

# **Connecting the ditches:**

*A spatial perspective on biodiversity in Dutch polder landscapes*

**Sven Teurlincx**

**Thesis committee:**

prof. dr. J.T.A. Verhoeven  
prof. dr. M.B. Soons  
prof. dr. ir. M.G. Vijver  
prof. dr. P.F.M. Verdonschot  
dr. J.M. Sarneel

**Illustrations:** Sven Teurlincx, Amber Heijboer, Michiel Verhofstad

**Photography:** Perro de Jong

**Cover design:** Julie Mazurek

**Printed by:** Printservice-Ede, proefschriftenprinten.nl

This thesis should be cited as: Teurlincx, S. (2019) Connecting the ditches: A spatial perspective on biodiversity in Dutch polder landscapes. PhD thesis, Utrecht University, the Netherlands

**ISBN:** 978-94-92679-85-7

# **Connecting the ditches:**

## ***A spatial perspective on biodiversity in Dutch polder landscapes***

### **Verbindingen tussen sloten:**

#### ***een ruimtelijke kijk op biodiversiteit in Nederlandse polderlandschappen***

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de  
rector magnificus, prof. dr. H.R.B.M. Kummeling, ingevolge het besluit van het college  
voor promoties in het openbaar te verdedigen op maandag 25 maart 2019 des  
middags te 12.45 uur

door

**Sven Teurlincx**

geboren op 8 december 1987 te Venlo

Promotor: prof.dr. E. van Donk

Copromotor: dr. S.A.J. Declerck

This thesis was financially supported through the Division for Earth and Life Sciences (ALW) from the Netherlands Organization for Scientific Research (NWO), by the Biodiversity Works Research Program (OBW) project no. 841.11.009.

# Table of contents

Chapter	Page
1 General introduction	8
2 Conservation of aquatic biodiversity in ditch networks requires a regional approach across spatial scales	19
3 Local functioning, landscape structuring: drivers of soil microbial community structure and function in peatlands	55
4 Evidence for a non-linear response of submerged plant tissue nitrogen and phosphorous to environmental nutrient availability	85
5 Species sorting and stoichiometric plasticity control community C:P ratio of first-order aquatic consumers	107
6 Managing successional stage heterogeneity to maximize landscape-wide biodiversity of aquatic vegetation in ditch networks	139
7 Effects of nutrient-load redistribution on the ecological state of an agricultural waterscape: Using simulation modelling to assess landscape scale impacts of nutrient management on ditch vegetation	167
8 General discussion	187
Reference list	201
Summary (English)	225
Samenvatting (Nederlands)	229
Acknowledgements	233
About the author	237



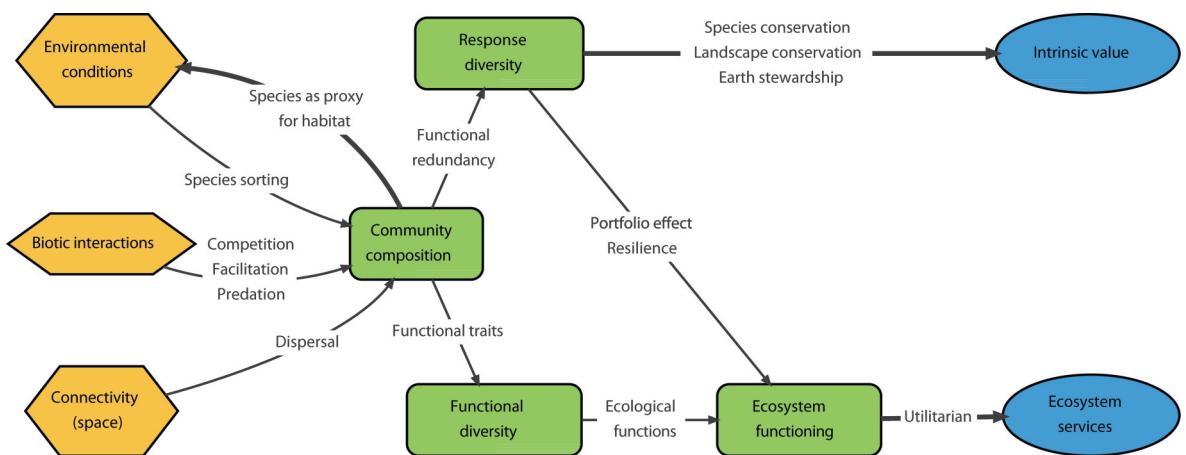
# 1

## General introduction

*Sven Teurlincx*

# The need for conserving biodiversity

Biodiversity loss is happening at an unprecedented rate, with current extinction rates estimated to be over a thousand times higher than natural background extinction rates (Pimm *et al.* 2014). Surprisingly, this global loss of biodiversity is not caused by a decrease in local diversity (Dornelas *et al.* 2014), but rather due to biotic homogenization (McGill *et al.* 2015). Due to the loss of species and range shifts of existing species, communities resemble each other more and more. The differences between communities, characterized by their  $\beta$ -diversity (i.e. Whittaker 1960), is lost. Halting the decline of biodiversity will require active management and conservation action, at both the local and regional scale.



**Figure 1.1:** A schematic representation of how factors (yellow) drive community composition and how this gives rise to different aspects of biodiversity (green) and end goals for conservation (blue).

All conservation and management of biodiversity starts from the community composition of the organismal group(s) under consideration (Figure 1.1). This community composition is influenced by environmental, biotic and spatial drivers. The community gives rise to diversity in functional traits (Figure 1.1), but also contains numerous species with similar functional traits (Kremen 2005). In turn, the functional diversity gives rise to the number of ecosystem functions that the community may support. Richness in functional diversity alone however, does not guarantee optimal ecological functioning, as species may vary in their efficiency in performing a function. A high response diversity (the number of species capable of carrying out a given function (Elmqvist *et al.* 2003), can secure or stimulate the functional output of a functional group (Loreau & Hector 2001). Along similar lines, the portfolio effect

suggests that a higher response diversity will increase the probability that a species with a high efficiency is present to carry out a given function (Schindler *et al.* 2015). Also, increasing response biodiversity will increase the functional redundancy and thereby the resilience of a system by increasing the probability of maintaining an ecological function after a severe perturbation of the ecosystem (Elmqvist *et al.* 2003).

The need for halting diversity decline and conserving biodiversity is generally acknowledged by a wide range societal actors (Escobar 1998; Brown *et al.*, 2010), though ideas on what aspect of biodiversity (e.g. functions, species, communities, habitats) requires protection may vary wildly (Buijs *et al.* 2010). Mace *et al.* (2012) defines two distinct reasons for why people want to conserve biodiversity, namely for its intrinsic value or for the ecosystem services they provide. Conservation of biodiversity for its intrinsic value may take on many forms such as the conservation of (flagship) species (Smith *et al.* 2012), conserving (cultural) landscapes, ecosystems and its associated diversity (Henle *et al.* 2008), and protection of the earth's biosphere as a whole (earth stewardship: (Ogden *et al.* 2013). Alternatively, the loss of diversity can have profound impacts on the functioning of ecosystems (Hines *et al.* 2015), and may directly affect a wide range of ecosystem functions and the services they provide for humankind (Walsh *et al.* 2016; Hilt *et al.* 2017; Orth *et al.* 2017). Hence, maintaining biodiversity is key to human wellbeing. To maintain these services and biodiversity as a whole there is a need for well-informed and effective conservation management (Clarke 2014), which requires knowledge on the main pressures impacting different communities across spatial scales.

## Biodiversity loss through changing land use

Biodiversity loss can be largely attributed to increasing anthropogenic pressure such as land use changes on our natural ecosystems (Newbold *et al.* 2015). In particular, freshwater ecosystems and their diversity have been impacted by a wide range of pressures such as climatic change, disturbance and modification of natural flow regimes, and land use changes in the surrounding landscape (Dudgeon *et al.* 2006). Changing land use can impact biodiversity by directly deteriorating, or improving, habitat quality (Foley *et al.* 2005b), thereby affecting the local community and thus affecting local diversity (e.g. McGoff and Irvine, 2009). Alternatively, land use change may promote or nullify gradients in the landscape responsible for turnover between communities (Donohue *et al.* 2009). When communities are composed of distinct sets of species, a loss of the gradients that lead to these distinct communities will negatively impact the overall diversity of the landscape (Heino 2009). For freshwater ecosystems in particular, land use change may involve direct hydromorphological reshaping of

waters (Brauns *et al.* 2007). Moreover, agricultural land use intensification adjacent to fresh waters leads to an increase in nutrient loading (Carpenter *et al.*, 1998). This nutrient loading causes eutrophication in water systems and can have disastrous ecological effects, e.g. by promoting toxic algal blooms (Paelrl *et al.* 2001), aquatic weed proliferation (Verhofstad & Bakker 2017) or fish kills (Moss *et al.* 2011). Beside direct impacts on the habitat, land use alterations may also affect connectivity within and between landscapes (Trombulak & Frissell 2000), thereby changing the diversity through limiting or improving dispersal between water bodies (Bornette *et al.*, 1998; De Bie *et al.* 2012).

## Ditch networks in the Netherlands: a case of landscapes heavily impacted by land use

The ditch networks in the typical Dutch polder landscapes are a prime example of aquatic ecosystems where diversity and ecological integrity of the water systems is under threat because of intense agricultural land use. With over 300.000 kilometres of this canalised drainage and irrigation water system in the Netherlands (Veraart *et al.* 2017), these systems constitute a large part of the fresh water surface of the country. Ditches are historic landscape elements in the Netherlands, used as early as the ninth century to drain peat bogs and make them habitable (TeBrake 2008). Due to this drainage and active peat excavation the peat bogs slowly subsided, lowering them to the point that flooding risk became increasingly common (TeBrake 2002). To avoid flooding, dikes with sluices were constructed around the landscapes, ushering in the polder landscapes of the Netherlands. Water management of these polder landscapes became increasingly intensive over time, with an increasing drainage capacity being realized by advancing pumping technology (e.g. wind powered, fossil fuel powered) (van Dam 2009). Water management of ditch networks was generally aimed at drying out and reclaim land from the sea, allowing it to be used for agricultural purposes (Herzon & Helenius 2008).

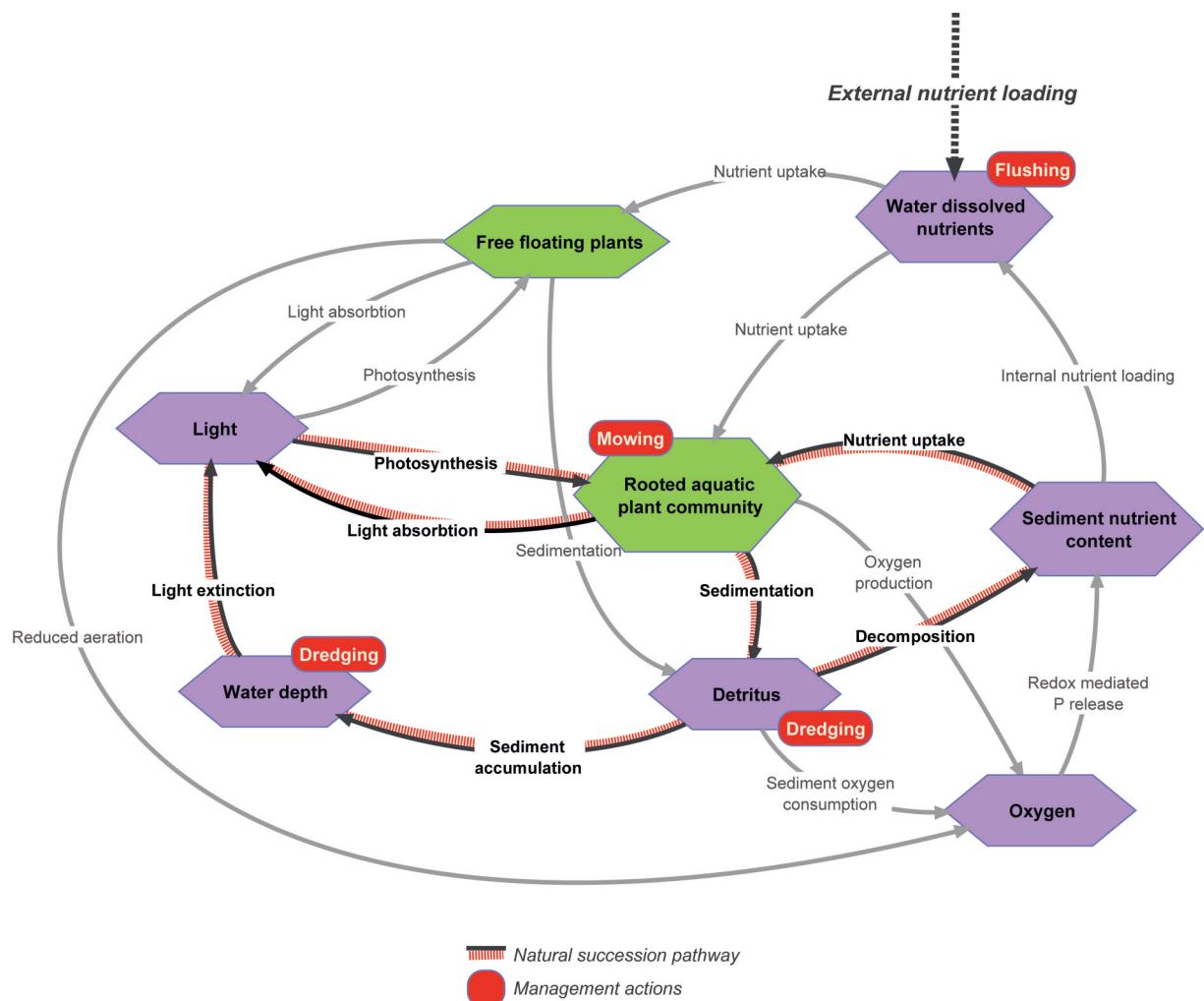
Ditch ecosystems are stagnant to slow flowing water systems. They are shallow (<2m), narrow (0.5-8.0m) and often highly productive systems (Olde Venterink *et al.* 2006). Ditches are strongly influenced by agricultural land use and the nutrients they supply (Vermaat & Hellmann 2010). However, ditches can harbour a diverse set of species and offer a myriad of important ecosystem services (Dollinger *et al.* 2015). While ditches are not generally considered to be biodiversity hotspots in themselves (Williams *et al.* 2003), the importance of small water bodies at the scale of entire landscapes is increasingly being recognized (Declerck *et al.* 2006; Leng *et al.* 2011; Whatley *et al.* 2014a). Moreover, being largely surrounded by agricultural or former agricultural land,

these ditches and their banks offer a refuge for a large share of the diversity of the entire landscape (Kleijn *et al.* 2011; Hill *et al.* 2016). Biodiversity in these systems is known to depend strongly on vegetation and the detrital matter it provides (Dollinger *et al.* 2015). Aquatic plants are a key biotic group within these systems, offering structure and habitat for other organisms (Whatley *et al.* 2014).

## Functioning of the local ditch reach

Ditches are complex ecosystems where vegetation is a key group mediating and facilitating a large part of the ecological processes taking place. When external nutrient supply is limited (i.e. mesotrophic conditions in the reach) the natural succession of the vegetation community will progress, with a varying composition of vegetation through time (Figure 1.2, red-black arrows) (Beltman *et al.* 1996). Natural succession in ditch systems is therefore an important process for species turnover. Due to continuous biomass build-up of vegetation increasing amounts of detrital matter will be deposited to the sediment. This sediment accumulation will lead to an increasingly shallow system while simultaneously increasing the sediment nutrient content. When left unmanaged, over time, ditch systems would slowly fill up with sediment and plants and develop into wetlands or eventually terrestrial habitats (Kirschner *et al.* 2001; Lamers *et al.* 2002). To halt this natural process and maintain their hydrological function, continual management in the form of mowing and dredging is required (Twisk *et al.* 2003).

Increasing eutrophic conditions are part of the natural succession of ditch systems, but excessive nutrient loading can lead to a deterioration of the ecological state and diversity of a ditch system (Janse & Van Puijenbroek 1998), independent of its successional stage (Figure 1.2, grey arrows). Analogous to the stable states of shallow lakes, the clear, submerged plant-dominated state can deteriorate to a less favourable ecological state (Scheffer *et al.* 2003). In contrast to the shallow lakes, the latter is not necessarily a turbid state dominated by phytoplankton, but often a state dominated by free-floating plants (e.g. lemnids). Dominance by free-floating plants reduce light (Gerven *et al.* 2015) and oxygen availability which both negatively impact the ecological quality of the ecosystem. Excessive external nutrient loading can disrupt the cycle of natural succession by increasing the dissolved nutrients in the water column. Free floating plants can only take up nutrients directly from the water column as they are unrooted and have no direct access to the sediment nutrient pools. Competition for light is also asymmetrical, and per definition the floating plant has an advantage here (Gerven *et al.* 2015). The success of free-floating plants is therefore associated with the dissolved nutrient availability.



**Figure 1.2:** Conceptual representation of the different processes that determine the ecological state of a ditch system. Central to the figure are the submerged aquatic plants that cycle nutrients within the system and slowly build up an organic sediment layer which will facilitate other submerged plant life (natural succession pathway). With increasing external nutrient load, another biotic group (free-floating plants) may proliferate and break the natural successional cycle. This group has the advantage of being effective at taking up nutrients directly from the water phase. The free-floating plants also benefit from being on top of the water column and thereby shading out submerged plants. Lastly, by hampering aeration of the water layer, free floating plants create hypoxic conditions under which P release from the sediment will occur, leading to more free nutrients in the water phase that may be taken up by the free floating plants. Management options of ditch systems are indicated in red with the components that they affect.

Recovery from a free-floating plant dominated state is difficult due to positive feedback loops that help to reinforce the free floating plant state (Scheffer *et al.* 2003; van Gerven *et al.* 2017). Apart from reducing the light availability, the free-floating plant layer also reduces aeration of the water layer by wind. Coupled with the low oxygen production in the water layer because of the decrease in algae and submerged macrophytes, this can cause hypoxic, or even anoxic, conditions in the water layer and sediment. Hypoxic or anoxic conditions reduce the binding capacity of phosphorus to (iron in) the sediment (Smolders *et al.* 2006), and thereby increase internal phosphorus loading. This again benefits the free floating plant community through increased nutrient availability (Boedeltje *et al.* 2005). This positive feedback loop may be strengthened by nutrient rich detrital matter of free-floating plants being deposited to the sediment. Decomposition of this nutrient rich detritus will cause additional internal loading (Søndergaard *et al.* 2003) increases dissolved nutrient concentrations of the water column. Due to these positive feedback loops, restoring and maintaining a sound ecological quality and associated biodiversity is challenging, especially as reducing nutrient loads from agricultural lands is in itself a challenge.

## The role of stoichiometry in local ditch functioning

While sustained excessive nutrient loading may suddenly result in a shift towards a degraded ecological state, nutrient loading may already impact community dynamics and diversity long before such a sudden ecosystem shift. When external loading is such that the gross nutrient flux to the sediment is in equilibrium with the nutrient flux out of the sediment, the system will stay in its current trophic state (Nürnberg 1991). However, when the external load is high, the release of nutrients outpaces its sedimentation; slowly shifting a system towards increasingly eutrophic conditions. With increasing nutrient loading, stoichiometrically flexible primary producers will increase their relative incorporation of nutrients into their tissues (lower C:N or C:P). Under nutrient depleted conditions additional nutrients will lead to enhanced growth and luxurious uptake of excess nutrients by producers (Sterner & Elser 2002; Velthuis *et al.* 2017a). A decrease in plant C:nutrient ratio will cause the sedimented detritus (i.e. senescent plant material) to be enriched in nutrients, and decay of this material may serve as a pump of nutrients from the sediment to the water (Bini *et al.* 2010). Enriched sediments will in turn give rise to even more nutrient rich plants, and thereby even richer sediments with higher internal loading potential (Nürnberg 2009). Shifts in vegetation along this gradient of eutrophication may have important feedbacks on the nutrient cycle of ditch systems, as species composition of aquatic plants can directly influence nutrient uptake (Barko *et al.* 1991), redox mediated nutrient release (Boros et

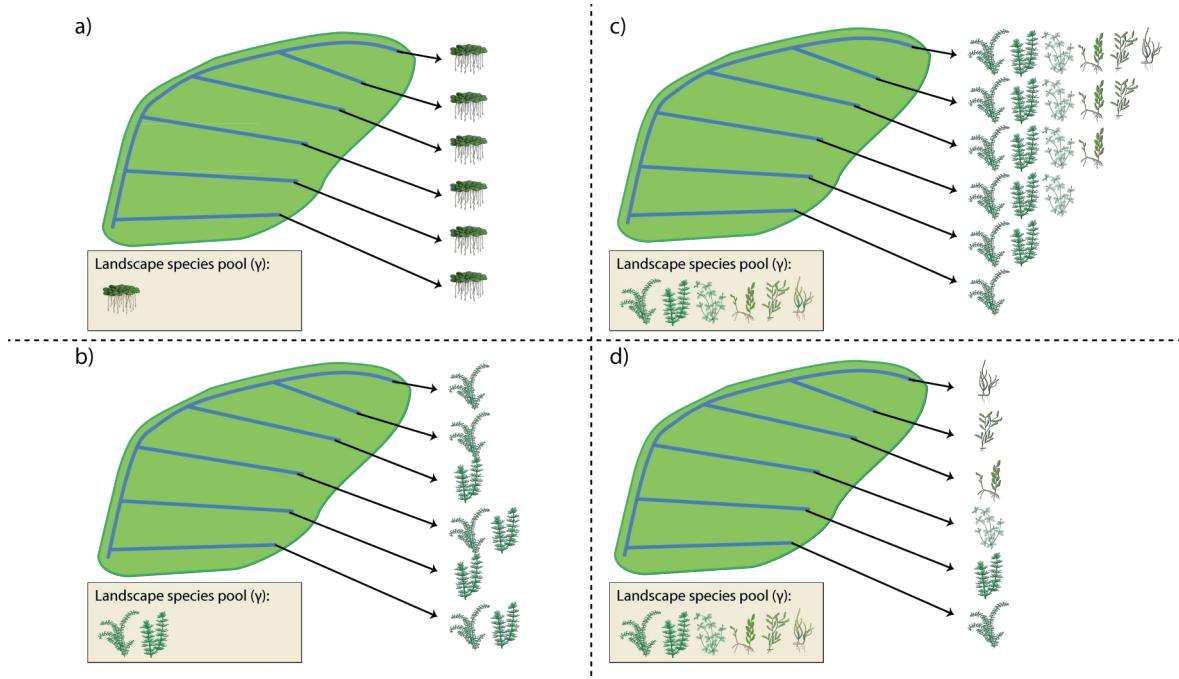
al. 2011) and denitrification (Veraart *et al.* 2017).

Changing nutrient content of primary producers may also have far-reaching consequences for the community composition and diversity of higher trophic levels. The theory of ecological stoichiometry (Sterner & Elser 2002) dictates that mismatches between a consumer and its food source will have negative effects on the consumer, as it is forced to use more energy to meet its essential nutrient demands (Hessen 2003). It is widely accepted that phytoplankton are highly variable in their elemental composition (Hessen *et al.* 2013). Flexibility in elemental composition in aquatic plants has traditionally been accredited to inter-species differences (Duarte 1992), though there is increasing evidence of intra-specific flexibility as well (Velthuis *et al.* 2017a). Furthermore, while traditionally consumers have been assumed to be strictly homeostatic, some flexibility in (first order) consumers has been found (Schoor & Boersma 2010; Declerck *et al.* 2015). Such phenotypic flexibility in somatic elemental composition may reflect a higher ability to cope with changes in relative nutrient supply. This allows species to remain competitive in a changing environment and persist within the community (Hillebrand *et al.* 2014). When species are not able to persist under changed conditions, they will disappear from the system. Depending on dispersal from nearby systems, resident populations and seed banks, they may be replaced by new species, leading to an altered community (Brederveld *et al.* 2011).

## Biodiversity partitioning across spatial scales

Biodiversity is organized across spatial scales and its conservation and management benefits from a perspective beyond the local ditch reach for maximum effectiveness. A hierarchical partitioning of landscape scale diversity ( $\gamma$ ) into components representing the local diversity ( $\alpha$ ) and dissimilarity between local communities ( $\beta$ ) may help to better understand how biodiversity is structured across levels of spatial scale. The local  $\alpha$ -component is strongly influenced by the local environmental quality whereas  $\beta$  components are driven by environmental heterogeneity. Ditch systems, despite being highly connected, are known to be strongly dissimilar in terms of their community composition within landscapes (Verdonschot *et al.* 2011; Goldenberg Vilar *et al.* 2014). In other words, the landscape level diversity is strongly determined by the  $\beta$ -diversity and less so by the local community diversity of individual ditch reaches ( $\alpha$ ). Such biotic heterogeneity (high  $\beta$ ) may be caused by both heterogeneous environment as well as variation in biotic interactions between localities (Hunter & Price 1992). High local diversity, in comparison, is driven strongly by the local habitat quality. Both environmental heterogeneity as well as quality in ditch systems is likely to be strongly linked to land use, both historic and current. Land use determines the fertilization

regime of adjacent lands (Vermaat *et al.*). Furthermore, land use will have impacts on local disturbance by cattle (Conroy *et al.* 2016), water level management and mowing and dredging regimes (van Strien *et al.* 1991).



**Figure 1.3:** Illustration of four different landscapes with a) a landscape filled with undesirable (free-floating plant) species, b) a system with dominance of a limited set of submerged plant species, c and d) a landscape with a wide range of submerged plant species. Landscape c and d are different in that in c) all species are presented in the most northern ditch reach and along a gradient there is a subsequent loss of species diversity where the species poor sets form nested subsets of the most species rich. In d) all ditch reaches contribute unique species to the overall landscape species pool (the  $\gamma$  diversity).

Knowing that  $\beta$ -diversity is high is, in itself, insufficient to properly inform conservation efforts (Socolar *et al.* 2016a). Beta diversity can also be generated by a variable degree of degradation of local habitat quality within the landscape (Podani & Schmera 2011), leading to a ditch reach with high species diversity and subsequent reaches with a subset of these species (Figure 1.3c). In this case the environmental gradients driving such  $\beta$ -diversity should be suppressed. Alternatively, true species replacement among ditch reaches may occur (Figure 1.3d), with distinct species pools per ditch that contribute unique (often rare) species to the landscape (Socolar *et al.* 2016b). In this

case conservation planning will need to conserve all of these complementary and unique localities by maintaining and promoting the underlying environmental gradients (Bergamin *et al.* 2017). The advantage of the biodiversity partitioning approach is that it is scale-independent and can thus be applied across a variety of spatial scales (e.g. Werner *et al.* 2007; Costa & Melo 2008; Alahuhta *et al.* 2017). Regional diversity of polder landscapes can thus easily be partitioned into a component of landscape diversity and an among landscape compositional dissimilarity as well.

The maintenance and conservation of large scale biodiversity requires knowledge of biodiversity architecture and its drivers across different levels of spatial scale (Socolar *et al.* 2016a; Alahuhta *et al.* 2017). At large spatial scales there are questions of selecting landscapes of top conservation priority (reserve selection) (Venter *et al.* 2014). At the landscape scale water level management can have pronounced effects on the transport of nutrients (Kröger *et al.* 2008, 2013) and organisms such as free-floating plants (van Gerven *et al.* 2017). Also, water level management such as flushing may have profound effects on critical nutrient loads (Liere *et al.* 2007) and phytoplankton species composition (Elliott 2010). Land use planning involved with extensification (e.g. through the implementation of agri-environmental schemes) acts at the landscape scale, but its implementation effects may be strongly localized. Similarly, ditch cleaning and dredging may have strong local impacts on diversity (van Strien *et al.* 1991; Twisk *et al.* 2003) that may lead to heterogeneity at larger spatial scales (Whatley *et al.* 2014a).

## Thesis outline

Conserving and managing biodiversity can be a challenge in systems experiencing strong anthropogenic pressures such as Dutch polder landscapes. To do so adequately management will benefit from an understanding of biodiversity structure across spatial scales as well as across different biotic functional groups. Moreover, an in-depth understanding of local processes and assessment of management impacts on biodiversity is imperative.

In the first part of this thesis, I examine the architecture of biodiversity and describe the ecological state and diversity of a large number of ditch ecosystems and their banks in the west of the Netherlands (Chapter 2, Chapter 3). Through empirical study I aim to elucidate some of the primary drivers of biodiversity at both local and landscape scales (Chapter 2, Chapter 3) as well as among landscapes (Chapter 2). In Chapter 3 I also study if community structure and functioning are driven by similar drivers and at multiple levels of spatial scale.

Next, I look into local impacts of changing nutrient supply on organisms and their nutrient content. The theory of ecological stoichiometry (Sterner & Elser 2002) dictates

that such changes can have far-reaching ecological effects on higher trophic levels. As primary producers are known to be most flexible, I study the flexibility in tissue elemental composition of aquatic plants that were collected along a wide range of environmental nutrient conditions in the field (Chapter 4). This chapter aims to show the inherent flexibility in aquatic plants and discusses its ecological consequences. In chapter 5, I subsequently test the effects of changing carbon to nutrient ratios of phytoplankton primary producers on a community of zooplankton consumers in a mesocosm system. Within this system I address the question if and how a changing carbon to nutrient ratio leads to shifts in species composition and to what extent consumers can cope with such changes.

Subsequently, I go into the effects of management on local ditch and landscape diversity. In chapter 6 I examine if a successional reset through dredging and management can be used to promote landscape level diversity. To do so I examined the complementarity of natural successional stages and simulate landscapes with varying heterogeneity in natural succession. Using a spatially explicit linked process-based hydrodynamical and ecological model I study the effects of changing land use configurations and intensity on functional aquatic plant proliferation (Chapter 7). Such an approach can aid conservation and restoration management in defining an optimal land use scheme to promote development of desirable vegetation types.

Finally, I discuss the implications of this work in the context of the multiple different meanings given to the concept of biodiversity and varying goals of conservation. I explain how local scale processes related to nutrient cycling can have landscape level impacts on biodiversity of different trophic levels. Furthermore, I discuss the need for modelling approaches to predict changing pressures and management impacts on biodiversity across spatial scales to aid decision making across levels of spatial scale and species groups. All in all, this thesis will contribute to maximisation of biodiversity in Dutch ditch systems across scales and functional groups through understanding of drivers of community composition and diversity and development of methodological approaches to analyse potential management scenarios.



# 2

## **Conservation of aquatic biodiversity in ditch networks requires a regional approach across spatial scales**

*Sven Teurlincx, Steven A. J. Declerck*

## Abstract

Diversity worldwide is being threatened by increasing anthropogenic pressures such as land use intensification and homogenization. To maintain high levels of biodiversity in an intensely managed agricultural region, the complex interaction of biodiversity and its drivers at multiple spatial scales needs to be considered. Understanding the drivers that lead to biodiverse landscapes and attention to the complementarity both within and between landscapes is key. In this study we investigate the underlying patterns of biodiversity and its drivers across spatial scales in ditch systems of Dutch polder landscapes. We use a large dataset of 21 landscapes with 24 ditch reaches each to reveal the primary drivers of diversity of four organism groups: bank vegetation, helophytes, hydrophytes and cladocera. Our results showed that diversity was largely shaped by compositional differences within and between landscapes (large  $\beta$  diversity). Further analyses revealed that intensive agronomical and urban land use had strong negative impacts on biodiversity of a landscape, suggesting that conservation efforts are best focussed on the least intensively used landscapes. These patterns were consistent among organism groups. However, to complicated conservation efforts, we also showed that complementarity between landscapes was relevant for the regional diversity. While intensely managed landscapes do have their own unique set of species that contribute to the regional species pool, we argue that intensely managed agricultural lands are unlikely to disappear from the region. Hence, conservation effort should focus its attention on preserving the most diverse landscapes by maintaining low intensity landscapes with sufficient connected open water area and heterogeneity in ditch morphology.

# Introduction

The establishment of protected areas for the preservation of freshwater biodiversity has long received limited attention, despite its effectiveness in terrestrial and marine realms (Hermoso *et al.* 2016), but has recently been brought back into focus (Venter *et al.* 2014) as a means of preserving the rapidly declining freshwater biodiversity (Collen *et al.* 2014). The establishment of protected landscapes for biodiversity is especially relevant given the increasing land use intensification (Davis *et al.* 2015). At a regional scale (~100 kilometers), this calls for an identification of landscapes (kilometer scale) with a high levels of biodiversity. This requires an inherent appreciation for the complexity of landscape diversity, which is housed by numerous water bodies with their own local diversity and ecology (Hill *et al.* 2018). This is especially relevant for landscapes with a multitude of smaller water bodies, which have been increasingly recognized for their importance to landscape scale biodiversity (Scheffer *et al.* 1999; Declerck *et al.* 2006; Lemmens *et al.* 2013). Identifying landscapes of high diversity is complicated by the fact that the local diversity (alpha diversity) of its comprising water bodies as well as the variation between their local communities (beta diversity) will contribute to the landscape diversity. These two components are controlled by different drivers. Alpha diversity is strongly controlled by local conditions, whereas the beta diversity may be controlled by the existence of environmental gradients within the landscape. Hence an understanding of the underlying architecture and drivers of landscape diversity is imperative for adequate conservation decision-making.

Land use changes are one of the prime pressures on aquatic systems and their diversity (Downing 2014). Land use intensification leads to increasing nutrient loads and eutrophication of aquatic systems (Carpenter *et al.* 1998) with a whole scale of negative consequences for ecology such as harmful algal blooms (Paerl *et al.* 2001), fish kills and biodiversity decline (Jeppesen *et al.* 1998). Moreover, land use changes may alter the connectivity among freshwater systems, hampering dispersal (Smith *et al.* 2015) or causing homogenization of aquatic communities (Strecker & Brittain 2017). There is evidence that changing farmland management can aid in the promotion of freshwater biodiversity (van Strien *et al.* 1989; Mantyka-Pringle *et al.* 2016). While changing land use will affect the local diversity through influencing habitat quality, it will also impact the landscape diversity through changing landscape wide gradients and associated beta diversity. There is clear evidence of the negative impacts of land use intensification causing homogenization of vegetation and thereby a decline in beta diversity (Karp *et al.* 2012). The homogenization of biodiversity becomes especially relevant within the context of the land sparing - land sharing debate surrounding agricultural landscapes (Koning *et al.* 2017). Agri-environmental schemes are

increasingly common ways of reducing some of the intensity of agricultural practice in comparison to tradition intensive farming. Both the intensity and heterogeneity in agricultural and nature management practice are relevant for the landscape diversity as a whole, driving the alpha and beta component respectively (Socolar *et al.* 2016a). As agricultural landscapes are increasingly managed for multifaceted use (Boelee *et al.* 2017), the spatial heterogeneity of land use intensity and the need for multi-scale approaches to biodiversity conservation will become increasingly relevant (Gonthier *et al.* 2014).

Conservation priorities should be set at a large spatial scale (i.e. regional). Knowledge of diversity and drivers of landscape level diversity is helpful to pinpoint the most diverse landscapes within the region. However, knowledge of the architecture of biodiversity at the regional scale is required to ensure that complementarity between landscapes is utilized and maintained for a maximal regional diversity (Socolar *et al.* 2016b). A consideration of the possible complementarity between landscapes is imperative when designing management plans for regional diversity (Brown *et al.* 2010), as high beta diversity among landscapes in itself is insufficient. High beta diversity can be caused by a degradation of landscape quality within the region, leading to a single landscape with high species diversity and subsequent landscapes with a subset of species. In this case conservation of the single landscape of high diversity would be sufficient. Alternatively, true species replacement among landscapes may occur, with distinct species pools per landscape that contribute unique (often rare) species to the region (Socolar *et al.* 2016b). In this case regional planning will need to conserve all distinct landscapes to maintain a maximal regional diversity (Bergamin *et al.* 2017). It is important to note that the drivers of the beta diversity between landscapes may be markedly different from those at smaller spatial scales (Barton *et al.* 2013). At the scale of the region, dispersal limitation as well as large scale environmental gradients in biogeographical history become increasingly relevant (Henriques-Silva *et al.* 2013).

While larger scale planning and management is essential from a governance perspective, the community and its ecology exists at the level of the locality (Heino *et al.* 2015). The local conditions capture the inherent state of the ecosystem in which the local community persists (i.e. nutrient conditions, morphology, biotic interactions). Landscape scale management efforts, such as hydrological management and land use practices can directly or indirectly affect these local conditions by influencing processes such as nutrient or hydraulic loading to the ecosystem or directly affecting species through removal (e.g. mowing, harvesting). Changing processes (e.g. nutrient loading) create local conditions (e.g. dissolved nutrient concentrations) to which species respond (e.g. increased growth of primary producers) and cause species sorting of a

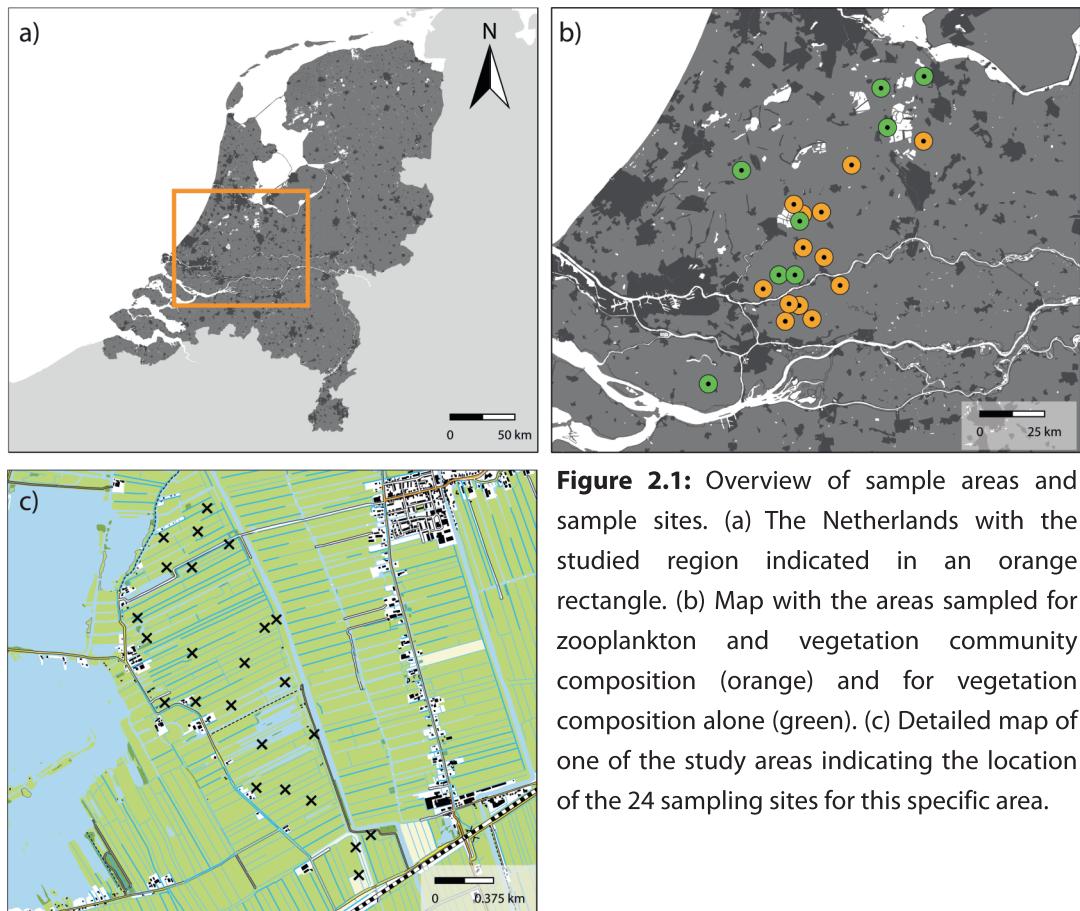
resident community. Effect of regional planning on the local community may seem less direct than those of landscape management, but may be pronounced nonetheless. Upstream changes along a catchment can drastically alter incoming water supply and quality, and urbanization and cityscape advancement may negatively impact biodiversity of nearby areas (Sun *et al.* 2018). Aligning regional policy, landscape level management and ecological demands of species is a challenging endeavor (Sayles & Baggio 2017) that benefits from an understanding of how biodiversity is structured at landscape and regional scale.

In order to understand biodiversity conservation at regional scales and inform conservation management and policy we study the diversity of multiple organism groups of vegetation and cladocera zooplankton across ditch networks in 21 polder landscapes in The Netherlands. These polder landscapes constitute distinct landscapes with their own land use configuration and hydrological management. We selected these landscapes along a gradient of land use intensity (intensive agriculture, varying agri-environmental schemes and nature management) and heterogeneity of these land use types. These landscapes consist of grasslands intersected by a network of drainage ditches, of which 24 ditch reaches per area were sampled. Our landscapes are situated within the Western Peat District region of the Netherlands. We first examined the underlying architecture of the regional biodiversity through biodiversity partitioning. Based on these results we analyzed the underlying drivers of landscape level diversity to elucidate the drivers that lead to highly diverse landscapes. Next we studied the drivers causing among landscape biodiversity differences. Lastly, we focused on complementarity in biodiversity between landscapes by analyzing the drivers specifically relevant for replacement patterns in species diversity. Combined, these analyses help to understand the importance of landscape level diversity in a regional context, aiding management effort and conservation prioritization for freshwater biodiversity.

## Methods

### Study landscapes and site selection

Our study took place in the peat meadow polder region situated in the west of The Netherlands (Figure 2.1a). The landscapes in this region were historically created through the drainage of peatlands to reclaim land for agriculture, creating the typical Dutch polder landscapes. In these landscapes narrow fields of land are intersected by a network of drainage ditches, with the whole system being surrounded by dykes. The water level within these landscapes is strictly controlled through weirs and pumps. Due



**Figure 2.1:** Overview of sample areas and sample sites. (a) The Netherlands with the studied region indicated in an orange rectangle. (b) Map with the areas sampled for zooplankton and vegetation community composition (orange) and for vegetation composition alone (green). (c) Detailed map of one of the study areas indicating the location of the 24 sampling sites for this specific area.

to their long time agricultural use and the related nutrient runoff most of these ditches would be characterized as eutrophic to hypereutrophic. The fields are used primarily for intensive cattle farming, although variation in land use intensity is generated by governmental subsidies (agri-environmental schemes) that stimulate farmers to cultivate land less intensively (Catry *et al.* 2017). Additionally, some fields are owned by nature conservation organisations and managed with the aim of creating and sustaining moist natural grassland vegetation and its associated biota. In all cases the ditches are regularly mown and dredged to sustain their hydrological function. In our study we selected 21 different polder landscapes of roughly 200 hectares each with varying land usage (Figure 2.1). Within each of these 21 landscapes, 24 ditch reaches of 100 meters were selected according to a stratified random design. For this a landscape was first divided into 24 parts with equal surface area and in each of them a site was randomly selected along the ditch network. (Figure 2.1c).

## Diversity data collection and partitioning

Vegetation surveys were carried out for each of the 504 ditch reaches in the summer (June-August) of 2012, 2013 or 2014. For each reach a survey was made along a 100 meter transect. The abundance of macrophyte species were assessed according to the Tansley scale (Tansley 1946), encompassing all species growing along the bank and within the water. The Tansley dominance classes were subsequently converted to percentage cover using Table S2.1. Subdivisions of the vegetation data were made to define different vegetation groups based on Ellenberg values for moisture (F: Hill, 1999), with dry bank vegetation being regarded as all species with an F value lower than 8, all helophytes having a value of 8 to 10 and all true hydrophytes having a value of 11 or 12. Also, at part of the ditch reaches visited in 2012 and 2013 (14 landscapes of 24 sites each, figure 2.1b), samples were taken for the determination of cladocera zooplankton community composition. 18L of water was collected using a tube sampler, thoroughly mixed and filtered over a 80 $\mu\text{m}$  plankton net (Hydrobios GmbH). The resulting filtrate ( $>80\mu\text{m}$ ) was fixed using an acidic Lugol's solution (5%) for later identification and enumeration of zooplankton with light microscopy. All cladocera zooplankton were identified to species level where possible (Amoros 1984; Flössner 2000). For each sample, subsamples were counted in groups of 100 individuals and counting was continued until no new species were found in the last group of 100 individuals.

## Diversity partitioning

For all macrophyte functional groups (bank vegetation, helophytes, hydrophytes) and for cladocera zooplankton we calculated two measures of species diversity, i.e. species richness (SR) and the exponent of Shannon diversity ( $H'$ ).  $H'$  represents the effective number of species derived from the Shannon entropy (Jost 2007b). Given an equal representation of abundance among all species, this diversity would be equal to the total number of species present in the landscape. With increasingly less equal representation (i.e. dominance of few species) the index will decrease to a minimum of one. This makes  $H'$  a straightforward measure of diversity which incorporates the abundance of species as well as their presence and absence. Applying the biodiversity partitioning framework of Jost (2007; 2010) we express the total regional diversity ( $\gamma$ ) into a component of local ( $\alpha$ ) diversity and a component representing the differences between communities. We then subdivided the differences between communities into a component attributable to within landscape differences between ditch reaches ( $\beta_1$ ) and an among landscape differences in community composition ( $\beta_2$ ) component. Furthermore, we partitioned the  $\beta_2$  component into two additional additive components, a component of 'true species replacement' ( $\beta_{\text{rep}}$ ) and a 'richness

difference' component ( $\beta_{\text{rich}}$ ) using the approach proposed by Podani and Schmera (2011). For presence/absence, the  $\beta_{\text{repl}}$  and  $\beta_{\text{rich}}$  partitions were calculated from a Jaccard-based multi-site  $\beta$ -diversity index (Ensing & Pither 2015) and for abundance data we partitioned the total variance of a Ruzicka dissimilarity matrix following Legendre (2014).

## Drivers of landscape diversity

We quantified four categories of variables that potentially affect landscape level diversity and species composition:

- **Variables related to nutrient management and agricultural land use (NUT):** variables describing the intensity and diversity of the grassland management of fields within the landscape as well as the nutrient loading towards the ditch system. Land use intensity and diversity were computed by scoring each management practice (SNL map, 2012-2014) on a scale of 1 to 5. A score of 1 is given to land use types consistent of unfertilized nature managed land, 2 to nature managed though lightly fertilized lands, 3 to agri-environmental management which has a strong impact on regular farming practice, 4 to agri-environmental management which has a smaller impact on regular farming practice and 5 to intensive agriculturally used lands. The overall contributions of all land use categories within each study landscape were calculated using ArcGIS 10.1 (ESRI) and subsequently used to calculate a weighted average intensity score analogues to much used indices such as the Anthropogenic Index (e.g. Larsen *et al.* 2010; Manfrin *et al.* 2016). Furthermore, the diversity of land use was calculated using the exponent of the Shannon index of the land use category with their respective fractions being used as the abundance weighing factor (Honnay *et al.* 2003). Runoff of nutrients from the agricultural fields to the water system were calculated at the landscape level using the nutrient runoff-model STONE (Wolf *et al.* 2003). Average yearly nutrient loads of both N and P of a 17 year period (1997-2013) were extracted from the model and used in subsequent analysis.

- **Variables representing local environmental conditions (ENV):** The environmental conditions in individual ditch reaches may determine the landscape level diversity through their impact on local reach diversity, as well as by generating community turnover among ditch reaches. The first is related to the environmental quality of the ditch reaches within the landscape, whereas the second is related to the environmental variability between reaches within the landscape. We quantified carbon, nitrogen and phosphorus pools in the water, soil and sediment. We also measured biotic variables such as concentration of phytoplankton Chl-a and fish abundance and measured morphological characteristics of the ditch reach on site (width, depth, thickness of fluffy sediment layer). Lastly, we characterized the underlying soil types of a ditch reach

based on soil maps of the Netherlands (PAWN). Given that many of these variables showed a high degree of collinearity, we applied standardized PCA to extract a low number of mutually independent explanatory factors representing important latent environmental gradients. We did this for each of the four organism groups separately. For each organism group we used a prior defined subset of selected environmental variables known to be important as potential drivers of community diversity and composition specifically for this particular group (Table S2.1). Each of the four PCA analyses was performed on the entire dataset, i.e. including information on all sites across all landscapes. For each group, the environmental quality of each landscape was characterized by calculating the average site scores of its reaches on the first two axes (PC1 and PC2) of the respective PCA analysis. In addition, variation of environmental quality within a landscape was calculated as the variance among its site scores. Differences between landscapes in terms of environmental quality were tested using a redundancy analysis (RDA) with dummy coded landscape identity being used as the explanatory variable of the environmental conditions of ditch reaches.

- **Landscape characteristics (CHARS):** Using GIS layers (Kadaster 2013: TOP10NL map) we calculated the fraction of urbanized land and open water surface within each landscape and the total length of the ditch network. Furthermore, we counted the number of hydrological obstructions (land bridges, dams, weirs) present within the ditch network.

- **Variables describing patterns of spatial autocorrelation (SPACE):** Spatial autocorrelation in among-landscape variation for species diversity and composition was modeled using Moran Eigenvector Map analysis (Dray *et al.* 2006; Peres-Neto *et al.* 2012). MEM variables represent a spectrum of spatial variables derived from the geographic configuration of sampling sites and range from coarse to fine grained (Legendre & Gauthier 2014). For their derivation, connections between pairs of landscapes were determined following a Gabriel graph whereas their weights were specified as the inverse of the distance between these landscapes (MEM; Dray *et al.* 2006). We proceeded to select only the significant MEM variables with a positive spatial autocorrelation (Dray *et al.* 2006)

## Explaining patterns in landscape diversity

We applied generalized linear models (glm) with a gamma distribution and log-link function to explain variation in landscape diversity by the drivers in each of the four categories of explanatory models. Diversity was defined here as species richness (i.e. the total number of species found in a landscape) and the exponent of the Shannon diversity, where the weight of each species was determined by the fraction of sampled ditch reaches in which it was found to occur within the landscape. For the latter we

chose to use this frequency of occurrence as weighting factor rather than total percentage cover, because the first gives a good representation of the degree to which species occupy the landscape whereas the second may be largely confounded by within-reach abundance. Glm models were subjected to a stepwise forward selection procedure, and the model with the lowest AICc score was selected.

## Explaining patterns of compositional diversity among landscapes

We applied distance based redundancy analysis (dbRDA; Legendre and Anderson, 1999) to explain  $\beta$ -diversity among different landscapes at the regional scale ( $\beta_2$ ). To this end we defined  $\beta$ -diversity as a Ruzicka dissimilarity index between landscapes. Similarly as with landscape diversity, we defined the weight of each species in this abundance-based dissimilarity measure by the fraction of ditch reaches in which it was found to occur within the landscape. A large dissimilarity value represents large compositional differences between a pair of landscapes whereas small values indicate more similar landscapes in terms of species composition. We subsequently carried out a variation partitioning analysis (Peres-Neto *et al.* 2006) using dbRDA to examine the unique contribution of the different models (NUT, ENV, CHAR, SPACE) explaining differences in  $\beta_2$  diversity. All variables of these models (NUT, ENV, CHAR, SPACE) were subjected to a forward selection procedure prior to variation partitioning (Blanchet *et al.* 2008). We assessed the marginal importance of individual selected variables within the models by examining the explained variation ( $R^2_{adj}$ ) of the selected variables in isolation (Teurlincx *et al.* 2018a).

## Conservation of regional biodiversity

For conservation planning of regional biodiversity, consideration of landscape richness and compositional complementarity among landscapes is of pivotal importance. To address true replacement patterns across landscapes we used an identity based dissimilarity index (Jaccard) to express the dissimilarity between each pair of landscapes that is caused by species replacement only ( $\beta_{2\ repl}$  partition) as per Legendre (2014). To examine the potential drivers of among landscape species replacement patterns this dissimilarity matrix was used as input for a dbRDA analysis and followed by a variation partitioning analysis, similar to analysis carried out for  $\beta_2$  diversity (see earlier).

To visualize the degree of complementarity between the different landscapes we calculated the relative contribution of each landscape to the replacement partition of  $\beta_2$  diversity (LCBD). In our analysis, this LCBD index is a relative measure of the degree to which a landscape contributes to the replacement component of overall beta

diversity. The LCBD is a relative measure, with all landscape LCBD values together being equal to 1. Hence, the average expected landscape LCBD can be calculated as one divided by the number of landscapes in the region. By centering the LCBD values around this average expected landscape LCBD value we obtained a scaled LCBD metric. This scaled metric is positive when a landscape is more unique in species composition than expected by chance, whereas a negative value indicates a lower than average complementarity of the landscape in comparison landscapes. We applied a correlation analysis to investigate if the richer landscapes are also the most complimentary. Furthermore, we examined correlations between the landscape richness and LCBD values of our different organism groups. This analysis may serve to reveal if landscapes are consistently more complementary or rich richness across organism groups.

All analyses were carried out in R 3.4.2 using the vegan (Oksanen *et al.* 2015) and ggplot2 library and the custom code supplied with Legendre (2014).

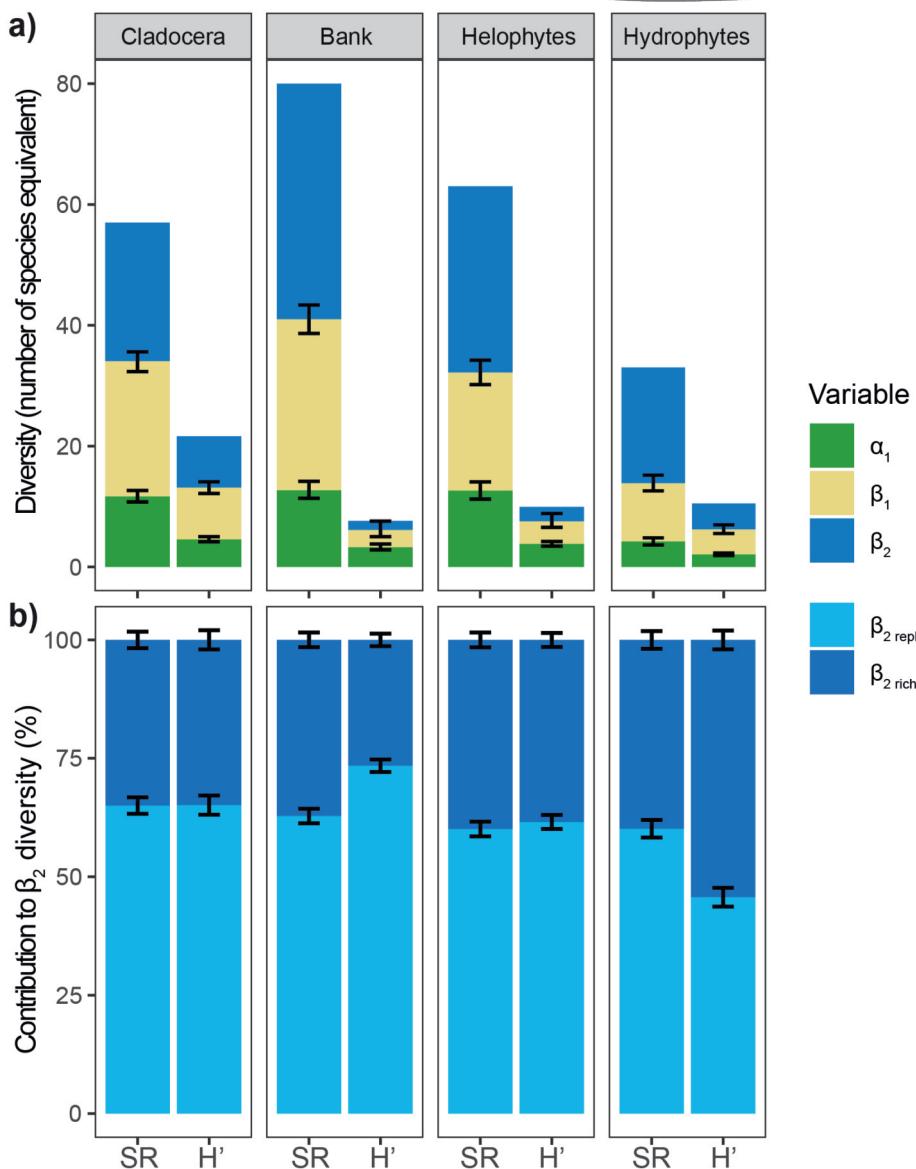
## Results

### Hierarchical diversity partitioning

Beta diversity was found to be the major component of the total regional diversity ( $\gamma$ ) for all organism groups (Figure 2.2a). Across organism groups and diversity metrics, alpha diversity, on average, accounted only for 23% of the total gamma diversity. Especially for species richness, differences in beta diversity were not just caused by within landscape differences ( $\beta_1$ ), but also by differences between landscapes ( $\beta_2$ ) (Figure 2.2a). Further partitioning showed that the largest part of  $\beta_2$  (Figure 2.2b) could be attributed to replacement of species among polders (average 65.6% over all organism groups) instead of to richness differences.

### Local environmental quality and variability

Landscapes differed strongly in the quality and variability of local environmental conditions (Figure S2.1). Each of the sets of environmental variables deemed relevant for the different groups was explained significantly by landscape identity ( $P < 0.001$ ), with  $R^2_{adj}$  ranging between 32 and 38% (Table S2.3). Table 2.1 summarizes for each organism group what the first two axes of PCA analysis on local environmental variables stand for. In brief, for all of the organism groups there was at least one PC axis that represented a gradient in nutrient availability (Table 2.1). Due to the inclusion of additional sediment nutrient variables, the first two PC axes represented two orthogonal gradients for N (PC1) and P (PC2) in helophytes and bank vegetation. PC2 of bank vegetation was also associated with soil type. For cladocera and hydrophytes, PC2



**Figure 2.2:** (a) Total observed diversity ( $\gamma$ ) for cladoceran zooplankton, bank vegetation, helophytes and hydrophytes partitioned into components of mean local diversity of ditch reaches ( $\alpha$ ), the difference in community composition between ditch reaches within polder landscapes ( $\beta_1$ ) and the difference in community composition among polder landscapes ( $\beta_2$ ). (b)  $\beta_2$ -diversity is further divided into a component attributable to the relative contribution of true species replacement ( $\beta_{2\text{ repl}}$ ) and a component of richness differences ( $\beta_{2\text{ rich}}$ ) (Legendre, 2014). Partitioning results are shown for both species richness (SR) and the exponent of the Shannon-Wiener index ( $H'$ ). Error bars reflect variation in diversity among reaches and landscapes and equal twice the standard error around the mean.

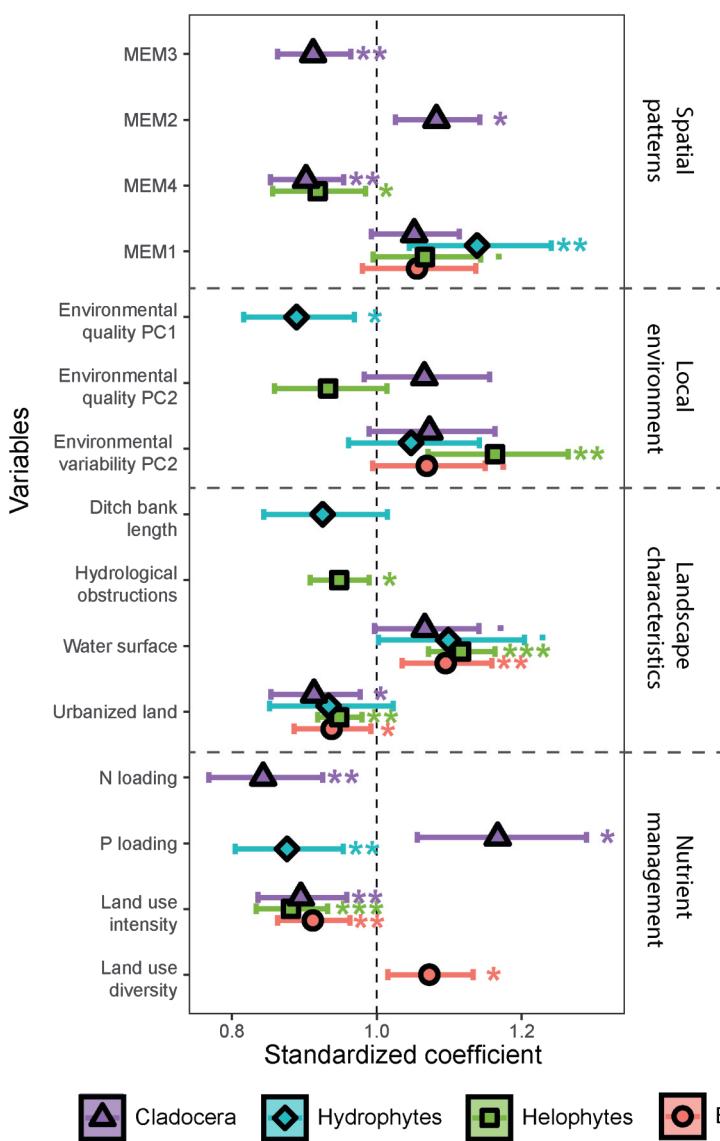
represented a gradient from turbid to clear water (PC2) as well as a gradient from small, shallow ditches to wide deep ditches.

**Table 2.1:** Description of the first two PCA axes and the gradients they represent used for determining landscape level metrics of environmental quality and variability given for the different species groups. See table S1 for a description of variables included in the different models.

Group	Axis	Gradient (negative to positive)
Cladocera	PC1	high to low nutrient availability
	PC2	turbid to clear waters; small to larger waters
<i>Hydrophytes</i>	PC1	low to high nutrient availability
	PC2	clear to turbid waters; larger to smaller waters
<i>Helophytes</i>	PC1	high to low nitrogen and carbon availability in sediments and soil
	PC2	low to high phosphorus availability in sediments and soil; shallow to deep waters
Bank	PC1	low to high nitrogen and carbon availability in sediments and soil; steep to shallow slopes
	PC2	low to high phosphorus availability in soils; clay to sand on peat

## Drivers of landscape diversity

Overall, the main drivers of landscape diversity ( $H'$ : Figure 2.3) and landscape richness (SR: Figure S2.2) were comparable, though variation in  $H'$  was generally better explained than that of SR (pseudo  $R^2$ -values of 45 and 23%, respectively). A number of drivers were found to impact  $H'$  of multiple organism groups in a very similar fashion (Figure 2.3). The proportional water surface was found to be positively associated with landscape diversity of helophytes and bank vegetation ( $p < 0.001$ , Figure S2.3, S2.4). Similar but marginally significant associations were also found between this variable and diversity of hydrophytes and cladocera ( $p < 0.1$ , Figure S2.5, S62.). Furthermore, the fraction of urbanized land had consistent negative effects on the landscape diversity of bank vegetation, helophytes and cladocera. Land use intensity was found to negatively impact landscape diversity of cladocera ( $p < 0.01$ ), helophytes ( $p < 0.001$ ) and bank vegetation ( $p < 0.01$ ). A number of drivers were found to be associated with landscape diversity of specific groups as well. Cladocera were found to be negatively associated by N loading specifically ( $p < 0.01$ ) and positively by P loading ( $P < 0.05$ ). On closer inspection of marginal relations (Figure S2.6), relations between landscape diversity and P loading alone appear to be limited. Hydrophyte diversity negatively associated to



**Figure 2.3:** Estimated standardized coefficients of variables selected from generalized linear models of cladocera, hydrophytes, helophytes and bank vegetation Shannon diversity ( $H'$ ) at the polder level explained by different drivers. Drivers have been grouped into four models, a spatial model, a local environmental model, a landscape characteristics model and a nutrient management model. Asterisks indicate significant coefficients (\*\*\*( $p<0.001$ ); \*\*( $p<0.01$ ); \*( $p<0.05$ )) with a coefficient above 1 being a positive and below 1 a negative correlation with the exponent of the shannondiversity. Note that PC1 and PC2 of the environmental model and the different MEM variables may represent different gradients (or scales thereof) for the different species groups.

P loading ( $P<0.01$ , Figure S2.5). Landscape diversity was also related to local environmental quality (Quality PC1, Figure S2.5), with a lower nutrient availability leading to more diversity. Landscape diversity of helophytes was associated negatively with the amount of hydrological obstructions in the landscape. Furthermore, helophyte diversity was positively associated with environmental variability (Variability PC2, Figure S2.4), showing a positive relationship with a more heterogeneous environment in terms of P availability in ditch depth. Bank vegetation was found to be positively associated with land use diversity ( $p<0.05$ , Figure S2.3). Landscape level diversity was also found to show signals of spatial autocorrelation for cladocera, hydrophyte and helophyte diversity (Figure 3). This spatial autocorrelations is indicative of the existence of spatial patterns in landscape diversity within the region.

## Drivers of compositional diversity among landscapes

Between-landscape compositional diversity ( $\beta_2$ ) of bank vegetation (Figure 2.4a) was significantly explained by nutrient management (NUT: 29.4%), environmental conditions (ENV: 13.3 %) and landscape characteristics (CHAR: 14.7%) whereas a strong spatial pattern (SPACE: 30.8%) was also suggested by the MEM model. The four variables related to nutrient loading (i.e. land use intensity, N and P loading and land use diversity) all explained  $\beta_2$  to variable degrees. With respect to local environmental conditions,  $\beta_2$  was found to be associated with within landscape variability in variables such as soil C, N and P content, ditch morphology and soil (Variability PC1 and PC2) and among-landscape variation in mean values of P availability and soil type (Quality PC2). Water surface area accounted for the entire effect of the landscape characteristics model on  $\beta_2$  (14.7%). Collinearity between some models was, however, high. A large proportion of the variation in  $\beta_2$  that was explained by nutrient loading was confounded with effects of landscape characteristics (10.5%) or spatially autocorrelated (12.1%). Effects of differences in local environmental conditions were associated with nutrient loading but also spatially structured (6.8%).

Helophyte compositional variation between landscapes was largely explained by the same drivers as bank vegetation (Figure 2.5b), especially by the NUT (33.8%) and SPACE (49%) models and to a somewhat lesser extent the ENV (28.1%) and CHAR (23.3%) models. Effects of the NUT model were mainly associated to P loading, land use intensity and N-loading. The ENV model represented effects of mean local environmental quality, especially in terms of water depth and the C, N and P content of sediments and soil (Quality PC1 and PC2) as well as variability in water depth and P content of sediments and soil (Variability PC2). The fraction of water surface (23.3%) was again the only variable representing landscape characteristics (CHAR). The spatial model accounted for almost half of the observed variation in  $\beta_2$  and remained

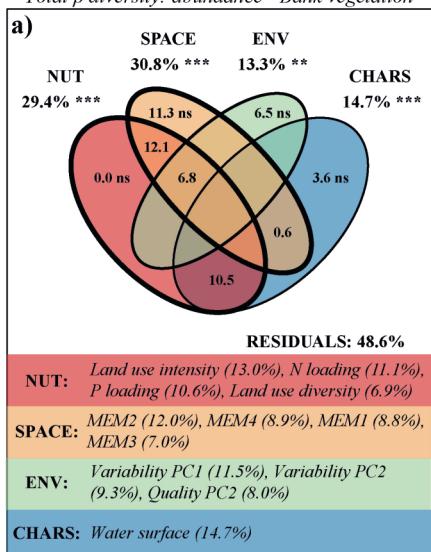
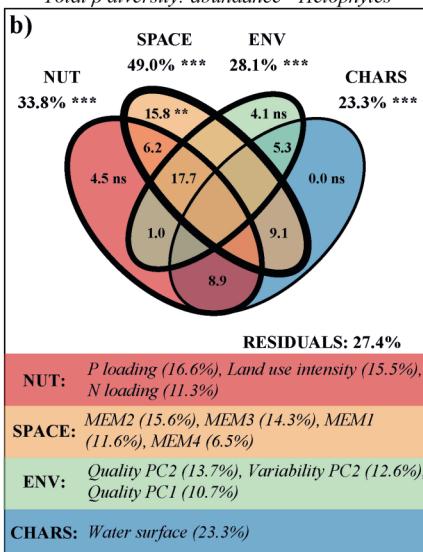
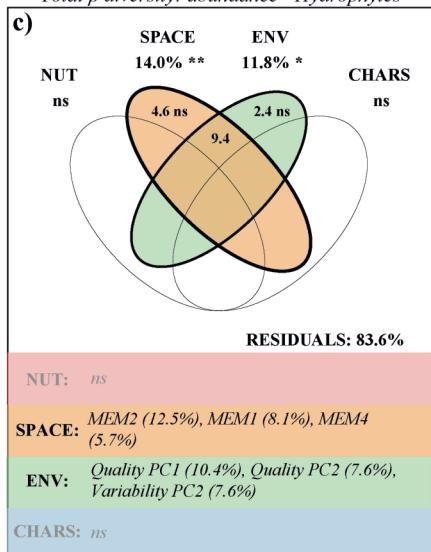
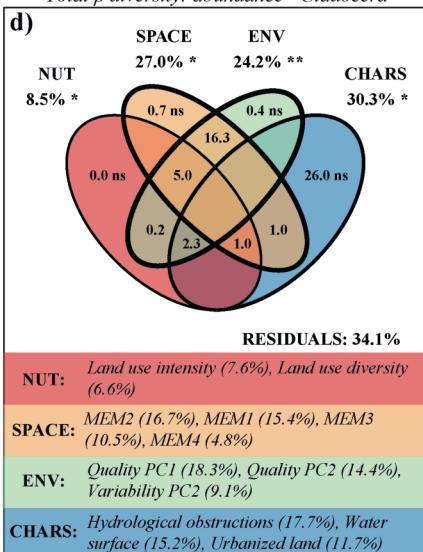
significant also when effects of the other models were accounted for (conditional effect: 15.8%). As with bank vegetation, collinearity between models was high. Effects of nutrient loading were confounded with those of landscape characteristics (8.9%), spatially structured environmental conditions (17.7%) or were spatially autocorrelated themselves (6.2%). Effects of landscape characteristics were also to a large extent spatially autocorrelated (9.1%).

Hydrophyte compositional differences among landscapes were found to be relatively poorly explained by our models (Figure 2.5c), with only SPACE (14%) and ENV (11.8%) being significant. We found that the differences in local environmental quality (Quality PC1: 10.4% and Quality PC2: 7.6%), especially those associated with nutrient availability (Quality PC1), water turbidity and depth (Quality PC2) were best associated with compositional variation of helophytes across landscapes. A large part of this explained variation was spatially structured (9.4%).

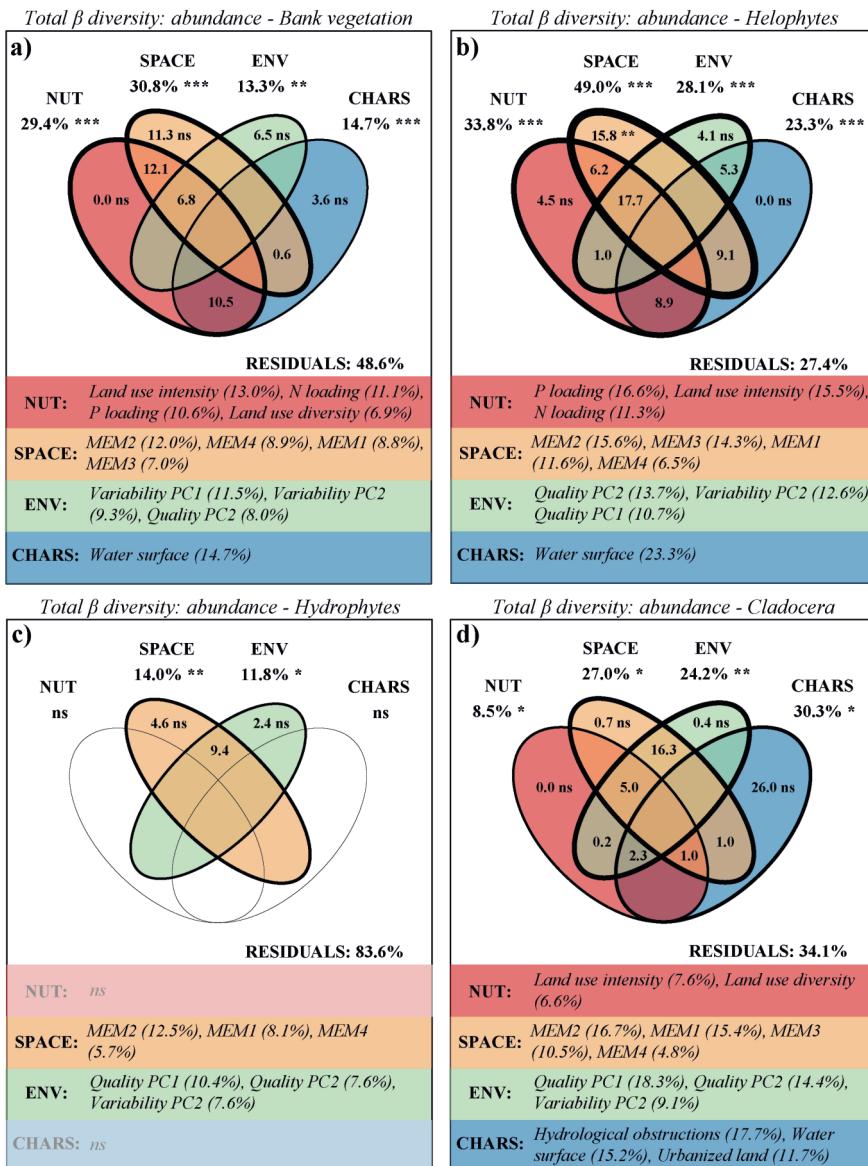
Between-landscape compositional diversity of cladocera (Figure 2.4d) was significantly associated with environmental conditions (ENV: 24.2 %) and landscape characteristics (CHAR: 30.3%) but also spatially structured (SPACE: 27%). In contrast, although significant, variables related to land use and nutrient loading (NUT: 8.5%) explained only a marginal part of  $\beta_2$ . The ENV model represented among-landscape variation in nutrient availability (Quality PC1), and among- as well as within-polder variation in water turbidity and water size (Quality PC2, Variability PC2). Environmental conditions were strongly spatially structured (21.3%). Of the variables in the CHAR model, differences among polders in the number of hydrological obstructions (17.7%), total water surface (15.2%) and also the fraction of urbanized land use (11.7%) explained cladocera  $\beta_2$ .

## Gradients of conservation relevance among landscapes

The replacement partition of species richness differences among landscapes showed consistent patterns for different organism groups (Figure 2.5). The total explained variation of the models explaining replacement partitions was drastically reduced compared to the models explaining variation in between-landscape compositional diversity (compare Figure 2.5 to Figure 2.4). Between-landscape compositional replacement of species ( $\beta_{2\text{ repl}}$ ) was significantly explained by nutrient management (NUT: 9.2%), local environmental quality (13.6%) and landscape characteristics (6.7%), with a clear spatial autocorrelation being present (SPACE: 16.2%). Nutrient loading of both P and N explained patterns in  $\beta_{2\text{ repl}}$ . The ENV model was primarily composed of variables associated with local ditch reach quality, namely the C, N (Quality PC1) and P supply (Quality PC2) in sediments and soils and variation in slopes of ditch banks (Quality PC1). Environmental variability in Variability PC2 (P supply, soil type) also

Total  $\beta$  diversity: abundance - Bank vegetationTotal  $\beta$  diversity: abundance - HelophytesTotal  $\beta$  diversity: abundance - HydrophytesTotal  $\beta$  diversity: abundance - Cladocera

**Figure 2.4:** Venn diagrams of a variation partitioning of the community compositional differences among polder landscapes (total  $\beta_2$  diversity) of (a) bank vegetation (b) helophytes, (c) hydrophytes and (d) cladocera. The partitioning is carried out using four dbRDA models (Ruzicka index): NUT (nutrient management), SPACE (spatial patterning), ENV (local environment) and CHAR (landscape characteristics). Asterisks indicate significant coefficients (\*\*p<0.001; \*\*p<0.01; \*p<0.05) with values representing adjusted R<sup>2</sup> values greater than 0 for the given partition. For more information on included variables per model see table S2.2. Below the Venn diagram the forward selected variables with their marginal adjusted R<sup>2</sup> of are presented.



**Figure 2.5:** Venn diagrams of a variation partitioning of the replacement component of the landscape level community differences ( $\beta_{2 \text{ rep}}$ ) of (a) bank vegetation (b) helophytes, (c) hydrophytes and (d) cladocera. The partitioning is carried out using four dbRDA models: NUT (nutrient management), SPACE (spatial patterning), ENV (local environment) and CHAR (landscape characteristics). Asterisks indicate significant coefficients (\*\*\*( $p < 0.001$ ); \*\*( $p < 0.01$ ); \*( $p < 0.05$ ) with values representing adjusted R<sup>2</sup> values greater than 0 for the given partition. For more information on included variables per model see table S2.2. Below the Venn diagram the forward selected variables with their marginal adjusted R<sup>2</sup> of are presented.

explained a small amount of the variation in  $\beta_2 \text{ repl}$ . Effects of the CHARS model were completely described by variation in the total length of ditch banks in the landscape.

