebalancing their structure or resilience the functioning. Phytoplankton communities are highly sensitive biotic groups, reflecting environmental changes even quicker than fish or macrophytes communities (Marzin et al., 2012). In addition, short-term hydrological variation factors had the greatest impact on phytoplankton characteristics from our previous findings (Qu et al.,

2018; Wu et al., 2016). The consistent identification strengthens our confidence in the relationship underlying model predictability. In future interdisciplinary studies of phytoplankton communities, we therefore recommend the consideration of the weekly and biweekly hydrological alteration indices.

Land cover by forest and pasture emerged as the top predictors of phytoplankton species richness (Fig. 6.2). Forest share had a positive effect while pasture affected species richness in a negative way (Fig. 6.3). Stream ecologists have long been aware of the strong dependence of streams on the surrounding terrestrial environment. Rivers receive subsidies to the food web in terms of particulate detritus and dissolved organic matter from the surrounding watershed (Polis et al., 1997). Exploitation and development of natural watersheds is continuing globally, which means that the substantial conversion of forest to agricultural land may potential modify or reduce natural biodiversity (Weijters et al., 2009). Researchers commonly reported that streams draining agricultural land support fewer species of sensitive taxa than streams draining forest catchments (Allan and Castillo, 2007; Quinn, 2000). Preservation of forests could provide an opportunity for increased macroinvertebrate biodiversity (Wilkins et al., 2015). Spatial patterns of fish biodiversity in tropical floodplain rivers were strongly positively associated with forest cover (Arantes et al., 2018). Rivers in the forest area are more to a pristine state with a series of multiple sloughs and backwater areas. It reported that deadwood in the area helped to create and maintain more complex aquatic habitats (Turunen et al., 2017; Wohl, 2017). While, in farmland areas, one main straightened channel usually provides simple and homogeneous habitat for aquatic biota. This causes a series of reactions which are negative to riverine ecosystem biodiversity across all taxa (Leitão et al., 2018; Piggott, 2013).

6.4.2 Combined impacts

Land-use factors are usually treated as indirect factors of algal growth, while physiochemical and hydrological factors, such as light, water temperature, nutrients concentration and flow regime, are classified as direct factors influencing the growth of algae (Hicks and Taylor, 2018). We conjecture that because of the land-use patterns reflect the combined and interactive effects of complex factors (Fuß et al., 2017). For instance, reduced riparian vegetation might increase the exposure to light, water temperature and nutrients enrichment, which is beneficial for the development of filamentous algae and diatoms but increase stress on “clear water” species (Quinn, 2000). On the other hand, homogeneous habitats support stable dominant species which are adapted to the flow regime condition. If these species do not provide food for upper consumers that will potentially reduce the biodiversity of macroinvertebrate and fish community along the food web (Hette-Tronquart et al., 2018; Lehtinen et al., 2017). Lowland rivers basins worldwide have undergone major land cover changes, with forest

loss projected to increase during the next decades (Navarro-Ortega et al., 2015). In our catchment area, land covered by forest constituted less than 10% of sub-catchments of all sampling sites. The catchment is mainly composed by agricultural crops (around 50%) and pastures (around 30%). Agricultural intensification can impose a variety of stressors on streams, including temperature extremes, nutrient peaks, augmented fine sediment inputs, increased frequency and magnitude of peak flows and lowered base flow patterns (Lange et al., 2016; Paul and Meyer, 2001). These effects may be magnified for small streams in farming areas (Walsh et al., 2005). Changes in global temperature and land-cover can interactively affect lake phytoplankton nutrient limitation status (Hayes et al., 2015). Forecasting changes in stream ecosystems in response to global change is difficult, due to the risk that the relationship will vary over time, owing to changes in specific practices or in the environment itself (Arthington et al., 2010). Riverine algal species patterns seem to shift in their timing, duration and magnitude because of these multiple stressor changes (Bernhardt et al., 2018; Qu et al., 2019). The implications of riverine primary producers are difficult to predict, in part because the direct impacts of these stressors on river can be antagonistic, and because the direct impacts may be mitigated or enhanced than the expected based on knowledge of the effects of individual stressors (Table 6.3). The variety of consequences for river environments makes it intricate to separate the individual and combined effects involved (Piggott et al., 2015). Even slight modifications in land-use may lead to changes of natural flow regime, and cause to significant changes in the structure of the riverine phytoplankton community (Tonkin et al., 2018). From our study, we would suggest that simultaneous measurement and modelling the river flow regimes and biological properties will be needed to predict the likely consequences of climate and land-use changes. The modeling methods for projecting biological responses are improving (Bussi et al., 2018). Our integrated modeling methods progress the integrative subjects of hydrology and ecology, increase model simulation accuracy, and improve projected results credibility (Fig. 6.4). Our emerging technique for projecting responses of riverine phytoplankton biodiversity regime opens new opportunities to apply integrated modeling methods as a diagnostic tool for river ecosystems management.

