Biodiversity and ecosystem functioning in created agricultural wetlands

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2009

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Cover illustration- Thomas Prade and Geraldine Thiere
Chapter photos and illustrations- Geraldine Thiere

Printed by E-huset tryck, Lund

ISBN: 978-91-7105-295-7

Biodiversity and ecosystem functioning in created agricultural wetlands

Geraldine Thiere

AKADEMISK AVHANDLING
som för avläggande av filosofie doktorsexamen vid Naturvetenskapliga fakulteten vid Lunds Universitet kommer att offentligen försvaras i Blå Hallen, Ekologihuset, Sölvegatan 37, Lund, fredagen den 24 april, klockan 10.00.

Fakultetens opponent: Professor Jos T.A. Verhoeven, Section of Landscape Ecology, Institute of Environmental Biology, Department of Biology, Utrecht University, The Netherlands

Avhandlingen kommer att försvaras på engelska.

Dissertation
Lund 2009

Organization	Document name
LUND UNIVERSITY Department of Ecology, Limnology Ecology Building SE-223 62 Lund Sweden	DOCTORAL DISSERTATION
	Date of issue April 24, 2009
	Sponsoring organization Wetland Research Centre, School of Business and Engineering, Halmstad University, Box 823 SE-30118 Halmstad, Sweden
Author(s) Geraldine Thiere	

Title and subtitle

Biodiversity and ecosystem functioning in created agricultural wetlands

Abstract. Wetland creation at large, regional scales is implemented as a measure to abate the biodiversity loss in agricultural landscapes and the eutrophication of watersheds and coastal areas by non-point source nutrient pollution (mainly nitrogen). The consequences of creating many new wetlands for biodiversity conservation and nutrient retention (ecosystem functioning) in agricultural landscapes are still relatively unknown, both on local (per wetland) and regional (per landscape) scales. In Sweden, wetland creation has progressed already since the 1990s, and by now larger numbers of created wetlands are present, mainly in the intensively farmed landscapes of southwestern Sweden. This thesis aimed to investigate the following aspects in these systems: (i) their large-scale effects on biodiversity, (ii) their functional diversity of bacterial denitrifiers, (iii) the abiotic and biotic influences on wetland ecosystem functioning, (iv) the potential for biodiversity-function links, and (v) the potential for functional links and joint functioning.

(i) Created wetlands hosted diverse assemblages of macroinvertebrates and plants. They maintained a similar composition and diversity as natural ponds in agricultural landscapes. The environmental conditions per wetland did hardly affect macroinvertebrate and plant assemblages, and the prerequisites for nutrient retention did neither. In landscapes were wetland creation efforts had increased the total density of small water bodies by more than 30%, macroinvertebrate diversity of created wetlands was facilitated on both local and regional scales. (ii) Diverse communities of denitrifying bacteria with the capacity for conducting different denitrification steps (functional types) were present in all investigated wetlands. The richness of denitrifying bacteria communities was affected by nitrate concentration and hydraulic loading rate, which may potentially be relevant for the nitrogen retention function of created wetlands. The diversity across different functional types of bacterial denitrifiers increased with nitrate concentration. (iii) Both abiotic and biotic factors influenced ecosystem functions of created wetlands. Variation in nitrogen retention was associated to nitrate load, but even to vegetation parameters. In wetlands with constant nitrate load, planted emergent vegetation facilitated nitrogen retention compared to other vegetation types. In wetlands with variable loads, nitrogen retention was facilitated if nitrate load was high and many different vegetation types were present; nitrogen load could explain the majority of the variation in nitrogen retention compared to vegetation parameters. Phosporus retention of created wetlands was best explained by vegetation parameters. Litter decomposition was inhibited at high nitrate to phosphorus ratios. Methane production increased with age and decreased with plant cover. (iv) Biodiversity may facilitate wetland ecosystem functions, particularly in dynamic wetland ecosystems. Nitrogen retention increased with vegetation type diversity, phosphorus retention capacity with plant richness, and litter decomposition with macroinvertebrate diversity. (v) Created wetlands have the capacity of sustaining several parallel ecosystem services. Some wetland functions were coupled; nitrogen retention increased with fast litter decomposition. On the other hand, methane emission and nitrogen retention were independent of each other, as were nitrogen and phosphorus retention.

In conclusion, created wetlands have the potential to at least partly abate the lost biodiversity and multifunctionality caused by the past extensive destruction of natural wetlands in agricultural landscapes.

Key words:
constructed ponds; eutrophication abatement; biodiversity conservation; functional diversity; macroinvertebrates; plants; bacterial denitrification; watershed scale; nitrogen removal; phosphorus retention

Classification system and/or index termes (if any):

Supplementary bibliographical information:	Language English
ISSN and key title:	ISBN 978-91-7105-295-7
Recipient's notes	Number of pages 145 / Price
	Security classification

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This thesis is based on the following papers:

Paper I is reprinted with permission from the publisher.

My contribution to the papers

I

I planned the wetland surveys together with Stefan Weisner (my supervisor). I conducted the macroinvertebrate sampling and identification with assistance of Göran Sahlén (Halmstad University). I and Susann Milenkovski (PhD student, Lund University) carried out the vegetation survey (in 36 wetlands). I compiled all data and carried out the analyses and interpretation of the data set. The final scope of the manuscript took shape during regular discussion meetings with the working group "Wetland Ecology & Biotechnology (WEB)", in which all coauthors are members. I wrote the manuscript, with contributions from the other authors.

II

I contributed to the study planning and sampling, the interpretation of data, and the development of the final scope of the manuscript as a member of the WEB group. I and S.M. carried out the vegetation and microbial biofilm surveys. S.M. analyzed all samples in the lab and compiled the microbial data. I compiled the other wetland data and carried out the analyses of the combined data set. S.M. wrote the manuscript in close cooperation with me and contributions from the other authors. This manuscript was included in the thesis of S.M.

III

I and S.W. contributed the data on N retention. Johanna Stadmark (Lund University) had the idea to this study and collected the methane samples. Together we developed the manuscript into its current form. I compiled and analyzed the data and wrote the paper in close cooperation with J.S. and S.W. This manuscript was included in the thesis of J.S.

IV

I planned and coordinated the biotic field sampling during the four study years. I conducted parts of the macroinvertebrate, and all of the plant identification. Chemical samples were taken and analyzed by Per Magnus Ehde (Halmstad University). S.W. designed the experimental setup of the experiment. I compiled the dataset and S.W. did the statistical analyses and wrote the paper in cooperation with me.

V

I and S.W. planned the study and sampling design. I coordinated and conducted most of the field work with assistance from undergraduate students. Chemical analyses were performed by Per Magnus Ehde (Halmstad University). I compiled and analyzed the data set. I wrote the manuscript with contributions from S.W.

BACKGROUND

Shortsighted decisions & wetland loss. The topic of my thesis (created wetlands, see definition Box 1) would be irrelevant if not for a common mistake in the history of mankind: shortsighted decisions. Such decisions do not consider the large-scale or long-term consequences of an action, but solely focus on the local-scale/short-term benefits.

An example of this is the past (and ongoing) destruction and reduction of natural wetland areas in many parts of the world. The primary aim, i.e. often the extension of areas suitable for agricultural production or other human needs, seemed to easily outweigh any potential benefits associated with the original wetland areas. As a consequence, regions with intensive agriculture have lost up to 90% of the historic wetlands (Finlayson & Spiers 1999; Mitsch & Gosselink 2000; Biggs *et al*. 2005) mainly during the last 200 years (Hoffmann *et al*. 2000). The excessive wetland destruction was realized at great financial expenses, and led to the installation of extensive drainage measures (e.g. pipe systems, river channeling, and groundwater level manipulation), effectively transforming wet ecosystems into productive agricultural, forested or urban land.

Box 1. Wetland definitions.

Mitsch & Gosselink (2000) define wetlands according to hydrology, physicochemical environment and prevalent biota: "*Wetlands are distinguished by the presence of water, either at the surface or within the root zone; they often have unique soil conditions (poorly aerated and/or water-saturated soil) that differ from adjacent uplands; wetlands support vegetation adapted to the wet conditions (hydrophytes) and, conversely, are characterized by an absence of flooding-intolerant vegetation*."

The Ramsar convention (www.ramsar.org) includes a wide variety of aquatic ecosystems in the term wetland: "*Wetlands are areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water not exceeding six metres depth*."

The wetlands described in this thesis are man-made, pond-like systems with an inlet and outlet (hence, connected to a watershed), which are located in landscapes with intensive agriculture and receive high nitrate concentrations.

The concept of wetland creation. Today, wetland functions are better understood (Mitsch & Gosselink 2000), and wetlands have finally established a reputation as one of the world's most productive ecosystem types providing services of invaluable ecological (e.g. biodiversity) and high economic value (e.g. nutrient retention, flood control, food production) (Costanza *et al*. 1997; Zedler 2005; Costanza *et al*. 2008). Consequently, wetland protection is now common in the industrialized world and wetlands of international importance are protected by the Ramsar convention (www.ramsar.org).

Apart from wetland protection, strategies to actively abate the loss of wetland ecosystem services involve wetland restoration and creation. Wetland restoration aims at restoring damaged sites which to some extent still serve as wetlands or have done so until rather recently. The concept of wetland creation (Fig 1) on the other hand, can include the establishment of wet areas from scratch, i.e. on land which has been under another type of usage for long periods of time.

Wetland creation is nowadays implemented at large spatial scales (Mitsch *et al*. 2001; Paludan *et al*. 2002; Zedler 2003; Hoffman & Baattrup-Pedersen 2007), and is financed by international (European Union) agri-environment schemes or national (e.g. Sweden, Denmark, USA) political entities. In Scandinavia and particularly Sweden, large-scale wetland creation was implemented early on, starting in the 1990s (Lindahl 1998). The national environmental objectives (www.

Fig 1. Created agricultural wetlands. (a) Illustration of the creation progress: After excavation, water is collected via an inlet (here: drainage pipe) slowly establishing a permanent water table. The wetland outlet discharges into a small agricultural stream. (b) A wetland three years after establishment.

miljomal.nu) state that 12,000 ha wetland area are to be created until 2010 (SJV 2000). Hence, by now large numbers of created wetlands are already established in Swedish agricultural landscapes and watersheds. The majority of these wetlands have been created in the intensively farmed landscapes stretching along the southwestern coast of Sweden (Scania/Halland). In these areas, historic landscape changes and habitat losses were drastic (up to 95% wetland losses; Krug 1993; Ihse 1995; Hoffmann *et al*. 2000) and today's nutrient export to the coast is nationally highest (www.ma.slu.se; Kyllmar 2006), as is the number of threatened species (Artdatabanken 2009).

Thesis scope. The background outlined above forms the base for research questions in the area of 'applied science', aimed to provide knowledge relevant for decision making, policy and management strategies for created agricultural wetlands. Applied research questions of this thesis deal with if and how created wetlands can compensate for the lost multifunctionality and biodiversity of natural wetlands.