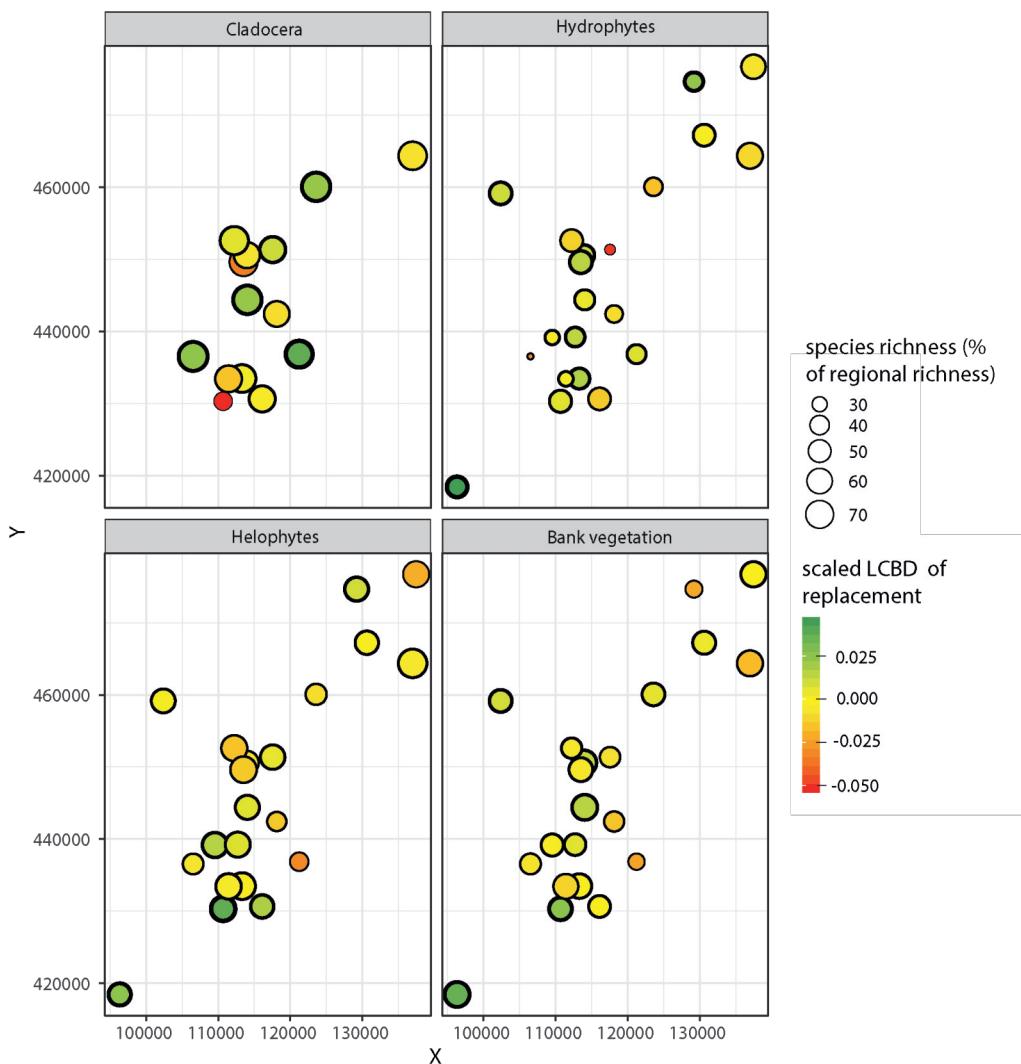
Replacement patterns of helophytes were described by variation in nutrient management (6.5%), local environment (6.7%) and again showed clear spatial patterning (SPACE: 8.8%). Gradients in P and N loading and land use diversity were driving patterns of helophyte replacement (NUT model). The ENV model was composed of variables describing local environmental quality, with gradients between landscapes in C, N (PC1) and P and water depth (PC2) explaining patterns in  $\beta_2 \text{ repl}$ .

Between landscape compositional replacement of hydrophytes was spatially structured (SPACE: 13.4%) but could not be explained by any other models. Cladocera  $\beta_2 \text{ repl}$  was explained by environmental quality alone (10.6%). The entire ENV model could be attributed to variation in water turbidity and ditch size between landscapes (PC2).

Examining the local contribution of different landscapes to  $\beta_2 \text{ repl}$  (LCBD: Figure 2.6, Figure S2.7), we found that the richness of landscapes and the relative contribution to the replacement component of  $\beta_2 \text{ repl}$  were unrelated. Landscapes with high richness but low LCBD values and vice versa exist for all organism groups. Moreover, landscapes with high LCBD values were not consistent between organism groups, with no clear correlations between LCBDs of organism groups being found with the exception of helophytes and hydrophytes ( $p < 0.05$ , Figure S2.8). The landscape richness of different organism groups, in comparison, did show clear correlations for nearly all combinations of groups (Figure S2.9). Richness of bank vegetation was significantly correlated to all other organism groups (Helophytes:  $p < 0.001$ , Hydrophytes:  $p < 0.05$ , Cladocera:  $p < 0.05$ ). Landscape richness of helophytes was also significantly correlated with hydrophyte ( $p < 0.05$ ) and cladocera landscape richness ( $p < 0.005$ ).

## Discussion

Regional biodiversity planning and reserve selection requires an understanding of drivers of landscape level diversity. It also requires a knowledge on existence and drivers of the complementarity between landscapes within a region. In our study we have shown that Dutch polder landscapes in a region of the Netherlands are distinct in terms of their environmental conditions as well as in their species composition and diversity of different organism groups. Increased agronomic and urban pressure was shown to negatively impact the landscape diversity of all organism groups. Organism groups showed to be strongly correlated to one another in terms of their landscape richness, suggesting that drivers of organisms are comparable and thus that conservation management that supports some groups is likely to support most others.



**Figure 2.6:** Spatial representation of the different landscapes and their relative species richness (expressed as the percentage of the total regional richness) and their complementarity (colour gradient) in terms of species composition. The complementarity is expressed using a local contribution to beta diversity (LCBD) metric of the replacement component of the landscape level community differences ( $\beta_{2\text{rep}}$ ) which was scaled in such a way that positive values indicate landscapes that are more complementary than expected by chance and negative values indicate landscapes that are complementary than randomly expected.

We found that diversity of the region was largely composed of a beta diversity component, the compositional dissimilarity between ditch reaches within a landscape and the dissimilarity between landscapes constituted a large part of the regional diversity. Complementarity between landscapes (replacement) was substantial,

indicating that landscapes harbor distinct species and diversity making a regional approach to conservation imperative. Examination of gradients underlying this replacement, in contrast to expectation, also identified nutrient management as a major driver. This implies that while landscape scale diversity may increase with decreasing nutrient loads and land use intensity (cf. Figure 2.3), the regional diversity may benefit from maintaining some heterogeneity in nutrient loads and land use as these drivers also increased complementarity among landscapes (cf. Figure 2.4 and 2.5). This complicates regional conservation, as only conserving the most diverse landscapes may thus have negative impacts on the regional diversity by diminishing complementarity.

## Landscape history as an explanation of collinearity

In our study we found a high collinearity between the different models explaining compositional dissimilarity among landscapes. Land use variation in itself can lead to a myriad of environmental changes in at multiple spatial scales, each with their own ecological impacts (for examples see: Carpenter *et al.* 1998; Guo & Gifford 2002; Pielke *et al.* 2007). The high collinearity between our different drivers of compositional diversity is not unexpected in this light. Within our study region local communities are influenced by land use directly as it will influence local environmental characteristics such as water and sediment nutrient content and the thickness of the organic sediment layer. Moreover, land use will also impact landscape scale properties such as the overall nutrient loading, ditch morphology and the amount of hydrological obstructions to facilitate more intensive water level management. The fact that most of these gradients appear to be spatially structured may be attributed to the history of the landscapes. Historically, these landscapes were peat bogs that were drained to make them suitable for human settlement and agriculture. Originally, ditches in these systems were able to drain the land by gravity alone. Depending on the thickness of the underlying peat and the rate of land subsidence at some point in history the landscapes had to be actively managed by means of windmills or pumps to control water levels (TeBraake 2008). These processes are now known to have massive impact on groundwater flows and ecology (de Mulder *et al.* 1994). Depending on the moment of settlement, different hydraulic solutions existed to deal with subsiding lands (i.e. sluices, dams, windmills, pumps). Depending on these solutions, ditch networks were designed differently, reflected by width of agricultural fields and the width of ditches (van Dam 2009). As landscape history will vary between the studied landscape, spatial gradients may be expected that show strong collinearity with other variables such as ditch width and water surface area. This study describes the major gradients that shape variation in diversity, composition and complementarity among landscapes. All the

while we should acknowledge that land use and geographic history have led to a complex set of confounded environmental and spatial gradients in which no singular gradient can be easily identified that controls diversity. Despite this complexity, we find compelling evidence for a number of relevant processes that shape landscape diversity and compositional differences within our study region.

## Land use drives landscape diversity and compositional dissimilarity

Intensity of agricultural land use has been directly linked to increased disturbance, fragmentation and eutrophication of ecological systems. Here we found patterns that suggest that increased agricultural intensity and associated nutrient loading and supply has clear negative impacts on landscape diversity of all organism groups. While it is hard to pinpoint specific processes underlying this general pattern of intensified agricultural, possible causes may be found in direct eutrophication of land and water systems (Leip *et al.* 2010), disturbances by cattle (Conroy *et al.* 2016) or use different mowing regimes (van Strien *et al.* 1989; Twisk *et al.* 2003). We found clear gradients of agricultural intensification explaining compositional variation among landscapes ( $\beta_2$ ) as well for bank vegetation, helophytes and cladocera. This is not purely a pattern of degradation (cf. nestedness: Soininen *et al.* 2018) as may be expected from our results that showed clear landscape diversity decline with increasing intensified land use. Agricultural land use related variables (i.e. land use intensity, nutrient loading, local quality axes related to nutrients in water and sediment) lead to (partial) complementarity between landscapes as indicated by their importance for landscape species replacement  $\beta_{2\text{ repl}}$ . Compositional patterns of hydrophytes were relatively ill explained by nutrient management. This may be attributed to large differences in local management practice in terms of mowing and dredging that can have massive impacts on hydrophyte community composition, both directly (Milsom *et al.* 2004; Teurlincx *et al.* 2018b) and by changing sediment and water nutrient dynamics (Dollinger *et al.* 2015). We also found hydrological obstructions to be relevant for decreasing helophyte landscape diversity and found obstructions to be associated with compositional variation among landscapes ( $\beta_2$ ) of cladocera. While hydrological obstructions can be dispersal barriers (Soomers *et al.* 2013), they may also impact nutrient dynamics (Kröger *et al.* 2013). Moreover, there is an explicit link between hydrological obstructions and water management for agriculture.

In addition to agricultural land use impacts on landscape diversity, we also found clear negative impacts of urban land use. Urban environments have a multitude of interacting pressures that may negatively impact aquatic ecosystems (Teurlincx *et al.* 2019). Urban areas may supply large nutrient pulses through sewage overflows

(Jeppesen *et al.* 1998) as well as through less obvious routes such as paved surface (Hobbie *et al.* 2017) and fertilized garden runoff (Toor *et al.* 2017). Moreover, major roads may be sources of a variety of micro pollutants (Hwang *et al.* 2016) such as heavy metals and micro and nanoplastics that could cause serious harm to aquatic ecosystems and filter feeders such as cladocera specifically (Cole *et al.* 2013). The pronounced impacts of these micro pollutants on filter feeders are a plausible explanation as to why compositional differences in cladocera among landscapes ( $\beta_2$ ) are partly driven by gradients in urbanization as well.

## Heterogeneity for cross scale diversity

Environmental heterogeneity has long been acknowledged as an important driver of the existence of high compositional dissimilarity at varying levels of spatial scale (Benton *et al.* 2003; Cadenasso *et al.* 2006; Alahuhta *et al.* 2017). Our study showed the importance of land use diversity in driving landscape diversity of bank vegetation. Helophyte landscape diversity was affected by local environmental variability in sediment phosphorus supply and water depth. Sediment phosphorus supply is a known driver of vegetation composition of ditches (van Zuidam & Peeters 2013) and water depth has long been acknowledged as a key factor driving terrestrialisation (succession) of ditch systems (Beltman *et al.* 1996). Furthermore, we found that local environmental variability could significantly explain part of the compositional differences between landscapes ( $\beta_2$ ) for all organism groups. As local diversity ( $\alpha$ ) and within landscape compositional differences ( $\beta_1$ ) represent a significant proportion of the total regional diversity (Figure 2), the regional diversity is clearly dependent upon sound environmental quality but also heterogeneity within the landscapes. The importance of local environmental quality and heterogeneity has important implications for management, as maintaining this local scale environmental heterogeneity may require continual effort (see Teurlincx *et al.*, 2018b). Moreover, as landscapes were also found to be complementary to one another, heterogeneity at the regional level will also need to be maintained to some extent. To achieve conservation within and among landscapes a multi-scale approach to biodiversity conservation is therefore required (Hodbod *et al.* 2016).

## Conservation focus: diverse landscapes or complimentary landscapes?

A multi-scale approach to biodiversity conservation that accounts for both diverse landscapes as well as complementary landscapes may fraud with pitfalls. Multiple actors (e.g. water managers, farmers, legislators) all act at their own spatial scale, and the spatial scale at which species themselves interact may be markedly different from

the scales of the actors (Sayles & Baggio 2017). The main focus of conservation within the region should be to maximize regional diversity, which at a minimum requires maintaining conditions that correlate with high landscape diversity in the region. Landscape diversity showed clear patterns which can be easily understood (i.e. low intensity land use leads to high diversity). Moreover, landscape diversity of different organism groups was strongly correlated (Figure S2.9), in contrast to the complementarity of different groups (Figure S2.8). This relation between landscape diversity of different groups may be caused by similar underlying drivers, or by direct facilitation of different biotic groups (Declerck *et al.* 2005). It does suggest that single organism groups can serve as proxies for landscape diversity of other groups, making monitoring easier and more cost effective. Whilst complementarity between landscapes is obviously important based on our results, this becomes especially relevant when the landscapes exhibit different species of interest for conservation (i.e. rare/unique species). It should be noted that our studied landscapes were chosen to maximize the land use diversity within the area. This is important as it also means that they do not represent the relative representation of landscapes within the region. The region is predominantly agricultural in nature, with intensive agriculture being the norm rather than the exception. Our results showed that even these more intensively managed landscapes contributed to the regional species pool. However, when conserving diversity within the region, intensive agriculture will generally persist. In fact, scenario analyses of land sparing practices for biodiversity conservation generally predict an increase in intensity of the surrounding landscapes (Reidsma *et al.* 2006). Hence, conserving rich landscapes with low intensive land use pressures in terms of agricultural nutrient loading as well as urbanization is a key step. Where resources allow, the conservation of rich and highly complementary landscapes should be considered to maintain the maximal landscape diversity.

## Restoration of degraded landscapes

Restoring degraded landscapes may be pivotal to increase the cross-scale response diversity (i.e. the number of species capable of delivering ecosystem functions), thereby increasing resilience of the overall region. Restoring landscapes does not increase the total diversity of a region per se, unless novel ecosystems for the region are created (Hobbs *et al.* 2014). It does however create additional populations of currently rare species to serve as source populations when their current landscapes are compromised. Furthermore, while our study focusses strongly on land use as a pressure that degrades extant biodiversity, other pressures such as climate change may also negatively impact the ecological quality and associated diversity of ditch ecosystems (Netten *et al.* 2011; Veraart *et al.* 2011). To assure the maintenance of current levels of

regional diversity in a changing future, we must bolster its resilience to both long term (i.e. climate change) and short term (i.e. sewage overflow) perturbations. To do so, restoration of degraded landscapes will be of vital importance. The results of our study can support the selection of landscapes for restoration. Depending on the exact goal of restoration, either the most species poor and least complementary landscapes that offer the most room for improving landscape diversity or the slightly degraded but low complementary landscapes will be selected. Restoring low quality, diversity poor systems offers the advantage of restoring key ecosystem functions in these systems such as nutrient retention (Vermaat & Hellmann 2010) that may improve water quality at the regional scale. On the other hand, restoration of slightly degraded systems may offer increased habitat for rare species, offering increased spatial insurance of the regional biodiversity against stochastic extinction events (Loreau *et al.* 2003). Regardless of the chosen landscapes, to effectively restore a landscape's diversity we require knowledge of processes underlying gradients that promote landscape diversity (i.e. less intensive land use decreases overland nutrient flows, decreases disturbance, increases dispersal pathways). Our study serves to define drivers that lead to rich landscapes, but an in-depth landscape specific system diagnosis including quantification of water and nutrient flows, management activities and ecological processes will be vital for successful restoration to take place (for an example see: Waagen *et al.*, 2016).

In conclusion, we have shown that a regional approach to biodiversity conservation is of vital importance, even in heavily managed agricultural landscapes (Gonthier *et al.* 2014). The marginal zones within our landscape (ditches and ditch banks) showed high potential for diversity, especially when considering the heterogeneity both within and between landscapes. Intensive agricultural and urban land use has clear negative impacts on landscape diversity (Petsch 2016), implying that land sparing practices (Koning *et al.* 2017) and reserve designation (Venter *et al.* 2014) are worthwhile approaches to maintaining regional diversity. Complementarity was found to be relevant, stressing the importance of intensive agricultural lands in maintaining its own distinct set of species. However, increasing protection of less-intensively managed landscapes is unlikely to cause a region wide disappearance of intensively managed agricultural lands and the species it holds. While conserving the extant diversity is necessary, in the light of global change increasing the resilience of currently degraded landscapes will be vital to maintain the current levels of regional diversity (Prober *et al.* 2015a). Restoring less diverse and complimentary landscapes by mitigating the negative impacts of land use can help to achieve a more resilient landscape with a high cross-scale diversity (Hodbod *et al.* 2016). Our approach offers an insight into how an understanding of the different components of biodiversity and its drivers can aid in

management and policy of biodiversity across spatial scales.

## Acknowledgements

The authors would like to thank Edwin van den Berg, Dennis Waasdorp, Erik Reichman, Marta Alirangues, Maik Janssen, Lilith Kramer, John O'Connor, Thomas Clay, Roos Plak, Lisa Freitag, Jeroen Jongerius, Marlies Gräwe and Annegreet Veeken for help in the field and Leo Renaud for help with the STONE model data. This work was supported by the Division for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO), more specifically by the Biodiversity Works Research Program (OBW) project no. 841.11.009.

## Chapter 2: Supplementary materials

**Table S2.1:** The locally measured variables and their units as included in the PCA analyses to determine landscape level measures for local environmental quality and variability. The used sets of variables differs for the different species groups (cladocera zooplankton, hydrophytes, helophytes and bank vegetation), with included variables indicated with an x.

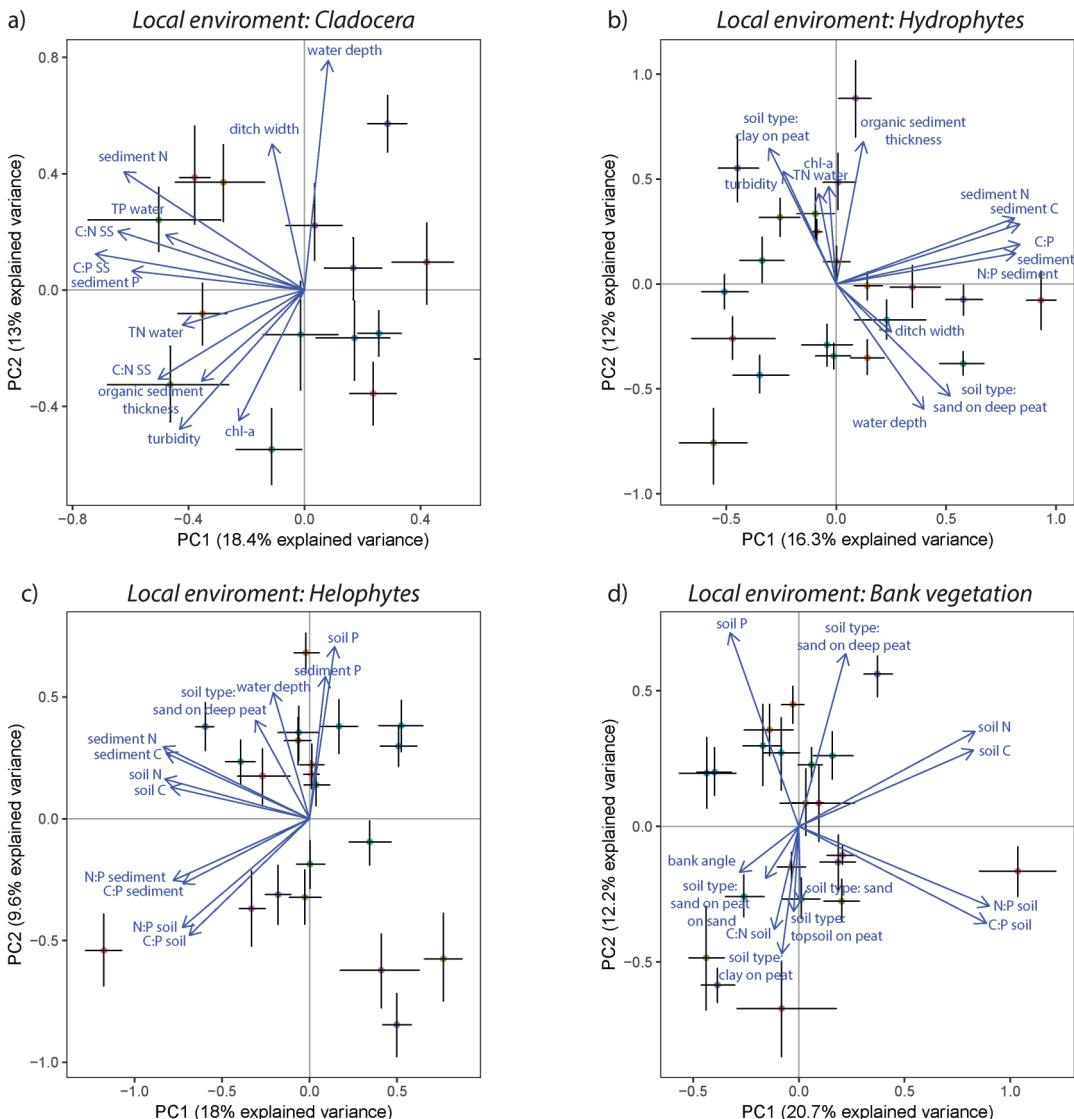
Variable	Unit	Cladocera	Hydrophyte	Helophyte	Bank
soil type	categorical		x	x	
water depth	m	x	x	x	
organic layer thickness	m	x	x	x	
ditch width	m	x	x	x	
bank width	m			x	x
bank angle	degrees			x	x
soil total carbon	mgC/gDW			x	x
soil total nitrogen	mgN/gDW			x	x
soil total phosphorus	mgP/gDW			x	x
soil C:N	molC/molN			x	x
soil C:P	molC/molP			x	x
soil N:P	molN/molP			x	x
sediment total carbon	mgC/gDW		x	x	
sediment total nitrogen	mgN/gDW	x	x	x	
sediment total phosphorus	mgP/gDW	x	x	x	
sediment C:N	molC/molN		x	x	
sediment C:P	molC/molP		x	x	
sediment N:P	molN/molP		x	x	
water total nitrogen	mgN/L	x	x	x	
water total phosphorus	mgP/L	x	x	x	
suspended solids C:N	molC/molN	x			
suspended solids C:P	molC/molP	x			
suspended solids N:P	molN/molP	x			
fetch	m	x	x	x	
turbidity	NTU	x	x	x	
chlorophyll-a	µg/L	x	x	x	
fish abundance	CPUE	x			

**Table S2.2:** The different models with their included variables and a short description of the variable.

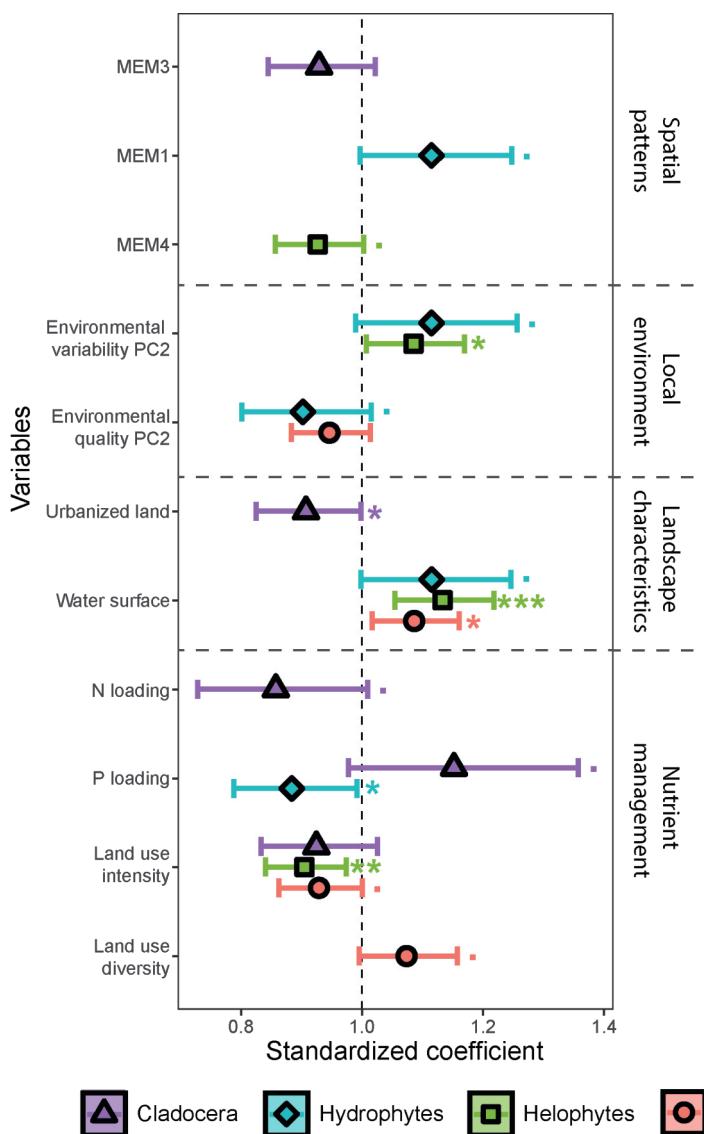
Model	Variable	Short description
<b>Spatial patterns</b>	MEM1	Spatial autocorrelation variable 1
	MEM2	Spatial autocorrelation variable 2
	MEM3	Spatial autocorrelation variable 3
	MEM4	Spatial autocorrelation variable 4
<b>Local environment</b>	Environmental quality PC1	centroid per landscape along PC1
	Environmental quality PC2	centroid per landscape along PC2
	Environmental variability PC1	variance per landscape along PC1
	Environmental variability PC2	variance per landscape along PC2
<b>Landscape characteristics</b>	Ditch bank length	m / ha of landscape
	Hydrological obstructions	no. / km of ditch
	Water surface	% of total landscape
	Urbanized land	% of terrestrial landscape
<b>Nutrient management</b>	N loading	kg/ha/y
	P loading	kg/ha/y
	Land use intensity	weighted average of land use categories
	Land use diversity	Shannon exponent of land use categories

**Table S2.3:** Results of RDA analyses on landscape identity explaining the variation in locally measured environmental variables.

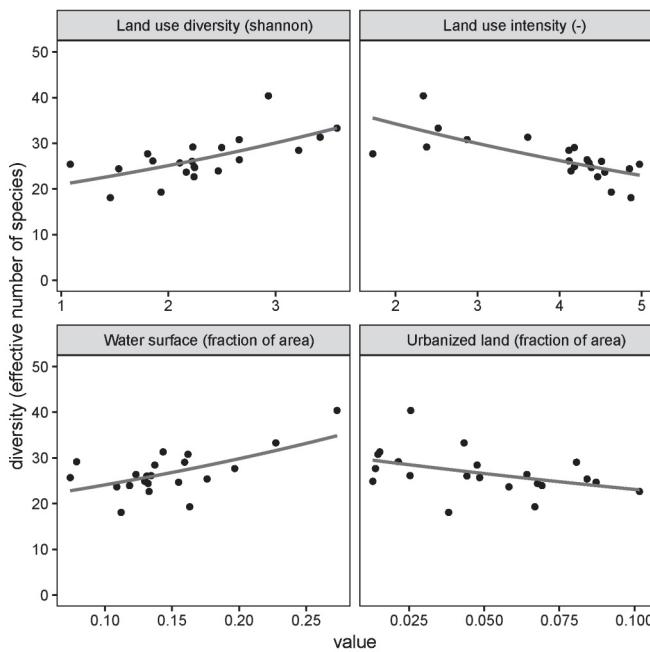
Species group	df	F	p	R <sup>2</sup> <sub>adj</sub> (%)
<i>Bank vegetation</i>	21	13.97	0.0001	36.9%
<i>Helophytes</i>	21	14.93	0.0001	38.5%
<i>Hydrophytes</i>	21	13.89	0.0001	36.8%
<i>Cladocera</i>	14	11.19	0.0001	31.5%



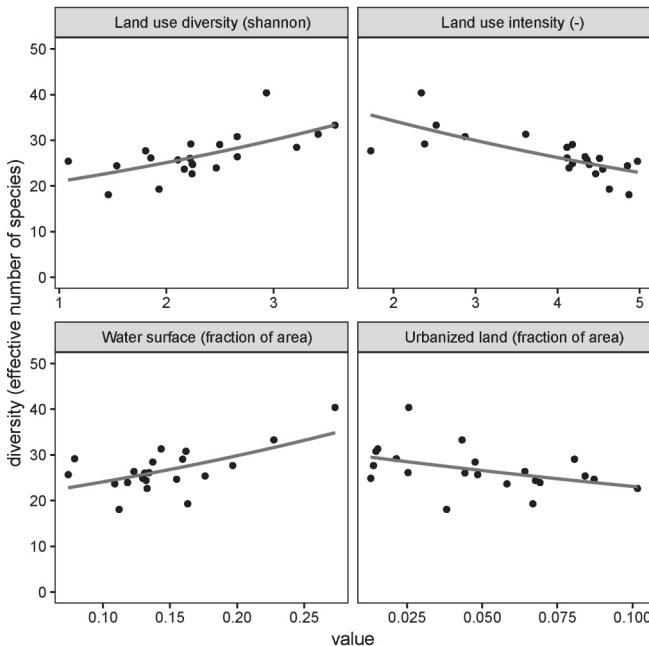
**Figure S2.1:** Biplotsof principal components analysis performed on environmental variables measured at the level of reaches. Plots present different sets of variables relevant for specific organism groups: a) cladocera zooplankton, b) hydrophytes, c) helophytes, d) bank vegetation. Centroids represent mean values of PCA-axis scores for polders whereas error bars represent twice the standard error of reaches within these polders.



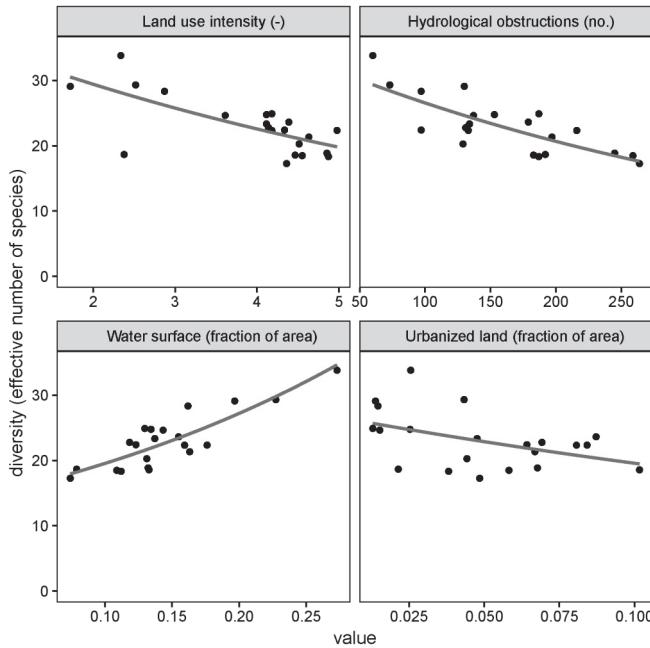
**Figure S2.2:** Estimated standardized coefficients of variables selected from generalized linear models of cladocera, hydrophytes, helophytes and bank vegetation species richness (SR) at the polder level explained by drivers. Drivers have been grouped into four models, a spatial model, a local environmental model, a landscape characteristics model and a nutrient management model. Asterisks indicate significant coefficients (\*\*\*( $p < 0.001$ ); \*\*( $p < 0.01$ ); \*( $p < 0.05$ ) with a coefficient above 1 being a positive and below 1 a negative correlation with the species richness.



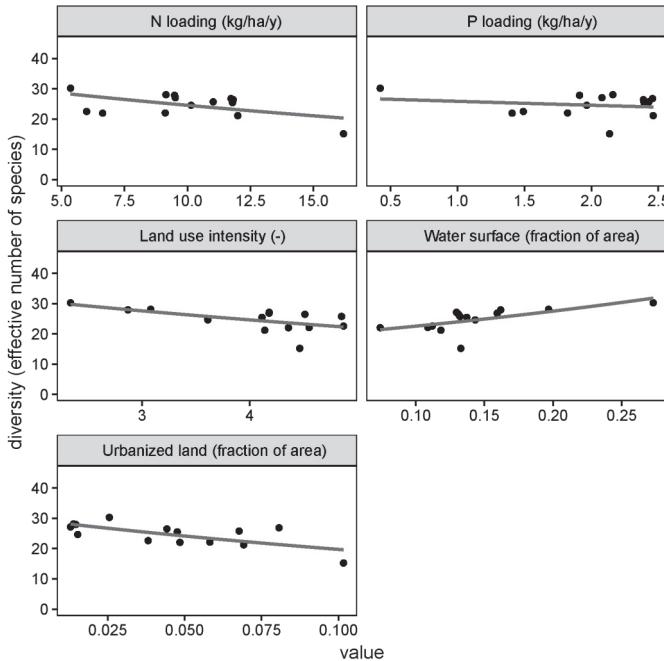
**Figure S2.3:** Relations between the different selected drivers and the Shannon diversity of bank vegetation at the landscape level.



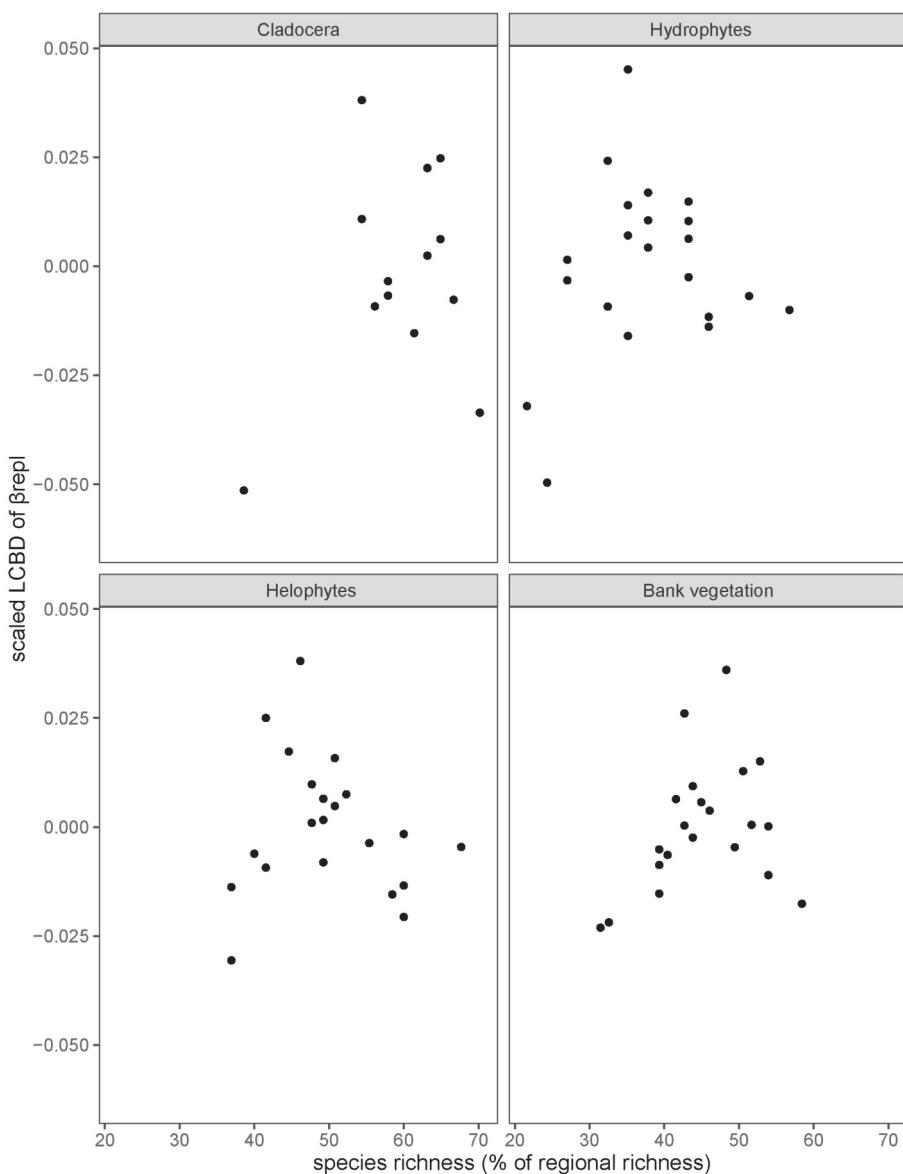
**Figure S2.4:** Relations between the different selected drivers and the Shannon diversity of helophyte vegetation at the landscape level.



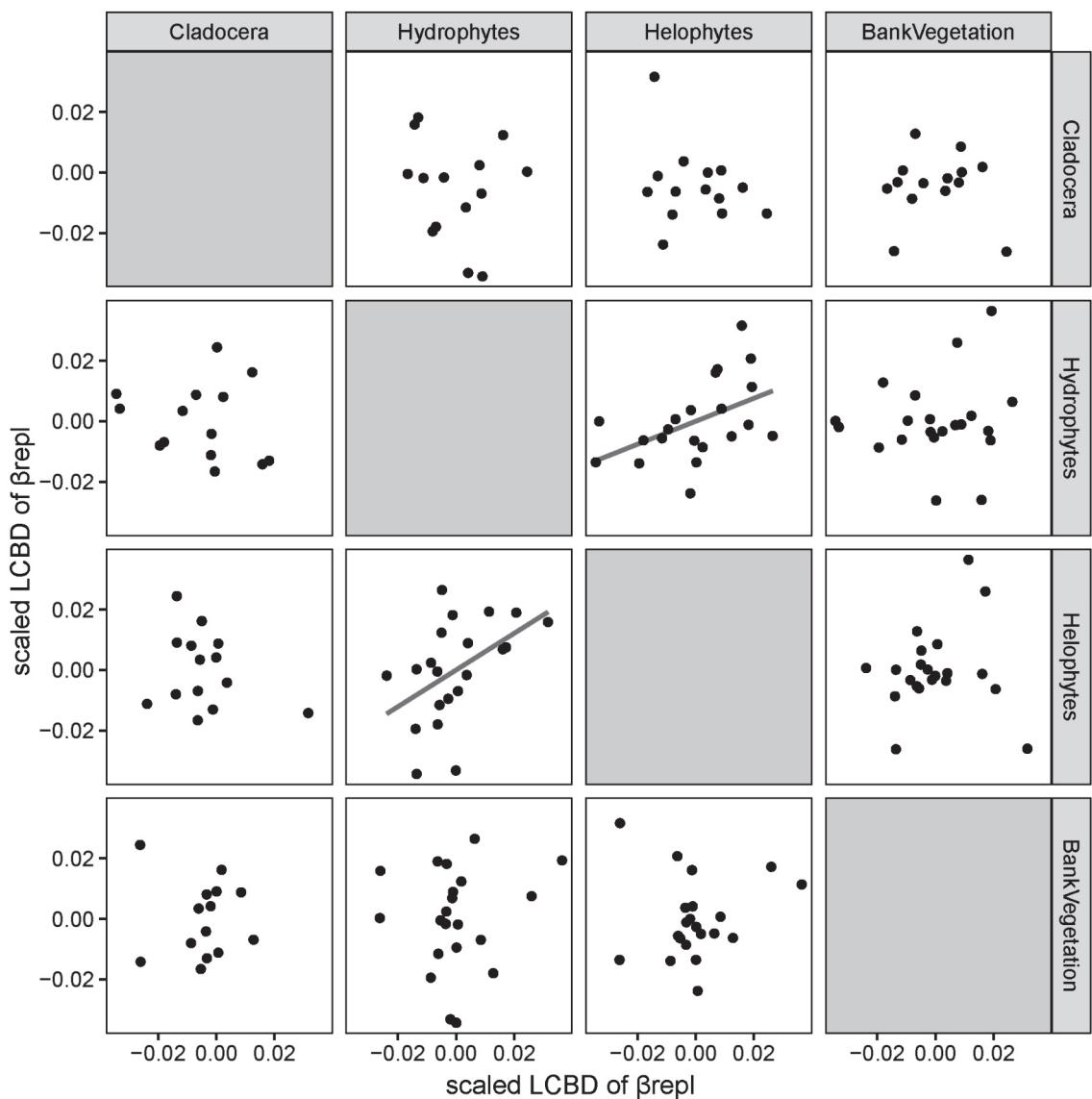
**Figure S2.5:** Relations between the different selected drivers and the Shannon diversity of hydrophyte vegetation at the landscape level.



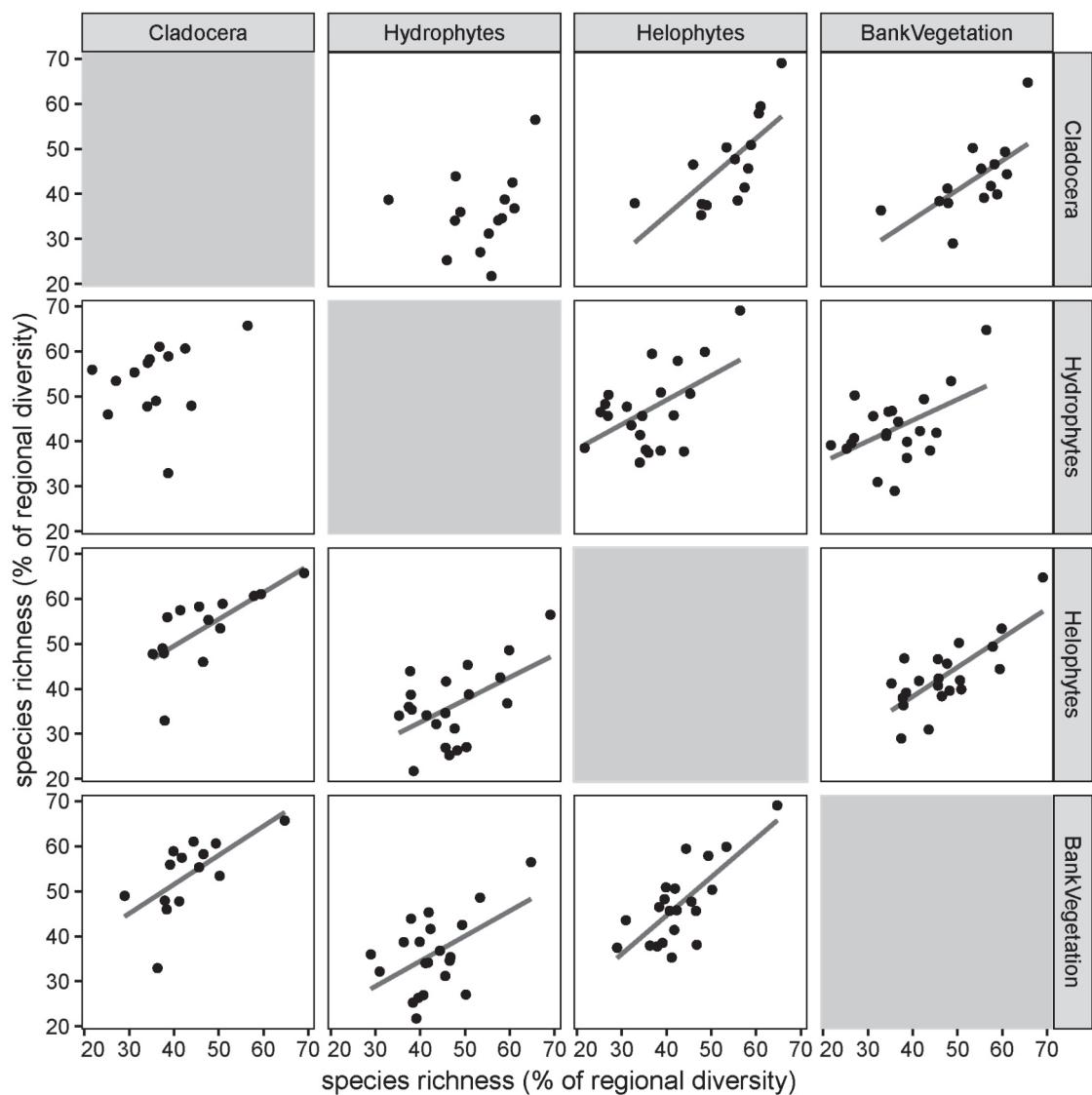
**Figure S2.6:** Relations between the different selected drivers and the Shannon diversity of cladoceran zooplankton at the landscape level.



**Figure S2.7:** Plots of the species richness (% of total regional richness) plotted against the scaled local contribution to beta diversity of the replacement component of  $\beta_2$ , a measure of complementarity between landscapes. No significant correlations were found between richness and LCBD.



**Figure S2.8:** Correlations between the landscape level complementarity, given as the LCBD of the  $\beta_{\text{repl}}$  of different species groups. Blue lines indicate significant correlations ( $p < 0.05$ ) between different species.



**Figure S2.9:** Correlations between the landscape level species richness (expressed as the percentage of the total region) different species groups. Blue lines indicate significant correlations ( $p < 0.05$ ) between different species.



# 3

## **Local functioning, landscape structuring: drivers of soil microbial community structure and function in peatlands**

**Sven Teurlincx\***, **Amber Heijboer\***, **Annelies J. Veraart**, **George A. Kowalchuk**,  
**Steven A.J. Declerck**

*\*Authors contributed equally to this work*

*This chapter is based on the paper: Teurlincx, S., Heijboer, A., Veraart, A. J., Kowalchuk, G. A., & Declerck, S. A. (2018). Local functioning, landscape structuring: drivers of soil microbial community structure and function in peatlands. *Frontiers in microbiology*, 9:2060.*

## Abstract

Agricultural peatlands are essential for a myriad of ecosystem functions and play an important role in the global carbon (C) cycle through C sequestration. Management of these agricultural peatlands takes place at different spatial scales, ranging from local to landscape management, and drivers of soil microbial community structure and function may be scale-dependent. Effective management for an optimal biogeochemical functioning thus requires knowledge of the drivers on soil microbial community structure and functioning, as well as the spatial scales upon which they are influenced. During two field campaigns, we examined the importance of different drivers (i.e. soil characteristics, nutrient management, vegetation composition) at two spatial scales (local vs landscape) for, respectively, the soil microbial community structure (determined by PLFA) and soil microbial community functional capacity (as assessed by CLPP) in agricultural peatlands. First, we show by an analysis of PLFA profiles that the total microbial biomass changes with soil moisture and relative C:P nutrient availability. Secondly, we showed that soil communities are controlled by a distinct set of drivers at the local, as opposed to landscape, scale. Community structure was found to be markedly different between areas, in contrast to community function which showed high variability within areas. We further found that microbial structure appears to be controlled more at a landscape scale by nutrient-related variables, whereas microbial functional capacity is driven locally through plant community feedbacks. Optimal management strategies within such peatlands should therefore consider the scale-dependent action of soil microbial community drivers, for example by first optimizing microbial structure at the landscape scale by targeted areal management, and then optimizing soil microbial function by local vegetation management.

# Introduction

Peatlands play an important role in Earth's biogeochemical cycles by storing about an estimated third of all terrestrial carbon (C) (Turetsky *et al.* 2002; Turunen *et al.* 2002). In Europe, the majority of peatlands is in use as agricultural land (Joosten & Clarke 2002). Despite their potential to sequester C, agricultural peatlands typically act as significant C sources. Worldwide drainage of such peatlands has increased the rates of peat oxidation and hence microbial decomposition, causing high rates of C losses and greenhouse gas emissions (Drösler *et al.* 2008). However, due to the large C sequestration potential of agricultural peatlands, they could play an important role in efforts to increase soil C storage, such as the recently launched '4 per 1000 initiative', which seeks to increase C storage in agricultural soils with 4‰ per year (Le Foll 2015).

Current peatland management influences microbe-mediated biogeochemical functions, for example by maintaining waterlogged conditions to prevent microbial peat oxidation and thereby reduce peat subsidence and CO<sub>2</sub> emissions (Kløve *et al.*, 2017). Restoration of peat ditches often seeks to optimize nutrient removal and reduce eutrophication, both of which have links to the microbial processes of nitrogen (N) and phosphorus (P) conversion. Microbial activities are clearly critical to the success of peatland management strategies, for instance for C storage, yet management practises rarely consider potential impacts on soil-borne microbial communities. With future climate change pressures in mind, the management of ecosystems for minimal microbial mediated CH<sub>4</sub> and N<sub>2</sub>O emission is expected to become ever more important (Taft *et al.* 2017).

Traditionally regarded as random noise, spatial variability in soil microbial communities is now widely acknowledged (Ettema & Wardle 2002), and it displays consistent and informative patterns at different spatial scales (O'Brien *et al.* 2016). With mounting evidence for scale-dependent ecological processes acting on microbial communities, the need for examining multiple spatial scales to understand the patterns in soil microbial communities has become apparent (Martiny *et al.* 2011). This has led to the study and discovery of clear examples of small scale (cm to m) patterns (e.g. Franklin and Mills 2003), as well as large continental (Waldrop *et al.* 2017) and global biogeographic patterns in soil microbial communities (Nemergut *et al.* 2011). These scales of study are not always in line with the scales at which the management of such ecosystems takes place. For agricultural peatlands, the most obvious scale of management is that of the field level, with local farmers carrying out customary management practices such as fertilization, grazing and mowing. Another relevant scale is that of the landscape level at which spatial planning and water level management occurs. Furthermore, the different components of the ecosystem may

also be organized at different spatial scales themselves (Yergeau *et al.* 2010). To adequately steer management towards optimization of microbial communities, there is a need to match the spatial scale of land management and the study of spatial microbial community patterns.

While identification of patterns in soil microbial composition is in itself relevant, there is a clear need to go beyond pattern description and towards identification of the underlying drivers of community structure and functioning (Martiny *et al.* 2011; Hanson *et al.* 2012). Soil microbial community structure and functioning are often assumed to be driven by the same factors (O'Brien *et al.* 2016). This would imply that management aimed at an optimal microbial structure will also result in the desired functioning of the ecosystem. Alternatively, soil microbes are often considered to have high functional redundancy (Strickland *et al.* 2009); and therefore drivers of soil microbial community structure may have minimal effect on soil microbial functioning. This perspective would imply that management practices designed to control only the drivers of community function would be sufficient to achieve the desired ecosystem functions.

Soil microbial community structure has been shown to be controlled by a wide spectrum of drivers: soil pH and C:N ratio (Lauber *et al.* 2008; Fierer *et al.* 2009, 2012; Kuramae *et al.* 2012; Zhang *et al.* 2013; Ramirez *et al.* 2014), vegetation (O'Donnell *et al.* 2001; de Vries *et al.* 2012), external nutrient load (O'Donnell *et al.* 2001; O'Brien *et al.* 2016), and soil moisture (Brockett *et al.* 2012). Although drivers of soil microbial community function have not been examined in as much detail, but it has been shown that soil microbial community functions are also controlled by soil moisture (Brockett *et al.* 2012), C:N ratio (Kuramae *et al.* 2014), and external nutrient load and pH (Wakelin *et al.* 2013). These different drivers of community structure and functioning differ with the spatial scale of examination. As land management is carried out at scales orders of magnitude larger than those experienced by microbes directly, it remains to be tested if and how microbial communities respond to changes in these drivers. If soil microbial community structure and functioning are influenced by scale-dependent drivers, information on the scale-dependency of these microbial community drivers could be useful for informing management designed to improve peatland functioning.

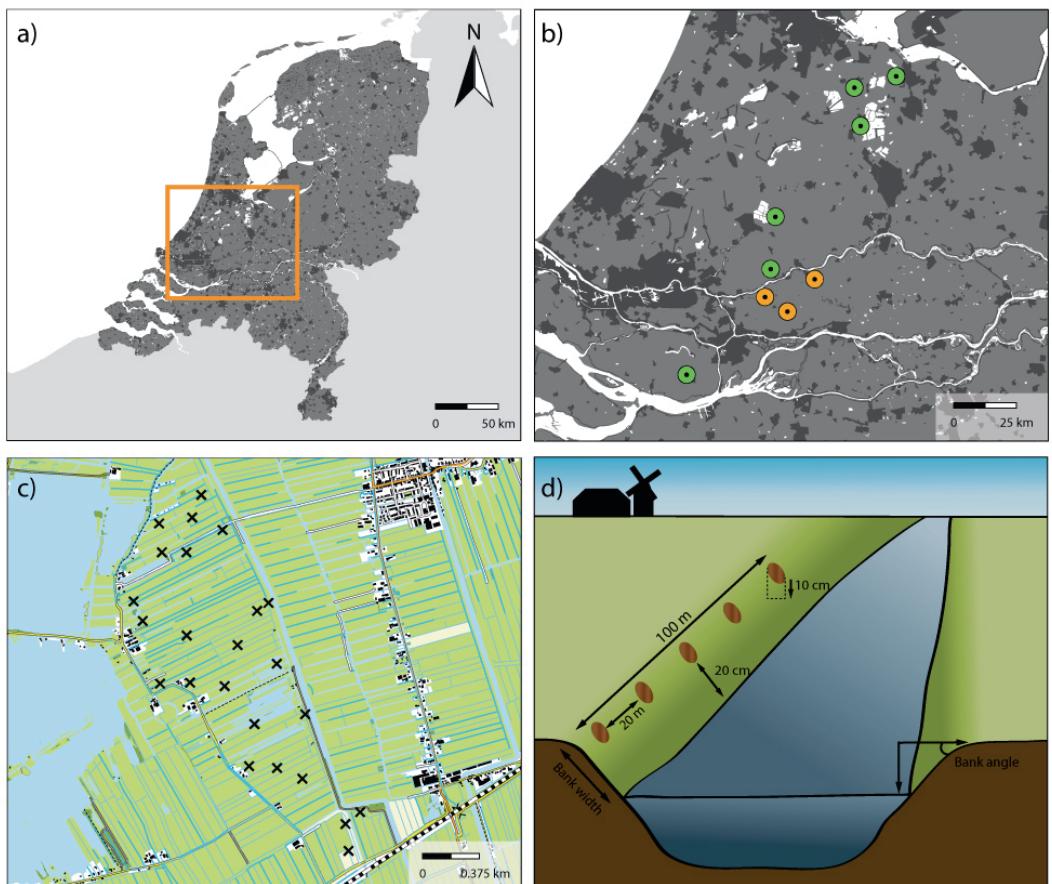
In this study, we assessed the impacts of several drivers of soil microbial structuring and functional capacity at local and landscape scales in agricultural peatlands. We combine data from two sampling campaigns across agricultural peatlands in The Netherlands (Figure 3.1a, 3.1b), one that examined drivers of soil microbial community structure, as determined by phospholipid fatty acid (PLFA) analysis, and one that examined drivers of soil microbial community functional capacity, as estimated by community level physiological profiling (CLPP). These patchwork agricultural landscapes are highly heterogeneous, making them effective model systems for

examining the effects of multiple environmental drivers on soil microbial communities (Vasseur *et al.* 2013). Comparison of samples within and between different polder areas made it possible to analyse drivers at two different spatial scales: local scale (sites within a sampled area) and landscape scale (differences between sampled polder areas).

## Methods

### Study sites and design

The field sites used in this study are situated in a peat area in the West of The Netherlands (Figure 3.1a, Table 3.1) and comprise nine  $\pm 200$  ha peatlands (Referred to as 'sampling areas', Figure 3.1b, each comprising between 18 and 24 sampling sites). All areas are characterized by a mixture of intensive and extensively managed peatlands intersected by ditches, resulting in a mosaic of land uses. In the summer of 2013, a total of three agricultural peatland areas were sampled, and another six areas were sampled in the subsequent summer of 2014. In each area, between 18 and 24 transects were laid out on field margins (referred to as 'sampling sites', Figure 3.1c), as such edges account for 96% of the total vegetation species richness of a field (Kleijn *et al.* 2001). Each transect had a total length of 100 meter, where the vegetation was surveyed for the sloping part of each transect up to the waterside (Figure 3.1d). Vegetation abundance was assessed using Tansley abundance classes (Tansley 1946), which were subsequently converted into abundance percentages (Table S3.1). To analyse soil physical-chemical properties and microbial community structure and functional capacity, five soil samples (10 cm deep) were taken in each transect, 20 meters apart from each other and 20 cm from the waterside (Figure 3.1d). Soil samples were mixed per transect after removal of the vegetation layer, sieved (2 mm mesh) and stored at -80°C as one composite sample.



**Figure 3.1:** Overview of sample areas and sample sites. a) The Netherlands with the studied region indicated in an orange rectangle. b) Map with the areas sampled in 2013 for PLFA analyses (orange) and in 2014 for CLPP analyses (green). c) Detailed map of one of the study areas indicating the location of the 24 sampling sites for this specific area. d) Schematic representation of how samples were collected along the waterside of ditches.

**Table 3.1:** Characteristics of the different study areas (ID) used for PLFA or CLPP. Land management of peatlands is given as the percentage of the management style to the total peatland. Estimated N and P application through fertilizers (organic and inorganic) and the total water area, peaty soil and clay soil percentages are also shown for each area.

	ID	Lon (°)	Lat (°)	Int. Agr. (%)	AES (%)	Nat. (%)	Org. N fertilizer (kg/ha/y)	Org. P fertilizer (kg/ha/y)	Inorg. N fertilizer (kg/ha/y)	Inorg. P fertilizer (kg/ha/y)	Water Area (%)	Peat (%)	Clay (%)
PLFA	H	4.75494	51.88792	50	24	26	167.2	62.7	36.9	11.7	13	95	5
	I	4.82294	51.86355	63	35	2	151.1	56.7	79.7	23.3	10	100	0
	O	4.89699	51.91930	90	9	1	169.3	66.5	86.2	26.3	10	93	7
CLPP	Q	4.53776	51.75151	5	9	86	160.9	63.9	0.0	0.0	7	61	39
	R	5.00921	52.25942	58	36	6	169.0	67.7	75.7	21.8	14	80	20
	S	5.03050	52.19264	44	0	56	134.7	68.7	19.8	6.5	12	83	17
	T	5.13003	52.27827	28	0	72	136.5	68.4	14.7	4.6	19	94	4
	U	4.77225	51.93984	65	9	26	167.5	62.8	36.0	11.6	13	94	6
	Z	4.78264	52.03339	3	8	90	92.0	48.6	0.0	0.0	16	100	0

## Phospholipid fatty acid (PLFA) analyses

Soil microbial community structure was determined by analysing Phospholipid Fatty Acids (PLFA) extracted from soil samples taken in the areas that were sampled in summer 2013 (three areas, 63 samples). PLFAs were extracted from 4 grams of soil per composite sample using an adapted protocol, following White *et al.* (1979) and Frostegård and Bååth (1996). Lipid fractionation took place over prepacked Bond Elut SI solid phase extraction columns, after which lipid extracts were identified by gas chromatography (GC-FID, 7890A, Agilent technologies, Delaware, USA). The (relative) abundance of fungi, Gram positive ( $G^+$ ) and Gram negative ( $G^-$ ) bacteria was characterized by the use of specific indicator PLFA biomarkers: fungi (18:2 $\omega$ 6),  $G^+$  bacteria (i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0) and  $G^-$  bacteria (16:1 $\omega$ 7, cy17:0 and 18:1 $\omega$ 7). Total bacterial biomass was determined by taking the sum of all bacterial biomarkers, including the general biomarker 15:0. Abundance of each PLFA biomarker was expressed as nmol PLFA g<sup>-1</sup> dry weight of soil.

## Community level physiological profiling (CLPP)

Functional diversity of the soil microbial community was determined in soil samples originating from the areas that were sampled in summer 2014 (six areas, 144 samples) by the use of Biolog EcoPlates (Biolog, Hayward, CA, USA). These 96-well plates contain three replicate sets of 31 ecologically relevant C substrates, along with a tetrazolium

redox dye (Insam, 1997). Microbial use of these substrates is reflected by colour change in each of the wells, as the redox dye is reduced to tetrazolium violet (Pohland & Owen, 2009). Eco-plate wells were inoculated with diluted soil slurries (150 µl), obtained by mixing 0.5 gram of soil with 49.5 mL of milli-Q water, shaken (200 rpm) for 30 minutes on an orbital shaker, and 10<sup>-4</sup> diluted by serial dilution. Three technical replicates were included for each of the 144 soil samples. Eco-plates were incubated in the dark at 25°C, and colour development was recorded as optical density (OD<sub>590</sub>, OD<sub>750</sub>) at the start and after 24, 48, 72, 96, 168 and 192 hours, on a Biotek Synergy HT plate reader (Biotek Instruments, Winooski, United States).

Conceptually, the function of Ecoplate-substrate utilisation through time consists of four distinct phases (Figure S3.1a). The substrate utilization function captures the signals of community respiration, but also that of the substrate consumed for community growth. For our purposes, we were interested only in the respiration of the originally sampled community. To remove the signal of reproduction from the data, we used a modified method of Brouns *et al.* (2016). The rationale behind this method is that by removing the exponential-growth signal from the exponential phase of the substrate-use function, only the substrate use of the initial community remains. The exponential phase is characterized by plentiful substrate where growth of organisms is not limited by its availability. By fitting a log-linear regression to the extracted exponential phase (Figure S3.1b), we determined the initial community substrate use (y-intercept). In contrast to the existing methodology, we determined the phase of true exponential growth from the second derivative of a polygonal curve fit. By finding the inflection point, the point where the second derivative changes from positive to negative, the convex, true exponential, part of the curve is determined. We also accounted for the possible existence of a lag phase by removing non-positive values (i.e. zeroes). We calculated the classical Average Well Colour Development (AWCD; Garland and Mills 1991) and diversity metrics of substrate utilization (e.g. Gomez *et al.* 2006), substrate richness, the exponent of the Shannon diversity and Pielou's evenness of the substrate utilization.

## Soil chemical analyses

Soil pH was measured after shaking a soil-water (1:2.5 w/v) suspension on an orbital shaker at 200 rpm for 2 hours. Total C, N and P analyses were performed on oven-dried (60°C, 96h) and ground (1.0 mm, Retsch SM 100, Haan, Germany) soil samples. Total C and N were determined using an Elemental Analyser (Thermo Electron, Milan, Italy). Total P was determined according to the method of Murphy and Riley (1962). Soil samples were ashed at 550 °C for 30 minutes, after which P was re-suspended by digestion with 2.5% (w/v) acid persulphate in an autoclave (30 minutes at 121°C). Total

P was measured colorimetrically, on a continuous flow analyser (SEAL analytical, Abcoude, The Netherlands). Soil moisture was determined as the percentage weight loss upon oven drying.

## Cartographic information

Soil typological information, yearly fertilizer use and land management were extracted from geographical maps (Alterra, PAWN; Natuur op Kaart, Kadaster 2013/14, SNL, IPO 2013/14) using ArcGIS 10.1. With this information, we determined fertilizer use and N and P loadings per hectare. In determining artificial and organic fertilizer (manure), we assumed that farmers used the maximum amount of admissible fertilizer based on national legislation. Fields with specific nutrient management schemes, such as areas with natural grassland management generally use less artificial fertilizer due to a resting period where no fertilizer can be applied or due to legal restrictions on artificial fertilizer use. Also, manure application may be constrained due to the resting period or further limited to solid manure application for certain types of nature management. In designated natural grassland sites, neither artificial nor organic fertilizer application is allowed. We estimated inorganic and organic N and P loadings per hectare (ha) per year for fields in each polder (Table 3.2, Table S3.2).

**Table 3.2:** Average and range [min ; max] of local soil conditions of the different areas (H, I, O) used in soil community structure analyses (PLFA).

Variables	H	I	O
pH	<b>4.51</b> [3.91 ; 5.4]	<b>4.93</b> [3.87 ; 6.64]	<b>4.34</b> [3.70 ; 5.10]
C (mg/g dry weight of soil)	<b>208.62</b> [158.86 ; 273.75]	<b>196.48</b> [92.9 ; 240.64]	<b>177.96</b> [105.13 ; 237.67]
N (mg/g dry weight of soil)	<b>14.95</b> [11.59 ; 18.74]	<b>14.68</b> [6.8 ; 18.04]	<b>12.89</b> [7.79 ; 16.57]
P (mg/g dry weight of soil)	<b>1.42</b> [0.75 ; 2.06]	<b>1.33</b> [0.84 ; 2.01]	<b>2.69</b> [2.04 ; 4.22]
Moisture (%)	<b>64.63</b> [40.23 ; 76.97]	<b>61.88</b> [49.96 ; 74.72]	<b>62.56</b> [37.78 ; 77.43]
Microbial biomass (nmol/g dry weight of soil)	<b>20.01</b> [4.81 ; 64.93]	<b>14.04</b> [4.93 ; 31.42]	<b>11.77</b> [3.45 ; 38.35]
FB_ratio (-)	<b>0.06</b> [0.03 ; 0.09]	<b>0.06</b> [0.03 ; 0.09]	<b>0.06</b> [0.03 ; 0.1]

## Data analysis

All analyses were performed in R version 3.2.1 using the vegan, KernSmooth, MASS, PCNM, packfor and VennDiagram packages. In this study, we use two separate datasets on soil microbial communities. One dataset contained data on soil microbial community structure (PLFA data), and another dataset contained data on soil microbial community functional capabilities (CLPP data). The PLFA data consisted of three areas containing 22, 18 and 23 sites, each. The CLPP data encompassed 6 distinct areas with 24 sites each. First, we examined general soil properties, biomass and PLFA and CLPP patterns in these datasets. We determined descriptive soil properties (soil C,N,P content, pH and moisture) and tested how microbial biomass changed along environmental gradients using generalized linear models with a gamma distribution and log link function to deal with deviations from normality. These models were run for the different proportions of biomass as calculated from the PLFA data as dependent variables and included all soil geochemical, as well as all land management-related variables and the polder area identity as explanatory variables. Second, to assess general patterns and clustering in polder areas we used a principal component analysis (PCA). We tested the importance of general drivers and the existence of polder level differences further using distance-based redundancy analysis models (dbRDA; Legendre and Anderson, 1999), where microbial community variation (in composition or functional capacity) between sites was expressed as an Odum's percentage difference distance. Thus, large distances indicate very different sites and small distances indicate comparable sites in terms of community structure or functioning. We defined two spatial scale levels for this analysis, the local field level within polders and between polder areas at the regional level. At both scale levels, we carried out a variation partitioning analysis (Peres-Neto *et al.* 2006) using dbRDA. Prior to variation partitioning, a dbRDA analysis on the full data set was carried out. Next, all models were subjected to a forward selection procedure prior to variation partitioning (Blanchet *et al.* 2008). We subsequently assessed the importance of underlying variables in shaping the microbial community structure and functional capacity at the two scale levels by examining the explained variation ( $R^2_{adj}$ ) of the selected variables in isolation.

## Local scale: model definition

Differences in community composition or functional capacity at the local scale may result from differences in environmental quality between field edges. To analyse patterns at the local level, we applied the approach described by Declerck *et al.* (2011). Briefly, dummy-coded polder identifiers delineate the different study areas. These polder identifiers were used as covariates in the analysis to control for large-scale

patterns in the data. By controlling for the polder identity, we could effectively study within polder patterns in community structure and function for multiple polders simultaneously. We distinguished four explanatory models at the polder level: a soil characteristics model (SOIL), a nutrient management model (NUT), a vegetation composition model (VEG) and a spatial model (SPACE).

The SPACE model was composed of Moran Eigenvector Map (MEM) variables that explain the spatial autocorrelation between sites in the landscape based on geographical distances (PCNM: Dray et al. 2006). By using staggered matrices of MEM eigenvectors (Declerck et al. 2011; Legendre et al. 2013), we described spatial autocorrelation among sites within polders. We only selected the eigenvectors with positive spatial correlations for analyses. The SPACE model represents small scale spatial patterning in community variation, large scale patterning already being excluded through the use of polder identity as a covariate. Our SOIL model consisted of variables describing the quality of the soil (pH, C, N and P content, C:N, C:N and N:P ratio, moisture), morphometric characteristics (bank angle, bank width) and soil typology. Our NUT model consisted of loadings of organic and inorganic N and P applied to the field along with dummy coded variables of the occurring nutrient management schemes (Table S3.4). Our VEG model consisted of a staggered matrix of the principal axes of a principal coordinate analysis (PCoA) per polder. Because many axes of a PCoA explained little to no variation, we selected for relevant axes based on a broken stick model of explained variation, with all axes before the break point being selected. The resulting axes were arranged into a staggered matrix (Legendre et al. 2013) with the goal of only representing within polder differences. All models were subjected to forward selection based on a double stopping criterion ( $R^2_{adj}$  and  $a>0.05$ ) and tested for significance using 99,999 permutations constrained within polder identity levels.

## Regional scale: model definition

Differences in community composition or functioning at the landscape scale may result from differences in environmental quality between polders. Environmental gradients existing at the spatial grain of the landscape may be markedly different from those at the field level. Hence, an examination of these gradients irrespective of the variation explained within polders is appropriate. To this end, we used an approach where we first constructed a statistical model explaining community variation by dummy-coded polder identity variables (Polder model). By taking the predicted values of this polder model, we obtained a matrix of community variation present between polder landscapes only. We used this matrix as our response matrix in subsequent analyses of drivers of community variation between polders, allowing us to make models that only

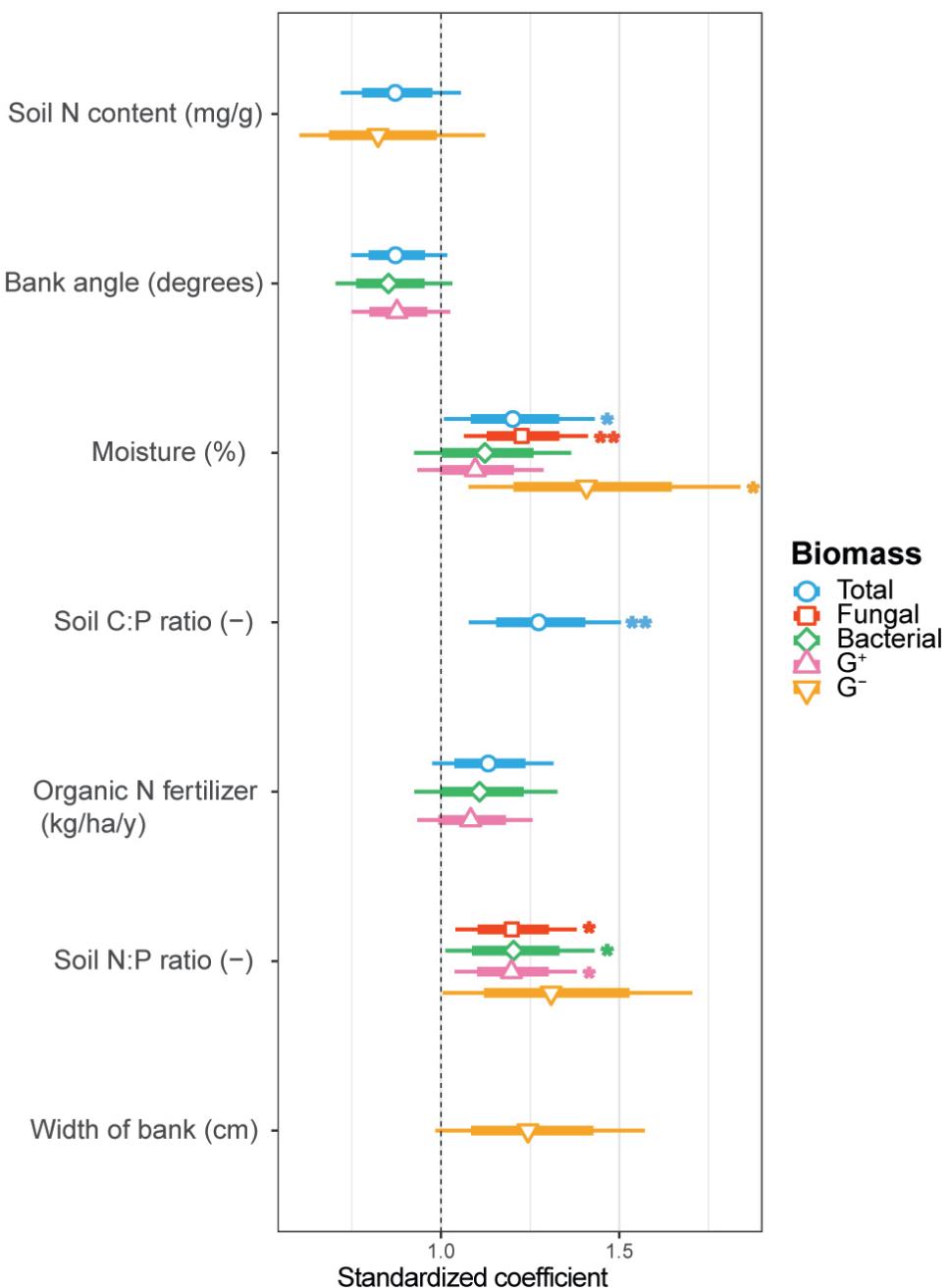
explain community variation encompassed by the polder model. Here, we constructed three explanatory models at the level of the landscape: a soil characteristics model (SOIL), a nutrient loading model (NUT) and a vegetation composition model (VEG). For the sake of interpretation, the explained variation of the models was rescaled to the total community variation captured by the polder model.

Our SOIL and NUT models consisted of the same variables as those used at the local scale. Our VEG model was created by transformation of an Odum's percentage dissimilarity matrix of the vegetation composition of all field edges within the respective data set by means of a principal coordinate analysis (PCoA). For the model explaining community variation, we made use of the resulting PCoA axes. This approach differs from the one used at the local scale in that we did not use PCoA axes per polder, but rather examined variation across all polders. As for the local model, we selected for the relevant PCoA axes based on a broken stick model of explained variation, with all axes before the break point being selected. The uniquely explaining part of the variation of the polder model, the part not explained by environmental drivers, may be interpreted as spatial patterning at the landscape level that is not directly related to the measured environment. A formal permutation test is not viable with the limited number of different polders. Hence, forward selection was carried out using the increase in  $R^2_{adj}$  as the only criterion. Additionally, when models were found to be non-significant in explaining patterns in the full data, irrespective of spatial scale, we disregarded the model in this analysis.

## Results

### Soil chemical properties and microbial biomass patterns

We examined soil chemical properties of our two data sets by calculating mean and spread of the soil chemical properties for all sample areas (Table 3.2 and Table 3.3). The sample areas used in the assessment of soil community structure analyses, showed a wide range in nutrient content (Table 3.2) as well as in soil moisture levels across the different sample areas. Areas also showed a wide range in microbial biomass (Table 3.2), which persisted across different groups (Fig S3.2). The microbial biomass increased with decreasing relative soil P-content (measured as molar C:P ratio and N:P) and was positively correlated to soil moisture levels (Figure 3.2, Table S3.3). The range of soil properties (nutrient content and soil moisture) was even greater for the samples examined by CLPP, though average soil chemical properties were within the same general range (Table 3.3). Substrate utilization was largely comparable between areas, though varied considerably within areas (Table 3.3, Figure S3.4 S3.5).