6.5 Conclusion

In this study, we observed how the response of phytoplankton species richness changed under extrinsic controls from spatial factors, physiochemical conditions, flow disturbances and land-use patterns over a year. Our results highlight that forest land-use in the catchment is necessary to maintain aquatic algal biodiversity. The alteration of weekly and biweekly hydrological alteration indices were better indicators of effects to the phytoplankton species richness pattern. We also found that

phytoplankton biodiversity decreased with higher temperature. The interactive scenarios demonstrate that additional temperature change can modulate the effects of land-use on biodiversity. Environmental changes act as multiple interacting stressors and pose a whole spectrum of management and conservation challenges that range from basic science to regulation, policy and governance. Our findings suggest that human impacts on lowland rivers are ubiquitous, and results from land-use related stressors and altered flow regime could interact with changing water temperature. Biological monitoring programs therefore require expansion to integrate characterization of our environmental surroundings. The integrated modeling method is highly suggested for better understanding the implications of river ecosystems under global change.

Chapter 7 General discussion

As linear-shaped sinks, rivers act as collectors of water, soluble, suspension and sediment, which are transported in unidirectional flows at different velocities (Birk, 2019). They are located lowest in the landscape and are strongly linked to their surroundings. Such features condition the vulnerability of river ecosystem to multiple stressors. This study disentangled the effects from a matrix of environmental filters on riverine phytoplankton community by integrated models. Under consideration of various aspects of the algal features, the key drivers were identified and their influences were quantitatively evaluated. With these efforts, the specific research questions formulated in Section 1.3.2 can now be answered.

7.1. Addressing of research questions

(1) How is the contribution of spatial factors in structuring the riverine phytoplankton communities compared to the local factors in the study area?

Based on the metacommunity theory (Heino et al., 2015), the observed community at a certain point is shaped by two broad categories of effects – local (i.e. physicochemical factors) and regional (i.e. spatial factors) effects. Local effects are largely caused by environmental constraints or species interactions, while spatial effects are driven by the flux of organisms from the regional species pool (Brown and Swan, 2010). Spatial factors can act as an important element in structuring the aquatic biotic communities, while their impact varies between different spatial gradients. Analyses of local and regional effects have been discussed around the world with different assemblages. Zbinden and Matthews (2017) found that local environmental factors accounted more than spatial factors for fish beta diversity, particularly in headwater streams, while the fish assemblages were mainly structured by environmental filtering. Another case study, whose catchments were located in southeast of China, showed that the variations of diatom assemblages were better explained by spatial factors. In this study, the results demonstrated that the local phytoplankton communities (described by species, traits composition as well as the species richness diversity) in Treene catchment were more affected by local effects (e.g., local hydrological and environmental variables) than spatial effects as indicated by spatial variables (see Chapter 2, Fig 2.6; Chapter 5, Fig. 5.2, and Chapter 6, Fig. 6.2).