Apart from that, the many small created water bodies appearing in the agricultural landscape may also serve as interesting model systems to answer questions of general ecological relevance, i.e. serving the purposes of 'fundamental science'. Wetland creation is an example of a large-scale manipulation of the agricultural management, providing an opportunity for landscape-scale experiments which are otherwise practically impossible to carry out (Herzog 2005). Such fundamental research questions deal with biodiversity and ecosystem functioning, their controls and interactions.

In my research on created agricultural wetlands, I tried to regard both applied and fundamental aspects. Further, I tried to avoid shortsighted recommendations, and I aimed to integrate several aspects and consequences of wetland creation instead. More specifically, I studied the following aspects in created wetlands:

- their large-scale effects on biodiversity (Papers I & II; unpublished data),
- their functional diversity of bacterial denitrifiers (Paper II),
- the abiotic and biotic influences on wetland ecosystem functioning (Papers III, IV & V),
- the potential for biodiversity-function links (Papers IV & V), and
- the potential for functional links and joint functioning (Papers III & V) in created wetlands.

TERMS & CONCEPTS

Wetland & watershed scales

Wetland destruction is associated with dramatic consequences for single wetlands, but also for entire watersheds. For the purpose of this thesis I will introduce two main environmental problems arising from the past diminishing of wetland areas, particularly in agricultural watersheds: (i) habitat destruction causing loss of species (biodiversity loss), and (ii) nutrient export from agricultural to aquatic systems (retention loss) causing eutrophication.

Biodiversity loss. The accumulated local loss of wetland habitat may affect the regional species pool, i.e. the metacommunities (Leibold *et al*. 2004) that are sustained by all the regional freshwater habitats together. Although local species diversity and composition of small isolated wetlands/ponds typically vary in space, over time, and with season, this habitat type is hypothesized to be particularly important for the local and regional diversity (Fig 2) of certain organism groups, including aquatic macroinvertebrates and plants (Scheffer *et al*. 2006). For maintaining regional diversity of these groups, small lentic water bodies are crucial as their heterogeneity and spatial turnover is high, i.e. local species assemblages are highly distinct from each other (Oertli *et al*. 2005; Robson & Clay 2005; Scheffer *et al*. 2006; Céréginho *et al*. 2008). Further, the regional species pools hosted by different aquatic environments (ponds, lakes, rivers, streams, and ditches) in the agricultural landscape, differ in quantity and quality: small ponds exceed regional diversity of all other water body types (Davies 2005), they harbor over 70% of all aquatic plant and macroinvertebrate species in agricultural landscapes (Williams *et al*. 2004), and also most rare species (Biggs *et al*. 2007; Davies *et al*. 2008). The local destruction of wetlands may thus have large impacts on regional scale, particularly as rare species loose habitat refuges and may disappear from the region. In southernmost Sweden, where extensive wetland loss occurred, diversity loss is particularly threatening. In Scania alone, 380 wetland- and freshwater species are red-listed (www.artdatabanken.se); this corresponds

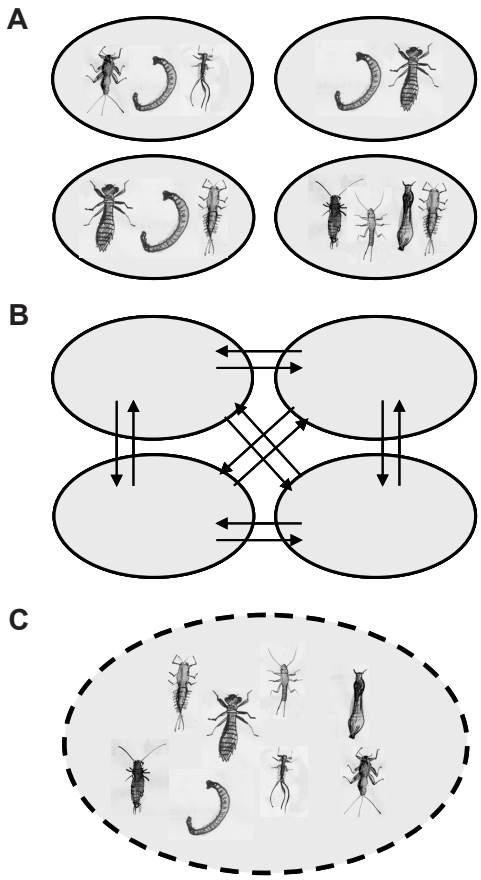

Fig 2. The spatial components of biodiversity. (A) Local or α diversity. Species number and composition per wetland is shown (four examples). (B) Spatial turnover or β diversity. The wetlands in a defined region can be compared pair-wise (arrows) with each other to determine the overall degree of differentation among wetland assemblages. (c) Regional or γ diversity. If the species present in all the wetlands of a region (the four examples in (a) are pooled, the cumulative richness can be determined.

to more than half of Sweden's endangered species of these habitat types, and greatly exceeds the average numbers (170 wetland and freshwater species) being red-listed in Swedish regions with less intensive agriculture and lower farmland proportions (www.artdatabanken.se).

Retention capacity loss. Similarly as above, the repeated loss of local wetland buffering capacity may affect the watershed-scale nutrient retention capacity. Wetlands act as transforming landscape units (Mitsch & Gosselink 2000) and are effective nutrient traps (Saunders & Kalff 2001; Zedler 2003). In agricultural areas, nutrient levels (nitrogen N and phosphorus P) and the associated eutrophication risk are often raised throughout entire watersheds (e.g. in Europe, Kroeze & Seitzinger 1998), and the excess nutrients are exported to the estuaries and coastal shelfs. Monitored watersheds in southern Sweden (7 to 15 km^2 in size) today sustain nutrient exports of 2,400 to 4,400 kg N km^{-2} yr^{-1}, and 30 to 60 kg P km^{-2} yr^{-1} (Kyllmar 2006), originating largely from anthropogenic agricultural use (Fig 3). The high regional nutrient efflux (Scania and Halland) constitutes up to one third of the total Swedish N and P emissions to the Baltic Sea (125,000 Mg N and 3,200 Mg P per year in 2007; www.ma.slu.se), ensuring Sweden a top per-capita emission rank among the countries in the Baltic watershed (Helcom 2004).

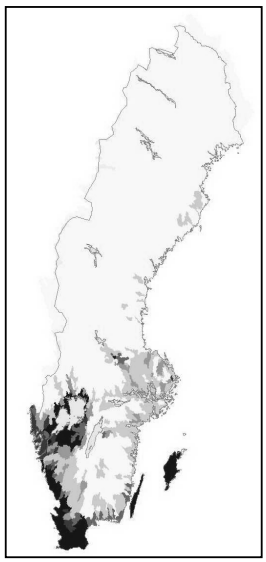

Fig 3. Anthropogenic N leakage from watersheds in Sweden. Shading denotes extent of N leakage; the darkest shade in the south indicates >500 kg km^{-2} of annual N export to the coast (www-nrciws.slu.se/TRK/).

The extent of nutrient export is proportional to the extent of wetland destruction in the watersheds (Mitsch *et al*. 2001). Apart from the increased eutrophication risk within agricultural watersheds, the N export in particular also states a threat for the marine recipients. Mitsch *et al*. (2001) link the N export (21,000,000 Mg N yr^{-1}) of the Mississippi river basin to the area with anoxic sea bottoms in the Bay of Mexico (2,000,000 ha). Similarly, anoxic bottoms along the Swedish and Danish coasts are increasing, and algal blooms are common in the Baltic Sea (Helcom 2004).

Thus, wetland loss had consequences for the biodiversity and functional integrity in natural wetlands, entire agricultural watersheds, and even the recipient coastal habitats. Consequently, as wetland creation is now implemented at large spatial scales, there is a need to assess the potential effects on diversity and function on local to regional scales (Wagner *et al*. 2008).

Biodiversity

Realizing the increasing threat arising from habitat and species loss, the European Commission has set the target to halt biodiversity loss in Europe until 2010, in order to avoid profound consequences for the natural world and human well-being (www.eea.europa.eu/multimedia/vnr-biodiversity). The term *biodiversity* covers many aspects of biological variation, ranging from genes and species over microhabitats to ecosystems (Gaston 1996). In common words it describes the living species in a defined space (often an area), that may vary from very small (e.g. a soil sample; a water droplet) to very large (e.g. a continent; the whole earth). Scientific definitions of biodiversity are complex and cover

'the variety of life forms, the ecological roles they perform, and the genetic diversity they contain' (Wilcox 1984; Murphy 1988).

For diversity investigations, this broad biodiversity term needs to be specified for differing applications and quantified with suitable measures. For this purpose, it is useful to distinguish between biodiversity components related to structural, functional, spatial and temporal aspects. *Structural* aspects account for different amounts of individual organisms present in a population or community, i.e. that species or genetic diversity is related to number (richness) and relative abundance (amount). *Functional* aspects regard (i) the specializations of individual organisms for their environment and their influence on the ecosystem, and (ii) the organism/species interactions (e.g. competition, trophic relations). The *spatial* components of biodiversity regard the variation of community structure among continents, landscapes, ecosystem types, and individual sites of the same ecosystem type. 'Landscapes' usually contain assemblages of different ecosystem types (e.g. forests, lakes, agricultural fields), which in turn are represented by several individual sites. Finally, the *temporal* aspects of biodiversity regard the variation of the structural, functional and spatial components over time. The species identity and number of organisms present in an ecosystem, and their interactions change on a daily, seasonal or annual basis, or on longer evolutionary time-scales.

All these biodiversity aspects are of relevance for created wetlands, and in the following section I introduce several specific research issues for the separate diversity components.

Structure & function. Diversity mechanisms depend on organism size (Finlay 2002; Cottenie 2005; Beisner *et al*. 2006), and biotic group/organisation level (Beisner *et al*. 2006; Prosser *et al*. 2007). To allow comparisons, I included several distinct biotic groups of differing organisation level in my diversity investigations on created wetlands, namely plants (primary producers), macroinvertebrates (consumers and predators), and bacteria (mainly decomposers).

Within the main biotic groups, further structural and functional aspects can be differentiated, e.g. by applying different concepts of defining the biotic units of diversity, either based on phylogeny (species/strains) or function (functional groups). Species are defined by close phylogenetic relationships of individual biotic units; for macroorganisms the 'biological species concept' applies (see Coyne & Orr 2004). For microbes, phylogeny can be evaluated based on genetic markers, universal, e.g. the eubacteria (16S ribosomal RNA gene), but specific for bacterial strains (Dahlöf 2002). In contrast, functional groups are defined by the presence of similar morphological or genetic features that are relevant for a defined function. Phylogenetically distant organisms may fulfill similar roles in an ecosystem (e.g. predators) or have similar prerequisites/genes for carrying out ecosystem processes (e.g. bacterial denitrifiers). Based on specific features supporting a function, they can be grouped together in a functional group.