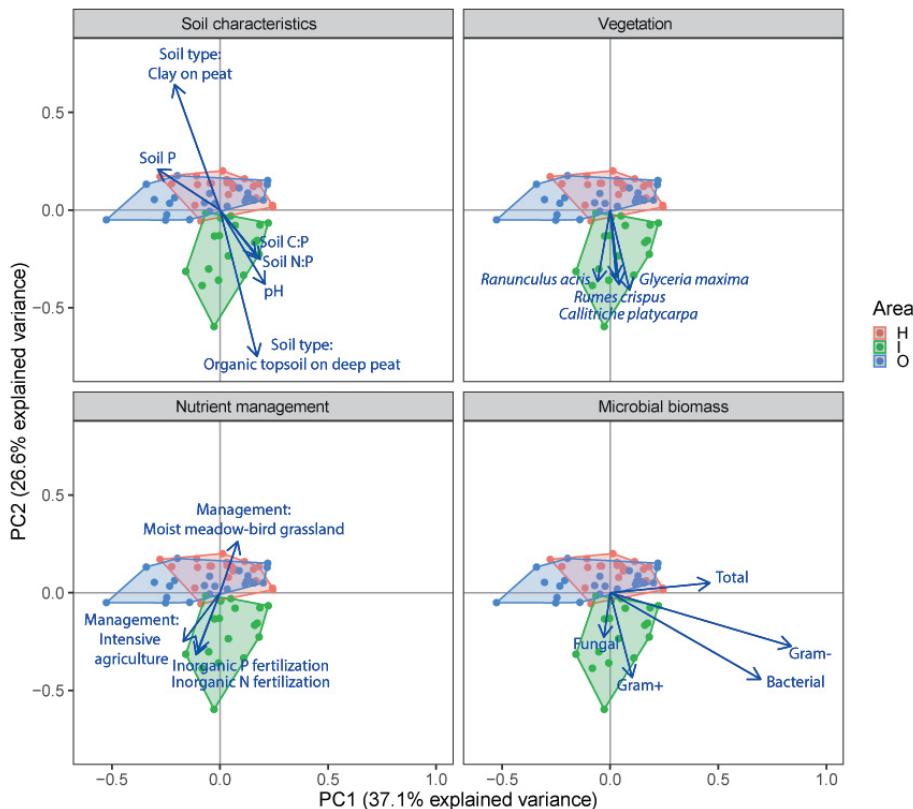
**Figure 3.2:** Estimated standardized coefficients for AIC selected generalized linear models of total, fungal, bacterial, G<sup>+</sup> bacterial and G<sup>-</sup>bacterial biomass explained by environmental drivers. Asterisks indicate significant coefficients (\*\*p<0.001; \*\*p<0.01; \*p<0.05.) with a coefficient above 1 being a positive and below 1 a negative correlation with the biomass component.

**Table 3.3:** Average and range [min ; max] of local soil conditions of the different sampling areas (Q,R,S,T,U,Z) used in soil community functioning analyses (CLPP).

Variables	Q	R	S	T	U	Z
pH	6.67 [4.74 ; 7.84]	5.44 [4.76 ; 6.4]	<b>5.34</b> [4.23 ; 6.62]	<b>5.30</b> [4.06 ; 6.24]	<b>6.03</b> [5.46 ; 7.43]	<b>5.55</b> [4.63 ; 6.03]
C (mg/g dry weight of soil)	121.17 [59.6 ; 273.9]	228.0 [152.2 ; 284.1]	<b>255.4</b> [167.7 ; 298.3]	<b>156.51</b> [47.9 ; 410.49]	<b>220.6</b> [27.2 ; 315.9]	<b>268.9</b> [159.0 ; 329.0]
N (mg/g dry weight of soil)	8.23 [4.45 ; 17.46]	16.6 [12.12 ; 20.53]	<b>19.55</b> [13.1 ; 22.18]	<b>10.05</b> [3.66 ; 22.67]	<b>16.54</b> [1.64 ; 23.06]	<b>21.31</b> [14.29 ; 25.48]
P (mg/g dry weight of soil)	1.00 [0.39 ; 1.77]	1.28 [0.94 ; 2.07]	<b>1.59</b> [1.21 ; 2.07]	<b>0.91</b> [0.42 ; 1.28]	<b>1.5</b> [0.75 ; 2]	<b>0.72</b> [0.49 ; 1.15]
Moisture (%)	<b>50.21</b> [14.83 ; 69.29]	68.62 [56.12 ; 87.12]	<b>68.44</b> [55.4 ; 76.8]	<b>58.13</b> [34.82 ; 80.83]	<b>65.37</b> [31.54 ; 78.54]	<b>72.33</b> [49.52 ; 79.41]
Substrate utilisation ( $\text{h}^{-1}$ )	0.0171 [0.006 ; 0.179]	0.0103 [0.005 ; 0.023]	0.010 [0.003 ; 0.025]	0.0085 [0.003 ; 0.020]	0.0102 [0.006 ; 0.018]	0.0107 [0.0038 ; 0.027]
AWCD	<b>0.73</b> [0.55 ; 0.89]	<b>0.64</b> [0.42 ; 0.9]	<b>0.73</b> [0.48 ; 0.89]	<b>0.5</b> [0.2 ; 0.78]	<b>0.65</b> [0.47 ; 0.88]	<b>0.64</b> [0.24 ; 0.99]
Substrate richness	<b>27</b> [22 ; 31]	<b>24.38</b> [16 ; 31]	<b>26.5</b> [23 ; 31]	<b>24.79</b> [19 ; 30]	<b>25.62</b> [21 ; 30]	<b>25.75</b> [19 ; 30]
Substrate diversity (Shannon)	<b>22.78</b> [17.59 ; 25.54]	<b>20.62</b> [13.48 ; 25.21]	<b>22.56</b> [19.2 ; 26.72]	<b>19.77</b> [14.23 ; 25.53]	<b>21.18</b> [17.26 ; 27.82]	<b>20.91</b> [14.45 ; 26.77]
Substrate evenness	<b>0.71</b> [0.55 ; 0.8]	<b>0.64</b> [0.42 ; 0.79]	<b>0.7</b> [0.6 ; 0.84]	<b>0.62</b> [0.44 ; 0.8]	<b>0.66</b> [0.54 ; 0.87]	<b>0.65</b> [0.45 ; 0.84]

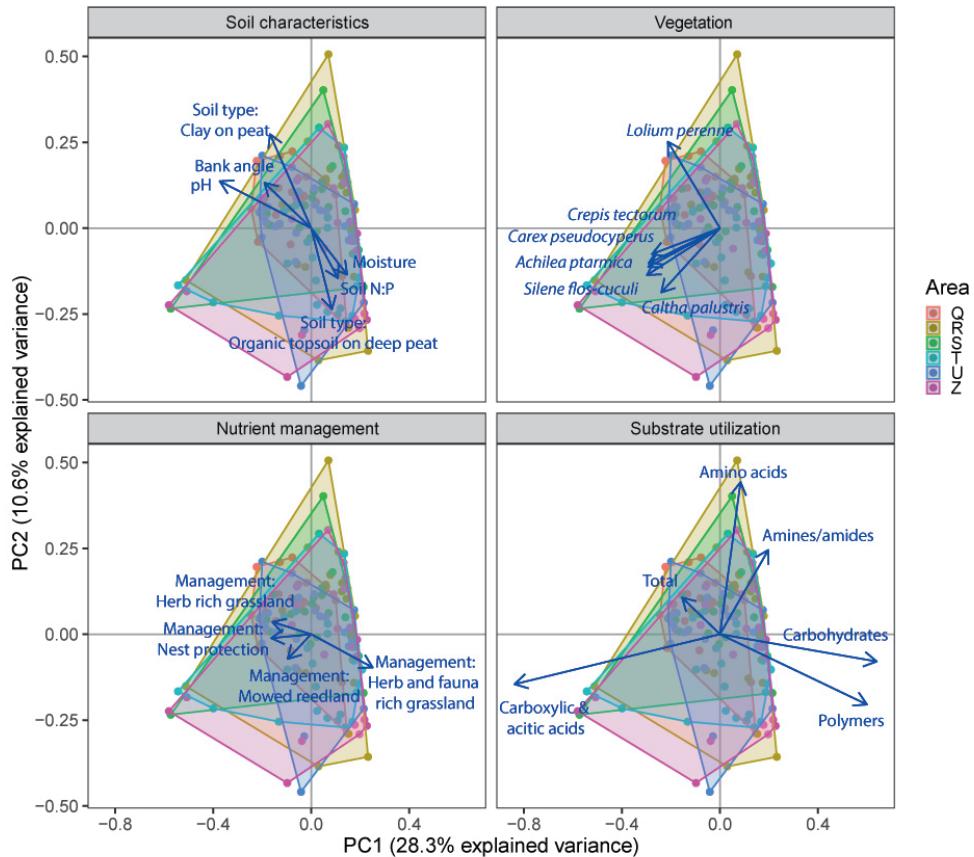
## Drivers of soil microbial community structure at different spatial scales

Soil microbial community structure was examined by PLFA fingerprinting. In a first examination of PCA results (Figure 3.3), we found clear differences between the different polder areas examined. A dbRDA of the PLFA data revealed that 19.8% of the community variation could be explained by differences between polders (Figure 3.5a; Table S3.4c) and a mere 4.0% could be explained within polders (Fig 3.5b; Table S3.4a). Nonetheless, we were able to identify consistent, significant gradients explaining community structure (Table S3.4). At the local scale (Figure 3.5b), only the NUT model proved to explain a significant portion of community variation ( $R^2_{\text{adj}}=4.0\%$ ,  $P<0.01$ ). This leaves large parts of the total variation explained at the level of the full dataset unaccounted for (Table S3.5). A part of this community variation was found to be explained at the landscape scale instead (Figure 3.5a) by means of the SOIL, NUT and VEG models. Only 2.9% (ns) of the variation was unique to the polder model, and not captured by one of the other models (Figure 3.5a, Table S3.4a). The SOIL model was the most important explaining environmental component ( $R^2_{\text{adj}}=16.1\%$ ), encompassing large parts of the explained variation of the NUT ( $2.7\%+1.1\%=3.8\%$ ) and VEG model ( $3.0\%+1.1\%=4.1\%$ ).



**Figure 3.3:** PCA plots of soil microbial structure data (PLFA) for the three different groups of drivers (Soil characteristics, Nutrient management and Vegetation) and shifts in total microbial biomass, fungi, gram-positive bacteria, gram-negative bacteria and total bacteria. Arrows are projected variables showing variables with the highest squared correlation coefficients. Different colours indicate the different sampled areas (H, I, O).

We ranked variables underlying the main drivers identified in the variation partitioning in terms of their importance (Table 3.4). At the landscape scale, PLFA structuring responded most strongly to nutrient-related variables, the soil P content (10.3%), inorganic N fertilization (3.1%), soil N content (1.7%), organic P (1.0%) and N fertilization (0.6%). In addition to nutrient-related parameters, soil type (7.8%), the presence of nature management schemes (2.6%), agri-environmental schemes (0.2%), and the resident vegetation composition (4.6%) were shown to be important variables in explaining landscape level community structure. At the local scale, less of the variation in PLFA data was explained, with organic P fertilization being the most pronounced driver (5.7%) of microbial community structure.

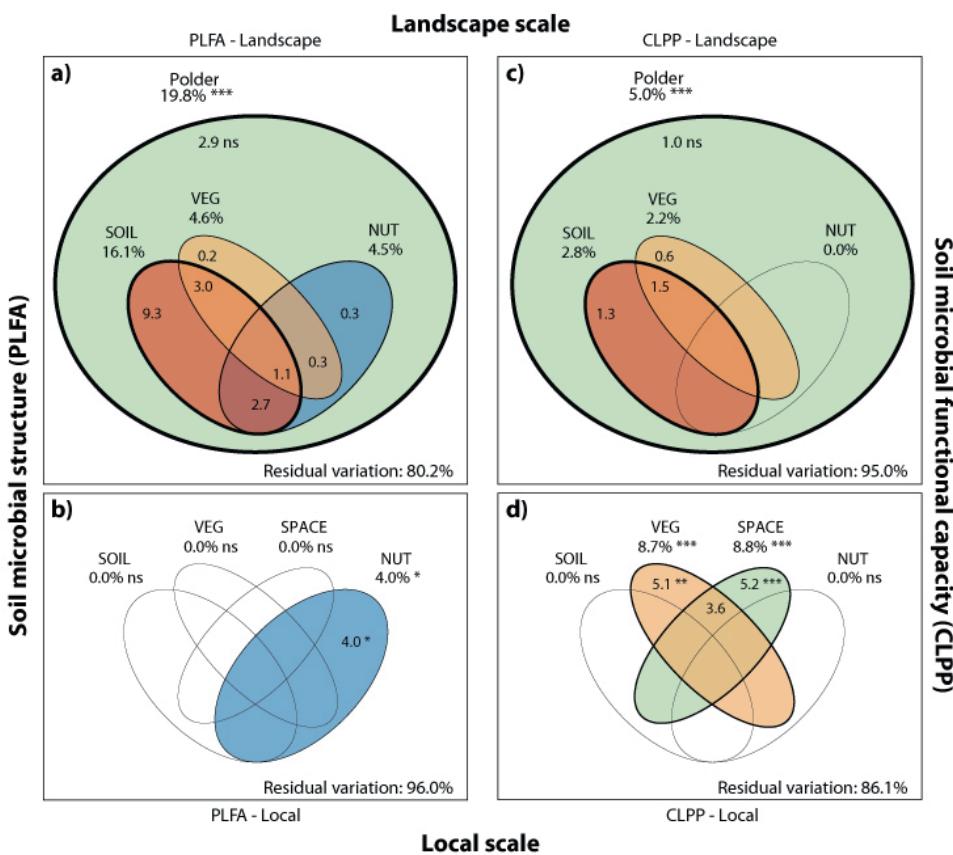


**Figure 3.4:** PCA plots of soil microbial functional capacity data (CLPP) for the three different groups of drivers (Soil characteristics, Nutrient management and Vegetation), with projections of the shifts in the utilization of specific substrate types. Arrows are projected variables showing factors variables with the highest squared correlation coefficients. Different colours indicate the different sampled areas (see Methods).

## Drivers of microbial community functional capacity at different spatial scales

Community level physiological profiling (CLPP) was used as a proxy for the functional capacity of the microbial community. In a first examination of PCA results (Figure 3.4), we found a strong overlap between sites of the different polder areas under examination. This was also reflected in RDA analyses of the data, with only 5.0% of the total variation in community functional capacity being explained by the polder model (Figure 3.5c). Despite this small part of the variation being explained, we did find that part of the CLPP variation between landscapes was associated with soil characteristics ( $R^2_{adj}=2.8\%$ ) and vegetation ( $R^2_{adj}=2.2\%$ ) (Figure 3.5c). Nutrient management was found

to be non-significant in explaining patterns in the full dataset (Table 3.3), and it therefore did not explain any of the variation encompassed by differences between polders. At a local scale (Figure 3.5d), we could explain a larger part of the variation (13.9%), which was attributed to the vegetation composition model ( $R^2_{adj}=8.7\%$ ,  $P<0.001$ ) and spatial patterns in community functional capacity ( $R^2_{adj}=8.8\%$ ,  $P<0.001$ ).



**Figure 3.5:** Drivers of microbial community structure and functioning on local and landscape scale. Venn diagrams showing the variation partitioning of different statistical dbRDA models: a soil characteristics model (SOIL), a nutrient management model (NUT) and a vegetation composition model (VEG) and a spatial model (SPACE). These models are used to explain soil microbial structure (PLFA) at the landscape (a) and local (b) scale, and functioning (CLPP) at the landscape (c) and local scale (d) by different drivers. Stars indicate significance and numbers express the adjusted  $R^2(\%)$  of the model partitions.

We identified the primary driving variables related to soil microbial community functional capacity (Table 3.4). At the landscape scale, soil pH (1.1%) and soil type (0.9%) and soil P ratios (soil N:P: 0.7% and soil C:P: 0.6%) were found to be most explaining for the variation in functional capacity. The local scale was explained by the vegetation community and a spatial MEM model based on geographical distance between field edges. The latter showed that most patterns were described by the highest order MEM variable (7.7%), indicative of a relatively coarse spatial patterning of community functioning.

**Table 3.4:** Importance of variables underlying soil microbial community structure (PLFA) at both scale levels (local and landscape).

Model	Variable	Explained variation*	
		Local	Landscape
Soil characteristics (SOIL)	Soil P content	-	10.3
	Soil type: Organic top soil on deep peat layer	-	7.8
	Soil N content	-	1.7
Nutrient management (NUT)	Organic P fertilization	5.7	1.0
	Management: Nature - Moist meadow-bird grassland	0.8	2.6
	Inorganic N fertilization	-	3.1
	Organic N fertilization	-	0.6
	Management: AES - Meadow-bird nest protection	-	0.2
Spatial patterns (SPACE)	ns	-	-
Vegetation composition (VEG)	Vegetation composition	-	4.6

#### Footnotes

\* Explained variation of each variable is given as R<sup>2</sup> (%) of the variable

- Variable was not selected in the forward selection of the specific model

## Comparing community structure and functional capacity

Comparing the two datasets, the two analyses of community variation yielded highly disparate results with respect to the scale at which different environmental factors

could explain variation in the data (Figure 3.5). Community structure data (PLFA) was associated with environmental factors between different polders, *i.e.* at a large landscape scale (Figure 3.5, Table S3.4). In contrast, functional data was poorly explained at this scale; rather environmental variation within polders offered the greatest level of explanatory power (Figure 3.5). Despite the difference in total explained variation, at the landscape scale the general partitioning and relative weight of the drivers was comparable for both PLFA structure and CLPP (Figure 3.5, Table S3.4). Both microbial community properties were most explained by the SOIL model with a small contribution of the variance being explained by VEG. Moreover, variation was highly collinear between the different models. On a local scale, patterns were markedly different between community structure and functional capacity.

**Table 3.5:** Importance of variables underlying soil microbial functional capacity (CLPP) at both scale levels (local and landscape).

Model	Variable	Explained variation*	
		Local	Landscape
Soil characteristics (SOIL)	Soil pH	-	1.1
	Soil type: Sand	-	0.9
	Soil N:P	-	0.7
	Bank angle	-	0.6
	Soil C:P	-	0.6
	Soil type: Clay on peat	-	0.6
	Soil N content	-	0.6
	Soil C:N	-	0.3
Nutrient management (NUT)	ns	-	-
Spatial patterns (SPACE)†	MEM1	7.7	-
	MEM2	3.1	-
	MEM3	1.4	-
Vegetation composition (VEG)	Vegetation composition	8.7	2.2

#### Footnotes

\* Explained variation of each variable is given as R<sup>2</sup> (%) of the variable

- Variable was not selected in the forward selection of the specific model

† Spatial patterns model is composed of Moran Eigenvector Map (MEM) variables based on geographical distance as per Dray et al., 2006. Variables of increasing order indicated decreasing scale of spatial patterning.

## Discussion

Understanding the drivers of soil microbial processes at relevant scales can help to improve management of agricultural peatlands to protect and improve desired ecosystem functioning. Through our analyses, we have examined the driving forces of microbial community structure and functioning in field margins along agricultural banks at two different scale levels; within polders (local) and between polders (landscape). We found local and landscape scale drivers to be distinct at different scale levels. The underlying variables were found to be largely different as well. This implies that the spatial scale of soil microbial studies is important when talking about driving forces of soil microbial community structure and functioning, enforcing the idea that the scale of soil management and the scale of study of soil microbial structure and functioning need to be well aligned.

### Local functioning, landscape structuring

While somewhat anecdotal due to the separate collection of the datasets, we showed that soil microbial community structure (PLFA) was more strongly regulated at the landscape scale, while functional capacity (CLPP) was more strongly driven at the local scale. Explained variation, while not being exceptionally high (15-20%), was comparable to other studies using similar multivariate community analysis approaches (Van der Gucht *et al.* 2007; Sayer *et al.* 2017). Future studies could consider integrated methods that address both structure and functioning conjointly (e.g.  $^{13}\text{C}$  PLFA, Yao *et al.* 2015). The inclusion of additional environmental drivers, such as specific fractions of bio-available nutrient pools, would potentially have increased the amount of explained variation. Across polder regions, e.g. at the landscape scale, the results indicate a driving role for soil characteristics, with vegetation being largely collinear with soil characteristics (for similar findings see: Kuramae *et al.* 2010). We therefore conclude that, with respect to soil microbial structure, differences in vegetation and nutrient management between polders are well reflected in the soil characteristics. Local microbial structure could only be led back to the applied nutrient management of the field and explained little variation. In contrast, variation in community functional capacity could be explained better by vegetation composition and spatial patterns at the local scale, with both explaining distinctly different parts of the community variation. The overlap in drivers at the landscape scale is likely due to the fact that the studied areas vary in land-use, land-history and management, which leads to landscape-scale vegetation and nutrient availability patterns that leave clear imprints in the soil. Locally, the small-scale heterogeneity of fields becomes more important in driving the specific microbial function. This mismatch in scale between structure and

function has been described previously for specific microbes and their functions (Veraart *et al.* 2017).

## Drivers of soil microbial community structuring and functional capacity

Drivers of community variation may differ strongly with scale (Yergeau *et al.* 2010; Prober *et al.* 2015b), and our analyses support this premise. At both scale levels, community structure was driven by nutrient management. The latter result is in agreement with previous research (O'Donnell *et al.* 2001; O'Brien *et al.* 2016) that has shown the importance of fertilization regimes for soil microbial communities. In turn, the supply and manner in which nutrients are added can have direct consequences for ecosystem functions such as nutrient retention and plant uptake (Heijboer *et al.* 2016). We, however, did find clear differences in underlying drivers of nutrient management of the within and between polder scales, with organic P loading and inorganic N loading being most important. This highlights the importance of identifying underlying drivers (Martiny *et al.* 2011). By focussing on a single scale level, important drivers may be overlooked and incorrect conclusions may arise, potentially leading to mismanagement of the agricultural landscape.

Our conclusions regarding landscape scale patterns are complicated by the lack of extensive replication at the landscape level, making formal testing of the drivers encapsulated within the polder model problematic. While we acknowledge these limitations within our study, our results are strengthened by the strong significant patterns found in tests of the entire data set (Table S3.4). As large parts of the total variation that can be explained by our models remain unexplained at the local scale (e.g. Figure 3.2b), it is reasonable to assume that this variation may be explained at the landscape scale.

A surprising similarity in soil characteristic drivers of soil microbial community structure and functioning can be found for nutrient-related drivers (soil N:P ratio, soil P and N content). Specifically, soil N content was found as the only variable that was important in determining landscape scale community structure, as well as the community functional capacity. Additionally, for community structure, specifically P-related processes were important drivers at a local (organic P fertilization) and at a landscape scale (soil P content, organic P fertilization). Soil nutrient content and the relative P availability compared to other nutrients were also primary drivers of microbial biomass. In existing literature, little attention has been paid to the effects of P on peatland microbial communities and functioning (Lin *et al.* 2014; Veraart *et al.* 2015). Our results suggest that these effects of P enrichment on peatland microbial communities deserve additional consideration.

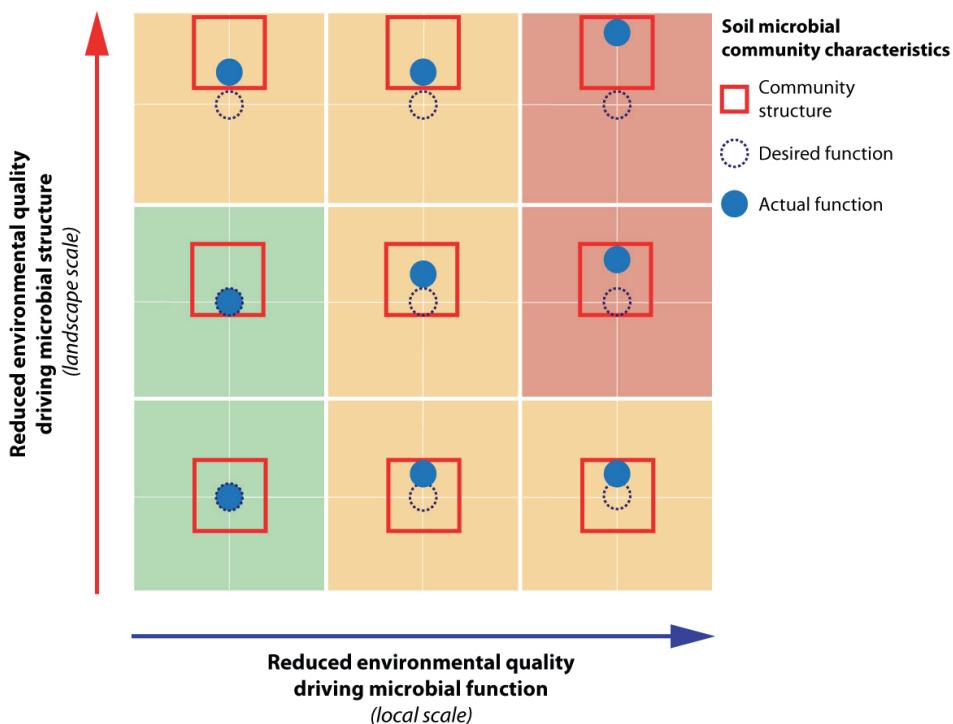
The relevance of the resident vegetation community for local microbial functional capacity, but not local microbial structure, is a noteworthy result. This could be caused by the study design in which we compare different polder areas with slightly different plant communities. An ecological explanation for this may be found in the stimulating role of plant presence and diversity on the function of soil microbes by (e.g. Zak *et al.*, 2003). Furthermore, a well-developed, species-rich riparian zone will influence water and nutrient retention (Hefting *et al.* 2005) and thereby microbial functioning (Korol *et al.* 2016). This development of a riparian zone depends strongly on local disturbance by mowing and cattle grazing. We did not directly quantify these factors, although they should in part be represented in the nutrient and land management schemes. However, within these schemes, there is room for variation in grazing and mowing regimes at the digression of the land manager. As land managers tend to own different nearby fields within a landscape, this variation in mowing and grazing is likely to be spatially structured. Our results, where vegetation and spatial structure explain local functional capacity, may thus be (partially) explained by these unmeasured differences in management regimes.

We found evidence for spatial patterns that could not be explained by any of the measured environmental drivers at the level of the local functional capacity (uniquely explained variation of the SPACE model), which may represent a possible signal of dispersal limitation (Dray *et al.* 2006). While dispersal-limitation has been shown to be plausible within microbial communities (Evans *et al.* 2017; Langenheder *et al.* 2017), it is rarely a significant driver of microbial community structure (Martiny *et al.* 2011; O'Brien *et al.* 2016). Hence, our observed spatial patterns are likely to be caused by spatially-structured environmental variables (e.g. light climate, soil redox conditions, readily available nutrient fractions, available substrates) that were not taken into account in this study (Martiny *et al.* 2006; Yao *et al.* 2011).

## Management of soil microbial communities in peatlands: an integrative approach

Our results suggest that microbial function is regulated by multiple different drivers that are distinct from those driving soil microbial structure, and that these drivers act at different spatial scales. This complicates the task of managing agricultural peatlands for desired ecological functioning. The traditional view maintains that environmental drivers influence community structure and that this structure in turn influences community functioning (Allison & Martiny 2008). However, this paradigm has been proven to be insufficient to explain microbial functional patterns in nature (Strickland *et al.* 2009; Weedon *et al.* 2017). Microbial functions have been shown to change independently of microbial community structure (Tian *et al.* 2016; Weedon *et al.* 2017)

and respond to different variables than structure (Boeddinghaus *et al.* 2015). However, disregarding community structure entirely and solely focussing on functioning is also clearly inappropriate, as microbial community structure serves as a constraint on the realized functioning of the community and the ecosystem as a whole (Pérez-Valera *et al.* 2015; Heijboer *et al.* 2016).



**Figure 3.6:** Schematic representation of the effects of reduced environmental quality on soil microbial community structure and functioning. This conceptual figure illustrates how reduced environmental quality of drivers relevant for functional capacity will directly lead to shifts of soil microbial functioning away from its desired function. Reduced environmental quality relevant for microbial structural composition will cause shifts in the soil microbial community structure box. This can ultimately also result in a shift in soil microbial community function through its constraint on microbial function. Within the context of the current study, the environmental drivers of microbial functioning were found to be manifest at the local scale, while the drivers shaping structure operated at the landscape scale.

We argue that for effective management of desired functioning to optimize the different societal benefits obtained from the landscape, both soil microbial structure and functioning need to be considered. Based on our study, environmental quality changes relevant for soil microbial functional capacity were most pronounced at the local scale. As local environmental quality shifts, this may lead to a direct shift in realized functioning away from the desired function (Figure 3.6, horizontal axis). However, the magnitude of this shift may be limited by the community structure, which constraints the extent of the shift in function (e.g. compare Figure 3.6, central-right and bottom-right, respectively unconstrained vs constrained situation). Changes in environmental drivers governing structure (Figure 3.6, vertical axis) were primarily found to manifest themselves at the level of the landscape within the context of this study. A change in environment at the landscape level may hamper realization of the desired function by constraining the realised function negatively as well (e.g Fig 3.6, top-left). Hence, a thorough understanding of the community structure and its potential to facilitate the desired function is an imperative first step in soil microbial management, followed by optimization of the conditions directly driving required soil microbial functioning. Throughout this process, the spatial scale at which microbial structure and functioning responds to these changes needs to be taken into account. Landscape measures, such as water level fluctuations and spatial planning set the constraints for the potential functioning (i.e. structure), and once this stage has been set, local management options such as mowing and fertilization regimes are decisive in determining if the desired functioning can be achieved.

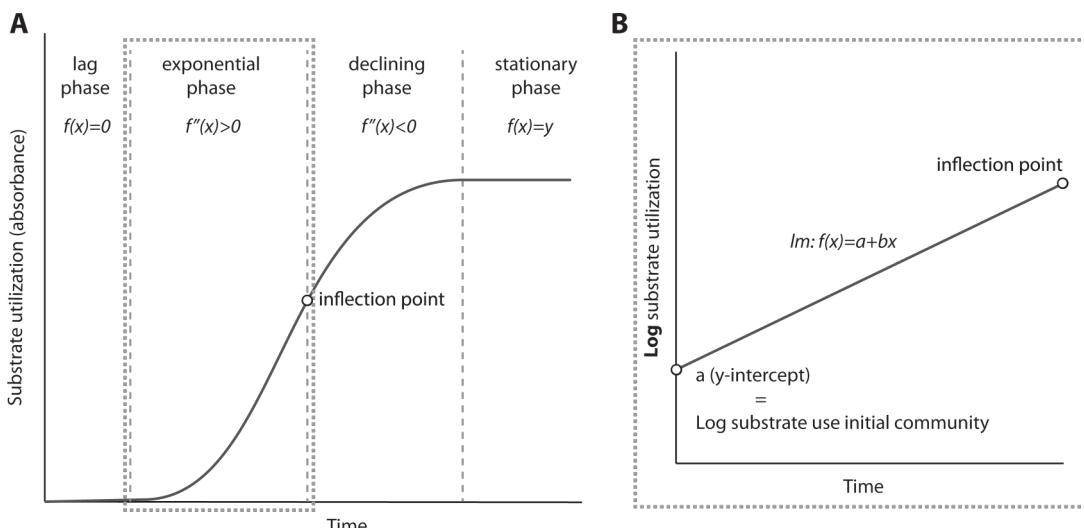
## **Concluding remarks**

Our study showed that soil microbial communities of agricultural peatlands are driven by different factors at distinct, management-relevant spatial scales. Furthermore, our study provides a first indication that soil community structure and function do not necessarily respond to the same factors, or at the same spatial scales. We argue that it is important to take both these soil microbial community characteristics (structure and function) into account for management of these important ecosystems. Based on this study, we suggest optimizing management of microbial ecosystem functioning in peatlands by first focussing on landscape restoration, followed by suitable local scale management optimization. This is directly related to recent initiatives such as the 4% initiative for increasing soil C storage in agricultural areas (Le Foll 2015) and efforts to optimize long-term biogeochemical functioning of agricultural peatlands.

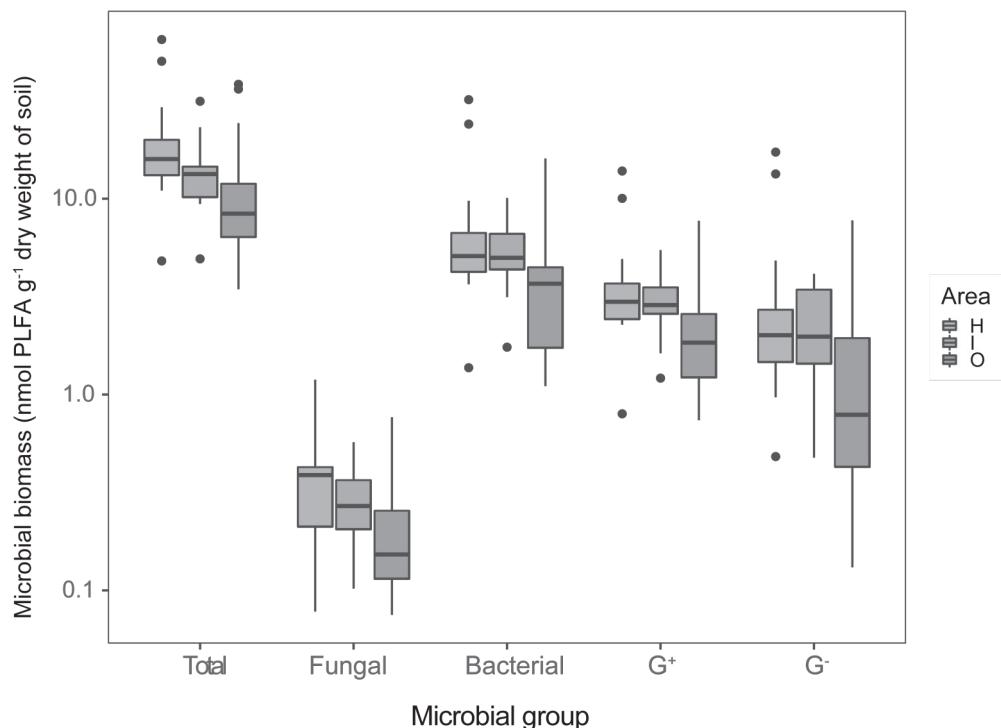
## Acknowledgements

The authors would like to thank Ciska Raaijmakers for technical assistance, Max Huitema, Daniela Sannino, Laura Vroom and Petra Reemst for practical assistance and Joost Keuskamp and Pedro Peres-Neto for advice on data analyses. This research was funded by the Netherlands Organization for Scientific Research (NWO) under projects 823.001.008 and (NWO; Biodiversiteit Werkt) 841.11.012 and 841.11.009.

## Chapter 3: Supplementary materials



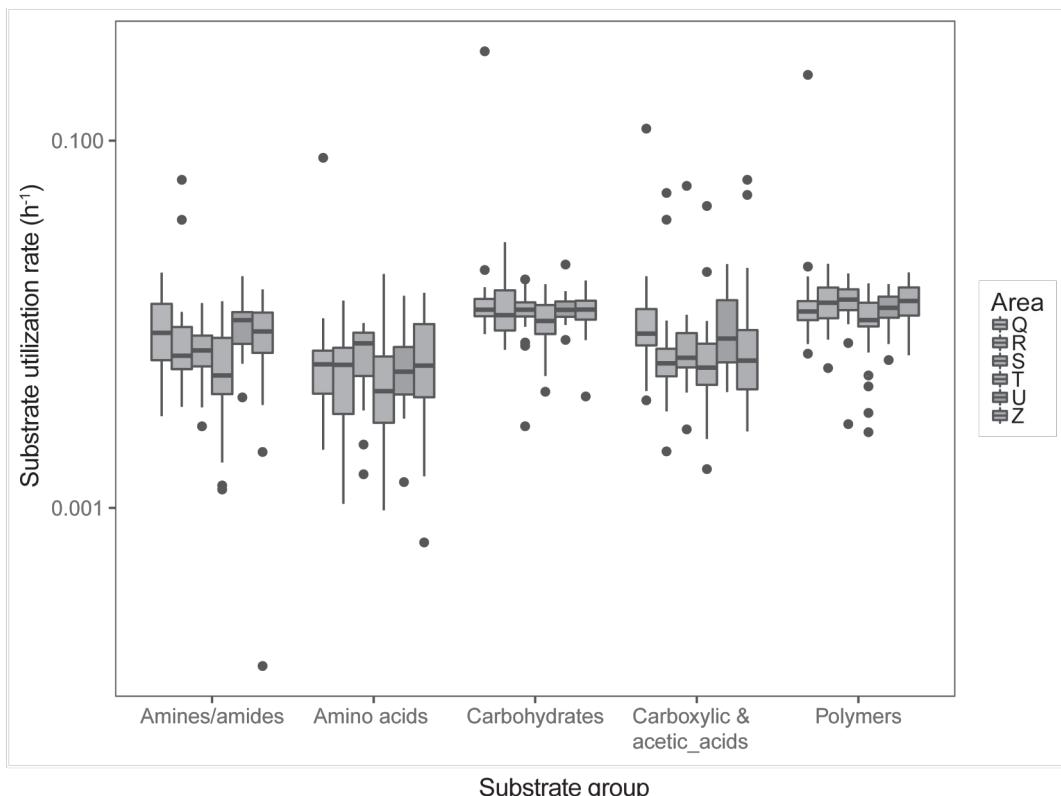
**Figure S3.1: Schematization of the steps to calculate substrate use by the initial microbial community (CLPP).** A) Conceptually, the function of substrate utilisation through time consists of four distinct phases. The lag phase in which no measurable amount of substrate is utilized, the exponential phase in which the function is convex ( $f''(x)>0$ ), the declining phase in which the function is concave and a stationary phase in which the substrate is depleted. B) By extracting the exponential phase of the function (delimited by positive y values and the inflection point) and fitting a log-linear model, the y-intercept is found. This yields the substrate use without growth, the substrate utilization of the initial community.



**Figure S3.2:** Boxplots of the microbial biomass of different sampling areas given for the different microbial groups as calculated from PLFA fingerprinting data.

**Table S3.1:** Conversion of Tansley abundance classes to numeric classes and percentage cover.

Tansley score	Tansley numeric score	Cover (%)
d	11	60
cd	10	40
ld	9	22
a	8	15
la	7	9
f	6	8
lf	5	3
o	4	2
lo	3	1
r	2	0.5
s	1	0.1



**Figure S3.3:** Boxplots of the substrate utilization rate of different areas given for the groups of the substrate types as present in the BiologEcoplate.

**Table S3.2:** Description of the different nutrient-management schemes and their respective N and P fertilization limits based on resting periods and legislation.

Nutrient management practice		$\mathbf{N}_{\text{organic}}$ limit	$\mathbf{N}_{\text{inorganic}}$ limit	P limit	Times mown (per year)	First mowing day
<b>Agri-environmental</b>	Meadow-bird grassland to June 1	170.0	18.8	67.7	4.0	153
	Meadow-bird grassland to June 15	109.2	0.0	39.1	3.0	167
	Meadow-bird grassland, pre-grazed	170.0	51.0	79.2	3.0	167
	Meadow-bird nest protection	170.0	58.6	82.0	4.0	134
	Herb-rich meadow-bird grassland	161.3	0.0	57.8	2.0	174
<b>Nature</b>	Swamp	0.0	0.0	0.0	0.0	-
	Mowed reed land	0.0	0.0	0.0	1.0	106
	Moist hay meadow	170.0	0.0	70.0	1.0	153
	Herb and fauna rich grassland	170.0	0.0	70.0	1.0	153
	Moist meadow-bird grassland	170.0	0.0	70.0	2.0	153
<b>Intensive agriculture</b>	Intensive agriculture	170.0	95.0	95.0	5.0	134

**Table S3.3:** Summary of regression analysis of microbial biomass components against environmental variables. Generalized linear models with a gamma distribution were selected using an AIC selection. Mean coefficients, 95% confidence limits and test statistics are given for each variable (where selected) with stars indicating significance: \*\*\*p<0.001; \*\*p<0.01; \*p<0.05.

	<b>Total</b>	<b>Fungal</b>	<b>Bacterial</b>	<b>Gram+</b>	<b>Gram-</b>
Soil N content (mg/g)	-0.14 [-0.33; 0.05] (-1.40)				-0.19 [-0.50; 0.12] (-1.23)
Bank angle (degrees)	-0.14 [-0.29; 0.02] (-1.74)		-0.16 [-0.35; 0.03] (-1.64)	-0.13 [-0.29; 0.03] (-1.64)	
Moisture (%)	0.18 * [0.01; 0.36] (2.05)	0.20 ** [0.06; 0.35] (2.83)	0.12 [-0.08; 0.31] (1.17)	0.09 [-0.07; 0.25] (1.12)	0.34 * [0.07; 0.61] (2.51)
Soil C:P ratio	0.24 ** [0.08; 0.41] (2.85)				
Organic N fertilizer (kg/ha/y)	0.12 [-0.02; 0.27] (1.63)		0.10 [-0.08; 0.28] (1.12)	0.08 [-0.07; 0.23] (1.06)	
Soil N:P ratio		0.18 * [0.04; 0.32] (2.51)	0.19 * [0.01; 0.36] (2.10)	0.18 * [0.04; 0.32] (2.48)	0.27 [0.01; 0.53] (2.00)
Width of bank (cm)					0.22 [-0.02; 0.45] (1.83)
p-value	0.0001	0.0001	0.0016	0.0011	0.0052
deviance	14.74	17.31	22.37	15.86	42.00
pseudo R2	0.36	0.19	0.28	0.28	0.25
df.residual	57.00	60.00	58.00	58.00	58.00

**Table S3.4:** Variation partitioning of soil microbial community drivers. Partial models are constrained for all variables contained in other models, giving the unique variation explained by the model under consideration, excluding the polder model where applicable. Dashes (-) indicate a model which was not tested due to limited replication at the landscape level.

**A) Variation partitioning of PLFA data at the landscape scale**

Model		R <sup>2</sup> adj (%)	F	p		DF model	DF res
Soil characteristics	full	16.07	-	-	-	3	59
	Partial	9.30	-	-	-	3	50
Vegetation community	Full	4.62	-	-	-	4	58
	Partial	0.16	-	-	-	4	50
Polder	Full	19.83	8.67	0.0002	***	3	59
	Partial	2.93	1.01	0.3658	ns	3	59
Nutrient management	Full	4.48	-	-	-	5	57
	Partial	0.33	-	-	-	5	50

**B) Variation partitioning of CLPP data at the landscape scale**

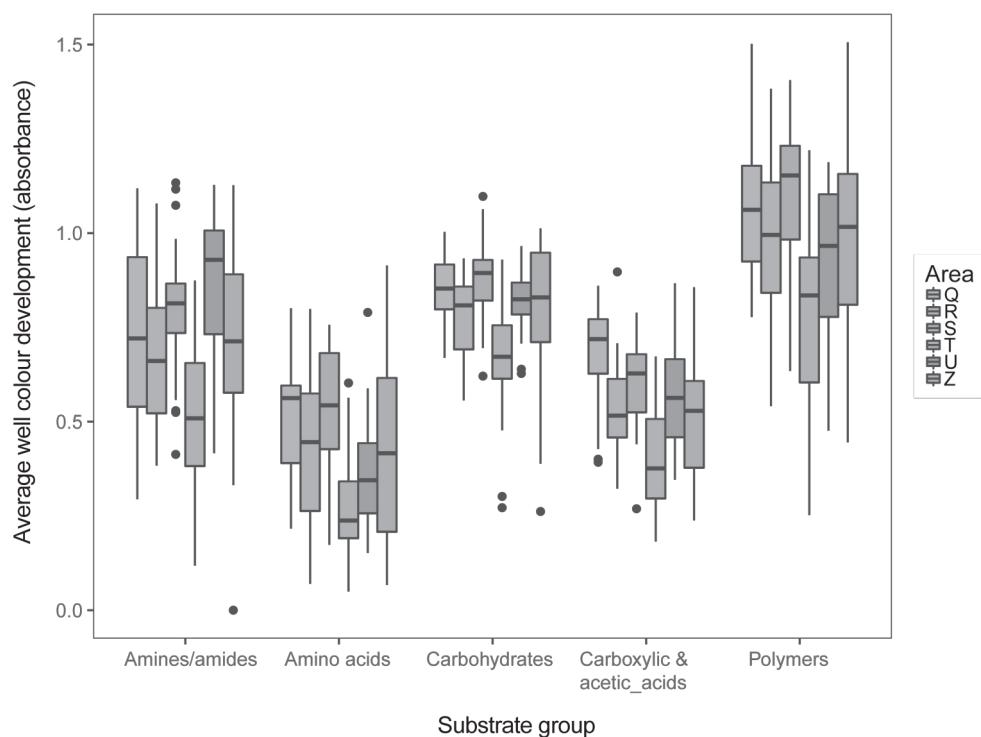
Model		R <sup>2</sup> adj (%)	F	p		DF model	DF res
Soil characteristics	Full	2.85	-	-	-	8	135
	Partial	1.27	-	-	-	8	130
Vegetation community	Full	2.17	-	-	-	5	138
	Partial	0.59	-	-	-	5	130
Polder	Full	4.99	29.10	0.0020	**	5	138
	Partial	1.04	10.21	0.4434	ns	5	119
Nutrient management	Full	0.00	-	-	-	NA	NA
	Partial	0.00	-	-	-	NA	NA

**C) Variation partitioning of PLFA data at the local scale**

Model		R <sup>2</sup> adj (%)	F	p		DF model	DF res
Soil characteristics	Full	0.00	NA	NA	ns	NA	NA
	Partial	0.00	NA	NA	ns	NA	NA
Vegetation community	Full	0.00	NA	NA	ns	NA	NA
	Partial	0.00	NA	NA	ns	NA	NA
Spatial patterns	Full	0.00	NA	NA	ns	NA	NA
	Partial	0.00	NA	NA	ns	NA	NA
Nutrient management	Full	4.02	2.59	0.0241	*	2	58
	Partial	4.02	2.59	0.0241	*	2	58

**D) Variation partitioning of CLPP data at the local scale**

Model		R <sup>2</sup> adj (%)	F	p		DF model	DF res
Soil characteristics	Full	0.00	NA	NA	ns	NA	NA
	Partial	0.00	NA	1.0000	ns	NA	NA
Vegetation community	Full	8.75	4.50	0.0010	***	4	134
	Partial	5.14	3.09	0.0051	**	4	128
Spatial patterns	Full	8.76	3.34	0.0010	***	6	132
	Partial	5.16	2.42	0.0007	***	6	128
Nutrient management	Full	8.76	NA	NA	ns	NA	NA
	Partial	0.00	NA	1.0000	ns	NA	NA



**Figure S3.4:** Boxplots of the average well colour development (AWCD) of different areas given for the groups of the substrate types as present in the BiologEcoplate.

**Table S3.5:** Importance of different drivers in explaining the full variation in soil microbial community structure (PLFA) and functional capacity (CLPP) irrespective of spatial scale.

	Model	R <sup>2</sup> adj (%)	F	p	DF res
<b>PLFA</b>	Soil characteristics	19.76	2.27	0.0034	** 50
	Vegetation composition	15.11	2.58	0.0069	** 55
	Polder	19.83	8.67	0.0002	*** 60
	Nutrient management	10.77	2.07	0.0276	* 55
<b>CLPP</b>	Soil characteristics	9.50	2.07	0.0131	* 129
	Vegetation composition	9.15	2.80	0.0001	*** 135
	Polder	4.99	2.50	0.0022	** 138
	Nutrient management	3.38	1.42	0.1309	ns 131

# 4

## Evidence for a non-linear response of submerged plant tissue nitrogen and phosphorous to environmental nutrient availability

Michiel J. J. M. Verhofstad\*, Sven Teurlincx\*, Steven A. J. Declerck and Elisabeth S. Bakker

\*Authors contributed equally to this work

## Abstract

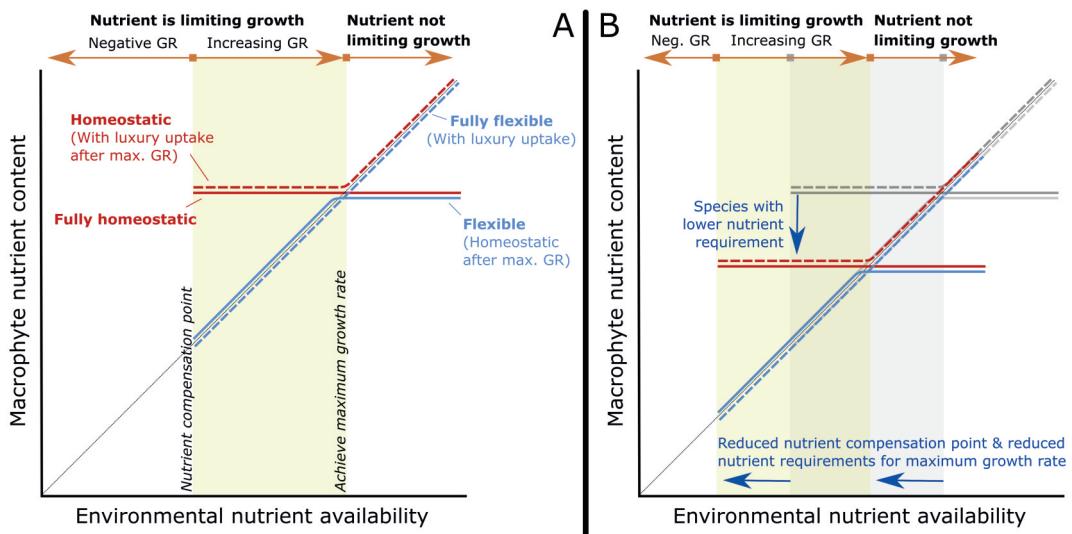
In shallow freshwater ecosystems, aquatic plants are an important component of the food web. Plant quality as food source for herbivores is partly determined by concentrations of essential elements including nitrogen (N) and phosphorous (P). Plant nutrient content is variable and may depend on environmental nutrient availability. Hence, changes in environmental nutrient availability may affect food quality for consumers, leading to altered rates of consumption and decomposition. However, the relationship between environmental nutrient availability and plant nutrient content is not immediately clear. We provide a framework, based on mechanisms underlying variation in plant nutrient stoichiometry within and among plant species, including homeostasis, nutrient utilization during nutrient limitation, and luxury uptake, in relation to environmental nutrient availability. We found that the combination of these mechanisms potentially results in both linear and non-linear associations between environmental nutrient availability and plant tissue nutrient content. Subsequently, we investigated the relationship between environmental nutrient availability (N and P) and tissue nutrient content in five widespread submerged macrophyte species in an extensive field survey across 193 sites in the Netherlands. As expected, we found high intra-specific variation in plant N and P concentrations. The plant's tissue P content increased significantly linearly with increasing environmental nutrient availability, while N content was unaffected. However, at high nutrient availability no further increase was observed in two plant species, providing compelling evidence that the relationship between plant and environmental nutrient content can be non-linear. This result is especially relevant for ecosystems undergoing eutrophication or oligotrophication, since plant nutrient content, may increase during eutrophication, whereas it may show a delayed response to lowering the nutrient status of a eutrophic ecosystem (i.e. oligotrophication), which has consequences for the food web interaction strength and nutrient recycling in these aquatic systems.

# Introduction

Submerged aquatic plants are at the base of the food web and play a keystone role in the functioning of shallow freshwater ecosystems (Carpenter & Lodge 1986; Phillips *et al.* 2016; Hilt *et al.* 2017). Recently, the importance of herbivory on aquatic vascular plants has been unequivocally confirmed (Bakker *et al.* 2016; Wood *et al.* 2016), indicating that aquatic plants are a vital component of aquatic food webs. The plant's nutrient content and stoichiometry can determine the intensity of herbivore impact, with stronger consumption of plants with high tissue nutrient content (Drenbosch and Bakker 2011; Bakker *et al.* 2013; Bakker and Nolet 2014). Autotrophs in general are considered to have a high degree of flexibility in their tissue elemental concentrations and ratios, which may depend on nutrient availability (Duarte 1992; Waal *et al.* 2012; Hessen *et al.* 2013; Zhang *et al.* 2018). This would suggest that changes in nutrient loading of aquatic systems, resulting in eutrophication or oligotrophication, can alter the nutrient content of aquatic plants, and thereby the food web interaction strength (e.g. Bakker *et al.* 2016; Hidding *et al.* 2016)). However, the relationship between environmental nutrient availability and plant nutrient content is not immediately clear.

Plant nutrient requirements and associated plant elemental composition will change along gradients of nutrient availability, with plants slowly shift from nutrient limited to light limited growth conditions (Duarte 1995). Aquatic plant growth is inherently linked to nutrient supply, with more nutrients supporting more biomass (Gerloff 1975). Aquatic plant community structure has also been linked to gradients of sediment nutrient availability (Chambers 2018). Conceptually, all plant species have a physiological constraint at which their nutrient supply is too low to support positive growth (Figure 1), the 'nutrient compensation point' (Fichtner & Schulze 1992). This point indicates the lower limit of the fundamental niche of the plant in relation to nutrient supply. Once the nutrient supply exceeds the 'nutrient compensation point', plant growth will become positive and increase with increasing nutrient supply (e.g. Farquhar *et al.* 1980). The growth rate will increase until the point where factors other than nutrients become limiting (Wersal & Madsen 2011; van de Waal *et al.* 2012), at which point increasing nutrient supply will no longer increase growth. Different plant species may be inherently different in their basal physiology, and thereby exhibit a different elemental composition (Gerloff 1975; Garbey *et al.* 2004; González *et al.* 2010). Physiological difference may reflect preferential investments of plants in structural tissues (Elser *et al.* 2010) or in root systems (Macek & Rejmánková 2007). This inherent difference between plant species in physiology can lead to species with different nutrient requirements (Figure 4.1 B: 'species with lower nutrient requirement') and thereby species turnover along a gradient of nutrient supply (Chambers 2018).

Besides inherent differences, the degree of homeostasis within a single species may not be uniform over the whole gradient of nutrient supply. Rather, its homeostasis may vary depending on the environmental nutrient availability (Meunier *et al.* 2014; Gulis *et al.* 2017; Heyburn *et al.* 2017). Species may range from being fully homeostatic, i.e. regulators, (Figure 4.1 panel A: solid red line) to being fully flexible, i.e. conformers, reflecting environmental nutrient availability (Figure 1A: dashed blue line) (Meunier *et al.* 2014). Flexible species may have a changing elemental composition for two distinct reasons: 1) increased nutrient uptake with increased growth (Duarte 1992; Ågren & Weih 2012) and 2) luxury uptake of nutrients while nutrients are not limiting growth (Madsen & Cedergreen 2002; Pietro *et al.* 2006; Li *et al.* 2015; Sistla *et al.* 2015). Theoretically, plants may exhibit luxury uptake without showing increased uptake during the growing phase (Figure 4.1: 'Flexible') or vice-versa (Figure 1: 'Homeostatic with luxury uptake after max. GR').



**Figure 4.1:** Conceptual framework showing possible responses of aquatic plant nutrient content to a gradient of environmental availability of that nutrient. Panel A: The lines only depict the extreme responses (i.e. fully flexible or fully homeostatic over the depicted ranges), but intermediate responses are also possible. GR stands for 'growth rate'. Panel B: Identical to panel A, but showing possible contrasting responses of plant species with different nutrient requirements (grey versus coloured, darker lines).

The concepts depicted in Figure 4.1 demonstrate that there can be both linear and non-linear positive associations between environmental nutrient availability and plant tissue nutrient content. Field surveys that simultaneously measure submerged macrophyte and environmental carbon (C), nitrogen (N), and phosphorous (P) concentrations are rare. The surveys that exist generally found only weak correlations between environmental nutrient availability and aquatic plant nutrient content and stoichiometry (Demars & Edwards 2007; Meyer *et al.* 2012; Xing *et al.* 2015). However, this is in contrast with controlled experiments that have suggested stronger positive relationships between nutrient addition and plant tissue nutrient content (Cronin & Lodge 2003; Xie *et al.* 2005; Bakker *et al.* 2013; Bakker & Nolet 2014). This discrepancy between experiments and field surveys may have been caused by the relatively low numbers of sampling sites (~1–20) per species in the field surveys, resulting in low statistical power and the potential omission of part of the environmental nutrient range in which species occur. Additionally, the existence of non-linear responses in submerged plant tissue nutrient content along environmental nutrient gradients (Robach *et al.* 1995; Sistla & Schimel 2012; Meunier *et al.* 2014) (Figure 4.1) may have obscured associations.

In this study, we investigated whether five widespread submerged macrophyte species show positive linear or non-linear associations between environmental nutrient availability (N and P) and tissue nutrient content in an extensive field survey with 20–135 sampled sites per species.

## Methods

### Field sites and sampling methods

We focussed on *Ceratophyllum demersum* and *Elodea nuttallii* as our main target species, because these are two of the most common and widespread submerged macrophyte species of temperate shallow water ecosystems both occurring over a wide range of aquatic habitats in the Netherlands (De Lyon and Roelofs, 1986). Additional samples of the common species *Myriophyllum spicatum*, *Potamogeton lucens*, and *Potamogeton pectinatus* were added to allow for more general conclusions on the variability in elemental composition of common and widespread submerged macrophyte species. We collected plant, water and sediment samples from 193 different sites in the Netherlands. Plant samples were collected from the topmost 15–20 cm of the shoot to ensure comparability between species. Most of the samples of *C. demersum* (108 out of 135) and *E. nuttallii* (83 out of 111) originated from drainage channels sampled from the end of May to mid-August of 2014. Additional samples of

these species (*E. nuttallii*: 27; *C. demersum*: 28) were collected from a variety of shallow freshwater ecosystems in June and July of 2008 and 2009, while also collecting samples of *M. spicatum*, *P. lucens*, and *P. pectinatus* (22, 21, 20 sites sampled, respectively).

To quantify environmental nutrient availability, we measured inorganic and total N and P in the water and total and extractable N and P in the sediment, as most submerged plants can take up nutrient from both media, depending on the relative nutrient availability (Barko *et al.* 1991).

## Plant analyses

After drying (60°C), plant dry mass (DW) was ground to a powder using a vegetation grinder with a 0.5 mm mesh (IKA®MF 10 basic, IKA Werke GmbH and Co. KG / Germany). To measure the C and N concentration of the plants, 0.5 mg of dry, ground plant material was inserted into tin capsules and analysed using a CN analyzer (FlashEA 1112 Series, Thermo Scientific, Waltham, MA, USA). Total phosphorous of the plants was measured by ashing (30 min at 550°C) 0.5 mg of dry, ground plant material, which was subsequently digested in an autoclave at 121°C for 30 minutes using a 2.5% persulphate solution. These samples were measured colourimetrically on an Auto-Analyser system (QuAAstro SFA, Seal Analytical, Norderstedt, Germany).

## Surface water analyses

At each site, a sample of the water column was taken for nutrient analysis. Water was filtered in the field over pre-washed GF/F filters (Whatman, 0.7 µm pore size), and the filtrate was analysed for dissolved inorganic nutrients ( $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and  $\text{NH}_4^+$ ) using a QuAAstro39 Auto-Analyser (SEAL Analytical Ltd.). The total nitrogen and phosphorous concentration was determined by analysing the suspended solids captured on the GF/F filter after drying at 60°C. Suspended solid nitrogen concentration was analysed using the CN elemental analyser. Suspended solid phosphorous was determined using the potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ; 2.5 % w/v) digestion method at 121 °C after a digestion step where samples are placed in a blast furnace at 550°C. Digested P (as  $\text{PO}_4^{3-}$ ) was measured on the Auto-Analyser. The total N and P concentrations were calculated by adding inorganic water nutrients to the suspended solids nutrients.

## Sediment analyses

The top 10 cm of the sediment was collected using a tube sampler (diameter=13.5 cm). Sediment samples were dried at 60°C for at least 96 h and analysed for total and available nitrogen and phosphorous. Sediments were put through a grinder (IKA® MF 10 basic, IKA Werke GmbH and Co. KG / Germany) with a 1mm sieve, resulting in a fine

powder. Total N was determined through analysis of a 1 mg powdered subsample using the CN elemental analyser. Total P was analysed using the same P-digestion method described above for particulate P determination, using a 5 mg subsample.

A KCl-extraction was performed on the dried sediment to estimate the amount of plant available N. 12.5 ml 1 M KCl was added to 2.5 g of dried sediment and subsequently shaken for 2.5 h at 250 rpm. 2 subsamples (2 ml each) were centrifuged for 10 min at 10,000 rpm and the supernatant was stored at -20°C for colourimetical nitrogen analysis on the autoanalyser system. Plant available orthophosphate in the sediment was estimated using an adapted P-Olsen protocol as follows. 50 ml 0.5 M NaHCO<sub>3</sub> (at pH 8.50) was added to 2.5 g of dried sediment and subsequently shaken for 30 min and immediately after that, the solution was poured over a filter (Whatman Grade 42, GE Healthcare Europe GmbH, Eindhoven, the Netherlands). Sulphuric acid (1.04 ml, 2.5 M) was added to 10 ml of the filtrate in an Erlemeyer flask. The flask was agitated until no more gas development was visible and the filtrate was filtered again (Whatman Grade 42) and stored at -20°C until analyses for phosphate concentration on the autoanalyser system. When not enough dried material was available for both extractions, the P-extraction was prioritized and a corresponding reduction in reagent volume was applied in order to keep the sediment:reagent-ratio equal between all samples if needed. See Appendix Table 1 for nutrient content of the plants, water and sediment.

## Statistical analyses

To assess whether and how the plant tissue nutrient content was associated with environmental nutrient availability, we performed regression analyses. Because we expected that this relationship would not be the same over the entire environmental nutrient gradient (see Figure 4.1), we compared the fit of two different models: (1) a linear regression model, and (2) a segmented linear regression model. The best model was selected by comparing the Akaike Information Criterions (AIC) of the models that explained a significant part of the variation in the data ( $p<0.05$ ). The statistically significant model with the lowest AIC was selected. To identify the best measure for the environmental nutrient availability we first performed PCA analyses on the water nutrient (i.e. inorganic and total N and P) and sediment nutrient variables (i.e. extractable and total N and P). The first PCA-axis (PC1) represented the environmental nutrient gradient best and captured 64 and 65 % of the variation of environmental N and P, respectively (Figure S4.1). In our analyses, we used these PC-axes as proxies for the environmental N and P availability, as the measured concentrations of N and P were positively correlated (P: Spearman's rho = 0.35-0.84,  $p<0.001$ ; N: Spearman's rho = 0.38-0.86,  $p<0.001$ ). The environmental nutrient data was scaled and centered for the PCA after removal of 3 outliers (i.e. all  $> 1.5 * \text{interquartile range}$ ) in the P-dataset.

Pairwise t-tests were used to analyse whether the shoot nutrient content differed among plant species when they were collected from the same site.

All statistical analyses were performed in R version 3.3.2 using additional *Hmisc*, *car*, *ggplot2*, *segmented*, and *vegan* packages.

## Results

### Association of plant and environmental nutrient content

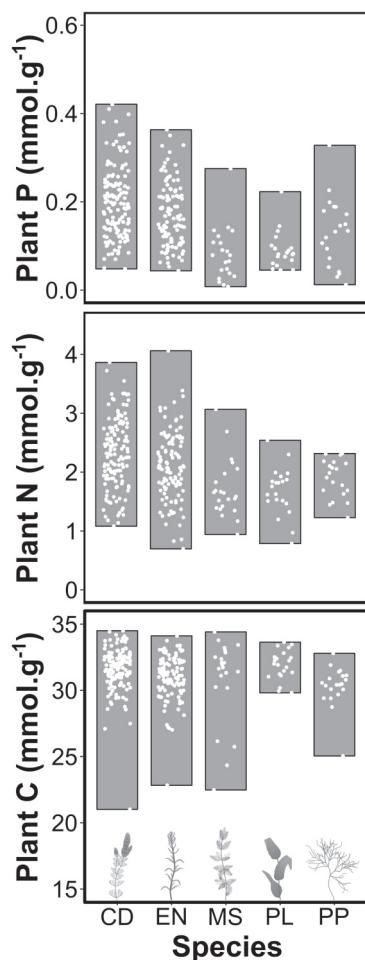
We found large variation in shoot C, N, and P content (Figure 4.2). N and P content almost exclusively determined shoot C:N and C:P stoichiometry, respectively (Figure S4.2, S4.3 & S4.4). All species showed a positive association between environmental P and plant P, at least over part of the environmental nutrient range (Figure 4.3; see Table S4.1 for ranges in plant and environmental N and P). For both *C. demersum* and *E. nuttallii* the segmented linear model fitted the data best. These models showed an increase in plant P content with increasing P availability in the lower range of the studied environmental nutrient gradient. Above a certain threshold value (i.e. the segmented model's breakpoint), plant P did not increase with environmental P (Figure 4.3). The breakpoint on the PC1-axis between the two lines of the segmented model was similar for both species:  $0.89 \pm 0.43$  (SE) and  $0.50 \pm 0.63$  (SE) for *C. demersum* and *E. nuttallii* respectively (Table S4.2). These breakpoints correspond to water P concentrations of approximately  $11 \mu\text{mol.L}^{-1}$  inorganic  $\text{PO}_4$  and approximately  $20 \mu\text{mol.L}^{-1}$  total P, and correspond to sediment P concentrations of approximately  $2.8 \mu\text{mol.g}_{\text{dw}}^{-1}$  Olsen-P and approximately  $30 \mu\text{mol.g}_{\text{dw}}^{-1}$  total P (i.e. mean concentrations from sites with a PC1 value of between 0-1). The other three species were all collected from sites in the lower part of the environmental nutrient gradient (Figure 4.3). The P concentration in *P. pectinatus* shoots significantly increased linearly over the sampled environmental P gradient. The segmented model did fit the data best for *M. spicatum* and *P. lucens* (Table S4.2). This was caused by only one data point: the site with the highest environmental P concentration (Figure 4.3). Without this point the linear model fit the data best for *M. spicatum* (slope=0.22,  $p=0.005$ , intercept=0.49), while neither the linear nor the segmented found a significant association between plant and environmental P for *P. lucens* ( $p > 0.05$ ).

In contrast to P, no associations between environmental N and plant N were found for any species except *M. spicatum* (Figure 4.3), even though the overall variation in both plant and environmental N was large (Figure 4.2; Table 4.1). As for P, the range in environmental N was much larger for *C. demersum* and *E. nuttallii* than for the other

three species.

## Inter-specific differences in elemental composition

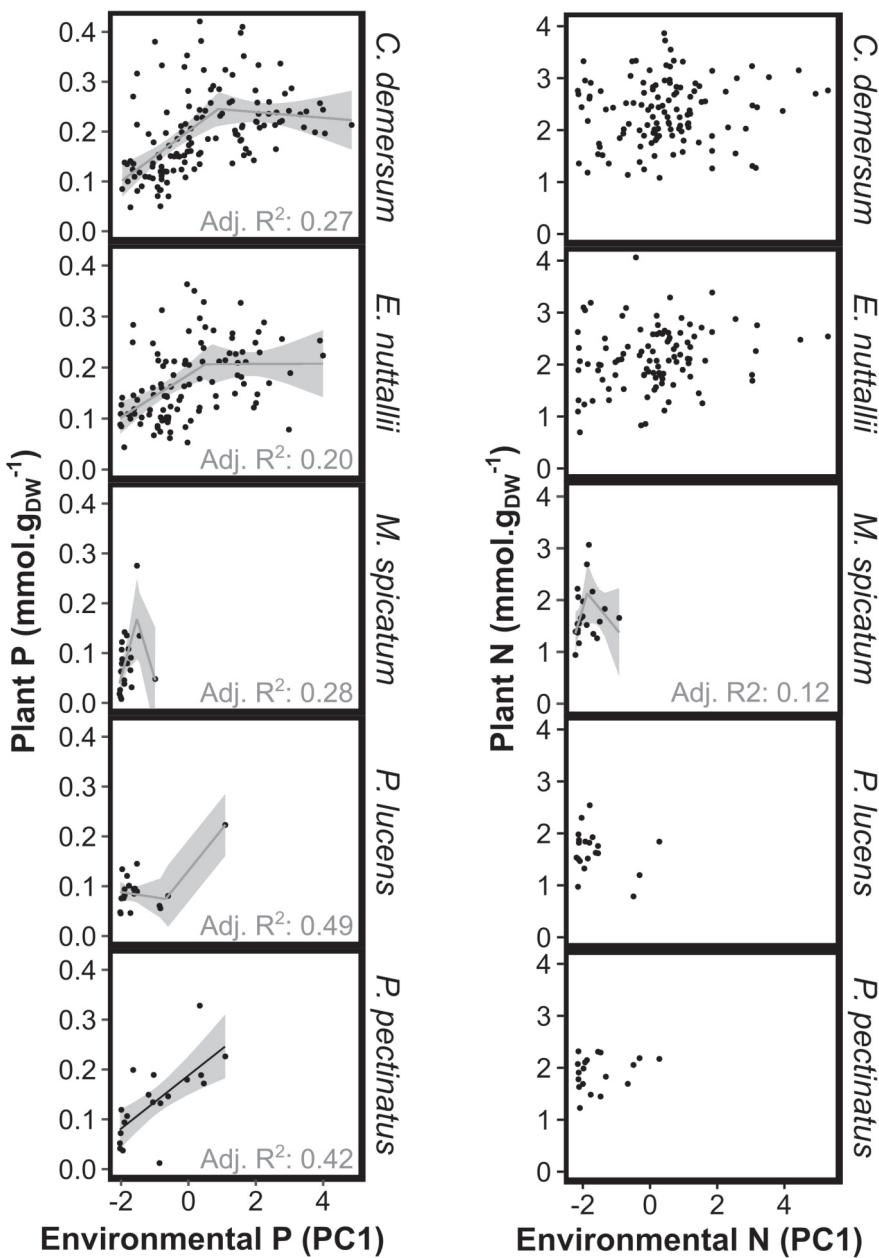
Significant within-site pairwise differences among species were more frequently found for shoot concentrations of P than for N (Table 4.1). Shoot P concentration of *E. nuttallii* was similar to that of *C. demersum*, but significantly higher than in all of the other species. Shoot P concentration in *C. demersum* was significantly higher than in *P. pectinatus*. *E. nuttallii*'s shoot N concentration was significantly higher than in *P. pectinatus* and similar to the remaining species.



**Figure 4.2:** Range of shoot phosphorous (P: top panel), nitrogen (N: middle panel), and carbon (C: bottom panel) concentration ( $\text{mmol.g}_{\text{dw}}^{-1}$ ) of the sampled macrophyte species. White dots show the individual data points, bars show the range. CD: *C. demersum*, EN: *E. nuttallii*, MS: *M. spicatum*, PL: *P. lucens*, and PP: *P. pectinatus*.

**Table 4.1:** Pairwise within-site differences in shoot nitrogen (N) and phosphorous (P) contents among five submerged aquatic plant species. The values show the mean differences in shoot nutrient content ( $\delta \text{ mmol.g}_{\text{dw}}^{-1}$ ) between the pair of species and the statistical significance of the difference (at  $p < 0.05$  \*). When the value is positive, the tissue nutrient content of the species in top row was higher than the contents of the species on the left. Bold values highlight statistically significant differences. The last panel (Cases) shows the number of sites in which each pair of species co-occurred. Species are abbreviated as: CD (*C. demersum*), EN (*E. nuttallii*), MS (*M. spicatum*), PL (*P. lucens*), and PP (*P. pectinatus*). The 'Grand mean' N and P contents ( $\text{mmol.g}_{\text{dw}}^{-1}$ ) of each species show the overall mean tissue nutrient content for each species sampled in this study. Different superscript letters indicate statistically significant differences in shoot nutrient content among species (ANOVA: N:  $F_{4,304}=11.8$   $p<0.001$ ; P:  $F_{4,304}=19.9$   $p<0.001$  with Tukey post-hoc tests).

N	CD	EN	MS	PL	PP
<b>EN</b>	0.03				
<b>MS</b>	-0.11	0.12			
<b>PL</b>	0.49	0.19	0.15		
<b>PP</b>	0.47	0.62*	0.03	-0.33	
<i>Grand mean</i> ( $\text{mmol.g}_{\text{dw}}^{-1}$ )	2.33 <sup>A</sup>	2.11 <sup>B</sup>	1.72 <sup>C</sup>	1.68 <sup>C</sup>	1.89 <sup>BC</sup>
P	CD	EN	MS	PL	PP
<b>EN</b>	0.004				
<b>MS</b>	-0.021	0.052*			
<b>PL</b>	0.054	0.029*	0.024		
<b>PP</b>	0.086*	0.059*	-0.050	0.010	
<i>Grand mean</i> ( $\text{mmol.g}_{\text{dw}}^{-1}$ )	0.197 <sup>A</sup>	0.170 <sup>B</sup>	0.082 <sup>C</sup>	0.091 <sup>C</sup>	0.130 <sup>BC</sup>
Cases	CD	EN	MS	PL	PP
<b>EN</b>	78				
<b>MS</b>	5	5			
<b>PL</b>	9	8	5		
<b>PP</b>	10	10	4	7	
<i>Grand total (n)</i>	135	111	22	21	20



**Figure 4.3:** Associations between the environmental phosphorous (P) or nitrogen (N) availability (i.e. axis 1 extracted from the PCA) and shoot P or N contents of the five submerged plant species. The dots show the unique data points, the lines show the model fit, and the grey area shows the 95% confidence limits of the model. Only the best fitting model is plotted (blue line for the linear model and red line for the segmented linear model). When environmental nutrient availability could not explain a significant part of the variation in plant tissue nutrient content (i.e.  $p > 0.05$ ), no line is drawn. For model details see Table S4.2.

## Discussion

### Variation in submerged plant tissue nutrient content

Autotroph flexibility in tissue elemental composition is generally considered to be high, as reported for phytoplankton (Elser & Urabe 1999; Hessen *et al.* 2013). While some common aquatic plant species have been described as being relatively homeostatic (Hao *et al.* 2013), our study clearly demonstrated high variability in tissue nutrient content under field conditions when sampled across large environmental nutrient gradients and with sufficient sampling intensity. We found a similar range in intra-specific submerged plant nutrient content in the field as reported in reviews for submerged plants in general (Duarte 1992; Garbey *et al.* 2004) and a slightly lower range than reported for phytoplankton in reviews (Duarte 1992; van de Waal *et al.* 2012). Our study also provides compelling evidence for the existence of non-linear responses of plant tissue nutrient content along a gradient of nutrient supply under field conditions (Figure 4.3). Additionally, we also found inter-specific within-site differences in tissue nutrient content among several of the aquatic plant species.

### Associations with environmental nutrient content

Figure 4.1 illustrates that the relationship between environmental and plant nutrient content may not be uniform over the entire environmental nutrient gradient for all aquatic plant species (also see: Robach *et al.* 1995; Sistla *et al.* 2015). We found significant associations between environmental nutrient availability and plant tissue nutrients for P, but less so for N. In our study, the shoot P contents of *C. demersum* and *E. nuttallii* were similar when growing in the same site and significantly increased with increasing environmental P concentrations up until halfway along the sampled environmental nutrient gradient (Figure 4.3). Both species showed a breakpoint in this trend at similar levels of nutrient availability in their environment. In the upper half of the nutrient gradient, the P concentration in the shoots of these two species showed no clear association with environmental nutrient availability, possibly because P was no longer limiting here. This closely matches the ‘Flexible’ strategy depicted in our conceptual framework (Figure 4.1: solid blue line). Due to the limited nutrient range sampled for the other three species, it was impossible to fully characterize their response pattern. The P concentration in *P. pectinatus* shoots increased linearly with increasing environmental P, indicating a considerable degree of plasticity, at least in the lower ranges of nutrient availability, which corresponds with one of the two ‘flexible’ strategies. The linear association between *P. pectinatus* tissue P and environmental P could indicate that *P. pectinatus* is ‘Fully flexible’ (Figure 4.1: dashed blue line). However,

high nutrient sites in the dataset are lacking, preventing us from being able to discern between a 'flexible' and a 'fully flexible' strategy for this species. The P concentration in the shoots of *P. lucens* and *M. spicatum* was significantly associated with environmental P concentrations and the non-linear model (i.e. segmented) fitted the data best, but in both cases this was due to an outlier. Without outliers, a linear association between plant and environmental P concentrations was found for *M. spicatum*, while no statistically significant association was found for *P. lucens*.

The difficulty to predict the plant nutrient content with high accuracy from the environmental availability may stem from the fact that true environmental nutrient availability is difficult to accurately measure and because factors other than environmental nutrient availability can affect plant nutrient content and stoichiometry, for example water depth, temperature, light availability, life-history stage or growth form (Cronin & Lodge 2003; Demars & Edwards 2007; Ventura *et al.* 2008; Elser *et al.* 2011; Xia *et al.* 2014; Xing *et al.* 2015; Zhang *et al.* 2016).