These findings also supported that the suitability of lowland riverine phytoplankton as bioindicator for local habitat changes. Historically, phytoplankton has been doubted as the bio-assessment organism for rivers: whether can they maintain populations in rivers and where do they

come from (Hötzel and Croome, 1999)? As a consequence, comparing to fish, aquatic plants, benthic invertebrates or periphyton, pelagic algae were less used as bio-indicators than other communities in the river. However, with this uncertainty, studies for disentangling the relative roles of local and spatial variables on spatial pattern of the community are a promising way to understand the source of pelagic algae communities. Recent studies (Centis et al., 2010; Huszar et al., 2015; Wu et al., 2017) showed that planktonic algal species do reproduce within rivers and many species develop substantial populations *in situ*. Especially for lowland rivers, algae can become abundant in slowly moving sections and linkage pond where their doubling rates exceed downstream losses. This study certainly concurs with Kelly (2013) that phytoplankton within consideration of soft-body algae should also be included in river ecological investigations for extending the assessment of riverine health.

Nevertheless, the Treene watershed is characterized by low hydraulic gradients (Kiesel et al., 2010; Pfannerstill et al., 2014), low altitude gradient and relatively small geographic scale. These characteristics may explain the low relevance of spatial factors. Consistently, directional processes and dispersal had a prevailing effect on algae metacommunity structuring rather than local physicochemical factors in a high mountain stream (Dong et al., 2016). Moreover, spatial factors have higher contribution to regional metacommunity distribution (Chapter 2, Table 2.1). Therefore, the contribution of spatial factors may vary among different historical and geographical situations, and depend on the characteristics of spatial extent of the case study area.

(2) How does the flow regime act in structuring the algal taxonomic and functional compositions?

Flow regime is regarded as one of the primary determinants of river ecosystems (Poff, 1997; Poff et al., 2010). A rapidly growing body of literature documents the overriding importance of high flow or low flow disturbances in structuring riverine primary producers (Atkinson et al., 2008; Bunn and Arthington, 2002; Elosegi and Sabater, 2013). Researches illustrated that the consequent impacts of altered flow regimes are linked with aquatic biodiversity in various aspects. Firstly, flow is a major determinant of physical habitat in streams, which in turn is a major determinant of biotic composition (Acreman, 2016). Secondly, aquatic species have evolved biological traits primarily in direct response to the natural flow regime (Lange et al., 2014b). Thirdly, maintenance of natural patterns of river connectivity is essential to the population and diversity of riverine species (Jowett and Biggs, 2009).

In this thesis, the flow regime has been discussed by the indices of hydrological alterations, which are based on the modeled daily discharge results (Guse et al., 2015b). Results illustrated that flow regime impact algal local and regional composition distribution (Chapter 2, Fig 2.6 & Table 2.1). The hydrological factors have a higher contribution in the high flow period than in the low flow period

(Chapter 4, Fig. 4.4). During the high flow period, both species and traits-based beta diversities were mostly related to hydrological and environmental heterogeneity with a high contribution of hydrological variables than of environmental variables (Chapter 5, Table 5.3). By the integrated model's analysis, the alteration of hydrological indices in a shorter period of time (e.g., skewness of 14 days flow, low flow pulse count for 14 days) has advantages in indicating effects on the pattern of phytoplankton (Chapter 6, Fig. 6.2 & Table 6.1).

There is still high uncertainty in quantifying and predicting ecological responses to altered flow regime, since water flow is relevant components of river health. Paralleling with our findings, the major taxa of lowland riverine phytoplankton was constrained by water depth and water flow rather than nutrients limiting in the San Joaquin River, California. The longitudinal variations of flow regime influence the development of phytoplankton being the major contributing factors to the species selection (Leland, 2003). Water level fluctuation also plays an important role in algal bloom prevention in Three Gorges Reservoir, China. Larger daily water level fluctuation and a longer duration of water level rise would enhance algal bloom (Ji et al., 2017). The relative findings confirmed that even slight modification to the natural flow regime could lead to profound impacts, and the correlated responses varied under flow alteration.

