Tracking phosphorus enrichment and ecosystem change in wetlands

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Cover photo

Peninsula Lake (top), Swamphen Lake (bottom) at Capel Wetlands Centre (K. Kauhanen).

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Kirsi J. Kauhanen 20 February 2019

Statement of Contribution by Others

This PhD project and the associated Capel Wetlands Centre wastewater trial were fully funded by Western Australian Water Corporation. They also supplied operational and monitoring data for the Capel Wastewater Treatment Plant as detailed in this thesis.

Dedication

This thesis is dedicated to my family - those far away who brought me up to take on challenges and persevere, and those close by who support me in doing that every day.

Thesis Abstract

This thesis describes how phosphorus (P) storage of ecosystem components and ecosystem structure change simultaneously as a wetland undergoes nutrient enrichment. Three experiments at Capel Wetlands Centre (Western Australia), where treated municipal effluent was discharged into P-limited wetlands, tracked progress of eutrophication within and between different functional zones (submerged bare sediment, submerged aquatic vegetation (SAV), emergent *Typha* stands), over different temporal scales: a mesocosm experiment (3-month), and seasonal (9-month) and annual (3-year) monitoring. Conceptual models were developed to describe the outcomes.

The experiments revealed several important aspects of the eutrophication process. Seasonal patterns in P-dynamics evident in unenriched lakes were overwhelmed by high P-loads, and progression to hypereutrophy occurred despite not all P-stores being saturated. Functional zones responded differently to enrichment, contingent on the type and number of P-stores with different uptake patterns. P concentration in stores responded more rapidly than mass, with both measures needed to assess the progress of eutrophication. Phytoplankton, periphyton and SAV responded rapidly to enrichment, but uptake was minimal, while topsoil, litter, floc and *Typha* responded more slowly but were larger P-stores. Bare sediment eutrophied most rapidly. Enrichment caused loss of SAV; then re-released P stimulated phytoplankton and floc production. Diverse stores in the *Typha* zone slowed the progress of eutrophication, but *Typha* itself declined due to increased depth and decreased water quality caused by wastewater discharge. Maintaining vegetation is recommended to maximise direct and indirect P uptake and biodiversity benefits. Litter and floc took up substantial P in early enrichment, but potentially become P-sources when water/sediment quality deteriorates. Their presence reduced soil/water interaction potentially limiting P-uptake by soil.

The holistic approach used here chronicles eutrophication as a changing flowpath of interrelated reactions between different ecosystem components over different temporal scales. It provides a functional and transferable understanding of eutrophication, necessary for successful wetland management.

List of Abbreviations

Al	Aluminium
ANZECC	Australian and New Zealand Environment Conservation Council
BOD	Biochemical oxygen demand
CWC	Capel Wetlands Centre
DO	Dissolved oxygen
EC	Electrical conductivity
Fe	Iron
FRP	Filterable reactive phosphorus
ID	Internal diameter
Mn	Manganese
N	Nitrogen
NH_4-N	Ammonium nitrogen
NOD	Nitrogenous oxygen demand
NO_x-N	Oxidised nitrogen (nitrate and nitrite)
ORP	Oxidation-reduction potential
P	Phosphorus
SCP	Swan Coastal Plain (in southwest Western Australia)
SS	Suspended solids
TN	Total nitrogen
TP	Total phosphorus

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CHAPTER 1: INTRODUCTION

1.1 The eutrophication conundrum

Nutrient enrichment and associated eutrophication of wetlands and other aquatic ecosystems remains a major environmental concern across the world (Davis and Koop 2006, Schindler 2006, Verhoeven *et al.* 2006, Smith and Schindler 2009, Hefting *et al.* 2012, Dodds and Smith 2016, Schindler *et al.* 2016) and is associated with a range of adverse ecosystem changes (Table 1-1). The problem has been extensively studied for decades and yet research interest appears to be increasing rather than decreasing (increased numbers of publications on eutrophication, Li and Nan 2017). Why is it that despite all the research undertaken we are not able to better manage wetlands undergoing eutrophication? What are we missing that could improve current management?

Eutrophication has been described as "the process of nutrient enrichment in waterbodies that causes excessive plant growth and accumulation of organic matter" (Boulton *et al.* 2014, p.253). Eutrophication is not a simple process but rather involves a multitude of interrelated reactions by different ecosystem components over different temporal scales (Howard-Williams 1985). The associated processes are temporally sensitive because: 1) some ecosystem components are able to take up nutrients nearly instantaneously while other components respond more slowly (Richardson and Vaithiyanathan 2009); 2) the storage period of nutrients before recirculation varies among ecosystem components (Vymazal 2007); 3) while all phosphorus (P) removal processes apart from soil accretion are considered finite, the time it takes to exhaust this capacity differs between ecosystem components (Richardson and Marshall 1986). Consequently, different nutrient storage and cycling pathways dominate at different points in time as eutrophication progresses and this is reflected in the timing of ecosystem changes (e.g. vegetation and water quality changes) (Richardson and Marshall 1986). Furthermore, the overall rate of eutrophication and thus the temporal scales of the processes involved are affected by the size and timing of nutrient inputs (e.g. whether a pulse or press disturbance) (Smith *et al.* 1999, Butzler and Chase 2009, Murphy *et al.* 2012, Tuya *et al.* 2015).

Understanding how eutrophication proceeds over time and how the associated processes respond and interact over time is crucial for effective management of wetlands. However research providing a holistic view of the processes involved at different temporal scales is rare (but see Florida Everglades (USA) research e.g. Noe *et al.* 2002, Childers *et al.* 2003, Noe *et al.*

2003, Gaiser *et al.* 2005, Noe and Childers 2007, Dierberg *et al.* 2012). Research to date commonly describes a selected few processes at a particular temporal scale (e.g. Menon and Holland 2014, Dunck *et al.* 2015, Olsen *et al.* 2015), rather than the naturally occurring interactive suite of processes over a range of temporal scales. While the reason for this is clear, the reductionism entailed by focussing on single elements of the issue precludes a holistic understanding of the eutrophication process. Synthesising a holistic view is also hindered by the fact that each of the studies has been undertaken in different systems with highly variable methods, system characteristics and circumstances.

Another gap in our understanding relates to spatial variation within wetlands. The occurrence of different ecosystem components in different wetland zones affects the nutrient uptake and storage pathways available, both spatially and temporally, and therefore the overall progress of eutrophication in wetlands. How does eutrophication proceed in wetlands containing a variety of different habitats (e.g. vegetated and unvegetated zones), all of which potentially react differently to enrichment? While studies have identified differences in the process of eutrophication in different zones (e.g. vegetated versus unvegetated, SAV versus emergent vegetation) (White *et al.* 2006, Sollie and Verhoeven 2008, Menon and Holland 2013, Di Luca *et al.* 2015), it remains unclear whether zones nested within a single system respond differently. Furthermore, interactions may occur where one zone has competitive advantage over another or where one zone protects another from the influence of nutrient enrichment. All of which would change the holistic wetland response to enrichment.

This thesis was designed to address these two knowledge gaps. As P is often the limiting nutrient in freshwater ecosystems (Reddy and DeLaune 2008), including the wetlands in this thesis, and thus the likely driver for eutrophication, this thesis investigated the temporal and spatial dynamics of P to better understand the process of eutrophication.

Table 1-1: Effects of eutrophication in wetlands (Correll 1998, Smith 2003, Verhoeven *et al.* 2006).

Effects of eutrophication
• Increased biomass of phytoplankton, suspended algae, benthic and epiphytic algae and macrophytes.
• Increased biomass of consumer species.
• Shifts to bloom-forming algal species that might be toxic or inedible.
• Changes in species composition of macrophytes.
• Increased incidence of fish kills.
• Reductions in species diversity.
• Decreased water transparency.
• Oxygen depletion.
• Shifts between stable ecosystem states.
• Taste, odour and drinking water treatment problems.
• Decreases in aesthetic value.

This thesis provides a rare, holistic account of how P storage of ecosystem components changes in different functional zones as a wetland undergoes nutrient enrichment, thereby addressing several limitations and gaps in existing knowledge (Table 1-2). Firstly, P storage through time was tracked across all ecosystem components simultaneously in contrast to previous research which commonly focussed on individual P stores and cycling processes. P entering wetlands will cycle through a range of potential stores simultaneously, but these processes have been rarely documented concurrently, so examples that clarify these processes are needed. Secondly, a multiple lines of evidence approach was used comprising three studies conducted at different temporal scales. This approach provided more robust conclusions than previous research limited to a particular temporal scale (e.g. Mustafa and Scholz 2011, Di Luca *et al.* 2015). Thirdly, ecosystem changes associated with nutrient enrichment are presented in the context of changing P storage (and vice versa) reflecting the interconnected nature of the two. This addresses a common limitation in previous research of presenting ecosystem changes only in the context of changes in ambient or incoming water quality with limited or no data on changes in TP storage within the system (Kagalou *et al.* 2008, Macek *et al.* 2010, Orth *et al.* 2010, Wentzell *et al.* 2016). Fourthly, the research explores differences in the processes and progress of nutrient enrichment and P storage between functional zones (unvegetated and differently vegetated zones) within a wetland. Although variation in P storage and cycling due to differences in vegetation type and cover among

wetlands has been studied, variation between the different zones within a single wetland is poorly understood.