In this thesis, plant- and macroinvertebrate assemblages were differentiated according to species or function. Further, eubacterial communities were differentiated based on genetic variation (within the 16S rRNA gene). The functionally important group of denitrifying bacteria was differentiated based on three enzyme genes (required for different denitrification steps) and and their genetic variation (within the different enzyme genes). I investigated both diversity and structural composition of the biotic assemblages in created wetlands.

Spatial aspects. As outlined before, large-scale wetland creation may affect diversity on local to regional scales (Fig 2). Local or alpha (α) diversity (per created wetland) can be measured by richness (number of distinct biotic units), or by the Shannon diversity index, integrating both the number of biotic units and their relative abundance (Spellerberg & Fedor 2003). Beta (β) diversity or

spatial turnover rates among several created wetlands can be assessed directly (degree of differentiation) or indirectly (degree of similarity) by pair-wise comparisons of assemblages between wetlands (Koleff *et al*. 2003). Gamma (γ) or regional diversity is the diversity that is cumulatively hosted by all wetlands, located in a defined geographic area (landscape). Integrating all three spatial aspects of diversity allows a basic evaluation of the biodiversity conservation value of created wetlands, but also to investigate the factors influencing diversity, at different spatial scales.

Temporal aspects. Species diversity is time-dependent and increases with observation period (Adler & Lauenroth 2003). To investigate temporal changes, repeated measurements in the same system are required; the time scale of observation needs to be adapted to the ecological question. The time frame of this thesis covers interannual variation of created wetlands during the first few years after establishment.

Habitat heterogeneity. The environmental conditions prevalent among individual farmland ponds/wetlands are often highly distinct; this habitat heterogeneity is hypothesized to be one of the mechanisms maintaining the high regional species diversity of small water bodies (Briers & Biggs 2005; Biggs *et al*. 2005; Scheffer *et al*. 2006). The environmental conditions in aquatic habitats may reflect the local assemblages of various biotic groups (Declerck *et al*. 2005; Lindström *et al*. 2005; Shade *et al*. 2008). In created wetlands, nutrient concentrations and loads can be expected to be high, as they are prerequistites to sustain the retention function (Kadlec & Knight 1996; Kadlec 2005). Thus, habitat heterogeneity of created wetlands could be lower than that of natural wetlands, and high local nutrient concentrations in constructed wetlands may interfere with simultaneous diversity aims (Hansson *et al*. 2005). In this thesis, I therefore study the extent of environmental influence in general, and of retention requirements in particular, on the diversity and composition of local assemblages.

Ecosystem functioning

Hooper *et al*. (2005) define ecosystem functioning as the sum of all processes provided by a given ecosystem. In this thesis, the general term ecosystem functioning is applied to cover all ecosystem functions sustained parallelly by a system (Fig 4). Natural wetlands are multifunctional ecosystems (Costanza *et al*. 1997; Zedler 2005) and there is a need to investigate if created agricultural wetlands restore lost multifunctionality. Also, small water bodies were traditionally assigned a minor role for functioning on regional to global scales (compared to large freshwaters); however, more recently, small waterbodies were suggested to exceed larger lakes both in number and cumulative area (Downing *et al*. 2006), thus being highly relevant for regional/global cycles. The ecosystem functioning of small water bodies in particular thus requires more research attention (Downing *et al*. 2006).

Each ecosystem function, i.e. its quality and absolute/relative quantity, relies on ecosystem properties and process rates; often several properties/processes contribute to a given ecosystem function, and properties/processes may influence each other. The flow chart (Fig 4) also illustrates that ecosystem process rates and properties in turn depend on (i) the type and quantity of abiotic ecosystem components (resource pool sizes), defined by external supplies, internal consumption, and physicochemical interactions of abiotic resources. Further, they depend on (ii) the abundance and activities of biotic units (i.e. species or functional groups) and their resource utilization rates, respectively. Interactions between biotic units (e.g. competition or predation) may directly or indirectly alter ecosystem process rates, properties, or functions, and consequently ecosystem functioning. The complexity and the temporal

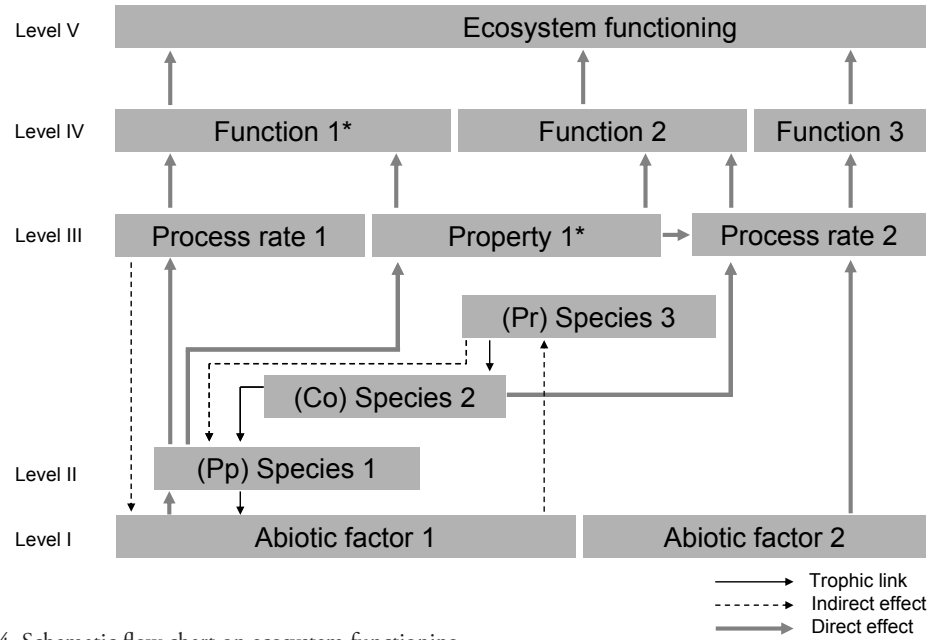

Fig 4. Schematic flow chart on ecosystem functioning.
Abiotic factor 1 is a resource to producer species 1, which sustains both process rate 1 and ecosystem property 1. Abundance/activity of species 1 is directly controlled by consumer species 2 and indirectly by predator species 3. Process rate 1 controls in turn the supply of abiotic factor 1. Process rate 1 and property 1 both contribute to ecosystem function 1; propperty 1 also contributes to function 2.
Abiotic factor 2 directly limits process rate 2, which is sustained by consumer species 2. Consumer species 2 is directly controlled by predator species 3, which in turn is indirectly controlled by abiotic factor 1. Property 1 serves as a resource for process rate 2. Process rate 2 sustains function 3 entirely and function 2 partly.
The whole system maintains three simultaneous ecosystem functions; functions and properties marked with * are of direct value to humans (ecosystem services). The complexity of level II (biotic units) determines the species diversity of the ecosystem.

(and spatial) stability of biotic interactions and activities depend on the composition and diversity maintained in the system itself, but also the conditions for external species recruitment and recolonization after disturbance.

Regarding the ecosystem functioning of created wetlands, these systems are created to serve one or several specified functions, or ecosystem services. The term ecosystem service is applied for an ecosystem function/process/property which contributes to human welfare (Boyd & Banzhaf 2007; Costanza 2008); an ecosystem service is a *desired capacity* of an ecosystem. Often, ecosystem services are assigned a direct economical value (Boyd & Banzhaf 2007), and include, for example, the food production by crops, the carbon dioxide assimilation by a forest, or the the nutrient retention by wetlands.

Man is trying to enhance desired or to decrease undesired ecosystem functions, particularly in managed ecosystems. By adjusting environmental conditions or ecosystem processes in man-made ecosystems, ecosystem functions can be directed in a desired way. Thereby, an ecosystem service might be established or optimized. The creation or management of ecosystems in order to achieve certain ecosystem services (or environmental goals) requires the assessment of potentially adverse consequences or unde-

sired functions, for example by cost-benefit analysis. Contrary to beneficial services, undesired ecosystem functions may sustain an environmental, ecological or human health risk.

Environmental influences

General ecological theories predict environmental factors to influence (i) biodiversity and (ii) ecosystem functioning. The following section introduces hypotheses on influencing factors, but outlines also where their direct application to small discrete waterbodies (such as created wetlands) is limited. In these cases, environmental factors that are likely to influence wetland diversity and function in particular, are specified.

Biodiversity influences. Species diversity of a given ecosystem is constrained by species colonization and extinction rates, as well as the rate of speciation; these rates in turn can be affected by factors operating on local (intrinsic), regional, or global (extrinsic) scales (Sarvala *et al*. 2005). Intrinsic factors like the local environmental conditions determine for example the stability/disturbance and the resource pool sizes of an ecosystem. Theories (corrobated by empirical observations) hypothesize positive relations of biodiversity to area (island geography; MacArthur & Wilson 1967) and time (Adler & Lauenroth 2003), a positive or hump-shaped relation of biodiversity to productivity (Mittelbach *et al*. 2001), and a facilitation of diversity at intermediate disturbance frequency (Connell 1978). Extrinsic controls include global properties (e.g. temperature range) and regional processes (e.g. climatic events, dispersal barriers) and the links between a local system to its landscape context (e.g. size and structure of the regional species pool; connectivity of habitats).

Regarding the biodiversity of pond-like systems in particular, habitat size is most important for large, dispersal-limited organisms (e.g. fish, Sarvala *et al*. 2005). For many other organism groups (plants, plant-eating birds, macroinvertebrates, amphibians, zooplankton), habitat size is either not of importance (Oertli *et al*. 2002), or small habitat sizes (and absence of fish) facilitate high local diversity (secondary habitat size effects, Scheffer *et al*. 2006). The biotic assemblages of small pond/wetland systems are subjected to strong environmental fluctuations (extreme events), thus are exposed to a higher risk of local extinction (Scheffer *et al*. 2006). Hence, local populations need internal and external strategies to compensate for losses, for e.g. by high reproduction rates or resting stages (bacteria, phyto- and zooplankton) or by good dispersal ability (flying macroinvertebrates, plant seeds). Larger organisms (e.g. fish) may be excluded from long-term establishment in ponds, if local disturbance frequency is too high to compensate extinction rate by (dispersal-limited) external recruitment (Sarvala *et al*. 2005).

Further, the external recruitment potential is determined by the landscape context, i.e. the situation of a local habitat in relation to (i) the quantity and quality of other habitats present within the dispersal range of its biota, and (ii) the size and structure of the regional species pool available for colonization (Leibold *et al*. 2004). Created wetlands are likely to be strongly dependent on the regional factors, particularly just after establishment. However, in landscapes with low densities of isolated habitats, wetland creation (on large scale) may also influence the species exchange rates among natural aquatic habitats, interconnecting habitats as 'stepping stones' for species dispersal.