In addition, flow regime strongly constrains spatial and temporal patterns of many ecological processes in rivers including the magnitude and timing of river ecosystem primary productivity (Bernhardt et al., 2018). In this thesis, skewness of 7 days' flow (Chapter 4, Fig. 4.5; Chapter 5, Table 5.2), 14 days flow (Chapter 6, Fig. 6.2 & Fig. 6.3), and low flow pulse count in 14 days (Chapter 5, Table 5.2; Chapter 6, Fig. 6.2 & Fig. 6.3) have been detected as the key hydrological variables. Two inferences can be obtained from these results. First of all, the key role in aquatic community is neither the contemporary velocity, discharge, water level as the previous ecological research nor the annual flow variation of the traditional hydrological research. It enlightened time lags for the phytoplankton community to rebalancing their structure or resilience the functioning, which should be taken into consideration for future studies. In addition, it also highlights the significant impacts of hydrograph skewness, extreme events, especially drought, on hydrological processes as well as community structure (Fleischmann et al., 2016; Guo et al., 2018). It has been reported that flood reduction with drought and flow homogenization both have greater simplifying community-wide consequences than increasing flooding (Tonkin et al., 2018b).

(3) How strongly is the downstream riverine phytoplankton community influenced by the upstream eutrophic lentic waterbodies?

In the lentic-lotic continuum system, lakes may take a role as a source, a link, or a sink of rivers, which is determined by the location of the lakes. They also act either a source or a sink for water, nutrients, algae species, as well as pollutions, which means their influences can lead to positive or negative consequences. Lakes act as regulators of river flow, which easing the impact of floods and droughts, trapping sediments and nutrients (Lampert and Sommer, 2007). On the one hand, a high diversity and abundance biomass of algae outputs from the lake benefit the downstream consumers. On the other hand, exceeding nutrients and pollutions inputs from the river, which overstep lake's purifying ability, can lead to opposite outcome. In our study area, as major lakes are located in the upstream, the dominant species always changed from lacustrine species to fluvial species along the longitudinal direction. There are significant dissimilar species compositions before and after a lake in the mainstream of the river, while non-significant differences for the downstream section. However, the impacts varied among different seasons, and are limited to a relatively short distance (with a distance of 6 km in September, 2015, while even shorter in other seasons, (Chapter 2, Fig. 2.2 & Fig. 2.3). The dominant species shift was in accordance with Yu et al. (2015; 2017) and Gillett et al. (2016) and illustrated the impacts of lakes on downstream of rivers in different catchments. For example, Yu et al. (2017) observed in Tanglan River (China) the toxic cyanobacteria from Dianchi Lake dominated at upper reach, but replaced by *Chlorococcales* (green algae) and centric diatoms in the lower reach. As a typical lacustrine species, *Microcystis spp.* was loaded to the river by the linkage waterbodies (Chapter 4, Fig 4.5). Similar to the lake-river system, reservoir-river systems also face a dominance of toxic algae, which are exported from the reservoir. *Microcystis* cells can withstand passage through hydroelectric installations and transport over distance on the downstream (Otten et al., 2015). The pressures from eutrophic lakes are determined by the magnitude, duration and diffusion condition. Pressures change under different climate and flow conditions. Eutrophication is eliminated by heavy precipitation, while it turns severe and long-lasting under dry periods, particularly continuous droughts (Mosley, 2015). Therefore, further bio-monitoring and bio-assessment are suggested to consider the impacts of linked lentic waterbodies, such as lakes and reservoirs. Implication refers to the necessity of investigation under catchment perspective, especially for dealing with eutrophication problems. It worth to identify the impacts under different scenarios of human activities and climate conditions in future studies.

(4) *Which are the causal relationships between abiotic factors and algal communities?*

One of the long-standing tasks in ecology is to explore the cause-effect relationship between biological organisms and their environmental surroundings. Based on empirical ecological

requirements, the directive causal relationships between environment and algae should include light, water chemistry, temperate and grazing. The catchment features such as land use, flow regime, river topography and geology, as well as climate features thus affect these proximate variables, which indicatively regulate the riverine algal community population and composition.