Overall, this research provides a small-scale but detailed example of the processes and progress of nutrient enrichment that supports a broader scale understanding of eutrophication. The knowledge produced by this research will assist wetland managers in better understanding nutrient enrichment and associated changes in P storage, and in designing more effective management and monitoring regimes to achieve optimal P retention and positive biodiversity outcomes. This research will also be of value to managers of treatment wetlands, as it goes beyond the 'black box' view (focussed on P inputs and outputs), instead exploring internal P storage and cycling processes responsible for treatment performance. Papers continue to be published that make long-term wetland management recommendations on the basis of short-term studies into the P removal capacity of selected ecosystem components, while failing to address the longer-term perspective of these removal processes and the wider context around them (e.g. Schaafsma *et al.* 1999, Lu *et al.* 2009, Tercero *et al.* 2017). In comparison, this research provides a holistic view of the system and addresses how the processes change over time.

Table 1-2: Existing research and its limitations (P = phosphorus).

	Main areas of research focus in existing literature (overlapping)		
	General P storage and cycling in wetlands	**Nutrient enrichment and eutrophication**	**Treatment wetlands**
Good understanding	Different forms and transformations of P in wetlands. Available P stores and cycling pathways. Short- versus long-term nature of storage. Factors regulating P storage and cycling (e.g. Howard-Williams 1985, Richardson 1985, Reddy *et al.* 1999, Reddy and DeLaune 2008, Vymazal 2016b)	Changes in wetland ecosystems as a result of nutrient enrichment. These changes are commonly linked to incoming nutrient concentrations and/or ambient nutrient concentrations in water (e.g. Scheffer *et al.* 1993, Smith *et al.* 1999, Smith 2003, Schindler 2006, Smith *et al.* 2006, Schindler and Vallentyne 2008, Smith and Schindler 2009)	P retention capacity (performance) of treatment wetlands under different environmental/input conditions. Constructed wetlands for wastewater treatment. P retention modelling. (e.g. Greenway 2005, Kadlec 2005, Vymazal 2007, Kadlec and Wallace 2009, Vymazal 2011, Walker and Kadlec 2011, Kadlec *et al.* 2012, Kadlec 2016, Land *et al.* 2016, Vymazal 2016b)
Limitations	Commonly focused on individual stores or cycling processes, with limited efforts to track P storage and cycling across all ecosystem components simultaneously and over time. Examples incorporating most ecosystem components include (e.g. Mitsch *et al.* 1979, Richardson and Marshall 1986, Noe *et al.* 2003).	Limited efforts to track P storage and cycling of all ecosystem components within a wetland undergoing nutrient enrichment and to relate this to observed ecosystem changes. Examples where P storage of ecosystem components has been sampled at different nutrient levels include (e.g. Noe *et al.* 2002, Noe and Childers 2007, Menon and Holland 2013, Di Luca *et al.* 2015).	Largely focussed on retention capacity (inputs-outputs) with "very limited information on internal processes and mass balances of P in wetlands" (Reddy and DeLaune 2008, p.401) Examples of treatment wetlands where P storage of ecosystem components has been tracked (at least partly) include e.g. Des Plaines River and Olentangy River constructed wetlands (e.g. Mitsch *et al.* 1995, Wang and Mitsch 2000, Mitsch *et al.* 2014), Everglades (e.g. Noe and Childers 2007), Houghton Lake (e.g. Richardson and Marshall 1986, Kadlec 2009a, b, Kadlec and Bevis 2009).
Limitations	Differences in P storage and cycling between functional zones (e.g. bare sediment and vegetation zones) within a wetland (e.g. Menon and Holland 2013)		
Limitations	Wetland P storage and cycling are impacted by a number of regulating factors resulting in a wide range of P storage and cycling environments. While a few environments have been well researched (e.g. Everglades USA), most have not, and particularly holistic case studies of P storage and cycling in different environmental settings are needed.		

1.2 Thesis aim and research questions

The aim of this thesis is to provide a holistic account of simultaneous changes in P storage by ecosystem components in different functional zones, as a wetland undergoes the process of nutrient enrichment, and also to track associated changes in ecosystem characteristics. Three experiments were used to test and refine conceptual models of the progression of eutrophication both within and between three different functional zones (submerged bare sediment, submerged aquatic vegetation (SAV), stands of the emergent macrophyte *Typha orientalis*), over different temporal scales: a short-term mesocosm experiment (3 months), seasonal (9 months) and annual (3 years) wetland studies. The two whole-wetland studies involved enrichment via addition of treated municipal effluent. This meant that the overall treatment effect combined the effects of nutrient addition and water addition. The overarching research questions are:

- How does phosphorus storage and cycling change as a wetland undergoes nutrient enrichment and how does this differ between functional zones?
- How is nutrient enrichment reflected in ecosystem change in the different functional zones?

1.3 Definition of terms and quantities

For the purposes of this thesis, TP storage is defined as the amount of total P stored per unit area (e.g. g/m^2), derived from TP concentration and mass per unit area for each ecosystem component. Changes in TP storage are related to changes in either TP concentration, store mass or both. Changes in P cycling were inferred from changes in TP storage of ecosystem components over time. Quantifying P flux along any particular pathway was beyond the scope of the thesis. The term functional zone refers to areas (zones) of different vegetation type (emergent or submerged) or submerged bare sediment. These zones operate (function) differently with regard to P storage and cycling as they have different P stores and cycling pathways. Assessment of ecosystem change addresses changes in vegetation characteristics and general water quality variables (chlorophyll α, turbidity, temperature, pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), electrical conductivity (EC)) that form the structural basis of the food web. Other effects on ecosystems caused by nutrient enrichment (e.g. changes in fauna) were outside the scope of this thesis. Such changes have been widely studied and are predictable based on the observed changes in vegetation and water quality.

1.4 Opportunity at Capel Wetlands Centre

In Western Australia, the state government owned Water Corporation embarked on a new project involving controlled discharge of treated municipal effluent into wetlands at the Capel Wetlands Centre (CWC). The CWC wetlands are rehabilitated mineral sand mining pits containing clay and silt (mineral sand processing fines) that are high in iron and manganese and readily take up P from the water column. This P is then stored in the sediment, limiting the P available for primary production and consequently the development of functional wetland ecosystems. The discharge of municipal effluent (rich in P) to the wetlands provided a unique opportunity to study nutrient enrichment because the wetlands would be artificially enriched in a controlled manner. Due to the historically P deficient state of the wetlands, they were particularly suitable for studying changes in P storage and cycling over the nutrient enrichment continuum.

1.5 Thesis structure

Following the General Introduction, Chapter 2 (Development of Conceptual Models and Research Approach) provides theoretical context for the thesis through a literature review of P storage, cycling and impacts of nutrient enrichment. The literature review informed the creation of conceptual models that illustrate expected changes in the different functional zones as nutrient enrichment progresses. Chapter 2 also describes more detailed research aims (sub-questions) and hypotheses developed to test and refine these conceptual models. Chapter 3 (Experimental Design) describes methodology applicable to all three independent but related studies in the thesis, including: study area, sampling, laboratory analysis and data processing.

Chapters 4-6 each present a study focussed on one of the temporal scales: a short-term mesocosm experiment (3 months), seasonal (9 months) and annual (3 years). Chapter 4 explores wetland P storage and cycling with a seasonal dataset over a nine-month period in one treatment wetland in the early stages of enrichment (receiving treated municipal effluent) compared with two unenriched wetlands. Chapter 5 extends that study to cover wetland P storage and cycling over three consecutive spring periods in the treatment wetland. Chapter 6 explores the research questions in a more controlled environment through a nutrient enrichment experiment using in-situ mesocosms. Chapter 7 (General Discussion) provides a synthesis of the research and discusses its implications for understanding and managing wetland ecosystems undergoing nutrient enrichment.

CHAPTER 2: DEVELOPMENT OF CONCEPTUAL MODELS AND RESEARCH APPROACH

2.1 Introduction

Non-quantitative conceptual models are commonly used to synthesise and communicate understanding of complex dynamics in environmental systems, by identifying key system components and interactions between them (Odum 1983, Gentile *et al.* 2001, Ogden *et al.* 2005, Sethi and Hollmen 2015). In this thesis, the development of conceptual models comprised two steps: 1) basic models showing the available P stores and cycling pathways in the different functional zones under stable nutrient conditions; 2) adjusting these basic models to reflect changes in P storage and cycling over the nutrient enrichment continuum. Detailed research aims and hypotheses that would address the knowledge gaps and assumptions identified by literature review were then derived from these conceptual models, to be tested in subsequent chapters using multiple lines of evidence from three investigations at different temporal scales.

The literature review and conceptual models focussed on the functional zones and key ecosystem components present at CWC. The functional zones were: bare sediment in open water; submerged aquatic vegetation (SAV) comprising macroalgae *Chara* and *Nitella* spp. (SAV was not present in all wetlands); stands of the emergent macrophyte, *Typha orientalis*. Ecosystem components were: surface water, soil (wetland sediment), floc (flocculent sediment), plant litter, SAV and *Typha*. Periphyton was included in the literature review as its contribution to mass balance was estimated in the mesocosm study, however it was not incorporated into the conceptual models.

2.2 Basic conceptual models of phosphorus storage and cycling

Within a wetland, P is present in organic and inorganic forms that can each be divided further into particulate and dissolved forms (Reddy and DeLaune 2008). These are cycled and stored through a range of physical, chemical and biological pathways involving wetland ecosystem components. A generalised P cycling model (Figure 2-1) developed from several models available in literature (Reddy and DeLaune 2008, Kadlec and Wallace 2009, Boulton *et al.* 2014, Mitsch and Gosselink 2015) shows inflows and outflows of P to/from the wetland ecosystem and key P storage and cycling pathways within the wetland. This initial model incorporates the different forms of P necessary to understand the processes involved and interpret changes in P

storage and cycling. However, for the purpose of this thesis, the model can be simplified to focus on total P storage and cycling. Some aspects of the model can be left out due to the characteristics of the study area (i.e. aspects that are not applicable at CWC).