In addition to the local environmental conditions reflecting the species assemblages (Declerck *et al*. 2005) in small pond-like habitats, mechanisms other than environmental factors may also explain assemblage variation. For example, stochastic events tend to have great influence on biotic assemblages, particularly in small water bodies (Scheffer *et al*. 2006). Composition of both plants and

macroinvertebrates also depends on the order in which species initially enter a community (priority or preemption effects, De Meester *et al*. 2002; Forbes & Cole 2002; Chadwell & Engelhardt 2008). Productivity (affecting local diversity) is even influencing diversity on larger, regional scale; assemblages of eutrophic waters are therefore highly heterogeneous (Chase & Leibold 2002).

Ecosystem functioning influences. As illustrated in Fig 4, ecosystem functioning in general depends on abiotic and biotic parameters of the system. Abiotic parameters can either control properties/process rates by resource pool size (e.g. nutrient supply for plant primary production), or regulate process efficiency via catalysing effects (e.g. temperature and pH affecting enzyme activity). Abiotic effects on ecosystem functioning are often mediated through the biotic compartments of the system; i.e. abiotic factors influence performance of biotic compartments which in turn regulate ecosystem processes/properties, and ultimatively functioning.

Each biotic unit (species; functional groups) present in an ecosystem may affect singular processes/properties/functions (Fig 4); the individual units are strongly affected by the abiotic parameters of the system, as well as the interactions with other biotic units (competition/predation).

Ecosystem functioning of wetlands in particular depends on both the abiotic and biotic prerequisites. The capacity for removing nutrients (N and P in various forms) is determined by the incoming concentration and hydraulic loading rate, i.e. the nutrient load (Kadlec & Knight 1996). However, even the 'biotic setup', i.e. the structure, composition, and extent of the biotic assemblages influence wetland performance capacity (Kadlec 2008; Thullen *et al*. 2008; Kallner Bastviken *et al*. 2009). However, there is need to outline the relative effect of biotic and abiotic parameters, particularly in aquatic environments (Gamfeldt & Hillebrand 2008). For example, in highly dynamic wetland systems, abiotic variation (disturbance) may overrule biotic influences. On the other hand, biotic effects may be comparably important in unstable environments, as biotic units are frequently replaced; the capacity to switch a certain function from one to another biotic unit would require species and functional redundancy, i.e. diversity.

Potential interactions

Interactions between biodiversity and function as well as among functions are of importance for understanding freshwater ecology (Gamfeldt & Hillebrand 2008). With regard to created wetlands in particular, they are also relevant for developing suitable management strategies.

Biodiversity–function links. Apart from effects mediated by singular biotic units, performance of biotic compartments also depends on the strength, stability, and complexity of biotic interactions between units. Put simply, the more biotic compartments present per ecosystem, the more complex the biotic interactions. This complexity or diversity is considered to be a major driver of ecosystem functioning itself (Loreau *et al*. 2001, Hooper *et al*. 2005). Theories hypothesize a positive effect of biodiversity on ecosystem functions (Loreau *et al*. 2001; Hooper *et al*. 2005), based on the mechanistic explanations, that more diverse communities are either (i) more resource-efficient due to resource complementarity and species facilitation (niche-differentiation effect), or (ii) have a higher chance of containing species performing above-average (sampling effect). Species diversity and functional redundancy may also provide an insurance against future environmental change (Hooper *et al*. 2002; Loreau *et al*. 2003).

Consistent with diversity-function predictions, diversity parameters can influence and enhance wetland functioning. P retention in equally loaded mesocosms increased

with submerged plant richness (and associated macroalgae biomass) (Engelhardt & Ritchie 2001; 2002), N accumulation or plant uptake increased with plant species richness (Chabrerie *et al*. 2001; Callaway *et al*. 2003), methane production decreased with functional richness of wetland plants (Bouchard *et al*. 2007), and decomposition rate increased both with shredder richness and biomass (Thullen *et al*. 2008). However, observations from other studies seem contradictive to the diversity-function hypothesis. Several studies have shown that monocultures of certain species may exceed diverse assemblages in performing the ecosystem function investigated, particularly if communities were non-randomly assembled (Smith & Knapp 2003; Schläpfer *et al*. 2005; Srivastava & Velland 2005; Sullivan *et al*. 2007; Lake *et al*. 2007). The created wetlands investigated in this thesis assemble their biotic communities by natural succession; species assembly is thus affected by non-random mechanisms (Weiher & Keddy 1995).

Functional coupling & joint functioning. Only recently carried out studies outline, that the more functions considered, the more apparent the importance of biodiversity for ecosystem functioning (Hector & Bagchi 2007; Gamfeldt *et al*. 2008; Gamfeld & Hillebrand 2008). In contrast, previous diversity-functioning research commonly assumed that one ecosystem function investigated at a time may serve as an estimate for overall ecosystem functioning (see Gamfeld *et al*. 2008) and diversity mechanisms where concluded mainly based on studies involving only one trophic level (often primary producers). These simplifications, however, may partly be the cause for confounding results on diversity-function links. Some specific ecosystems may be sustained without involving any biotic species; or mediated by one so-called 'keystone' species alone; in these cases biodiversity could be of minor importance. Other functions however, may involve more complex food web interactions; in these cases biodiversity effects may become apparent. By randomly choosing one specific ecosystem function to represent overall (joint) ecosystem functioning, biodiversity links may or may not become apparent. Further, single ecosystem processes and properties may affect each other, and the strength, stability and complexity of their interaction (functional coupling) may also affect overall ecosystem functioning. These aspects need thus further research attention, particularly in freshwater habitats (Gamfeldt & Hillebrand 2008).

Summary: Created agricultural wetlands

Wetland creation - a potential solution?
As a potential solution to (partially) abate the habitat/species loss and eutrophication caused by historic wetland loss, the restoration or creation of wetland areas at large, watershed scales is suggested (Mitsch *et al*. 2001; Paludan *et al*. 2002; Zedler 2004; Chapman & Reed 2006; Mitsch *et al*. 2006; Olde Venterink *et al*. 2006). The idea is that an increase of the aquatic habitat in monotonous agricultural landscapes with intensive production, may benefit species diversity or retention. Wetland creation with pure biodiversity aims has been successful in the United States (Galatowitsch & van der Valk 1996; Seablom *et al*. 2001; Seablom & van der Valk 2003; Balcombe *et al*. 2005a, 2005b), with local and regional diversity of specific wetland habitat types (e.g. prairie potholes) being restored. The creation of small permanent water bodies may be suitable for sustaining both the diversity of aquatic as well as transitional wetland species, which has been demonstrated for man-made agricultural ponds (Declerck *et al*. 2006; Abellan *et al*. 2006; Céréghino *et al*. 2008).

With regard to eutrophication, nutrient export from farming areas to aquatic habitats can be abated using different strategies (Mitsch *et al*. 2001; Zedler 2004; Olde Ven-

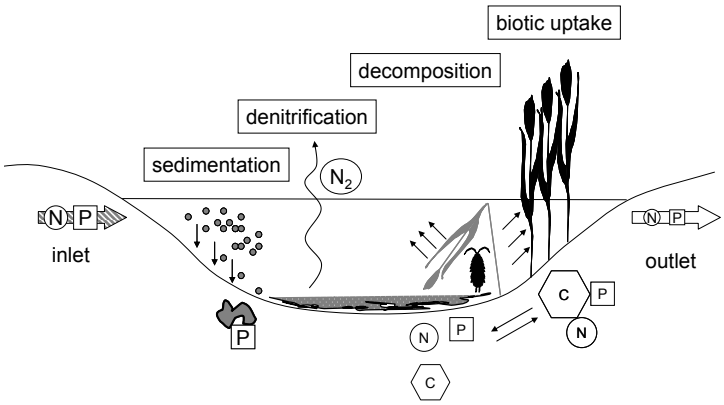

Fig 5. Schematic view of a created wetland and its main ecosystem functions. High concentrations of nutrients enter at the wetland inlet; nutrients are then processed via different pathways: sedimentation of particle-associated phosphorus, bacterial denitrification of nitrate-N to N_2, biotic uptake and seasonal storage of both nitrate-N and phosphate-P, and delayed nutrient release via litter decomposition.

terink *et al*. 2006): (i) a change of nutrient application and soil preparation practices may reduce the amount of applied fertilizers and decrease the risk of leaching and runoff exports, (ii) the creation of buffer zones (e.g. floodplain restoration, buffer strips) between farming areas and streams/ditches may hinder nutrients from entering the aquatic system, and (iii) the installation of overflow areas or permanent pond-like water bodies may slow down the transport velocity within the watershed, and allow processing of nutrients which have already entered the aquatic system. These strategies target a decrease in nutrient (N and P) concentrations and loads, i.e. aim to increase the retention capacity of the watershed. The types of measures may differ in efficiency of N or P retention (Mitsch *et al*. 2001). Created wetlands with permanent water bodies are considered particularly suitable to remove N (Fleischer *et al*. 1994; Leonardson 1994; Mitsch *et al*. 2001; Paludan *et al*. 2002; Hey 2002; Kadlec 2005; Mitsch *et al*. 2005; Olde Venterink *et al*. 2006), by providing required conditions for denitrification. High nitrate, low oxygen content (Knowles 1982), and high macrophyte biomass, i.e. litter as carbon sources and surface for biofilms (Weisner *et al*. 1994) facilitate denitrification; these conditions can be sustained in pond-like wetlands created in agricultural landscapes.

Multiple purposes. This type of created wetlands may have the potential for abating part of either the biodiversity loss or eutrophication problem, or both simultaneously. However, the simultaneous targeting of biodiversity and nutrient retention purposes in created wetlands requires close evaluation, as high nutrient concentrations may interfere with biodiversity goals (Hansson *et al*. 2005). Wetland creation may also increase the risk for other undesired environmental consequences, as wetlands sustain a comparably high risk for climate gas emissions (Mitsch *et al*. 2001; Verhoeven *et al*. 2006; Stadmark & Leonardson 2005, 2007) or host organisms potentially hazardous to human health (e.g. mosquitos, Dale & Knight 2008). On the other hand, wetlands created for nitrogen abatement may also sustain several ancillary beneficial functions (e.g. P retention, Tonderski *et al*. 2005), and potential links between functions, and multifunctionality may be of relevance for restoration management (Findlay *et al*. 2002; Euliss *et al*. 2008).

Studied ecosystem functions & services. The target ecosystem services N retention and biodiversity (species and functional diversity) of created wetlands are the focus of this thesis. Nitrogen retention and biodiversity conservation are stated aims of the Swedish

environmental objectives (www.miljomal.nu), and are functions desired to be achieved by wetland creation. Further, the ecosystem functions P retention (ancillary service), litter decomposition and methane emission (environmental risk), are considered in the papers of this thesis; firstly to investigate targeted and ancillary benefits as well as environmental risks of wetland creation, and secondly to investigate functional coupling and joint ecosystem functioning.