## Implications for ecology

We want to stress that understanding how aquatic plants respond to environmental nutrient availability is challenging, but very important for limnology. Whereas phytoplankton and terrestrial plants mainly take up nutrients from respectively the water column and the soil (but see: Farquhar *et al.* 1980), aquatic plants can use multiple sources for resource acquisition, depending on their growth form (Lacoul & Freedman 2006). Rooted submerged plants, as we mostly used, are able to take up nutrients from both the water column and from the sediment. As a consequence, the relationship between environmental nutrient availability and plant nutrient content can be more complex compared to other groups of primary producers.

We showed that changing environmental nutrient availability may lead to variation in tissue nutrient content of submerged plants, but perhaps not over the entire nutrient gradient or for all elements in the same way (P versus N in our study), indicating P limitation along our student nutrient supply gradient. Due to inherent differences in nutrient requirements among species and species-specific habitat preferences, changes in environmental nutrient availability may also lead to a different species composition of the aquatic vegetation (Lacoul & Freedman 2006; Hirzel & Le Lay 2008; Bornette & Puijalon 2010). Changes in nutrient content of macrophytes at the community level (e.g. Demars & Edwards 2007; Frost & Hicks 2012) that result from changes in the availability of nutrients in the environment are therefore likely to be determined by a combination of changes in species composition, differences in nutrient content among these species and pronounced species-specific elemental plasticity (Teurlincx *et al.* 2017).

When plant tissue increases in nutrient content this can have clear impacts on nutrient cycling of aquatic ecosystems. Higher plant nutrient content will increase the release of nutrients to the water column (Granéli & Solander 1988). Sedimentation of more nutrient rich material will lead to an increase in sediment nutrient release (c.f. internal loading) (Hupfer & Lewandowski 2008) causing turbid conditions and eventual disappearance of submerged aquatic plants (Carpenter 1981a). This increased internal nutrient loading can hamper recovery of aquatic systems from eutrophication for extended periods of time (Søndergaard *et al.* 2003) and prevent development of species rich aquatic vegetation types (Hilt *et al.* 2018).

Variation in the carbon to nutrient stoichiometry of aquatic vegetation may affect its palatability (e.g. Dorenbosch & Bakker 2011) and therefore the resource uptake efficiency and performance of its consumers (e.g. Miler & Straile 2010). This, in turn, may have large effects on systems undergoing eutrophication or oligotrophication (van Altena *et al.* 2016) through changes at the food web level (Sardans *et al.* 2012; Hessen *et al.* 2013; Bakker *et al.* 2016). Because we found no association between environmental nutrient availability and plant tissue nutrient content in the higher part of the sampled environmental nutrient range, some plants may become more nutrient rich during eutrophication, but only up until a certain level of eutrophication. During continued eutrophication plant nutrient content and thus plant palatability may thus potentially remain unaffected by the increased nutrient availability. Alternatively, during oligotrophication, the plants' nutrient content may not be affected by the reduced nutrient availability at first, but might decrease after continued oligotrophication of eutrophic ecosystems. This suggests that at first plants can contain relatively high nutrient content in ecosystems subject to oligotrophication, which may result in high palatability and consumption, potentially inhibiting of plant growth and colonization (c.f. van Altena *et al.* 2016).

## Conclusions

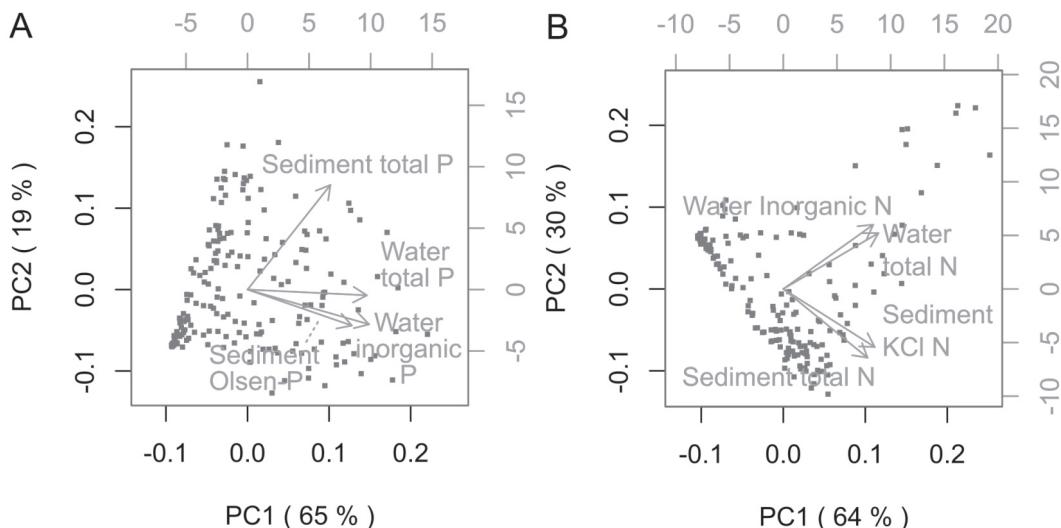
We have shown that submerged aquatic plants can be very flexible in their intra-specific N, and P concentration, and that this variation within several species was associated with nutrient availability in the field, for P, but not for N in most species. We show that this relationship can be linear, as well as non-linear in aquatic plants. We found that P concentration in several plant species increased with environmental availability in the lower environmental nutrient range, but not at high environmental nutrient availability. Moreover, we showed that field surveys aimed at assessing possible relationships between environmental and plant elemental nutrient concentrations under field conditions should include plant samples over the entire

range of environmental nutrient availability present in the species' niche. Together, this will help to better understand true intraspecific flexibility in tissue elemental composition and thereby help to better understand the consumer-resource dynamics of the food web of shallow freshwater ecosystems and its repercussions on the ecosystem level under changing environmental nutrient availability.

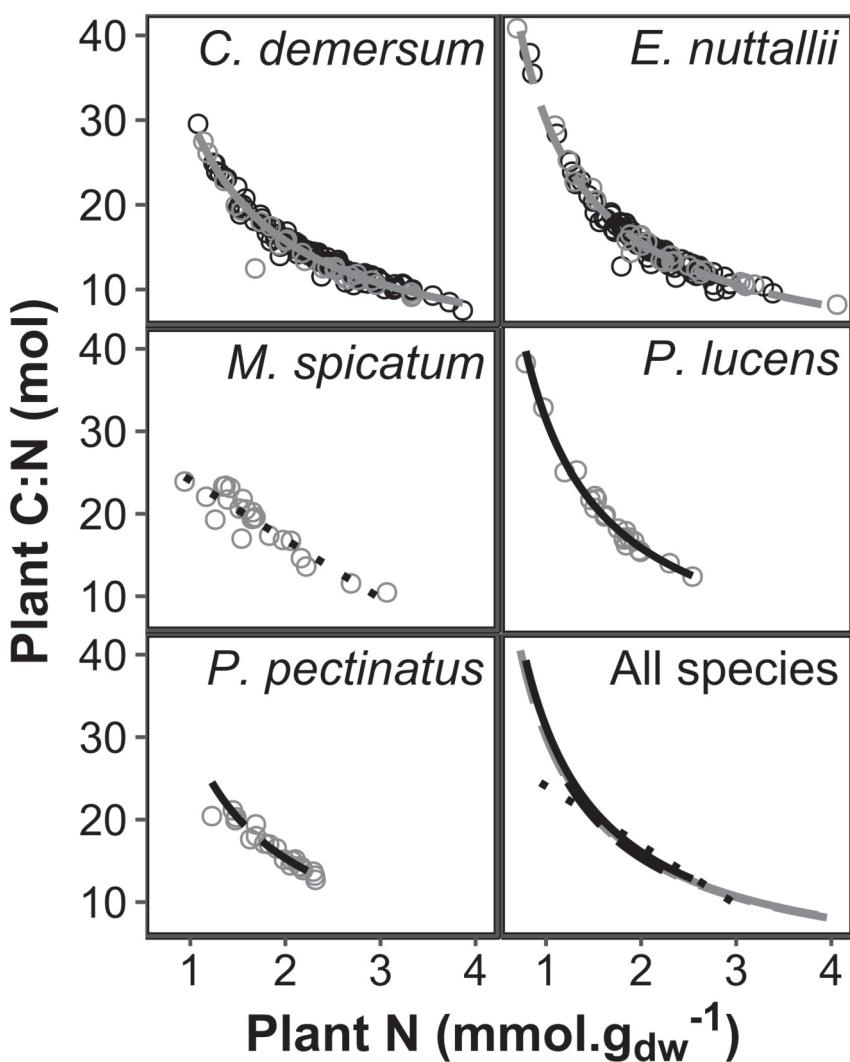
## Acknowledgements

We would like to thank Dennis Waasdorp and Erik Reichman for their valuable assistance with the analyses of the plant samples. We also wish to thank Marlies Gräwe, Marta Alirangues, and Annegreet Veeken for their tireless assistance with the field work. S. Teurlincx was financially supported by the Division for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO), by the Biodiversity Works Research Program (OBW) project no. 841.11.009. E.S. Bakker was funded by the Netherlands Organization for Scientific Research (NWO), VENI grant No. 863.07.006).

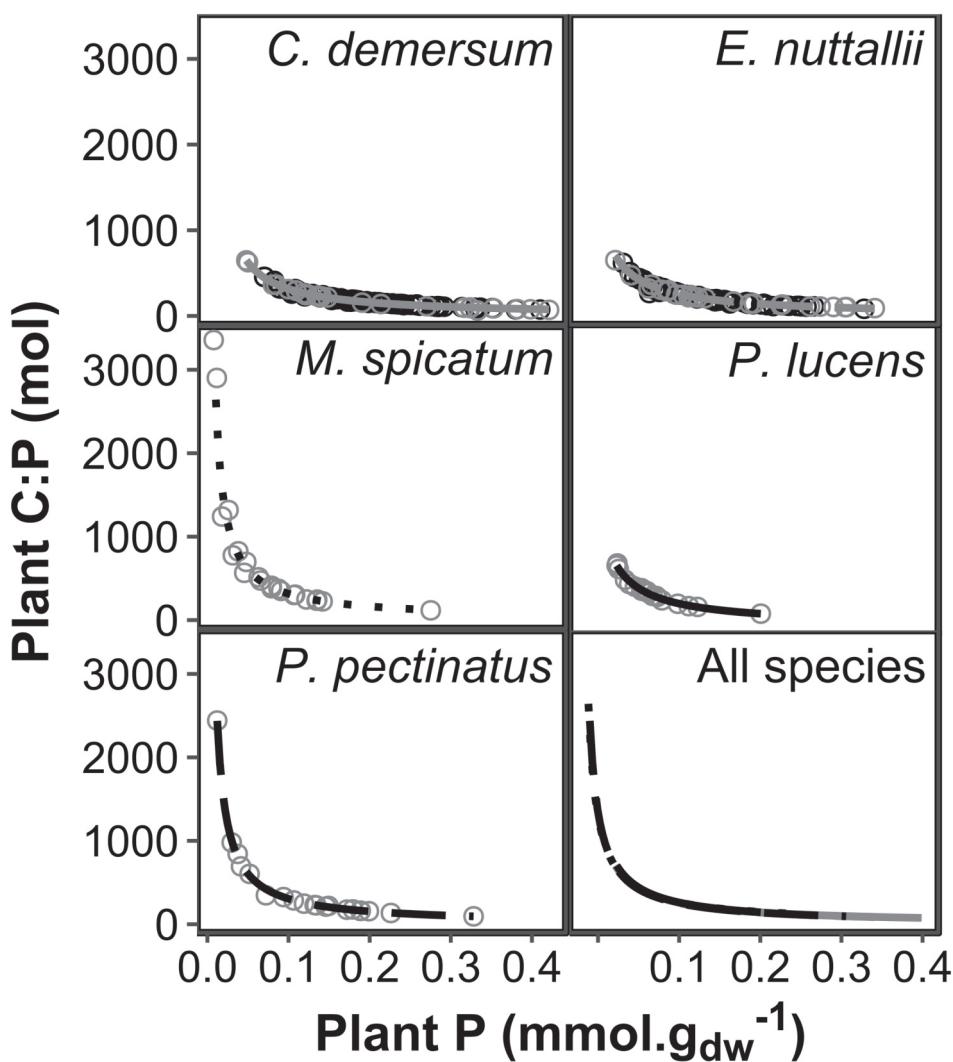
## Chapter 4: Supplementary materials



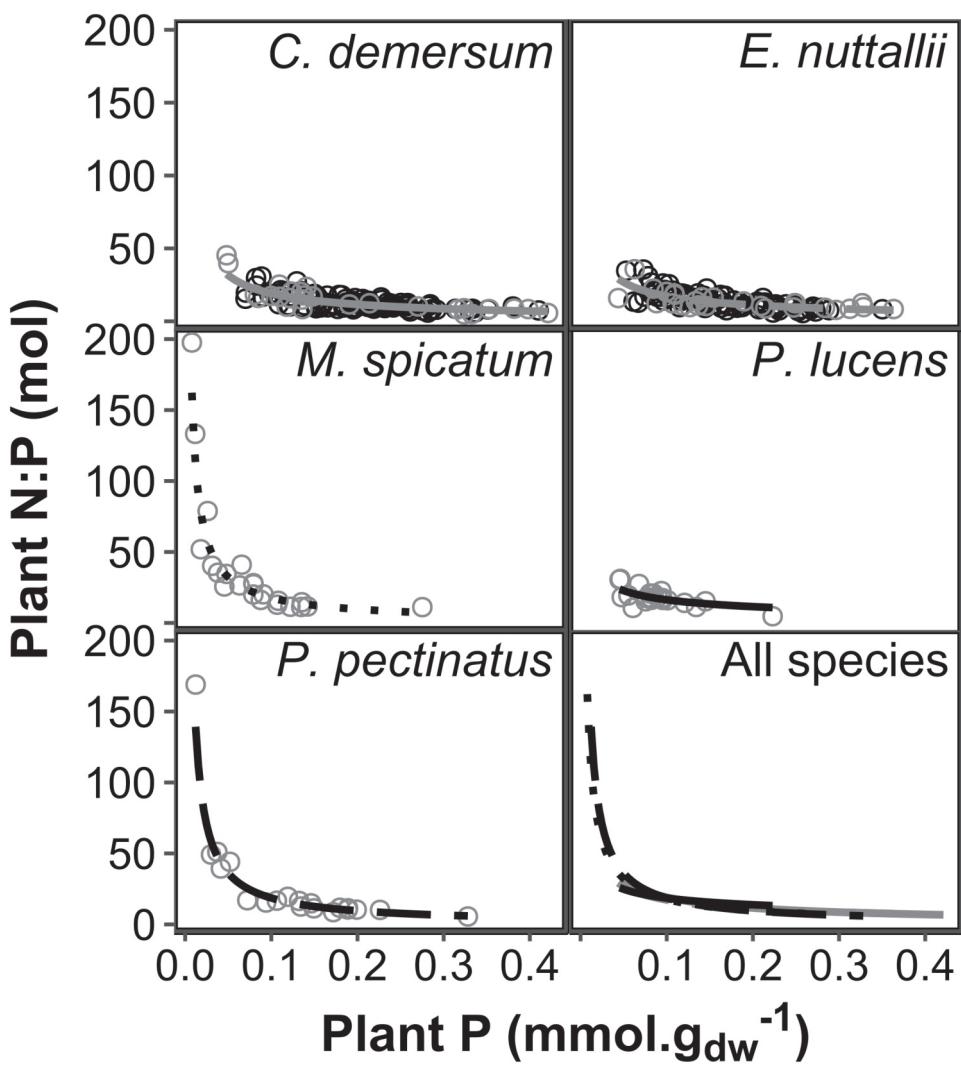
**Figure S4.1:** Principal component analyses of environmental phosphorous (P: panel A) and nitrogen (N: panel B) parameters (PCA analyses on all data, scaled and centered). Percentages between brackets in the axis title indicate the amount of variation in the data captured by the PC axis. Black axis labels are for the dots and red axis labels for the arrows. PC1 was used as a proxy for environmental nutrient availability in this study.



**Figure S4.2:** Plant carbon to nitrogen (C:N) ratios in relation to plant N or P concentration. The relationships between C:N and N were highly significant for all species ( $p < 0.001$ ; Adjusted  $R^2$ : 0.86-0.99).



**Figure S4.3:** Plant carbon to phosphorous (C:P) ratios in relation to plant N or P concentration. The relationships between C:P and P were highly significant for all species ( $p < 0.001$ ; Adjusted R<sup>2</sup>: 0.86-0.99).



**Figure S4.4:** Plant carbon to nitrogen (C:N), carbon to phosphorous (C:P), and nitrogen to phosphorous (N:P) ratios in relation to plant N or P concentration. The relationships between C:N and N and C:P and P were highly significant for all species ( $p < 0.001$ ; Adjusted  $R^2$ : 0.86-0.99). Shoot N:P was mainly related to the shoot's P concentration ( $p < 0.009$ ; Adjusted  $R^2$ : 0.29-0.90) and less so to N.

**Table S4.1:** Range (min-max) and mean  $\pm$  standard deviation of the carbon (C) concentration of the shoots of the plants and nitrogen (N), and phosphorous (P) concentration of the plants, water and sediment. For water and sediment, both plant available (free) and total amounts are shown. Number between brackets indicates sample size (n). Exponent indicates the applied power of 10, for example:  $9^{-3} = 9 \times 10^{-3} = 0.009$ .

		<i>Ceratophyllum demersum</i>	<i>Elodea nuttallii</i>	<i>Myriophyllum spicatum</i>	<i>Potamogeton lucens</i>	<i>Potamogeton pectinatus</i>
Plant	C (mmol.g <sub>dw</sub> <sup>-1</sup> )	21.0-34.5 31.6 $\pm$ 1.67 (135)	22.8-34.1 <b>31.0<math>\pm</math>1.65</b> (111)	22.5-34.4 <b>30.8<math>\pm</math>3.20</b> (22)	29.8-33.6 <b>31.9<math>\pm</math>1.12</b> (21)	25.0-32.8 <b>30.3<math>\pm</math>1.55</b> (20)
	N (mmol.g <sub>dw</sub> <sup>-1</sup> )	1.08-3.86 <b>2.33<math>\pm</math>0.59</b> (135)	0.70-4.06 <b>2.11<math>\pm</math>0.58</b> (111)	0.94-3.07 <b>1.72<math>\pm</math>0.49</b> (22)	0.79-2.54 <b>1.68<math>\pm</math>0.41</b> (21)	1.23-2.32 <b>1.89<math>\pm</math>0.33</b> (20)
	P (mmol.g <sub>dw</sub> <sup>-1</sup> )	4.80 <sup>-2</sup> -4.21 <sup>-1</sup> 1.97 <sup>-1</sup> $\pm$ 7.99 <sup>-2</sup> (135)	4.40 <sup>-2</sup> -3.63 <sup>-1</sup> 1.70 <sup>-1</sup> $\pm$ 7.20 <sup>-2</sup> (111)	7.80 <sup>-3</sup> -2.75 <sup>-1</sup> 8.16 <sup>-2</sup> $\pm$ 5.97 <sup>-2</sup> (22)	4.53 <sup>-2</sup> -2.23 <sup>-1</sup> 9.05 <sup>-2</sup> $\pm$ 4.04 <sup>-2</sup> (21)	1.20 <sup>-2</sup> -3.28 <sup>-1</sup> 1.30 <sup>-1</sup> $\pm$ 7.84 <sup>-2</sup> (20)
Surface water	Free N (mmol.L <sup>-1</sup> )	5.00 <sup>-4</sup> -4.24 <sup>-1</sup> 6.09 <sup>-2</sup> $\pm$ 8.89 <sup>-2</sup> (135)	0-3.96 <sup>-1</sup> 5.16 <sup>-2</sup> $\pm$ 7.95 <sup>-2</sup> (111)	2.50 <sup>-4</sup> -4.50 <sup>-2</sup> 3.78 <sup>-3</sup> $\pm$ 9.37 <sup>-3</sup> (22)	4.40 <sup>-4</sup> -6.72 <sup>-2</sup> 7.98 <sup>-3</sup> $\pm$ 1.80 <sup>-2</sup> (21)	2.80 <sup>-4</sup> -6.72 <sup>-2</sup> 9.56 <sup>-3</sup> $\pm$ 1.93 <sup>-2</sup> (20)
	Free P (mmol.L <sup>-1</sup> )	0-1.57 <sup>-1</sup> 1.53 <sup>-2</sup> $\pm$ 2.02 <sup>-2</sup> (135)	0-1.57 <sup>-1</sup> 1.15 <sup>-2</sup> $\pm$ 2.08 <sup>-2</sup> (111)	0-9.11 <sup>-4</sup> 4.93 <sup>-5</sup> $\pm$ 1.96 <sup>-4</sup> (22)	0-2.34 <sup>-2</sup> 1.16 <sup>-3</sup> $\pm$ 5.10 <sup>-3</sup> (21)	0-2.52 <sup>-2</sup> 5.81 <sup>-3</sup> $\pm$ 8.82 <sup>-3</sup> (20)
	Total N (mmol.L <sup>-1</sup> )	6.00 <sup>-3</sup> -5.06 <sup>-1</sup> 9.34 <sup>-2</sup> $\pm$ 1.02 <sup>-1</sup> (135)	2.80 <sup>-3</sup> -4.44 <sup>-1</sup> 8.00 <sup>-2</sup> $\pm$ 8.77 <sup>-2</sup> (111)	2.00 <sup>-3</sup> -5.37 <sup>-2</sup> 1.35 <sup>-2</sup> $\pm$ 1.10 <sup>-2</sup> (22)	4.00 <sup>-4</sup> -6.72 <sup>-2</sup> 1.67 <sup>-2</sup> $\pm$ 1.71 <sup>-2</sup> (21)	4.30 <sup>-3</sup> -6.88 <sup>-2</sup> 2.04 <sup>-2</sup> $\pm$ 1.81 <sup>-2</sup> (20)
Sediment	Total P (mmol.L <sup>-1</sup> )	3.00 <sup>-4</sup> -1.66 <sup>-1</sup> 2.08 <sup>-2</sup> $\pm$ 1.99 <sup>-2</sup> (135)	2.00 <sup>-4</sup> -1.66 <sup>-1</sup> 1.87 <sup>-2</sup> $\pm$ 2.32 <sup>-2</sup> (111)	2.15 <sup>-4</sup> -1.46 <sup>-3</sup> 6.31 <sup>-4</sup> $\pm$ 2.98 <sup>-4</sup> (22)	2.10 <sup>-4</sup> -2.63 <sup>-2</sup> 2.05 <sup>-3</sup> $\pm$ 5.58 <sup>-3</sup> (21)	2.10 <sup>-4</sup> -2.71 <sup>-2</sup> 8.04 <sup>-3</sup> $\pm$ 1.02 <sup>-2</sup> (20)
	Free N (mmol.g <sub>dw</sub> <sup>-1</sup> )	5.00 <sup>-4</sup> -2.43 <sup>-2</sup> 1.15 <sup>-2</sup> $\pm$ 5.56 <sup>-3</sup> (132)	3.00 <sup>-4</sup> -2.43 <sup>-2</sup> 1.03 <sup>-2</sup> $\pm$ 6.15 <sup>-3</sup> (107)	1.20 <sup>-4</sup> -4.10 <sup>-3</sup> 1.44 <sup>-3</sup> $\pm$ 1.14 <sup>-3</sup> (22)	6.00 <sup>-5</sup> -1.28 <sup>-2</sup> 2.56 <sup>-3</sup> $\pm$ 3.20 <sup>-3</sup> (20)	2.20 <sup>-4</sup> -1.28 <sup>-2</sup> 2.85 <sup>-3</sup> $\pm$ 3.37 <sup>-3</sup> (19)
	Free P (mmol.g <sub>dw</sub> <sup>-1</sup> )	1.50 <sup>-4</sup> -7.52 <sup>-3</sup> 2.46 <sup>-3</sup> $\pm$ 1.79 <sup>-3</sup> (134)	1.00 <sup>-4</sup> -7.52 <sup>-3</sup> 1.82 <sup>-3</sup> $\pm$ 1.70 <sup>-3</sup> (108)	3.90 <sup>-5</sup> -1.69 <sup>-3</sup> 3.49 <sup>-4</sup> $\pm$ 3.64 <sup>-4</sup> (22)	9.60 <sup>-5</sup> -2.79 <sup>-3</sup> 8.14 <sup>-4</sup> $\pm$ 7.97 <sup>-4</sup> (20)	4.60 <sup>-5</sup> -2.79 <sup>-3</sup> 9.31 <sup>-4</sup> $\pm$ 9.23 <sup>-4</sup> (19)
Sediment	Total N (mmol.g <sub>dw</sub> <sup>-1</sup> )	0-1.78 1.20 $\pm$ 5.30 <sup>-1</sup> (123)	0-1.78 1.05 $\pm$ 5.54 <sup>-1</sup> (104)	0-1.14 2.05 <sup>-1</sup> $\pm$ 3.21 <sup>-1</sup> (22)	0-1.81 3.40 <sup>-1</sup> $\pm$ 5.45 <sup>-1</sup> (21)	0-1.81 4.26 <sup>-1</sup> $\pm$ 6.34 <sup>-1</sup> (20)
	Total P (mmol.g <sub>dw</sub> <sup>-1</sup> )	2.80 <sup>-3</sup> -1.78 <sup>-1</sup> 2.96 <sup>-2</sup> $\pm$ 1.91 <sup>-2</sup> (135)	1.10 <sup>-32</sup> -1.78 <sup>-1</sup> <b>2.89<sup>-2</sup><math>\pm</math>2.05</b> (111)	1.13 <sup>-3</sup> -2.55 <sup>-2</sup> 6.43 <sup>-3</sup> $\pm$ 6.38 <sup>-3</sup> (22)	1.10 <sup>-3</sup> -3.35 <sup>-2</sup> 1.03 <sup>-2</sup> $\pm$ 1.06 <sup>-2</sup> (21)	1.00 <sup>-3</sup> -3.35 <sup>-2</sup> 1.39 <sup>-2</sup> $\pm$ 1.26 <sup>-2</sup> (20)

**Table S4.2:** Associations between plant and environmental nutrient concentrations as described by the different statistical models used (i.e. linear and segmented linear). For the segmented model, Slope 2 - 1 indicates the difference between the slope of the second segment and the slope of the first segment. The segmented model could not identify any breakpoint in the data of *E. nuttallii*'s N concentration, indicated by 'NA'.

Nutrient	Model	Parameters	Estimate	SE	t-value	p-value	Adj. R2	AIC
<i>Ceratophyllum demersum</i>	Null	Intercept	2.329	0.051				243.1
	Linear	Intercept	2.325	0.058	39.80	<0.001	0.00	225.9
		Slope	0.040	0.037	1.07	0.287		
		Intercept	1.270	2.629	0.48	0.630		
	Segmented linear	Slope 1	-0.589	1.346	-0.44	0.662	0.00	228.4
		Slope 2 - 1	0.655	1.347	0.49	0.628		
<i>P</i>		Breakpoint	-1.556	0.871				
	Null	Intercept	0.197	0.007				-296.1
	Linear	Intercept	0.186	0.007	28.36	<0.001	0.20	-315.8
		Slope	0.023	0.004	5.78	<0.001		
		Intercept	0.201	0.009	21.19	<0.001		
	Segmented linear	Slope 1	0.050	0.010	4.83	<0.001	0.27	-325.5
<i>E. nuttallii</i>		Slope 2 - 1	-0.056	0.015	-3.74	<0.001		
		Breakpoint	0.886	0.430				
	Null	Intercept	2.112	0.055				197.5
	Linear	Intercept	2.126	0.059	35.99	<0.001	0.02	181.6
		Slope	0.076	0.041	1.85	0.067		
		Intercept	NA	NA	NA	NA	NA	NA
<i>M. spicatum</i>	Segmented linear	Slope 1	NA	NA	NA	NA	NA	NA
		Slope 2 - 1	NA	NA	NA	NA		
		Breakpoint	NA	NA				
	Null	Intercept	0.170	0.007				-266.2
	Linear	Intercept	0.170	0.006	26.19	<0.001	0.17	-267.2
		Slope	0.022	0.005	4.72	<0.001		
<i>N</i>		Intercept	0.186	0.011	16.67	<0.001		
	Segmented linear	Slope 1	0.042	0.011	3.83	<0.001	0.20	-269.1
		Slope 2 - 1	-0.041	0.017	-2.37	0.020		
		Breakpoint	0.500	0.629				
	Null	Intercept	1.716	0.105				34.3
	Linear	Intercept	2.068	0.633	3.27	0.004	-0.03	36.0
<i>M. spicatum</i>		Slope	0.188	0.332	0.56	0.579		
		Intercept	6.161	2.403	2.56	0.020		
	Segmented linear	Slope 1	2.188	1.166	1.88	0.077	0.12	34.0
		Slope 2 - 1	-2.985	1.324	-2.25	0.037		
		Breakpoint	-1.842	0.133				

**Table S4.2 (continued)**

Nutrient	Model	Parameters	Estimate	SE	t-value	p-value	Adj. R2	AIC
<i>Myriophyllum spicatum</i>	P	Null	Intercept	0.082	0.013			-58.6
		Linear	Intercept	0.208	0.095	2.18	0.042	0.04 -58.5
		Slope	0.069	0.052	1.33	0.197		
	N	Segmented linear	Intercept	0.548	0.200	2.74	0.014	
		Slope 1	0.248	0.105	2.36	0.030	0.28	-63.2
		Slope 2 - 1	-0.474	0.161	-2.95	0.009		
<i>Potamogeton lucens</i>	P	Null	Breakpoint	-1.528	0.119			
		Linear	Intercept	1.678	0.089			24.71
		Slope	-0.154	0.136	-1.13	0.273	0.01	24.7
	N	Segmented linear	Intercept	0.974	0.456	2.14	0.049	
		Slope 1	-0.382	0.242	-1.58	0.134	0.05	25.5
		Slope 2 - 1	1.476	0.988	1.49	0.155		
<i>Potamogeton pectinatus</i>	P	Null	Breakpoint	-0.382	0.393			
		Linear	Intercept	0.091	0.009			-72.2
		Slope	0.140	0.018	7.84	<0.001	0.30	-74.0
	N	Segmented linear	Intercept	0.032	0.011	3.00	0.008	
		Slope 1	-0.010	0.020	-0.52	0.612	0.49	-78.9
		Slope 2 - 1	0.094	0.032	2.97	0.009		
<i>P</i>	N	Null	Breakpoint	-0.678	0.398			
		Linear	Intercept	1.890	0.073			14.8
		Slope	2.078	0.175	11.89	0.000	0.01	14.2
	P	Segmented linear	Intercept	0.108	0.103	1.05	0.309	
		Slope 1	-19.293	23.735	-0.81	0.429		
		Slope 2 - 1	-9.980	11.149	-0.90	0.385	-0.01	16.1
<i>Potamogeton pectinatus</i>	N	Breakpoint	10.147	11.150	0.91	0.377		
		Null	-2.110	0.028				
		Linear						
	P	Intercept	0.130	0.018				-42.1
		Intercept	0.188	0.018	10.26	0.000	0.46	-51.4
		Slope	0.053	0.013	4.04	0.001		
<i>P</i>	N	Intercept	0.487	0.594	0.82	0.425		
		Segmented linear	Slope 1	0.210	0.302	0.69	0.498	0.42 -48.6
		Slope 2 - 1	-0.170	0.303	-0.56	0.583		
	P	Breakpoint	-1.757	0.434				



# 5

## Species sorting and stoichiometric plasticity control community C:P ratio of first-order aquatic consumers

**Sven Teurlincx, Mandy Velthuis, Dominika Seroka, Lynn Govaert, Ellen van Donk,  
Dedmer B. Van de Waal, Steven A.J. Declerck**

*This chapter is based on the paper: Teurlincx, S., M. Velthuis, D. Seroka, L. Govaert, E. van Donk, D. B. Van de Waal, and S. A. J. Declerck. 2017. Species sorting and stoichiometric plasticity control community C:P ratio of first-order aquatic consumers. Ecology Letters 20:751-760.*

## Abstract

Ecological stoichiometry has proven to be invaluable for understanding consumer response to changes in resource quality. Although interactions between trophic levels occur at the community level, most studies focus on single consumer species. In contrast to individual species, communities may deal with trophic mismatch not only through elemental plasticity but also through changes in species composition. Here we show that a community of first order consumers (e.g. zooplankton) is able to adjust its stoichiometry (C:P) in response to experimentally induced changes in resource quality, but only to a limited extent. Furthermore, using the Price equation framework we show the importance of both elemental plasticity and species sorting. These results illustrate the need for a community perspective in ecological stoichiometry, requiring consideration of species-specific elemental composition, intra-specific elemental plasticity and species turnover.

# Introduction

Several main drivers of global change, such as eutrophication and increasing atmospheric CO<sub>2</sub>-concentrations, result in strong alterations of the amounts and ratios of essential elements available to ecosystems (Falkowski *et al.* 2000; Waal *et al.* 2010). Ecological stoichiometry (Sterner & Elser 2002; Hessen *et al.* 2013) represents a powerful research avenue that may contribute to a better understanding of how such anthropogenic alterations may affect the functioning of ecosystems (Elser *et al.* 2009). Although ratios of elements such as carbon (C), nitrogen (N) and phosphorus (P) vary widely in nature, the elemental composition of organisms is confined more strictly. Organisms tend to be stoichiometrically homeostatic, because they are composed of biomolecules with specific elemental ratios (e.g., proteins, lipids, carbohydrates, nucleic acids) and because they need to create a stable internal environment suitable for essential cellular processes (Meunier *et al.* 2014). Nevertheless, primary producers are known to be considerably more flexible in their C:N:P ratios than heterotrophs (Sterner & Elser 2002; Persson *et al.* 2010). Such plasticity may lead to mismatches with the elemental composition of consumers, reduce consumer performance and have implications for population dynamics and food web interactions (Andersen *et al.* 2004; Hall *et al.* 2004; Hessen *et al.* 2013). Additionally, high stoichiometric flexibility of producers may affect a variety of stoichiometry-regulated ecosystem functions, such as biogeochemical cycling and carbon sequestration (Mack *et al.* 2004; Dickman *et al.* 2006; Sistla *et al.* 2013). For this reason, there has been a long tradition of research on aspects of producer stoichiometric plasticity, including its main drivers (Goldman *et al.* 1979; Klausmeier *et al.* 2004) and potential ecosystem consequences (Sterner *et al.* 1997; Dickman *et al.* 2006; Van Donk *et al.* 2008; Mette *et al.* 2011; Sardans *et al.* 2012; Sistla & Schimel 2012; Plum *et al.* 2015).

So far, considerably less attention has been given to the potential roles of stoichiometric plasticity of consumers. An important reason for this is that consumers have traditionally been considered as being fixed in their elemental ratios (Andersen & Hessen 1991; Sterner & Elser 2002; Andersen *et al.* 2004). This view has been challenged by studies that demonstrate stoichiometric plasticity of primary consumers when exposed to nutrient-limited food (Demott & Pape 2005; Persson *et al.* 2010). Such observations have recently sparked an interest in the potential consequences of consumer plasticity for ecosystem functioning. Along with changes in biochemical quality (e.g. composition of fatty acids or sterols), plasticity in the elemental composition of consumers may be an important factor affecting the efficiency of energy transfer to higher food levels (Malzahn *et al.* 2007; Boersma *et al.* 2008; Rowland *et al.* 2015). Such quality-driven bottom-up effects may affect the biomass and

productivity of higher trophic levels and ultimately determine important ecosystem services like fish or lobster production (Dickman *et al.* 2006; Schoo *et al.* 2012). Consumers play a central role as nutrient recyclers and have the potential to influence the biomass, stoichiometry and community composition of producers through grazing and via regulation of nutrient supply (Elser *et al.* 2000, 2001; Vanni 2002; Hall 2009). The relative rates at which elements are excreted by consumers are largely dictated by the mismatch between the stoichiometric requirements of consumers and the elemental composition of their food source (Sterner 1990; Elser & Urabe 1999). The question of how nutrient-mediated feedback mechanisms between grazers and producers may be altered by elemental plasticity of the consumer communities is still poorly explored empirically, although recent theoretical work has pointed out its potential importance (Mulder & Bowden 2007; Wang *et al.* 2012).

Theoretical and experimental studies on the consequences of plasticity in consumer stoichiometry so far have mostly emphasized plasticity of single species in linear food webs (Plum *et al.* 2015). Evaluation of stoichiometric constraints for ecosystem functioning nevertheless requires consideration of stoichiometric plasticity at the level of entire communities. Upscaling from organismal to community level requires consideration of the fact that communities may consist of species with different elemental composition, both constitutively as well as in terms of their plasticity (Andersen & Hessen 1991; Persson *et al.* 2010). Changes in the elemental limitation of the food source may affect the relative performance of species in a community, which by itself may be function of body elemental composition (Danger *et al.* 2008). For example, taxa with high growth rates need to contain high levels of P in order to sustain high rates of protein synthesis via P-rich ribosomes (Elser *et al.* 1996; Main *et al.* 1997). Compared to low-P consumer taxa, they are expected to have superior performance when feeding on P-rich food but suffer stronger fitness reductions when food is P deficient. For this reason the prediction is that increases in the P content of the food source will result in community compositional shifts towards a dominance of fast growing P-rich consumers, whereas reductions in food P content are expected to result in relative increases of P-poor consumers (Sterner *et al.* 1997; Hall *et al.* 2004; Urabe *et al.* 2010). Stated more generally, along a gradient of environmental change, the stoichiometric response of a trophic level to a change in the stoichiometry of its resources is not only function of the elemental plasticity at the level of its individual constituent species, but also of potential shifts in species composition along the gradient (Schade *et al.* 2005; Dickman *et al.* 2006; Plum *et al.* 2015). Consequently, for a given trophic level, pronounced among-species variability in elemental composition may result in a degree of elemental plasticity that is larger than predicted from phenotypic variability at the organismal level alone (see also Danger *et al.* 2008).

Surprisingly, few attempts have been made to combine information on within- and among-species variability in the analysis of community level elemental ratios. Probably, this is due to lack of suitable datasets and an appropriate analytical framework that allows the simultaneous evaluation of the role of species-level phenotypic responses and community compositional change. One such framework may be found in Price's theorem that was recently extended for use in research on the relationship between biodiversity and ecosystem functioning (e.g. Loreau & Hector 2001; Winfree *et al.* 2015). The extended Price equation (Fox 2006; Fox & Kerr 2012) is a modified version of the original Price equation (Price 1970) and is developed to explain variation in an observed ecosystem function among a pair of sites by species gains, species losses and species' specific context-dependent contributions to the function. Actually, it can be applied to any additive community property, such as total community C and P content, and be used for the analysis of community elemental ratios (e.g. C:P). As such, the extended Price equation provides a promising tool for the study of the mechanisms underlying changes in stoichiometry at the community level. Here we demonstrate its value for the analysis of community elemental plasticity with data of zooplankton herbivore communities that were experimentally exposed to a gradient of seston stoichiometry in absence of predators. In outdoor mesocosms, we created different levels of seston C:P by manipulating P supply and light intensity in a multifactorial design. Our experiment is unique in that we assessed zooplankton community composition simultaneously with the elemental composition of all individual constituent species in each of the experimental treatments. Through application of the Price framework, our approach allows us to address the extent of community level elemental plasticity and to evaluate the impact of its major underlying drivers, i.e. organismal plasticity, and community assembly processes such as species' losses and gains and changes in the relative abundance of species.

## Methods

### Mesocosm experiment

In June of 2014 twenty outdoor mesocosms were filled with tap water (180L). The experimental design consisted of a 2 x 2 factorial combination of light and phosphorus (P), with 5 replicates per multifactorial combination. The goal of these experimental manipulations was to create variation in the stoichiometry of primary producers (phytoplankton). The factor levels for light were full day light ('Full light') or shade ('Shade'). Shade was created by placing layers of cloth over the mesocosm which reduced incoming photosynthetic active radiation (PAR) by 70%. The factor levels of P consisted of high (HP) or low (LP) P availability. At day 1, HP mesocosms initially received 71.03 µmol N/L (0.995 mg N/L) and 14.20 µmol P/L (0.44 mg P/L); LP mesocosms received 71.03 µmol N/L and 1.420 µmol P/L. This corresponded to initial N:P molar ratios of 5:1 in HP and 50:1 in the LP mesocosms. Throughout the experiment, 10% of the initial nutrient amounts were added twice a week to compensate for nutrient loss through sedimentation in the system (Hall *et al.* 2004). The experiment ran for a total of 11 weeks from 30-06-2014 to 12-09-2014. The mesocosms were inoculated with phytoplankton and zooplankton from 6 water bodies on day 6 and day 13, respectively yielding a total mesocosm volume of 183.5L. Seston and zooplankton samples were collected at the end of the experiment (day 74). Cladocerans were counted and identified at the species level, copepods at the order level. In addition, we measured the elemental C and P content of the seston and of each individual zooplankton species. For detailed information about experimental set-up, sampling and sample analysis, see Supplement S5.1.

### Calculation of total C, total P and community C:P ratio

We calculated total zooplankton community C and P content in each mesocosm as

$$C = \sum_i^n \bar{C}_i \cdot N_i \text{ and } P = \sum_i^n \bar{P}_i \cdot N_i$$

with  $N_i$  being the population density and  $C_i$  and  $P_i$  being the mean individual C and P content of species  $i$ , respectively. The two components were used to calculate the community C:P ratio.

### Explaining changes in total community C and P by compositional turnover and elemental plasticity

The main aim of our analysis was to investigate the response of zooplankton

community C:P to increasing seston C:P and evaluate if such response was caused by species turnover or by plasticity of species-specific elemental ratios. To this end, we applied the extended version of the Price equation by Fox and Kerr (2012). Application of this equation mathematically partitions the change in any additive community property among two mesocosms into at least three independent components (Fox & Kerr 2012): (1) a 'species richness effect' of gained and lost species ( $SRE = SRE_G + SRE_L$ ) which represents the extent by which the community property has changed as a result of random gain and loss of species, independent of the identity or traits of these species; (2) a 'species composition effect' of gained and lost species ( $SCE = SCE_G + SCE_L$ ) which quantifies the extent by which the community property has changed due to non-random species losses or gains, explicitly taking into account the specific contributions to this property of species that are lost and gained. (3) The 'context dependent effect' (CDE), unlike the SRE and SCE, represents between-mesocosm differences in contributions of species that are present in both mesocosms. CDE itself can be partitioned into three components (Fox 2006): a component resulting from changes in species' abundances between mesocosms ( $CDE_h$ ), a component resulting from individual species' phenotypic changes ( $CDE_p$ ) and a component caused by the altered abundance of species that also exhibit phenotypic change ( $CDE_i$ ). Although the partitioning of the CDE component has received little attention so far, it proves very useful for the purpose of our study because it allows disentangling the effects of changes in species abundance from phenotypic trait responses on community elemental content and ratios. We regrouped the Price partitions to focus on four components, i.e. net effects of species gains and losses ( $SRE + SCE$ ), contribution of species abundance changes ( $CDE_h$ ), effect of phenotypic changes ( $CDE_p$ ) and interaction between abundance and phenotypic change ( $CDE_i$ ).

Application of the Price equation requires that one mesocosm (the 'comparison') is compared to a reference mesocosm or 'baseline'. In our analyses, we evaluated the responses of community C and P to P reduction within light treatment levels using HP tanks as baseline and LP tanks as comparison. Similarly, responses to increasing light intensity within P treatment levels were studied by taking shaded tanks as baseline and full light tanks as comparison.

## Evaluating the impact of compositional turnover and plasticity on changes in community elemental ratios

Changes in total C and P content equal the sum of their Price components

$$\Delta C = \sum_j^4 \Delta C_{pj} ; \text{ resp. } \Delta P = \sum_j^4 \Delta P_{pj}$$

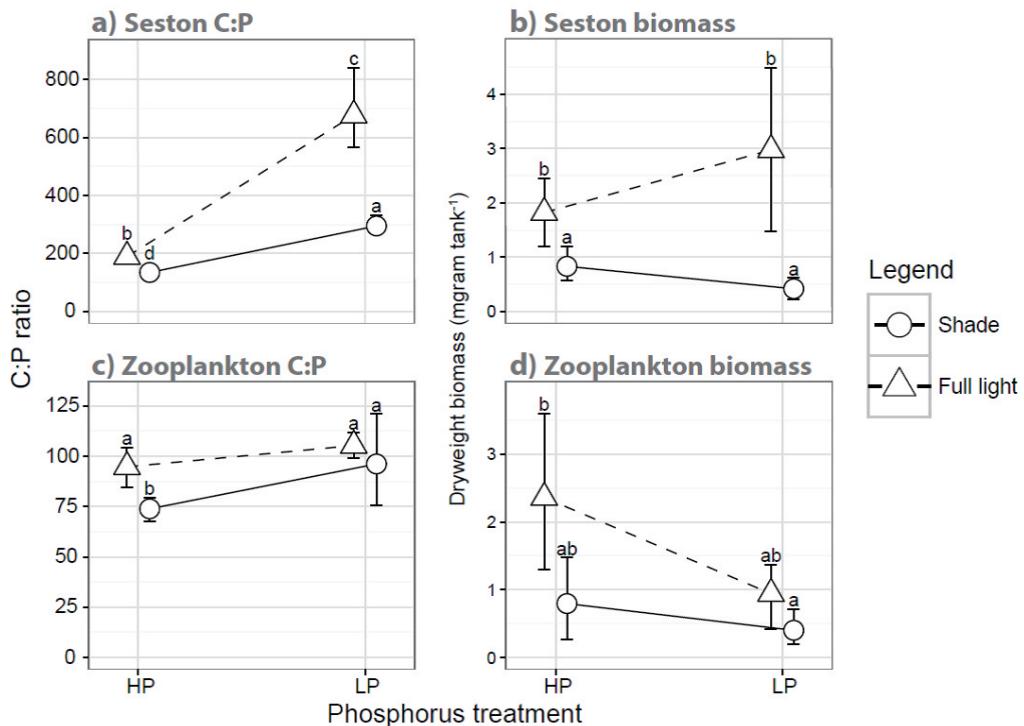
where  $\Delta C_{pj}$  and  $\Delta P_{pj}$  refer to changes in community C and P due to the individual Price component  $pj$ . Direct application of the Price equation to community C:P is not possible because the partitions of a ratio do not add up. Nevertheless, we can evaluate the relative effect of each Price component on the community C:P ratio separately, which requires the assumption of all else being equal. For each Price component  $pj$  the community content of C and P in the comparison can be estimated as  $C_{comp,pj} = C_{base} + \Delta C_{pj}$  and  $P_{comp,pj} = P_{base} + \Delta P_{pj}$  where  $C_{base}$  and  $P_{base}$  represent the community C and P content in the baseline. Hence, the expected unique effect of Price component  $pj$  on the community C:P in the comparison site can be evaluated as  $\Delta C:P_{pj} = C_{comp,pj} / P_{comp,pj} - C_{base} / P_{base}$ . Note that  $\Delta C_{pj}$  or  $\Delta P_{pj}$  of SRE+SCE and CDE<sub>n</sub> values can each be affected by changes in the relative abundance of its species as well as by total community abundance.  $\Delta C:P_{pj}$ -values, however, are not affected by a change in total abundance as C and P content are tied together within the individual organisms making up the community. Thus, increasing total abundance without changing species abundances relative to one another would increase both  $\Delta C_{pj}$  and  $\Delta P_{pj}$  proportionately and thereby cancel out effects in  $\Delta C:P_{pj}$ -values.

## Statistical analyses

Seston and zooplankton community biomass and C:P differences were analyzed with a full-factorial generalized linear model ( $\alpha=0.05$ ) with a gamma distribution and a log-link function to deal with heteroscedasticity. Post-hoc comparisons of treatments were carried out using Tukey contrasts, applying a correction for multiple comparisons (Westfall 1997). Differences between zooplankton taxa in C:P were assessed using a K-sample permutation test with 99,999 Monte-Carlo resamplings. The relationship between seston C:P and zooplankton community C:P was analyzed using a nonlinear logistic model. Community shifts in relative abundance due to experimental treatments were tested with a distance-based redundancy analysis (dbRDA, Legendre & Anderson 1999) using Odum's percentage difference dissimilarity. For any pair of contrasting treatments we tested whether Price components were significantly different from the components calculated among random combinations of replicates within these treatments using a two-sample location test (further details given in Supplement S5.1). All analyses were carried out in R (3.2.1) using a modified code of Winfree et al. (2015) and the packages coin, vegan and ggplot2 (Hothorn et al. 2006; Oksanen et al. 2015). All R code is archived in the Dryad depository belonging to this paper.

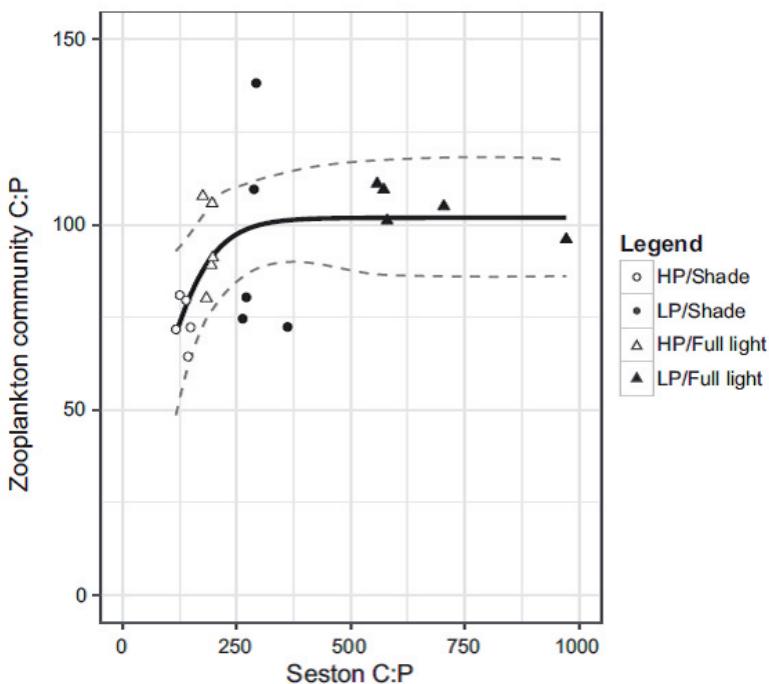
# Results

Our manipulations of phosphorus and light availability strongly influenced the C:P ratio and biomass of seston (Figure 5.1, Table S5.2.1), with response patterns being robust throughout the experiment (Figure S5.3.1). Seston C:P increased with P reduction and light availability (Figure 5.1). The increase in seston C:P with light was stronger under conditions of low compared to high P availability: under HP conditions, molar seston C:P increased from 135 to 189 with increasing light, whereas under LP conditions, seston C:P increased from 295 to 677. Seston biomass increased with increasing light availability but showed no significant response to P availability (Table S5.2.1). The phytoplankton communities were primarily composed of small cells (0-2 $\mu$ m and 2-30 $\mu$ m) of eukaryote phytoplankton and showed no significant differences in functional group composition between treatments (Figure S5.3.4).



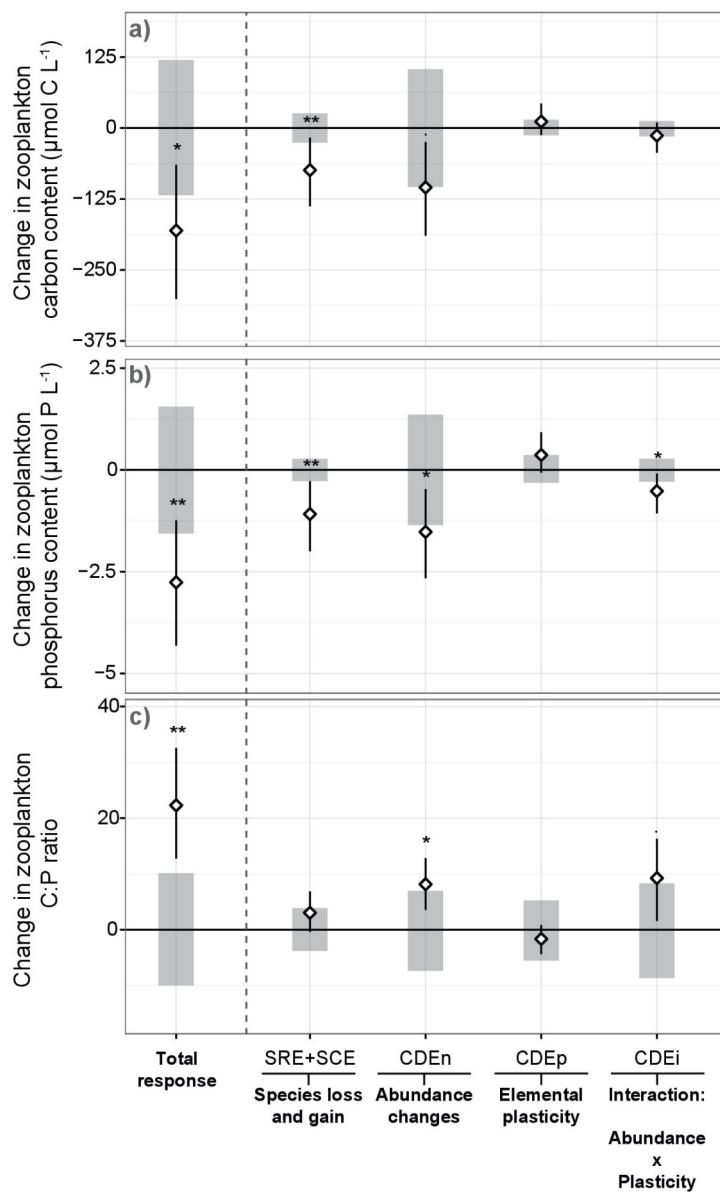
**Figure 5.1:** Reaction norm plots representing responses of seston (a), and zooplankton (c) C:P ratios and seston (b) and zooplankton (d) total tank biomass (mg dry weight) to phosphorus addition (HP, LP) and light treatments (triangles, dotted line: full light; circles, continuous line: shaded). Error bars indicate 97.5% confidence limits. Letters indicate significant pairwise differences.

Community level zooplankton C:P increased with decreasing P availability and increased with increasing light availability (Figure 5.1; Table S5.2.1). Zooplankton C:P responded in a non-linear fashion to variation in seston C:P (Figure 4.2). Starting from low levels, zooplankton C:P levels increased with increasing seston C:P, but levelled off beyond a seston C:P of 270 (Nonlinear logistic least square regression:  $R^2_{adj}=18.3\%$ , Asym=101.9, Xmid=69.3, scal=58.4, Table S5.2.4). A shift from low to high light under HP conditions resulted in a 29% increment of seston C:P (from 135 to 189; Figure 5.1a) and a 22% gain of the mean zooplankton C:P (from 74 to 95; Figure 5.1c). A decrease of P availability under shaded conditions caused a 54% augmentation of seston C:P (from 135 to 295) and a 22% increase of zooplankton C:P (i.e. from 74 to 95). In contrast, although seston C:P increased 3.6 fold (from 189 to 677) in response to a decrease in P availability under full light conditions, zooplankton C:P increased only 10% (from 95 to 105). Similarly, a 2.3 fold increase of seston (from 295 to 664) with increased light intensity under LP conditions was followed by a zooplankton C:P raise of 10% only (from 95 to 105). Zooplankton community biomass responded positively to increases in P and light availability (Figure 5.1d). Under conditions of full light, the increase in seston C:P associated with reduced P availability coincided with a strong decline in zooplankton biomass.

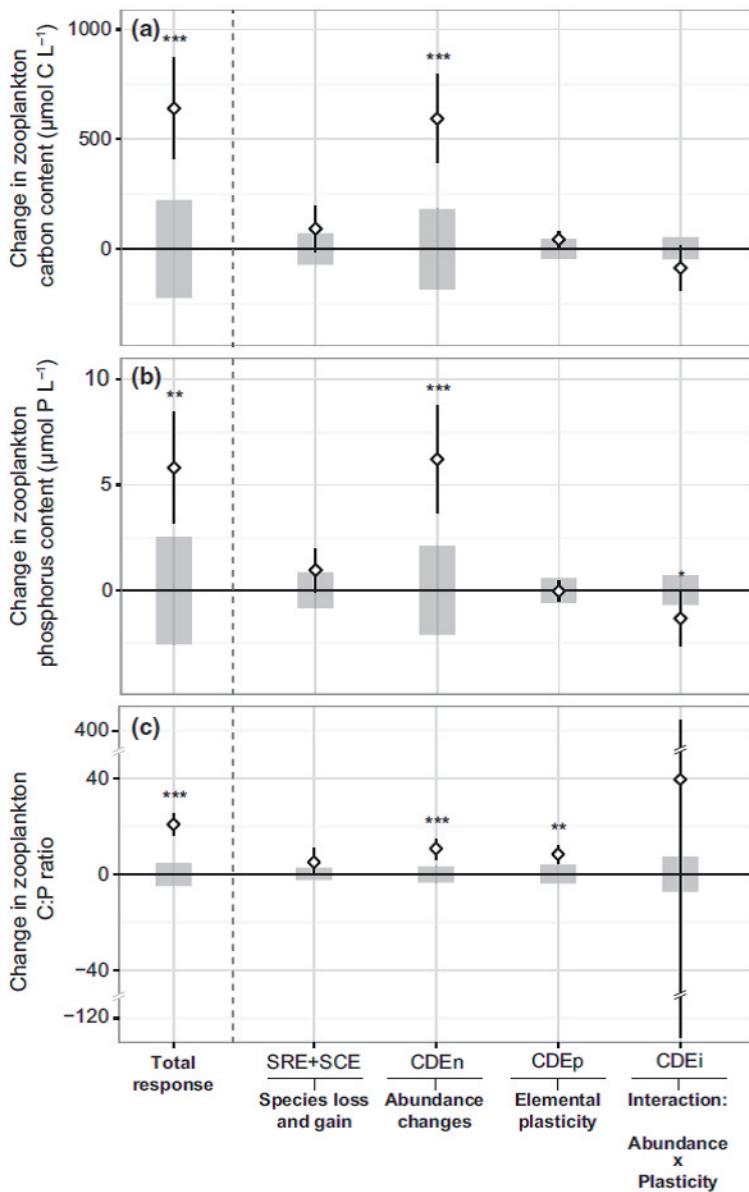


**Figure 5.2:** Response of community zooplankton C:P ratio to variation in the seston C:P ratio. The continuous line represents a nonlinear logistic regression curve with its 97.5% confidence interval (dashed lines).

Experimental treatment combinations for which significant shifts in zooplankton community C:P were observed (i.e. from HP to LP in the shade and from Shade to Full light in HP) were partitioned using the Price equation (Figure 5.3 and Figure 5.4, respectively). For both comparisons, we detected an increase in zooplankton community C:P of around 22 (Figure 5.3c & Figure 5.4c,f. Total response). With a reduction of P supply under shaded conditions, the net effect of species losses and gains resulted in reduced community C and P (Figure 5.3; SRE+SCE) but both effects canceled each other out and were therefore inconsequential for zooplankton community C:P. Declines in total abundance of resident species resulted in strong reductions of both community C and P content (Figure 5.3;  $CDE_n$ ). Here, changes in the relative abundances of species caused a proportionally stronger reduction of P than C content, and had a significant, positive impact on community C:P (Figure 5.3c). Plastic responses of species to a reduction of P availability were relatively weak (Figure S5.1) and had no influence on community C:P (Figure 5.3;  $CDE_p$ ), although a marginally significant effect of the  $CDE_i$  component (i.e. the interaction between  $CDE_p$  and  $CDE_n$ ) suggests that a change in the abundance of some plastic species may have influenced the community C:P.

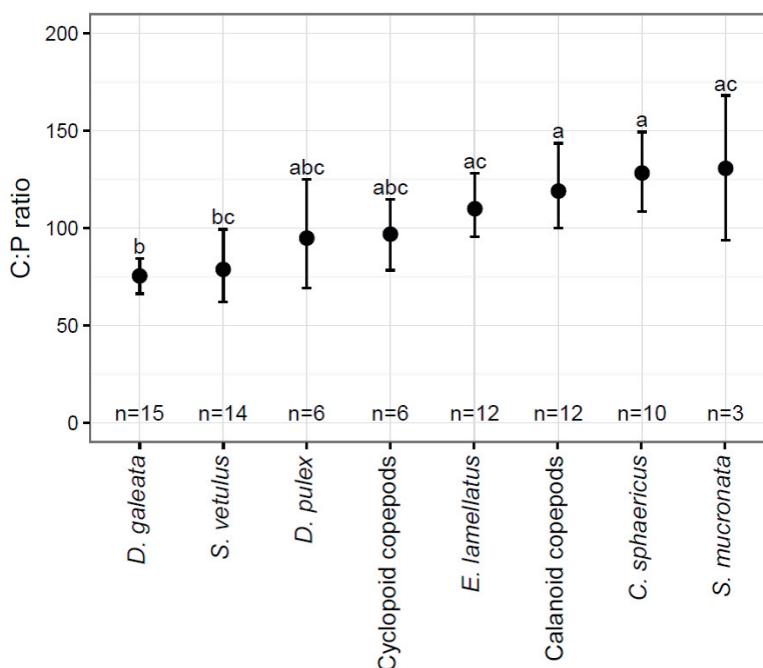


**Figure 5.3:** Price partitions of changes in the zooplankton community elemental content between HP and LP treatments under shaded conditions (baseline condition: HP/Shade treatment; comparison condition: LP/Shade treatment) and their effects on community elemental ratio. a) community C content; b) community P content; c) community C:P elemental ratio. For a detailed explanation on the individual Price partitions (SRE+SCE, CDE<sub>n</sub>, CDE<sub>p</sub> and CDE<sub>i</sub>), we refer to the Supplement S5.1. Error bars represent 97.5 % confidence limits. Grey bars represent the zero centered null distribution of values obtained by random permutations within treatments. Significance levels were assessed using a permutation based location test: \*\*\*:p<0.001, \*\*:p<0.01, \*:p<0.05, ::p<0.10.



**Figure 5.4:** Price partitions of changes in the zooplankton community elemental content between Shade and Full light under HP conditions (baseline condition: Shade/HP; comparison condition: Full light/HP) and their effects on community elemental ratio. a) community C content; b) community P content; c) community C:P elemental ratio. For a detailed explanation on the individual Price partitions (SRE+SCE, CDE<sub>n</sub>, CDE<sub>p</sub> and CDE<sub>i</sub>), we refer to Supplement S5.1. Error bars represent 97.5% confidence limits. Grey bars represent the zero centred null distribution of values obtained by random permutations within treatments. Significance levels were assessed using a permutation based location test: \*\*\*: $p < 0.001$ , \*\*: $p < 0.01$ , \*: $p < 0.05$ , : $p < 0.10$ .

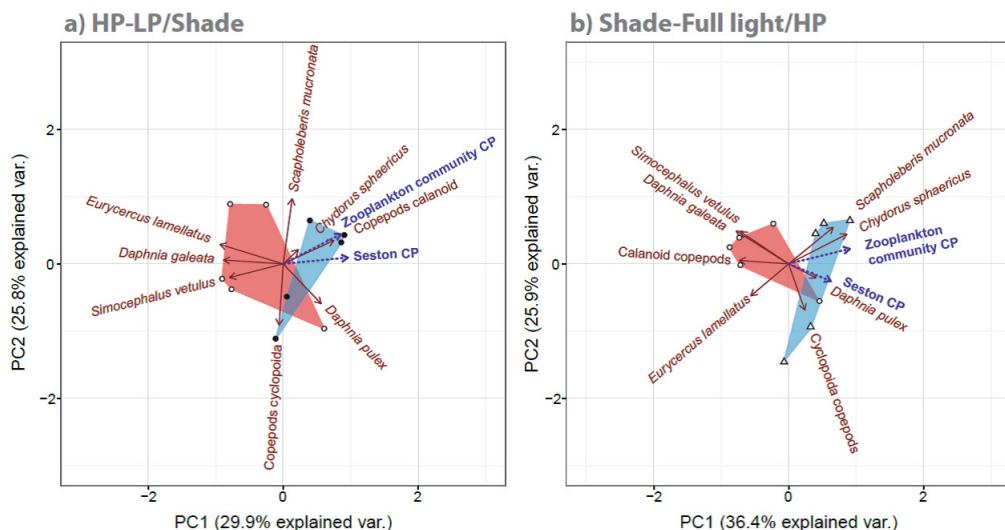
With an increase in light intensity under HP conditions, the combined effect of species losses and gains did not influence community C and P (Figure 5.4; SRE+SCE) although the gain component of SRE was found to have a positive contribution when considered in isolation. Total zooplankton abundance increased strongly and resulted in augmented community C and P content (Figure 5.4a-b;  $CDE_n$ ). A significant, positive impact of the  $CDE_n$  component on community C:P indicates a compositional shift towards more C-rich species. Plasticity ( $CDE_p$ ) had a significant impact on community C:P, where species per capita C content increased disproportionately compared to per capita P content (Figure 5.4).



**Figure 5.5:** Mean C:P ratio of different zooplankton taxa calculated across treatments. Error bars represent 97.5% confidence limits around the mean. Letters indicate significant pairwise differences.

The importance of community compositional shifts underlying zooplankton community C:P changes as revealed by Price partitioning is confirmed by additional analyses. The C:P ratio of species showed constitutive differences across treatments, with mean values ranging from 75 (*Daphnia galeata*) to 128 (*Chydorus sphaericus*, *Scapholeberis mucronata*) (Figure 5.5; Table S5.2.2). Furthermore, dbRDA analyses revealed significant shifts in zooplankton composition but only for the treatment combinations for which significant responses in zooplankton community C:P ratios

were observed (Table S5.2.3), i.e. the HP-LP comparison in shade (Figure 5.6a:  $R^2\text{adj} = 13.8\%$ , F: 2.446; P = 0.039) and the shade-full light comparison at HP (Figure 5.6b:  $R^2\text{adj} = 14.8\%$ , F: 2.561; P = 0.038). This pattern seemed to be mainly driven by the response of *D. galeata* to treatments associated with high seston C:P (see Figure S5.4.1 and Table S5.4.1). We observed an overall low responsiveness of body elemental composition of individual species to experimental treatments (Figure S5.1).



**Figure 5.6:** PCA plots of the zooplankton community composition along experimental treatments of a) HP to LP under shaded conditions and b) Shaded to Full light under HP conditions. Seston C:P and zooplankton community C:P ratio are plotted in blue (dashed arrow) as neutral variables. Open symbols indicate HP tanks and closed symbols LP tanks, circles represent shaded tanks whereas triangles represent full light tanks.

## Discussion

The question, to what extent elemental plasticity of consumers may affect food web interactions and ecosystem functions, has so far mainly been based on an evaluation of the phenotypic response of single species in simple linear food webs (Plum *et al.* 2015). In natural systems, however, consumer communities may consist of multiple species differing in elemental composition. As a result, along gradients of changing relative resource supply, community compositional shifts may result in community plasticity greater than that expected by the plasticity of individual species alone (Danger *et al.* 2008). This idea is confirmed by the results of our Price-based analysis which

demonstrated a relatively large impact of compositional shifts on community C:P ( $CDE_n$ -components in Figure 5.3c and Figure 5.4c) relative to phenotypic elemental plasticity of its constituent species ( $CDE_p$ -components in Figure 5.3c and Figure 5.4c). Indeed, species were found to differ in body elemental composition (Figure 5.5) and multivariate analysis demonstrated consistent shifts in community composition across seston C:P gradients (Figure 5.6).

Our results support the prediction of ecological stoichiometry, in that shifts towards food sources with high P should result in compositional shifts towards a dominance of fast growing, P-rich consumers, whereas reductions in food P content should result in a dominance of P-poor consumers that are less sensitive to P limitation (Sterner *et al.* 1997). The P-rich taxon *Daphnia galeata*, for example, responded nicely according to that prediction and dominated in the treatment combination with lowest seston C:P (i.e. under low light with high nutrient concentrations), while its relative abundance decreased in the treatment combinations with higher seston C:P. However, no significant trends in the relative abundance of other taxa were found. A similar observation was made by Hall *et al.* (2004) who also reported a dominance of *Daphnia* at the lowest seston C:P levels, but were unable to relate elemental composition of other taxa with their abundance response along experimental resource supply gradients. One explanation for this is that the relative performance of zooplankton along a food C:P gradient is determined by more traits than elemental composition alone (Hall *et al.* 2004). Taxa may differ in their P acquisition, assimilation and retention efficiencies, their ability to graze selectively or in their resource allocation. In fact, even within one species, micro-evolutionary adaptation to LP food has been shown to involve changes in sexual investment of a plankton consumer without demonstrable changes in body elemental composition (Declerck *et al.* 2015). In communities without predators such as ours, we expect that the capacity to exploit limiting resources should be a better predictor of relative performance than P related growth rate (Iwabuchi & Urabe 2012a, b). Nevertheless, our Price-based analyses confirm that an increase of seston C:P has resulted in a net increase of zooplankton C:P via a shift towards a dominance of C-rich taxa, a conclusion that we would not have easily reached from the consideration of the responses of individual taxa alone.

In our multifactorial experiment, we observed a strong interaction effect of light- and P-supply on phytoplankton C:P (see also Dickman *et al.* 2006). This response of phytoplankton C:P was strongly in accordance with the light-nutrient hypothesis (Sterner *et al.* 1997; Elser *et al.* 2003; Hall *et al.* 2004; Urabe *et al.* 2010), which predicts high phytoplankton C to nutrient ratios when light supply is high relative to nutrients. At low P, zooplankton biomass failed to respond to an increase in phytoplankton biomass caused by increased light availability. It is unlikely that this lack of response is

due to shifts in the functional composition of the phytoplankton community because all treatments in the experiment were dominated by edible phytoplankton (Geller & Müller 1981). More likely it reflects a stoichiometry-driven reduction of herbivore resource use efficiency (Urabe & Sterner 1996; Urabe *et al.* 2010). It is noteworthy that in higher ranges of producer C:P values (C:P > 270), strong increases in seston C:P (up to 677; due to increased light at low P supply or reduced P availability at full day light) resulted in no further increase in zooplankton C:P. For these treatment combinations, dbRDA indicated no significant changes in zooplankton community composition. These observations altogether indicate that seston C:P had surpassed a threshold above which zooplankton communities were unable to respond via phenotypic plasticity or species sorting.

Our Price-based analysis suggests qualitative differences in the way zooplankton community stoichiometry responded to different factors. In shade the increase of community C:P observed in response to a decreased P-availability could only be explained by changes in the relative abundance of species ( $CDE_n$ ). In contrast, the community C:P increase observed in response to an augmentation of light intensity at high P levels was not only realized through compositional shifts but also via phenotypic elemental plasticity ( $CDE_p$ ). The absence of plasticity in the first case may reflect a stronger tendency of zooplankton organisms to remain homeostatic than in the second case. Invertebrates are known to maintain elemental homeostasis in the face of nutrient limitation, for example by increasing P uptake (e.g. compensatory or selective grazing), P assimilation (e.g. through increased gut passage time or production of digestive enzymes), and P retention. These strategies are energetically costly, resulting in enhanced respiration and therefore loss of carbon in the form of  $CO_2$ , which may also contribute to maintaining the C:P balance. In contrast, the elemental plasticity observed with varying light intensity at high P levels may reflect a tolerance to a higher body C content when P is saturating, for example under the form of lipid storage (Becker & Boersma 2005). To summarize, our results show that different mechanisms (P limitation or excess of light) may result in similar increases in seston C:P to which zooplankton C:P responds. Although the zooplankton C:P responses to both environmental factors were of comparable magnitude, the Price-based analysis revealed differences in the mechanisms underlying these responses (see also Supplement S5.3).

There has been a long tradition of research into the relationship between biodiversity and ecosystem functioning (Loreau *et al.* 2001). Although the potential importance of stoichiometric plasticity is increasingly recognized as a determinant of ecosystem functioning (Hall *et al.* 2007b; Vanni *et al.* 2008; Rowland *et al.* 2015), it remains unclear to what extent this plasticity is determined by species composition and diversity. Hall

et al. (2007) broadened the bottom-up perspective of the light-nutrient hypothesis by emphasizing the potential importance of grazer-induced changes in the taxonomic composition of producer communities. Similarly, Liess and Kahlert (2009) and Mette *et al.* (2011) studied the stoichiometric response of producer communities to light and nutrient gradients in the presence and absence of grazers and reported associations between phytoplankton community composition and seston C:P. In these studies, however, the relative importance of compositional changes and of intraspecific stoichiometry plasticity was not disentangled. Recently, a few studies have addressed the relationship between species diversity and stoichiometry at the base of the food web. Striebel *et al.* (2009) suggested that spectral niche complementarity associated with high species diversity may enhance phytoplankton C:P via an increase in resource use efficiency. With laboratory and field experiments, (Striebel *et al.* 2009b) demonstrated a positive association between phytoplankton diversity and algal C:P ratios. Plum *et al.* (2015) demonstrated that a richness-induced enhancement of phytoplankton C:P resulted in a decrease of food quality large enough to negatively affect herbivore growth. In these studies, diversity is treated as a factor independent of resource supply whereas, in reality, diversity itself often responds to gradients in relative resource supply. Due to such covariation, it may prove difficult to disentangle the effects of diversity change from other mechanisms that affect community stoichiometry along such gradients. Along a resource supply gradient, new species may establish whereas others may disappear. Some species may become relatively more abundant whereas others become scarce. Provided that species differ in body elemental composition, each of these changes will have its effects on community stoichiometry, in addition to the effects of phenotypic plasticity in elemental composition. The strength of our Price-based approach lies in its ability to reveal the independent impact of species gains, species losses, compositional change and phenotypic plasticity along gradients of relative resource supply.

Although initial communities were composed of zooplankton mixtures from multiple sources, species sorting was likely constrained. For this reason, we may regard the observed community elemental plasticity as conservative. It is expected that stronger shifts in community elemental plasticity would be observed in systems more connected to a regional species pool as the latter would allow the colonization and establishment of species with a broader range of elemental phenotypes (cf. 'boundary species', Danger *et al.* 2008). Through our Price-based approach such a hypothesis may be tested, effectively linking community stoichiometry to metacommunity dynamics.

The Price equation has already been successfully applied in studies addressing the role of biodiversity in ecosystem functioning (e.g. Winfree *et al.* 2015). We extend its use to the analysis of community level stoichiometry, and propose a statistical framework for

the analysis of its partitions in replicated community experiments. Our approach has the limitation that the sum of the proportional components is not equal to the total; ratios may only be evaluated and tested in isolation. Still, the approach can yield fundamental insights into the mechanisms that shape community stoichiometry in a reproducible way, as is illustrated by our experimental results.

## Conclusion

Gradients in seston C:P were shown to lead to C:P shifts in the consumer community larger than expectations based on observed levels of elemental plasticity within individual species. Therefore, studies of single-species' consumer plasticity in response to food stoichiometry are likely to underestimate the potential of whole-community plasticity in natural systems. Our results illustrate that species sorting is a major factor in governing community level stoichiometry shifts in response to food quality. As such, species sorting may dampen negative effects of stoichiometric mismatches on important ecosystem functions such as grazing and nutrient cycling by consumers (Danger *et al.* 2008).

## Acknowledgements

The authors would like to thank Chiara Carotenuto, Daniela Sannino, Giulia Ucello and Koos Swart for practical assistance with the experiment and Nico Helmsing for help with chemical analyses. This work benefited greatly from discussions of SAJD with other members of the sDiv-funded working group 'sCAFE', especially Colin Kremer, Thomas Koffel, Mathew Leibold and Katherine Bannar-Martin. We thank three anonymous reviewers and the editor, Punidan Jeyasingh, for their constructive comments that helped to improve the manuscript. This work was partly supported by the Division for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO), more specifically by the Biodiversity Works Research Program (OBW) project no. 841.11.009, by the Gieskes-Strijbis Foundation and by the Flemish Institute for the Promotion of Scientific Technological Research in Industry (IWT) through a PhD fellowship awarded to LG (project no. 131454).