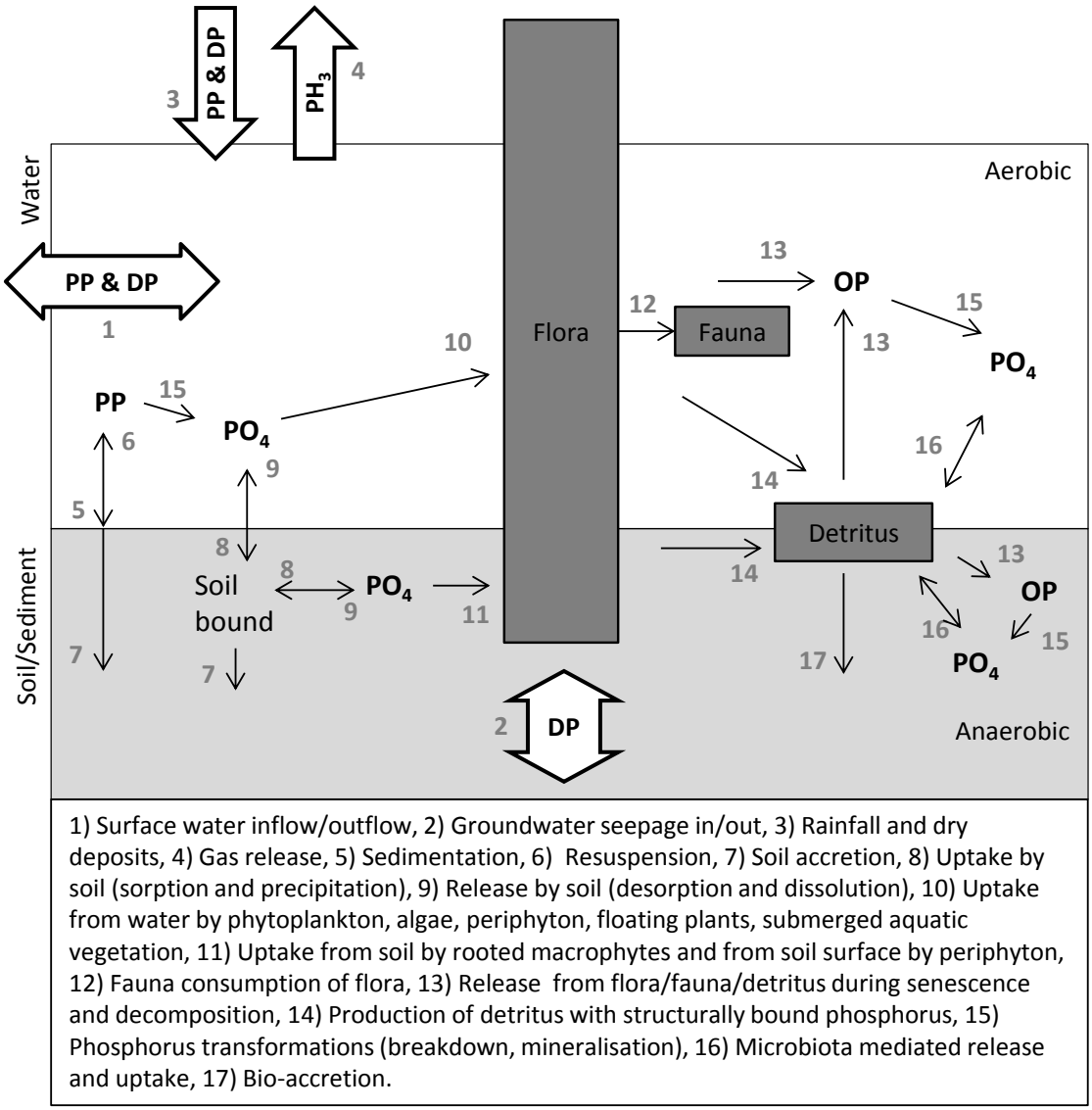

Figure 2-1: Model of phosphorus (P) cycling in wetlands. Developed by Kauhanen from several existing models (Reddy and DeLaune 2008, Kadlec and Wallace 2009, Boulton *et al.* 2014, Mitsch and Gosselink 2015). Abbreviations include PP = particulate P, DP = dissolved P, OP = organic P, PO_4 = dissolved inorganic P, PH_3 = gaseous P (phosphine).

P cycling within a wetland or a functional zone varies depending on the number and type of P stores available and pathways between them. In the bare sediment zone, P storage and cycling is mostly limited to water, sediment and potentially suspended or benthic algae, whereas the SAV and *Typha* zones provide more potential stores and cycling pathways through the presence of vegetation and larger amounts of litter (Figure 2-2).

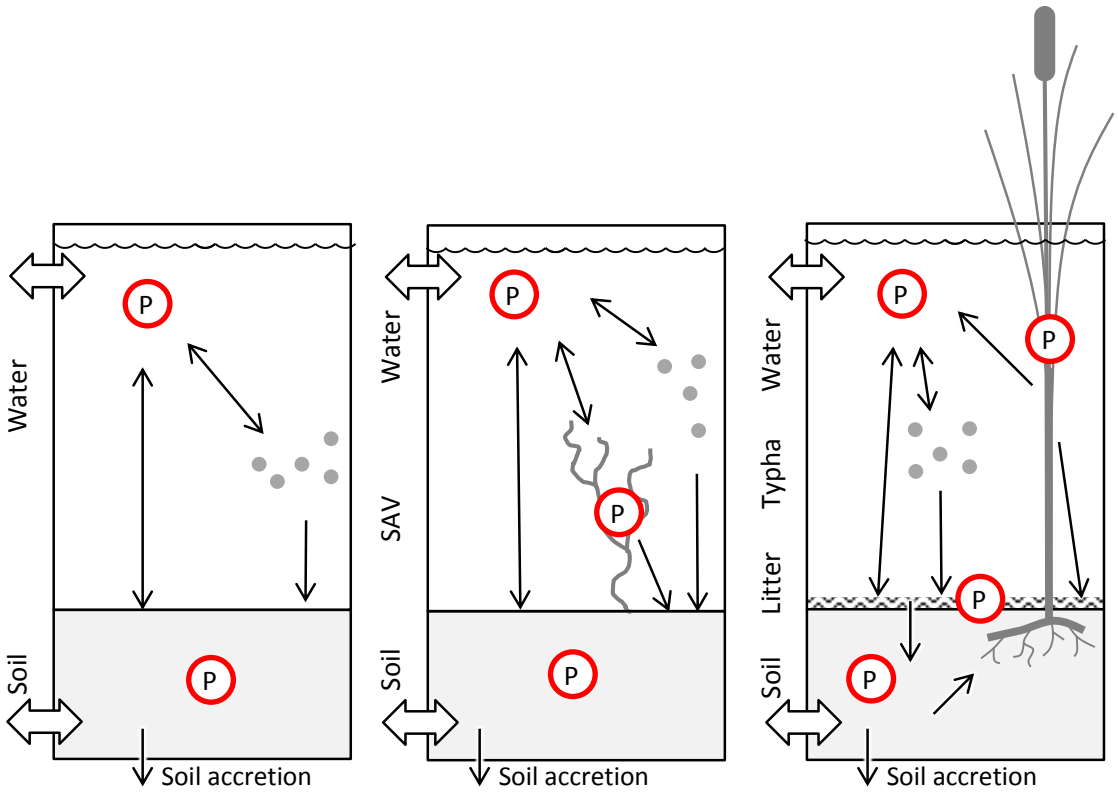

Figure 2-2: Conceptual models of basic phosphorus storage and cycling in bare sediment, SAV and *Typha* zones. Bulky arrows indicate movement of phosphorus in and out of the system, narrow arrows indicate phosphorus cycling pathways within the system and P in circles indicate the stores available.

P enters a wetland via surface or groundwater flow or to a lesser extent via rainfall and atmospheric dry deposits. P exits a wetland via surface or groundwater flow (Reddy *et al.* 1999). Gaseous loss of P (phosphine) may occur in systems that have very high P levels, however the topic remains poorly researched (Kadlec and Wallace 2009). P inputs via rainfall and atmospheric dry deposits and P outputs via gaseous losses were considered likely to be negligible at the study area so were not included in the conceptual models (Figure 2-2).

Particulate P is removed from the water column largely by sedimentation or entrapment in vegetation. Some particulate organic P may be transformed into dissolved organic P and thence into dissolved inorganic P (PO_4), returning it to circulation (Reddy and DeLaune 2008). While some resuspension of sediments may occur (e.g. bioturbation or wind driven), net flux is generally towards the soil. Sedimentation results in gradual soil accretion, representing a long-term P sink (Richardson and Vaithiyanathan 2009). In the conceptual models, sedimentation of particulate P and potential resuspension is illustrated by the two-way arrow between water

and soil that also covers other soil-related P uptake and release. Gradual soil accretion is illustrated by a one-way arrow at the bottom of the models (Figure 2-2).