Ecosystem services and functions present in created wetlands are summarized in Fig 5. Certain wetland functions can be passively facilitated by the physicochemical conditions (e.g. particle and P sedimentation due to reduced current), others are actively sustained by biotic compartments (e.g. N retention due to bacterial denitrification plus plant nutrient uptake; litter decomposition by microbes and shredder macroinvertebrates). Wetland processes/properties depend on the prevalent abiotic characteristics (e.g. hydraulic turbulence, pH). In addition, the abiotic environment mediates indirect effects via the biotic compartments; abiotic conditions influence the composition of biotic assemblages (e.g. absolute amount of nutrient supply excludes/facilitates species), and the biotic interactions between biota (e.g. competition of plants and bacteria for nutrients), and thereby ecosystem rates and properties. In turn, the ecosystem properties themselves (e.g. plant biomass) may affect the abiotic environment (e.g. flow patterns or shading/UV radiation) or ecosystem processes (e.g. amount of plant nutrient uptake), thus indirectly or directly affect wetland functions.

Thesis relevance. The effect of created wetlands on regional diversity is still largely unknown, and with regard to combining wetlands for several environmental goals, impacts on diversity need to be considered (Paper I). Previous diversity investigations in wetlands focus almost exclusively on higher organisms; in contrast, information on microbial assemblages is scarce, although major wetland processes are mediated by bacterial communities, e.g. denitrification (Paper II). Despite the multifunctionality of wetlands, studies that compare (usually very few) created wetlands for their functional capacity are often limited to one ecosystem function at a time, or else, the potential for interactions/relations between ecosystem functions has not been considered (Papers III & V). Also, temporal aspects on the functioning and biodiversity of wetlands need to cover longer time scales, to test if biodiversity-function interactions interact with time (Paper IV). Biotic parameters are mostly not considered when wetland functions are assessed/predicted, and their relative effect in highly dynamic wetland environments is unknown (Paper V). Further, research on biodiversity–function links that considers non-random species assemblages (Paper IV), and diversity effects in dynamic environments (Paper V) are needed.

RESULTS & DISCUSSION

The investigated created wetlands as a whole provided several ecosystem services; Table 1 summarizes ecosystem services studied in the five papers of this thesis. Please observe that ecosystem services were measured with different methods, limiting direct comparability. I included Table 1 to provide an overview of my results, and as a guide for which of the five papers to consult for details.

Biodiversity results

The requirements for nutrient retention in created wetlands are not an obstacle for biodiversity.

Incoming nutrient concentrations and hydraulic loading rate (i.e. retention capacity indicators, Kadlec & Knight 1996; Kadlec 2005) were not associated to composition or diversity of aquatic macroinvertebrates in

Table 1. Ecosystem services related to nutrient retention and biodiversity that are provided by created agricultural wetlands. The table gives an overview on the ecosystem service(s) stucied in each of the five thesis papers, respectivly.

		Biodiversity		Functional diversity		Retention function	
		Plant richness (Species number)	**Macroinv. richness** (Species number)	**Denitrif. richness*** (DGGE band number)		**Nitrogen** (kg ha^{-1} yr^{-1})	**Phosphorus** (kg ha^{-1} yr^{-1})
Paper I							
Field, n=36	mean		33				
2004	range		6 – 51				
Vegetation season	cumulative		176				
Paper II				*nir*	*nos*		
Field, n=32	mean	14		11	6		
2004	range	5 – 22		7 – 16	2 – 13		
Vegetation season	cumulative	84		53	21		
Paper III							
Model, n=36	mean					567	
2004	range					419 – 2135	
Annual	cumulative					8505 (15 ha)	
Paper IV							
Exp. wetlands, n=18	mean	10	16			821	
2003–2006	range	3 – 21	6 – 29			675 – 1068	
Summer	cumulative	62**	95**			nm	
Paper V							
Field, n=14	mean	22	34			840	10
2004–2006	range	12 – 30	19 – 47			135 – 2156	-77 – 89
Vegetation season	cumulative	111**	113**			nm	nm

**nir*/*nos*. Bacterial denitrifying enzyme genes *nir*K+*nir*S and *nos*Z coding for enzymes needed for different steps in the denitrification chain (see also Fig 7A). DGGE (see Paper II). **all years/wetlands. nm not measured.

created wetlands (Table 2; Paper I). Macroinvertebrate and plant assemblages were instead constrained by parameters determining potential colonization success (wetland age, distance and connectivity to potential source habitats, Table 2). In general, large variation fractions of richness and composition were unexplained by environmental parameters (Table 2). Similarly, only low variation fractions could be assigned to environmental factors in previous studies on diversity and assemblage composition of macroinvertebrates (Lundqvist *et al*. 2001; Van de Meutter *et al*. 2008), plants (Edvardsen *et al*. 2006) and bacteria (Langenheder & Ragnarsson 2007). Further, a study comparing lake bacteria, phytoplankton, zooplankton, and fish assemblages indicated that dispersal predictors are more important than local environment for the two latter, larger and less motile groups (Beisner *et al*. 2006). The large unexplained variation may also be due to factors which where unaccounted for in Papers I and II; these include for example stochastic effects (Scheffer *et al*. 2006), preemption/priority effects (De Meester *et al*. 2002; Forbes & Cole 2002; Chadwell & Engelhardt 2008) and productivity effects operating on regional scale (Chase & Leibold 2002), which are particularly relevant for small water bodies.

I also found minor effects of retention capacity indicators on richness and composition of wetland plants (unpublished data). Plant composition varied with N:P ratio, and submerged plant richness decreased with P and suspended solid levels (Table 2). Submerged macrophytes may be effectively suppressed by high P and turbidity levels (Scheffer 1998; Jeppesen *et al*. 2000). The high diversity of small isolated ponds is partly assigned to their higher likelihood of being in a macrophyte-dominated state (compared to larger lakes with fish; Scheffer *et al*. 2006). If high P levels in created wetlands would cause shifts from a macrophyte to a phytoplankton state, this may ultimately lead to a decrease in macroinvertebrate diversity (Declerck *et al*. 2005). Very high P levels were associ-

ated with low macroinvertebrate richness in other Swedish created wetlands (Hansson *et al*. 2005). The phosphorus levels observed in created wetlands in Papers I-V were low to moderate by comparison. Further, the P levels were highly heterogeneous, i.e. negative P effects are likely to operate on local scale only. Regarding nitrogen, no adverse effects on richness of plants or macroinvertebrates were found, despite the very high ambient concentrations (3-20 mg l^{-1}), i.e. regionally elevated N levels.

Composition of eubacterial biofilm communities (Table 2) and overall richness of bacterial and denitrifying communities (Paper II), were more strongly influenced by the functional requirements of retention wetlands. Nitrate concentration explained part of the DGGE (denitrifying gradient gel electrophoresis; see Paper II) band structure of eubacteria (Table 2). The majority of the explained variation was accounted for by biotic parameters, i.e. richness of emergent and submerged plants (Table 2; Paper II). Eubacterial community structure was earlier shown to be influenced by vegetation state (Langenheder & Prosser 2008), water chemistry (Hewson *et al*. 2003), and wetland morphology (Hewson *et al*. 2007), and similar magnitudes of influence as in Paper II have been reported for bacterial communities from other aquatic habitats (Beisner *et al*. 2006; Langenheder & Ragnarsson 2007).

In conclusion, environmental parameters seem to have only minor effects on the assemblages of macroinvertebrates and plants; accordingly, the prerequisites for a simultaneous retention function of created wetlands did not hinder the establishment of diverse local and regional assemblages in these systems. In comparison, the bacterial assemblages seemed to be influenced mainly by

Table 2. Influence of abiotic and biotic wetland parameters on biodiversity of created wetlands.

		Plants		Macroinvertebrates		Eubacteria (16S)*		Denitrifying enzyme genes**	
Wetland characteristics		Composition	Richness	Composition	Richness	Composition	Richness	Composition	Richness
ABIOTIC									
Nutrients	NO_3					x			+ (*nos*), – (*nir*)
	P		– (Su)					x (*nir*K)	
	N:P	x							
Morphohydrology	q								+ (*nos*)
	depth					x		x (*nos*)	
	area								
Physicochemistry	pH			x				x (*nir*S)	
	T								
	TSS		– (Su)						
Other	age	x		x	+				
	connect	x		x					
	agric	x							
Regional	forest			x					
	north	x							
BIOTIC									
Species richness	Em					x			
	Su					x			
	Fl								+ (*nir*K)
TOTAL EXPL		28%	ns (total)	19%	r_S = 0.44	19%	ns	< 10% each	17 – 34%

*Eubacteria were targeted by applying the 16S rDNA primer (Paper II). **The denitrification enzyme genes *nir*K, *nir*S and *nos*Z were sampled from bacterial biofilm. Wetland characteristics are abbreviated as **NO_3** nitrate-N and **P** total phosphorus concentration, **N:P** total nitrogen:phosphorus ratio, **q** hydraulic loading rate, **depth** mean water depth, **area** wetland size, **T** water temperature, **TSS** total suspended solids, **age** time since wetland creation, **connect** lotic surface water connectivity, **agric** dominance of agricultural use in direct vicinity, **forest** distance to forested inland area, **north** northing within the region, **Em** emergent plants, **Su** submerged plants, **Fl** floating and floating-leaved plants. **x** effect present, **+** – positive/negative direction. **TOTAL EXPL** total variation fractions explained by all parameters. **ns** not significant. **r_S** Spearman Rank correlation.

vegetation and water quality, i.e. factors that may also affect the N retention capacity of wetlands (Kadlec 2005; Kadlec 2008).

Created wetlands are equally valuable habitats as natural ponds and large-scale wetland creation has the potential to enhance regional diversity.

Created agricultural wetlands sustained similar local and regional macroinvertebrate richness and overall species pools when compared to natural ponds in the same region (Paper I) and in agricultural landscapes elsewhere (Williams *et al*. 2004; Robson & Clay 2005; Biggs *et al*. 2007; Davies *et al*. 2008). In created wetlands, insects clearly dominated local assemblages as well as the regional pool; the most diverse orders being aquatic beetles, dragon-and damselflies, caddisflies, and water bugs (Paper I). Compared to natural ponds in the same region, created wetlands hosted more lotic groups (mayflies, stoneflies), probably as a result of higher connectivity to the watersheds.

Created wetlands sustained highly individual plant and macroinvertebrate assemblages, i.e. their spatial heterogeneity and β diversity was high (Paper I; unpublished data). Wetlands located in landscapes with extensive wetland creation (hence, high total aquatic habitat density; Paper I) hosted richer local plant and macroinvertebrate assemblages (Fig 6A; Paper I). With regard to macroinvertebrates, a positive density effect prevailed even on regional scale: the cumulative macroinvertebrate species pool sustained in the high density landscape was greater (110 compared to about 90 species, Fig 6B) than that of the other two regions. In small isolated ponds, the richness of some organism groups (including macroinvertebrates and plants) may be promoted by second order effects of habitat size (Scheffer *et al*. 2006), implying that several small wetland sites likely sustain more species than one large site of equal area (Oertli *et al*. 2002).