## Chapter 5: Supplementary materials

### S5.1: Methodological details of mesocosm inoculation, sampling and sample analysis

#### Initial setup and inoculation of mesocosms

We aimed to start the experiment with a diverse plankton community to prevent that results would be constrained by a limited number of initial species. Samples for the initial inoculation were collected from six drainage canals in a naturally managed polder area in the Netherlands (Westbroekse Zodden: N52.163000, E5.114000). On 6-7-2014 (experimental day 6), we collected 20L of water at each site, filtered it over an 80µm mesh and added 1.5L (total cell density of inoculum: 57,000 cells/mL) of the mixed inoculum to each mesocosm. At day 13 (13-7-2014), after growth of the algae was observed by means of Chl-a fluorescence, an additional 500L water was collected from the same sites, but now with a 30µm plankton net (Hydrobios GmbH). A concentrated sample of 2L containing the equivalent of 9L of the filtered water with zooplankton was added to each mesocosm on the same day. Additionally, we added a number of cultured zooplankton species (*Daphnia pulex*, *Daphnia galeata*, *Scapholeberis mucronata*) to further increase the diversity of the starting community. Throughout the experiment, all mesocosms were covered with a fine mesh (0.5mm) to avoid aerial contamination. Mesocosms were not mixed. As a result, organic material could sink to the bottom together with associated nutrients during the course of the experiment. Through our nutrient enrichment which was carried out twice per week we aimed at compensating for such nutrient loss. The water level in the mesocosms stayed relatively constant because losses due to evaporation and sampling were compensated by rainfall.

#### Sampling and sampling analysis

Seston and zooplankton samples were collected by taking depth-integrated water samples from the mesocosms and filtering 15L of water over a 80µm zooplankton net (Hydrobios GmbH). The filtrate (<80µm) was used to measure seston dry weight and seston C and P content by concentrating 50 to 100mL on a Whatmann GF/F filter (0.7µm). Sestonic chlorophyll-a concentrations were monitored throughout the experiment using a PhytoPAM with an Optical Unit ED-101US/MP (Heinz Walz GmbH, Effeltrich, Germany), with a 0.2µm filtered water sample for background correction. At the end of the experiment a 4mL sample of the filtrate was fixed with a paraformaldehyde-glutaraldehyde solution of 1% (v/v) for phytoplankton community

composition analysis using flow cytometry (MoFlo Legacy Cell Sorter; Beckman Coulter, Miami, Florida, USA). The used method is described in further detail in Velthuis *et al.* 2017. The zooplankton sample (>80µm) was fixed using an acidic Lugol's solution (5%) for later identification and enumeration of zooplankton with light microscopy. All cladoceran zooplankton were identified to species level and copepods were identified to the order level (Amoros 1984; Flössner 2000). For each sample, subsamples were counted until no new species were found in the last group of 100 individuals.

To determine C and P content of zooplankton species we haphazardly collected individuals for each species separately. Immediately upon sampling, they were immersed in demiwasser for 1.5h to allow clearance of the digestive system. For each species, multiple individuals were picked haphazardly (5-35 individuals, depending on body size) and placed in a pre-weighed tin cup or glass vial for C and P analysis, respectively. Each of these samples was weighed again after drying. C determinations were carried out using a FLASH 2000 organic elemental analyzer (Interscience B.V., Breda, the Netherlands). P was determined using the potassium persulphate ( $K_2S_2O_8$ ; 2.5% w/v) digestion method at 121°C after a destructionstep where samples are placed in a blast furnace at 550°C. P-content was determined using a QuAAstro segmented flow autoanalyser (Beun de Ronde, Abcoude, the Netherlands). C and P content were expressed in molar mass per unit dry weight of the sampled zooplankton.

## Testing significance of the Price components

To calculate the Price equation results between treatments we first defined a baseline tank from the reference treatment and a comparison tank from the other treatment (also see Methods section in main text). We calculated the change in the community property between baseline and comparison tank for all combinations between tanks within the two treatments, thereby assessing the difference caused by environmental changes between the two treatments. To determine the changes in community properties within treatments we defined both baseline and comparison tanks from within the same treatment (replicates). Any shifts in community property found here were caused by random variation within our treatments, which may be stemming from measurement error, natural variation within the communities or stochastic processes unrelated to the treatment effect itself. By comparing the distribution of within treatment shifts in community property to those between treatments we were able to determine if the Price equation components were significant ( $p<0.05$ ). Specifically, our method was based on testing the change in a community property between two contrasting treatments from being different to changes found within the treatments.

To statistically assess this difference we used a two-sample location test based on 99,999 Monte-Carlo re-samplings. The first sample was composed of all the between

treatment tank combinations. The second sample was composed of all within treatment tank combinations. The test compares the mean difference of the two samples to zero, given the empirical distribution of both samples. When significant, we may conclude that changes between treatments were markedly different from those expected by random variation within treatments. This approach may be used to test changes in any community additive property (in this study: zooplankton community C and P) and combinations thereof (here: community C:P).

## S5.2: Details of the statistical analyses

**Table S5.2.1:** Summary of Anova results using a glm model with a gamma distribution testing the effects of P and light treatment levels on seston and zooplankton C:P and biomass. Significant differences are indicated by \*\*\*:p<0.001, \*\*:p<0.01, \*:p<0.05, :p<0.10.

Model		df	Deviance	t	P	
<i>Seston C:P</i>	P	1	5.763309	-7.127	0.000	***
	Light	1	1.964911	-3.395	0.004	**
	P x Light	1	0.002989	0.351	0.730	
	Residual	16	0.349834			
<i>Seston Biomass</i>	P	1	0.302695	1.953	0.069	.
	Light	1	8.371595	-2.173	0.045	*
	P x Light	1	1.532785	-2.238	0.040	*
	Residual	16	4.578845			
<i>Zooplankton C:P</i>	P	1	0.158425	-2.485	0.024	*
	Light	1	0.130894	-2.347	0.032	*
	P x Light	1	0.043979	1.265	0.224	
	Residual	16	0.414565			
<i>Zooplankton Biomass</i>	P	1	3.545032	1.340	0.199	
	Light	1	4.382352	-1.903	0.075	.
	P x Light	1	0.207052	-0.600	0.557	
	Residual	16	9.634481			

**Table S5.2.2:** Pairwise test results of a K-sample permutation test on differences in C:P ratio between different zooplankton taxa.

Taxa 1	Taxa 2	Test statistic (W)	Adjusted P-value
<i>Ceriodaphnia reticulata</i>	<i>Chydorus sphaericus</i>	-1.378	0.3729
<i>Ceriodaphnia reticulata</i>	<i>Calanoid copepods</i>	-0.8317	0.5832
<i>Ceriodaphnia reticulata</i>	<i>Cyclopoid copepods</i>	0.3851	0.7315
<i>Ceriodaphnia reticulata</i>	<i>Daphnia galeata</i>	2.246	0.08582
<i>Ceriodaphnia reticulata</i>	<i>Daphnia pulex</i>	0.3925	0.8169
<i>Ceriodaphnia reticulata</i>	<i>Eury cercus lamellatus</i>	-0.4756	0.7315
<i>Ceriodaphnia reticulata</i>	<i>Scapholeberis mucronata</i>	-1.116	0.3795
<i>Ceriodaphnia reticulata</i>	<i>Simocephalus vetulus</i>	1.303	0.3795
<i>Chydorus sphaericus</i>	<i>Calanoid copepods</i>	0.6525	0.6614
<i>Chydorus sphaericus</i>	<i>Cyclopoid copepods</i>	1.939	0.1567
<i>Chydorus sphaericus</i>	<i>Daphnia galeata</i>	3.724	0.00018 ***
<i>Chydorus sphaericus</i>	<i>Daphnia pulex</i>	1.823	0.207
<i>Chydorus sphaericus</i>	<i>Eury cercus lamellatus</i>	1.451	0.3653
<i>Chydorus sphaericus</i>	<i>Scapholeberis mucronata</i>	-0.1176	0.9118
<i>Chydorus sphaericus</i>	<i>Simocephalus vetulus</i>	2.998	0.01377 *
<i>Calanoid copepods</i>	<i>Cyclopoid copepods</i>	1.338	0.3729
<i>Calanoid copepods</i>	<i>Daphnia galeata</i>	3.287	0.00018 ***
<i>Calanoid copepods</i>	<i>Daphnia pulex</i>	1.325	0.3729
<i>Calanoid copepods</i>	<i>Eury cercus lamellatus</i>	0.7093	0.6454
<i>Calanoid copepods</i>	<i>Scapholeberis mucronata</i>	-0.5152	0.7315
<i>Calanoid copepods</i>	<i>Simocephalus vetulus</i>	2.613	0.03084 *
<i>Cyclopoid copepods</i>	<i>Daphnia galeata</i>	2.204	0.08582
<i>Cyclopoid copepods</i>	<i>Daphnia pulex</i>	0.1265	0.9118
<i>Cyclopoid copepods</i>	<i>Eury cercus lamellatus</i>	-1.023	0.4632
<i>Cyclopoid copepods</i>	<i>Scapholeberis mucronata</i>	-1.558	0.3397
<i>Cyclopoid copepods</i>	<i>Simocephalus vetulus</i>	1.221	0.3795
<i>Daphnia galeata</i>	<i>Daphnia pulex</i>	-1.681	0.2429
<i>Daphnia galeata</i>	<i>Eury cercus lamellatus</i>	-3.252	6.00E-04 ***
<i>Daphnia galeata</i>	<i>Scapholeberis mucronata</i>	-3.036	0.02693 *
<i>Daphnia galeata</i>	<i>Simocephalus vetulus</i>	-0.3599	0.8006
<i>Daphnia pulex</i>	<i>Eury cercus lamellatus</i>	-1.017	0.4632
<i>Daphnia pulex</i>	<i>Scapholeberis mucronata</i>	-1.337	0.3729
<i>Daphnia pulex</i>	<i>Simocephalus vetulus</i>	0.9925	0.4632
<i>Eury cercus lamellatus</i>	<i>Scapholeberis mucronata</i>	-1.111	0.4547
<i>Eury cercus lamellatus</i>	<i>Simocephalus vetulus</i>	2.372	0.07169
<i>Scapholeberis mucronata</i>	<i>Simocephalus vetulus</i>	2.145	0.08582

**Table S5.2.3:** Results of multivariate tests for community shifts in zooplankton based on a redundancy analysis of zooplankton abundance for different subsets of the data. Data is split into response of the community between different treatment comparisons. P values in bold indicate significant community shifts ( $p < 0.05$ ) and the F column shows the associated test statistic.

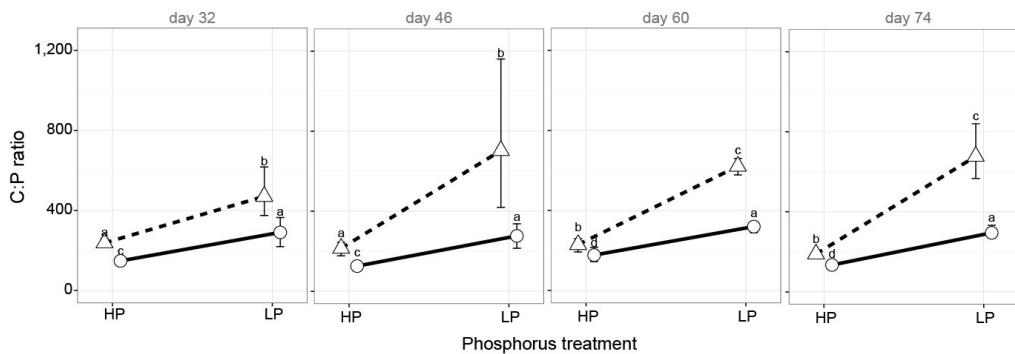
Treatment comparison		P-value	F	R <sub>adj</sub> (%)
HP-LP	Full light	0.549	0.828	-1.943
	Shade	<b>0.039</b>	2.446	13.839
Shade-Full light	HP	<b>0.038</b>	2.561	14.778
	LP	0.078	2.279	12.440

**Table S5.2.4:** Comparison of different regression models for assessing the relation between zooplankton community C:P and seston C:P ratio. Models are ordered by their respective AICc score.

Model	AICc	df	R <sub>adj</sub>	Terms	Estimate	Test statistic
Logistic nonlinear least square regression	173.28	3	18.3%	Asym	101.857	***
				Xmid	69.263	1.222
				scal	58.39	1.246
Segmented linear regression	175.17	4	18.8%	y0	33.52	0.278
				$\beta_1$	0.314	*
				$\beta_2$	0.009	0.061
Linear regression	175.35	2	11.2%	y0	81.732	***
				$\beta$	0.032	.
Linear null model (intercept only)	176.76	1	-	y0	92.020	***
						22.07

## S5.3: Supporting figures

### Patterns in seston C:P through time



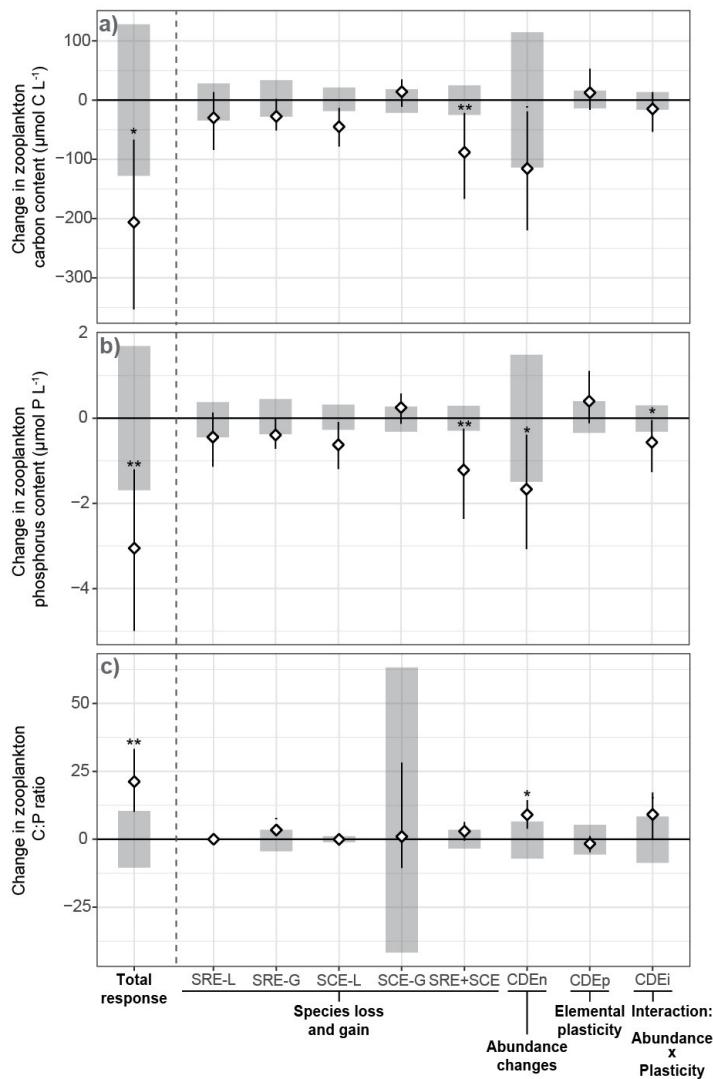
**Figure S5.3.1:** Reaction norm plots of seston C:P to phosphorus addition (HP, LP) and light treatments (triangles, dotted line: full light; circles, continuous line: shaded) at multiple time points during the experiment. Error bars indicate 97.5% confidence limits. Letters indicate significant pairwise differences.

### Further division of Price equation components

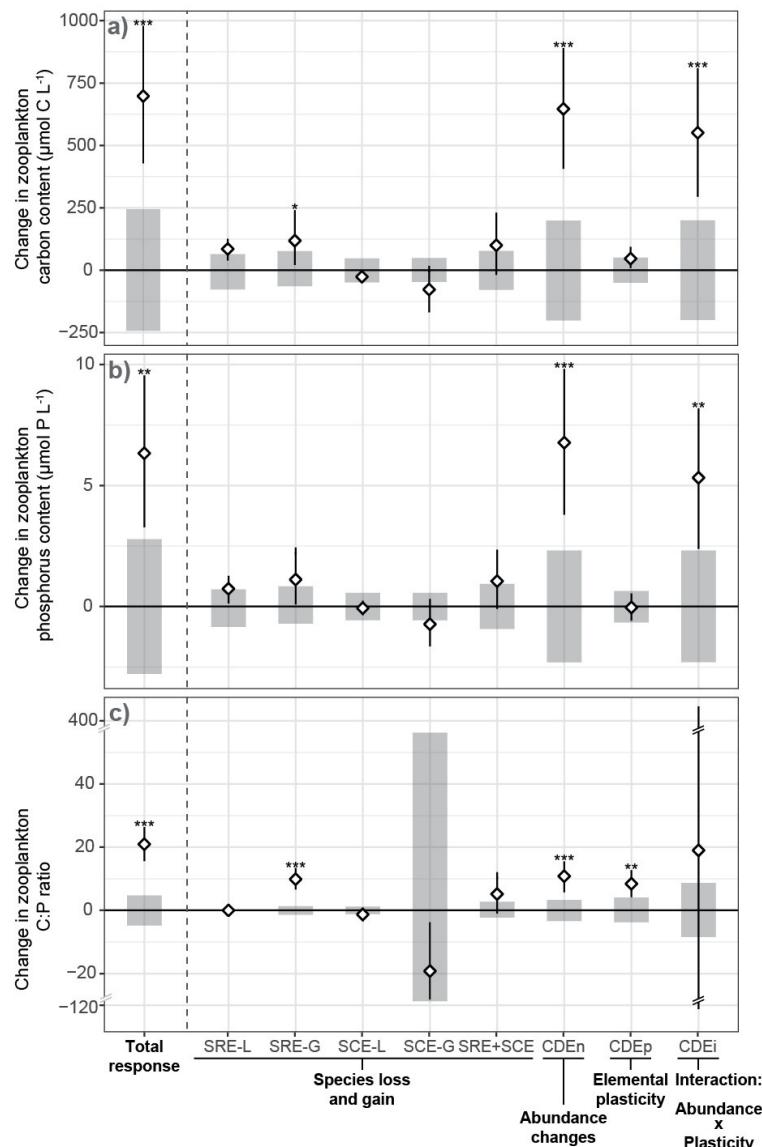
Figure S5.3.2 and S5.3.3 are expanded versions of Figure 5.3 and 5.4 found in the main text. In these figures a further subdivision of the Price equation partition SRE+SCE into the components (SRE-L, SRE-G, SCE-L and SCE-G; following Fox and Kerr (2012)) is given. The SRE describes the community property change due to the random loss or gain of species, independent of species identity. In this context SRE informs on changes in C, P and C:P due to the loss (SRE-L) or gain (SRE-G) of species in general. The SCE is a component that accounts for the specific properties of the species that are lost or gained and informs on the change in community C, P or C:P due to the loss (SCE-L) and gain (SCE-G) of species from the community between the treatment combinations. Mathematical details on the calculation of these components can be found in Fox and Kerr (2012).

For the treatment comparison of HP-LP/Shade we found no significant changes in community C:P for any of the subdivided partitions. In the Shade-Full light:HP combination the SRE-G partition of change in zooplankton C:P ratio was significantly positive. This result indicates that there is a gain of species when going from HP to LP under shaded conditions that leads to a shift in the C:P of the zooplankton community. The C:P average across all species equals 104 (see Figure 5.5 main text). As the HP/Shade community C:P is far below this value at a C:P of 75 (Figure 5.1, main text), a

gain of any random draw of species would generally lead to an increase of the community C:P. Regardless, no net effect of species gain and loss (SRE+SCE) as a whole was found though, illustrating that this change is compensated through the other components of species loss and gain.



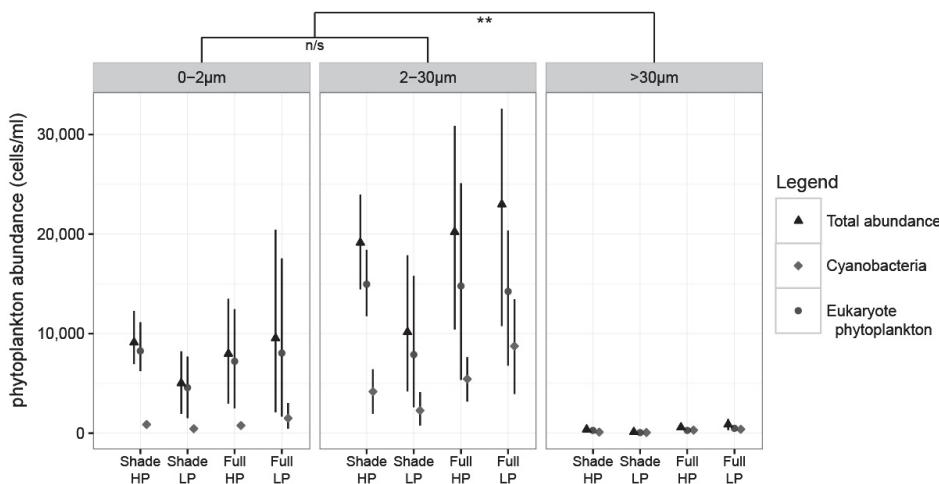
**Figure S5.3.2:** Price partitions of changes in the zooplankton community elemental content compared between HP-Shade (baseline) and LP-Shade (comparison) conditions and their effects on community elemental ratio. a) community C content; b) community P content; c) community C:P elemental ratio. Error bars represent 97.5% confidence limits. Grey bars represent the zero centered null distribution of values obtained by random within treatment combinations. Significance levels were assessed using a permutation based location test: \*\*\*: $p<0.001$ , \*\*: $p<0.01$ , \*: $p<0.05$ , :: $p<0.10$ .



**Figure S5.3.3:** Price partitions of changes in the zooplankton community elemental content compared between Shade-HP (baseline) and Full light-HP (comparison) conditions and their effects on community elemental ratio. a) community C content; b) community P content; c) community C:P elemental ratio. Error bars represent 97.5% confidence limits. Grey bars represent the zero centered null distribution of values obtained by random within treatment combinations. Significance levels were assessed using a permutation based location test: \*\*\*:p<0.001, \*\*:p<0.01, \*:p<0.05, ·:p<0.10.

## Phytoplankton community analyses

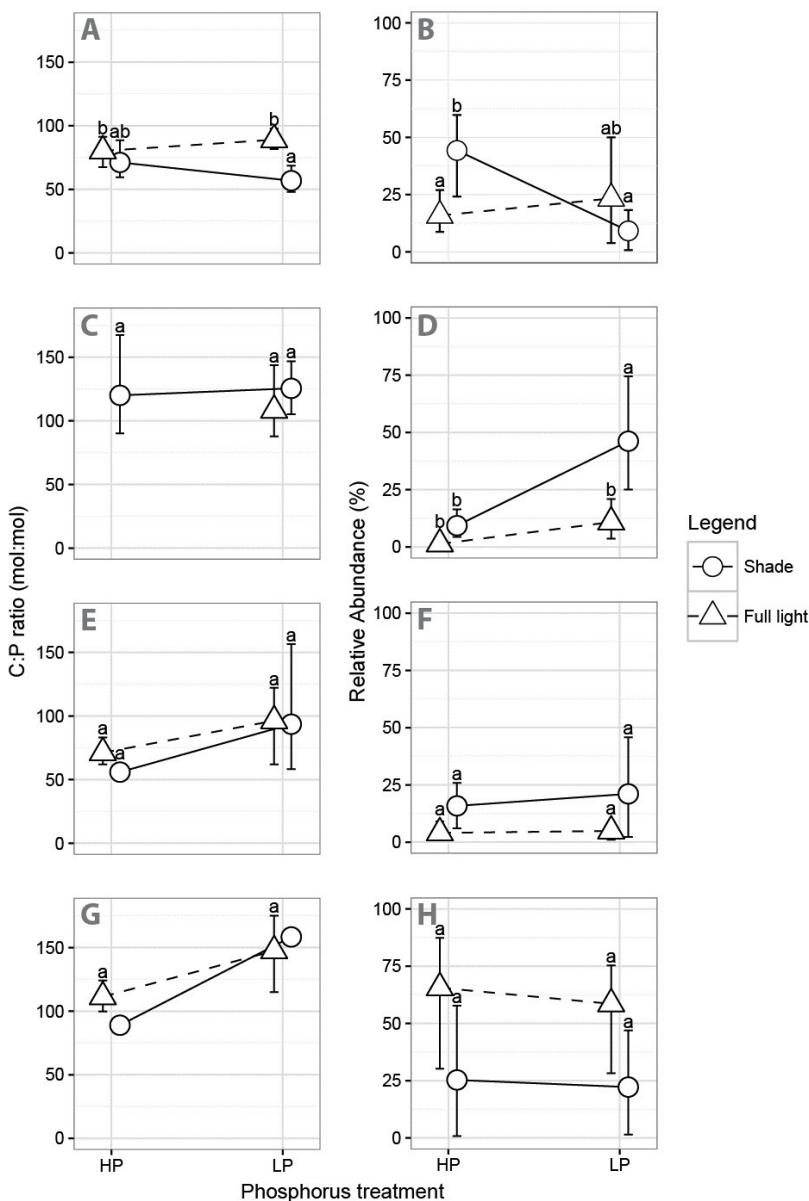
Analyses of the phytoplankton community using flow cytometry showed that overall phytoplankton densities were relatively low. The extant community was dominated by eukaryote phytoplankton in the low size ranges ( $0\text{-}2\mu\text{m}$  and  $2\text{-}30\mu\text{m}$ ). Larger cells ( $30\text{-}80\mu\text{m}$ ) were only present in very low abundances (on average  $<1000$  cells/mL). Cyanobacteria tend to increase in the LP/Full light treatment, though the relative abundance of cyanobacteria within the community did not prove to change significantly between the different treatments (quasi-binomial GLM:  $P=0.083$ ,  $\chi^2=12.81$ ).



**Figure S5.3.4:** Phytoplankton abundance per size categories and major groups (blue diamonds: cyanobacteria, green circles: eukaryote phytoplankton) and their combined total abundance (black triangles) as determined using flowcytometry. Statistical differences between the total abundance in different size classes is shown above. Error bars represent 97.5% confidence limits.

## S5.4: Response of individual zooplankton taxa to the experimental treatments

We observed qualitative differences in the compositional response of the zooplankton communities to seston C:P gradients (Figure S5.1, Table S5.1). Although compositional shifts to increased seston C:P were highly comparable between HP and LP treatment comparison in the shade and along the Shade to Full light comparison at high P, there were also some important differences. Whereas the HP-LP comparison was associated with a reduced relative abundance of *D. galeata* (Figure S5.1B) and a trend towards an increased abundance of calanoid copepods (Figure S5.1D), populations of this taxon collapsed along the Shade-Full light comparison. The disappearance of calanoids cannot be explained by competition with *Daphnia*, because *Daphnia* also decreased along the latter gradient. More likely, the observed discrepancies in the response of calanoid copepods to increases in seston C:P that are similar in range but generated by different experimental treatment combinations rather reflect the differential impact of light and phosphorus availability on other aspects of food quality to which calanoids may be sensitive, such as the functional or biochemical composition of the phytoplankton community (Müller-Navarra 1995, Arts *et al.* 2009).



**Figure S5.4.1:** Responses of body elemental C:P ratio and the relative population abundance of the most abundant species to the experimental treatments. *Daphnia galeata* (A,B), calanoid copepods (C,D), *Simocephalus vetulus* (E,F) and *Chydorus sphaericus* (G,H) relative abundance and species specific C:P are shown with letters indicating the pairwise significance after multiple comparison correction ( $p<0.05$ ). HP and LP indicate high and low phosphorus treatment, respectively. Symbols indicate light treatments (triangles, dotted line: Full sunlight; circles, continuous line: Shaded).

**Table S5.4.1:** Anova table of the results of a glm model testing the effects of P and light availability treatment combinations on zooplankton community C:P and relative abundance.

Treatment	Species	Treatment	Df	Deviance	t	P-value
<i>Community C:P</i>	Calanoid copepods	Light availability	1	0.0369012	0.5811	0.5754
		P availability	1	0.0007384	-0.2037	0.8431
		P x Light	0	-	-	-
		Residuals	9	0.8062671		
<i>Chydorus sphaericus</i>	<i>Chydorus sphaericus</i>	Light availability	1	0.0031926	-1.0877	0.3185
		P availability	1	0.2823226	-2.2270	0.0675
		P x Light	1	0.0393624	1.1287	0.3021
		Residuals	6	0.1877957		
<i>Daphnia galeata</i>	<i>Daphnia galeata</i>	Light availability	1	0.258762	-0.9720	0.3520
		P availability	1	0.0001445	1.6521	0.1268
		P x Light	1	0.1049729	-1.8416	0.0926
		Residuals	11	0.3178688		
<i>Simocephalus vetulus</i>	<i>Simocephalus vetulus</i>	Light availability	1	0.043336	-0.9580	0.3606
		P availability	1	0.6144543	-1.9949	0.0740
		P x Light	1	0.0591179	0.7336	0.4801
		Residuals	10	1.1391841		
<i>Relative abundance</i>	Calanoid copepods	Light availability	1	2.52864	-1.25144	0.228761
		P availability	1	4.498783	3.082893	0.007131 **
		P x Light	1	2.528345	0.04417	0.965315
		Residuals	16	6.571403		
<i>Chydorus sphaericus</i>	<i>Chydorus sphaericus</i>	Light availability	1	9.765187	1.692049	0.110016
		P availability	1	12.85627	-0.15744	0.876867
		P x Light	1	9.761082	-0.08757	0.931306
		Residuals	16	12.90847		
<i>Daphnia galeata</i>	<i>Daphnia galeata</i>	Light availability	1	4.809123	-2.0436	0.057819 .
		P availability	1	4.956122	-2.48498	0.0244 *
		P x Light	1	3.585302	2.273775	0.037107 *
		Residuals	16	5.499622		
<i>Simocephalus vetulus</i>	<i>Simocephalus vetulus</i>	Light availability	1	2.59535	-1.49158	0.155261
		P availability	1	3.627399	0.549037	0.590561
		P x Light	1	2.593245	-0.11829	0.907313
		Residuals	16	3.672704		

# 6

## Managing successional stage heterogeneity to maximize landscape-wide biodiversity of aquatic vegetation in ditch networks

**Sven Teurlincx\***, Michiel J.J.M. Verhofstad\*, Elisabeth S. Bakker, Steven A.J. Declerck

\*Authors contributed equally to this work.

*This chapter is based on the paper: Teurlincx, S., Verhofstad, M. J., Bakker, E. S., & Declerck, S. A. (2018). Managing successional stage heterogeneity to maximize landscape-wide biodiversity of aquatic vegetation in ditch networks. Frontiers in plant science, 9.*

## Abstract

The presence of a high diversity of different successional stages in a landscape may help to conserve and promote landscape-wide biodiversity. A strategy to achieve this is using Cyclic Rejuvenation through Management (CRM), an approach employed in a variety of different ecosystems. CRM periodically resets the successional stages in a landscape. For aquatic systems this constitutes vegetation removal and dredging. For this approach to be useful (a) successional stages are required to be different in community composition and (b) these differences need to be caused by true replacement of species between stages. While potentially valid, these assumptions are not generally tested prior to application of CRM. In this study we test these assumptions to explore the usefulness of managing on successional stage heterogeneity for maximizing landscape-wide aquatic plant diversity. We carried out vegetation surveys in the ditch networks of 21 polder landscapes in The Netherlands, each containing 24 ditch reaches. Using a clustering approach combined with insight from literature on vegetation succession in these systems we assigned our sampled communities to defined successional stages. After partitioning landscape diversity into its alpha and beta components, we quantified the relative importance of replacement among successional stages. Next, through scenario analyses based on simulations we studied the effects of reducing successional stage heterogeneity on landscape-wide biodiversity. Results showed that differences in community composition among successional stages were a potentially important factor contributing to landscape diversity. Early successional stages were characterized by higher replacement of species compared to late successional stages. In a scenario of gradual decrease of heterogeneity through the systematic loss of the earliest successional stages we found 20% of the species richness in a polder was lost, pointing towards the importance of maintaining early successional stages in a polder. This makes a compelling case for application of CRM within agricultural drainage ditch landscapes to maximize regional aquatic plant diversity. While applied to drainage ditch systems, our data-driven approach is broadly applicable to other systems and may help in providing first indications of the potential of the CRM approach. We argue that CRM may maintain and promote regional biodiversity without compromising the hydrological function of the systems.

# Introduction

Land use intensification and global change have led to decreasing biotic diversity (Foley *et al.* 2005a). Global species numbers show clear negative trends, though different levels of spatial scale show very different trends (McGill *et al.* 2015). Much of this biodiversity loss is caused by increasing homogenisation of communities (i.e. biotic homogenisation), and not necessarily by loss of local diversity (Dornelas *et al.* 2014). For the landscape scale this may imply that landscape diversity may decrease mainly due to the disappearance of differences between local communities (Smart *et al.* 2006), rather than locally detectable declining species numbers (McGill *et al.* 2015). As declines in biodiversity threaten the multifaceted functioning of ecosystems at both local and landscape scales (van der Plas *et al.* 2016), its conservation and restoration requires appropriate landscape-wide management strategies.

The process of ecological succession has long been acknowledged as a primary driver of biodiversity (Sousa 1979). Different stages of ecological succession may exhibit different species richness levels. A classic example on forest succession shows higher species numbers in intermediate stages of succession (Odum 1969, Whittaker 1970). Furthermore, different successional stages may harbour very different sets of species. Thus, the landscape diversity is not a function of the local diversity alone, but also of the complementarity between stages present in the landscape. Hence, to maximize the diversity of a landscape, both the local diversity of successional stages, as well as the difference between communities of the different stages needs to be considered. This line of thought is well represented in the classical partitioning of the landscape diversity ( $\gamma$ ) into a local component ( $\alpha$ ) and a turnover component ( $\beta$ ) (Whittaker 1960; Jost *et al.* 2010). Periodic resets of unidirectional succession through naturally occurring disturbance events in different parts of the landscape may contribute to the maintenance of a mosaic of successional stages (Sousa 1984). In natural systems, examples of such disturbances include fire (Vandvik *et al.* 2005), scouring by peak river discharges (Tockner *et al.* 2000) or landslides (Walker *et al.* 1996). In absence of such natural dynamics, for example due to human interventions, there is a risk of loss of successional stage heterogeneity within the landscape (Baptist *et al.* 2004).

To obtain biodiversity within a landscape that has lost its natural dynamics due to human influence, management efforts need to be directed towards maintaining a landscape with a variety of successional stages present. Such management has been widely applied in a variety of different ecosystems (Baptist *et al.* 2004; Vandvik *et al.* 2005) and is known by different names, e.g. cyclic rejuvenation, rotational management and periodic ecosystem reset. Here we use the term cyclic rejuvenation through management (CRM), which is the practice of periodically resetting part of the

habitat in a landscape to a (mostly) bare state in order to create a spatially dynamic mosaic of habitat patches in different stages of succession (Hinsch and Poethke 2007). CRM in floodplain management, aimed at systematic removal of part of the floodplain forests, increased biodiversity in channels where natural reset of succession was absent (Baptist *et al.* 2004). Likewise, controlled fire management can help preserve grassland diversity through CRM (Richards *et al.* 1999). While good results have been shown, the inherent success of this management approach rests strongly on the assumption that successional stages are complementary to one another. The more unique stages are with respect to their community composition, the larger the gain to the regional species pool and thus overall landscape diversity. Conversely, when successional stages are highly similar, CRM will have little effect on landscape diversity.

Manmade water systems such as agricultural drainage ditches are a good example of anthropogenic ecosystems which are under continuous management to protect the hydrological drainage of agricultural land (Hill *et al.* 2016). However, their value for conservation of biodiversity is increasingly recognized as well (Armitage *et al.* 2003; Herzon & Helenius 2008; Clarke 2014). Reshaping ditch banks, removing vegetation and dredging organic sediment helps to maintain hydrological functioning, but may also support ecological function and diversity (Twisk *et al.* 2003). Conventional management of these waterways results in the constant resetting of succession (van Strien *et al.* 1991; Clarke 2014; Hill *et al.* 2016), making an often unorganized form of CRM the norm in these systems. Formal CRM potentially offers an approach for this management to take place, while also realising a biodiversity increase in comparison to existing management.

In human dominated landscapes, management primarily aims at maintaining provisioning services (e.g. water storage, food production (Power 2010)). For ditch ecosystems, the application of CRM has been suggested as a promising way to increase diversity at a landscape scale (Watson & Ormerod 2004; Clarke 2014; Hill *et al.* 2016) while maintaining associated services. Landscape level diversity ( $\gamma$ ) in these ecosystems has been shown to be largely caused by differences in community composition between individual sites (Goldenberg Vilar *et al.* 2014; Whatley *et al.* 2014a), as indicated by a large  $\beta$ -diversity component, stressing the importance of landscape heterogeneity. Despite the claims on the potential usefulness of CRM (Clark, 2015), so far no formal evaluation of its potential has been made, nor has it been widely adopted in practice in ditch management. A first step in the evaluation and adoption of such a management practice is to illustrate the importance of successional stage heterogeneity for landscape-wide diversity. Differences in community composition between sites may be caused by two inherently different underlying patterns, namely one of species richness difference and one of species replacement (Baselga 2010;

Legendre 2014). Differences in community composition between sites ( $\beta$ -diversity) may be partitioned into a richness difference and species replacement component (Baselga, 2010; Podani & Schmera, 2011; Legendre, 2014). Species replacement refers to the simultaneous gain and loss of species along an ecological gradient (Legendre, 2014). A large replacement component is indicative for high levels of complementary between sites in terms of species composition. Alternatively, compositional differences among sites may also be generated merely by differences in species numbers (richness difference, *sensu* Legendre, 2014, also see Podani & Schmera, 2011). In contrast to the latter, it is the replacement component that will contribute to  $\gamma$ -diversity. Translating this to ditch networks, the degree to which successional stage diversity will be important in generating a high  $\gamma$ -diversity at the landscape scale will largely depend on the relative importance of the replacement component in the  $\beta$ -diversity among successional stages. For CRM to truly be useful for increasing landscape biodiversity, species replacement between different successional stages is required (i.e. complementarity) and not difference in richness alone.

In this study we performed 504 vegetation surveys in ditch reaches spread over 21 different polder landscapes in the Netherlands to study the importance of maintaining successional stage diversity on the landscape-wide species diversity of aquatic vegetation. We characterised the dissimilarity among successional stages and its underlying patterns to assess the complementarity of communities of different successional stages and their contribution to landscape biodiversity. In addition, we tested the potential effect of landscape-wide heterogeneity in successional stages on the landscape-wide diversity. This was done by calculating the biodiversity of simulated landscapes with different combinations of successional stages. Based on these results we evaluate the hypothesized merit of CRM on promoting landscape-wide diversity in ditch systems (Clarke, 2015) through proliferation of a diverse spatial-temporal mosaic of habitats.

## Methods

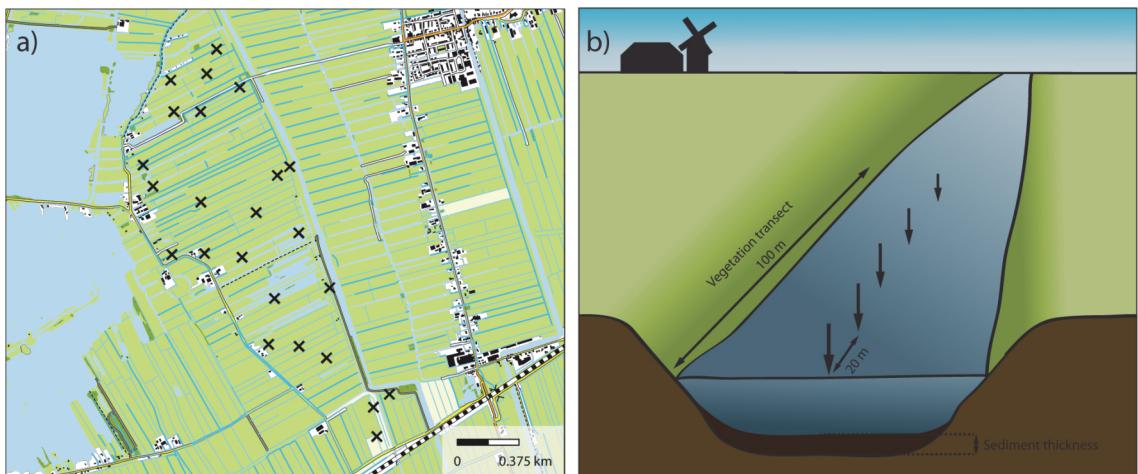
### Study landscapes and site selection

Our study took place in the peat meadow polder landscapes in the west of The Netherlands (coordinates given in Table S6.1). These landscapes were historically created by drainage of peatlands to reclaim land for agriculture. This has led to the typical Dutch polder landscapes in which long, narrow fields are intersected by a network of drainage ditches, with the whole system being surrounded by dykes. Water level fluctuations within these landscapes are strictly controlled. The ditches in these

systems are generally eutrophic to hypertrophic due to their long time agricultural use. The fields are used primarily for intensive cattle farming, although some variation in land use intensity is generated by governmental subsidies (agri-environmental schemes) that stimulate farmers to cultivate land less intensively (Catry *et al.* 2017). Additionally, some fields are owned by nature management organisations and managed more extensively with the aim of creating and sustaining moist natural grassland vegetation and its associated biota. In all cases the ditches need to be regularly managed to sustain their hydrological function. This is done through removal of plant biomass (e.g. mowing) and dredging of organic sediments from the ditch bottom. In our study we selected 21 different polder landscapes of roughly 200 hectares each. Agricultural land use intensity in these landscapes varied greatly, ranging from relatively extensive (low density, organic dairy farming, nature conservation area) to very intensive (e.g., high density cattle stocks). Within each of these 21 landscapes we selected 24 ditch reaches. Each landscape was first divided into 24 equal subparts. Within each of these subparts one ditch reach of 100m length was selected randomly. In this way, reaches were spread more or less evenly across each landscape while their exact location was still selected on a random basis within the confines of each subpart (Figure 6.1a). Vegetation surveys were carried out along each ditch reach and the relative abundance of all plant species growing in the water was assessed according to the Tansley scale (Tansley 1946). These Tansley dominance classes were converted to cover percentages using Table S6.2. Furthermore, the thickness of the organic sediment layer was measured at five locations (every 20 m) in the ditch reach in the centre of the ditch (Figure 6.1b).

## Defining successional stages

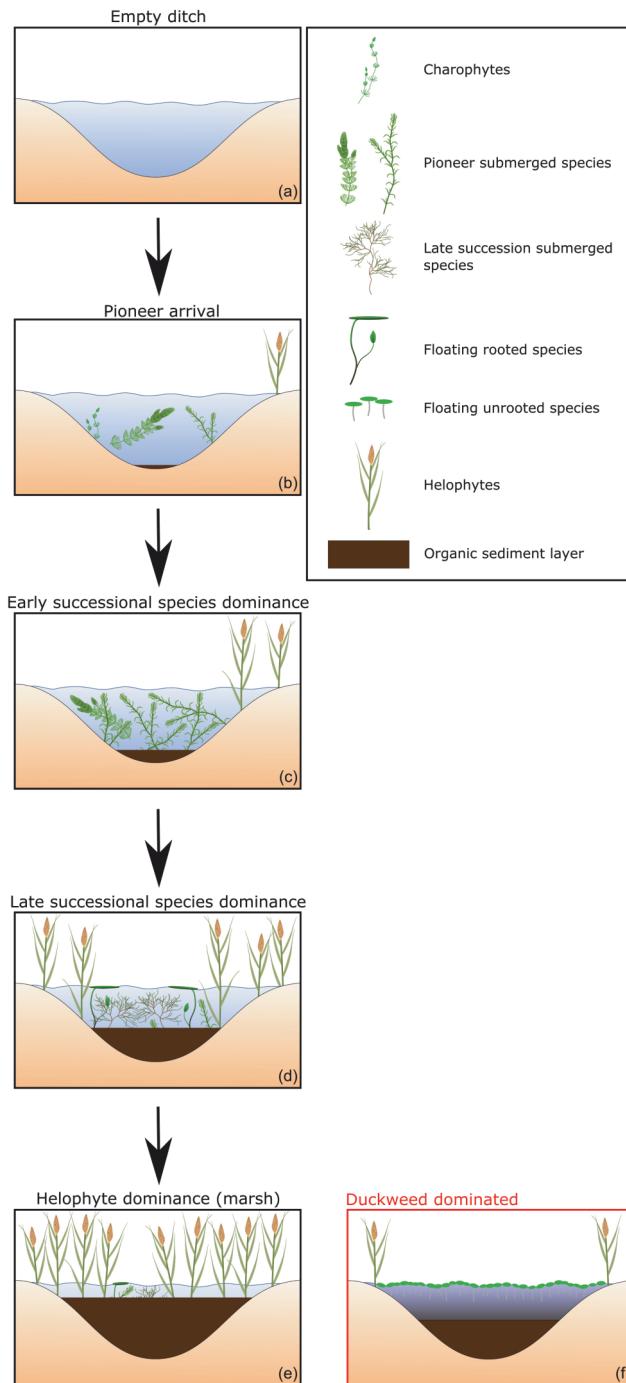
Succession of aquatic vegetation in ditches is known to follow a general trajectory (Caspers & Heckman 1981; Barendregt *et al.* 1992; Portielje & Roiackers 1995; Lamers *et al.* 2002; Watson & Ormerod 2004). After a short barren stage (Figure 6.2a), succession starts off with sparse patches of early successional submerged species, such as charophytes and vascular plants such as *Ceratophyllum demersum* and *Elodea nuttallii* (Figure 6.2b). Due to the production and subsequent sedimentation of plant material the sediment will become covered by a thin layer of organic matter. The fastest growing species eventually become dominant. Due to a higher biomass production the organic sediment will build up (Figure 6.2c). With increasing organic sediment layer thickness, the internal nutrient release will increase as well. This increase in nutrient status will favour different species groups, submerged groups such as *Potamogeton* sp. and rooted floating species such as *Nuphar lutea* (Figure 6.2d). Eventually, the organic sediment layer will build up to the point that helophytes are able to occupy the centre of the



**Figure 6.1:** a) Example of one of the 21 studied polder landscapes. Crosses indicate the predetermined locations where ditch reaches were sampled. b) Illustration of the ditch reach with the vegetation transect (100m) and the five points at which organic sediment thickness was measured.

ditch (Figure 6.2e). If left unmanaged a carr will remain. Under some conditions, succession deviates from this typical trajectory. Given high nutrient loading, the water surface may become entirely covered with duckweed (Figure 6.2f). Dominance by duckweed is believed to represent an alternative stable state which stabilizes itself via its impact on the aquatic environment. For example, high cover by duckweed results in anoxic conditions and a strongly reduced solar irradiance (Scheffer *et al.* 2003). This hampers the establishment of submerged and rooted floating plants (Gerven *et al.* 2015) and as such prevents the progression of succession.

We defined functional types of vegetation known to be indicative for different successional stages (Barendregt *et al.* 1992; Lamers *et al.* 2002; Watson & Ormerod 2004): charophytes, submerged pioneer vegetation, submerged late successional vegetation, rooted floating vegetation, unrooted floating vegetation (e.g. duckweeds) and helophyte vegetation (Figure 6.2). Through cluster analysis we grouped reaches according to successional stage properties (relative cover by vegetation functional types and sediment thickness). To select the most suitable cluster technique and the optimal number of clusters we compared k-means-, fuzzy-, self-organizing tree algorithm- and model based clustering approaches with the *clValid* package in R (Brock *et al.* 2011) using silhouette width and connectivity as the selection criteria. Based on functional type composition and sediment thickness, we ranked the clusters along the successional stage continuum as described above (Figure 6.2). All reaches were assigned to a cluster which represents their successional stage for further analyses.



**Figure 6.2:** Schematic representation of the progression in the composition of functional vegetation types and organic sediment accumulation characteristic for vegetation succession in drainage ditches.

## Successional stages and diversity

In this study, the two focal biodiversity indices are species richness and the species numbers equivalent of the Shannon diversity ( $H'$ ). These two measures of diversity were chosen as they can be correctly partitioned into alpha and beta components in an additive fashion (Jost 2007a).  $H'$  is calculated as the exponent of the Shannon-Wiener index and may be interpreted as a measure of the richness of abundant species in the community. For each of both indices, using the diversity partitioning framework of Jost (2007) and Jost *et al.* (2010), landscape diversity ( $\gamma$ ) for each polder was partitioned into the average local diversity of ditch reaches ( $\alpha$ - diversity) and a  $\beta$ -diversity component describing community dissimilarity among reaches. If different successional stages differ in community composition, then successional stage variation among ditch reaches will affect  $\gamma$ -diversity through the  $\beta$ -component. To evaluate the impact of such among-stage compositional differences on  $\gamma$ -diversity  $\beta$  diversity was further partitioned into a component reflecting differences among communities within successional stages ( $\beta_{\text{within}}$ ), and a component reflecting community compositional differences among successional stages ( $\beta_{\text{between}}$ ).  $\beta_{\text{within}}$  was calculated as the average of  $\beta$ -diversity values calculated for each successional stage in each polder.  $\beta_{\text{between}}$  was calculated across successional stages within the polder. For species richness this was done using the list of species present in each stage (presence/absence data). For  $H'$ , we used the average abundances of species calculated across ditch reaches within successional stages instead.

To investigate whether compositional differences among successional stages arise from true species replacement patterns we partitioned the  $\beta_{\text{between}}$  component into two additional additive components, a component of ‘true species replacement’ ( $\beta_{\text{repl}}$ ) and a ‘richness difference’ component ( $\beta_{\text{rich}}$ ) using the approach proposed by Podani & Schmera (2011). For richness, the repl and rich partitions were calculated from a Jaccard-based multi-site  $\beta$ -diversity index (Ensing & Pither 2015). To our knowledge, no such indices have been developed for abundance data. Instead, for the latter type of data we partitioned the total variance of a Ruzicka dissimilarity matrix following Legendre (2014b).

We also tested for differences in community composition among all and between combinations of successional stages using distance-based Redundancy Analysis (dbRDA, Legendre and Anderson 1999). Presence/absence data were analyzed using a Jaccard dissimilarity matrix among reaches within landscapes. Similarly, abundance data were analyzed using the Ruzicka dissimilarity index. dbRDAs were also performed on the species replacement ( $\beta_{\text{repl}}$ ) and richness difference ( $\beta_{\text{rich}}$ ) components of these indices (Legendre 2014).

Typically, successional stages were represented by different numbers of reaches within landscapes. As differences in sample size may result in biased estimations of  $\beta$  diversity components and incorrect significance values of RDA analyses (Anderson & Walsh 2013) we applied a random resampling procedure ensuring an equal representation of successional stages in each of these analyses. First, the stage with the least number of reaches in a polder was identified. Then  $\beta$  diversity components were estimated and RDA analyses were performed on 1000 equally sized random draws of reaches from the different successional stages in the polder. Per polder, the resulting  $\beta$  diversity values and RDA-associated p-values and test statistics were averaged across these permutations.

## Scenario analyses through simulations: evaluating the effect of successional stage heterogeneity on $\gamma$ -diversity

To test for the effect of removing successional stages from the landscape on  $\gamma$ -diversity we defined two main scenarios related to ditch management. The 'Selective Management Scenario' represents a management that gives priority to resetting succession in the late successional stages (e.g. through dredging). For this scenario we simulated seven sub scenarios. Together these sub-scenarios represent a gradient of increased management intensity along which the most advanced successional stages (late in the successional trajectory) are successively being removed. One extreme end of this gradient thus represents a situation where all stages are present whereas the other extreme pertains to a landscape that only contains reaches with stage 1 (earliest stage in the successional trajectory). The second scenario, further referred to as the 'No Management Scenario' represents a situation in which management has stopped taking place and where the succession of the vegetation in each site progresses through the subsequent successional stages until it reaches the most advanced stage. Also for this scenario, we simulated 7 sub scenarios. In contrast to the Selective Management Scenario the earliest stages are progressively removed from the landscape, starting from stage 1 and working forward (e.g. removing stage 1 and 2, stage 1 to 3, etc.) up to the point that a landscape consists solely of reaches with succession stage 7 (the latest in the successional trajectory).

Simulations were performed for each of the main scenarios separately. For each of the sub scenarios of a given main scenario, we assigned 12 ditches to each of 21 simulated landscapes. This was done by taking random draws of reaches from our vegetation dataset but respecting the constraints that define each of the sub-scenarios. This approach makes the explicit assumption of complete interchangeability of all reaches in the dataset, irrespective of polder identity. This implies that in our simulated landscape habitat suitability for species is determined by the successional stage alone.

Furthermore, all species are deemed capable of reaching all the sites in the landscape (i.e. no dispersal limitation). For each landscape x sub-scenario combination, we partitioned  $\gamma$  into its  $\alpha$  and  $\beta$  components and  $\beta$  into its richness and replacement components. For each of these end point variables, we studied its association with successional stage heterogeneity using linear regression of the form  $y=a*x+b$  with  $y$  being the diversity end point variable,  $x$  being the successional stage heterogeneity of the sub scenario. This process was repeated 2000 times and we calculated average p value and coefficients for the resulting 2000 regression lines. To study the diversity of individual successional stages we employed the same simulation strategy with each sub-scenario consisting of 12 randomly drawn ditch reaches of the same successional stage.

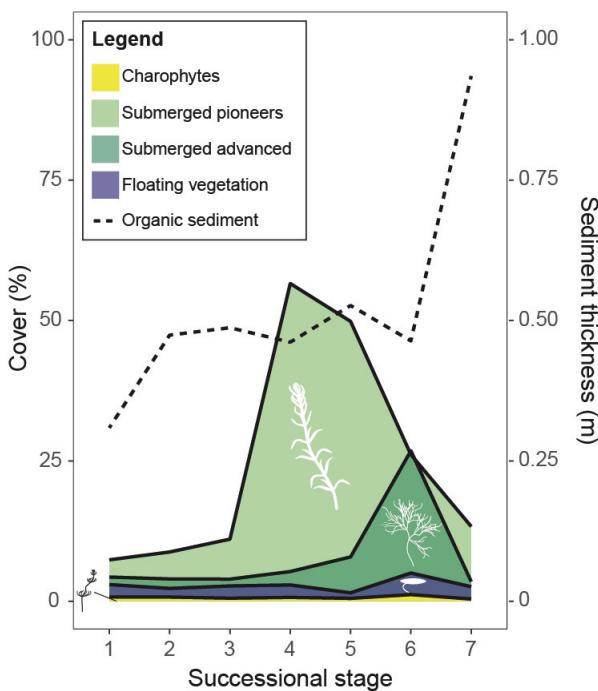
All analyses and simulations were performed in R version 3.3.2 using the *ggplot2* and *vegan* (Oksanen *et al.* 2015) packages and the custom code supplied with Legendre (2014).

## Results

### Identifying successional stages

Fuzzy clustering yielded a solution with 9 clusters and was found to provide the best fit (connectivity: 192.8; silhouette width: 0.241). Based on functional type composition of the vegetation and sediment thickness, seven of these clusters could be identified as successional stages and ranked along the succession gradient as described in Figure 6.2 (Figure 6.3, Figure S6.1). Stage 1 was characterized primarily by low abundance of submerged vegetation and a thin organic sediment layer. Compared to Stage 1, Stage 2 and 3 had a thicker sediment layer and showed an increased abundance of submerged pioneering species. Stages 2 and 3 were very similar and differed mainly in cover by floating unrooted vegetation (Figure S6.1). Compared to the previous stages, Stage 4 showed a strong increase in the cover by submerged pioneers. Similarly to Stage 4, Stage 5 contained a relatively large population of submerged pioneers but also had a larger share of late successional submerged plants. In Stage 6 late successional submerged species became dominant, floating rooted plants increased while early pioneers decreased in abundance. Ditch reaches belonging to Stage 7 were characterised by a relatively thick layer of organic sediment and an overall decline of true aquatic vegetation. Very advanced successional stages with a predominance of helophytes in the middle of the ditch did not occur in our dataset, most probably as the result of current management. Generally, helophytes were growing along the ditch margins in all successional stages and proved a poor indicator of successional stage

(not shown in Figure 6.3). One cluster corresponded best with a duckweed dominated alternative stable state and could as such not be categorized as a successional stage. Another cluster was characterized by a relatively thick organic sediment layer and overall low vegetation cover, possibly caused by high local sedimentation rates or a combination of frequent vegetation removal with lack of dredging. This stage is also rather atypical and was not categorized as a successional stage either.

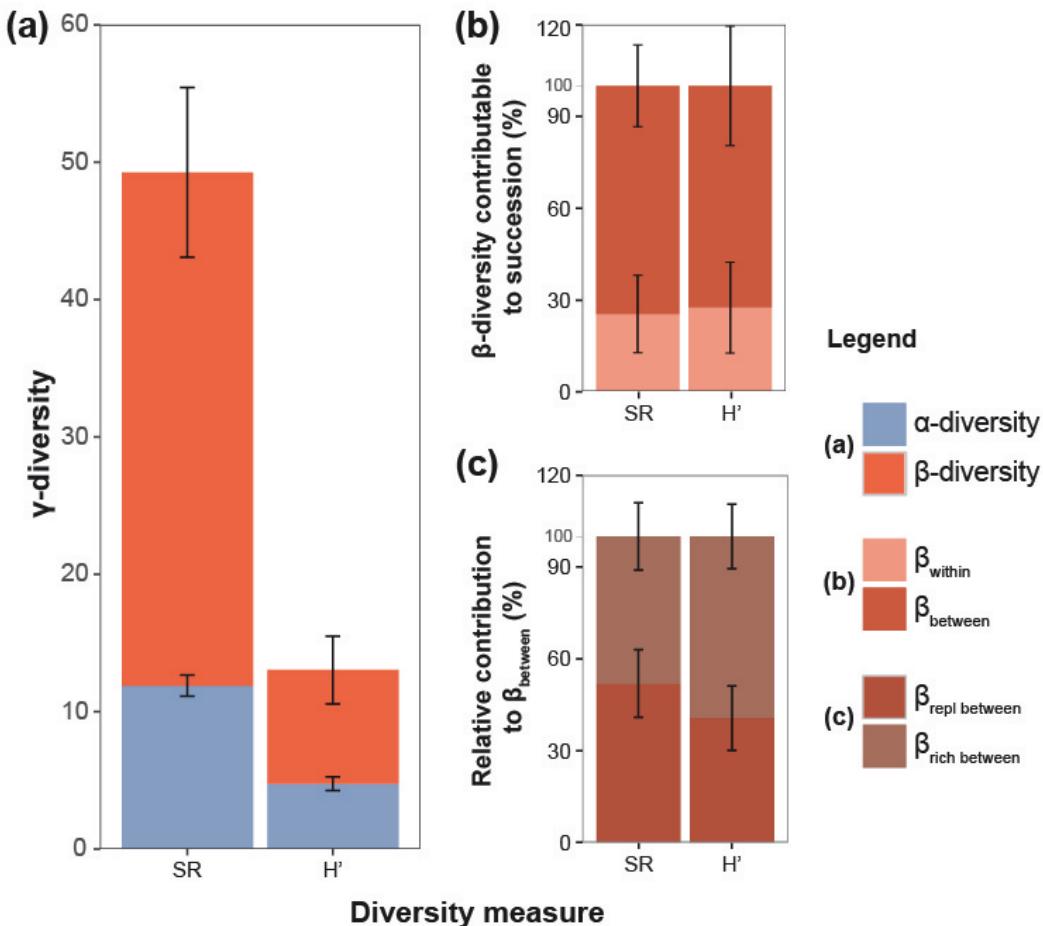


**Figure 6.3:** Progression of plant functional group cover (%) and organic sediment thickness (m) through the different identified successional stages (1-7). Averages are given of ditch reaches per identified successional stage. For data on relative cover for each of the species: see Figure 6.1.

## Diversity in successional stages: partitioning results

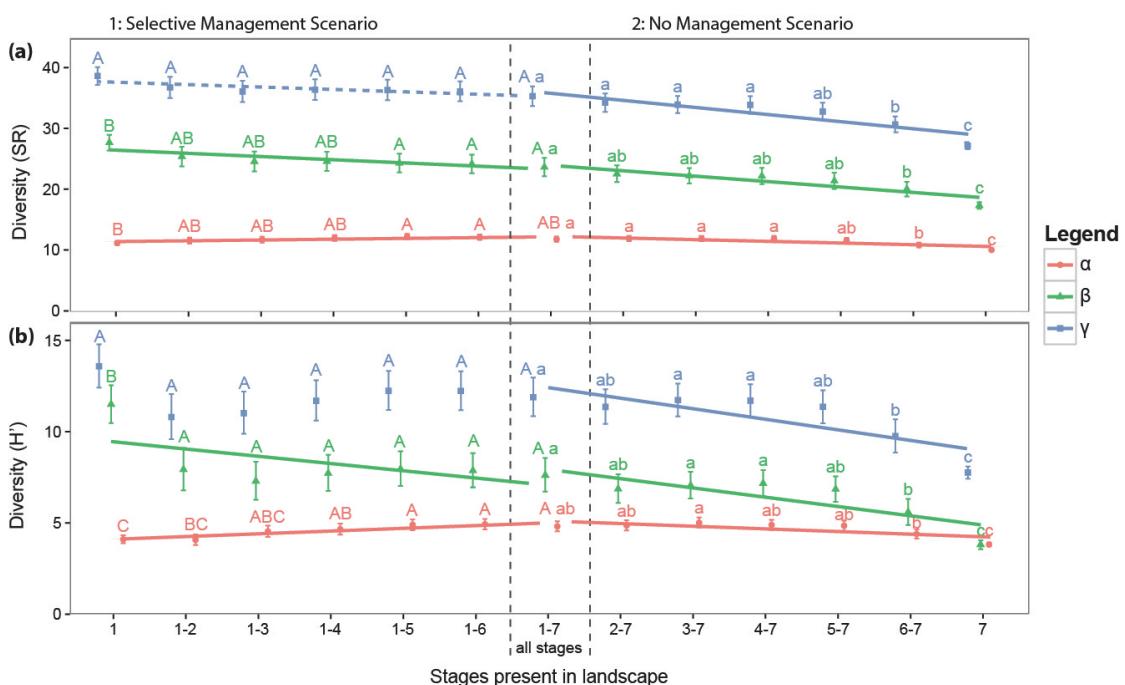
The partitioning of  $\gamma$ -diversity into its  $\alpha$  and  $\beta$  components (Figure 6.4a) revealed that only a small part of the landscape-wide diversity ( $\gamma$ ) could be attributed to the local diversity ( $\alpha$ ) of ditch reaches ( $SR=11.9$ ,  $H'=4.8$ ). In contrast, the contribution of the  $\beta$  component was much higher ( $SR=75\%$ ,  $H'=60\%$ ), indicating large differences in community composition among ditch reaches within polders. A large part of the  $\beta$ -diversity could be attributed to differences between successional stages ( $\beta_{\text{between}}$ :  $SR=80.4\%$ ,  $H'=77.3\%$ , Figure 6.4b) and about half of this  $\beta$ -diversity ( $\beta_{\text{between}}$ ) represented true replacement of species ( $SR: 51.7\%$ ,  $H'= 40.4\%$ ; Figure 6.4c).

The dbRDA-analyses showed significant differences in the community composition of successional stages (Jaccard: mean  $R^2_{adj}=5.2\%^{***}$ , Ruzicka: mean  $R^2_{adj}=25.6\%^{***}$ ). Species replacement was significantly associated with differences between successional stages (Jaccard:  $R^2_{adj}=4.4\%^{***}$ , Ruzicka:  $R^2_{adj}=8.6\%^{***}$ ). Likewise, species richness difference was significantly explained by successional stage (Jaccard:



**Figure 6.4:** (a) Total observed diversity ( $\gamma$ ) of ditch vegetation within polders partitioned into components of: 1) mean local diversity of ditch reaches ( $\alpha$ ), 2) difference in community composition between reaches within polder landscapes ( $\beta$ ). (b)  $\beta$ -diversity is further divided into a component attributable to compositional variation among reaches belonging to the same successional stages ( $\beta_{within}$ ) and variation among reaches from different successional stages ( $\beta_{between}$ ). (c) Relative contribution of species replacement ( $\beta_{rep}$ ) and richness differences ( $\beta_{rich}$ ) (Legendre 2014). Partitioning results are shown for both species richness (SR) and the exponent of the Shannon-Wiener index ( $H'$ ). Error bars reflect variation among polders and equal twice the standard error around the mean.

$R^2_{adj}=6.2\%^{***}$ , Ruzicka:  $R^2_{adj}=62.3\%^{***}$ ). When comparing successional stages pairwise (Figure S6.2, Figure S6.3), we found varying underlying patterns of these two components of  $\beta$ -diversity. For the species presence/absence (Jaccard) data we found that  $\beta$ -diversity is caused by higher replacement among the earlier successional stage combinations compared to later successional stage combinations. For the abundance based analyses (Ruzicka) these patterns were less clear, though very high replacement as well as high richness difference occurred between some pairs of successional stages.



**Figure 6.5:** Simulation results of scenario analyses showing the effects of decreasing landscape-wide successional stage heterogeneity on the landscape diversity (y) and its partitions ( $\alpha$ ,  $\beta$ ) for both species richness (a) and true Shannon diversity  $H'$  (b). On the left hand side the Selective Management scenario is shown, where late successional stages are progressively removed from the landscape. On the right hand side the No Management scenario is shown, where early successional stages are progressively removed from the landscape. Error bars show twice the standard error around the landscape-wide mean diversity values based on 21 simulated landscapes with 12 ditch reaches per landscape. Letters indicating pairwise significance between successional group means. Lines show significant trends ( $p<0.05$ ) along the gradient of management based on a linear model and dashed lines show marginally significant trends ( $p<0.10$ ).

## Effects of management scenario's on diversity

With scenario analyses we assessed potential changes in the  $\gamma$ -diversity through management associated reductions in landscape-wide successional stage heterogeneity. Landscapes containing all seven successional stages had a mean  $\gamma$ -diversity of 35 species (Figure 6.5). In the Selective Management Scenario, where late successional stages were progressively removed from the landscape, we did not find a significant change in the  $\gamma$ -diversity (LM: intercept=35.21, slope=0.40,  $p=0.0502$ ,  $R^2_{adj}=6.8\%$ ). In contrast, the gradual disappearance from the landscape of early successional stages in the No Management Scenario resulted in a significant reduction of the  $\gamma$ -diversity with a total loss of on average 7 species or about 20% of the species in a landscape (LM: intercept=36.36, slope=-1.17,  $p<0.001$ ,  $R^2_{adj}=36.5\%$ ). Although this trend was associated with a reduced  $\alpha$ -diversity, it seemed to be mainly caused by a reduction in  $\beta$ -diversity (Figure 6.5b, Table S6.3).

To further elucidate the diversity decline observed for the No Management Scenario, we partitioned the  $\beta$ -diversity between successional stages ( $\beta_{between}$ ) into its replacement ( $\beta_{repl}$ ) and richness difference ( $\beta_{rich}$ ) components. Figure S6.4 (Table S6.4) shows that the decline in  $\beta$ -diversity of the reaches in the landscape ( $\beta_{between}$ ) was entirely driven by a decline in  $\beta_{repl}$ , i.e. complementarity among successional stages within the landscape (LM: intercept=0.39, slope=-0.045,  $p<0.001$ ,  $R^2_{adj}=33.7\%$ ) and not by the loss of richness differences among successional stages,  $\beta_{rich}$  ( $p=0.401$ ). Similar analysis on species abundance, giving greater weight to highly abundant or dominant species, showed no effect on the total  $\beta_{between}$  ( $p=0.124$ ). Results of species richness and Shannon diversity were similar (Table S6.4).

By running sub-scenarios with landscapes consisting of only one successional stage we were able to assess how successional stages differed from each other in terms of their diversity. A landscape consisting of just the first successional stage is very divers with respectively 38 and 14 species for SR and H' (Figure 6.6). Landscapes consisting of the later successional stages are less divers with a landscape consisting of stage 7 alone consisting of only respectively 27 and 7 species for SR and H'. The local diversity ( $\alpha$ ) of the different stages is relatively similar, ranging from 10 to 11 species for SR and 4 to 5 for H'. The  $\beta$ -diversity of the different stages varied widely (SR: from 29 to 19 species and H': 12 to 4 species) with the earliest stage showing the greatest within-stage heterogeneity among ditch reaches.

## Discussion

Our diversity partitioning analyses show that  $\beta$ -diversity forms a very important component of the  $\gamma$ -diversity of macrophytes in ditch networks of polder systems. These results are in agreement with several other studies that have shown large differences in community composition among sites for a variety of organism groups in agricultural ditches, including macroinvertebrates, diatoms and helophytes and aquatic vegetation (Leng *et al.* 2011; van Zuidam & Peeters 2013; Clarke 2014; Goldenberg Vilar *et al.* 2014). New to our analyses is that more than 70% of this  $\beta$ -diversity seems to arise from differences in successional stages of ditch vegetation and that at least 40% of this  $\beta$ -diversity stems from complementarities in species composition between successional stages. Our results thus clearly demonstrate that heterogeneity in successional stages contribute strongly to the regional diversity of macrophytes in the ditch network of a landscape.

The importance of landscape heterogeneity for biodiversity has been shown in a variety of biomes such as boreal forests, grasslands and rivers (respectively, Niemala *et al.*, 1996; Richards *et al.*, 1999; Ward *et al.*, 2002). Here we showed similar results for aquatic plants in agricultural drainage ditches within polder landscapes, illustrating the importance of maintaining landscape heterogeneity. The large contribution of  $\beta$ -diversity to  $\gamma$ -diversity observed in our study emphasizes the necessity for a focus of management on diversity at the landscape scale rather than at the scale of local ditch reaches. Given that differences among successional stages form an important part of the landscape heterogeneity influencing the  $\beta$ -diversity in our polder landscapes, the creation and maintenance of successional heterogeneity may be an important way through which management may be able to enhance and sustain landscape wide biodiversity. In the absence of any management or natural disturbances, heterogeneity among communities of different sites will gradually be reduced as succession progresses (Vandvik *et al.* 2005). The landscape would increasingly be dominated by a limited number of late successional stages. As the 'No Management Scenario' of our simulations illustrate, this is expected to result in a gradual decrease of  $\beta$ - and  $\gamma$ -diversity through time, as the early successional stages are lost from the landscape.

Currently, management in the agricultural ditch networks of The Netherlands is almost entirely focused on the maintenance of their hydrological function and largely involves regular dredging, vegetation removal and bank reshaping (Twisk *et al.* 2003). Given that these measures involve a reset of succession, they contribute to the rejuvenation of vegetation (Wade 1993) and may as such also contribute to the maintenance of successional stage heterogeneity and  $\gamma$ -diversity. However, the impact of these measures on the latter variables will also depend on the frequency and the scale of

their application. The frequent and simultaneous application of these measures over large spatial areas will inevitably result in the disappearance of late successional stages. According to our diversity partitioning results this would be expected to result in a reduced  $\beta$ - and  $\gamma$ -diversity as well. However, such expectation is poorly supported by our simulation results which suggest that a predominance of young successional stages would be of little consequence to  $\gamma$ -diversity. Indeed, in these simulations, the progressive loss of later successional stages resulted in a minor reduction of  $\alpha$ -diversity, but this was entirely compensated by an increase in  $\beta$ -diversity. Across the entire dataset,  $\beta$ -diversity of early successional stages was relatively high compared to late successional stages (Figure S6.5). The high compositional variability among communities of early successional stages likely reflects a large impact of stochasticity during community assembly. Compared to late successional stages, interspecific interactions in young habitat patches are still weak and the composition of pioneering communities is likely more determined by coincidental dispersal and colonization events than by the outcome of competition (Tilman 1994).

When interpreting the simulation results it is, however, important to note that diversity indices were calculated based on random draws from the entire dataset. Communities of ditch reaches were thus regarded to be freely interchangeable among polders. This implicitly assumes absence of any dispersal limitation and ignores historic trajectories of metacommunities in individual polders. This may have led to an underestimation of the negative effects of late successional stage loss. By removing late successional stages, their function as source of populations recolonizing the earlier stages may be compromised. The diversity of early successional stages in the absence of late successional stages may therefore have been overestimated in our simulations. In reality the reassembly of communities after the reset of succession may largely depend on colonization, which may take 3-4 years to recover full vegetation diversity (Milsom *et al.* 2004). Colonization by dispersing propagules from proximate source communities is also likely to be more influential than from more distant ones (Brederveld *et al.* 2011; Van Dijk *et al.* 2014). Furthermore, the remaining seed bank can also play an important role in community reestablishment (Sarneel *et al.* 2014; Van Leeuwen *et al.* 2014), although it is unknown to what extent dredging activities reduce local community resilience by removing large parts of this reservoir. If management resets succession in large parts of the landscape simultaneously, recolonization of empty sites may be severely impeded by dispersal limitation reducing overall  $\beta$ -and  $\gamma$ -diversity of the landscape. Although the contribution of early successional stages to  $\gamma$ -diversity may potentially be disproportionately high, the role of nearby mid and late successional stages as source communities of dispersal propagules should not be ignored in real plant metacommunities.

Conceptually, CRM is likely the type of management scheme that best guarantees the presence of a range of successional stages in the landscape. However, much depends on the specific parameters of management, namely the delay and frequency of visit by management (Hinsch & Poethke 2007). The delay is defined as the time that each site is left to develop naturally before it is revisited (Morris 2000). Frequency of visits by management to the landscape is defined as the proportion of sites being managed during each visit. More frequent visits lead to a lower proportion of sites being managed per visit, but creating a higher variation in successional age within the landscape (Hinsch & Poethke 2007). A low delay (the duration of a few years) may entirely exclude some late successional stages from the landscape and therefore eliminate potential sources for recolonization of recently managed ditch reaches. In contrast, high delays (decades) may result in a predominance of late successional stages that may interfere with the hydrological function of the ditches and contribute less to  $\gamma$ -diversity. When a large number of ditch reaches are visited at once (low frequency), a landscape will likely consist of large, homogenous blocks with relatively low successional stage diversity across the landscape. Low frequency management is typically found in the currently employed, large scale dredging operations where large swaths of the landscape are managed at the same time. We advocate that application of CRM management schemes with more frequent visits and possibly also higher delays would lead to more heterogeneous mosaic-like landscape patterns (see Niemala *et al.* 1996 for an example in boreal forests). Such mosaic may be further elaborated through a spatial planning that is focused on minimizing distance between recently managed and other reaches as to facilitate dispersal and recolonization (Watson *et al.* 2012; Van Dijk *et al.* 2014).

Although the potential advantages of CRM for biodiversity conservation in agricultural ditch networks are obvious, it is difficult to make generalizations about what should be the optimal CRM scheme. Ideally, CRM schemes are based on knowledge about the development rates of the different successional stages. Succession rates appear to be highly variable as the time needed for succession to turn an empty ditch into a carr may range between 10 and 27 years (Bakker *et al.* 1994). Succession rates in aquatic systems are highly context specific and depend on several factors such as sedimentation rates, flow velocity (Sousa 1979), sediment characteristics, intensity of land use and associated nutrient loading (Carpenter 1981). Obviously, more productive systems with high rates of sediment loading will require CRM schemes with shorter delays than less productive systems. Ideally, CRM schemes are adapted and improved based on information acquired through the monitoring of succession in parts of the ditch network that have been managed, a form of adaptive management (Haney & Power 1996). Using social-benefit analyses (e.g. Fliervoet *et al.* 2013) the benefits to

farmers (reduced management effort and cost), water quantity managers (maintained drainage capacity) and nature water quality organizations (increased ecological diversity) may be quantified in detail to arrive at an optimal management plan for a specific landscape.

It's important to note that the ditch networks considered in our study have historically been associated with intensive land use. They are biotically impoverished in comparison to ditch networks in areas with less intensive agriculture (Leng *et al.* 2011). High nutrient loading is likely to enhance the intensity of interspecific competition especially in the later successional stages, leading to monotonous vegetation (van Zuidam & Peeters 2013). This may have contributed to the relatively high degree of homogeneity of these stages in the landscape. Furthermore, conclusions of this study only pertain to the successional stages that were encountered in our field study. Current management practices in the Dutch agricultural polder landscape prevent ditches from containing successional stages that are older and more developed than the ones observed in our studies (e.g. alder carrs, floating fens). Our analyses and simulations do not take into account the potential contribution of such successional stages to  $\beta$ - and  $\gamma$ -diversity. Although such stages likely interfere with the drainage function of the ditch network, they would potentially have a unique and important contribution to regional diversity. Hence it would be interesting to also evaluate such stages in future work and consider how they can be maintained in the landscape.