Phosphate (PO_4) is removed from water by soil/sediment through sorption (adsorption and absorption) and precipitation (Boulton *et al.* 2014). Physical adsorption is the movement of soluble inorganic P from soil pore water to the soil mineral surface where it accumulates without penetrating the structure and maintains equilibrium with P in soil pore water. Chemical adsorption is similar but involves chemical bonding at the solid surface (Reddy and DeLaune 2008). The adsorbed P is only loosely bound to the soil and may be released if water and sediment chemistry change (Aldous *et al.* 2005). High clay content and high concentrations of iron (Fe) and aluminium (Al) oxides provide for stronger adsorption (Vymazal 2007, Douglas *et al.* 2016). Absorption is the diffusion (penetration) of the adsorbed P into the solid phase. Precipitation refers to the reaction of phosphate ion with metallic cations such as Fe, Al, calcium (Ca) or magnesium (Mg) to form amorphous precipitates. Over time these may turn into crystalline forms that are more stable. Dissolution of precipitates may occur when concentrations of any of the reactants fall below the solubility product of the compound (Reddy and DeLaune 2008). Movement of water down the soil profile may occur as a result of hydraulic pressure gradients, groundwater movement or 'pumping' by emergent vegetation (Boulton *et al.* 2014). These processes transport P deeper into the soil where further sorption, precipitation or plant uptake occur (Reddy and DeLaune 2008, Kadlec and Wallace 2009). In the conceptual models, uptake and release of P from soil is represented by the two-way arrow between water and soil (Figure 2-2). In this thesis, P in soil pore water was not measured separately but as part of the soil store.

Redox potential (ORP) and pH affect the solubility of P (Reddy and DeLaune 2008, Palmer-Felgate *et al.* 2011). Soils rich in Fe and Al form stable precipitates under acidic conditions but solubilities increase with increasing pH. Iron minerals associated with P are stable in aerobic conditions but as the soil becomes anaerobic and ORP decreases, reduction of ferric iron to ferrous iron results in increased solubilisation of P (Reddy and DeLaune 2008). Macrophytes may help to bind P to the sediment as some of the oxygen they transport to their roots for respiration leaks into the rhizosphere creating aerobic and oxidising conditions (Aldridge and Ganf 2003, Stottmeister *et al.* 2003).

Phosphate is the most bioavailable form of P whereas organic P and particulate inorganic P generally need to undergo transformations to become bioavailable (Reddy *et al.* 1999). Phytoplankton, periphyton, floating plants or submerged macrophytes are able to assimilate

PO_4 directly from the water column. Submerged macrophytes also take up PO_4 from the soil root zone. In contrast, emergent macrophytes such as *Typha* take up PO_4 solely from the root zone (Reddy *et al.* 1999), although their adventitious roots may also use nutrients from the bottom of the water column (Rejmánková and Snyder 2008). Periphyton may also assimilate organic P (Bentzen *et al.* 1992). All these plants store extracted P as organic P in their growing biomass.

The storage of P in plant biomass is mostly short-term as biota release a significant portion of stored P into water during senescence, litter fall and decomposition (Davis 1991, Kröger *et al.* 2007, Menon and Holland 2014, Wang *et al.* 2018). In the case of algae, floating and submerged plants the majority of stored P is released upon their death, with a small fraction contributing to long-term storage via bio-accretion (Kadlec and Wallace 2009). A greater portion (up to 50%) of P is retained in emergent vegetation, as P in above-ground parts is translocated back to underground roots and rhizomes when they senesce (Gopal and Sharma 1984, Garver *et al.* 1988, Reddy and DeLaune 2008). Emergent vegetation also has more robust and refractory supportive tissue than floating and submerged vegetation, resulting in greater accumulation of litter, and a greater contribution to P losses via bio-accretion (Reddy and DeLaune 2008). While decomposition is commonly associated with release of various forms of P, microbial colonisation of the decomposing material may also result in net sequestration of P in nutrient-enriched sites (Qualls and Richardson 2000, Cheesman *et al.* 2010). In the conceptual models, arrows indicate P uptake from water by plants (suspended algae, SAV, *Typha*) and litter (mainly *Typha* detritus), release to water by plants and litter, uptake from soil by *Typha*, and detrital contribution to sediment or litter layer (Figure 2-2).

The basic conceptual models (Figure 2-2) do not include a flocculent sediment layer (floc) as none was present at CWC prior to the commencement of the wastewater addition. Floc is poorly defined in the literature and terminology used to describe it varies (e.g. ooze, muck, gelatinous material) but it has generally been described as unconsolidated, low density material that can be easily disturbed and can be poured out of a sampling corer (Kadlec and Wallace 2009). Floc likely contains a significant microbial component as well as algal and macrophyte detritus (Droppo 2001, Neto *et al.* 2006, Kadlec and Wallace 2009), linking floc into P cycling pathways involving biota. Potential microbial colonisation of detrital matter would provide avenues for P uptake (as described above for litter). When floc was observed during the three studies, it was sampled as a separate store (defined as the portion of sediment that could be poured out of corer) providing an additional P store to inform the models.

Microbiota (bacteria, fungi, nematodes) are crucial for P cycling through mineralisation of organic P. Most microbial biomass in wetlands resides in the soil and in the decomposing litter layer (Reddy and DeLaune 2008) however some microbiota would be present in all of the wetland ecosystem components (Boon *et al.* 2014). Microorganisms assimilate some of the mineralised PO_4 into their own biomass, thus regulating the release of P into the water column, and playing a significant role in short-term P storage (Reddy *et al.* 1999). However, as the lifecycle of these organisms is short, the majority of the stored P is rapidly released back into circulation (Kadlec and Wallace 2009). Microorganisms are known to contribute to the creation of refractory organic compounds, thus removing some of the P from active cycling (Reddy *et al.* 1999). ORP has been found to affect microbial P uptake. In organic soils microbial P accounted for up to 20% of total P under aerobic/high ORP conditions but less than 10% of total P under anaerobic/low ORP conditions (McLatchey and Reddy 1998). In the conceptual models, microbiota were not considered separately, but rather as part of the parent material (e.g. soil) due to practical considerations of field sampling (Figure 2-2).

Fauna are actively involved in the cycling of P through consuming biota and bioturbation. Some P is incorporated into faunal biomass, however this storage is short-term and the majority of stored P is released during decomposition, with some contributions to refractory long-term storage. The role of fauna in P cycling is poorly understood (Howard-Williams 1985, Land *et al.* 2016) and is mostly excluded from studies into wetland P storage. There were no fish or other aquatic macrofauna at CWC that would have had a major impact on P storage or cycling. Invertebrate fauna was not sampled separately but rather as part of host material (e.g. in litter or plants) thus contributing to TP storage of that component. Therefore, fauna were not included in the conceptual models (Figure 2-2).

2.3 Conceptual models of phosphorus storage and cycling over nutrient the enrichment continuum

2.3.1 The nutrient enrichment continuum

The nutrient enrichment continuum assumes that a system accumulating nutrients will gradually become increasingly enriched and move along trophic categories from oligotrophic towards eutrophy. Very low levels of P accumulation may fall within the natural P assimilation capacity of a wetland, meaning that the wetland can sustainably transform and absorb the added P without significant changes to ecosystem structure or function or increases in

outgoing P concentrations (Richardson and Qian 1999). Average sustainable P assimilative capacity of 0.5 g/m^2/year (Vymazal 2007, Hefting *et al.* 2012) or 1 g/m^2/year (Richardson and Qian 1999) has been proposed for natural wetlands whereas slightly higher range of 0.5-5.0 g/m^2/year (Mitsch *et al.* 2000) or 4.0 g/m^2/year (Hefting *et al.* 2012) has been proposed for constructed wetlands. The effect of incoming P load is also dependent on hydraulic retention time (time that effluent remains in the wetland) as longer retention time increases P removal (Tanner *et al.* 1995, Toet, Van Logtestijn*, et al.* 2005) and could consequently result in faster exhaustion of overall P uptake capacity.

Furthermore, ecosystem changes may develop from the upstream (i.e. close to P input) portion of a wetland, but then stabilise into a new equilibrium able to assimilate incoming P in the long-term without further ecosystem changes or increases in outflowing P concentration (Figure 2-3) (Richardson and Qian 1999, Richardson and Vaithiyanathan 2009). Anything higher than the long-term P assimilation capacity of a wetland is expected to result in gradual nutrient enrichment and associated ecosystem changes (Figure 2-3) (Richardson and Vaithiyanathan 2009). Time and eutrophication may be represented as showing a linear relationship at medium-high nutrient loads to reflect the generally gradual nature of this pathway (Figure 2-3). It is not intended to suggest that all eutrophication-related changes occur gradually, as some of them may be subject to catastrophic shifts once ecosystem resistance has been sufficiently eroded (Scheffer and Carpenter 2003). It is important to note that the scale of the P load (low, medium, high) is relative to the P assimilation capacity of a wetland and varies greatly between wetlands (Figure 2-3) (Richardson and Vaithiyanathan 2009). Furthermore, these models (Figure 2-3) focus on P load as the driver for change and ignore the impact of other, potentially equally significant stressors (e.g. climate change). The majority of constructed wetlands operate with P loads higher than estimates of their long-term assimilative capacity (Braskerud *et al.* 2005, Kadlec and Wallace 2009) and would thus also be expected to progress along the nutrient enrichment continuum.