In this study, the causes to the variation pattern of phytoplankton community have been investigated by phytoplankton functional groups (PFG). Group TB, which mainly represent by benthic pennales (for example *Navicula*, *Achnanthes*, *Surirella*), is the most common PFG in the whole catchment, and was the dominant group for most of the sites (Chapter 3, Fig. 3.3). According to the analysis of RDA, sulfate and winter pasture land cover have a positive correlation with TB (Chapter 3, Fig. 3.4). Additionally, the structural equation model detected that skewness of 7 days flow mediated through sulfate and dissolved oxygen impact on TB (Chapter 4, Fig. 4.5). Detailed analyses also conduct for group M which composed by *Microcystis*. It has been a dominant group in some part of the study area (Chapter 3, Fig. 3.3). Despite waterbody (lake) land-cover contributes as a direct reason for their appearance, the concentration of phosphate ($\text{PO}_4\text{-P}$) also acts as the direct shaping factor (Chapter 3, Fig. 3.4; Chapter 4, Fig. 4.5). Moreover, analysis result also reveals the water temperature fostered the community development in the low flow period (Chapter 4, Fig. 4.5).

In this study, analysis has mainly focused on group TB and M since they are dominant groups in the study area. However, group W1 and W2 have shown significant positive relationship with agriculture land-use (Chapter 3, Fig. 3.4). Group W1 and W2, mainly composed of *Euglena*, *Phacus* and *Trachelomonas*, are described with habitat preference of mesotrophic, organic ponds. These groups of species can survive without light, while on a diet of organic matters. The reasons for their ascendancy probably relate to the relatively higher organic matter pollutions in the upstream intensive cropland of the study area. The percentage of agricultural land-use is more pronounced in the upstream of the river, where manure, fertilizer and pesticide enhance the loads of organic matter into the nearby rivers and linking ponds (Hayes et al., 2015; Ulrich et al., 2018). Therefore, further studies on the timing and transportation of pesticides and fertilizers usage in this area will help to better explain the pattern of group W1 and W2, and more importantly, to unravel the ecological status in relation to the land-use pattern, which increasingly demanded for sustainable watershed management regarding to aquatic biota.

Despite the wide used ecological methods, such as variation partitioning and redundancy analysis for clarifying the biotic and abiotic factors relationship, SEM disentangles the cause-effect relationship of multiple stressors and riverine phytoplankton community in this study. The approach brings novel insights into the complex pathways of joint impacts of natural and anthropogenic descriptors (Lefcheck,

2016; Tang et al., 2017). It is a useful tool to improve the understanding of the causality of biodiversity response to the changing environment, and suggest to be used for further studies.

(5) *What are possible responses of phytoplankton to interactive stressors?*

In river ecosystems, stressors rarely occur alone in the environment, where they act jointly to produce complex responses. Accordingly, phytoplankton respond to a diverse array of stressors in aquatic systems. The impacts of the multiple stressors can amplify (synergistic interaction) or buffer (antagonistic interaction) on the riverine phytoplankton community (Sabater et al., 2019b). The observations illustrate that spatial factor, alteration of flow regime, land-use and physicochemical condition jointly modulate the phytoplankton community pattern. In this study, it has found that analyzing interactions leads to better explanations than only analyzing single drivers (Chapter 2, Fig. 2.6; Chapter 4, Fig. 4.4; Chapter 5, Fig. 5.2). In the mainstream of the river, the decrease of the nitrogen and phosphorous ratio may potentially cause phytoplankton density increase (Chapter 2, Fig. 2.4). Lower biodiversity corresponds to the combination of less nitrogen concentration and high flow alteration (Chapter 2, Fig. 2.5). Species richness is most sensitive to forest land-use change (Chapter 6, Fig. 6.2).