The same case can be made for other groups of organisms that make their home in the ditch systems. Despite the important structural and functional role of macrophytes in the aquatic habitat, conservation management in ditch networks should also aim at supporting biodiversity of other organism groups. Extrapolation of our conclusions regarding the contribution of successional stages to macrophyte biodiversity should therefore be done with prudence. Although the diversity of macrophytes may be an important determinant for the diversity of some associated groups (Whatley *et al.* 2014b), such association is not unequivocally strong for all aquatic groups. Producers are shown to be more strongly driven by patterns of replacement than higher trophic groups (Soininen *et al.* 2018). Therefore, studies on the impact of succession on the  $\beta$ - and  $\gamma$ -diversity of those other groups and how these can be adequately managed is vital. Our data driven approach may be easily applied to other species groups to assess the potential efficacy of a CRM scheme. Herein lays the power of our approach, all it requires is data on species composition of different sites in a landscape and a conceptual notion of successional trajectories. This type of data is generally gathered by nature- and water managers in their standard monitoring schemes. These data may serve as an ideal first step in evaluating the inherent requirements (differences in, and replacement of species between successional stages) for useful application of CRM,

before extensive management activities are carried out that may be hit-or-miss. Further development of a specific management scheme could benefit from specific deterministic models (see Perona *et al.* 2009 for CRM in floodplains and Larocque *et al.* 2013 for an overview of similar models in forests).

In conclusion, we have shown the importance of succession as a driving force in agricultural drainage ditches, non-natural aquatic habitats that nonetheless can contribute to the biodiversity of the landscape (Davies *et al.* 2008; Verdonschot *et al.* 2011). Promoting successional heterogeneity is possible through cyclic rejuvenation of parts of the landscape through management. As management of ditch systems is needed to preserve hydrological functioning (Herzon & Helenius 2008), the change to a system-specific, spatially explicit management scheme employing the principles of CRM can be both viable and cost-effective. This combination of drainage and ecological function fits well within the context of reconciliation ecology (Rosenzweig 2003), allowing for coexistence of relatively high levels of biodiversity in a seemingly inhospitable landscape of anthropogenic agricultural activity.

## Acknowledgements

The authors would like to thank Dennis Waasdorp, Erik Reichman, Edwin van den Berg, Marta Alirangues, Maik Janssen, Lilith Kramer, John O'Connor, Thomas Clay, Roos Plak, Lisa Freitag, Jeroen Jongerius, Marlies Gräwe and Annegreet Veeken for help in the field and Amber Heijboer for illustrations. This work was supported by the Division for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO), more specifically by the Biodiversity Works Research Program (OBW) project no. 841.11.009.

# Chapter 6: Supplementary materials

**Table S6.1:** Landscape coordinates (WGS84) in degrees.

Landscape number	Latitude (degrees)	Longitude (degrees)
1	52.12791	4.92838
2	52.04209	4.78927
3	51.88817	4.78154
4	51.98641	4.79103
5	51.85967	4.74393
6	52.16753	5.12449
7	51.88792	4.75494
8	51.86355	4.82294
9	51.96902	4.85145
10	52.04824	4.83968
11	52.05982	4.76403
12	51.91468	4.68527
13	52.11776	4.61890
14	51.91930	4.89699
15	51.93908	4.72608
16	51.75151	4.53776
17	52.25942	5.00921
18	52.19264	5.03050
19	52.27827	5.13003
20	51.93984	4.77225
21	52.03339	4.78264

**Table S6.2:** Conversion of Tansley abundance classes to numeric classes and percentage cover.

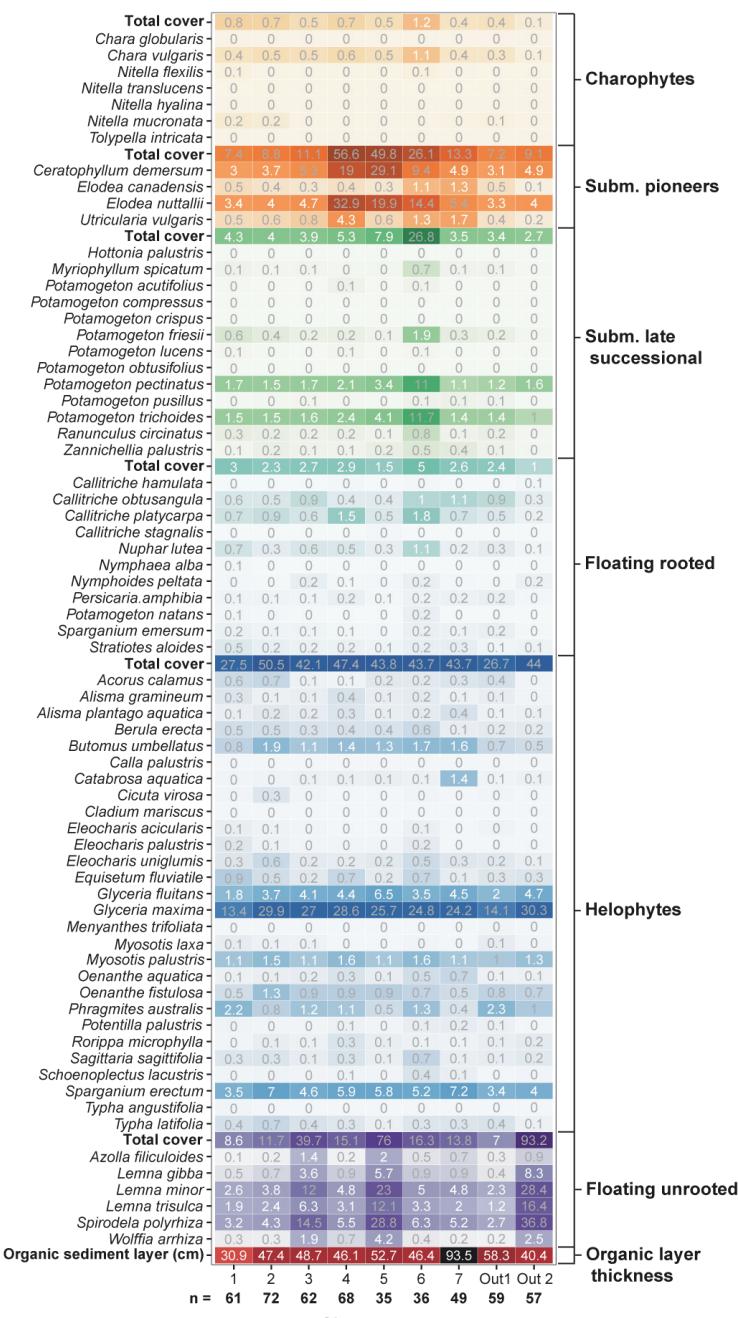
Tansley score	Tansley numeric score	Cover (%)
d	11	60
cd	10	40
Id	9	22
a	8	15
la	7	9
f	6	8
If	5	3
o	4	2
lo	3	1
r	2	0.5
s	1	0.1

**Table S6.3:** Summary table of the different regression models and their parameters showing the trends present in the scenario analyses outlined in Figure 6.5.

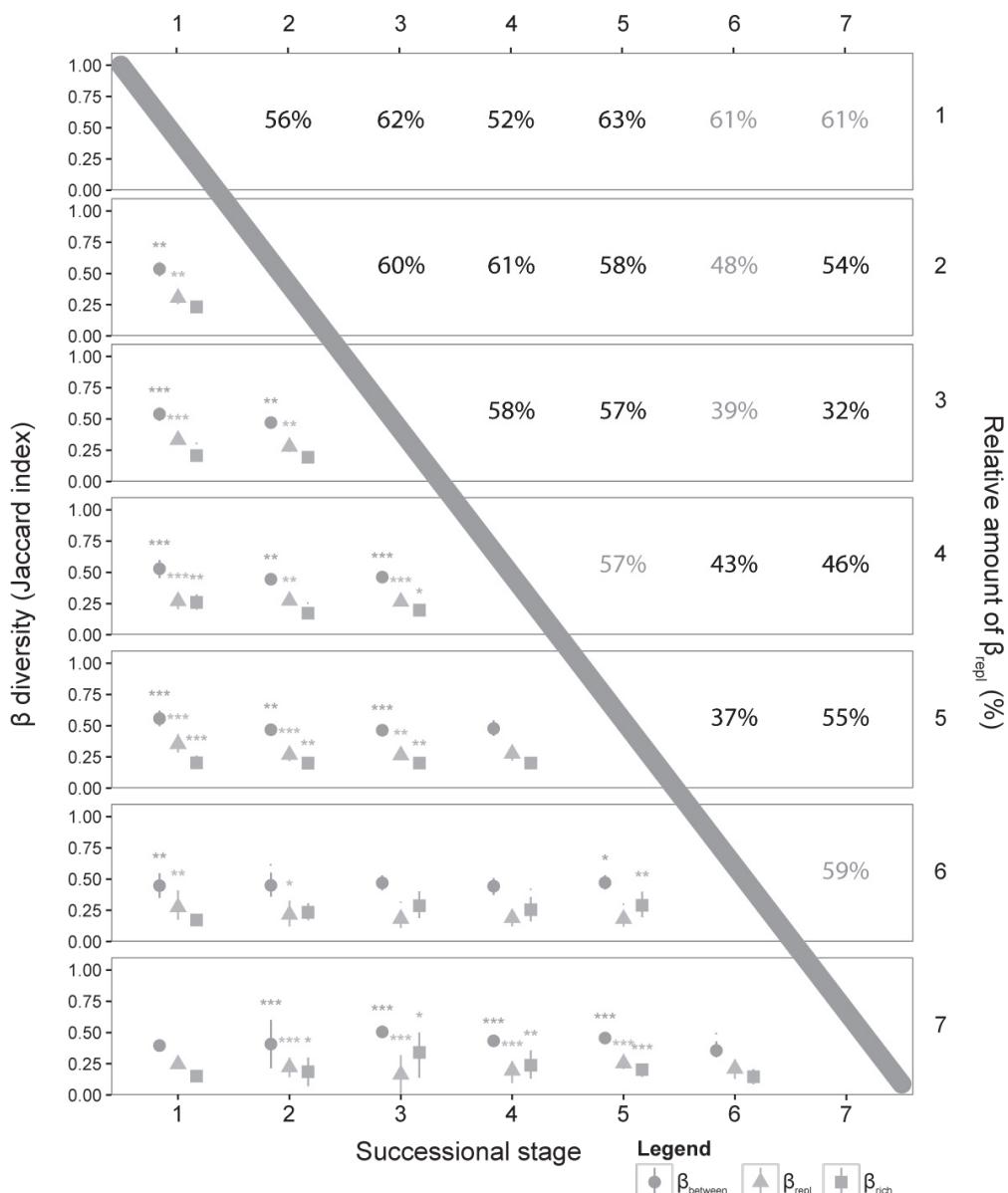
Partition	Scenario	q	parameter	Value	±	se	DF	t-value	p-value
$\alpha$	1: Selective management	SR	intercept	12.31	±	0.18	125	67.52	7.82E-93
			slope	-0.13	±	0.04	125	-3.24	0.019
			adjusted R <sup>2</sup>	8.35					
	H'	SR	intercept	5.17	±	0.12	125	44.83	1.72E-71
			slope	-0.15	±	0.03	125	-5.89	1.73E-05
			adjusted R <sup>2</sup>	20.29					
	2: No management	SR	intercept	12.28	±	0.40	125	35.91	2.07E-59
			slope	-0.28	±	0.04	125	-7.12	1.61E-07
			adjusted R <sup>2</sup>	26.18					
$\beta$	1: Selective management	SR	intercept	22.91	±	0.62	125	37.26	5.79E-63
			slope	0.53	±	0.14	125	3.88	0.005
			adjusted R <sup>2</sup>	10.95					
	H'	SR	intercept	6.68	±	0.45	125	15.04	6.32E-25
			slope	0.40	±	0.10	125	4.02	0.003
			adjusted R <sup>2</sup>	10.69					
	2: No management	SR	intercept	24.08	±	1.17	125	26.02	6.22E-45
			slope	-0.89	±	0.11	125	-7.81	2.48E-08
			adjusted R <sup>2</sup>	30.06					
$\gamma$	1: Selective management	SR	intercept	35.22	±	0.67	125	53.13	2.78E-81
			slope	0.40	±	0.15	125	2.71	0.050
			adjusted R <sup>2</sup>	6.76					
	H'	SR	intercept	11.78	±	0.47	125	24.98	3.38E-44
			slope	0.04	±	0.10	125	0.34	0.494
			adjusted R <sup>2</sup>	1.79					
	2: No management	SR	intercept	36.36	±	1.31	125	34.06	1.92E-57
			slope	-1.17	±	0.13	125	-9.12	6.72E-11
			adjusted R <sup>2</sup>	0.37					
	H'	SR	intercept	12.45	±	0.86	125	19.34	4.25E-32
			slope	-0.58	±	0.08	125	-6.93	9.35E-07
			adjusted R <sup>2</sup>	25.17					

**Table S6.4 :**Summary table of the different regression models and their parameters showing the trends present in the scenario analyses outlined in Figure S6.4.

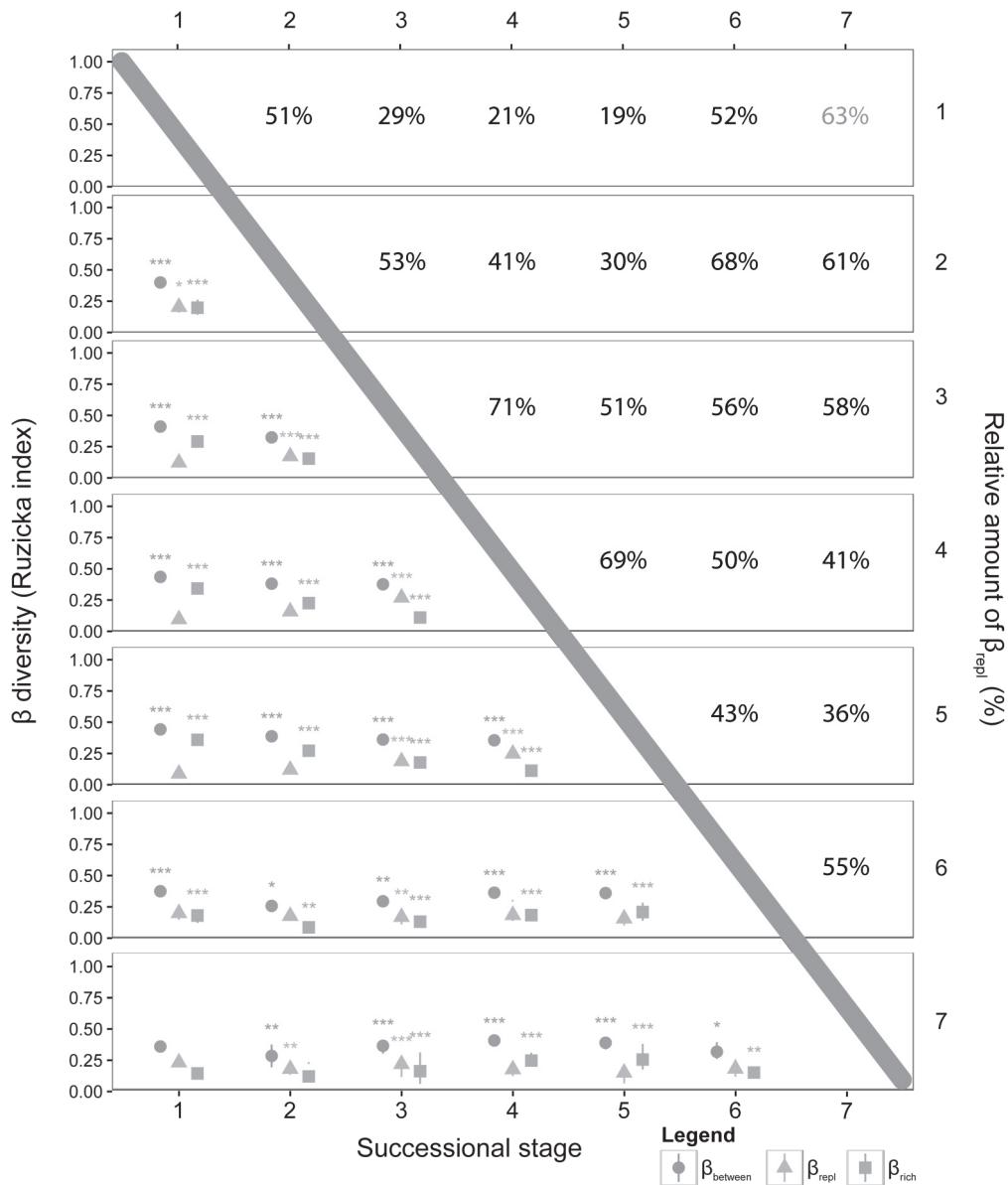
Partition	q	parameter	Value	± se	DF	t-value	p-value
$\beta_{\text{between D}}$	SR	intercept	0.77	± 0.04	102	26.69	7.79E-41
		slope	-0.05	± 0.00	102	-11.52	2.2E-14
		adjusted R <sup>2</sup>	51.90				
$\beta_{\text{between repl}}$	H'	intercept	0.41	± 0.02	102	21.52	1.53E-33
		slope	0.00	± 0.00	102	-2.24	0.123656
		adjusted R <sup>2</sup>	6.38				
$\beta_{\text{between rich}}$	SR	intercept	0.39	± 0.06	102	12.71	3.80E-17
		slope	-0.04	± 0.01	102	-7.77	1.41E-07
		adjusted R <sup>2</sup>	33.76				
	H'	intercept	0.17	± 0.03	102	7.92	1.28E-07
		slope	-0.01	± 0.00	102	-3.91	1.17E-02
		adjusted R <sup>2</sup>	13.25				
	H'	intercept	0.24	± 0.04	102	3.99	4.76E-03
		slope	0.01	± 0.00	102	1.84	1.97E-01
		adjusted R <sup>2</sup>	5.64				



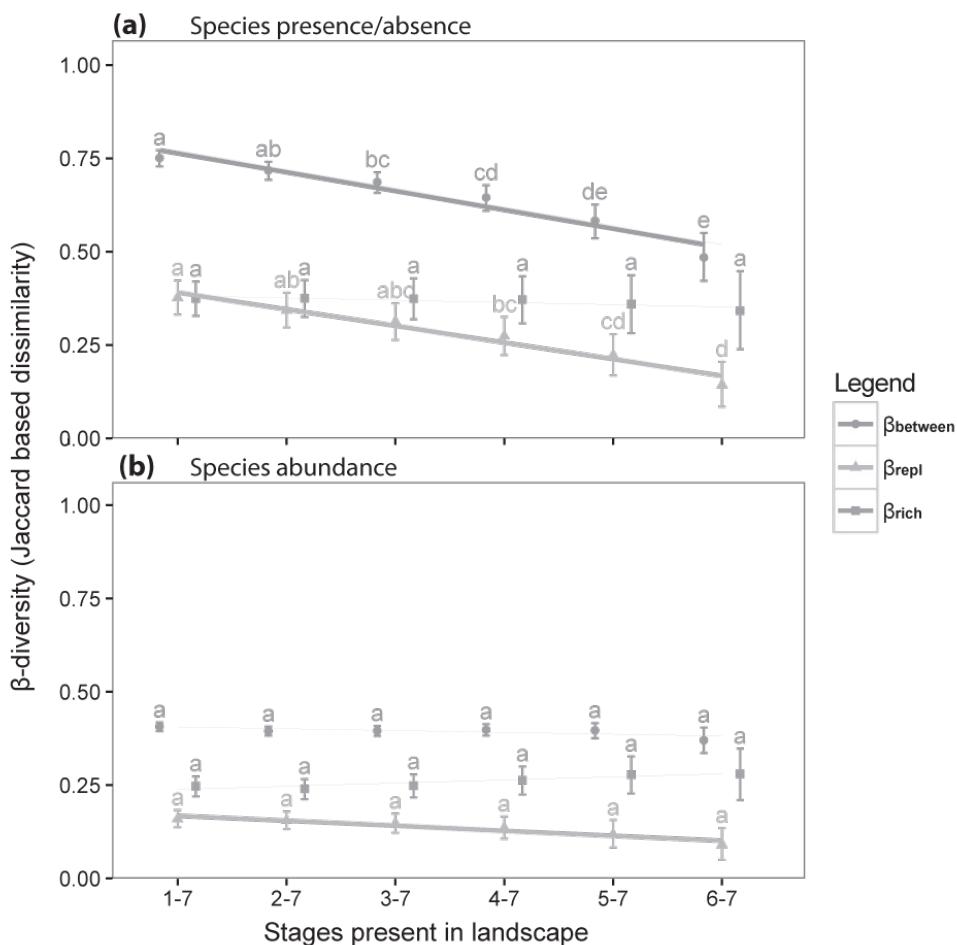
**Figure S6.1:** Heatmap of clusters (columns) and ranked along the successional gradient (except for groups 8 and 9; see text). Species (rows) are grouped by functional type (colours). Numbers in cells and colour intensity represent the relative cover (%) averaged over sites within clusters. Total cover: sum of cover of all species belonging to specified functional groups. Clusters Out1 and Out2 were not considered as part of the natural progression of succession and disregarded in further analyses.



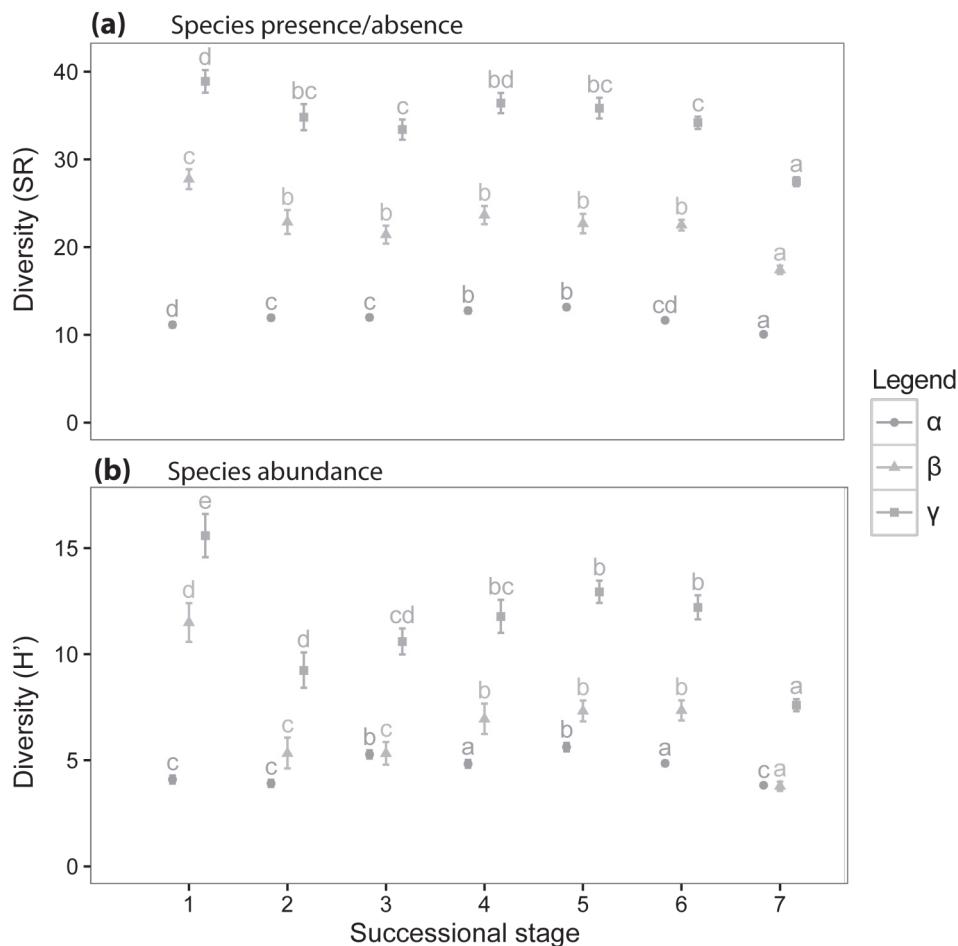
**Figure S6.2:**  $\beta$ -diversity (expressed as a multi-site Jaccard dissimilarity index) between communities of different successional stages ( $\beta_{\text{between}}$ ) and its partitions of species replacement ( $\beta_{\text{rep}}$ ) and richness difference patterns ( $\beta_{\text{rich}}$ ) for presence/absence data. All possible pairwise combinations among successional stages (1-7) are shown. Significance against random difference in community composition based on a dbRDA analysis is indicated using symbols (\*\*:  $p < 0.01$ , \*\*:  $p < 0.05$ , \*:  $p < 0.10$ ) and error bars give twice standard error around the polder mean. The values given in the upper triangle refer to the mean percentage of  $\beta$ -diversity which may be attributed to replacement. Values given are given in grey when the dbRDA model was not significant.



**Figure S6.3:**  $\beta$ -diversity (expressed as the total variance of a Ruzicka index distance matrix) between communities of different successional stages ( $\beta_{\text{between}}$ ) and its partitions of species replacement ( $\beta_{\text{repl}}$ ) and richness difference patterns ( $\beta_{\text{rich}}$ ) for presence/absence data. All possible pairwise combinations among successional stages (1-7) are shown. Significance against random difference in community composition based on a dbRDA analysis is indicated using symbols (\*\*\*( $p < 0.001$ ), \*\*( $p < 0.01$ ), \*( $p < 0.05$ ), .( $p < 0.10$ )) and error bars give twice standard error around the polder mean. The values given in the upper triangle refer to the mean percentage of  $\beta$ -diversity which may be attributed to replacement. Values given are given in grey when the dbRDA model was not significant.



**Figure S6.4:** Effects of decreasing landscape-wide successional stage heterogeneity on the between successional  $\beta$ -diversity ( $\beta_D$ ) and its partitions ( $\beta_{\text{repl}}$ ,  $\beta_{\text{rich}}$ ) based on a scenario of No Management (scenario 2). (a) Changes in diversity based on species incidence data using a multi-site Jaccarddissimilarity index. (b) Changes in diversity based on species abundance data using the total variance of a Ruzicka (abundance based Jaccard) dissimilarity matrix. Error bars show the 97.5% confidence limits around the landscape-wide mean diversity values based on 21 simulated landscapes with 12 sites per landscape. Letters indicating pairwise significance between successional group means. Lines show significant trends ( $p < 0.05$ ) along the gradient of management based on a linear model and dashed lines show marginally significant trends ( $p < 0.10$ ).



**Figure S6.5:** Diversity of successional stages in isolation, based on simulated landscapes of 12 randomly selected sites of the given successional stage. Twice the standard error around the landscape-wide mean diversity values based on 21 simulated landscapes. Letters indicating significance ( $p < 0.05$ ) of post-hoc comparisons between successional stages in terms of their diversity indeces.

# 7

## Effects of nutrient-load redistribution on the ecological state of an agricultural waterscape

*Using simulation modelling to assess landscape scale impacts of nutrient management on ditch vegetation*

**Sven Teurlincx, Annette B.G. Janssen, Luuk van Gerven, Jan Kuiper, Lilith Kramer,  
Annegreet Veeken, Leo Renaud, Steven A. J. Declerck**

## Abstract

Agricultural drainage ditches are aquatic systems that are highly impacted by surrounding agricultural land use. With suitable management they can be hotspots of biodiversity in the landscape, however when loaded excessively with nutrients their ecological state deteriorates quickly. Extensification of land use and associated reductions of nutrient loads help to restore ditch systems, but is often difficult to achieve and is expensive. Redistribution of nutrient loadings within the landscape may facilitate improvements in water quality in parts of the landscape and promote desired submerged vegetation. Using the ecological model PCDitch coupled to a hydrodynamic model SOBEK and fed with nutrient loading from runoff model STONE, we made projections of the effects of nutrient load reduction and redistribution in a polder landscape situated in the west of the Netherlands. Our results showed that lowering nutrient loads to a level of extensified land use helped to improve the ecological state of the polder landscape, from floating species to a submerged plant state. However, the achieved nutrient reduction would not lead to occurrence of desired charophyte vegetation type. Redistribution of nutrients was able to achieve this, with the most persistent patches being found when loading was lowered in the most isolated part of the landscape and increased near the pour and the pump (water in- and outlet). However, when redistributing nutrient pressure through land use changes, the ditch reaches which receive a higher nutrient loading can exhibit a deteriorated ecological state, elucidating a clear tradeoff. We conclude that our approach can be used as a first projection of the effects of a wide variety of landscape level management options, prior to engaging in costly and possibly irreversible application in the field.

# Introduction

To sustain ever growing human populations, agricultural landscapes are generally managed intensively to produce high yield of industrial crops (Haberl *et al.* 2004). Large parts of these landscapes are basically dedicated to the ecosystem service of food provisioning only (Nelson *et al.* 2009; Landis 2017). Marginal landscape features such as field edges and adjacent waterways are, however, generally sparsely used for production, leaving room for nature (Hietala-Koivu *et al.* 2004). Drainage ditches and their banks are often overlooked landscape features despite their potentially high value to overall biodiversity (Chester & Robson 2013) and their contribution to other ecosystem services such as water storage and purification (Herzon & Helenius 2008; Dollinger *et al.* 2015). The absence of food production in ditches and their banks leaves room for other ecosystem services to be developed. This leads to a multifaceted landscape with different ecosystem functions contributing to overall sustainability and resilience of the area (Fischer *et al.* 2017).

For optimal delivery of multiple ecosystem services the ecological quality and state of waters is vital (Hilt *et al.* 2017). Due to their proximity to agricultural fields, drainage ditches are directly impacted by nutrient pollution from agricultural activities (Janse & Van Puijenbroek 1998). Eutrophication of water systems can have dramatic effects on the ecological state (Liere *et al.* 2007). As eutrophication increases, submerged vegetation, associated with clear water and high biodiversity (Carpenter & Lodge 1986), is outcompeted by free-floating plants such as lemnids (Gerven *et al.* 2015). Dense free-floating plant coverage leads to oxygen depletion of the water and hence a loss of life under water, resulting in a loss of biodiversity and an impoverished ecological state (Scheffer *et al.* 2003). However, even though highly productive agricultural landscapes are often regarded as monotonous at the macro level, both the land- and waterscape are inherently heterogeneous. Ditches may vary in width and depth and have different respective locations in the landscape compared to the dominant water flows. Moreover, the organization and history of land uses within a agricultural landscape lead to spatial differences in nutrient loading of drainage ditches (Kröger *et al.* 2013). Not surprisingly, a close inspection of the ecology of ditches in an agricultural landscape will likely reveal noticeable spatial differences in the occurrence of plant groups and the composition of the plant community. This may range from desireable submerged charophytevegetation (Beltman & Allegrini 1997) to systems overgrown with submerged plants such as *Elodea* or *Ceratophyllum* (van Zuidam & Peeters 2013) or systems covered by dense lemnid vegetation (van Zuidam 2013). Understanding the causal links between spatial distributions of land use, environmental pressures and ecology in a highly connected but heterogeneous landscapes remains an active

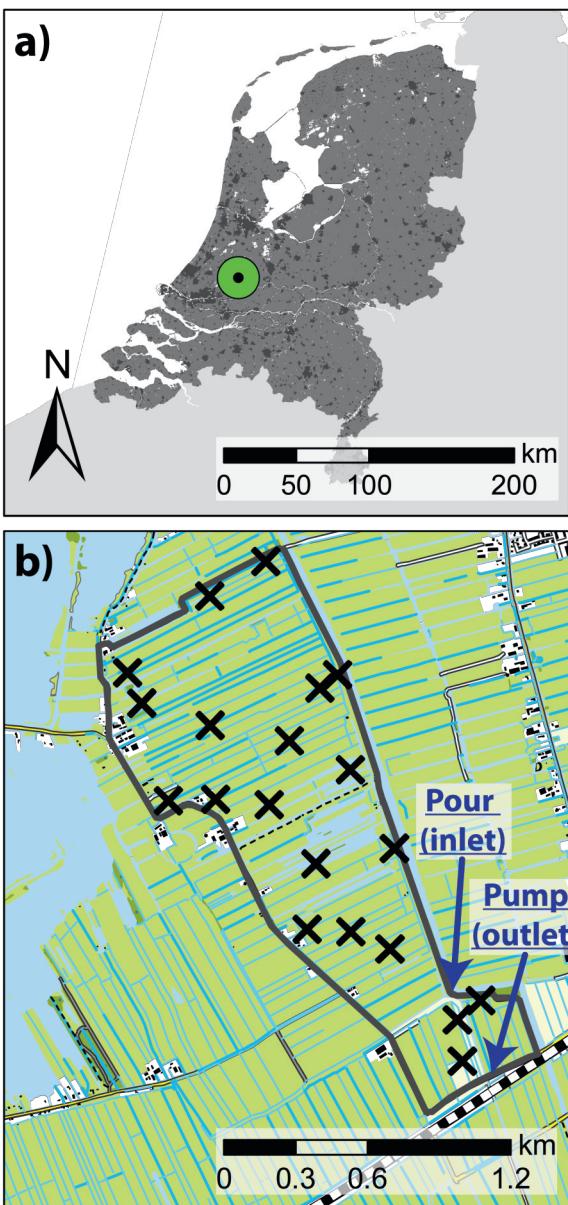
research frontier in contemporary ecology (e.g. Kovács-Hostyánszki *et al.* 2017; Staveley *et al.* 2017; Dunham *et al.* 2018)

The most straightforward and arguably most effective strategy to mitigate eutrophication of freshwater ecosystems is to reduce the total nutrient loading (Janse *et al.* 2008). However, such a measure tends to provoke resistance among farmers because it requires a profound change of land use, which is usually costly because of reduced productivity or investments in innovations to make agricultural practices more nutrient efficient (Paerl *et al.* 2016). A potentially cheaper option is to change the distribution of nutrient loading in the landscape, without changing the total nutrient loading. Unfortunately, very little is known about the importance of spatial distribution of nutrient loading for the ecological state of the landscape, and how effective it is relative to an actual reduction of the nutrient loading.

Experimentation with nutrient redistribution at the landscape scale is difficult for a number of reasons. First, landscapes are unique in biogeographic history, having had specific land use in the past, they create environmental and dispersal legacies that constrain current vegetation communities (Foster 2002; Foster *et al.* 2003). Furthermore, the current land and water use is highly dependent on the societal context, inhabitants, spatial planning decisions and societal mind-set all being major determinants of the land and waterscape (Lebel *et al.* 2006). Lastly, it is simply very costly to experiment with different nutrient distribution schemes at the level of a landscape as lack of success of a scheme leads to both societal backlash and ecological damage (Kingsford *et al.* 2011; Yalcin & Leroux 2018). As such, simulation modelling, using a spatially explicit ecosystem model may be a useful alternative. This approach allows for the testing of many different land usemanagement scenarios in a relatively short time frame for a fraction of the costs without the risk of ecological damage.

Here we use a dynamic aquatic ecosystem model for ditches, PCDitch (Janse 1998), to model the ecological state of ditches in a polder landscape (Oukoop) located in the Western Peat District of the Netherlands (Figure 7.1). We couple PCDitch to a spatially explicit hydrodynamic flow model, SOBEK, to allow for realistic flow of water and nutrients between ditches, and model the network configuration of coupled ditches (van Gerven *et al.* 2015). We determine nutrient loadings from the surrounding agricultural fields using the nutrient runoff-model STONE (Wolf *et al.* 2005). The goal of this modelling study is to determine if and how nutrient load reduction and redistribution can benefit the ecological state. We differentiate between ecological states that: 1) have abundant presence of desirable submerged vegetation in the form of charophytes, 2) less desirable submerged vegetation in the form of elodeids and ceratophyllids and 3) highly undesirable floating lemnid vegetation or phytoplankton blooms. To this end we vary both the intensity and the spatial distribution of nutrients

throughout the landscape.



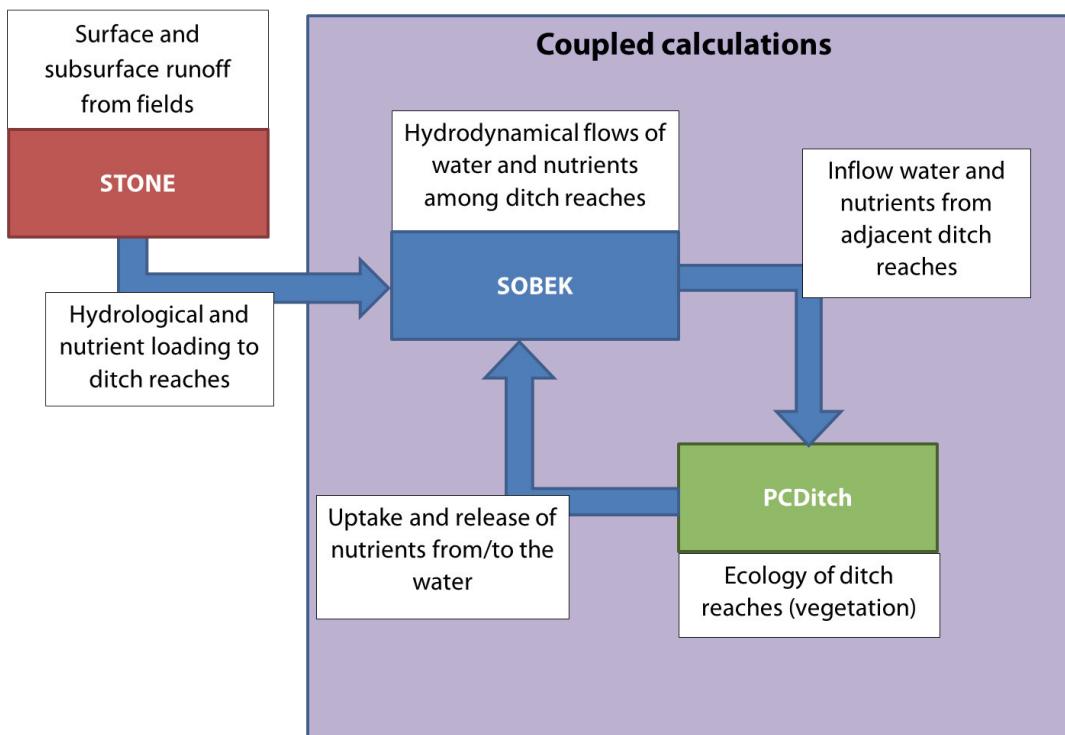
**Figure 7.1:** Map of the Netherlands with the studied landscape in green (a) and a cutout of the study landscape (b). Arrows indicate the pour where water is let into the ditch system from the nearby canal and the pump where water is let out. The 18 sites where vegetation composition was assessed for model validation are indicated with an X.

## Methodology

### Study site description

For our study we make use of a real world polder landscape in the Netherlands, Polder Oukoop (lat: 52.032695, lon: 4.784238; Figure 7.1). The landscape consists of grasslands

intersected by ditch reaches of varying width and depth. As of 2012, the land use intensity of the polder is being reduced in a transition to a more nature-inclusive farming practice. This process has led to a reduction of the cattle in the area with an expected reduction of phosphorus loading of 48% (Table S7.2). The water system of the polder is highly controlled by the regional water authority, maintaining a largely constant water level by letting in and pumping out water actively from the nearby canal. During water-scarcity water is let in from a pour situated on the south-west of the polder and during periods of high rainfall water is pumped out of the system through an outlet situated in the far south of the polder.



**Figure 7.2:** A schematic representation of the information flow between models. The STONE model estimates the water and nutrient runoff from fields and provides input to the SOBEK model. The SOBEK model models the flow of water and nutrients between the individuals reaches in the ditch network. For each of these reaches PCDitch calculates the ecological status and the local nett uptake/release of nutrients. As such the PCDitch model also provides input to the SOBEK model for estimating the transport of nutrients to the next downstream ditch reach.

## The PCDitch model

To model the ecological state of ditches within the landscape we make use of the ecosystem model PCDitch (Janse 1998). This dynamic model includes a range of ecologically relevant processes such as oxygen and nutrient dynamics and competition between different functional groups of aquatic plants and algae (Figure S7.1). The model excels at predicting critical nutrient loads of ditches (Liere *et al.* 2007), where a transition from a submerged vegetated state to a free-floating plant dominance occurs.

With the default parameter settings of the model various groups of vegetation do not proliferate, limiting the assessment of ecological quality within submerged vegetated state. To remedy this, we manually adjusted a number of vegetation parameters based on literature and internal model validation (Table 7.1). With this new parameter set the model predicts a shift from charophytes to elodeids, followed by ceratophyllids and an eventual shift to a lemnid dominated state as nutrient loads increase (Figure S7.1). These shifts in vegetation are in accordance to ecological states of permanently water holding drainage ditches as described in literature (Beltman *et al.* 1996; Lamers *et al.* 2002; Teurlincx *et al.* 2018b).

## Spatially explicit modelling setup

We estimated the flow of nutrients and water from the adjacent fields to each ditch reach using the STONE model (Figure 7.2), a nutrient runoff model that uses climate, soil and land use data to determine surface and subsurface water, nitrogen and phosphorus loading from fields. Hydrological and nutrient loads used for model calculations were based on an average of a 17 year period (1997-2013). To estimate the loading to each ditch we assumed that each adjacent field drains to all of its surrounding ditch reaches proportionally (by ditch length). The hydrological and nutrient loading was used as input to the SOBEK model to calculate hydrodynamic and nutrient transport throughout the ditch network. The schematization of the ditch network, the spatial configuration of the ditch reaches and their connection to one another, was based on existing map material (Kadaster: TOP10NL) complemented with information from field visits. For simplicity we assumed a constant water level in the system where water shortages were compensated through inlet water at the pour (with a TP of 0.51 mg/L and TN of 2.27 mg/L, as based on measurements by the local water authority) and water surplus pumped out at the outlet pump. The coupled SOBEK-PCDitch model (van Gerven *et al.* 2017) allowed for feedback between nutrient transport and retention through changing ecological configuration of the ditches in the network, thereby calculating the ecological state on the level of each ditch reach within the landscape. For full technical details of this dynamic coupling we refer to van

Gerven *et al.* (2015, 2017). We made use of the standard settings of the model for peat meadow ditches (Janse 1998), differentiating in depth and width of the ditches (table S7.1), and using the updated parameter values for submerged plants (table 7.1). As initial state values of each ditch are unknown, we run our model to equilibrium. In our case, due to the high spatial complexity, this involved running the model for 70 years.

## Model simulations

Using the explained model configuration, we predicted the spatial distribution of macrophyte vegetation types across the ditch network in the Oukoop polder following two different types of scenarios. In a first series of scenarios we evaluated the effects of different levels of total nutrient loading, corresponding to three contrasting land management scenarios: 1) conventional agriculture, 2) extensive agriculture, and 3) intensified agriculture. In a second series of scenarios we evaluated the effects of differences in the spatial distribution of nutrient loading.

We used the STONE model to determine the nutrient loading of the *conventional agriculture scenario* by calculating the nutrient loading of the landscape as it was before the land use extensification measures were taken (Table S7.2), resulting in a value of 4.17 kgP/ha/y. For the *extensive agriculture scenario*, we multiplied this load by a factor 0.52 to obtain the nutrient load that is consistent with the expected reduction of 48% following the established reduction of the cattle density (Table S7.2), resulting in 2.23 kgP/ha/y. For the *intensified agriculture scenario* we assumed a nutrient load of 1.5 times the conventional agriculture scenario (6.26 kgP/ha/y).

Next, we considered alternative spatial distributions of the nutrient loading within the polder landscape. In the *homogeneous land use scenario* we assume an equal cattle and fertilizer pressure per area for each field, and hence an equal nutrient loading from all fields in the landscape. We contrast this land use scenario with two alternative configurations with heterogeneous land use distributions, that is, where nutrient loading per area is lower in part of the landscape and higher in other parts. Such spatial differences may arise from changes in the operation of valves and pumping stations, managing where livestock spends time, and changing the location of stables. We distinguish between a configuration where loading is most reduced in the Northern fields and slowly increases towards the Southernmost fields (*North-South scenario*) and a scenario with an opposite gradient where the Southern fields have the most reduced nutrient loads and it slowly increases towards the North (*South-North scenario*). We implemented this by subdividing the area into 5 connected parts of equal area along the North-South axis of the landscape. Each subarea is assigned a loading change of respectively 75%, 25%, 0%, -25% and -75% compared to the total loading of the area. Important to note is that the total loading of the landscape is kept constant,

**Table 7.1:** Changes in the parameter settings for the different functional vegetation groups in the PCDitch model. Changed parameter estimates from the default are indicated with a symbol referring to the basis of the change with: a (Fair & Meeke 1983), b (Owens 1974), c (Van et al. 1976), d (Best & van der Werf 1986), e (Sand-Jensen & Madsen 1991), f (Gerloff 1975), g (Smart 1980), h (Dorenbosch & Bakker 2011), i (Wu & Yu 2004), j (Brock et al. 1983), + (Janse 1998), \* internal model validation/expert judgement.

Parameter	Elodeids	Cerato-phyllids	Charophytes	Lemnids	Unit	Description
$cMuMaxSpec$	0.32+	0.28*	0.22+	0.4+	day-1	maximum growth rate of vegetation at 20 °C
$hLRefSpec$	32+	30a	19+	7.5+	W/m <sup>2</sup> /s PAR	half-sat. light at 20 °C
$cExtSpSpec$	0.01+	0.016b	0.01+	0.01+	W/m <sup>2</sup> /s PAR	specific extinction
$kDRespSpec$	0.024+	0.009c	0.025+	0.03+	day-1	dark respiration rate of vegetation at 20 °C
$cQ10RespSpec$	1.5+	1.3d	1.2+	3+	-	temperature quotient of respiration
$kMortSpecSum$	0.005+	0.009e	0.005+	0.02+	day-1	vegetation mortality rate in Spring and Summer at 20 °C
$cAffNUptSpec$	0.2+	0.1*	0.3+	0.02+	m3/gDW	initial N uptake affinity vegetation
$cAffPuptSpec$	0.2+	0.1*	0.3*	0.02+	m3/gDW	initial P uptake affinity vegetation
$cNDSpecMax$	0.035+	0.03f	0.013h	0.1+	gN/gDW	maximum N/DW ratio vegetation
$cNDSpecMin$	0.01+	0.013g	0.008h	0.04+	gN/gDW	minimum N/DW ratio vegetation
$cPDSpecMax$	0.004+	0.004+	0.002*	0.026+	gP/gDW	maximum P/DW ratio vegetation
$cPDSpecMin$	0.0008+	0.001f	0.001*	0.004+	gP/gDW	minimum P/DW ratio vegetation

meaning that a decrease in loading in some fields is counteracted by an increase in other fields. To understand how these scenarios, and their combinations, relate to the nonlinear response of the ecological state to changing nutrient loading - represented by transitions between dominant plant groups - we modelled another 23 nutrient

loading levels along an eutrophication axis, ranging from a 900% increase and 90% decrease of the loading (Table S7.3).

As output we show the landscape average plant coverage (% of water area) for charophytes, elodeids, ceratophyllids and lemnids, as well spatial maps showing which plant group is dominating in each ditch segment in the polder landscape.

## Model validation

To validate model outcomes we make use of vegetation data collected in the summer of 2012 and 2014 in 18 ditch reaches within the study area (Figure 7.1). As these field observations are gathered just after extensification began we assumed a loading of 3.80 kgP/ha/y (10% reduction from the conventional agricultural scenario). Furthermore, as fields in the southern part of the polder were used more intensively due to proximity to the stables (pers. comm. A. de Goey, Farmer), we assumed a *North-Southscenario* load distribution. We tested the occurrence of lemnids and charophytes in these ditch reaches against the occurrence of these same groups in our model results. For charophytes we used a low cut-off value of 0.01% cover to avoid extremely low biomass in the model to be erroneously counted as having charophytes. As the occurrence of lemnids in small quantities has little ecological relevance we made use of a higher cut-off value of 25% cover to assign them as being present (read: dominant) at the ditch reach. Using logistic regression we assessed if occurrence patterns in the field matched those predicted by the model setup. We determined the distance to the southernmost point of the polder of each ditch reach. By testing the occurrence of lemnids or charophytes to this distance we could test the South-North spatial occurrence of vegetation groups.

## Results

### Validation by field observations

To assess the validity of our model predictions we validated our model results based on plant occurrence as found in the field. In field observations we found significant patterns in both charophyte and lemnid occurrence along a south-north axis across the polder in both field data and modelled data (Table 7.2). Charophytes were found to occur primarily towards the Northern tip of the polder landscape (Figure 7.3a, Table 7.2). This pattern was also found in our model predictions (Figure 7.3b, Table 7.3), although the section of the landscape in which charophytes were found was much more narrow (>2000m from southernmost point). The presence of lemnids showed an inverse pattern to the charophytes, both in the field and in the model, with an

increasing chance of finding lemnids towards the southern regions of the polder (Figure 7.3c and d). Here the field observations and model predictions matched very well, with coefficients being markedly similar (Table 7.2).

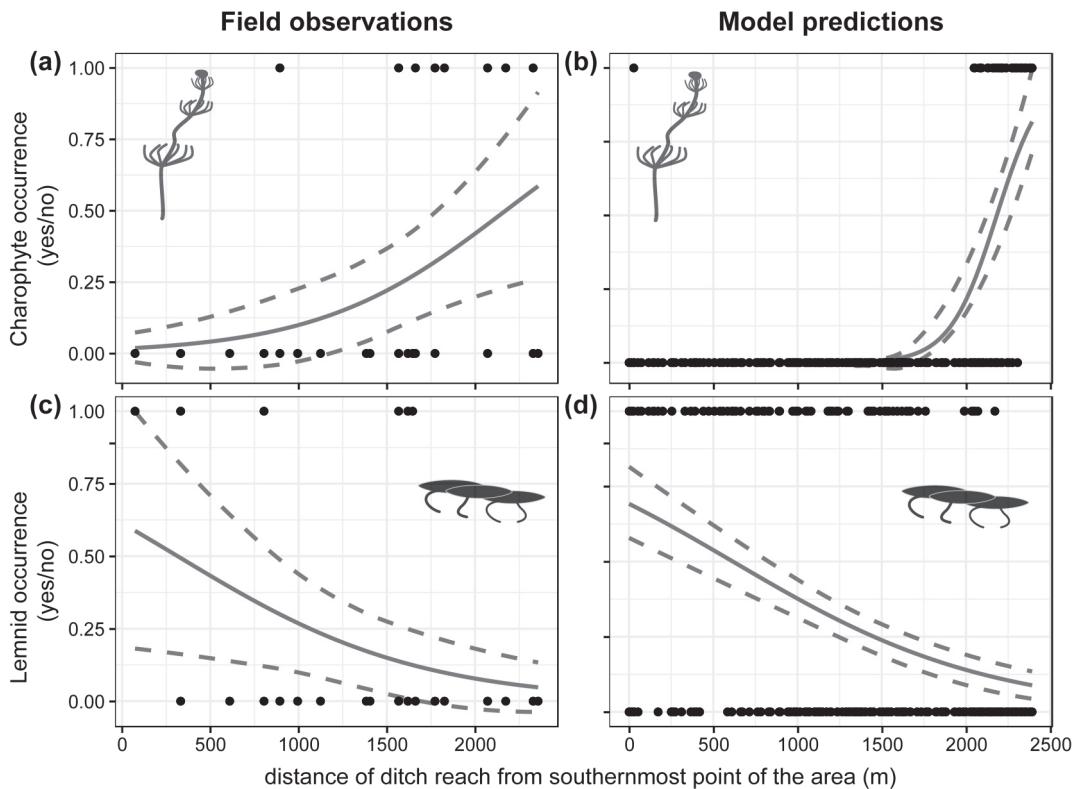
## Shifting plant dominance at different nutrient levels

Running the model for a wide range of nutrient loadings enabled us to determine transitions in vegetation groups and relate them to the three agricultural land use scenarios under study (Figure 7.4). Examining the *homogeneous land use scenario* (Figure 7.4, shaded area) we found that under conventional agricultural land use (orange line) the landscape is dominated by a mix of elodeids (Figure 7.4b, ~25% coverage), ceratophyllids (Figure 7.4c, ~25% coverage), and lemnids (Figure 7.4d, ~50% coverage). Charophytes are (almost) completely absent from the landscape (Figure 7.4a). When increasing loading further to a level of 6.26 kgP/ha/y under an *intensified agriculture scenario* (Figure 7.4, red line), we found a stark increase in the coverage of lemnids to over 80% (Figure 7.4d). An extensive agriculture scenario (2.23 kgP/ha/y) lead to an almost complete disappearance of Lemnids and an increase of elodeids, but not to an increase of charophytes (Figure 7.4, green line).

The modelling results showed that heterogeneous nutrient loading (*North-South or South-North scenarios*) lead to a change of vegetation occurrence along the axis of nutrient loading compared to the *homogeneous land use scenario*. The charophytes are able to persist in the landscape with higher nutrient loads, even under the conventional land use scenario (Figure 7.4a, orange line). This is most pronounced for the *North-South scenario* where they exist even under intensified land use conditions, albeit at low density. Ceratophyllids and elodeids were also found to persist at higher nutrient loads compared to a homogeneously spread nutrient loading. However, the maximal cover found for all three plant groups was diminished somewhat (Figure 7.4a, b, c). Also, lemnids were found within the landscapes at markedly lower nutrient loading levels compared to the homogeneous scenario (Figure 7.4d).

## Spatial patterns in plant dominance at different nutrient loads

Our three different scenarios of nutrient loadings led to variation in the spatial expression of the different functional plant groups in the landscape (Figure 7.5). We found that under a nutrient load corresponding to intensified land use (2.23 kgP/ha/y) under homogeneous distribution of nutrients most ditches in the landscape were dominated by elodeids and ceratophyllids. Only very rare and small patches of lemnids and charophytes were found in the modelled landscape. Increasing the nutrient load to 4.17 kgP/h/y leads to a clear increase in floating plant cover (lemnids) and a near



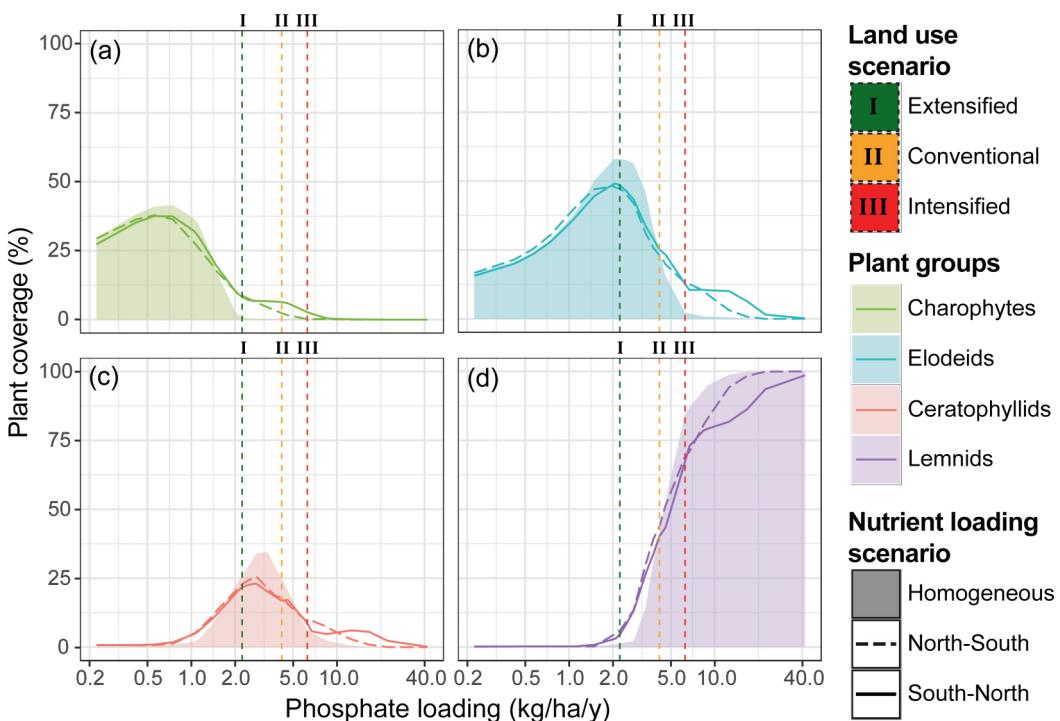
**Figure 7.3:** Logistic regression curves for charophyte (a, b) and lemnid (c, d) occurrence (0/1) against the distance to the most southern point of the polder area as found in the field (a, c) and simulated using the PCDitch model (b, d). Dashed lines indicate a 95% confidence limit around the mean logistic regression curve. For details on statistics see table 7.2.

**Table 7.2:** Summary statistics of logistic regressions of observed and predicted presence/absence of charophytes and lemnids along an axis of spatial distance from the southernmost tip of the study landscape. Significance is assessed via a Chi-Square test of the model against a null model with \*\*\*: $p<0.001$ , \*\*: $p<0.01$ , \*: $p<0.05$ , : $p<0.10$ .

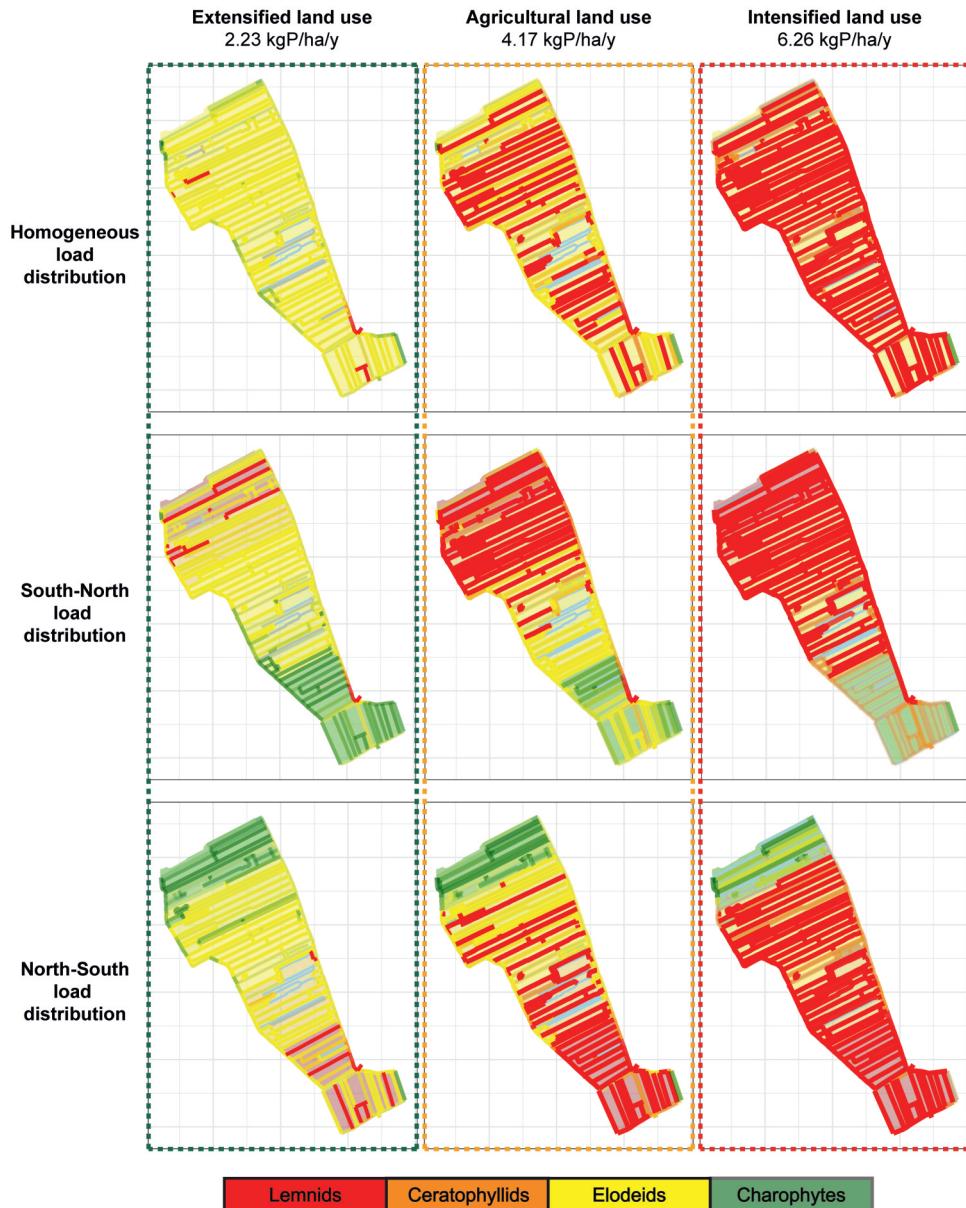
Group	Data	Coefficient	$\pm$ Std. Error	R2 (%)	df	deviance reduction
Charophytes	Field	0.0019	$\pm$ 0.00086	14.83	38	6.67 ***
	Model	0.0071	$\pm$ 0.00128	50.28	319	130.83 ***
Lemnids	Field	-0.0015	$\pm$ 0.0007	12.78	38	5.11 *
	Model	-0.0013	$\pm$ 0.0002	12.23	319	48.11 ***

complete disappearance of charophyte patches. A further increase in nutrient loading resulted in a system which is almost entirely covered by lemnids.

When applying a heterogeneously distributed load to the landscape (*South-North* and *North-South scenarios*) the model predicted a dominance of charophytes under extensified land use conditions in the sections of the landscape with the lowest loading. In contrast, the sections of the landscape with high loads were found to exhibit lemnid dominance. When increasing the loading to agricultural levels, charophytes were able to persist in some of the ditch reaches receiving lower levels of nutrient inflow from adjacent fields. In the case of the *North-South scenario* load distribution, ditches dominated by charophytes were still present in the northern reaches of the landscape under *intensified land use scenario*.



**Figure 7.4:** The landscape average plant coverage (% of water area) at different levels of phosphorus loading (kg/ha/y) for (a) ceratophyllids, (b) charophytes, (c) elodeids and (d) lemnids as predicted by the PCDitch-1D model setup. The shaded area shows the plant coverage under a homogeneous-, the dashed line under a North-South (low-high) and the continuous line under a South-North (low-high) nutrient loading scenario. Vertical dashed lines indicate the landscape load under extensive agriculture scenario (green; 2.23 kgP/ha/y), conventional agricultural scenario (orange; 4.17 kgP/ha/y) and intensified agricultural scenario (red; 6.26 kgP/ha/y).



**Figure 7.5:** Maps depicting the spatial variation in dominance of functional plant groups in different ditches within the landscape with charophytes in green, elodeids in yellow, ceratophyllids in orange and lemnids in yellow. A distinction is made between three levels of nutrient loading (land use scenarios): extensive land use (green; 2.23 kgP/ha/y), agricultural land use (orange; 4.17 kgP/ha/y) and intensified land use (red; 6.26 kgP/ha/y) with three different spatial distributions of the loading over the landscape (Homogeneous, South- North and North-

## Discussion

Changing the land use of a landscape and its associated changes in nutrient runoff is one of the first and most pivotal steps in restoring and improving aquatic ecosystem quality (Bohn & Kershner 2002; Withers *et al.* 2014). In agricultural landscapes the improvement of the fringe aquatic habitats such as ponds and ditch networks is an overlooked but important pathway towards increasing nature value, ecological quality and biodiversity of the landscape (Verdonschot *et al.* 2011; Lemmens *et al.* 2013; Hill *et al.* 2018). However, reducing or redistributing land use in a landscape is costly, both financially as well as socially. This study illustrates how a modelling approach is capable of informing management of the likely outcomes of different scenarios of land use intensity reduction and redistribution. In agreement with other studies, we find that excessive nutrient loadings lead to undesirable vegetation types dominated by lemnids, which cause hypoxic conditions and offer little structural habitat for other organisms (Scheffer *et al.* 2003). Reducing nutrient loads leads to more favourable submerged vegetation types dominated by elodeids and with sufficient nutrient reduction even charophytes. However, the levels of reduction required to reach a dominance of charophyte vegetations in the entire landscape (a further 65% reduction from extensive land use loading) are likely to be unrealistic when combined with any form of agricultural practice.

Our model performed remarkably well in predicting general patterns of vegetation occurrence when compared to field data (cf. Figure 7.3). We were able to replicate the patterns of lemnid occurrence, showing their occurrence throughout the landscape, but concentrated in the southern parts. While showing similar general patterns, the model predicted a more stringent boundary of charophyte occurrence. This can be explained as our model is a representation of an equilibrium condition, in which the landscape vegetation patterns would look like if loading and hydrology are kept at stable levels over many years. The field condition however represents a transient state in which nutrient loads are in the process of being reduced, hydrology and weather conditions change from year to year and stochastic dispersal events are common. For example, through adaptive management activity such as dredging and mowing, brief windows of opportunity may be created for the dispersal of charophytes (Beltman & Allegrini 1997; Teurlincx *et al.* 2018b). However, the system over the long term may prove unsuitable for the establishment of charophytes due to excess nutrient loading. Hence, our model would not predict the occurrence of charophytes whereas we may find them on occasion in the field. Overall though, we are able to replicate the broad-strokes patterns of both lemnids and charophytes, which is important to inspire confidence in the projections presented here.

It is well known that nutrient load is one of the driving forces of ecological quality and associated vegetation in ditch ecosystems (Janse & Van Puijenbroek 1998; Dollinger *et al.* 2015). However, reaching a sufficiently high nutrient load reduction to achieve the desired ecological quality and associated vegetation is a challenging endeavour, especially in agricultural landscapes (Needelman *et al.* 2007). This is well illustrated in our landscape, where given a homogeneous distribution of nutrients, charophytes remain largely absent from the landscape even under an extensive land use scenario. By redistributing nutrient loading of the landscape we are able to reduce nutrient pressure in part of our studied landscape to levels where desirable vegetation from a nature management standpoint (i.e. charophytes) can occur. Not surprisingly, the lower the total nutrient loading to the entire landscape, the more ditches will exhibit desirable vegetation. However, the exact distribution of nutrients over the landscape is essential (illustrated by *North-South vs South-North scenarios*). With the *North-South scenario* of nutrient load distribution (lower nutrients in the North) resistance of the patches of charophytes to nutrient load increase may be increased, allowing them to persist even under nutrient loads associated with conventional agricultural usage. The spatial configuration of the landscape (i.e. location of pumps and pours) and associated hydrological flows which limit the spread of nutrient rich water to the north of the landscape is a prime cause for this increased resilience of charophyte vegetation. Creating an ecologically more resilient agricultural landscape is relevant (Hodbod *et al.* 2016), even when overall nutrient loads are reduced, as it creates a natural capacity to deal with occasional perturbations (e.g. a sewage overflow) and future deterioration of ditch ecology under climate change (Netten *et al.* 2011).

Despite the charophyte persistence there is a clear trade-off to nutrient load redistribution, namely the increase of unwanted vegetation (i.e. lemnids) in ditches in the higher loaded part of the landscape. There are clear ecological and societal problems associated with the floating plant cover such as lemnids. They cause oxygen depletion of the water column, reduce biodiversity, increase greenhouse gas emissions, lead to foul odours and reduce attractiveness of open water (Scheffer *et al.* 2003; Veraart *et al.* 2015; Kosten *et al.* 2016). The importance of this trade-off is highly context dependent though. From the perspective of biodiversity maximisation the occurrence of a limited amount of low divers and floating plant infested ditches may be acceptable if the landscape as a whole will benefit in terms of biodiversity. Of course, the perspective and usage (e.g. drinking water for cattle) by the local inhabitants living close to these low quality ditches needs to be taken into account here. In other cases it is highly undesirable to create a niche for floating plants irrespective of potential benefits in low loaded regions for other species. For example, when the floating plants are highly invasive exotic species (Coetzee & Hill 2012). This serves to illustrate that the

model outcomes can aid managers, but should always be assessed with local knowledge of the system and the multifaceted interests of the landscape in mind.

The ecological state of aquatic systems is strongly influenced by both nutrient loads from runoff, as well as by water and nutrient transport from connected headwaters (Carpenter *et al.* 1998; Li *et al.* 2019). Hence, to assess and project future water quality conditions under changing land use and climate conditions a link between terrestrial runoff dynamics, water and nutrient transport and ecological processes is imperative. While links between terrestrial and water systems have been implemented on many occasions (see Shin *et al.* 2015 for a recent review), most of these models lack a link to mechanistic ecological models. Spatially explicit ecological models, linked to water transport models have progressed in recent years (Bruggeman & Bolding 2014; Janssen *et al.* 2017; Nielsen *et al.* 2017), but lack realistic runoff models at a suitable scale level. Our study offers a first tentative step into exploring linking all three components in a realistic setting. Our study shows that these models together may be used in a landscape setting to assess effects of land use changes. The application of the model toolbox could be applied to wide range of ecological questions such as assessing effects of climatic changes (e.g. changes in temperature and rainfall) or hydrology based management such as increasing flushing rates on ditch ecological quality (Couture *et al.* 2018; Hutchins *et al.* 2018; Molina-Navarro *et al.* 2018).

Ditch ecosystems are generally considered to be fringe habitats which are primarily designed for a drainage function (Herzon & Helenius 2008). They are limited in water surface area and due to their adjacency to agriculture, generally suffering from high nutrient loading. Despite these seemingly undesirable qualities, these systems have potential for biodiversity at the landscape level due to a highly dissimilar community between local ditch reaches (Watson & Ormerod 2004; Goldenberg Vilar *et al.* 2014; Whatley *et al.* 2014a). Also, due to the low diversity of adjacent agricultural fields ditch reaches and their banks are a prime source of biodiversity within these landscapes (Manhoudt *et al.* 2007; Leng *et al.* 2011). Moreover, promoting desired ecological states in these systems can have pronounced effects on the larger waterscape. Nutrients may be retained in these systems through emergent and submerged plant growth (Vermaat & Hellmann 2010), allowing outflowing water nutrient status to improve (Kröger *et al.* 2008). They may also supply a spawning ground for fish species, being shallow, well vegetated systems. Adequate, well informed, management of these systems is essential to achieve any of these functions though. As landscape level management and planning is costly, a modelling approach as outlined here can serve as a first step in exploring possible outcomes of adjusting and redistributing nutrient loading within these landscapes.

## Acknowledgements

We would like to acknowledge Wolf Mooij and Bob Brederveld for fruitful discussions. Also, we would like to thank Edwin van den Berg, Marlies Gräwe, Marta Alirangues and Maik Janssen for invaluable assistance in the field. ST and LG were financially supported by the Netherlands Organization for Scientific Research (NWO) Biodiversity Works Research Program (OBW) Application fund, project number 841.11.009. ABGJ is funded by the KNAW project SURE+, project number PSA-SA-E-01. JJK is funded by the Marianne and Marcus Wallenberg foundation research exchange program on natural capital, resilience and biosphere stewardship.

## Chapter 7: Supplementary materials

**Table S7.1:** Ditch types included in SOBEK schematisation.

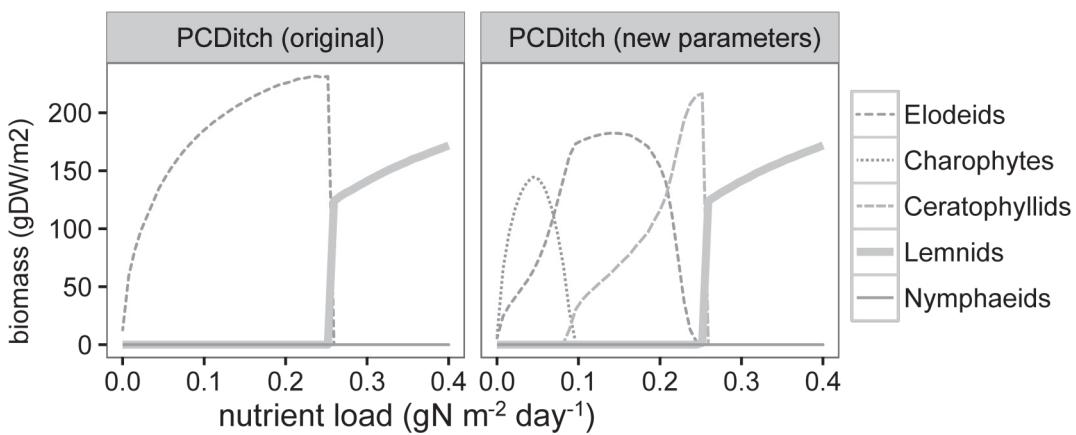
type	Diepte (m)	Breedte (m)
<i>Peat pit 'Petgat'</i>	2.5	25
<i>Main reach</i>	1	15
<i>Secondary reach</i>	0.7	5
<i>Tertiary reach</i>	0.5	2.5

**Table S7.2:** Cattle densities in polder Oukoop in intensive (<2012) and extensified situation (>2012) (after: Mouissie *et al.* 2013). Resulting phosphate (P) loading from cattle was calculated based on numbers used in national manure legislation (RVO, 2015).

	Cattle density (numbers)		P loading from cattle (kg/year)	
	Intensive	Extensive	Intensive	Extensive
<b>Cows</b>	361	200	15162	8400
<b>Sheep</b>	62	15	235.6	57
<b>Pigs</b>	110	0	462	0

**Table S7.3:** Mean landscape loading of N and P used within the model simulations.

Run	Mean P load (kg/ha/y)	Mean N load (kg/ha/y)	Run	Mean P load (kg/ha/y)	Mean N load (kg/ha/y)
1	0.223368	2.230867	14	3.350525	33.463
2	0.417469	4.169424	15	3.795171	37.90385
3	0.558421	5.577167	16	4.174688	41.69424
4	0.744561	7.436222	17	4.467367	44.61734
5	1.043672	10.42356	18	4.592157	45.86366
6	1.116842	11.15433	19	6.262032	62.54136
7	1.391563	13.89808	20	6.701051	66.92601
8	1.489122	14.87245	21	8.349376	83.38848
9	2.030621	20.28061	22	8.934734	89.23468
10	2.087344	20.84712	23	12.52406	125.0827
11	2.233684	22.30867	24	16.69875	166.777
12	2.457052	24.53954	25	22.33684	223.0867
13	2.783125	27.79616	26	41.74688	416.9424



South).

**Figure S7.1:** A plot of the vegetation development (dry weight) along a nutrient loading axis for the original PCDitchmodel (left) and the model with new parameter settings (right).



# 8

## General discussion

*Sven Teurlincx*

Ecology has developed into a spatially oriented science over the past three decades since the seminal paper of Wiens (1989) and later Levin (1992). While local communities respond to environmental conditions experienced, these conditions in themselves may be spatially structured (Turner 1989). Spatial heterogeneity in environmental conditions will lead to community turnover and thereby an increase in community dissimilarity (Whittaker *et al.* 2001). Moreover, space is an imperative requisite to coexistence of species in a landscape with a single limiting resource (Tilman 1994a). Biodiversity across spatial scales may lead to an insurance effect (Loreau *et al.* 2003; Isbell *et al.* 2018), allowing for nearby localities to repopulate a site after local extinction (stochastic or anthropogenic). Furthermore, local communities may be largely determined by metacommunity processes, such as source-sink dynamics, mass effects and dispersal limitation, that can have important impacts on the larger scale functioning of ecosystems (Bond & Chase 2002).

In a world where spatial processes are so clearly relevant to the local community, conservation and management of biodiversity cannot be seen outside of this spatial context (Wallington *et al.* 2005). In this thesis I have examined biodiversity in a region of intense anthropogenic pressures across levels of spatial scale and across organism groups. In this section, I will first start by examining the common denominators in patterns and drivers of these groups for the different levels of spatial scale. I continue by exploring and discussing causes for the found patterns. I focus on how ecological processes interact with the abiotic environment, thereby facilitating a diverse habitat mosaic within these landscapes. Next, I explore if ecological stoichiometry may have a function in disturbing or stabilizing ecological processes. With the knowledge gathered, I propose a more adaptive management strategy to preserve biodiversity across spatial scales in ditch systems in Dutch polder landscapes. I end with a treatise on the importance of integral biodiversity assessment, management across scales, organisms and stakeholders.

## Biodiversity patterns in a region of polder landscapes

Conserving biodiversity starts with a solid basis in understanding the organisation of communities across spatial scales. In this thesis I have shown that biodiversity of ditch systems within Dutch polder landscapes and within the studied region as a whole is strongly dependent on biotic heterogeneity (high  $\beta$ -diversity). This high within and between landscape  $\beta$ -diversity is found across different functional groups of vegetation and zooplankton (Chapter 2). Also, microbial communities were found to be distinctly different between landscapes (Chapter 3). Similar patterns have been

described for diatoms (Goldenberg Vilar *et al.* 2014) and macroinvertebrates (Verdonschot *et al.* 2011), where high community dissimilarity was also observed across spatial scales. Chapter 2 further shows that there is not just a difference in community composition, but that these differences lead to complementarity between landscapes. The existence of this complementarity at the level of the ditch reach and landscape suggests strong heterogeneity in underlying environmental drivers. In chapter 2 I illustrated that polder landscapes are strongly dissimilar in their environmental characteristics. These environmental characteristics did, in part, explain landscape and regional level diversity of different organism groups (Chapter 2 and Chapter 3). Land use, its intensity and signals of eutrophication (e.g. nutrient loading, fertilizer application, water and sediment nutrient concentrations) and heterogeneity therein were important drivers of among landscape community dissimilarity for all of the organism groups. Variation in successional stages was found to be an important driver for within landscape community dissimilarity as well (Chapter 6).