The reason why P assimilation capacity varies greatly between wetlands is related to the inherent diversity of wetland P storage and cycling processes that are affected by a range of interrelated regulating factors (Reddy *et al.* 1999, Reddy and DeLaune 2008, Kadlec and Wallace 2009, Land *et al.* 2016). Awareness of these factors underpins the understanding of differences between wetlands and transferability of research findings. Key regulating factors include: 1) presence or absence of different biotic stores and their characteristics (e.g. floating, submerged or emergent plants; their life cycles, density and cover) (Vymazal 2007, Kadlec and Wallace 2009); 2) characteristics of litter/detritus and rate of accumulation (Debusk and Reddy

2005); 3) soil physicochemical properties (Hogan *et al.* 2004); 4) water physicochemical properties (Reddy and DeLaune 2008); 5) hydrological regime (Aldous *et al.* 2005, Noe and Childers 2007); 7) inputs and outputs of nutrients, organic matter and sediments, 8) wetland geomorphology (Hansson *et al.* 2005); 9) climate (Kadlec and Wallace 2009); 10) wetland age (Hansson *et al.* 2005, Zemanová *et al.* 2010); 11) land use history (Hogan and Walbridge 2009) and 12) management measures (e.g. harvesting of biomass) (Kim and Geary 2001, Verhofstad, Poelen*, et al.* 2017).

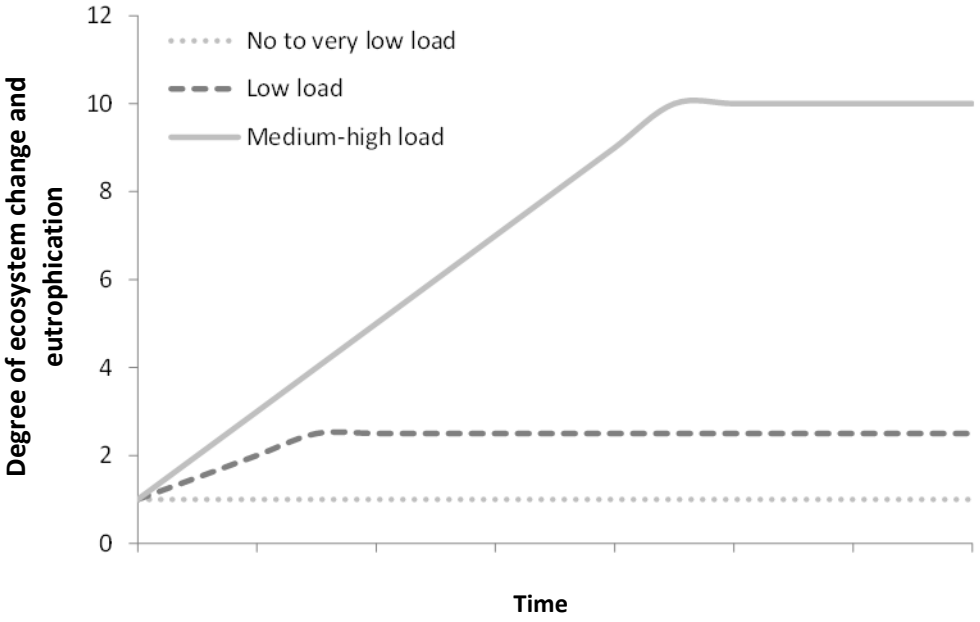

Figure 2-3: Conceptual model of influence of phosphorus load (relative to the phosphorus assimilation capacity of the wetland) on the degree of ecosystem change and eutrophication over time.

This thesis focussed on the medium-high load scenario, where assimilative capacity is exceeded. To develop conceptual models, the nutrient enrichment continuum was divided into three sequential stages: pre-enrichment, functional P uptake and hypereutrophy (Table 2-1). The rate of progress along the nutrient enrichment continuum depends on the P load and the P uptake and storage capacity of the wetland. The higher the load and the smaller the storage capacity, the faster the rate of progress along the continuum.

Stage 1: the pre-enrichment stage is characterised as oligotrophic-mesotrophic with low water TP concentration and rapid uptake of available P by ecosystem components. Water is clear because the chlorophyll α concentration (suspended algal production) is low. The majority of P present is stored in the soil and limited P is available for plant growth. There is likely some seasonal cycling of P between ecosystem components, for example due to changes in water

temperature, depth or due to uptake and release by biota during their lifecycles. However, overall accumulation of P in the ecosystem is low.

Stage 2: the functional P uptake stage covers the period of enrichment when ecosystem components are actively removing P from water and storing it through increased TP concentration and biomass. TP concentration of water may increase as the capacity of the other stores to remove P gradually decreases. Organisms sensitive to higher nutrient concentrations (or their effects) or that are less competitive in enriched conditions start to decline and may be lost.

Stage 3: the hypereutrophic end stage is characterised by more rapid accumulation of P in water as the P removal capacity of other ecosystem components is exhausted. Any further P retention is likely limited to accretion of new soil. Some ecosystem components (e.g. soil) may begin to release previously stored P back into the water column if water and sediment quality deteriorate. Algal blooms are likely to persist causing high turbidity. Loss of sensitive organisms continues and eventually only taxa suited to hypereutrophic conditions remain.

Table 2-1: Three key stages of phosphorus (P) storage and cycling along the nutrient enrichment continuum.

Nutrient enrichment continuum →

Stage 1: Pre-enrichment (Oligotrophic -Mesotrophic)	**Stage 2: Functional phosphorus uptake (Eutrophic)**	**Stage 3: Hypereutrophy (end stage)**
• TP concentration low in water with rapid uptake by other ecosystem components. • Low chlorophyll α concentration in water (few suspended algae), resulting in clear water. • P mostly bound in soil. • Some seasonal P cycling occurs (e.g. related to changes in temperature, water levels, biota lifecycles) but little accumulation overall.	• Incoming P readily taken up by ecosystem components that have not yet reached full storage capacity. • TP concentration in water may rise as uptake capacity of other components gradually decreases. • Decline and loss of taxa sensitive to eutrophication or less competitive organisms starts.	• P uptake capacity of ecosystem components exhausted. • P accumulates more rapidly in water fuelling persistent algal blooms. • Mechanisms for long-term permanent P removal limited to soil accretion. • Potential release of P to water from other ecosystem components (e.g. sediment and litter) if DO and ORP of water/sediment decrease. • Only organisms suited for hypereutrophic conditions will survive.

2.3.2 Creation of conceptual models for each zone

Current understanding of the progress of nutrient enrichment and eutrophication as described by literature was used to construct conceptual models of P storage and cycling in bare sediment, SAV and *Typha* zones over the nutrient enrichment continuum (Figure 2-4, Figure 2-5, Figure 2-6; bullet points described further in Section 2.3.3):

- TP concentration of ecosystem components will increase.
- Biomass of ecosystem components associated with primary production will increase.
- TP storage of ecosystem components (derived from TP concentration and biomass) will increase.
- Height, biomass and density of emergent plants will increase (i.e. *Typha*).
- SAV will be lost.
- Algal blooms (i.e. chlorophyll *a* > 30 µg/L) will develop.
- Water DO and ORP close to the sediment surface will decrease.

The conceptual models (Figure 2-4, Figure 2-5, Figure 2-6) predict changes in P storage by different ecosystem components and associated cycling pathways as enrichment progresses. Nutrient enrichment may alter wetland P cycling through loss of some pathways or zones (e.g. loss of SAV), gain of new pathways or zones (e.g. potential development of floc) and changes in the dominant pathways (e.g. increased cycling of P through suspended algae). The conceptual models show only the main pathways at each of the three key stages of the enrichment continuum. Whilst other pathways may be present, their influence on P movement is anticipated to be negligible. For example, during the second stage (functional P uptake), the dominant P pathway between water and soil is expected to be the uptake of P by soil (Figure 2-4). Some P may be released from soil (e.g. by resuspension) but this was considered a minor pathway at this stage, overwhelmed by the P uptake by soil, and thus is not shown in the model (Figure 2-4). In contrast, during stage 3, soil uptake is less significant and release from soil may become more dominant (e.g. due to lower ORP resulting release of Fe bound P) so it is represented by a double-headed arrow in the conceptual model (Figure 2-4).

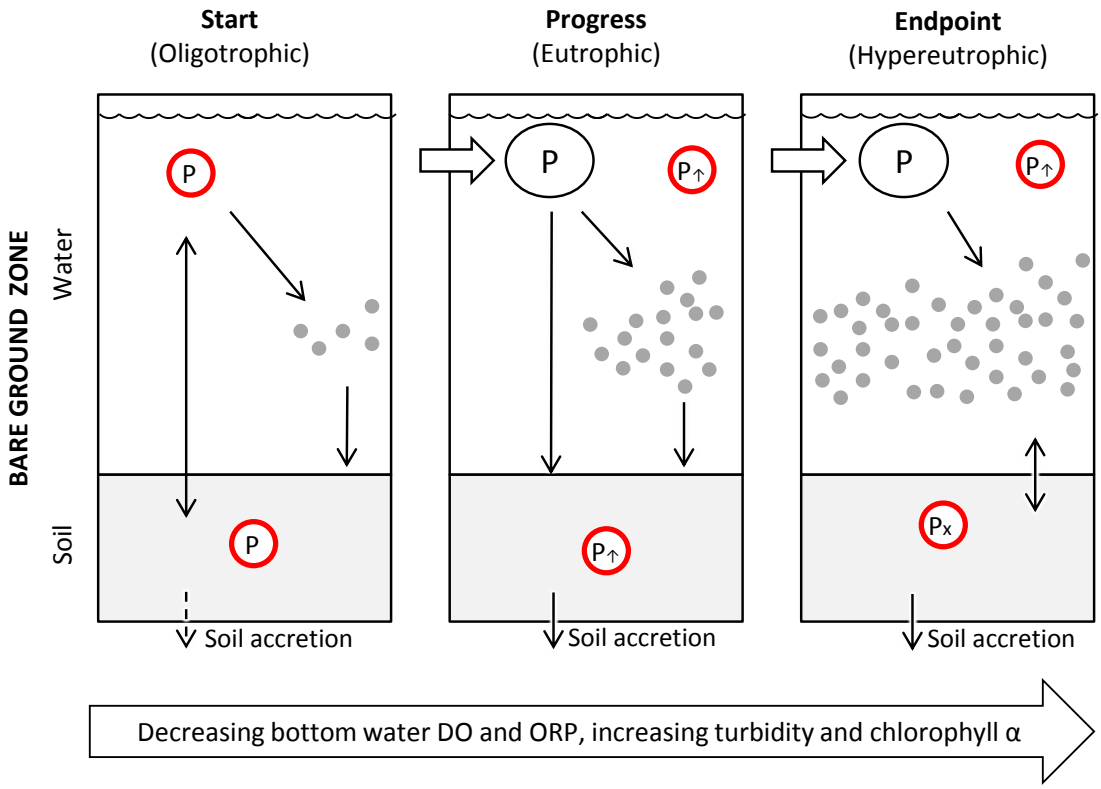

Figure 2-4: Conceptual model of changes to key P stores and cycling pathways in bare sediment zone as a wetland becomes increasingly enriched. Changes in TP concentration are not presented directly but are reflected in changes in TP storage. Grey dots show algal bloom intensity. Arrows represent movement of P between the ecosystem components. For simplicity, models show only the main pathways at each stage. P in red circle shows anticipated change in P storage in that component during that stage of enrichment. Change in TP storage is indicated by ↑ = increase, ↓ = decrease and x = storage capacity reached.