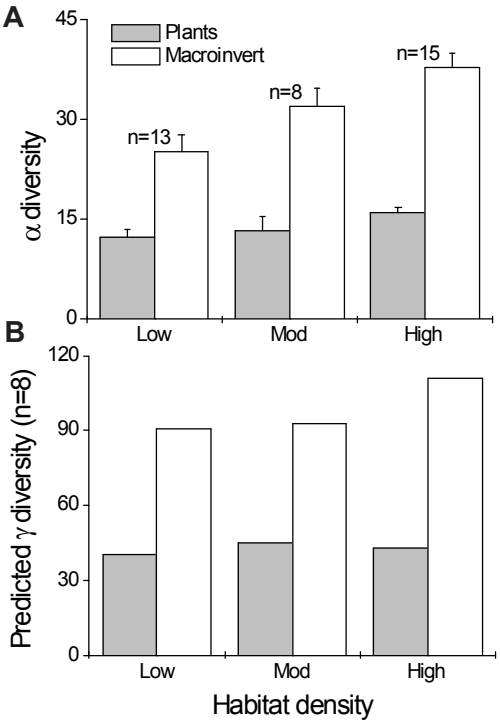

Fig 6. Effects of habitat density on local (A) and regional (B) species diversity of plants and macroinvertebrates. Density of aquatic habitats per landscape (Low, Mod, High) varied due to differences in wetland creation efforts (0.18, 0.24, and 0.35 wetlands per km^{-2}); 15% (i.e. n=13, 8, and 15 wetlands) of all created wetlands were investigated per landscape (Paper I).

In conclusion, created wetlands serving simultaneous diversity and N retention purposes have similar capacities for biodiversity conservation than natural agricultural ponds. The creation of many small created wetlands may promote both local and regional diversity of macroinvertebrates, particularly if wetland creation efforts raise total aquatic habitat densities by more than 30% (Paper I).

Functional diversity results

Created wetlands sustain functionally diverse denitrifier communities; the local environment is closely linked to bacterial denitrifier richness.

The three studied denitrifying enzyme genes (Paper II) were present in all wetlands, indicating that denitrifier communities with the potential for processing early and late denitrification steps (Fig 7A) were ubiquitously established (Table 1). Complexity and diversity of denitrifying enzyme gene composition of created wetlands were also comparable to that of agricultural soils (Throbäck *et al*. 2004). Further results from DNA sequencing of the denitrifying communities from the same created wetlands confirm that these systems host highly diverse denitrifier communities (Milenkovski 2009).

In created wetlands, the *nir*K and *nir*S enzyme genes decoding for an early denitrification step were more diverse on local and regional scales, than the *nos*Z enzyme gene coding for the last denitrification step (Table 1). The finding that diversity was greatest for the *nir* genes and lowest for *nos*Z is supported by a general relationship of lower diversity for *nos*Z compared to both *nir*K and *nir*S (Wallenstein *et al*. 2006). Generally, the band structure of DGGE patterns for the three enzyme genes was only weakly related to environmental conditions of created wetlands (Table 2) and unrelated to eubacterial diversity (Paper II), however similarly low explanation fractions have been reported from other systems (Langenheder & Ragnarsson 2007).

Interestingly, the spatial variation of denitrifying enzyme gene richness among wetlands was linked to factors which are known (Kadlec & Knight 1996; Kadlec 2005) to affect N retention, i.e. inlet nitrate concentration and hydraulic loading rate. Further, the enzyme gene richness of the early (*nir*) and last denitrification step (*nos*) responded differently to nitrate concentration; while

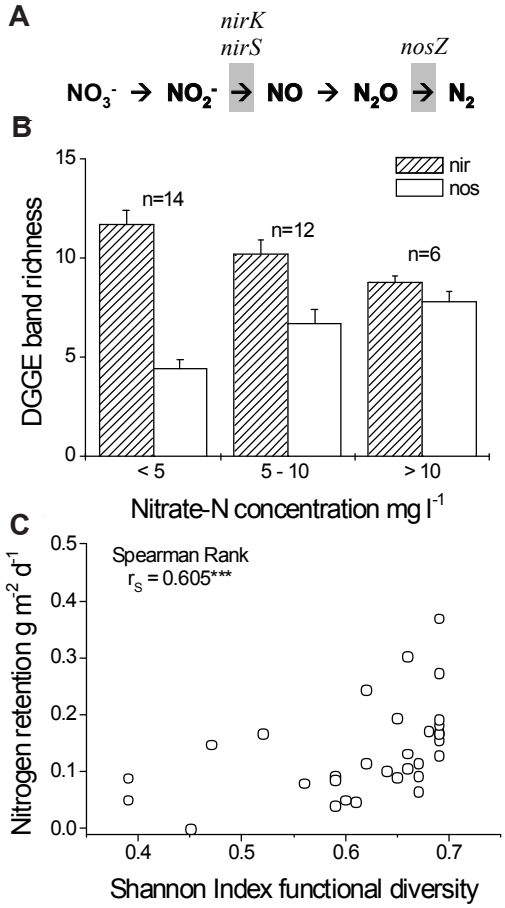

Fig 7. Denitrifying functional diversity. (A) Denitrification chain: reaction steps catalyzed by the *nir* and *nos* enzyme types are highlighted; the community composition of the enzyme genes *nir*K, *nir*S and *nos*Z of 32 created wetlands was investigated in Paper II. (B) Richness of bacterial denitrification enzyme genes coding for the second (*nir*) and last (*nos*) step in the denitrification chain was affected by nitrate concentration. At high nitrate concentrations, both enzyme genes became equally rich, otherwise the *nir* type (*nir*K + *nir*S) enzyme genes dominate. (C) Shannon functional diversity (based on DGGE band numbers of the *nir* and *nos* enzyme genes, data from Paper II) correlated ($p<0.0005$) with nitrogen retention of created wetlands (annual predictions from Paper III).

the *nir*K+*nir*S band richness decreased with nitrate concentration, the *nos*Z richness increased (Fig 7B). Similar to our findings, Kjellin *et al*. (2007) found that the *nos*Z enzyme gene composition varied with nitrogen and hydraulic loading rates. In contrast however, the *nos*Z diversity was lowest at high nitrogen and hydraulic loads (Kjellin *et al*. 2007).

Previous research (Wallenstein *et al*. 2006; Kandeler *et al*. 2006) suggests that denitrifier community structure and abundance in soils is primarily controlled by factors other than nitrate supply (e.g. carbon content, degree of water saturation, pH), despite the fact that the denitrification rate is stimulated by N/nitrate (Kjellin *et al*. 2007). Nitrate did not affect DGGE band pattern composition or richness within single enzyme genes in Paper II (Table 2); however, the overall complexity of bacterial denitrifier assemblages was influenced. Earlier studies of environmental effects on bacterial denitrifier communities have not compared functionally different enzyme genes (Braker *et al*. 1998; Hallin & Lindgren 1999; Braker & Tiedje 2003; Hannig *et al*. 2006; Bremer *et al*. 2007), and largely assumed that effects on one gene can represent effects on the overall denitrifier community. The results from Paper II strongly suggest that environmental effects (particularly nitrate supply) on denitrifier richness, and to a lesser extent composition, differ for functionally different enzyme genes. Hence, the use of single enzyme genes will not suffice to characterize the environmental influence on the overall bacterial denitrifier community in created wetlands.

Ecosystem functioning results

Ecosystem functions measured in this thesis included the (target and ancillary) ecosystem services N retention and P retention (Tables 1 & 3) and further, the wetland functions methane production and litter decomposition (Table 3, Papers III & V). Also studied were the abiotic and biotic controls (Table 3), potential links between functions (Papers III & V), and temporal changes of N retention (Fig 8). In the final Paper V, joint ecosystem functioning (i.e. simultaneous performance of N retention, P retention and litter decomposition, Fig 9) was studied.

Biotic factors affect the functioning of highly dynamic created wetlands and biotic influences partly differ between functions.

In Paper V, ecosystem functions in created wetlands with variable abiotic and biotic characteristics (dynamic environments) were investigated. Variation occured spatially (among wetlands; controlled for by parallel investigations in 14 wetlands) and temporally (over seasons and years; controlled for by repeated sampling). This setup allowed to test if biotic variables explained differences in three ecosystem functions (N retention, P retention and litter decomposition) additionally to abiotic factors, in dynamic environments.

Biotic parameters affected the processes/properties underlying the three functions (Table 3), and models containing both abiotic and biotic factors, explained more functional variation than abiotic factors alone (Paper V). Similarly, in Paper III, the variation in methane production was best explained by including biotic and abiotic explanatory variables in the model (Table 3). Prediction models for wetland functions (e.g. N and P retention, Kadlec & Knight 1996; methane production, Bastviken *et al*. 2004) are commonly based on the abiotic, physicochemical dynamics. The inclusion of biotic parameters may significantly improve predictability. Nevertheless, abiotic factors explained larger variation fractions for most functions; the abiotic factors which were found to influence the wetland functions (Table 3) agreed with previous studies (see detailed discussions in Papers III & V).

The biotic parameters in Paper V covered influences of two biotic groups (plants or macroinvertebates), and distinguished be-

Table 3. Influence of abiotic and biotic wetland parameters on ecosystem functions of created wetlands.

		Nitrogen retention				Phosphorus retention				Litter Decomposition		Methane production
		*k*N* ($m\ d^{-1}$)	N rate ($g\ m^{-2}\ d^{-1}$)	*k*NO_3* ($m\ d^{-1}$)	NO_3 rate ($g\ m^{-2}\ d^{-1}$)	*k*P ($m\ d^{-1}$)	P rate ($g\ m^{-2}\ d^{-1}$)	*k*PO_4 ($m\ d^{-1}$)	PO_4 rate ($g\ m^{-2}\ d^{-1}$)	*k*D 1mm (d^{-1})	*k*D 5mm (d^{-1})	CH_4 * ($g\ m^{-3}$)
ABIOTIC												
Nutrients	NO_3		+	–						–		–
	P											+
	PO_4										+	
	PO_4 load							+				
Morphohydrology	q	+	+	+	+							
	depth											
	area											
Physicochemistry	pH							–				
	T											
Other	age											+
	surface											+
BIOTIC												
Diversity	Pl rich					+		+	(+)			
	Pl fuDiv		+		+							
	M div										+	
	M shr rich											
Abundance	Pl cover									–	–	–
	Pl biom				+	–	(–)	–				
	M shr abu											
TOTAL EXPL		49%	72%	60%	70%	66%	ns	55%	ns	48%	58%	39%

*at reference temperature. Ecosystem processes/properties include the removal rate coefficients (***k*N**, ***k*NO_3**, ***k*P**, ***k*PO_4**) and areal removal rates (**N rate**, **NO_3 rate**, **P rate**, **PO_4 rate**) for total nitrogen, nitrate-N, total phosphorus, and phosphate-P; the litter decomposition rate coefficient in 1 and 5 mm mesh size bags (***k*D_{1mm}**, ***k*D_{5mm}**); the dissolved concentration of methane (**CH_4**). Wetland characteristics are abbreviated as **NO_3** nitrate-N, **P** total phosphorus, and **PO_4** phosphate-P concentration, **PO_4 load** phosphate-P load, **q** hydraulic loading rate, **depth** mean water depth, **area** wetland size, **T** water temperature, **age** time since construction, **surface** inlet type: surface-fed. **Pl rich** Plant richness, **Pl fuDiv** Plant functional diversity, **M shr rich** Macroinvertebrate shredder richness, **Pl cover** Plant cover, **Pl biom** Plant biomass, **M shr abu** macroinvertebrate shredder abundance. **TOTAL EXPL** total variation fraction explained by all parameters. **+ –** positive/negative influence. **ns** not significant.

tween (i) diversity and (ii) abundance effects. The significant diversity effects that were found, were positive (Table 3). At least one process involved in N retention, P retention and litter decomposition, respectively, increased with diversity of either plants (retention) or macroinvertebrates (decomposition). The abundance (biomass, cover) of plants on the other hand, seemed to influence ecosystem functions differently (Table 3; Papers V & III). Plant biomass was linked to increasing N retention, plant cover to decreasing P retention, litter decomposition, and methane production.