## Understanding mechanisms underlying large scale patterns

Understanding the mechanisms underlying observed large scale spatial patterns in biodiversity is key (Levin, 1992), especially for management of biodiversity. Large scale patterns in biodiversity always have their origin at the level of the local community. The local communities within a ditch reach are influenced by availability of resources and disturbances such as removal through mechanistic means or by predators/grazers. Local environmental differences between ditches that influence these key resources may be found in fertilization of adjacent lands and associated runoff of nutrients (Wolf *et al.* 2003), thereby increasing nutrient availability for organisms. Moreover, the local ditch morphology (e.g. water depth) may impact the light availability for rooted plant growth (Middelboe & Markager 1997). Ditch morphology may also limit plant growth when steep slopes are present that make rooting difficult (Duarte & Kalf 1986). Disturbance of aquatic organisms by cattle (Conroy *et al.* 2016) and removal of vegetation through mechanical management (van Strien *et al.* 1991) or grazing (Middleton *et al.* 2006) are the most common drivers that may vary between localities. Beside local habitat conditions, dispersal/immigration from adjacent communities may cause inflow of new species with a suite of possible biotic interactions associated with them (e.g. competition, predation, facilitation) (Leibold *et al.* 2004). Furthermore, organisms themselves may have feedbacks on their abiotic environment. An example of this is the succession of vegetation which leads to increasingly more shallow systems due to sedimentation of plant material (Chapter 7).

Changing nutrient supply is one of the more elusive pressures, as nutrients in dissolved form can be transported across spatial scales through hydrological connectivity (Freeman *et al.* 2007). Agricultural land use intensity has clear links to nutrient runoff. While external nutrient loading did not always drive communities directly (Chapter 2), all studied organism groups did have clear connections with nutrients, measured either in sediments, water or soils. I found that nutrient supply had negative impacts on landscape-wide diversity of organisms (Chapter 2). However, I also found that heterogeneity in nutrients could lead to replacement of species between landscapes (complementarity) in at least some organism groups (Chapter 2). This may seem paradoxical at first. However, during ecological succession ditch systems get slowly enriched in their sediment nutrient content (Caspers & Heckman 1981; Beltman *et al.* 1996). As shown in Chapter 6, diversity of successional stages can increase landscape diversity. Hence, observed heterogeneity in nutrient content may reflect the presence of different successional stages in a landscape and thereby complementarity in communities. However, excess nutrient loading may also cause ecosystem collapse (another trajectory of succession) into a free-floating plant dominated state (Scheffer *et al.* 2003; Liere *et al.* 2007). This free-floating plant dominated state may also cause biodiversity decline through light and oxygen depletion (Clare & Edwards 1983). Thus, nutrient supply may lead to successional gradients facilitating biodiversity, but also cause state shifts which may lead to biodiversity decline.

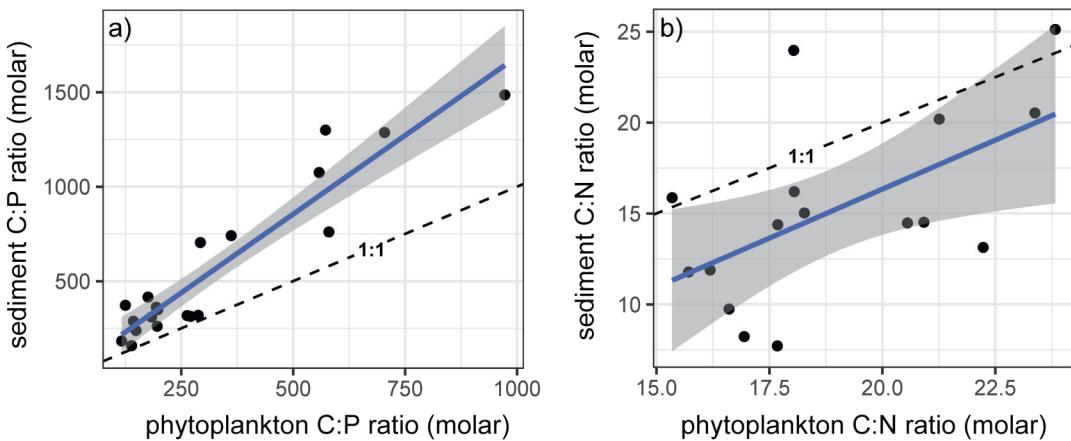
## Nutrient cycling and transport in ditch systems

### Vegetation-sediment dynamics (bottom-up)

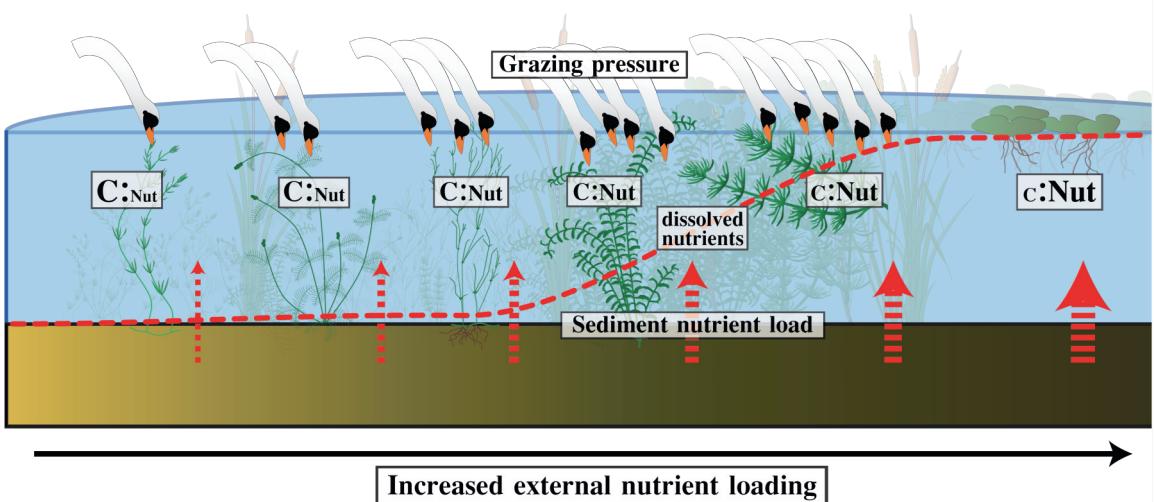
Plants play a key role in the nutrient cycling in ditches and connect sediment and water layers through nutrient uptake by roots (Barko *et al.* 1991b; Bini *et al.* 2010). They also serve as a food source and habitat structure for higher trophic levels (Whatley *et al.* 2014b; Bakker *et al.* 2016). I showed in chapter 4 that plants are able to adjust their internal stoichiometry in response to environmental nutrient supply (P specifically). As nutrient loads increase, plant nutrient uptake will increase and subsequently their detrital matter will be enriched in nutrients as well. In figure 8.1 I give an example of how primary producer carbon to nutrient ratios gives rise to similar sediment carbon to nutrient ratios. This example comes from data gathered in the mesocosm study of Chapter 5, where phytoplankton rather than aquatic plants were the primary producers. Nonetheless, as I have shown in chapter 4 that plants exhibit large stoichiometric variation as well, similar patterns may be expected in plants.

With an enriched sediment, internal loading potential increases (Søndergaard *et al.*

2003; Hupfer & Lewandowski 2008). The internal loading potential caused by nutrient enriched sediments may be controlled through sediment redox processes (Smolders *et al.* 2006; Hupfer & Lewandowski 2008), which is mediated by plant identity and abundance. An obvious example of this is the increased P release under anoxic conditions mediated by floating vegetation cover (Scheffer *et al.* 2003). Cover of submerged plants may also influence redox potential and associated internal nutrient loading though (Boros *et al.* 2011). In relation, nutrient status of the sediment has been linked directly to dominance of dense monocultures of rooted submerged plants (van Zuidam & Peeters 2013). Dissolved nutrient concentrations in the water column typically increase with increasing internal loading, leading to shifts in community composition towards species capable of taking up nutrients directly from the water. Lemnids take up nutrients from the water directly and are known to be highly nutrient rich (Körner & Vermaat 1998). Hence, with increasing dissolved nutrients, sediments may be enriched more quickly through sedimentation of increasingly rich plant material. This in turn will cause a further increase in dissolved nutrients through enhanced decomposition, accelerating the potential for undesired ecosystem state shifts.



**Figure 8.1:** Relationships between a) primary producer (phytoplankton) C:P ratio and sediment C:P ratio and b) primary producer (phytoplankton) C:N ratio and sediment C:N ratio. Data was gathered in the mesocosm experiment described in chapter 5. Sediment was collected at the end of the experiment and measured similarly to phytoplankton as described in chapter 5. Blue lines indicate significant linear relationships between phytoplankton and sediment C:P ( $p<0.001$ ) and phytoplankton and sediment C:N ( $p<0.05$ ) with grey areas indicating one standard error around the mean.



**Figure 8.2:** Illustration of the effects of land use intensification (cf. increased nutrient loading) on the local ditch system. As nutrient loading increases, plant carbon to nutrient ratio is decreased leading to: 1) increased grazing pressure, 2) increasing sediment nutrient loads due to enriched detrital matter from plants and 3) more dissolved nutrients in the water due to an increased sediment nutrient loading. These factors cause a shift in vegetation diversity and density along this gradient of increasing nutrient loading. *Illustrations are courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science ([ian.umces.edu/symbols/](http://ian.umces.edu/symbols/))*

## Vegetation-consumer dynamics (top-down)

Plants also serve as food for higher trophic levels. As plant carbon to nutrient ratios increase, plant quality as a food source for herbivores will increase as well (Elser *et al.* 2001; Sterner & Elser 2002). As food quality increases, top-down control on primary producers will increase through increased grazing pressure (Lampert *et al.* 1986; Bakker & Nolet 2014) (Figure 8.2). In chapter 5 I showed that changing producer stoichiometry can also impact the community composition of higher trophic levels, thereby impacting their diversity. For plant consumers restricted to the local ditch reach these impacts are likely to be comparable. However, a large part of the consumers such as birds (Kleyheeg *et al.* 2017), fish (Verhelst *et al.* 2018), crayfish, and even macro-invertebrates (Gall *et al.* 2017) are not restricted to the local ditch reach, having movement capacity reach far beyond the local ditch. Thereby these species are unlikely to experience strong species sorting in response to changing food quality. However, aquatic plants play a strong role in the habitat provisioning for higher trophic levels while simultaneously serving as a food resource (Burks *et al.* 2006). Motile organisms may, through movement between patches, create a spatial segregation in habitat and food resource usage. Generally, such a spatial mismatch in trophic interactions can lead

to an increased heterogeneity of both producers and consumers, which is mediated through decreasing local species richness while increasing  $\beta$ -diversity (Jabot & Bascompte 2012). Within the context of ditch systems, grazers may thus increase plant community  $\beta$ -diversity by selectively grazing patches of the best food quality.

## Nutrient transport and retention

The local functioning of ditch ecosystems and its associated nutrient cycling cannot be seen outside of its spatial context of the landscape. Nutrients are easily transported throughout the ditch system. However, large parts of the nutrients within aquatic ecosystems are bound in sediments and organisms (Vanni 2002). These processes are integrated in the nutrient spiralling concept developed for river and stream ecosystems (Newbold *et al.* 1981). This concept explicitly refers to organisms as temporal sinks of nutrients, limiting the downstream flow of nutrients by incorporation into organismal biomass (Ensign & Doyle 2006). This process has cascading effects for downstream sections as nutrients are not transported downstream when captured in certain organism groups (e.g. epiphytes). When changing pressures disrupt production and thereby nutrient fixation in upstream regions, this has direct impacts for downstream regions as well. Alternatively, the incorporation of nutrients in biomass of motile organisms has the potential to transfer nutrients over much larger spatial scales than those scales at which dissolved nutrients could be transported by water flow alone (Naiman *et al.* 2002; Bauer & Hove 2014; Hessen *et al.* 2017) and may even allow nutrient transport across water-land interfaces (Bartrons *et al.* 2013).

The nutrient spiralling concept may also be applied to ditches (Smith 2009), with nutrients being temporarily retained in ditch sections (nutrient spiralling) limiting further propagation through the system. In contrast to results from nutrient spiralling work in rivers (Webster *et al.* 2016), for ditch systems vegetation-detritus-sediment interactions form the backbone of the complex nutrient sorption dynamics (Nguyen & Sukias 2002). Different plants have varying capacities for nutrient uptake (Chapter 4, Dierberg *et al.* 2002). Hence, different vegetation communities will retain different amounts of nutrients and allow for transport of others. Transport and retention of nutrients will rely strongly on stoichiometric regulation. While the limiting nutrient for growth may be depleted entirely, the non-limiting nutrient will be partly transported to adjacent reaches (Small *et al.* 2009). In chapter 4 I showed strong responses of submerged rooted plants to environmental P, but limited responses to environmental N, indicative of P limitation of the studied species. These species would thus retain most of the P during their lifecycle, but release large parts of the external N loading to adjacent reaches. Moreover, consumer communities exhibit much smaller plasticity in their elemental stoichiometry (Chapter 5) and will therefore exhibit much higher

excretion of an excess nutrient under nutrient limitation (Small *et al.* 2009). These stoichiometric processes are essential to understand the retention and transport of nutrients across scales. Furthermore, nutrient retention within a ditch reach may be strongly reduced when the critical nutrient threshold is reached and a state shift to a free-floating plant state occurs (Liere *et al.* 2007). This will increase internal loading in this ditch due to anoxia. Subsequently, the transport of nutrients from this ditch to an adjacent ditch will increase, thereby increasing its total loading. This may cause for that ditch system to also experience a critical transition into a free-floating plant dominated state, which again leads to that ditch passing on more nutrients to its neighbouring ditches than it did in its submerged plant dominated state. Therefore, there is potential for domino effects through these local state shifts that may propagate throughout the landscape (van Nes & Scheffer 2005).

## Management for successional diversity without free-floating plant dominance

Avoiding state shifts towards an undesirable state where free-floating plants dominate is key to maintain a high diversity at the landscape level. However, landscape diversity was shown to be strongly influenced by a gradient of ecologic succession within the landscape (Chapter 6). This gradient implies an inherent heterogeneity in nutrient status between ditch reaches. Management should strive to maintain this heterogeneity, all the while avoiding excessive nutrient loads causing unwanted state shifts. Van Gerven *et al.* (2017) showed in a modelling study that a heterogeneous ditch system (in ditch dimensions and/or external nutrient loading) can lead to spatial heterogeneity in critical nutrient loads at which a state shift occurs. In the field situation, heterogeneity in both external nutrient loading and ditch dimensions is sooner a given than an exception (see Chapter 2). Hence, a singular critical nutrient load is unlikely to exist, complicating management. In chapter 7 I used a similar model setup to van Gerven *et al.* (2017) to model vegetation groups in a real-world ditch system. Free-floating plant dominance was indeed found to be spatially heterogeneous across the ditch system. This may be the result of environmental heterogeneity (i.e. ditch depth), heterogeneous nutrient pressures as well as possible feedbacks of vegetation on nutrient transport. Regular management with a clear spatial design (Chapter 6) may serve to reset succession. Mowing vegetation may also be used as a tool to remove excess nutrients from aquatic systems (Kuiper *et al.* 2017). However, regular management and especially sediment disturbance may also favour the proliferation of free-floating species (van Zuidam *et al.* 2012). Our work in chapter 6 and 7 illustrates the importance of a spatial perspective on management of ditch systems for diversity and ecological quality. However, to date, integration of management

effects on ditch systems (e.g. mowing, dredging) in a spatially explicit context that accounts for the clearly relevant biotic feedbacks of nutrient retention and transport is lacking. In systems where management is the norm these type of studies warrant immediate attention.

## Using models to inform adaptive management of biodiversity

Preserving diversity across spatial scales is a challenging endeavour for managers and policy makers, especially in a changing world. Where knowledge of the current state of diversity and drivers thereof may be sufficient to conserve diversity in a stationary world, in a dynamic world processes that govern diversity are constantly changing (e.g. water flows: Milly *et al.* 2008). This requires a view of management as a continuous process, rather than a predefined singular endpoint (Stein *et al.* 2013). The concept of adaptive management (Walters & Hilborn 1978) fits into such a mind-set, continuously updating and reassessing management efforts with new information on the ecological state of the system (Williams 2011). Cyclic rejuvenation through management (Baptist *et al.* 2004) as a method to maximize landscape diversity in ditch systems (Chapter 6) would likely have to rely on such an approach, as the speed of successional turnover is generally unknown. Kingsford *et al.* (2011) have further expanded on the concept by launching the framework of strategic adaptive management, which has an increased social focus and sets a clear end goal for management. Despite these efforts, adaptive management may be hampered by the lack of large scale experiments testing management effects in the field (Kingsford 2011). It is both difficult and costly for society to carry out these experiments which have far-reaching impacts on local stakeholders, a point also raised in relation to land use changes in chapter 7. Modelling scenarios may offer an alternative exploration of these costly management actions. Ferrier & Drielsma (2010) describe a generic modelling framework aimed at predicting landscape scale biodiversity changes for conservation purposes. Their framework consists of three components: 1) a spatially explicit prediction of expected (future) habitat conditions, 2) the expected level of persistence of each entity (e.g. species) given this new habitat and 3) the aggregation of all entities to derive the overall level of persistence expected for biodiversity as a whole (e.g. community compositional changes).

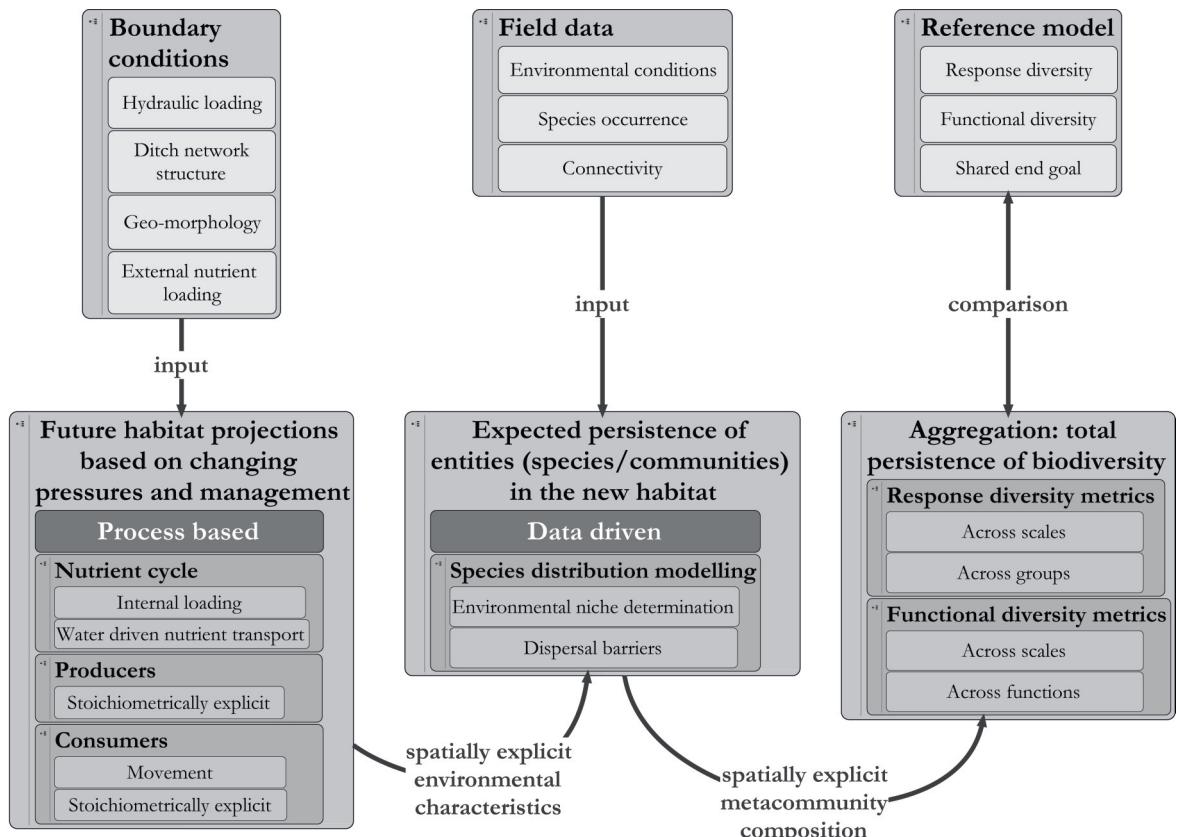
All of the components of the framework of Ferrier & Drielsma (2010) would benefit from being estimated using process-based models. Process-based or mechanistic models have the advantage of being able to perform beyond their calibration domain (Janssen *et al.* 2019), and are thus able to predict outcomes of complex and interacting pressures and management scenarios. This is especially relevant in a fast changing world that is likely to combine stressors in ways that have never been experienced before in nature (Downing 2014). However, the current state of biodiversity modelling has largely been confined to data-driven community models and dynamic single species models (D'Amen *et al.* 2017). Dynamic multi-species models are rare (for a review see D'Amen *et al.* 2017, for examples see: Wootton 2002; Lischke *et al.* 2006) and tend to model communities of limited diversity. Their fit to experimental data is promising though (Wootton 2004). Likewise, aquatic ecosystem models are plenty (Janssen *et al.* 2015), but they tend to include only functional group diversity, if any (see Chapter 7 for an example of functional group vegetation modelling). Modelling community composition and thereby species level biodiversity is a difficult task due to a lack of knowledge on the attributes that determine the fundamental and realized niche of different species (Mokany & Ferrier 2011). Without this knowledge, incorporating processes and setting parameters for a mechanistic community model is hampered (Webb *et al.* 2010). Combined approaches of process- and data-driven-modelling are likely the way forward (D'Amen *et al.* 2017), as it is unlikely that data and knowledge gaps can be properly identified and tackled within the timeframes needed to take action to conserve biodiversity.

Estimation of future habitat conditions under different pressures and management is a key step in determining biodiversity change. Within the Dutch polder landscapes nutrient cycling within the ditch reach (including sediment adsorption and release) and nutrient transport between reaches are important determinants of both functional (see chapter 3 and 7) and response diversity (see chapter 2, 3 and 6). Moreover, nutrient load reduction is a prime measure for restoring aquatic systems (Søndergaard *et al.* 2007). As ecological feedbacks on nutrient cycling and nutrient spiralling are pronounced, the nutrient cycle cannot be disconnected from primary producers and their elemental composition (see chapter 4 and Figure 8.2). Moreover, major consumers of primary producers and their community level elemental plasticity could further impact nutrient cycling (see chapter 4 and 5). To adequately predict nutrient conditions, the explicit nutrient transport through the landscape by both water flows (chapter 7) and movement of animals (see above) needs to be considered as well. These processes, although complex, may all be captured in a coherent process based model. The PCDitch-1D model setup used in chapter 7 offers a starting point for this and could be expanded by including more food web components as well as the

movement through the landscape of some of the higher level consumers (e.g. birds, fish). In addition, mechanistic animal movement and grazing models are well established (Fryxell *et al.* 2004; Baveco *et al.* 2011); and multiple trophic levels (Janse 1997) and stoichiometric constraints on grazing (van Altena *et al.* 2016) have been established within the closely related PCLake model. Inclusion of these processes into the existing model framework would allow for a process based prediction of habitat conditions under a wide range of interacting pressures and management actions (see Figure 8.3).

The suggested model may already inform on much of the functioning (and functional diversity) of ditch landscapes, but it does not inform on the extant response diversity of the landscape or region. Complex metacommunity models would be most suitable to predict persistence of each species under expected environmental conditions throughout the landscape, accounting for spatial configuration of the landscape, dispersal capacity and species environmental niches. However, to date these models have only been applied in theoretical settings without the need to parameterize real-world species and landscapes (Fournier *et al.* 2017). Alternatively, species environmental niches could be calculated to determine species distribution maps under the predicted habitat (see Broennimann *et al.* 2012 for a suite of options for niche determination). These species distribution maps could be aggregated into hypothetical communities for the landscape. Another option would be to link predicted habitat conditions to ecological community classifications that have been related to measured environmental conditions (e.g. Verdonschot & Higler 1989). Similarly, a simulation using environmental and community compositional data could be used to do permutation based replacements of closely matching sites based on the predicted habitat conditions (see chapter 6).

After application of any of these approaches there will be a predicted set of communities across the different reaches of the landscape. Based on this predicted metacommunity, a hierarchical partitioning of diversity (Jost *et al.* 2010), calculation of diversity metrics (e.g. landscape richness, complementarity) and occurrence of target species could be assessed. It is important to note that all of the model approaches have their own assumptions that are unlikely to ever hold completely under natural conditions. For example, our approach in chapter 6 assumes a lack of dispersal limitation, which is unlikely to be true in practice. Nonetheless, by comparing baseline and management scenarios (Figure 8.3), the impacts on biodiversity at multiple levels of spatial scale can be estimated. These type of predictions are vital to inform managers and facilitate discussion with stakeholders (Bohnet *et al.* 2011).



**Figure 8.3:** A scheme depicting an example of an implementation of the framework of Ferrier & Drielsma (2010) for ditch system diversity across scales. In this example a set of boundary characteristics of the ditch system and the landscape are used as inputs to a process based model to predict future habitat conditions. This prediction gives a set of spatially explicit environmental characteristics describing the future habitat conditions. Using species distribution modelling, species niche limits are determined based on a data set gathered in the field and applied to the future scenario while accounting for dispersal barriers. This leads to a spatially explicit community composition that can be aggregated to cross-scale functional and response diversity metrics. By comparing scenarios of no management action (reference model) to those where management actions are applied the direction of impact of management actions and changing pressures on biodiversity may be estimated.

## Managing competing biodiversity goals

A clearly defined shared end goal among stakeholders is essential for strategic adaptive management (Kingsford *et al.* 2011). Within this thesis I have talked extensively about conserving, promoting and managing both response and functional diversity, across different levels of spatial scale (local, landscape and regional). However, the rationale for conserving biodiversity may vary widely between different actors (Escobar 1998). This raises the question if maximal biodiversity in ditch systems is the shared end goal in Dutch peat meadow landscapes. The current setup of agri-environmental schemes is focussed strongly on terrestrial plant diversity and waterfowl and grassland bird conservation. With many of these birds being migratory species, they move over large spatial scales using the polder landscapes in the Netherlands largely as a breeding ground or overwintering grounds (e.g. Alves *et al.* 2010). Hence, they operate at a whole different spatial scale (continental) compared to the local, landscape and regional scales considered within this thesis.

The premise of conserving birds may lead to conflicts with conserving aquatic ditch biodiversity such as vegetation. Waterfowl may hamper development of aquatic vegetation through grazing (Bakker & Nolet 2014; Bakker *et al.* 2018). Moreover, creating suitable habitat for grassland birds through re-wetting agricultural lands can lead to increased nutrient release from these formerly agricultural soils (Olde Venterink *et al.* 2002; Tiemeyer *et al.* 2007). Although re-wetting is an effective management tool for meadow bird habitat creation and conservation (Kahlert *et al.* 2007; Verhulst *et al.* 2011), it may have negative impacts for water quality and the aquatic diversity of ditch systems. Both bird conservation as well as water quality goals currently coexist in Dutch polder landscapes, though they are often divided across different societal actors (e.g. nature conservation agencies and water managers respectively). Conservation of bird communities is quite clearly based on the intrinsic value of biodiversity, whereas the conservation of ditch diversity may lean more on maintaining its ecological services (utilitarian). While conflicts between birds and aquatic biodiversity can exist, synergies may also be found. For example, riparian zones offer shelter and foraging habitat for birds and at the same time retain nutrients and harbour other plant and animal diversity (Hefting *et al.* 2013). For effective biodiversity management, it is important that such potential conflicts are identified, and that prioritized goals are agreed upon.

## Concluding remarks

Adaptive biodiversity management across spatial scales is of vital importance in landscapes experiencing high anthropogenic pressure (Hodbod *et al.* 2016) such as the Dutch polder landscapes. Cross-scale response diversity is needed for a resilient landscape, yielding a spatial insurance effect that protects against extinction events (Loreau *et al.* 2003). Moreover, functional diversity may constrain or facilitate transport of nutrients throughout the landscape. To facilitate sound decision making, quantitative and integrative estimates of changing pressures and management actions across scales and biotic groups are needed. While conceptual frameworks to integrate such knowledge are plenty (e.g. Ferrier & Drielsma 2010; Webb *et al.* 2010), there is a need to move towards quantitative tools to inform management (Mantyka-Pringle *et al.* 2016). Moreover, acknowledging that no projection is true, an adaptive and continuous process in which projections are updated with information from standard monitoring schemes is ideal. These tools should include all aspects and levels of biodiversity that may be of concern to or affect different societal actors. Only then can trade-offs between management actions focussed on different aspects of biodiversity become obvious. The suggested adaptive and integrated approach will facilitate identification of possible management synergies and thereby contribute to biodiversity maximisation across scales and organism groups.

# R

## Reference list

## A

- Ågren, G.I. & Weih, M. (2012). Plant stoichiometry at different scales: element concentration patterns reflect environment more than genotype. *New Phytol.*, 194, 944–952.
- Alahuhta, J., Kosten, S., Akasaka, M., Auderset, D., Azzella, M.M., Bolpagni, R., et al. (2017). Global variation in the beta diversity of lake macrophytes is driven by environmental heterogeneity rather than latitude. *J. Biogeogr.*, 44, 1758–1769.
- Allison, S.D. & Martiny, J.B.H. (2008). Colloquium paper: resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U. S. A.*, 105 Suppl, 11512–9.
- van Altena, C., Bakker, E.S., Kuiper, J.J. & Mooij, W.M. (2016). The impact of bird herbivory on macrophytes and the resilience of the clear-water state in shallow lakes: a model study. *Hydrobiologia*, 777, 197–207.
- Alves, J.A., Lourenço, P.M., Piersma, T., Sutherland, W.J. & Gill, J.A. (2010). Population overlap and habitat segregation in wintering Black-tailed Godwits *Limosa limosa*. *Bird Study*, 57, 381–391.
- Amoros, C. (1984). Crustacés cladocères. Introduction pratique à la systématique des organismes des eaux continentales françaises. *Bull. Mens. la Société Linnéenne Lyon*, 53, 1–63.
- Andersen, T., Elser, J.J. & Hessen, D.O. (2004). Stoichiometry and population dynamics. *Ecol. Lett.*, 7, 884–900.
- Andersen, T. & Hessen, D.O. (1991). Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology Oceanogr.*, 36, 807–814.
- Armitage, P.D., Szoszkiewicz, K., Blackburn, J.H. & Nesbitt, I. (2003). Ditch communities: A major contributor to floodplain biodiversity. *Aquat. Conserv. Mar. Freshw. Ecosyst.*, 13, 165–185.

## B

- Bakker, E.S., Dobrescu, I., Straile, D. & Holmgren, M. (2013). Testing the stress gradient hypothesis in herbivore communities: facilitation peaks at intermediate nutrient levels. *Ecology*, 94, 1776–1784.
- Bakker, E.S. & Nolet, B.A. (2014). Experimental evidence for enhanced top-down control of freshwater macrophytes with nutrient enrichment. *Oecologia*, 176, 825–836.
- Bakker, E.S., Veen, C.G.F., Ter Heerdt, G.J.N., Huig, N. & Sarneel, J.M. (2018). High Grazing Pressure of Geese Threatens Conservation and Restoration of Reed Belts. *Front. Plant Sci.*, 9, 1–12.
- Bakker, E.S., Wood, K.A., Pagès, J.F., Veen, G.F.F. (Ciska) C., Christianen, M.J.A.A., Santamaría, L., et al. (2016). Herbivory on freshwater and marine macrophytes: a review and perspective. *Aquat. Bot.*, 135, 18–36.
- Bakker, S.A., van den Berg, N.J. & Speleers, B.P. (1994). Vegetation transitions of floating wetlands in a complex of turbars between 1937 and 1989 as determined from aerial photographs with GIS. *Vegetatio*, 114, 161–167.
- Baptist, M.J., Penning, W.E., Duel, H., Smits, A.J.M., Geerling, G.W., van der Lee, G.E.M., et al. (2004). Assessment of the effects of cyclic floodplain rejuvenation on flood levels and biodiversity along the Rhine river. *River Res. Appl.*, 20, 285–297.
- Barendregt, A., Stam, S.M.E. & Wassen, M.J. (1992). Restoration of fen ecosystems in the Vecht River plain: cost-benefit analysis of hydrological alternatives. *Hydrobiologia*, 233, 247–258.
- Barko, J.W., Gunnison, D. & Carpenter, S.R. (1991a). Sediment interactions with submersed macrophyte growth and community dynamics. *Aquat. Bot.*, 41, 41–65.
- Barko, J.W., Gunnison, D. & Carpenter, S.R. (1991b). Sediment interactions with submersed macrophyte growth and community dynamics. *Aquat. Bot.*, 41, 41–65.
- Bartrons, M., Papeş, M., Diebel, M.W., Gratton, C. & Vander Zanden, M.J. (2013). Regional-Level Inputs of Emergent Aquatic Insects from Water to Land. *Ecosystems*, 16, 1353–1363.
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Glob. Ecol. Biogeogr.*, 19, 134–143.
- Bauer, S. & Hoye, B.J. (2014). Migratory animals couple biodiversity and ecosystem functioning worldwide. *Science* (80-.), 344.
- Baveco, J.M., Kuipers, H. & Nolet, B.A. (2011). A large-scale multi-species spatial depletion model for overwintering waterfowl. *Ecol. Modell.*, 222, 3773–3784.
- Becker, C. & Boersma, M. (2005). Differential effects of phosphorus and fatty acids on *Daphnia magna* growth and reproduction. *Limnol. Oceanogr.*, 50, 388–397.

- Beltman, B. & Allegrini, C. (1997). Restoration of lost aquatic plant communities: New habitats for Chara. *Netherl. J. Aquat. Ecol.*, 30, 331–337.
- Beltman, B., Van Den Broek, T., Van Maanen, K. & Vaneveld, K. (1996). Measures to develop a rich-fen wetland landscape with a full range of successional stages. *Ecol. Eng.*, 7, 299–313.
- Benton, T.G., Vickery, J.A. & Wilson, J.D. (2003). Farmland biodiversity: Is habitat heterogeneity the key? *Trends Ecol. Evol.*, 18, 182–188.
- Bergamin, R.S., Bastazini, V.A.G., Vélez-Martin, E., Debastiani, V., Zanini, K.J., Loyola, R., et al. (2017). Linking beta diversity patterns to protected areas: lessons from the Brazilian Atlantic Rainforest. *Biodivers. Conserv.*, 26, 1557–1568.
- Best, E.P. & van der Werf, A.K. (1986). Respiration in relation to reserve substances in the submerged macrophyte *Ceratophyllum demersum*. *Aquat. Bot.*, 26, 235–246.
- de Bie, T., De Meester, L., Brendonck, L., Martens, K., Goddeeris, B., Ercken, D., et al. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecol. Lett.*, 15, 740–747.
- Bini, L.M., Thomaz, S.M. & Carvalho, P. (2010). Limnological effects of *Egeria najas* Planchon (Hydrocharitaceae) in the arms of Itaipu Reservoir (Brazil, Paraguay). *Limnology*, 11, 39–47.
- Blanchet, F.G., Legendre, P. & Borcard, D. (2008). FORWARD SELECTION OF EXPLANATORY VARIABLES. *Ecology*, 89, 2623–2632.
- Boeddinghaus, R.S., Nunan, N., Berner, D., Marhan, S. & Kandeler, E. (2015). Do general spatial relationships for microbial biomass and soil enzyme activities exist in temperate grassland soils? *Soil Biol. Biochem.*, 88, 430–440.
- Boedeltje, G., Smolders, A.J.P., Lamers, L.P.M. & Roelofs, J.G.M. (2005). Interactions between sediment propagule banks and sediment nutrient fluxes explain floating plant dominance in stagnant shallow waters. *Arch. für Hydrobiol.*, 162, 349–362.
- Boelee, E., Janse, J., Le Gal, A., Kok, M., Alkemade, R. & Ligtvoet, W. (2017). Overcoming water challenges through nature-based solutions. *Water Policy*, 19, 820–836.
- Boersma, M., Aberle, N., Hantsche, F.M., Schoo, K.L., Wiltshire, K.H. & Malzahn, A.M. (2008). Nutritional limitation travels up the food chain. *Int. Rev. Hydrobiol.*, 93, 479–488.
- Bohn, B.A. & Kershner, J.L. (2002). Establishing aquatic restoration priorities using a watershed approach. *J. Environ. Manage.*, 64, 355–363.
- Bohnet, I.C., Roebeling, P.C., Williams, K.J., Holzworth, D., van Grieken, M.E., Pert, P.L., et al. (2011). Landscapes Toolkit: An integrated modelling framework to assist stakeholders in exploring options for sustainable landscape development. *Landsc. Ecol.*, 26, 1179–1198.
- Bond, E.M. & Chase, J.M. (2002). Biodiversity and ecosystem functioning at local and regional spatial scales. *Ecol. Lett.*, 5, 467–470.
- Bornette, G., Amoros, C. & Lamouroux, N. (1998). Aquatic plant diversity in riverine wetlands: The role of connectivity. *Freshw. Biol.*, 39, 267–283.
- Bornette, G. & Puijalon, S. (2010). Response of aquatic plants to abiotic factors: a review. *Aquat. Sci.*, 73, 1–14.
- Boros, G., Søndergaard, M., Takács, P., Vári, Á. & Tátrai, I. (2011). Influence of submerged macrophytes, temperature, and nutrient loading on the development of redox potential around the sediment-water interface in lakes. *Hydrobiologia*, 665, 117–127.
- Brauns, M., Garcia, X.F., Walz, N. & Pusch, M.T. (2007). Effects of human shoreline development on littoral macroinvertebrates in lowland lakes. *J. Appl. Ecol.*, 44, 1138–1144.
- Brederveld, R.J., Jähning, S.C., Lorenz, A.W., Brunzel, S. & Soons, M.B. (2011). Dispersal as a limiting factor in the colonization of restored mountain streams by plants and macroinvertebrates. *J. Appl. Ecol.*, 48, 1241–1250.
- Brock, G., Pihur, V., Datta, S. & Datta, S. (2011). clValid, an R package for cluster validation. *J. Stat. Softw.*, 25.
- Brock, T.C., Bongaerts, M.C.M., Heijnen, G.J.M.A. & Heijthuijsen, J.H.F.G. (1983). Nitrogen and phosphorus accumulation and cycling by *Nymphaoides peltata* (Gmel.) O. Kuntze (Menyanthaceae). *Aquat. Bot.*, 17, 189–214.

- Brockett, B.F.T., Prescott, C.E. & Grayston, S.J. (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biol. Biochem.*, 44, 9–20.
- Broennimann, O., Fitzpatrick, M.C., Pearman, P.B., Petitpierre, B., Pellissier, L., Yoccoz, N.G., et al. (2012). Measuring ecological niche overlap from occurrence and spatial environmental data. *Glob. Ecol. Biogeogr.*, 21, 481–497.
- Brouns, K., Keuskamp, J.A., Potkamp, G., Verhoeven, J.T.A. & Hefting, M.M. (2016). Peat origin and land use effects on microbial activity, respiration dynamics and exo-enzyme activities in drained peat soils in the Netherlands. *Soil Biol. Biochem.*, 95, 144–155.
- Brown, L.E., Mitchell, G., Holden, J., Folkard, A., Wright, N., Beharry-Borg, N., et al. (2010). Priority water research questions as determined by UK practitioners and policy makers. *Sci. Total Environ.*, 409, 256–266.
- Bruggeman, J. & Bolding, K. (2014). A general framework for aquatic biogeochemical models. *Environ. Model. Softw.*, 61, 249–265.
- Buijs, A.E., Fischer, A., Rink, D. & Young, J.C. (2010). Looking beyond superficial knowledge gaps: Understanding public representations of biodiversity Looking beyond superficial knowledge gaps: Understanding public, 1604.
- Burks, R.L., Mulderij, G., Gross, E., Jones, I., Jacobsen, L., Jeppesen, E., et al. (2006). Center stage: the crucial role of macrophytes in regulating trophic interactions in shallow lake wetlands. In *Wetlands: functioning, biodiversity conservation, and restoration* (pp. 37–59). In: *Wetlands: functioning, biodiversity conservation, and restoration*. Springer Berlin Heidelberg, pp. 37–59.
- C
- Cadenasso, M.L., Pickett, S.T.A. & Grove, J.M. (2006). Dimensions of ecosystem complexity: Heterogeneity, connectivity, and history. *Ecol. Complex.*, 3, 1–12.
- Carpenter, S.R. (1981a). Submersed Vegetation: An Internal Factor in Lake Ecosystem Succession. *Am. Nat.*, 118, 372–383.
- Carpenter, S.R. (1981b). Submersed Vegetation - an Internal Factor in Lake Ecosystem Succession. *Am. Nat.*, 118, 372–383.
- Carpenter, S.R., Caraco, N.F., Correll, D.L., W.Howarth, R., Sharpley, A.N. & Smith, V.H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.*, 8, 559–568.
- Carpenter, S.R. & Lodge, D.M. (1986). Effects of submersed macrophytes on ecosystem processes. *Aquat. Bot.*, 26, 341–370.
- Caspers, H. & Heckman, C.W. (1981). Ecology of orchard drainage ditches along the freshwater section of the Elbe Estuary. *Arch. für Hydrobiol.*, 43, 347–486.
- Catry, I., Marcelino, J., Franco, A.M.A. & Moreira, F. (2017). Landscape determinants of European roller foraging habitat: implications for the definition of agri-environmental measures for species conservation. *Biodivers. Conserv.*, 26, 553–566.
- Chambers, P.A. (1987). Light and Nutrients in the Control of Aquatic Plant Community Structure. II. In Situ Observations. *J. Ecol.*, 75, 621.
- Chester, E.T. & Robson, B.J. (2013). Anthropogenic refuges for freshwater biodiversity: Their ecological characteristics and management. *Biol. Conserv.*, 166, 64–75.
- Clare, P. & Edwards, R.W. (1983). The macroinvertebrate fauna of the drainage channels of the Gwent Levels, South Wales. *Freshw. Biol.*, 13, 205–225.
- Clarke, S.J. (2014). Conserving freshwater biodiversity: The value, status and management of high quality ditch systems. *J. Nat. Conserv.*, 24, 93–100.
- Coetzee, J.A. & Hill, M.P. (2012). The role of eutrophication in the biological control of water hyacinth, *Eichhornia crassipes*, in South Africa. *BioControl*, 57, 247–261.
- Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., et al. (2013). Microplastic ingestion by zooplankton. *Environ. Sci. Technol.*, 47, 6646–6655.
- Collen, B., Whitton, F., Dyer, E.E., Baillie, J.E.M., Cumberlidge, N., Darwall, W.R.T., et al. (2014). Global patterns of freshwater species diversity, threat and endemism. *Glob. Ecol. Biogeogr.*, 23, 40–51.

- Conroy, E., Turner, J.N., Rymszewicz, A., O'Sullivan, J.J., Bruen, M., Lawler, D., et al. (2016). The impact of cattle access on ecological water quality in streams: Examples from agricultural catchments within Ireland. *Sci. Total Environ.*, 547, 17–29.
- Costa, S.S. & Melo, A.S. (2008). Beta diversity in stream macroinvertebrate assemblages: Among-site and among-microhabitat components. *Hydrobiologia*, 598, 131–138.
- Couture, R.M., Moe, S.J., Lin, Y., Kaste, Ø., Haande, S. & Lyche Solheim, A. (2018). Simulating water quality and ecological status of Lake Vansjø, Norway, under land-use and climate change by linking process-oriented models with a Bayesian network. *Sci. Total Environ.*, 621, 713–724.
- Cronin, G. & Lodge, D.M. (2003). Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia*, 137, 32–41.
- D**
- D'Amen, M., Rahbek, C., Zimmermann, N.E. & Guisan, A. (2017). Spatial predictions at the community level: from current approaches to future frameworks. *Biol. Rev.*, 92, 169–187.
- van Dam, P. (2009). Water and Dry Land. *BMGN-Low Ctries. Hist. Rev.*, 124, 459–467.
- Danger, M., Daufresne, T., Lucas, F., Pissard, S. & Lacroix, G. (2008). Does Liebig's law of the minimum scale up from species to communities? *Oikos*, 117, 1741–1751.
- Darchambeau, F., Faerøvig, P.J. & Hessen, D.O. (2003). How Daphnia copes with excess carbon in its food. *Oecologia*, 136, 336–346.
- Davies, B., Biggs, J., Williams, P., Whitfield, M., Nicolet, P., Sear, D., et al. (2008). Comparative biodiversity of aquatic habitats in the European agricultural landscape. *Agric. Ecosyst. Environ.*, 125, 1–8.
- Davis, J., O'Grady, A.P., Dale, A., Arthington, A.H., Gell, P.A., Driver, P.D., et al. (2015). When trends intersect: The challenge of protecting freshwater ecosystems under multiple land use and hydrological intensification scenarios. *Sci. Total Environ.*, 534, 65–78.
- Declerck, S., De Bie, T., Ercken, D., Hampel, H., Schrijvers, S., Van Wichelen, J., et al. (2006). Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. *Biol. Conserv.*, 131, 523–532.
- Declerck, S., Vandekerckhove, J., Johansson, L., Muylaert, K., Conde-Porcuna, J.M., Van Der Gucht, K., et al. (2005). Multi-Group Biodiversity in Shallow Lakes along Gradients of Phosphorus and Water Plant. *Ecology*, 86, 1905–1915.
- Declerck, S.A.J., Coronel, J.S., Legendre, P. & Brendonck, L. (2011). Scale dependency of processes structuring metacommunities of cladocerans in temporary pools of High-Andes wetlands. *Ecography (Cop.)*, 34, 296–305.
- Declerck, S.A.J., Malo, A.R., Diehl, S., Waasdorp, D., Lemmen, K.D., Proios, K., et al. (2015). Rapid adaptation of herbivore consumers to nutrient limitation: Eco-evolutionary feedbacks to population demography and resource control. *Ecol. Lett.*, 18, 553–562.
- Demars, B.O.L. & Edwards, A.C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: High inter-taxon differences and low phenotypic response to nutrient supply. *Freshw. Biol.*, 52, 2073–2086.
- Demott, W.R. & Pape, B.J. (2005). Stoichiometry in an ecological context: testing for links between Daphnia P-content, growth rate and habitat preference, 20–27.
- Dickman, E.M., Newell, J.M., Gonzalez, M.J. & Vanni, M.J. (2008). Light, nutrients, and food-chain length constrain planktonic energy transfer efficiency across multiple trophic levels. *Proc. Natl. Acad. Sci.*, 105, 18408–18412.
- Dickman, E.M., Vanni, M.J. & Horgan, M.J. (2006). Interactive effects of light and nutrients on phytoplankton stoichiometry. *Oecologia*, 149, 676–689.
- Dierberg, F.E., DeBusk, T.A., Jackson, S.D., Chimney, M.J. & Pietro, K. (2002). Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: Response to hydraulic and nutrient loading. *Water Res.*, 36, 1409–1422.
- van Dijk, W.F.A., Van Ruijven, J., Berendse, F. & de Snoo, G.R. (2014). The effectiveness of ditch banks as dispersal corridor for plants in agricultural landscapes depends on species' dispersal traits. *Biol. Conserv.*, 171, 91–98.

- Dollinger, J., Dagès, C., Bailly, J.S., Lagacherie, P. & Voltz, M. (2015). Managing ditches for agroecological engineering of landscape. A review. *Agron. Sustain. Dev.*, 35, 999–1020.
- van Donk, E., Hessen, D.O., Verschoor, A.M. & Gulati, R.D. (2008). Re-oligotrophication by phosphorus reduction and effects on seston quality in lakes. *Limnologica*, 38, 189–202.
- Donohue, I., Jackson, A.L., Pusch, M.T. & Irvine, K. (2009). Nutrient enrichment homogenizes lake benthic assemblages at local and regional scales. *Ecology*, 90, 3470–3477.
- Dorenbosch, M. & Bakker, E.S. (2011). Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. *Freshw. Biol.*, 56, 1783–1797.
- Dornelas, M., Gotelli, N.J., McGill, B., Shimadzu, H., Moyes, F., Sievers, C., et al. (2014). Assemblage time series reveal biodiversity change but not systematic loss. *Science* (80-.), 344, 296–299.
- Downing, J.A. (2014). Limnology and oceanography: Two estranged twins reuniting by global change. *Inl. Waters*, 4, 215–232.
- Dray, S., Legendre, P. & Peres-Neto, P.R. (2006). Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecol. Modell.*, 196, 483–493.
- Drösler, M., Freibauer, A., Christensen, T.R. & Friberg, T. (2008). Observations and status of peatland greenhouse gas emissions in Europe. In: *The continental-scale greenhouse gas balance of Europe*. Springer New York, pp. 243–261.
- Duarte, C.M. (1992). Nutrient concentration of aquatic plants: Patterns across species. *Limnol. Oceanogr.*, 37, 882–889.
- Duarte, C.M. (1995). Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, 41, 87–112.
- Duarte, C.M. & Kalff, J. (1986). Littoral slope as a predictor of the maximum biomass of submerged macrophyte communities. *Limnol. Oceanogr.*, 31, 1072–1080.
- Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., et al. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biol. Rev. Camb. Philos. Soc.*, 81, 163–182.
- Dunham, J.B., Angermeier, P.L., Crausbay, S.D., Cravens, A.E., Gosnell, H., McEvoy, J., et al. (2018). Rivers are social-ecological systems: Time to integrate human dimensions into riverscape ecology and management. *Wiley Interdiscip. Rev. Water*, 5, e1291.
- E**
- Elliott, J.A. (2010). The seasonal sensitivity of Cyanobacteria and other phytoplankton to changes in flushing rate and water temperature. *Glob. Chang. Biol.*, 16, 864–876.
- Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B., et al. (2003). Response diversity, ecosystem change, and resilience. *Front. Ecol. Environ.*
- Elser, J.J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., et al. (2003). Growth rate-stoichiometry couplings in diverse biota. *Ecol. Lett.*, 6, 936–943.
- Elser, J.J., Acquisti, C. & Kumar, S. (2011). Stoichiogenomics: the evolutionary ecology of macromolecular elemental composition. *Trends Ecol. Evol.*, 26, 38–44.
- Elser, J.J., Andersen, T., Baron, J.S., Bergström, A., Jansson, M., Kyle, M., et al. (2009). Shifts in lake N: P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. *Science* (80-.), 326, 835–837.
- Elser, J.J., Dobberfuhl, D.R., MacKay, N. a. & Schampel, J.H. (1996). Organism size, life history, and N: P stoichiometry: towards a unified view of cellular and ecosystem processes. *Bioscience*, 46, 674–684.
- Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swenson, N.G. & Enquist, B.J. (2010). Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytol.*, 186, 593–608.
- Elser, J.J., Hayakawa, K. & Urabe, J. (2001). Nutrient Limitation Reduces Food Quality For Zooplankton: Daphnia Response To Seston Phosphorus Enrichment. *Ecology*, 82, 898–903.
- Elser, J.J., Sterner, R.W., Galford, A.E., Chrzanowski, T.H., Findlay, D.L., Mills, K.H., et al. (2000). Pelagic C: N: P Stoichiometry in a Eutrophied Lake: Responses to a Manipulation, 293–307.
- Elser, J.J. & Urabe, J. (1999). The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. *Ecology*, 80, 735–751.

- Ensign, S.H. & Doyle, M.W. (2006). Nutrient spiraling in streams and river networks, 111, 1–13.
- Ensing, D.J. & Pither, J. (2015). A novel multiple-site extension to pairwise partitioned taxonomic beta diversity. *Ecol. Complex.*, 21, 62–69.
- Escobar, A. (1998). Whose Nature? Biodiversity, Globalization and Sustainability in Latin America and the Caribbean. *J. Polit. Ecol.*, 5, 53–82.
- Ettema, C.H. & Wardle, D.A. (2002). Spatial soil ecology. *Trends Ecol. Evol.*, 17, 177–183.
- Evans, S., Martiny, J.B.H. & Allison, S.D. (2017). Effects of dispersal and selection on stochastic assembly in microbial communities. *ISME J.*, 11, 176–185.
- F
- Fair, P. & Meeke, L. (1983). Seasonal variations in the pattern of photosynthesis and possible adaptive response to varying light flux regimes in *Ceratophyllum demersum* L. *Aquat. Bot.*, 15, 81–90.
- Falkowski, P.G., Scholes, R.J., Boyle, E., Canadell, J., Canfield, D., Elser, J., et al. (2000). The global carbon cycle: a test of our knowledge of earth as a system. *Science* (80-.), 290, 291–296.
- Farquhar, G.D., Firth, P.M., Wetselaar, R. & Weir, B. (1980). On the Gaseous Exchange of Ammonia between Leaves and the Environment: Determination of the Ammonia Compensation Point. *PLANT Physiol.*, 66, 710–714.
- Ferrier, S. & Drielsma, M. (2010). Synthesis of pattern and process in biodiversity conservation assessment: A flexible whole-landscape modelling framework. *Divers. Distrib.*, 16, 386–402.
- Fichtner, K. & Schulze, E.D. (1992). The effect of nitrogen nutrition on growth and biomass partitioning of annual plants originating from habitats of different nitrogen availability. *Oecologia*, 92, 236–241.
- Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., et al. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl. Acad. Sci.*, 109, 21390–21395.
- Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A. & Cleveland, C.C. (2009). Global patterns in belowground communities. *Ecol. Lett.*, 12, 1238–1249.
- Fischer, J., Abson, D.J., Bergsten, A., French Collier, N., Dorresteijn, I., Hanspach, J., et al. (2017). Reframing the Food–Biodiversity Challenge. *Trends Ecol. Evol.*, 32, 335–345.
- Fliervoet, J.M., Van den Born, R.J.G., Smits, A.J.M. & Knippenberg, L. (2013). Combining safety and nature: A multi-stakeholder perspective on integrated floodplain management. *J. Environ. Manage.*
- Flössner, D. (2000). *Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas*. 1st edn. Backhuys Publishers, Leiden.
- Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., et al. (2005a). Global Consequences of Land Use. *Science* (80-.), 309, 570–574.
- Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., et al. (2005b). Global Consequences of Land Use. *Science* (80-.), 309, 570–574.
- Le Foll, S. (2015). *A New Program for Carbon Sequestration in Agriculture*. Paris, France.
- Foster, D., Swanson, F., Aber, J., Burke, I., Brokaw, N., Tilman, D., et al. (2003). The Importance of Land-Use Legacies to Ecology and Conservation. *Bioscience*, 53, 77–88.
- Foster D.R. (2002). Insights from historical geography to ecology and conservation: lessons from the New England landscape. *J. Biogeogr.*, 29, 1269–1275.
- Fournier, B., Mouquet, N., Leibold, M.A. & Gravel, D. (2017). An integrative framework of coexistence mechanisms in competitive metacommunities. *Ecography* (Cop.), 40, 630–641.
- Fox, J.W. (2006). Using the Price Equation to partition the effects of biodiversity loss on ecosystem function. *Ecology*, 87, 2687–2696.
- Fox, J.W. & Kerr, B. (2012). Analyzing the effects of species gain and loss on ecosystem function using the extended Price equation partition. *Oikos*, 121, 290–298.
- Franklin, R.B. & Mills, A.L. (2003). Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiol. Ecol.*, 44, 335–346.
- Freeman, M.C., Pringle, C.M. & Jackson, C.R. (2007). Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. *J. Am. Water Resour. Assoc.*, 43, 5–14.

Frost, P.C. & Hicks, A.L.L. (2012). Human shoreline development and the nutrient stoichiometry of aquatic plant communities in Canadian Shield lakes. *Can. J. Fish. Aquat. Sci.*, 69, 1642–1650.

Frostegård, Å. & Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils*, 22, 59–65.

Fryxell, J.M., Wilmshurst, J.F. & Sinclair, A.R.E. (2004). Predictive Models of Movement. *Ecology*, 85, 2429–2435.

## G

Gall, M. Le, Chaput-Bardy, A. & Husté, A. (2017). Context-dependent local movements of the blue-tailed damselfly, *Ischnura elegans*: effects of pond characteristics and the landscape matrix. *J. Insect Conserv.*, 21, 243–256.

Garbey, C., Murphy, K.J., Thiebaut, G. & Muller, S. (2004). Variation in P-content in aquatic plant tissues offers an efficient tool for determining plant growth strategies along a resource gradient. *Freshw. Biol.*, 49, 346–356.

Garland, J.L. & Mills, A.L. (1991). Classification and characterisation of heterotrophic microbial communities on the basis of pattern of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.*, 57, 2351–2359.

Geller, W. & Müller, H. (1981). The filtration apparatus of Cladocera: Filter mesh-sizes and their implications on food selectivity. *Oecologia*, 49, 316–321.

Gerloff, G.C. (1975). *Nutritional ecology of nuisance aquatic plants*. National Environmental Research Center, Office of Research and Development, US Environmental Protection Agency.

van Gerven, L.P.A., Brederveld, R.J., de Klein, J.J.M., DeAngelis, D.L., Downing, A.S., Faber, M., et al. (2015a). Advantages of concurrent use of multiple software frameworks in water quality modelling using a database approach. *Fundam. Appl. Limnol. / Arch. für Hydrobiol.*, 186, 5–20.

van Gerven, L.P.A., de Klein, J.J.M., Gerla, D.J., Kooi, B.W., Kuiper, J.J. & Mooij, W.M. (2015b). Competition for Light and Nutrients in Layered Communities of Aquatic Plants. *Am. Nat.*, 186, 72–83.

van Gerven, L.P.A., Kuiper, J.J., Janse, J.H., Janssen, A.B.G., Jeuken, M., Mooij, W.M., et al. (2017). How Regime Shifts in Connected Aquatic Ecosystems Are Affected by the Typical Downstream Increase of Water Flow. *Ecosystems*, 20, 733–744.

Goldenberg Vilar, A., Van Dam, H., Van Loon, E.E., Vonk, J.A., Van Der Geest, H.G. & Admiraal, W. (2014). Eutrophication decreases distance decay of similarity in diatom communities. *Freshw. Biol.*, 59, 1522–1531.

Goldman, J., McCarthy, J. & Peavey, D. (1979). Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature*, 279, 210–215.

Gomez, E., Ferreras, L. & Toresani, S. (2006). Soil bacterial functional diversity as influenced by organic amendment application. *Bioresour. Technol.*, 97, 1484–1489.

Gonthier, D.J., Ennis, K.K., Farinas, S., Hsieh, H.Y., Iverson, A.L., Batáry, P., et al. (2014). Biodiversity conservation in agriculture requires a multi-scale approach. *Proc. R. Soc. B Biol. Sci.*, 281, 20141358.

González, A.L., Kominoški, J.S., Danger, M., Ishida, S., Iwai, N. & Rubach, A. (2010). Can ecological stoichiometry help explain patterns of biological invasions? *Oikos*, 119, 779–790.

Granéli, W. & Solander, D. (1988). Influence of aquatic macrophytes on phosphorus cycling in lakes. *Hydrobiologia*, 170, 245–266.

Van der Gucht, K., Cottenie, K., Muylaert, K., Vloemans, N., Cousin, S., Declerck, S., et al. (2007). The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. *Proc. Natl. Acad. Sci. U. S. A.*, 104, 20404–20409.

Gulis, V., Kuehn, K.A., Schoettle, L.N., Leach, D., Benstead, J.P. & Rosemond, A.D. (2017). Changes in nutrient stoichiometry, elemental homeostasis and growth rate of aquatic litter-associated fungi in response to inorganic nutrient supply. *ISME J.*, 11, 2729–2739.

Guo, L.B. & Gifford, R.M. (2002). Soil carbon stocks and land use change: A meta analysis. *Glob. Chang. Biol.*, 8, 345–360.

## H

- Haberl, H., Schulz, N.B., Plutzar, C., Erb, K.H., Krausmann, F., Loibl, W., et al. (2004). Human appropriation of net primary production and species diversity in agricultural landscapes. *Agric. Ecosyst. Environ.*, 102, 213–218.
- Hall, S.R. (2009). Stoichiometrically Explicit Food Webs: Feedbacks between Resource Supply, Elemental Constraints, and Species Diversity. *Annu. Rev. Ecol. Evol. Syst.*, 40, 503–528.
- Hall, S.R., Leibold, M. a., Lytle, D. a & Smith, V.H. (2007). Grazers, producer stoichiometry, and the light:nutrient hypothesis revisited. *Ecology*, 88, 1142–52.
- Hall, S.R., Leibold, M.A., Lytle, D.A. & Smith, V.H. (2004). Stoichiometry and planktonic grazer composition over gradients of light, nutrients, and predation risk. *Ecology*, 85, 2291–2301.
- Haney, A. & Power, R.L. (1996). Adaptive management for sound ecosystem management. *Environ. Manage.*, 20, 879–886.
- Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C. & Martiny, J.B.H. (2012). Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.*, 10, 497–506.
- Hao, B., Wu, H., Shi, Q., Liu, G. & Xing, W. (2013). Facilitation and competition among foundation species of submerged macrophytes threatened by severe eutrophication and implications for restoration. *Ecol. Eng.*, 60, 76–80.
- Hefting, M.M., Clement, J.C., Bienkowski, P., Dowrick, D., Guenat, C., Butturini, A., et al. (2005). The role of vegetation and litter in the nitrogen dynamics of riparian buffer zones in Europe. *Ecol. Eng.*, 24, 465–482.
- Hefting, M.M., van den Heuvel, R.N. & Verhoeven, J.T.A. (2013). Wetlands in agricultural landscapes for nitrogen attenuation and biodiversity enhancement: Opportunities and limitations. *Ecol. Eng.*, 56, 5–13.
- Heijboer, A., ten Berge, H.F.M., de Ruiter, P.C., Jørgensen, H.B., Kowalchuk, G.A. & Bloem, J. (2016). Plant biomass, soil microbial community structure and nitrogen cycling under different organic amendment regimes: a 15N tracer-based approach. *Appl. Soil Ecol.*, 107, 251–260.
- Heino, J. (2009). Biodiversity of Aquatic Insects: Spatial Gradients and Environmental Correlates of Assemblage-Level Measures at Large Scales. *Freshw. Rev.*, 2, 1–29.
- Heino, J., Melo, A.S., Siqueira, T., Soininen, J., Valanko, S. & Bini, L.M. (2015). Metacommunity organisation, spatial extent and dispersal in aquatic systems: Patterns, processes and prospects. *Freshw. Biol.*, 60, 845–869.
- Henle, K., Alard, D., Clitherow, J., Cobb, P., Firbank, L., Kull, T., et al. (2008). Identifying and managing the conflicts between agriculture and biodiversity conservation in Europe – A review, 124, 60–71.
- Henriques-Silva, R., Lindo, Z. & Peres-Neto, P.R. (2013). A community of metacommunities: Exploring patterns in species distributions across large geographical areas. *Ecology*, 94, 627–639.
- Hermoso, V., Abell, R., Linke, S. & Boon, P. (2016). The role of protected areas for freshwater biodiversity conservation: challenges and opportunities in a rapidly changing world. *Aquat. Conserv. Mar. Freshw. Ecosyst.*, 26, 3–11.
- Herzon, I. & Helenius, J. (2008). Agricultural drainage ditches, their biological importance and functioning. *Biol. Conserv.*, 141, 1171–1183.
- Hessen, D.O., Elser, J.J., Sterner, R.W. & Urabe, J. (2013). Ecological stoichiometry: An elementary approach using basic principles. *Limnol. Oceanogr.*, 58, 2219–2236.
- Hessen, D.O., Tombre, I.M., van Geest, G. & Alfsnes, K. (2017). Global change and ecosystem connectivity: How geese link fields of central Europe to eutrophication of Arctic freshwaters. *Ambio*, 46, 40–47.
- Heyburn, J., McKenzie, P., Crawley, M.J. & Fornara, D.A. (2017). Effects of grassland management on plant C:N:P stoichiometry: Implications for soil element cycling and storage. *Ecosphere*, 8.
- Hidding, B., Bakker, E.S., Hootsmans, M.J.M. & Hilt, S. (2016). Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. *Oikos*, 125, 1489–1495.
- Hietala-Koivu, R., Lankoski, J. & Tarmi, S. (2004). Loss of biodiversity and its social cost in an agricultural landscape. *Agric. Ecosyst. Environ.*, 103, 75–83.
- Hill, M., Mountford, J.O., Roy, D.B. & Bunce, R.G.H. (1999). *Ellenberg's indicator values for British plants*. ECOFACT Vol. 2, Tech. Annex.

- Hill, M.J., Chadd, R.P., Morris, N., Swaine, J.D. & Wood, P.J. (2016). Aquatic macroinvertebrate biodiversity associated with artificial agricultural drainage ditches. *Hydrobiologia*, 1–12.
- Hill, M.J., Hassall, C., Oertli, B., Fahrig, L., Robson, B.J., Biggs, J., et al. (2018). New policy directions for global pond conservation. *Conserv. Lett.*, 1–8.
- Hillebrand, H., Cowles, J.M., Lewandowska, A., Van de Waal, D.B. & Plum, C. (2014). Think ratio! A stoichiometric view on biodiversity-ecosystem functioning research. *Basic Appl. Ecol.*, 15, 465–474.
- Hilt, S., Alirangues Nuñez, M.M., Bakker, E.S., Blindow, I., Davidson, T.A., Gillefalk, M., et al. (2018). Response of Submerged Macrophyte Communities to External and Internal Restoration Measures in North Temperate Shallow Lakes. *Front. Plant Sci.*, 9.
- Hilt, S., Brothers, S., Jeppesen, E., Veraart, A.J. & Kosten, S. (2017). Translating Regime Shifts in Shallow Lakes into Changes in Ecosystem Functions and Services. *Bioscience*, 67, 928–936.
- Hines, J., van der Putten, W.H., De Deyn, G.B., Wagg, C., Voigt, W., Mulder, C., et al. (2015). *Towards an integration of biodiversity-ecosystem functioning and food web theory to evaluate relationships between multiple ecosystem services*. *Adv. Ecol. Res.* 1st edn. Elsevier Ltd.
- Hinsch, M. & Poethke, H.J. (2007). Consequences of cyclic vegetation management for arthropod survival: Simulation experiments. *Basic Appl. Ecol.*, 8, 321–331.
- Hirzel, A.H. & Le Lay, G. (2008). Habitat suitability modelling and niche theory. *J. Appl. Ecol.*, 45, 1372–1381.
- Hobbie, S.E., Finlay, J.C., Benjamin, D., Nidzgorski, D.A., Millet, D.B., Lawrence, A., et al. (2017). Correction for Hobbie et al., Contrasting nitrogen and phosphorus budgets in urban watersheds and implications for managing urban water pollution. *Proc. Natl. Acad. Sci.*, 114, E4116–E4116.
- Hobbs, R.J., Higgs, E., Hall, C.M., Bridgewater, P., Chapin, F.S., Ellis, E.C., et al. (2014). Managing the whole landscape: Historical, hybrid, and novel ecosystems. *Front. Ecol. Environ.*, 12, 557–564.
- Hodbod, J., Barreteau, O., Allen, C. & Magda, D. (2016). Managing adaptively for multifunctionality in agricultural systems. *J. Environ. Manage.*, 183, 379–388.
- Honnay, O., Piessens, K., Van Landuyt, W., Hermy, M. & Gulinck, H. (2003). Satellite based land use and landscape complexity indices as predictors for regional plant species diversity. *Landscape Urban Plan.*, 63, 241–250.
- Hothorn, T., Hornik, K., Wiel, M.A. van de & Zeileis, A. (2006). A Lego System for Conditional Inference. *Am. Stat.*, 60, 257–263.
- Hunter, M.D. & Price, P.W. (1992). Playing Chutes and Ladders: Heterogeneity and the Relative Roles of Bottom-Up and Top-Down Forces in Natural Communities. *Ecology*, 73, 724–732.
- Hupfer, M. & Lewandowski, J. (2008). Oxygen controls the phosphorus release from lake sediments - A long-lasting paradigm in limnology. *Int. Rev. Hydrobiol.*, 93, 415–432.
- Hutchins, M.G., Abesser, C., Prudhomme, C., Elliott, J.A., Bloomfield, J.P., Mansour, M.M., et al. (2018). Combined impacts of future land-use and climate stressors on water resources and quality in groundwater and surface waterbodies of the upper Thames river basin, UK. *Sci. Total Environ.*, 631–632, 962–986.
- Hwang, H.M., Fiala, M.J., Park, D. & Wade, T.L. (2016). Review of pollutants in urban road dust and stormwater runoff: part 1. Heavy metals released from vehicles. *Int. J. Urban Sci.*, 20, 334–360.
- I
- Isbell, F., Cowles, J., Dee, L.E., Loreau, M., Reich, P.B., Gonzalez, A., et al. (2018). Quantifying effects of biodiversity on ecosystem functioning across times and places. *Ecol. Lett.*, 21, 763–778.
- Iwabuchi, T. & Urabe, J. (2012a). Competitive outcomes between herbivorous consumers can be predicted from their stoichiometric demands. *Ecosphere*, 3, art7.
- Iwabuchi, T. & Urabe, J. (2012b). Food quality and food threshold: implications of food stoichiometry to competitive ability of herbivore plankton. *Ecosphere*, 3, art51.
- J
- Jabot, F. & Bascompte, J. (2012). Bitrophic interactions shape biodiversity in space. *Proc. Natl. Acad. Sci.*, 109, 4521–4526.
- Janse, J.H. (1997). A model of nutrient dynamics in shallow lakes in relation to multiple stable states. *Hydrobiologia*, 342/343, 1–8.

- Janse, J.H. (1998). A model of ditch vegetation in relation to eutrophication. *Water Sci. Technol.*, 37, 139–149.
- Janse, J.H. & Van Puijenbroek, P.J.T.M. (1998). Effects of eutrophication in drainage ditches. *Environ. Pollut.*, 102, 547–552.
- Janse, J.H., De Senerpont Domis, L.N., Scheffer, M., Lijklema, L., Van Liere, L., Klinge, M., et al. (2008). Critical phosphorus loading of different types of shallow lakes and the consequences for management estimated with the ecosystem model PCLake. *Limnologica*, 38, 203–219.
- Janssen, A.B., Janse, J.H., Beusen, A.H., Chang, M., Harrison, J.A., Huttunen, I., et al. (2019). How to model algal blooms in any lake on earth. *Curr. Opin. Environ. Sustain.*, 36, 1–10.
- Janssen, A.B.G., Arhonditsis, G.B., Beusen, A., Bolding, K., Bruce, L., Bruggeman, J., et al. (2015). Exploring, exploiting and evolving diversity of aquatic ecosystem models: a community perspective. *Aquat. Ecol.*, 49, 513–548.
- Janssen, A.B.G., de Jager, V.C.L., Janse, J.H., Kong, X., Liu, S., Ye, Q., et al. (2017). Spatial identification of critical nutrient loads of large shallow lakes: Implications for Lake Taihu (China). *Water Res.*, 119, 276–287.
- Jeppesen, E., Søndergaard, M., Jensen, J.P., Mortensen, E. & Hansen, A.-M. (1998). Cascading Trophic Interactions from Fish to Bacteria and Nutrients after Reduced Sewage Loading: An 18-Year Study of a Shallow Hypertrophic Lake. *Ecosystems*, 1, 250–267.
- Joosten, H. & Clarke, D. (2002). *Wise use of mires and peatlands- background and principles including framework for decision-making*. International Mire Conservation Group/International Peat Society, Saarijärvi, Finland.
- Jost, L. (2007a). Partitioning diversity into independent Alpha and Beta components. *Ecol. Soc. Am.*, 88, 2427–2439.
- Jost, L. (2007b). Partitioning Diversity Into Independent Alpha and Beta Components. *Ecology*, 88, 2427–2439.
- Jost, L., Devries, P., Walla, T., Greeney, H., Chao, A. & Ricotta, C. (2010). Partitioning diversity for conservation analyses. *Divers. Distrib.*, 16, 65–76.
- K**
- Kahlert, J., Clausen, P., Hounisen, J.P. & Petersen, I.K. (2007). Response of breeding waders to agri-environmental schemes may be obscured by effects of existing hydrology and farming history. *J. Ornithol.*, 148.
- Karp, D.S., Rominger, A.J., Zook, J., Ranganathan, J., Ehrlich, P.R. & Daily, G.C. (2012). Intensive agriculture erodes β-diversity at large scales. *Ecol. Lett.*, 15, 963–970.
- Kingsford, R.T. (2011). Conservation management of rivers and wetlands under climate change - a synthesis. *Mar. Freshw. Res.*, 62, 217.
- Kingsford, R.T., Biggs, H.C. & Pollard, S.R. (2011). Strategic Adaptive Management in freshwater protected areas and their rivers. *Biol. Conserv.*, 144, 1194–1203.
- Kirschner, A.K.T., Riegl, B. & Velimirov, B. (2001). Degradation of emergent and submerged macrophytes in an oxbow lake of an embanked backwater system: Implications for the terrestrialization process. *Int. Rev. Hydrobiol.*, 86, 555–571.
- Klausmeier, C.A., Litchman, E. & Levin, S.A. (2004). Phytoplankton growth and stoichiometry under multiple nutrient limitation. *Limnol. Oceanogr.*, 49, 1463–1470.
- Kleijn, D., Berendse, F., Smit, R. & Gilissen, N. (2001). Agri-environment schemes do not effectively protect biodiversity in Dutch agricultural landscapes. *Nature*, 413, 723–725.
- Kleijn, D., Rundlöf, M., Schepers, J., Smith, H.G. & Tscharntke, T. (2011). Does conservation on farmland contribute to halting the biodiversity decline? *Trends Ecol. Evol.*, 26, 474–481.
- Kleyheeg, E., van Dijk, J.G.B., Tsopoglou-Gkina, D., Woud, T.Y., Boonstra, D.K., Nolet, B.A., et al. (2017). Movement patterns of a keystone waterbird species are highly predictable from landscape configuration. *Mov. Ecol.*, 5, 2.
- Klöve, B., Berglund, K., Berglund, Ö., Weldon, S. & Maljanen, M. (2017). Future options for cultivated Nordic peat soils: Can land management and rewetting control greenhouse gas emissions? *Environ. Sci. Policy*, 69, 85–93.

- Koning, A.A., Moore, J., Suttidate, N., Hannigan, R. & McIntyre, P.B. (2017). Aquatic Ecosystem Impacts of Land Sharing Versus Sparing: Nutrient Loading to Southeast Asian Rivers. *Ecosystems*, 20, 393–405.
- Körner, S. & Vermaat, J.E. (1998). The relative importance of *lemona gibba* l., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Water Res.*, 32, 3651–3661.
- Korol, A.R., Ahn, C. & Noe, G.B. (2016). Richness, biomass, and nutrient content of a wetland macrophyte community affect soil nitrogen cycling in a diversity-ecosystem functioning experiment. *Ecol. Eng.*, 95, 252–265.
- Kosten, S., Piñeiro, M., de Goede, E., de Klein, J., Lamers, L.P.M. & Ettwig, K. (2016). Fate of methane in aquatic systems dominated by free-floating plants. *Water Res.*, 104, 200–207.
- Kovács-Hostyánszki, A., Espíndola, A., Vanbergen, A.J., Settele, J., Kremen, C. & Dicks, L. V. (2017). Ecological intensification to mitigate impacts of conventional intensive land use on pollinators and pollination. *Ecol. Lett.*, 20, 673–689.
- Kremen, C. (2005). Managing ecosystem services: what do we need to know about their ecology? *Ecol. Lett.*, 8, 468–479.
- Kröger, R., Dunne, E.J., Novak, J., King, K.W., McLellan, E., Smith, D.R., et al. (2013). Downstream approaches to phosphorus management in agricultural landscapes: Regional applicability and use. *Sci. Total Environ.*, 442, 263–274.
- Kröger, R., Holland, M.M., Moore, M.T. & Cooper, C.M. (2008). Agricultural Drainage Ditches Mitigate Phosphorus Loads as a Function of Hydrological Variability. *J. Environ. Qual.*, 37, 107.
- Kuiper, J.J., Verhofstad, M.J.J.M., Louwers, E.L.M., Bakker, E.S., Brederveld, R.J., van Gerven, L.P.A., et al. (2017). Mowing Submerged Macrophytes in Shallow Lakes with Alternative Stable States: Battling the Good Guys? *Environ. Manage.*, 59, 619–634.
- Kuramae, E.E., Gamper, H.A., Yergeau, E., Piceno, Y.M., Brodie, E.L., Desantis, T.Z., et al. (2010). Microbial secondary succession in a chronosequence of chalk grasslands. *ISME J.*, 4, 711–715.
- Kuramae, E.E., Yergeau, E., Wong, L.C., Pijl, A.S., Van Veen, J.A. & Kowalchuk, G.A. (2012). Soil characteristics more strongly influence soil bacterial communities than land-use type. *FEMS Microbiol. Ecol.*, 79, 12–24.
- Kuramae, E.E., Zhou, J.Z., Kowalchuk, G.A. & van Veen, J.A. (2014). Soil-Borne Microbial Functional Structure across Different Land Uses. *Sci. World J.*, 2014, 216071.
- L**
- Lacoul, P. & Freedman, B. (2006). Environmental influences on aquatic plants in freshwater ecosystems. *Environ. Rev.*, 14, 89–136.
- Lamers, L.P.M., Smolders, A.J.P. & Roelofs, J.G.M. (2002). The restoration of fens in the Netherlands. *Hydrobiologia*, 478, 107–130.
- Lampert, W., Fleckner, W., Rai, H. & Taylor, B.E. (1986). Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. *Limnol. Oceanogr.*, 31, 478–490.
- Landis, D.A. (2017). Designing agricultural landscapes for biodiversity-based ecosystem services. *Basic Appl. Ecol.*, 18, 1–12.
- Langenheder, S., Wang, J., Karjalainen, S.M., Laamanen, T.M., Tolonen, K.T., Vilmi, A., et al. (2017). Bacterial metacommunity organization in a highly connected aquatic system. *FEMS Microbiol. Ecol.*, 93, 1–9.
- Larocque, G.R., Luckai, N., Adhikary, S.N., Groot, A., Bell, F.W. & Sharma, M. (2013). Competition theory — science and application in mixed forest stands: review of experimental and modelling methods and suggestions for future research. *Environ. Rev.*, 21, 71–84.
- Larsen, S., Sorace, A. & Mancini, L. (2010). Riparian bird communities as indicators of human impacts along mediterranean streams. *Environ. Manage.*, 45, 261–273.
- Lauber, C.L., Strickland, M.S., Bradford, M.A. & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem.*, 40, 2407–2415.
- Lebel, L., Anderies, J.M., Campbell, B., Folke, C. & Hatfield-Dodds, S. (2006). Governance and the Capacity to Manage Resilience in Regional Social-Ecological Systems . Marine Sciences Faculty Scholarship Governance and the Capacity to Manage Resilience in Regional Social-Ecological Systems. *Ecol. Soc.*, 11, 1–19.