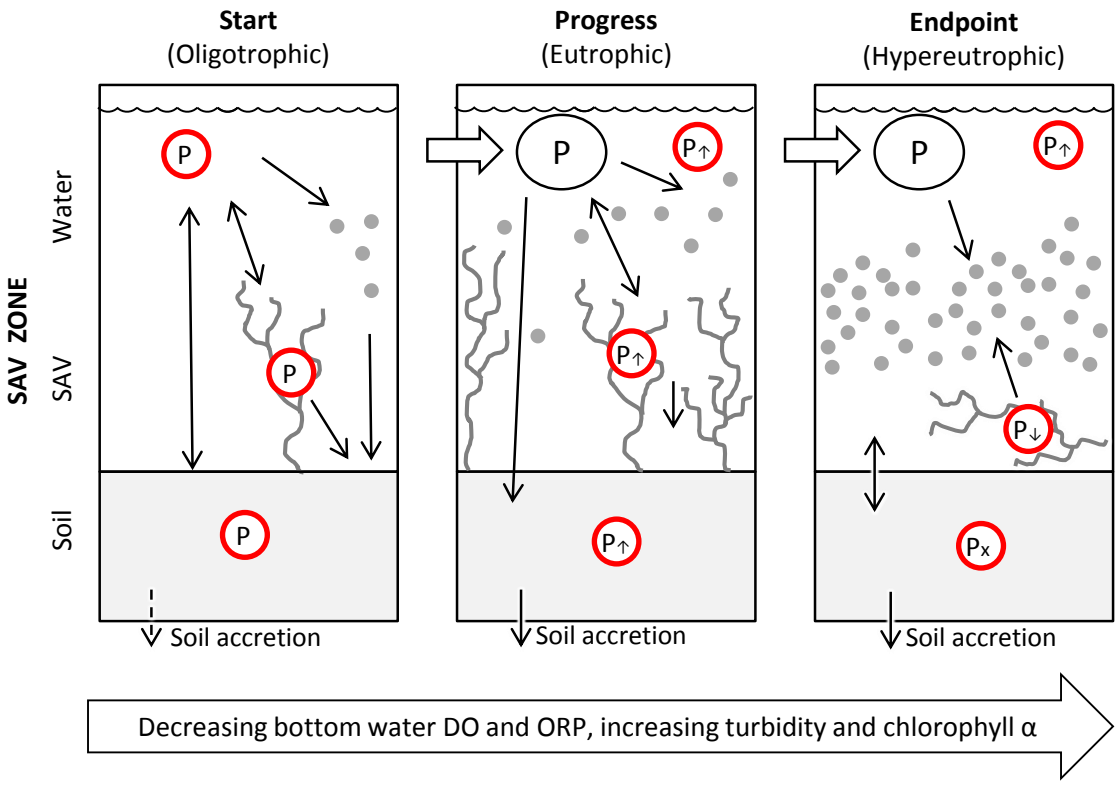

Figure 2-5: Conceptual model of changes to key P stores and cycling pathways in submerged aquatic vegetation (SAV) zone as a wetland becomes increasingly enriched. Changes in TP concentration are not presented directly but are reflected in changes in TP storage. Grey dots show algal bloom intensity. Arrows represent movement of P between the ecosystem components. For simplicity, models show only the main pathways at each stage. P in red circle shows anticipated change in P storage in that component during at that stage of enrichment. Change in TP storage is indicated by ↑ = increase, ↓ = decrease and x = storage capacity reached.

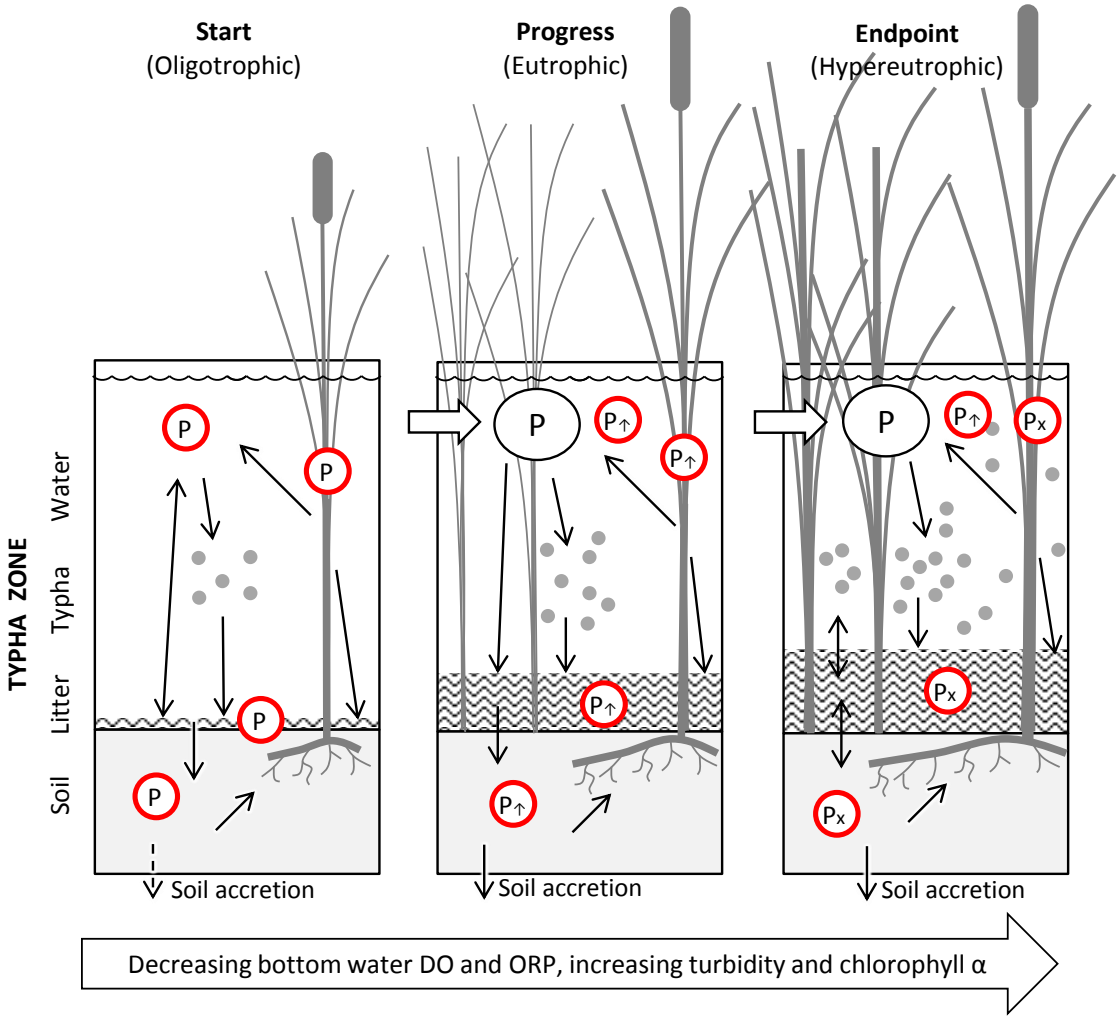

Figure 2-6: Conceptual model of changes to key P stores and cycling pathways in *Typha* zone as a wetland becomes increasingly enriched. Changes in TP concentration are not presented directly but are reflected in changes in TP storage. Grey dots show algal bloom intensity. Arrows represent movement of phosphorus between the ecosystem components. For simplicity, models show only the main pathways at each stage. P in red circle shows anticipated change in phosphorus storage in that component during at that stage of enrichment. Change in TP storage is indicated by ↑ = increase, ↓ = decrease and x = storage capacity reached.

2.3.3 Processes occurring over an enrichment continuum

Increase in TP concentration and TP storage of ecosystem components

TP concentration and TP storage by ecosystem components increases with nutrient enrichment as the stores assimilate the added P (Howard-Williams 1985, Kadlec and Wallace 2009) (Figure 2-7). TP storage is also influenced by expected increases in biomass of stores (as described later). The potential increase in TP concentration and TP storage of ecosystem components is finite and soil accretion is seen as the only P removal pathway sustained in the

long-term (Table 2-2). This results in the commonly (but not always) observed decrease in P removal capacity over time in wetlands (e.g.Nichols 1983, Richardson 1985, Mitsch *et al.* 2012, Dzakpasu *et al.* 2015, Griffiths and Mitsch 2017).