In conclusion, biotic parameters need to be accounted for in order to predict wetland functioning. Management of vegetation succession in created wetlands needs to balance between strategies facilitating plant richness and biomass to sustain desired functions; alternatively, one wetland function could be prioritized above others, and vegetation management adopted accordingly.

Biodiversity - function links

Functional diversity of denitrifiers correlates to N retention capacity of created wetlands.

Denitrification is the major pathway of N removal in nitrate-rich environments (Seitzinger *et al*. 2006; Beaulieu *et al*. 2008), and has the potential to limit the N retention function of created wetlands. The denitrification chain is a series of reaction steps, in which bacterial denitrifiers play a crucial role in expressing the enzymes needed to catalyze the three reductions from nitrite to dinitro-

gen gas (Zumft 1997; Fig 7A). Richness and structure of the bacterial enzyme gene assemblage may affect these steps, as denitrifier populations differ in physiological properties, e.g. their affinity for electron acceptors and donors or the relative reaction rates of denitrification steps (Phillipot & Hallin 2005). Results from previous studies (Cavigelli & Robertson 2000, 2001; Holtan-Hartwig *et al*. 2001, 2002; Rich *et al*. 2003) suggest that differences in the community composition of soil-denitrifying bacteria may explain differences in denitrification rates. A skewed community composition of the enzyme genes (*nir* compared to *nos*) may potentially limit one of the denitrification reactions, i.e. form a bottleneck in the denitrification process. Based on data from Paper II, I calculated the Shannon index for richness of the functionally different enzyme genes *nir* and *nos* per wetland (i.e. assuming equal importance of band richness for early and late denitrification steps), to assess denitrifier functional diversity. I then compared functional diversity with the predicted annual nitrogen retention (case study predictions from Paper III) for the 32 wetlands, which were included in both studies. I found that denitrifier functional diversity was positively correlated to predicted annual N retention (Fig 7C). Bell *et al*. (2005) suggest that bacterial community structure affects ecosystem functioning, and that species richness has positive effects on bacterial ecosystem functioning; Jayakumar *et al*. (2004) linked *nir*S diversity to high denitrification rates. In contrast to my results, Kjellin *et al*. (2007) observed highest denitrification rates at sites with lowest *nos*Z diversity (compared to other sites in the same wetland). Rich *et al*. (2004) found differences in denitrification rates between wetland and upland soils, but no structural or diversity differences in the *nos*Z gene. However, earlier studies on enzyme genes did not cover more than one denitrification step and focussed on richness *within* single enzyme genes; my results indicate that N retention may be highest if functional diversity *across* genes is high.

Vegetation type rather than diversity affects N retention.

In Paper IV, biodiversity and N retention were investigated in experimental wetlands with controlled abiotic conditions. This allowed to investigate the effect of vegetation state and diversity-function links (Fig 8). Regarding N retention, wetlands with a vegetation state of high biomass (tall emergent plants) exhibited continuously higher N removal during four years (Fig 8E), although plant Shannon diversity in these systems decreased over time and in relation to other vegetation states (submerged or freely-developed vegetation; Fig 8B). Thus, the N retention function in experimental wetlands seems closely linked to effects mediated by plant identity or vegetation state, while it was indifferent to vegetation diversity. Latest diversity-function research suggests that diversity effects are often associated to a 'sampling effect', i.e. ecosystem function is facilitated if species with high performance capacity are present (e.g. Bracken & Stachowicz 2006; Cardinale *et al*. 2006).

In Paper V, three parallel ecosystem functions (N and P retention and litter decomposition) and biodiversity were investigated in highly dynamic, full-scale created wetlands. Biodiversity influences on ecosystem functioning, particularly regarding plant diversity, became apparent after accounting for abiotic factors (Table 3). Plant diversity parameters affected both P retention (plant richness) and N retention (functional plant diversity) positively (Paper V). This suggests that in highly dynamic created wetland environments, plant diversity may be important to assure functioning over time. Biodiversity is hypothesized to serve as an insurance against disturbance and to stabilize ecosystem functioning (Hooper *et al*. 2002, Loreau *et al*. 2003). Also, while N retention seemed indifferent to species loss within functionally uniform plant assemblages (e.g. emergent plants, Paper IV), freely assembled vegetation in agricultural created wetlands (Paper V) could consist of up to five different functional groups (submerged, rooted

floating-leaved, free-floating, emergent, and woody wetland species). Created wetlands did not maintain extensive cover of emergent vegetation; large parts exceeded depth limits tolerable by emergent plants (average depth 1 m, Paper I). These parts of the water body were instead vegetation-free or colonized by aquatic (obligate hydrophytic) vegetation. The positive influence of functional plant diversity on N retention found in paper V may therefore rather be interpreted as a positive effect of vegetation as such (compared to vegetation-free), or as an effect of an even distribution among all the functional plant groups, ensuring that emergent plants are abundant (among others).

Temporal trends are apparent for biodiversity, but not for N retention; time effects depend on vegetation type.

In Paper IV, biodiversity and N retention were investigated over a four year period (Fig 8) to investigate effects of time and succession. N retention varied among years (likely due to temperature effects), but no trend across years was apparent (Fig 8E). Only in the first year after creation, wetlands without planted vegetation performed somewhat lower N retention than in the following years.

Diversity of macroinvertebrates and plants undergoes temporal changes as created wetlands mature (Paper IV; age effect in Paper I). Planted wetlands had higher initial plant diversity than unplanted wetlands, but in all vegetation states plant and macroinvertebrate species numbers increased over time (Fig 8A, C). Over a four-year period, however, plant Shannon index decreased in planted emergent wetlands, as a few plant species became highly dominant (Fig 8B). Hence, there were significant interactions of time and vegetation state. In contrast, the initial differences in macroinvertebrate diversity between vegetation states leveled out over time (Fig 8D).

These results suggest that time effects on biodiversity differ between (i) diversity measures (species richness/Shannon index),

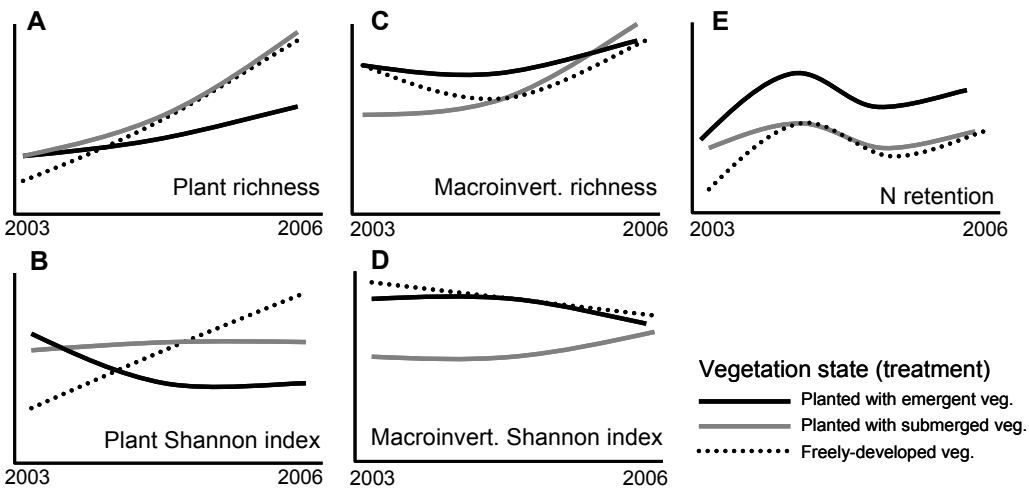

Fig 8. Temporal variation in biodiversity and nitrogen retention of experimental wetlands with differing vegetation states (n=6 each; Paper IV). Temporal trends (across years) for biodiversity (A-D) depend on vegetation state (legend), biotic group (plants or macroinvertebrates), and applied diversity measure (richness or Shannon index); time and vegetation state interactions were significant (Paper IV). Nitrogen retention (E) function differs between years and between vegetation states, but no continuous trend across years and no interaction were observed.

(ii) biotic groups (plants/macroinvertebrates), and (iii) vegetation states (emergent/submersed/freely-developed).

In conclusion, for an assessment of created wetland services, across-year trends need to be regarded, particularly for biodiversity. Species accumulation of both plants and macroinvertebrates is prevailing during at least 4 years after establishment, indicating ongoing external recruitment. Relative species abundance is changing faster in planted than unplanted wetlands, but differs between emergent and submerged vegetation states, suggesting that competition impacts on diversity are prevailing already after two years if emergent vegetation dominates.

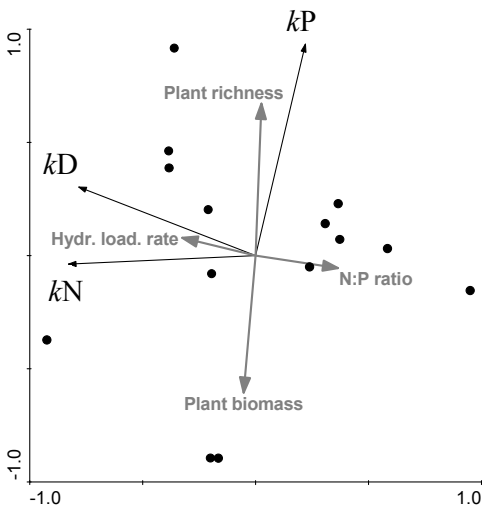

Fig 9. Joint ecosystem functioning in created wetlands. Three ecosystem functions, nitrogen retention, phosphorus retention, and litter decomposition, described by the process rate coefficients *k*N, *k*P and *k*D (black arrows), respectively, were simultaneously employed as multiple response variables in redundancy analysis RDA (Paper V); a set of 10 environmental parameters was tested for how much variation in joint functioning was explained among 14 created wetlands (black circles). The graph shows the final ordination diagram. Significant environmental gradients (grey arrows) delineate strength and direction of environmental influence by arrow length and direction (analogous interpretation for response variables; black arrows). Proximity between circles delineates similarity of environmental characteristics and ecosystem function of wetlands. In total, 67% (p=0.002) of the functional variation was explained.