The responses of phytoplankton in this study have further analyzed under considering the key functional groups and biological traits. Functional groups and traits variation have a higher explanation than species composition by abiotic factors (Chapter 5, Fig. 5.2). The functional group TB is an assemblage of species have siliceous cell wall, turbulence and mesotrophic preference, short residence time for the development, and low risk to be “undesirable” for the riverine ecosystem (Borics et al., 2007). Under such characteristics, it is not surprising to find with a dominance of low total biomass during high flow period (Chapter 4, Fig. 4.3). They sensitively respond to the interactions from hydrological alteration and physicochemical condition (Chapter 4, Fig. 4.5). On the contrary, functional group M prefer hypertrophic status and slightly lotic condition (Borics et al., 2007). It is dominant with high biomass in low flow period (Chapter 4, Fig. 4.3). Also, a close relationship with interactions of land-use pattern and physicochemical conditions has been detected, which have not been fully explained at present. However, we assume that because of local farmers fertilized crops and applied pesticides extensively in the study region (Ulrich et al., 2018). Cyanobacteria had been reported for a higher tolerance to herbicides than other phytoplankton taxa, such as Chlorophyta (Bérard et al., 1999), particularly under status of enhanced nutrient supply (Harris and Smith, 2016), indicating pesticides might potentially stimulated the dominance of group M under drought situation. On community level, pressures of different modes of action might play a

role in favoring one bioindicator while suppressing others. The mechanism may reflect on their various biological traits to overcome changing environment. The phytoplankton biological traits, for example the algal guild (classified to “low profile”, “high profile”, “motile taxa”, “planktonic taxa”) are sensitive to flow alteration (B-Béres et al., 2016; Wang et al., 2018). The large-bodied, non-attached filamentous algae that dominated under high farming intensities have limited dispersal abilities but may scope with unfavorable conditions through the formation of spores (Lange et al., 2016). Functional traits-based diversity exhibits the most consistent positive causal effects on phytoplankton resource efficiency, and thereafter improve our understanding on trophic transfer and nutrient cycling in aquatic ecosystems (Ye et al., 2019).

Phytoplankton species richness are mainly sensitive to forest and pasture land-use change (Chapter 6, Fig. 6.2, Table 6.3), and the value of forest presence exerted stronger effects than pasture changes in antagonistic effects scenarios (Chapter 6, Table 6.2). Land-use pattern itself brings interactive effects. As a reason of fact, land-use can disrupt the surface water balance and the partitioning of precipitation into evapotranspiration, runoff and ground waterflow (Costa and Botta 2003). In addition, temperature and land-use change interact with flow regulation to alter the light, thermal and flow regime that ultimately shape the biotic regime of rivers. Land-use change plays a critical role in enhancing or controlling the impact of climate change (Bussi et al., 2016). In this study, water temperature change influences lead to less negative but larger positive additive effects to the land-use changes (Chapter 6, Table 6.3), which consistent with a previous finding. Climate can modulate the effects of land-use on species biodiversity, but still lowered resistance of the community (Peters et al., 2019).

7.2. Outlook

Multiple stressors have been discussed in the study area. However, there are still several important factors uncovered in this research. First of all, it is undeniable that the underwater light condition has a vital influence on the algal community composition as well as the biomass and productivity. Most evidence suggests that there are differences in the responses of the major groups of algae to irradiance. Green algae usually are associated with high light levels, and diatoms and cyanobacteria appear to require lower light intensities than green algae (Hill 1996). Motile algae are able to avoid extremes by movement along the light gradient, while non-motile, prostrate taxa may decrease in abundance when light levels decline as a result of shading by overgrowth of the algal community. However, the light regime of rivers is less easy to be linked to mean temperature, precipitation or other widely available

environmental data. Quantifying the light level underwater is a complex task. Energy available for phytoplankton growth is strongly regulated by underwater light availability which depends on the shading condition from the riparian zone, instream critical mixing depth and fluctuating rate related to flow regime. Relative variables such as maximum photosynthetic rates, surface irradiance and some measure of underwater light attenuation can reflect the lighting circumstance.