- van Leeuwen, C.H.A., Sarneel, J.M., van Paassen, J., Rip, W.J. & Bakker, E.S. (2014). Hydrology, shore morphology and species traits affect seed dispersal, germination and community assembly in shoreline plant communities. *J. Ecol.*, 102, 998–1007.
- Legendre, P. (2014). Interpreting the replacement and richness difference components of beta diversity. *Glob. Ecol. Biogeogr.*, 23, 1324–1334.
- Legendre, P. & Anderson, M.J. (1999a). Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Experiments. *Ecol. Monogr.*, 69, 1–24.
- Legendre, P. & Anderson, M.J. (1999b). Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. *Ecol. Monogr.*, 69, 1–24.
- Legendre, P., Cáceres, M. De, Borcard, D. & Url, S. (2013). Community surveys through space and time: testing the space — time interaction in the absence of replication Community surveys through space and time: the space? time interaction in the absence of testing replication, 91, 262–272.
- Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., et al. (2004). The metacommunity concept: A framework for multi-scale community ecology. *Ecol. Lett.*, 7, 601–613.
- Leip, A., Leach, A., Musinguzi, P., Davis, K.F., Yu, K., Herrero, M., et al. (2010). Impacts of European livestock production: nitrogen , sulphur , phosphorus and greenhouse gas emissions , land-use , water eutrophication and biodiversity. *Environ. Res. Lett.*, 10, 115004.
- Lemmens, P., Mergeay, J., de Bie, T., Van Wichelen, J., de Meester, L. & Declerck, S.A.J. (2013). How to Maximally Support Local and Regional Biodiversity in Applied Conservation? Insights from Pond Management. *PLoS One*, 8, 1–13.
- Leng, X., Musters, C.J.M. & de Snoo, G.R. (2011). Spatiotemporal variation of plant diversity on ditch banks under different management regimes. *Basic Appl. Ecol.*, 12, 38–46.
- Levin, S.A. (1992). The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture. *Ecology*, 73, 1943–1967.
- Li, W., Cao, T., Ni, L., Zhu, G., Zhang, X., Fu, H., et al. (2015). Size-dependent C, N and P stoichiometry of three submersed macrophytes along water depth gradients. *Environ. Earth Sci.*, 74, 3733–3738.
- Li, X., Janssen, A.B.G., de Klein, J.J.M., Kroese, C., Strokal, M., Ma, L., et al. (2019). Modeling nutrients in Lake Dianchi (China) and its watershed. *Agric. Water Manag.*, 212, 48–59.
- Liere, L., Janse, J.H. & Arts, G.H.P. (2007). Setting critical nutrient values for ditches using the eutrophication model PCDitch. *Aquat. Ecol.*, 41, 443–449.
- Liess, A. & Kahlert, M. (2009). Gastropod grazers affect periphyton nutrient stoichiometry by changing benthic algal taxonomy and through differential nutrient uptake. *J. North Am. Benthol. Soc.*, 28, 283–293.
- Lin, X., Tfaily, M.M., Green, S.J., Steinweg, J.M., Chanton, P., Imvittaya, A., et al. (2014). Microbial metabolic potential for carbon degradation and nutrient (nitrogen and phosphorus) acquisition in an ombrotrophic peatland. *Appl. Environ. Microbiol.*, 80, 3531–3540.
- Lischke, H., Zimmermann, N.E., Bolliger, J., Rickebusch, S. & Löffler, T.J. (2006). TreeMig: A forest-landscape model for simulating spatio-temporal patterns from stand to landscape scale. *Ecol. Modell.*, 199, 409–420.
- Loreau, M. & Hector, a. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412, 72–6.
- Loreau, M., Mouquet, N. & Gonzalez, A. (2003). Biodiversity as spatial insurance in heterogeneous landscapes, 100, 12765–12770.
- Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., et al. (2001). Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. *Science* (80-. ), 294, 804–808.
- M**
- Mace, G.M., Norris, K. & Fitter, A.H. (2012). Biodiversity and ecosystem services: a multilayered relationship, 27, 19–26.
- Macek, P. & Rejmánková, E. (2007). Response of emergent macrophytes to experimental nutrient and salinity additions. *Funct. Ecol.*, 21, 478–488.
- Mack, M.C., Schuur, E. a G., Bret-Harte, M.S., Shaver, G.R. & Chapin, F.S. (2004). Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature*, 431, 440–443.

- Madsen, T.V. & Cedergreen, N. (2002). Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshw. Biol.*, 47, 283–291.
- Main, T.M., Dobberfuhl, D.R. & Elser, J.J. (1997). N:P stoichiometry and ontogeny of crustacean zooplankton: A test of the growth rate hypothesis. *Limnol. Oceanogr.*, 42, 1474–1478.
- Malzahn, A.M., Aberle, N. & Clemmesen, C. (2007). Nutrient limitation of primary producers affects planktivorous fish condition, 52, 2062–2071.
- Malzahn, A.M., Hantzsch, F., Schoo, K.L., Boersma, M., Aberle, N. & Boersma, M. (2010). Differential effects of nutrient-limited primary production on primary, secondary or tertiary consumers. *Oecologia*, 162, 35–48.
- Manfrin, A., Bombi, P., Traversetti, L., Larsen, S. & Scalici, M. (2016). A landscape-based predictive approach for running water quality assessment: A Mediterranean case study. *J. Nat. Conserv.*, 30, 27–31.
- Manhoudt, A.G.E., Visser, A.J. & de Snoo, G.R. (2007). Management regimes and farming practices enhancing plant species richness on ditch banks. *Agric. Ecosyst. Environ.*, 119, 353–358.
- Mantyka-Pringle, C.S., Martin, T.G., Moffatt, D.B., Udy, J., Olley, J., Saxton, N., et al. (2016). Prioritizing management actions for the conservation of freshwater biodiversity under changing climate and land-cover. *Biol. Conserv.*, 197, 80–89.
- Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J. a, Green, J.L., et al. (2006). Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.*, 4, 102–112.
- Martiny, J.B.H., Eisen, J.A., Penn, K., Allison, S.D. & Horner-Devine, M.C. (2011). Drivers of bacterial β-diversity depend on spatial scale. *Proc. Natl. Acad. Sci. U. S. A.*, 108, 7850–7854.
- McGill, B.J., Dornelas, M., Gotelli, N.J. & Magurran, A.E. (2015). Fifteen forms of biodiversity trend in the anthropocene. *Trends Ecol. Evol.*, 30, 104–113.
- McGoff, E. & Irvine, K. (2009). A test of the association between Lake Habitat Quality Assessment and macroinvertebrate community structure. *Aquat. Conserv. Mar. Freshw. Ecosyst.*, 19, 520–533.
- Mette, E.M., Vanni, M.J., Newell, J.M. & González, M.J. (2011). Phytoplankton communities and stoichiometry are interactively affected by light, nutrients, and fish. *Limnol. Oceanogr.*, 56, 1959–1975.
- Meunier, C.L., Malzahn, A.M. & Boersma, M. (2014). A New Approach to Homeostatic Regulation: Towards a Unified View of Physiological and Ecological Concepts. *PLoS One*, 9, e107737.
- Meyer, A., Combroux, I. & Trémolières, M. (2012). Dynamics of Nutrient Contents (Phosphorus, Nitrogen) in Water, Sediment and Plants After Restoration of Connectivity in Side-Channels of the River Rhine. *Restor. Ecol.*, 21, 232–241.
- Middelboe, A.L. & Markager, S. (1997). Depth limits and minimum light requirements of freshwater macrophytes. *Freshw. Biol.*, 37, 553–568.
- Middleton, B. a, Holsten, B. & van Diggelen, R. (2006). Biodiversity management of fens and fen meadows by grazing, cutting and burning. *Appl. Veg. Sci.*, 9, 307–316.
- Miler, O. & Straile, D. (2010). How to cope with a superior enemy? Plant defence strategies in response to annual herbivore outbreaks. *J. Ecol.*, 98, 900–907.
- Milly, P.C.D., Betancourt, J., Falkenmark, M., Hirsch, R.M., Kundzewicz, Z.W., Lettenmaier, D.P., et al. (2008). Stationarity Is Dead: Whither Water Management? *Science* (80-. ), 319, 573–574.
- Milsom, T.P., Sherwood, A.J., Rose, S.C., Town, S.J. & Runham, S.R. (2004). Dynamics and management of plant communities in ditches bordering arable fenland in eastern England. *Agric. Ecosyst. Environ.*, 103, 85–99.
- Mokany, K. & Ferrier, S. (2011). Predicting impacts of climate change on biodiversity: A role for semi-mechanistic community-level modelling. *Divers. Distrib.*, 17, 374–380.
- Molina-Navarro, E., Andersen, H.E., Nielsen, A., Thodsen, H. & Trolle, D. (2018). Quantifying the combined effects of land use and climate changes on stream flow and nutrient loads: A modelling approach in the Odense Fjord catchment (Denmark). *Sci. Total Environ.*, 621, 253–264.
- Morris, M.G. (2000). The effects of structure and its dynamics on the ecology and conservation of arthropods in British grasslands. *Biol. Conserv.*, 95, 129–142.
- Moss, B., Kosten, S., Meerhoff, M., Battarbee, R.W., Jeppesen, E., Mazzeo, N., et al. (2011). Allied attack: climate change and eutrophication. *Inl. Waters*, 1, 101–105.

- Moussie, A.M., Voet, T. van der & Veldhuizen, J.E. van. (2013). *Milieueffectrapport Polder Oukoop en Negenviertel*. Alkmaar.
- de Mulder, E.F.J., van Bruchem, A.J., Claessen, F.A.M., Hannink, G., Hulsbergen, J.G. & Satijn, H.M.C. (1994). Environmental impact assessment on land reclamation projects in The Netherlands: A case history. *Eng. Geol.*, 37, 15–23.
- Mulder, K. & Bowden, W.B. (2007). Organismal stoichiometry and the adaptive advantage of variable nutrient use and production efficiency in Daphnia. *Ecol. Model.*, 202, 427–440.
- Müller-Navarra, D.C. (1995). Biochemical versus mineral limitation in Daphnia. *Limnol. Oceanogr.*, 40, 1209–1214.
- Murphy, J. & Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. ACTA*, 27, 31–36.

**N**

- Naiman, R.J., Bilby, R.E., Schindler, D.E. & Helfield, J.M. (2002). Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems*, 5, 399–417.
- Needelman, B.A., Kleinman, P.J.A. & Allen, A.L. (2007). Improved management of agricultural drainage ditches for water quality protection: An overview. *J. Soil Water Conserv.*, 62, 171–178.
- Nelson, E., Mendoza, G., Regetz, J., Polasky, S., Tallis, H., Cameron, D.R., et al. (2009). Modeling multiple ecosystem services, biodiversity conservation, commodity production, and tradeoffs at landscape scales. *Front. Ecol. Environ.*, 7, 4–11.
- Nemergut, D.R., Costello, E.K., Hamady, M., Lozupone, C., Jiang, L., Schmidt, S.K., et al. (2011). Global patterns in the biogeography of bacterial taxa. *Environ. Microbiol.*, 13, 135–144.
- van Nes, E.H. & Scheffer, M. (2005). Implications Of Spatial Heterogeneity For Catastrophic Regime Shifts In Ecosystems. *Ecology*, 86, 1797–1807.
- Netten, J.J.C., van Zuidam, J., Kosten, S. & Peeterz, E.T.H.M. (2011). Differential response to climatic variation of free-floating and submerged macrophytes in ditches. *Freshw. Biol.*, 56, 1761–1768.
- Newbold, J.D., Elwood, J.W., O'Neill, R. V. & Winkle, W. Van. (1981). Measuring Nutrient Spiralling in Streams. *Can. J. Fish. Aquat. Sci.*, 38, 860–863.
- Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., et al. (2015). Global effects of land use on local terrestrial biodiversity. *Nature*, 520, 45–50.
- Nguyen, L. & Sukias, J. (2002). Phosphorus fractions and retention in drainage ditch sediments receiving surface runoff and subsurface drainage from agricultural catchments in the North Island, New Zealand. *Agric. Ecosyst. Environ.*, 92, 49–69.
- Nielsen, A., Bolding, K., Hu, F. & Trolle, D. (2017). An open source QGIS-based workflow for model application and experimentation with aquatic ecosystems. *Environ. Model. Softw.*, 95, 358–364.
- Niemelä, J., Haila, Y. & Punttila, P. (1996). The importance of small-scale heterogeneity in boreal forests: variation in diversity in forest-floor invertebrates across the succession gradient. *Ecography (Cop.)*, 19, 352–368.
- Nurnberg, G.K. (2009). Assessing internal phosphorus load - Problems to be solved. *Lake Reserv. Manag.*, 25, 419–432.
- Nurnberg, G.K. (1991). Phosphorus from Internal Sources in the Laurentian Great Lakes, and the Concept of Threshold External Load. *J. Great Lakes Res.*, 17, 132–140.
- O**
- O'Brien, S.L., Gibbons, S.M., Owens, S.M., Hampton-Marcell, J., Johnston, E.R., Jastrow, J.D., et al. (2016). Spatial scale drives patterns in soil bacterial diversity. *Environ. Microbiol.*, 18, 2039–2051.
- O'Donnell, A.G., Seasman, M., Macrae, A., Waite, I. & Davies, J.T. (2001). Plants and fertilisers as drivers of change in microbial community structure and function in soils. *Plant Soil*, 232, 135–145.
- Odum, E.P. (1969). The Strategy of Ecosystem Development. *Science* 164, 262–270.
- Ogden, L., Heynen, N., Oslender, U., West, P., Kassam, K. & Robbins, P. (2013). Global assemblages, resilience, and Earth Stewardship in the Anthropocene. *Front. Ecol. Env.*, 11-7, 341-347.

- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., et al.(2015). vegan: Community Ecology Package.
- Olde Venterink, H., Davidsson, T.E., Kiehl, K. & Leonardson, L. (2002). Impact of drying and rewetting on carbon cycling in a northern fen. *Plant Soil*, 243, 119–130.
- Olde Venterink, H., Vermaat, J.E., Pronk, M., Wiegman, F., van der Lee, G.E.M., van den Hoorn, Martin W. Higler, L.W.G., et al.(2006). Importance of sediment deposition and denitrification for nutrient retention in floodplain wetlands. *Appl. Veg. Sci.*, 9, 163–174.
- Orth, R.J., Dennison, W.C., Lefcheck, J.S., Gurbisz, C., Hannam, M., Keisman, J., et al. (2017). Submersed aquatic vegetation in chesapeake bay: Sentinel species in a changing world. *Bioscience*, 67, 698–712.
- Owens, M. (1974). Measurements on non-isolated natural communities in running waters. In: *A manual on methods for measuring primary production in aquatic environments*. Blackwell Scientific Publications, Oxford, pp. 111–119.
- P**
- Paerl, H.W., Fulton, R.S., Moisander, P.H. & Dyble, J. (2001). Harmful Freshwater Algal Blooms, With an Emphasis on Cyanobacteria. *Sci. World J.*, 1, 76–113.
- Paerl, H.W., Gardner, W.S., Havens, K.E., Joyner, A.R., McCarthy, M.J., Newell, S.E., et al. (2016). Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. *Harmful Algae*, 54, 213–222.
- Peres-Neto, P.-R., Leibold, M. & Dray, S. (2012). Assessing the effects of spatial contingency and environmental filtering on metacommunity phylogenetics. *Ecology*, 93, 14–30.
- Peres-Neto, P.R., Legendre, P., Dray, S. & Borcard, D. (2006). Variation partitioning of species data matrices: Estimation and comparison of fractions. *Ecology*, 87, 2614–2625.
- Pérez-Valera, E., Goberna, M. & Verdú, M. (2015). Phylogenetic structure of soil bacterial communities predicts ecosystem functioning. *FEMS Microbiol. Ecol.*, 91, 1–9.
- Perona, P., Camporeale, C., Perucca, E., Savina, M., Molnar, P., Burlando, P., et al. (2009). Modelling river and riparian vegetation interactions and related importance for sustainable ecosystem management. *Aquat. Sci.*, 71, 266–278.
- Persson, J., Fink, P., Goto, A., Hood, J.M., Jonas, J. & Kato, S. (2010). To be or not to be what you eat: Regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos*, 119, 741–751.
- Petsch, D.K. (2016). Causes and consequences of biotic homogenization in freshwater ecosystems. *Int. Rev. Hydrobiol.*, 101, 113–122.
- Phillips, G., Willby, N. & Moss, B. (2016). Submerged macrophyte decline in shallow lakes: What have we learnt in the last forty years? *Aquat. Bot.*, 135, 37–45.
- Pielke, R.A., Adegoke, J., Beltrán-Przekurat, A., Hiemstra, C.A., Lin, J., Nair, U.S., et al. (2007). An overview of regional land-use and land-cover impacts on rainfall. *Tellus, Ser. B Chem. Phys. Meteorol.*, 59, 587–601.
- Pietro, K.C., Chimney, M.J. & Steinman, A.D. (2006). Phosphorus removal by the Ceratophyllum/periphyton complex in a south Florida (USA) freshwater marsh. *Ecol. Eng.*, 27, 290–300.
- Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., et al. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science* (80-.), 344.
- van der Plas, F., Manning, P., Soliveres, S., Allan, E., Scherer-lorenzen, M., Verheyen, K., et al.(2016). Biotic homogenization can decrease landscape-scale forest multifunctionality. *Proc. Natl. Acad. Sci.*, 113, 3557–3562.
- Plum, C., Hüsener, M. & Hillebrand, H. (2015). Multiple vs. single phytoplankton species alter stoichiometry of trophic interaction with zooplankton. *Ecology*, 96, 3075–3089.
- Podani, J. & Schmera, D. (2011). A new conceptual and methodological framework for exploring and explaining pattern in presence - absence data. *Oikos*, 120, 1625–1638.
- Portielje, R. & Roijackers, R.M.M. (1995). Primary succession of aquatic macrophytes in experimental ditches in relation to nutrient input. *Aquat. Bot.*, 50, 127–140.
- Power, A.G. (2010). Ecosystem services and agriculture: Tradeoffs and synergies. *Philos. Trans. R. Soc. B Biol. Sci.*, 365, 2959–2971.
- Price, G.R. (1970). Selection and Covariance. *Nature*, 227, 520–521.

Prober, S.M., Byrne, M., McLean, E.H., Steane, D.A., Potts, B.M., Vaillancourt, R.E., et al. (2015a). Climate-adjusted provenancing: a strategy for climate-resilient ecological restoration. *Front. Ecol. Evol.*, 3, 1–5.

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., et al. (2015b). Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol. Lett.*, 18, 85–95.

## R

Ramirez, K.S., Leff, J.W., Barberan, A., Bates, S.T., Betley, J., Crowther, T.W., et al. (2014). Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proc. R. Soc. B Biol. Sci.*, 281, 20141988–20141988.

Reidsma, P., Tekelenburg, T., Van Den Berg, M. & Alkemade, R. (2006). Impacts of land-use change on biodiversity: An assessment of agricultural biodiversity in the European Union. *Agric. Ecosyst. Environ.*, 114, 86–102.

Richards, S.A., Possingham, H.P. & Tizard, J. (1999). Optimal Fire Management for Maintaining Community Diversity. *Ecol. Appl.*, 9, 880–892.

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

Rosenzweig, M.L. (2003). Reconciliation ecology and the future of species diversity. *Oryx*, 37, 194–205.

Rowland, F.E., Bricker, K.J., Vanni, M.J., González, M.J. & González, M.J. (2015). Light and nutrients regulate energy transfer through benthic and pelagic food chains. *Oikos*, 124, 1648–1663.

## S

Sand-Jensen, K. & Madsen, T.V. (1991). Minimum Light Requirements of Submerged Freshwater Macrophytes in Laboratory Growth Experiments. *J. Ecol.*, 79, 749–764.

Sardans, J., Rivas-Ubach, A. & Peñuelas, J. (2012). The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. *Perspect. Plant Ecol. Evol. Syst.*, 14, 33–47.

Sarneel, J.M., Janssen, R.H., Rip, W.J., Bender, I.M.A. & Bakker, E.S. (2014). Windows of opportunity for germination of riparian species after restoring water level fluctuations: A field experiment with controlled seed banks. *J. Appl. Ecol.*, 51, 1006–1014.

Sayer, E.J., Oliver, A.E., Fridley, J.D., Askew, A.P., Mills, R.T.E. & Grime, J.P. (2017). Links between soil microbial communities and plant traits in a species-rich grassland under long-term climate change. *Ecol. Evol.*, 855–862.

Sayles, J.S. & Baggio, J.A. (2017). Social–ecological network analysis of scale mismatches in estuary watershed restoration. *Proc. Natl. Acad. Sci.*, 114, E1776–E1785.

Schade, J.D., Espeleta, J.F., Klausmeier, C. a, McGroddy, M.E., Zhang, T. & Zhang, L. (2005). A conceptual framework for ecosystem stoichiometry: balancing resource supply and demand. *Oikos*, 109, 40–51.

Scheffer, M., Management, W.Q., Zimmer, K., Jeppesen, E., Butler, M.G., Alle, O.W., et al. (1999). Small habitat size and isolation can promote species richness;. 227–231.

Scheffer, M., Szabo, S., Gragnani, A., van Nes, E.H., Rinaldi, S., Kautsky, N., et al. (2003). Floating plant dominance as a stable state. *Proc. Natl. Acad. Sci.*, 100, 4040–4045.

Schindler, D.E., Armstrong, J.B. & Reed, T.E. (2015). The portfolio concept in ecology and evolution. *Front. Ecol. Environ.*, 13, 257–263.

Schoo, K.L., Aberle, N., Malzahn, A.M. & Boersma, M. (2012). Food Quality Affects Secondary Consumers Even at Low Quantities: An Experimental Test with Larval European Lobster. *PLoS One*, 7, e33550.

Shin, M.J., Guillaume, J.H.A., Croke, B.F.W. & Jakeman, A.J. (2015). A review of foundational methods for checking the structural identifiability of models: Results for rainfall-runoff. *J. Hydrol.*, 520, 1–16.

Sistla, S.A., Appling, A.P., Lewandowska, A.M., Taylor, B.N. & Wolf, A.A. (2015). Stoichiometric flexibility in response to fertilization along gradients of environmental and organismal nutrient richness. *Oikos*, 124, 949–959.

Sistla, S.A., Moore, J.C., Simpson, R.T., Gough, L., Shaver, G.R. & Schimel, J.P. (2013). Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature*, 497, 615–8.

Sistla, S.A. & Schimel, J.P. (2012). Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. *New Phytol.*, 196, 68–78.

- Small, G.E., Helton, A.M. & Kazanci, C. (2009). Can consumer stoichiometric regulation control nutrient spiraling in streams? *J. North Am. Benthol. Soc.*, 28, 747–765.
- Smart, M.M. (1980). Annual changes of nitrogen and phosphorus in two aquatic macrophytes (*Nymphaea tuberosa* and *Ceratophyllum demersum*). *Hydrobiologia*, 70, 31–35.
- Smart, S.M., Thompson, K., Marrs, R.H., Le Duc, M.G., Maskell, L.C. & Firbank, L.G. (2006). Biotic homogenization and changes in species diversity across human-modified ecosystems. *Proc. R. Soc. B*, 273, 2659–2665.
- Smith, D.R. (2009). Assessment of in-stream phosphorus dynamics in agricultural drainage ditches. *Sci. Total Environ.*, 407, 3883–3889.
- Smith, R.F., Venugopal, P.D., Baker, M.E. & Lamp, W.O. (2015). Habitat filtering and adult dispersal determine the taxonomic composition of stream insects in an urbanizing landscape. *Freshw. Biol.*, 60, 1740–1754.
- Smith, R.J., Ver, D., Isaac, N.J.B. & Jones, K.E. (2012). Identifying Cinderella species: uncovering mammals with conservation flagship appeal, 5, 205–212.
- Smolders, A.J.P., Lamers, L.P.M., Lucassen, E.C.H.E.T., Van Der Velde, G. & Roelofs, J.G.M. (2006). Internal eutrophication: How it works and what to do about it - A review. *Chem. Ecol.*, 22, 93–111.
- Socolar, J.B., Gilroy, J.J., Kunin, W.E. & Edwards, D.P. (2016a). How Should Beta-Diversity Inform Biodiversity Conservation? *Trends Ecol. Evol.*, 31, 67–80.
- Socolar, J.B., Gilroy, J.J., Kunin, W.E. & Edwards, D.P. (2016b). Sparse Data Necessitate Explicit Treatment of Beta-Diversity: A Reply to Bush et al. *Trends Ecol. Evol.*, 31, 338–339.
- Soininen, J., Heino, J. & Wang, J. (2018). A meta-analysis of nestedness and turnover components of beta diversity across organisms and ecosystems. *Glob. Ecol. Biogeogr.*, 27, 96–109.
- Søndergaard, M., Jensen, J.P. & Jeppesen, E. (2003). Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia*, 506–509, 135–145.
- Søndergaard, M., Jeppesen, E., Lauridsen, T.L., Skov, C., Van Nes, E.H., Roijackers, R., et al. (2007). Lake restoration: Successes, failures and long-term effects. *J. Appl. Ecol.*, 44, 1095–1105.
- Soomers, H., Karssenberg, D., Soons, M.B., Verweij, P.A., Verhoeven, J.T.A. & Wassen, M.J. (2013). Wind and Water Dispersal of Wetland Plants Across Fragmented Landscapes. *Ecosystems*, 16, 434–451.
- Sousa, W.P. (1979). Experimental Investigations of Disturbance and Ecological Succession in a Rocky Intertidal Algal Community. *Ecol. Monogr.*, 49, 227–254.
- Sousa, W.P. (1984). The Role of Disturbance in Natural Communities. *Annu. Rev. Ecol. Syst.*, 15, 353–391.
- Staveley, T.A.B., Perry, D., Lindborg, R. & Gullström, M. (2017). Seascape structure and complexity influence temperate seagrass fish assemblage composition. *Ecography (Cop.)*, 40, 936–946.
- Stein, B.A., Staudt, A., Cross, M.S., Dubois, N.S., Enquist, C., Griffis, R., et al. (2013). Preparing for and managing change: Climate adaptation for biodiversity and ecosystems. *Front. Ecol. Environ.*, 11, 502–510.
- Sterner, R.W. (1990). The Ratio of Nitrogen to Phosphorus Resupplied by Herbivores: Zooplankton and the Algal Competitive Arena. *Am. Nat.*, 136, 209–229.
- Sterner, R.W. & Elser, J.J. (2002). *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press.
- Sterner, R.W., Elser, J.J., Fee, E.J., Guildford, S.J. & Chrzanowski, T.H. (1997). The Light: Nutrient Ratio in Lakes: The Balance of Energy and Materials Affects Ecosystem Structure and Process. *Am. Nat.*, 150, 663–684.
- Strecker, A.L. & Brittain, J.T. (2017). Increased habitat connectivity homogenizes freshwater communities: historical and landscape perspectives. *J. Appl. Ecol.*, 54, 1343–1352.
- Strickland, M.S., Lauber, C., Fierer, N. & Bradford, M.A. (2009). Testing the functional significance of microbial community composition. *Ecology*, 90, 441–451.
- Striebel, M., Behl, S., Diehl, S. & Stibor, H. (2009a). Spectral Niche Complementarity and Carbon Dynamics in Pelagic Ecosystems. *Am. Nat.*, 174, 141–147.
- Striebel, M., Behl, S. & Stibor, H. (2009b). The coupling of biodiversity and productivity in phytoplankton communities: consequences for biomass stoichiometry. *Ecology*, 90, 2025–2031.
- van Strien, A.J., van der Burg, T., Rip, W.J. & Strucker, R.C.W. (1991). Effects of Mechanical Ditch Management on the Vegetation of Ditch Banks in Dutch Peat Areas. *J. Appl. Ecol.*, 28, 501–513.

van Strien, A.J., van de Linden, J. r, Melman, T.C.P. & Noordervliet, M.A.W. (1989). Factors Affecting the Vegetation of Ditch Banks in Peat Areas in the Western Netherlands. *J. Appl. Ecol.*, 26, 989–1004.

Sun, J., Hunter, P.D., Tyler, A.N. & Willby, N.J. (2018). The influence of hydrological and land use indicators on macrophyte richness in lakes – A comparison of catchment and landscape buffers across multiple scales. *Ecol. Indic.*, 89, 227–239.

## T

Taft, H.E., Cross, P.A., Edwards-Jones, G., Moorhouse, E.R. & Jones, D.L. (2017). Greenhouse gas emissions from intensively managed peat soils in an arable production system. *Agric. Ecosyst. Environ.*, 237, 162–172.

Tansley, A.G. (1946). *Introduction to plant ecology*. Allen & Unwin, London.

TeBraake, W.H. (2008). Review: Managing Water in an Urban Land: A New History of Rijnland. *BMGN-Low Ctries. Hist. Rev.*, 123, 71–78.

TeBraake, W.H. (2002). Taming the Waterwolf: Hydraulic Engineering and Water Management in the Netherlands during the Middle Ages. *Technol. Cult.*, 43, 475–499.

Teurlincx, S., Heijboer, A., Veraart, A.J., Kowalchuk, G.A. & Declerck, S.A.J. (2018a). Local Functioning, Landscape Structuring: Drivers of Soil Microbial Community Structure and Function in Peatlands. *Front. Microbiol.*, 9, 1–14.

Teurlincx, S., Kuiper, J.J., Hoevenaar, E.C., Lurling, M., Brederveld, R.J., Veraart, A.J., et al. (2019). Towards restoring urban waters: understanding the main pressures. *Curr. Opin. Environ. Sustain.*, 36, 49–58.

Teurlincx, S., Velthuis, M., Seroka, D., Govaert, L., van Donk, E., Van de Waal, D.B., et al. (2017). Species sorting and stoichiometric plasticity control community C:P ratio of first-order aquatic consumers. *Ecol. Lett.*, 20, 751–760.

Teurlincx, S., Verhofstad, M.J.J.M., Bakker, E.S. & Declerck, S.A.J. (2018b). Managing Successional Stage Heterogeneity to Maximize Landscape-Wide Biodiversity of Aquatic Vegetation in Ditch Networks. *Front. Plant Sci.*, 9, 1–12.

Tian, J., Wang, J., Dippold, M., Gao, Y., Blagodatskaya, E. & Kuzyakov, Y. (2016). Biochar affects soil organic matter cycling and microbial functions but does not alter microbial community structure in a paddy soil. *Sci. Total Environ.*, 556, 89–97.

Tiemeyer, B., Frings, J., Kahle, P., Köhne, S. & Lennartz, B. (2007). A comprehensive study of nutrient losses, soil properties and groundwater concentrations in a degraded peatland used as an intensive meadow - Implications for re-wetting. *J. Hydrol.*, 345, 80–101.

Tilman, D. (1994). Competition and biodiversity in spatially structured habitats. *Ecology*, 75, 2–16.

Tockner, K., Malard, F. & Ward, J. V. (2000). An extension of the flood pulse concept. *Hydrolog. Process.*, 14, 2861–2883.

Toor, G.S., Occhipinti, M.L., Yang, Y.Y., Majcherek, T., Haver, D. & Oki, L. (2017). Managing urban runoff in residential neighborhoods: Nitrogen and phosphorus in lawn irrigation driven runoff. *PLoS One*, 12, 1–17.

Trombulak, S.C. & Frissell, C.A. (2000). Review of Ecological Effects of Roads on Terrestrial and Aquatic Communities. *Conserv. Biol.*, 14, 18–30.

Turetsky, M., Wieder, K., Halsey, L. & Vitt, D. (2002). Current disturbance and the diminishing peatland carbon sink. *Geophys. Res. Lett.*, 29, 7–10.

Turner, M.G. (1989). Landscape Ecology: The Effect of Pattern on Process. *Ecology*, 20, 171–197.

Turunen, J., Tomppo, E., Tolonen, K. & Reinikainen, A. (2002). Estimating carbon accumulation rates of undrained mires in Finland – application to boreal and subarctic regions. *The Holocene*, 12, 69–80.

Twisk, W., Noordervliet, M.A.W. & Ter Keurs, W.J. (2003). The nature value of the ditch vegetation in peat areas in relation to farm management. *Aquat. Ecol.*, 37, 191–209.

## U

Urabe, J., Naeem, S., Raubenheimer, D. & Elser, J.J. (2010). The evolution of biological stoichiometry under global change. *Oikos*, 119, 737–740.

Urabe, J. & Sterner, R.W. (1996). Regulation of herbivore growth by the balance of light and nutrients. *Proc. Natl. Acad. Sci.*, 93, 8465–8469.

## V

- Van, T.K., Haller, W.T. & Bowes, G. (1976). Comparison of the Photosynthetic Characteristics of Three Submersed Aquatic Plants. *PLANT Physiol.*, 58, 761–768.
- Vandvik, V., Heegaard, E., Måren, I.E. & Arrestad, P.A. (2005). Managing heterogeneity: The importance of grazing and environmental variation on post-fire succession in heathlands. *J. Appl. Ecol.*, 42, 139–149.
- Vanni, M.J. (2002). Nutrient Cycling by Animals in Freshwater Ecosystems. *Annu. Rev. Ecol. Syst.*, 33, 341–370.
- Vasseur, C., Joannon, A., Aviron, S., Burel, F., Meynard, J.M. & Baudry, J. (2013). The cropping systems mosaic: How does the hidden heterogeneity of agricultural landscapes drive arthropod populations? *Agric. Ecosyst. Environ.*, 166, 3–14.
- Velthuis, M., van Deelen, E., van Donk, E., Zhang, P. & Bakker, E.S. (2017a). Impact of Temperature and Nutrients on Carbon: Nutrient Tissue Stoichiometry of Submerged Aquatic Plants: An Experiment and Meta-Analysis. *Front. Plant Sci.*, 8, 1–11.
- Velthuis, M., De Senerpont Domis, L.N., Frenken, T., Stephan, S., Kazanjian, G., Aben, R., et al. (2017b). Warming advances top-down control and reduces producer biomass in a freshwater plankton community. *Ecosphere*, 8.
- Venter, O., Fuller, R.A., Segan, D.B., Carwardine, J., Brooks, T., Butchart, S.H.M., et al. (2014). Targeting Global Protected Area Expansion for Imperiled Biodiversity. *PLoS Biol.*, 12.
- Ventura, M., Liboriussen, L., Lauridsen, T., Søndergaard, M., Søndergaard, M. & Jeppesen, E. (2008). Effects of increased temperature and nutrient enrichment on the stoichiometry of primary producers and consumers in temperate shallow lakes. *Freshw. Biol.*, 53, 1434–1452.
- Veraart, A.J., Dimitrov, M.R., Schrier-Uijl, A.P., Smidt, H. & de Klein, J.J.M. (2017). Abundance, Activity and Community Structure of Denitrifiers in Drainage Ditches in Relation to Sediment Characteristics, Vegetation and Land-Use. *Ecosystems*, 20, 928–943.
- Veraart, A.J., de Klein, J.J.M. & Scheffer, M. (2011). Warming can boost denitrification disproportionately due to altered oxygen dynamics. *PLoS One*, 6, 2–7.
- Veraart, A.J., Steenbergh, A.K., Ho, A., Kim, S.Y. & Bodelier, P.L.E. (2015). Beyond nitrogen: The importance of phosphorus for CH<sub>4</sub> oxidation in soils and sediments. *Geoderma*, 259–260, 337–346.
- Verdonschot, P.F.M. & Higler, L.W.G. (1989). Macroinvertebrates in Dutch ditches: A typological characterization and the status of the Demmerik ditches. *Hydrobiol. Bull.*, 23, 135–142.
- Verdonschot, R.C.M., Keizer-vlek, H.E. & Verdonschot, P.F.M. (2011). Biodiversity value of agricultural drainage ditches: A comparative analysis of the aquatic invertebrate fauna of ditches and small lakes. *Aquat. Conserv. Mar. Freshw. Ecosyst.*, 21, 715–727.
- Verhelst, P., Reubens, J., Pauwels, I., Buysse, D., Aelterman, B., Van Hoey, S., et al. (2018). Movement behaviour of large female yellow European eel (*Anguilla anguilla* L.) in a freshwater polder area. *Ecol. Freshw. Fish*, 27, 471–480.
- Verhofstad, M.J.J.M. & Bakker, E.S. (2019). Classifying nuisance submerged vegetation depending on ecosystem services. *Limnology*, 20, 55–68.
- Verhulst, J., Kleijn, D., Loonen, W., Berendse, F. & Smit, C. (2011). Seasonal distribution of meadow birds in relation to in-field heterogeneity and management. *Agric. Ecosyst. Environ.*, 142, 161–166.
- Vermaat, J.E., Goosen, H. & Omtzigt, N. (2007). Do biodiversity patterns in Dutch wetland complexes relate to variation in urbanisation, intensity of agricultural land use or fragmentation? *Biodivers. Conserv.*, 16, 3585–3595.
- Vermaat, J.E. & Hellmann, F. (2010). Covariance in water- and nutrient budgets of Dutch peat polders: What governs nutrient retention? *Biogeochemistry*, 99, 109–126.
- de Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., et al. (2012). Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecol. Lett.*, 15, 1230–1239.

## W

- Waagen, G., van Oosterhout, F., Douglas, G. & Lürling, M. (2016). Geo-engineering experiments in two urban ponds to control eutrophication. *Water Res.*, 97, 69–82.

- van de Waal, D.B., Boersma, M., Van De Waal, D.B. & Boersma, M. (2012). Ecological stoichiometry in aquatic ecosystems. *EoLss*, 1–37.
- van de Waal, D.B., Verschoor, A.M., Verspagen, J.M.H., Donk, E. Van, Huisman, J., Van De Waal, D.B., et al.(2010). Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. *Front. Ecol. Environ.*, 8, 145–152.
- Wade, P.M. (1993). The influence of vegetation pre-dredging on the post-dredging community. *J. Aquat. Plant Manag.*, 31, 141–144.
- Wakelin, S.A., Barratt, B.I.P., Gerard, E., Gregg, A.L., Brodie, E.L., Andersen, G.L., et al. (2013). Shifts in the phylogenetic structure and functional capacity of soil microbial communities follow alteration of native tussock grassland ecosystems. *Soil Biol. Biochem.*, 57, 675–682.
- Waldrop, M.P., Holloway, J.M., Smith, D.B., Goldhaber, M.B., Drenovsky, R.E., Scow, K.M., et al. (2017). The interacting roles of climate, soils, and plant production on soil microbial communities at a continental scale. *Ecology*, 98, 1957–1967.
- Walker, L.R., Zarin, D.J., Fether, N., Myster, R.W. & Johnson, A.H. (1996). Ecosystem Development and Plant Succession on Landslides in the Caribbean. *Biotropica*, 28, 566–576.
- Wallington, T.J., Hobbs, R.J. & Moore, S.A. (2005). Implications of Current Ecological Thinking for Biodiversity Conservation: a Review of the Salient Issues. *Ecol. Soc.*, 10, art15.
- Walsh, J.R., Carpenter, S.R. & Vander Zanden, M.J. (2016). Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proc. Natl. Acad. Sci.*, 113, 4081–4085.
- Walters, C.J. & Hilborn, R. (1978). Ecological Optimization and Adaptive Management. *Annu. Rev. Ecol. Syst.*, 9, 157–188.
- Wang, H., Sterner, R.W. & Elser, J.J. (2012). On the “ strict homeostasis ” assumption in ecological stoichiometry. *Ecol. Modell.*, 243, 81–88.
- Ward, J. V., Tockner, K., Arscott, D.B. & Claret, C. (2002). Riverine landscape diversity. *Freshw. Biol.*, 47, 517–539.
- Watson, A.M. & Ormerod, S.J. (2004). The microdistribution of three uncommon freshwater gastropods in the drainage ditches of British grazing marshes. *Aquat. Conserv. Mar. Freshw. Ecosyst.*, 14, 221–236.
- Watson, S.J., Taylor, R.S., Nimmo, D.G., Kelly, L.T., Clarke, M.F. & Bennett, A.F. (2012). The influence of unburnt patches and distance from refuges on post-fire bird communities. *Anim. Conserv.*, 15, 499–507.
- Webb, C.T., Hoeting, J.A., Ames, G.M., Pyne, M.I. & LeRoy Poff, N. (2010). A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecol. Lett.*, 13, 267–283.
- Webster, J.R., Newbold, J.D. & Lin, L. (2016). *Chapter 5—Nutrient Spiraling and Transport in Streams: The Importance of In-Stream Biological Processes to Nutrient Dynamics in Streams*. Stream Ecosyst. A Chang. Environ.
- Weedon, J.T., Kowalchuk, G.A., Aerts, R., Freriks, S., R??ling, W.F.M. & van Bodegom, P.M. (2017). Compositional stability of the bacterial community in a climate-sensitive Sub-Arctic Peatland. *Front. Microbiol.*, 8, 1–11.
- Werner, E.E., Yurewicz, K.L., Skelly, D.K. & Relyea, R.A. (2007). Turnover in an amphibian metacommunity: The role of local and regional factors. *Oikos*, 116, 1713–1725.
- Wersal, R.M. & Madsen, J.D. (2011). Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.) Verdc. *Hydrobiologia*, 665, 93–105.
- Westfall, P.H. (1997). Multiple Testing of General Contrasts Using Logical Constraints and Correlations. *J. Am. Stat. Assoc.*, 92, 299–306.
- Whatley, M.H., van Loon, E.E., van Dam, H., Vonk, J.A., van der Geest, H.G. & Admiraal, W. (2014a). Macrophyte loss drives decadal change in benthic invertebrates in peatland drainage ditches. *Freshw. Biol.*, 59, 114–126.
- Whatley, M.H., van Loon, E.E., Vonk, J.A., van der Geest, H.G. & Admiraal, W. (2014b). The role of emergent vegetation in structuring aquatic insect communities in peatland drainage ditches. *Aquat. Ecol.*, 48, 267–283.
- White, D.C., Davis, W.M., Nickels, J.S., King, J.D. & Bobbie, R.J. (1979). *Oecologia. Oecologica*, 40, 51–62.

- Whittaker, A. (1960). Vegetation of the Siskiyou Mountains , Oregon and California. *Ecol. Monogr.*, 30, 279–338.
- Whittaker, R.J. (1970). *Communities and Ecosystems*. 1st edn. Macmillan, New York, NY.
- Whittaker, R.J., Willis, K.J. & Field, R. (2001). Scale and species richness: towards a general, hierarchical theory of species diversity. *J. Biogeogr.*, 28, 453–470.
- Wiens, J.A. (1989). Spatial Scaling in Ecology. *Funct. Ecol.*, 3, 385.
- Williams, B.K. (2011). Adaptive management of natural resources-framework and issues. *J. Environ. Manage.*, 92, 1346–1353.
- Williams, P., Whitfield, M., Biggs, J., Bray, S., Fox, G., Nicolet, P., et al. (2003). Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. *Biol. Conserv.*, 115, 329–341.
- Winfree, R., Fox, J.W., Williams, N.M., Reilly, J.R. & Cariveau, D.P. (2015). Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. *Ecol. Lett.*, 18, 626–635.
- Withers, P.J.A., Neal, C., Jarvie, H.P. & Doody, D.G. (2014). Agriculture and eutrophication: Where do we go from here? *Sustain.*, 6, 5853–5875.
- Wolf, J., Beusen, A.H.W., Groenendijk, P., Kroon, T., Rötter, R. & Van Zeijts, H. (2003). The integrated modeling system STONE for calculating nutrient emissions from agriculture in the Netherlands. *Environ. Model. Softw.*, 18, 597–617.
- Wolf, J., Broeke, M.J.D.H. Ten & Rötter, R. (2005). Simulation of nitrogen leaching in sandy soils in the Netherlands with the ANIMO model and the integrated modelling system STONE. *Agric. Ecosyst. Environ.*, 105, 523–540.
- Wood, K.A., O'Hare, M.T., McDonald, C., Searle, K.R., Daunt, F. & Stillman, R.A. (2016). Herbivore regulation of plant abundance in aquatic ecosystems. *Biol. Rev.*, 92, 1128–1141.
- Wootton, J.T. (2002). Mechanisms of successional dynamics: Consumers and the rise and fall of species dominance. *Ecol. Res.*, 17, 249–260.
- Wootton, J.T. (2004). Markov chain models predict the consequences of experimental extinctions. *Ecol. Lett.*, 7, 653–660.
- Wu, Z. & Yu, D. (2004). The effects of competition on growth and biomass allocation in *Nymphaoides peltata* (Gmel.) O. Kuntze growing in microcosm. *Hydrobiologia*, 527, 241–250.
- X**
- Xia, C., Yu, D., Wang, Z. & Xie, D. (2014). Stoichiometry patterns of leaf carbon, nitrogen and phosphorous in aquatic macrophytes in eastern China. *Ecol. Eng.*, 70, 406–413.
- Xie, Y., An, S. & Wu, B. (2005). Resource allocation in the submerged plant *Vallisneria natans* related to sediment type, rather than water-column nutrients. *Freshw. Biol.*, 50, 391–402.
- Xing, W., Wu, H., Shi, Q., Hao, B., Liu, H., Wang, Z., et al. (2015). Multielement stoichiometry of submerged macrophytes across Yunnan plateau lakes (China). *Sci. Rep.*, 5.
- Y**
- Yalcin, S. & Leroux, S.J. (2018). An empirical test of the relative and combined effects of land-cover and climate change on local colonization and extinction. *Glob. Chang. Biol.*, 24, 3849–3861.
- Yao, H., Campbell, C.D. & Qiao, X. (2011). Soil pH controls nitrification and carbon substrate utilization more than urea or charcoal in some highly acidic soils. *Biol. Fertil. Soils*, 47, 515–522.
- Yao, H., Chapman, S.J., Thornton, B. & Paterson, E. (2015). <sup>13</sup>C PLFAs: a key to open the soil microbial black box? *Plant Soil*, 392, 3–15.
- Yergeau, E., Bezemer, T.M., Hedlund, K., Mortimer, S.R., Kowalchuk, G.A. & van der Putten, W.H. (2010). Influences of space, soil, nematodes and plants on microbial community composition of chalk grassland soils. *Environ. Microbiol.*, 12, 2096–2106.
- Z**
- Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D. & Tilman, D. (2003). Plant Diversity , Soil Microbial Communities , and Ecosystem Function: Are There Any Links? *Ecology*, 84, 2042–2050.

- Zhang, B., Liang, C., He, H. & Zhang, X. (2013). Variations in Soil Microbial Communities and Residues Along an Altitude Gradient on the Northern Slope of Changbai Mountain, China. *PLoS One*, 8.
- Zhang, P., Bakker, E.S., Zhang, M. & Xu, J. (2016). Effects of warming on *Potamogeton crispus* growth and tissue stoichiometry in the growing season. *Aquat. Bot.*, 128, 13–17.
- Zhang, P., van Den Berg, R.F., van Leeuwen, C.H.A., Blonk, B.A. & Bakker, E.S. (2018). Aquatic omnivores shift their trophic position towards increased plant consumption as plant stoichiometry becomes more similar to their body stoichiometry. *PLoS One*, 13, 1–13.
- van Zuidam, J.P. (2013). *Macrophytes in drainage ditches: functioning and perspectives for recovery*.
- van Zuidam, J.P. & Peeters, E.T. (2013). Occurrence of macrophyte monocultures in drainage ditches relates to phosphorus in both sediment and water. *Springerplus*, 2, 564.
- van Zuidam, J.P., Raaphorst, E.P. & Peeters, E.T.H.M. (2012). The Role of Propagule Banks from Drainage Ditches Dominated by Free-Floating or Submerged Plants in Vegetation Restoration. *Restor. Ecol.*, 20, 416–425.



S

## **Summary (*English*)**

Biodiversity loss is happening at an unprecedented rate, with current extinction rates estimated to be over a thousand times higher than natural background extinction rates. This loss of global diversity is caused by loss of small scale biotic heterogeneity, which leads to a decrease in diversity at the regional and landscape scale. Loss of diversity can have profound impacts on the functioning of ecosystems and may directly affect a wide range of ecosystem services key to human wellbeing. However, conserving and managing diversity is challenging, especially in intensively used agricultural landscapes.

The iconic Dutch ditch networks in polder landscapes are a prime example of aquatic ecosystems where biodiversity and ecological integrity are under threat because of intense agricultural land use. With over 300.000 kilometres of these canalised drainage and irrigation water systems in the Netherlands, they constitute a large part of the fresh water surface of the country. Due to their heavily connected nature, conservation and management of biodiversity may benefit from a more landscape or regional perspective on biodiversity. Within this thesis I studied the biodiversity (vegetation, zooplankton, microbes) and environmental quality of 21 polder landscapes in a region in the west of the Netherlands. Previous research had shown that there are large differences between communities of ditch reaches in a landscape (i.e. high  $\beta$ -diversity). My work in this thesis (**Chapter 2**, **Chapter 3**) confirmed the high dissimilarity in community composition between ditch reaches across different species groups. Furthermore, I show that at different polder landscapes may also harbour different communities (**Chapter 2**) and that complementarity between landscapes exists.

Intensity of land use has clear relations with the nutrient runoff to ditch banks and ditches. Hence, it is not unsurprising that we found land use and nutrient loading and their derivatives (e.g. water and sediment nutrients) to be important predictors of biodiversity across levels of spatial scale (local to regional) and across species groups (**Chapter 2 and 3**). Eutrophication of aquatic systems can lead to changing nutrient incorporation in stoichiometrically flexible primary producers (primarily plants in the context of ditches). In **chapter 4** I illustrate that aquatic plants are partly flexible in their nutrient incorporation and tissue nutrient content responds strongly to environmental conditions. I also showed that different plants may exhibit a different tissue elemental content under similar environmental nutrient conditions.

In contrast to primary producers, consumers tend to be largely homeostatic in their elemental composition. Mismatches in the elemental composition of producers and their consumers can have large impacts on consumer community structure and functioning. In **chapter 5** I study if and how such elemental mismatches impact consumer community composition using a phytoplankton-zooplankton community as a model system in outdoor mesocosms. Here I showed that consumer communities

where able to cope with mismatches in phytoplankton food quality through both shifts in species composition and intra-specific elemental plasticity. Extrapolating these results to field conditions, they suggest that changing nutrient availability due to land use changes may have impacts on consumer biodiversity and thereby ecosystem functioning. Also, producer nutrient enrichment led to an enrichment of sedimenting detrital matter, which in field situations would lead to an enriched sediment layer.

Sediment enrichment is an important part of the natural succession of vegetation in ditch systems. Through regular management (i.e. dredging, mowing) succession in ditches is reset, allowing for the redevelopment of successional stages and their associated communities. I examined the impact of successional stage heterogeneity on the landscape-wide biodiversity (**Chapter 6**). As may be expected, allowing for many successional stages to exist in space throughout the landscape is beneficial for overall diversity of a landscape, as successional stages were found to be partly complementary to one another. Especially the reset of late successional stages was found to be important for maintaining landscape-wide diversity.

While nutrient enrichment may be part of natural succession, excessive enrichment may cause a destabilization of the local ditch system and lead to dominance of free floating plants (e.g. *Lemna sp.*). Intensive land use is often associated with high nutrient loading to ditch systems, nutrients that (when not retained by plants) may travel through the landscape through water flows. In **chapter 7** I used a spatially explicit ecological simulation model to examine the effects of land-use configurations on functional vegetation group dominance in a polder landscape. Results indicated that the location of intensively and extensively used fields in the landscape can strongly impact what vegetation groups proliferate and where. Furthermore, the model helps to inform on the effort needed in reducing nutrient loading to improve ecological quality of the ditch system. While this model is restrained to functional groups, I present an outlook on how to use such models as ways to predict future habitat conditions that may affect species persistence under changing pressures and management (**General discussion**).

Finally, I discuss the importance of maintaining successional stage diversity and its heterogeneity in nutrient status, while avoiding state shifts to free-floating plant dominated conditions which are also linked to nutrient supply. I explain how biological feedbacks on nutrient cycling on a local scale can have landscape level impacts on biodiversity of different trophic levels. Here I note that integral assessment of biodiversity across groups is required, as management actions aimed at conserving or promoting specific organism groups may not necessarily benefit and possibly even negatively impact other organism groups. To do so a joint end goal for biodiversity in these Dutch polder landscapes is required that is shared among stakeholders.

Development of quantitative tools that can assess possible impacts of management actions and changing pressures across spatial scales is key to facilitate decision making. All in all, this thesis contributes to maximisation of biodiversity in Dutch ditch systems across scales and functional groups through understanding of drivers of community composition and diversity and development of methodological approaches to analyse potential management scenarios.

S

## **Samenvatting (*Nederlands*)**

De biodiversiteit van de wereld gaat momenteel met sprongen achteruit, waarbij soorten naar schatting meer dan duizend keer sneller uitsterven dan natuurlijk is. Dit verlies van wereldwijde diversiteit wordt veroorzaakt door verlies van kleinschalige heterogeniteit in levensgemeenschappen. Dit leidt tot een afname van diversiteit op een regionale- en landschapsschaal. Verlies van biodiversiteit kan grote gevolgen hebben voor het functioneren van ecosystemen en kan direct van invloed zijn op een breed scala aan ecosysteemdiensten die van cruciaal belang zijn voor het menselijk welzijn. Het behouden en beheren van deze biodiversiteit is echter een uitdaging, vooral in intensief gebruikte landbouwgebieden.

De iconische Nederlandse slootnetwerken in polderlandschappen zijn een goed voorbeeld van aquatische ecosystemen waar biodiversiteit en ecologische integriteit bedreigd worden door intensief landgebruik. Met meer dan 300.000 kilometer van deze gekanaliseerde drainage- en irrigatiewatersystemen in Nederland, vormen ze een groot deel van het zoetwateroppervlak van het land. Vanwege hun sterk verbonden aard kan behoud en beheer van biodiversiteit baat hebben bij een meer landschaps- of regionaal perspectief op biodiversiteit. In dit proefschrift bestudeerde ik de biodiversiteit van vegetatie, zooplankton en microben en de omgevingskwaliteit van 21 polderlandschappen in een regio in het westen van Nederland. Eerder onderzoek had aangetoond dat er grote verschillen zijn tussen levensgemeenschappen van sloten binnen een landschap (hoge  $\beta$ -diversiteit). Mijn werk in dit proefschrift (**Hoofdstuk 2, Hoofdstuk 3**) bevestigde de grote verscheidenheid in gemeenschapssamenstelling tussen sloten. Dit patroon herhaalde zich voor de verschillende soortgroepen die ik heb bestudeerd. Verder laat ik zien dat in verschillende polderlandschappen ook verschillende gemeenschappen kunnen zijn gehuisvest (**Hoofdstuk 2**) en dat er complementariteit in soortensamenstelling tussen deze landschappen bestaat.

Intensiteit van het landgebruik heeft duidelijke relaties met de afvoer van voedingsstoffen naar slotenkanten en sloten. Daarom is het niet verrassend dat we landgebruik en nutriëntenbelasting en hun afgeleiden hiervan (bijv. beschikbare voedingsstoffen in water en sediment) als belangrijke voorspellers van biodiversiteit terug vinden op verschillende niveaus van ruimtelijke schaal (lokaal tot regionaal) en voor verschillende soortgroepen (**Hoofdstuk 2 en 3**). Eutrofiëring (d.w.z. verusting) van aquatische systemen kan leiden tot veranderende nutriëntenopname in en een veranderende biochemische samenstelling in primaire producenten (voornamelijk planten in de context van sloten). In **hoofdstuk 4** illustreer ik dat waterplanten deels flexibel zijn in de opname van voedingsstoffen en het voedingsstoffengehalte van de weefsels sterk reageert op de omgevingsomstandigheden. Ik heb ook laten zien dat plantensoorten onder vergelijkbare nutriëntencondities in hun leefomgeving desondanks sterk verschillend

kunnen zijn in hun interne elementaire samenstelling (hoeveelheden nutriënten per eenheid plantenmateriaal).

In tegenstelling tot primaire producenten hebben consumenten de neiging om grotendeels gelijk te blijven in hun elementaire samenstelling bij veranderend nutriëntenaanbod in de leefomgeving (homeostase). Een discrepantie in de samenstelling van producenten (voedsel) en hun consumenten (de eter) kunnen grote gevolgen hebben voor de levensgemeenschappen van consumenten. In **hoofdstuk 5** onderzoek ik of en hoe een verhoogde koolstof tot fosfor verhouding van fytoplankton invloed heeft op de gemeenschapssamenstelling van zooplankton. Hier liet ik zien dat consumenten in staat zijn om met een veranderende koolstof:fosfor verhouding om te gaan doordat de gemeenschap verschuift in soortensamenstelling. Daarnaast passen de individuele soorten ook hun eigen elementaire ratio van koolstof tot fosfor aan in respons op de verandering. Als deze resultaten worden geëxtrapoleerd naar het veld, suggereren ze dat het veranderen van de beschikbaarheid van nutriënten als gevolg van veranderingen in landgebruik van invloed kan zijn op de biodiversiteit van de consument en daarmee op het functioneren van het ecosysteem. Daarnaast leidde de verrijking met nutriënten tot een nutriëntrijkere producent wat leidt tot een verrijking van detritus, wat in veldsituaties zou leiden tot een rijkere sedimentlaag (bagger).

Sedimentverrijking is een belangrijk onderdeel van de natuurlijke opeenvolging van vegetatie in slootsystemen. Door regelmatig beheer (dat wil zeggen baggeren, maaien) wordt de ecologische successie in sloten teruggezet, waardoor de herontwikkeling van successiestadia en de bijbehorende gemeenschappen mogelijk wordt. In **hoofdstuk 6** heb ik onderzocht in hoeverre heterogeniteit van successiestadia in sloten de biodiversiteit van het gehele slotenlandschap beïnvloed. Een grote ruimtelijke diversiteit aan successiestadia had, zoals verwacht, een positieve invloed op de biodiversiteit van het landschap. Dit komt doordat successiestatie gedeeltelijk complementair zijn in hun soortensamenstelling. Vooral het periodiek herstarten van late successiestadia was belangrijk voor het behoud van landschapswijde diversiteit, aangezien deze latere stadia minder complementair zijn dan vroegere successiestadia.

In **hoofdstuk 7** heb ik met behulp van een ecologisch simulatiemodel de ruimtelijke implicaties van landgebruiksveranderingen op de dominantie van verschillende functionele vegetatiegroepen onderzocht. Deze ruimtelijke computersimulaties tonen aan dat de locatie van intensief en minder intensief gebruikte velden in het landschap van grote invloed kan zijn op wat vegetatiegroepen prolifereren en waar. Bovendien helpt het model om in te schatten waar het reduceren van voedingsstoffen de grootste ecologische kwaliteitsverbetering kan bewerkstelligen in het slootsysteem. Hoewel dit model beperkt is tot functionele groepen van planten, presenteert ik een doorkijk naar het gebruik van dergelijke modellen om toekomstige habitatomstandigheden te

voorspellen die van invloed kunnen zijn op het voorkomen van soorten in een snel veranderende wereld (**algemene discussie**).

Ten slotte bespreek ik het belang van behoud van de diversiteit aan successiestadia en de daarmee samenhangende heterogeniteit in rijkdom in voedingstoffen. Echter, een omslag naar een ecologisch slecht ontwikkeld systeem bedekt met een laag kroos is ook sterk gelinkt aan de toevoer van voedingsstoffen. Ik leg uit hoe biologische feedback op nutriëntenkringlopen op lokale schaal impact op het landschap kan hebben op de biodiversiteit van verschillende trofische niveaus. Hier merk ik op dat een integrale beoordeling van biodiversiteit tussen groepen nodig is, omdat managementacties gericht op het behoud of de verbetering van specifieke organismegroepen niet noodzakelijkerwijs profiteren en mogelijk zelfs een negatieve invloed hebben op andere organismegroepen. Om dit te bereiken is een gezamenlijk einddoel voor biodiversiteit in deze Nederlandse polderlandschappen noodzakelijk, waarbij stakeholder betrokkenheid en een gedeelde visie een centraal thema zijn. Ontwikkeling van kwantitatieve instrumenten en tools die de impact van veranderend beheer, klimaat en landgebruik op verschillende schaalniveaus kunnen voorspellen zijn van cruciaal belang om de besluitvorming te ondersteunen. Afsluitend, de bijdrage van dit proefschrift aan het maximaliseren van de biodiversiteit van Nederlandse slootstelsystemen op verschillende schaalniveaus kan gevonden worden in het identificeren van verschillende belangrijke factoren die gemeenschapssamenstelling en biodiversiteit beïnvloeden en in het ontwikkelen van methodologische benaderingen die beheer en beleid kunnen ondersteunen.

# A

## Acknowledgements

This thesis is the accumulation of many years of work. At times it was highly exciting, at other times thoroughly exhausting. More than anything though, it has been one of the most educational experiences of my life, both professionally and personally. Such roads are seldom travelled alone and this is the place for me to express my thanks, admirations, appreciations and whatnot to those involved over the years. But first, as a disclaimer, seeing I am bound to forget someone (with thanks to **Alena** for the quote):

**"I don't know half of you half as well as I should like; and I like less than half of you half as well as you deserve"**

--*J.R.R. Tolkien*--

With that out of the way, I'll start with my co-promotor and promotor, Steven and Ellen. **Steven**, I have learned a lot from working with you that I will carry with me for the rest of my life. I appreciate your critical take on science and our discussions on the broader topics of science in general. I have learned much from you in these years that I will carry with me, always. Thank you for that. **Ellen**, thank you for all the support you've given me over these years. I feel we've always managed to have a very open and honest communication, which was very nice. Thank you also for the freedom you've given me to explore my own interests in education and science.

Next, of course, my co-authors. Thank you all for your ideas, support, interest and the fruitful discussions on the paper(s) we've written together. That many more may follow in productive collaboration. Special mention goes out to two of my senior scientist co-authors at NIOO here: **Dedmer**, I've always been impressed by your energy and enthusiasm for science. It is both intoxicating (which is indeed a great tongue-in-cheek remark to make about a cyano-phile) and impressive. **Liesbeth**, your pragmatic approach to science combined with your inherent drive for nature have been and still are an inspiration. Also, of course my microbial partners: **Amber**, who would have thought that the 'koekblik' would lead to writing papers together. **Annelies**, thank you for your friendship and confidence in me. And lastly, **Michiel**, writing some of the papers of both our thesis's together has been a pleasure, thank you.

Thanks are also warranted to all of my many, many students, without whom a lot of this work (and a lot of the work thereafter) would never have been carried out. In order of their respective first emails to me: **Lilith, Karel, Maik, John, Paul, Roos, Tom, Lisa, Jeroen, Max, Judy, Marlies, Laura, Annegreet, Chiara, Daniela, Dominika, Marleen, Tirza, Sam, Nienke, Tian, Abby, Anissa, Lora** and **Ellen**. It was both a pleasure and an honour to supervise you all and I hope you learned a lot from your time with me. I know I learned a lot from my interaction with all of you.

Likewise, my thanks go out to all of the technical staff that has helped me out over the years. **Nico, Dennis, Eric, Suzanne and Michaela**, thanks for the help in the field, the lab and all sorts of assorted odd jobs that I have surely forgotten about but I am sure were there. You make a great team! Also, **Koos**, thank you for your help with the mesocosm experiment. **Peter**, thank you for all the early morning coffees, GIS conversations, the beers and BBQs. **Marta**, I had a great time with you in the field and continue to enjoy your company and our ongoing scientific cooperation, though we did deviate away from ditches a wee bit. **Edwin**, I am sure my macrophyte identification would not be half of what it is today without you, and I was both lucky and thankful that I had you along in my first field season. Of course, beside a great 'veldtechnieker' you are also an amazing friend who's company I continue to cherish.

This project would not have been possible without the cooperation of a lot of farmers, nature managers and land owners who allowed me to pass over, study their ditch and generally learn and talk about farming and ecological water quality. Beside these people, my stakeholder meetings have been invaluable in learning about and developing this work in directions that matched with management reality. **John**, your knowledge of Oukoop and hands on nature development was essential in starting up this project. **Bas, Maarten**, our trip to Berlin and surroundings was both hugely educational and all in all just great fun and really showed me the importance of hands on systems diagnosis, thank you for the opportunity!

Some of the members of my thesis committee have played a big part in my development as an aquatic ecologist. **Merel**, thank you for all the many inspiring discussions we've had along the way. **Jos**, looking back you've been instrumental in my scientific life choices. My first interest in the aquatic environment started in the second year of my Bachelor studies in your Wetlands course. My Bachelor thesis on the importance of small habitats, inspired by some of the work done on ponds, cemented that interest and led to a research project at Deltares during my Master under your supervision once again. It is a pleasure to also have you here for this next step in my scientific career. And of course **Judith**, your excursion with a lot of recently started PhDs into Westbroek was one of my early highlights at NIOO.

NIOO has always been a great place to work. It has constantly changed over the years but has always managed to maintain a good atmosphere. To all my NIOO colleagues, both past and present, thank you for the great times. When I started my PhD we had a great gang of PhDs at Aquatic Ecology that I will always remember fondly and would like to thank specifically for all the great coffee breaks, evenings with beer and pizza, birthdays at the zoo, happy lakes conferences and whatnot. **Bart**, my Biodiversity Works partner in crime, it was a pleasure. **Luuk**, I will cherish the memories of your 'cluster bombs' and will always value that you took the time to help me out with

learning the basics of PCDitch and SOBEK. **Jan**, from the first days of the 'Koekblík' to writing papers together, talking about life in general with some beers or whiskey, it has been and continues to be a true pleasure to count you among my friends. **Annette**, as they would say on Texel, Bed-dankt. From our early papers to many long discussions near the NIOO coffee machine, I have always been impressed by your creativity and structural thinking. I am happy to have you beside me for this occasion as my paronymph.

My fellow AKWAnauts (both past and present) also deserve special reference: **Laura**, it's been a pleasure to share an office with you over the last years and share part of the PhD experience. **Maggie**, you're bouncy personality is a pleasure to have in the team! **Daphne**, your caring and hard working attitude was a real blessing and I wish you all the best in your new job. **Nandini**, as our most recent recruit all I can say is, be yourself, you should be fine if you do. And of course **Lisette**, thank you for your faith in me without which I would not have been where I am today. Your confidence, support and advice has been instrumental in finishing my thesis. I value our widely diverging and usually eventually converging discussions. I hope to have many more years with close collaboration with you on a wide diversity of topics.

**Wolf**, thank you for introducing me to modelling, but more relevant perhaps to the social side of science. Our many talks about triangles, egos, spheres and other somewhat odd social concepts underlying our interactions in the work and occasionally non-work place have helped me to grow as a person. I value our cooperation together with **Manqi** and **Dianneke** and hope to continue under the careful gaze of **Kangi** for many more years.

This brings me to all the friends and family. **Akke**, thank you for proof-reading and all the squash games and diners over the years. **Julie**, *merci* for the wonderful cover design. **Mamma, Pappa**, thank you for always believing in me and inspiring me from a young age to be creative and develop myself. **Rik, Luyuan, Luna, Kai, Oma, Sonja, Albert, Vincent en de Oma's**, bedankt dat jullie er altijd voor me zijn. And of course the *Duvels*: **Anoeska, Edwin, Koen, Niek, Mara en Rascha**. You've always been there for me during these years, with advice, questions, beer, games, beer, saboteur, talks about science of course friendship. But more than anything thank you for being you, all of you.

Last, but definitely not least, **Mandy**, from our first paper together to the final layout and proof-reading work on this thesis you have always been here for me. Through the hard times, the wins, the tears and the laughter you were always there to talk to for advice, a smile, a kiss and a solution (even when I just wanted to whine about it all). I couldn't ask for a better partner in both science and life. Falafel!

# CV

## About the author



**Sven Teurlincx** was born on the 8<sup>th</sup> of December in Venlo, The Netherlands. He grew up in Panningen, a small town in the south of the Netherlands in a part that is most definitely not known for its polders or ditch systems. Nonetheless, nature and agriculture were there aplenty and early on Sven developed an interest in understanding the natural world.

After a successful finishing his VWO study in Panningen, Sven moved to Utrecht where he started his BSc in Biology at *Utrecht University*. Here he

came into contact with peatlands and ditch systems the second year of his studies in a course on Wetlands. After successfully completing his BSc in 2009, he started an MSc education at *Utrecht University* in Environmental Biology. Despite having a broad interest, water was a clear overarching theme in his studies. His first research project took place at Deltres, working on aquatic vegetation in side channels of the river Rhine. Here he developed an affinity for GIS and community compositional analysis. His second research project focused on submergence tolerance of plants at Utrecht University.

After completing his MSc with a cum laude distinction, a PhD position in Aquatic Ecology at *NIOO-KNAW* came along that caught his interest. During his PhD work Sven worked on spatial patterns of biodiversity in ditch systems in Dutch Polder landscapes under the supervision of Steven Declerck. The project had a strong focus on working with stakeholders, which Sven found to be very stimulating. During this project he also discovered an affinity for mechanistic ecosystem models as a way to understand and upscale the functioning of aquatic ecosystems. He currently works at *NIOO-KNAW* in the knowledge group *AKWa (Aquatisch Kenniscentrum Wageningen)*. Here he is able to combine interactions with stakeholders such as water- and nature managers with ecosystem level modelling and empirical research.

## Scientific publications

- Janssen, A.B.G., **Teurlincx, S.**, An, S., Janse, J.H., Paerl, H.W. & Mooij, W.M. (2014). Alternative stable states in large shallow lakes? *J. Great Lakes Res.*, 40, 813–826.
- Kuiper, J.J., Janse, J.H., **Teurlincx, S.**, Verhoeven, J.T.A. & Alkemade, R. (2014). The impact of river regulation on the biodiversity intactness of floodplain wetlands. *Wetl. Ecol. Manag.*, 22, 647–658.
- Mooij, W.M., Brederveld, R.J., de Klein, J.J.M., DeAngelis, D.L., Downing, A.S., Faber, M., ... **Teurlincx, S.**, ... et al. (2014). Serving many at once: How a database approach can create unity in dynamical ecosystem modelling. *Environ. Model. Softw.*, 61, 266–273.
- Janssen, A.B.G., Arhonditsis, G.B., Beusen, A., Bolding, K., Bruce, L., Bruggeman, J., ... **Teurlincx, S.**, ... et al. (2015). Exploring, exploiting and evolving diversity of aquatic ecosystem models: a community perspective. *Aquat. Ecol.*, 49, 513–548.
- van Gerven, L.P.A., Brederveld, R.J., de Klein, J.J.M., DeAngelis, D.L., Downing, A.S., Faber, M., ... **Teurlincx, S.**, ... et al. (2015). Advantages of concurrent use of multiple software frameworks in water quality modelling using a database approach. *Fundam. Appl. Limnol. / Arch. für Hydrobiol.*, 186, 5–20.
- Agtmaal, M. van, Straathof, A., Termorshuizen, A., **Teurlincx, S.**, Hundscheid, M., Ruyters, S., et al. (2017). Exploring the reservoir of potential fungal plant pathogens in agricultural soil. *Appl. Soil Ecol.*, 121, 152–160.
- Teurlincx, S.**, Velthuis, M., Seroka, D., Govaert, L., van Donk, E., Van de Waal, D.B., et al. (2017). Species sorting and stoichiometric plasticity control community C:P ratio of first-order aquatic consumers. *Ecol. Lett.*, 20, 751–760.
- Hilt, S., Alirangues Nuñez, M.M., Bakker, E.S., Blindow, I., Davidson, T.A., Gillefalk, M., ... **Teurlincx, S.**, ... et al. (2018). Response of Submerged Macrophyte Communities to External and Internal Restoration Measures in North Temperate Shallow Lakes. *Front. Plant Sci.*, 9.
- Mantzouki, E., Campbell, J., van Loon, E., Visser, P., Konstantinou, I., Antoniou, M., ... **Teurlincx, S.**, ... et al. (2018). A European Multi Lake Survey dataset of environmental variables, phytoplankton pigments and cyanotoxins. *Sci. Data*, 5, 180226.
- Mantzouki, E., Lürling, M., Fastner, J., de SenerpontDomis, L., Wilk-Woźniak, E., Koreivienė, J., ... **Teurlincx, S.**, ... et al. (2018). Temperature effects explain continental scale distribution of cyanobacterial toxins. *Toxins (Basel)*, 10, 156.
- Seelen, L.M.S., Flaim, G., Keuskamp, J., **Teurlincx, S.**, Font, R.A., Tolunay, D., et al. (2018). An affordable and reliable assessment of aquatic decomposition: Tailoring the Tea Bag Index to surface waters. *Water Res.*, 31–43.
- Teurlincx, S.**, Heijboer, A., Veraart, A.J., Kowalchuk, G.A. & Declerck, S.A.J. (2018). Local Functioning, Landscape Structuring: Drivers of Soil Microbial Community Structure and

Function in Peatlands. *Front. Microbiol.*, 9.

**Teurlincx, S.**, Verhofstad, M.J.J.M., Bakker, E.S. &Declerck, S.A.J. (2018). Managing Successional Stage Heterogeneity to Maximize Landscape-Wide Biodiversity of Aquatic Vegetation in Ditch Networks. *Front. Plant Sci.*, 9, 1–33.

Janssen, A.B.G., **Teurlincx, S.**, Beusen, A.H.W., Huijbregts, M.A.J., Rost, J., Schipper, A.M., et al.(2019). PCLake+: A process-based ecological model to assess the trophic state of stratified and non-stratified freshwater lakes worldwide. *Ecol. Modell.*, 396, 23–32.

Janssen, A.B., Janse, J.H., Beusen, A.H., Chang, M., Harrison, J.A., Huttunen, I., ... **Teurlincx, S.**, ... et al. (2019). How to model algal blooms in any lake on earth. *Curr. Opin. Environ. Sustain.*, 36, 1–10.

Manfrin, A., **Teurlincx, S.**, Lorenz, A.W., Haase, P., Marttila, M., Syrjänen, J.T., et al. (2019). Effect of river restoration on life-history strategies in fish communities. *Sci. Total Environ.*

**Teurlincx, S.**, Kuiper, J.J., Hoevenaar, E.C., Lurding, M., Brederveld, R.J., Veraart, A.J., et al.(2019). Towards restoring urban waters: understanding the main pressures. *Curr. Opin. Environ. Sustain.*, 36, 49–58.

Mooij, W.M., van Wijk, D., Beusen, A.H., Brederveld, R.J., Chang, M., Cobben, M.M., ... **Teurlincx, S.** (2019). Modeling water quality in the Anthropocene: directions for the next-generation aquatic ecosystem models. *Curr. Opin. Environ. Sustain.*, 36, 85-95.