Function - function links

The risk for methane emission is independent from the N retention capacity of created wetlands.

Methane emission is an example for an environmental risk (climate gas emission) which may increase due to large-scale wetland creation (Mitsch *et al*. 2001; Verhoeven *et al*. 2006; Stadmark & Leonardson 2005, 2007). N retention is often the primary environmental goal targeted by large-scale wetland creation (Fleischer *et al*. 1994; Leonardson 1994; Mitsch *et al*. 2001; Paludan *et al*. 2002; Hey 2002; Kadlec 2005).

Methane emission (diffusional flux) from created wetlands was found to be generally low, although all wetlands had quantifiable methane production (Paper III). The extent of production could be predicted by wetland characteristics (Table 3; Paper III), of which some (nitrate concentration, plant cover) were shown to affect N retention processes of created wetlands (Table 3; Paper V). While nitrate concentration and plant cover can be expected to support N retention (Weisner *et al*. 1994; Kadlec 2005; Paper V), they tend to seem to suppress methane production (Paper III). Methane emission was not correlated to the N retention predictions (Table 1) for created wetlands. In conclusion, the investigated environmental risk and benefit of created wetlands can be managed independently and there is potential to optimize N retention.

Certain wetland functions are coupled: N retention increases with fast litter decomposition.

In accordance to the literature, the regression models for N retention processes (Table 3) indeed predicted 40-70% of the functional variation based on parameters known to affect denitrifier activity or composition: *hydraulic load* (Phipps & Crumpton 1994; Kjellin *et al.* 2007; Paper II), *nitrate concentration* (Ka-

dlec 2005; Seitzinger *et al* 2006; Beaulieu *et al*. 2008; Paper II), *plant biomass* (Weisner *et al*. 1994; Eriksson & Weisner 1997, 1999; Lin *et al*. 2002) and *plant diversity* (Ruiz-Rueda *et al*. 2008). However, in addition to these factors, the model fit and explanatory power improved further (60-90%; Paper V), if the decomposition rate coefficient *k*D was included as an independent variable. Litter decomposition rate limits the turnover of assimilated carbon (i.e. plant biomass) for heterotrophic consumption (Webster & Benfield 1986; McKie *et al*. 2006). For created wetlands, not only *carbon amount* (resource pool size: plant biomass), but also efficiency of *carbon processing* (litter decomposition) facilitate N retention. Hence, different process rates may be coupled in created wetlands.

In conclusion, if N retention and decomposition rate are linked, wetland management needs to develop strategies to optimize several functions simultaneously.

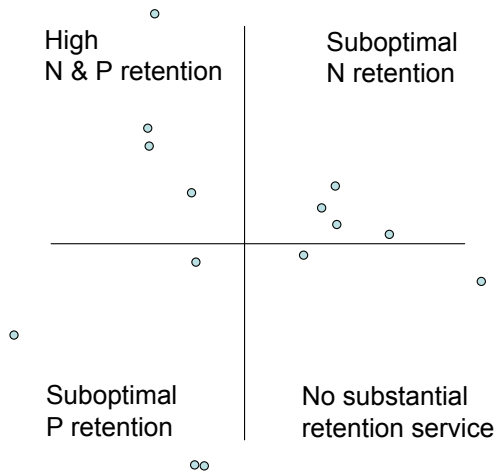

Fig 10. Multiple ecosystem services in created wetlands. Most wetlands (circles as in Fig 9) have suboptimal capacity of either N or P retention. Some wetlands, however, are capable of performing substantial N and P retention simultaneously (upper left corner). Furthermore, plant richness (an ancillary ecosysystem service) could also be sustained parallelly in these systems, considering the environmental parameters distinguishing joint ecosystem functioning among created wetlands (grey arrows in Fig 9).

Joint ecosystem functioning: Created wetlands can sustain multiple ecosystem services.

In Paper V, joint ecosystem functioning, i.e. the simultaneous performance of the wetland functions N retention, P retention and litter decomposition, was investigated (Fig 9) to assess the factors which distinguish the capacity for multifunctionality.

Variation in joint ecosystem functioning was explained by abiotic and biotic wetland characteristics, i.e. N:P ratio and hydraulic loading rate, as well as plant richness and plant biomass. Two thirds of the total functional variation were explained; biotic factors accounted for more than one third of the explainable variation.

The functions responded differently to environmental parameters (Fig 9). P retention increased with plant richness and decreased with plant biomass. N retention and litter decomposition varied independently from plant richness and biomass; these two functions were constrained by hydraulic load (+) and increasing N:P ratio (-) instead (Fig 9).

These results indicate that only some wetlands were capable of performing the ecosystem services N and P retention simultaneously and substantially (Fig 10), while most wetlands performed one service suboptimally. Wetlands with high simultaneous N and P retention tended to have higher hydraulic loading rate, lower N:P ratio, higher plant richness and lower plant biomass, compared to the average over all 14 wetlands. Suboptimal capacities of either service were distinguished by very high hydraulic load in combination with high plant biomass (suboptimal P retention); alternatively (suboptimal N retention) by high N:P ratio, thus slow litter decomposition, and low hydraulic loads. However, with constellations of low hydraulic load and plant richness, combined with high N:P ratio and plant biomass, created wetlands may run the risk of providing no substantial nutrient retention service.

SYNTHESIS

What explains diversity and ecosystem function of created wetlands?

Both diversity and functions of created wetlands were influenced by abiotic, vegetation, spatial (regional), and temporal parameters, and the magnitude and direction of the influence differed. Further, two types of interactions were observed, between diversity and function and among functions.

Local environment. Generally, the explained variation fractions for composition and diversity of any of the three investigated biotic groups (plants, macroinvertebrates, bacteria) were low, usually below 30% (Table 2), whereof minor proportions were accounted for by abiotic, water quality parameters (Papers I & II; unpublished data).

In contrast, major variation fractions of ecosystem functioning (single and joint functioning) were explained by the local environment (40% to 70%), with abiotic parameters exceeding the proportions explained by biotic ones, for most functions (Table 3).

Vegetation. Plant diversity influenced bacterial diversity and composition (Paper I), and more so than abiotic parameters. Vegetation states with high biomass affected plant diversity and evenness negatively in the long-term (Paper IV).

Vegetation effects on ecosystem functioning were related to diversity and abundance (Table 3) as well as vegetation state (Fig 8). Vegetation diversity had positive or no effects on functions (Table 3; Paper IV), while vegetation abundance was positively related to some functions (N retention) and negatively to others (P retention, litter decomposition, methane production). Vegetation effects were of subordinate importance compared to abiotic parameters in dynamic wetland environments for all functions but P retention. In more stable abiotic environments, N retention capacity differed with vegetation state, being highest in highly productive states.

Regional watershed/landscape. Spatial factors (including regional and location parameters) were rather important for biodiversity, explaining more than the local abiotic/biotic environment, at least for larger biota (plants and macroinvertebrates). For these groups, local assemblage establishment seemed to depend on the landscape context (connectivity, distance to source habitats) and the total regional habitat pool (habitat density). Similar patterns have been shown for lake assemblages; small organisms depending on environmental, larger organisms on dispersal conditions (Beisner *et al*. 2006).

Spatial parameters were not of directly related to ecosystem functioning. However, the location of created wetlands in a watershed is a management decision; spatial location determines the magnitude of load and concentration (which are important for N retention) received by a given wetland; thus spatial factors may indirectly influence function.

Time. Biodiversity was also affected by time, i.e. ongoing succession/aging over the first few years after establishment. The number of species increased (at least over 4 years), while the compositional diversity and evenness developed depending on the initial species constellations. If planted wetlands serve as models for 'late succession stages', diversity of plants is likely to decrease, when created wetlands reach the later succession stages.

Temporal succession trends seemed less important for the ecosystem service N retention, however daily and seasonal variations were observed (Papers IV & V). Retention function (N) has been reported to be annually variable (Kadlec & Knight 1996; Kadlec 2005), but variation is often related to seasonal/interannual variation in flow, concentration, and temperature, rather than to aging/succession effects. Wetland age could partly be relevant for methane production, i.e. the risk for climate gas emission may increase in older wetlands. Although not investigated

here, aging effects may also be relevant for litter decomposition and P retention. Earlier studies showed that litter decomposition increases with wetland age (Atkinson & Cairns 2001; Spieles & Mora 2007) mainly as a result of litter shortage during the first years, and that P retention decreases in older systems (Kadlec & Knight 1996; Braskerud *et al*. 2005), mainly as a result of saturated P binding capacities.

Interactions. Diversity-function links were shown to be particularly relevant for highly dynamic environments, where positive effects of diversity parameters on process rates involved in N retention, P retention and litter decomposition were observed after accounting for abiotic variation (Table 3). Functional diversity among bacterial denitrifying genes was positively correlated to N retention (Fig 7). N retention in experimental wetlands with low environmental variation, seemed indifferent to succeeding biodiversity loss (Fig 8). Function-function links were found for N retention and litter decomposition; N retention processes were facilitated when litter decomposition was fast (Paper V).

IMPLICATIONS & APPLICATIONS

(1) The ecosystem services provided by created agricultural wetlands are comparable to natural systems or to other constructed wetland types; created wetlands may thus contribute to biodiversity conservation and eutrophication abatement on watershed scales.
(2) Ecosystem functioning of created wetlands was more clearly linked to environmental conditions than biodiversity. Functioning may thus be managed primarily by wetland design and location in the watershed. Biodiversity management seems most efficient on regional scale; benefits due to increasing total habitat densities per watershed by more than 30% seem very likely. Optimizing the abiotic prerequisites for N retention (N concentration and hydraulic load) does not seem to be contradictory to biodiversity aims.
(3) Apart from wetland design/placement, management of vegetation type and extent is important for ecosystem functioning and biodiversity. Vegetation management affects ecosystem services differently: a high plant biomass favors N retention, but may inhibit plant diversity, and also P retention.
(4) Diversity parameters were positively related to ecosystem functions; a given function was enhanced by diversity of a particular biotic group or by functional diversity. This suggests, that if created wetlands are aimed at sustaining several parallel ecosystem functions (and services), biodiversity should be promoted.
(5) Functional diversity and composition of denitrifiers is influenced by retention prerequisites (N concentration and hydraulic load); high functional diversity may be linked to higher N retention capacity of created wetlands.
(6) Wetland creation contributes to (yet suboptimal) N retention, while the risk for simultaneous methane emission is low. An optimization of the N retention function is unlikely to increase methane emission.
(7) Rates of N retention and litter decomposition were functionally coupled. Created wetlands may require management for multifunctionality, in order to sustain a specific ecosystem service.

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