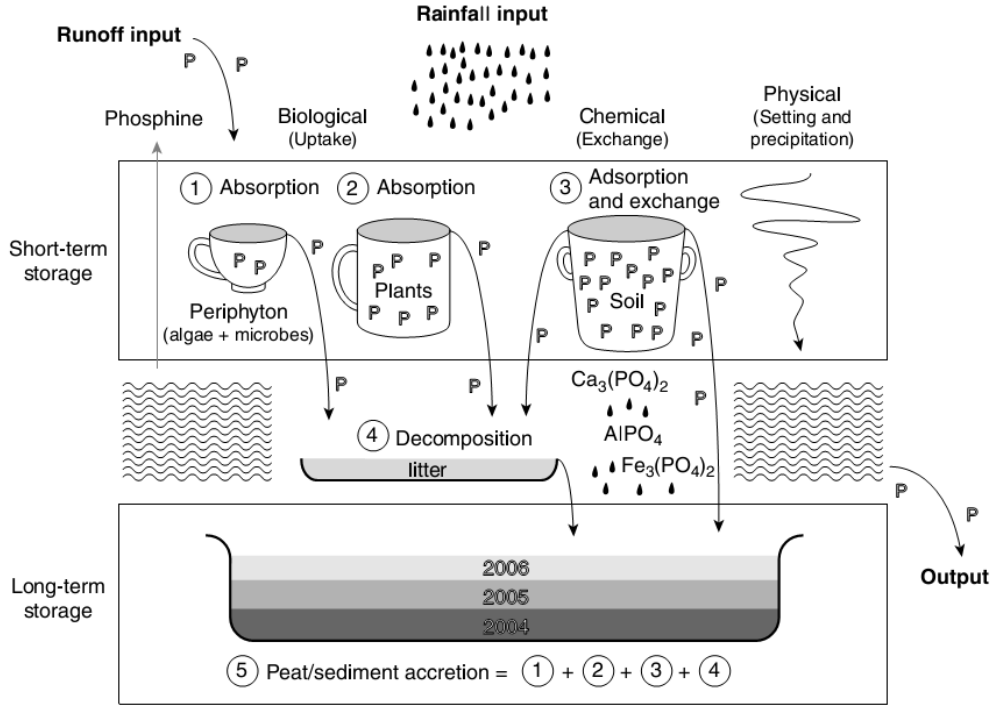

Figure 2-7: Conceptual model by Richardson and Vaithiyanathan (2009) of phosphorus storage and cycling in wetlands, showing only major reservoirs.

Table 2-2: Mechanisms controlling phosphorus retention in wetlands (Richardson and Vaithiyanathan 2009, modified by adding sustainability).

Mechanism	**Timescale**	**Sustainability for P removal**	**Magnitude**	**Rate**
Soil accretion	Long-term	Sustainable	High	Very slow
Soil adsorption	Long-term	Finite	Low/Moderate	Moderate
Precipitation	Long-term	Finite	Moderate	Fast
Plant uptake	Short-term	Finite	Low/Moderate	Slow
Detritus sorption	Short-term	Finite	Low	Fast
Microbial uptake	Short-term	Finite	Very low	Very fast

Potential increases in TP concentration and storage due to enrichment can be an order of magnitude or higher (Table 2-3, Table 2-4). Differences between ecosystem components are

more evident in TP storage than TP concentration. The conceptual models (Figure 2-4, Figure 2-5, Figure 2-6) show only changes in TP storage but TP concentration contributes to these. The duration of TP storage differs between uptake pathways (Table 2-2).

Soil: Maximum sorbed TP concentration of soil can be estimated with sorption isotherms (e.g. Langmuir), however these represent theoretical maxima rather than maxima in field conditions (Reddy and DeLaune 2008). Clay soils and soils with high iron and aluminium content have high sorption capacity (Richardson 1985, Reddy and DeLaune 2008, Douglas *et al.* 2016). Soil/sediment uptake of P is one of the main pathways of P retention (e.g.Wu *et al.* 2013). In treatment wetland scenarios, the capacity of soil to take up P may be reached rapidly, possibly within a few years (Richardson 1985, Kadlec and Wallace 2009).

Plants: TP concentration of plants (Table 2-3) is affected by enrichment, but also varies naturally between different plant parts and changes over the growing season. TP concentration of *Typha* leaves is generally highest early in the growing season and then declines (Boyd 1970). In contrast, TP concentration of rhizomes starts low and then begins increasing half way through the growing season (Bayly and O'Neill 1972), presumably as P from leaves is transported to rhizomes for storage. Nutrient enrichment has been shown to increase TP concentration of both above and below-ground parts of macrophytes (Dolan *et al.* 1981).

Litter: Nutrient enrichment increases TP concentration of litter (Dolan *et al.* 1981, Davis 1991, Grace *et al.* 2008). This occurs because enrichment increases the TP concentration of plants, thereby increasing TP concentration of the litter they produce. Furthermore, litter supports a microbial community that takes up additional P. Net P sequestration by microbiota in litter is higher in nutrient enriched than nutrient limited conditions (Qualls and Richardson 2000, Cheesman *et al.* 2010). As with litter, TP concentration of floc is expected to increase with enrichment due to higher TP concentration of incoming detrital material and potential uptake by microbiota (Table 2-3).

Table 2-3: TP concentrations of components of wetland ecosystems. Not applicable = na.

Ecosystem component	TP concentration (mg/g)			Source
	None to low enrichment	**Moderate to high enrichment**	**Includes both enriched and unenriched**	
Soil/Sediment	0.3-0.5	up to 1.0-2.0 up to 5.2 (maximum based on Langmuir sorption isotherms)	na	Kadlec and Wallace (2009)
	na	0.2 - 6.3	na	Maddison *et al.* (2009)
Periphyton	na	na	0.1 - 4.5	Reddy and DeLaune (2008)
	na	na	2.0 - 20.0	Vymazal (1995)
Charophytes (SAV)	na	na	0.2 - 4.2	review by Kufel and Kufel (2002)
Macrophytes	na	na	<1 - 7.0	Reddy and DeLaune (2008)
Typha	na	na	0.5 - 5.0	Kadlec and Wallace (2009)
	na	1.6 - 4.4 (leaves) 0.9 - 8.6 (roots/rhizomes)	na	Maddison *et al.* (2009)
	1.0 - 2.0	up to 11 (leaves)	na	Di Luca *et al.* (2015)
Litter	0.4 - 2.0	na	na	Chimney and Pietro (2006)
	na	0.3 - 1.8	na	Debusk and Reddy (2005)
	1.0	3.6	na	Kadlec and Bevis (2009)
	na	0.2 - 3.8	na	Maddison *et al.* (2009)
Floc	na	na	0.7 - 4.0	Kadlec and Wallace (2009)
	0.3 - 1.2	na	na	Noe *et al.* (2002)

Table 2-4: TP storage of components of wetland ecosystems. Not applicable = na.

Ecosystem component	TP storage (g/m^2)			Source
	None to low enrichment	**Moderate to high enrichment**	**Includes both enriched and unenriched**	
Soil/Sediment	1.8 - 29.7 (0 - 5 cm)	4.4 - 41.0 (0 - 5 cm)	na	Kadlec and Wallace (2009)
	1.5 - 4.0 (0 - 10 cm)	na	na	Noe *et al.* (2002)
	From 2.5 (0 - 10 cm)	up to 7.3 (0 - 10 cm)	na	Noe and Childers (2007)
Periphyton	0.02 - 0.14	na	na	Noe *et al.* (2002)
	From 0.02	up to 0.18	na	Noe and Childers (2007)
Charophytes (SAV)	na	na	0.3 - 2.8	review by Kufel and Kufel (2002)
Typha	na	0.4 - 10.2 (leaves) 0.5 - 7.2 (roots/rhizome)	na	Maddison *et al.* (2009)
	na	2.8 - 3.3 (leaves)	na	Hernández-Crespo *et al.* (2016)
	na	≈ 3.0 - 6.0 (leaves)	na	Toet, Bouwman*, et al.* (2005)
Litter	na	0.01 - 2.0	na	Maddison *et al.* (2009)
	0.1	9.14	na	Kadlec and Bevis (2009)
Floc	0.5 - 4.4	up to 46.5	na	Kadlec and Wallace (2009)
	0.5 - 1.7	na	na	Noe *et al.* (2002)
	From 0.5	up to 1.2	na	Noe and Childers (2007)
		up to 6.4		Kadlec (2016)

Table 2-5: Dry mass (per unit area) of components of wetland ecosystems. Not applicable = na.