Secondly, grazing rate has as expected important influence on the primary producer as expected. Grazers can reduce algal biomass and influence community composition by selectively eliminating certain species and growth forms (Rosemond et al. 2000), and also can affect nutrient content and diversity in fluvial ecosystems (Hillebrand et al. 2004). Studies reported that chlorophytes and cyanobacteria communities would mainly be grazed when herbivores were removed, instead by overgrown diatoms (Poff and Ward, 1995). The thesis considered the whole year condition and focused on two distinguished hydrological seasons, which were mainly in the winter and summer-autumn time. However, the springtime was less discussed when numerous zooplankton and highest diversity have observed during that time. Zooplankton, as one of the important consumers, and bioindicator of the ecological status, is less discussed. The multiple stressors, despite the environmental factors, interactions among species also act significant role in structuring the phytoplankton communities, for instance, the grazing pressures from zooplankton, as well as invertebrates and fishes. Further studies on metacommunity level may increase the capability to gain more information about the biotic interactions from regional species pool. Further application of food web modeling may show its advantage when linking the whole process of biotic assemblages and their surrounding habitat.

Thirdly, a rising number of chemicals utilized nowadays and thus more unknown stressors are affecting river ecosystems. As our study catchment is located in a rural area, one of the major threats would be the application of pesticides. Organic pollutions were reported with negative impacts on algae growth. However, we are not so clear about each species tolerances to them. It is worth to complete the investigation on agrochemical pollutions of surface water bodies which may provide important supportive information for the progress of phytoplankton structure, and then offer meaningful implications for the river management and policy.

Under intensive global change effects, interactions of multiple stressors are serious challenge for both researchers and river basin managers. The effects of combined stressors can often result in the sum of the single stressor effects, though in many other cases, synergistic or antagonistic interactions hamper the prognosis of stressor effects. This complexity aggravates the perplexity of unforeseen consequences by multiple stressors among riverine ecosystem. Despite interdisciplinary subjects of hydrology and ecology, integrating ecological study with socioeconomic study can be placed into

interdisciplinary context. Science and society must be more active in communicating the evidence of multiple stressors affecting rivers, as well as the risks associated with the ecosystem services they provide. Under social perspectives, stakeholders can be engaged in water quality modeling for controlling the multiple stressors challenge efficiently.

7.3. Conclusion

The work presented in this dissertation offers an insight into the dynamics of the phytoplankton community in responses to multiple environmental stressors, which includes both natural and anthropogenic alterations. In general, studies have distinguished the key structuring factors by considering flow regime, land use pattern and physicochemical conditions. The responses were assessed and evaluated with various features of the riverine phytoplankton community across different space and time. The summary of the key results and relevant achieving of this thesis is given below:

- (1) Application of riverine phytoplankton as bioindicators encompasses structural and functional variables of the river ecosystem. Trait-based biological factors can sensitively detect the effects of multiple stressors.
- (2) Taking into consideration the watershed land cover can better explain the structure of the species distribution. Monitoring is essential to set according to flow regime, as it appears as a key stressor. The interdisciplinary hydro-ecological modeling approach was applied to disentangle the complexity of effects.
- (3) It is beneficial to clarify the most relevant stressors according to various biological factors. The study allows for an attribution of causality and threshold of the environmental factors from local and regional scale.

The knowledge of the thesis contributes to the lowland riverine phytoplankton investigation, implementing the application of integrated ecohydrological modeling and improve our understanding of the interactions of changing environment on algal population, biodiversity and functioning. The approaches illustrated in this thesis are worth to be extended to other area and longer time series. Further investigations on other stressors and their interactions can give a comprehensive outlook for river management.

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Declaration

I, Yueming Qu, hereby declare that the dissertation submitted, entitled “Assessing potential impacts of multiple stressors on riverine phytoplankton community by integrated models” was written independently by me. The content and design of this thesis, apart from the supervisor’s guidance, is my own work. The thesis has not been submitted either partially or wholly as a part of a doctoral degree to another examining body and is my first and only doctoral procedure. Chapter 2, 3, 4, 5 of the thesis have published in the peer reviewed journals. The authors' share of the manuscripts is explained in the section "Author contributions". This work has been prepared respecting the Rules of Good Scientific Practice of the German Research Foundation. I have not been deprived of an academic degree.

Kiel, July, 2019

Yueming Qu