Ecosystem component	Dry mass (g/m^2)			Source
	None to low enrichment	**Moderate to high enrichment**	**Includes both enriched and unenriched**	
Soil/Sediment	na	na	2500 - 32500 (0 - 5cm)	Kadlec and Wallace (2009)
Periphyton	up to 1409 (floating and benthic periphyton)	na	na	DeBusk *et al.* (2004)
	up to 237 (floating mat)	na	na	Noe *et al.* (2002)
	na	≈ 30 (attached to plants)	na	Kadlec and Wallace (2009)
Charophytes (SAV)	na	0.7 - 500.0	na	review by Kufel and Kufel (2002)
Typha	From 1000	up to 6000	na	Kadlec and Wallace (2009)
	na	1235 (live leaves) 1072 (dead leaves)	na	Kadlec and Bevis (2009)
	na	up to 2090 (above-ground)	na	Toet, Bouwman*, et al.* (2005)
	na	370 - 1760 (leaves), 610 - 1310 (roots/rhizome)	na	Maddison *et al.* (2009)
	na	up to ≈ 1600 (above-ground)	na	Mitsch *et al.* (2015)
Litter	300-600	up to 3000	na	Kadlec and Bevis (2009)
	na	up to 1580	na	Christensen *et al.* (2009)
Floc	na	na	266 - 11630	Kadlec and Wallace (2009)
		average 8100		Kadlec (2016)
	1500 - 2900	na	na	Noe *et al.* (2002)

Increase in biomass of ecosystem components

Nutrient enrichment generally results in increased biomass production in primary producers (Smith 2003, Kadlec and Wallace 2009), although the capacity for biomass to increase is finite (Table 2-5). The impact of enrichment on plant biomass may differ among species depending on preferred environmental conditions. For example, while *Typha domingensis* biomass responded positively to increases in soil and water TP in the Florida Everglades, *Cladium jamaicense* (a species adapted to low nutrient environments) had a negative biomass response (Vymazal and Richardson 2003).

Biomass does not remain stable but follows growth cycles, so increases associated with enrichment refer to peak biomass reached during growth. In macrophytes, above-ground biomass follows a seasonal cycle (increasing from the start of growing season, peaking and then declining during senescence, Hill 1987, Sayer *et al.* 2010) whereas below-ground biomass generally follows a longer (non-seasonal) cycle (Hill 1987). For algae, phytoplankton and periphyton, biomass turnover is quicker and controlled by nutrient concentration, light, temperature and grazing (Mahdy *et al.* 2015, McCall *et al.* 2017). The conceptual models (Figure 2-5, Figure 2-6) show overall change in biomass of SAV and *Typha* as a result of enrichment, but do not include seasonal changes.

Eutrophication, through increased primary production, results in increased fall of fresh litter (Emsens *et al.* 2016). However, because rates of decomposition may also change as a result of nutrient enrichment (e.g. due to changes in microbial community and litter composition), the actual mass of litter might not increase (Emsens *et al.* 2016). For example, in wetlands with annual *Typha* production ranging from 289-1900 g/m^2, litter mass was found to vary between 83 - 1580 g/m^2, with high *Typha* production not necessarily resulting in high litter mass (Christensen *et al.* 2009). For the purposes of the *Typha* zone conceptual models (Figure 2-6), it is predicted that litter mass would increase.

Nutrient enrichment may increase floc mass through higher detrital inputs caused by increased biomass, however this remains poorly researched and available findings are conflicting. In Sacramento, constructed wetlands receiving secondary treated effluent accumulated a substantial floc layer 11-17 cm thick in 2.6 years ≈ 9700 g/m^2 dry floc (Kadlec and Wallace 2009 based on data by Nolte and Associates 1997). However, in a low concentration nutrient addition (up to 30 µg/L) experiment in the Everglades, Noe *et al.* (2002) found no differences between control and treatment systems, with former containing 1500 - 2800 g/m^2 and latter

1700 - 2900 g/m^2 of floc. Although floc was not included in the conceptual models, when floc appeared it was examined to refine the models.

Soil accretion through settling of organic and inorganic particulates and burial of plant and animal detritus generally increases with eutrophication (Craft and Richardson 1993). This will likely change soil/sediment composition over time, particularly in newly created wetlands or wetlands with bare mineral soils (Anderson *et al.* 2005, Kadlec and Wallace 2009, Mitsch *et al.* 2012). Nutrient enrichment may thus change soil mass per unit area, but it may increase or decrease depending on changes in composition. Soil commonly has higher mass per unit area than other ecosystem components which contributes to it being the dominant store of P in wetlands (Table 2-5). Changes in soil mass were not addressed in the conceptual models but were sampled as part of the three studies.

Changes in vegetation cover

Eutrophication may more than double stem density of *Typha* (Miao and Sklar 1997, Rejmánková 2001, Kadlec and Wallace 2009, Macek *et al.* 2010). For example, *Typha latifolia* stem density was 71 ± 23 culms/m^2 under enriched conditions compared to 35 ± 22 culms/m^2 under low nutrient conditions (Michigan USA, Kadlec and Wallace 2009). Nutrient enrichment has also been associated with increased plant height, including *Typha* (Newman *et al.* 1996, Rejmánková *et al.* 2008, Macek *et al.* 2010). Consequently, increased *Typha* density and height are illustrated in the conceptual model (Figure 2-6), in addition to the increase in *Typha* TP storage.

While enrichment is generally associated with increased plant biomass, loss of SAV has been commonly recorded when enrichment results in significant increases in turbidity (Blindow 1992, Scheffer *et al.* 2001, Kufel and Kufel 2002, Morris *et al.* 2003, Romo *et al.* 2004, Ferriol *et al.* 2016). SAV may also be adversely impacted by dense epiphytic algal growth (Phillips *et al.* 1978, Asaeda *et al.* 2004, Phillips *et al.* 2016).

Changes to water quality

In P limited systems, a strong correlation has been recorded between water column TP concentration, total chlorophyll α concentration (indicator for phytoplankton blooms) and turbidity (Carlson 1977, Reddy and DeLaune 2008). Thus, boundaries for wetland trophic status can be based on these variables (Table 2-6). The stages of the conceptual models refer to these trophic states and increases in suspended algae have also been visualised in the models.

Table 2-6: Trophic status classification (modified OECD 1982).

Trophic category	Mean TP	Mean Chlorophyll α	Max Chlorophyll α	Mean Secchi depth	Min Secchi depth
	µg/L	µg/L	µg/L	m	m
Oligotrophic	<10	<2.5	<8	>6	>3
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypereutrophic	>100	>25	>75	<1.5	<0.7

Enrichment often reduces water DO and ORP close to the sediment surface (Figure 2-4, Figure 2-5, Figure 2-6) (McCormick and Laing 2003, Palmer-Felgate *et al.* 2011). Accordingly, in most treatment wetlands, DO is expected to decrease to 1-2 mg/L due to incoming biochemical oxygen demand (BOD) and nitrogenous oxygen demand (NOD) loads (Kadlec and Wallace 2009). Nutrient enrichment has also been associated with decreased sediment ORP, likely due to increased organic matter accumulation raising consumption of oxygen and other electron acceptors during decomposition (Mortimer 1971, Qualls *et al.* 2001). Reduced sediment ORP can influence solubility of sediment bound P (Reddy and DeLaune 2008, Erler *et al.* 2011) and also reduce growth of macrophytes (Pezeshki *et al.* 1996, Pezeshki and DeLaune 2012); both decrease P storage capacity of wetlands.

Differences between functional zones

Vegetated wetlands generally remove more added P from water than unvegetated wetlands (Tanner *et al.* 1995, Huett *et al.* 2005, Greenway and Lucas 2008, Menon and Holland 2013). Consequently, I expected water TP concentration to be lower in vegetated (SAV and *Typha*) than unvegetated (bare sediment) functional zones and that total TP storage would be higher in vegetated than unvegetated zones. Furthermore, if TP concentration is lower in vegetated zones and there is shading by *Typha*, chlorophyll α concentration should be lower in vegetated than unvegetated zones.

2.3.4 Knowledge gaps

Rate of response of ecosystem components

Some stores like microbiota, phytoplankton and algae are able to respond to enrichment immediately and have been found to assimilate added P within hours (Havens *et al.* 1999, Noe *et al.* 2003, Kadlec and Wallace 2009). In contrast, other stores appear to respond more

slowly. For example, in a five year study looking at cascading ecological effects of low-level P enrichment (30 μg/L) in the Florida Everglades, Gaiser *et al.* (2005) found that TP concentration of periphyton and floc increased significantly over the first year of treatment, soil TP concentration during the second, macrophyte TP concentration during the third and water TP concentration during the fifth year. Further, in a P radiotracer study of oligotrophic *in situ* mesocosms in Everglades National Park, Noe *et al.* (2003) found that over an 18 day period, very little ^{32}P made its way into live emergent macrophyte tissue, while the ^{32}P was found in soil, floc, epiphyton, metaphyton and consumers. Richardson and Vaithiyanathan (2009) described general response rates of different P uptake mechanisms from very fast (microbial uptake), fast (detritus sorption, precipitation), moderate (soil adsorption), slow (plant uptake) to very slow (soil accretion). However, we do not know about whether wetland components show consistent rates of response to P enrichment across wetland types and geographic regions. Yet, such knowledge is vital for understanding both eutrophication processes and the functioning of treatment wetlands. Consequently, this study aimed to understand differences in the rate of response to enrichment between ecosystem components in the CWC wetlands.

Changes in proportional phosphorus storage

Although, the largest store of P in wetlands is generally soil (Richardson and Marshall 1986, Verhoeven 1986, Kadlec and Wallace 2009), nutrient enrichment is expected to change the proportional distribution of total P between different ecosystem components over the enrichment continuum. For example, under oligotrophic conditions in the Florida Everglades (USA), proportional P stores were: surface soil ≈ 75%, floc ≈ 20% and other stores 1% or less of total P, whereas under enriched conditions, surface soil stored ≈ 60%, floc ≈ 35% and periphyton ≈ 2% (Noe *et al.* 2002). P enrichment may reduce the proportion of P in topsoil (Diluca *et al.* 2015), and increase the proportion stored in macrophytes (Wang and Mitsch 2000, Noe and Childers 2007). The proportional share of total P is also expected to differ between functional zones due the availability of different stores. As with rates of response to P enrichment, little is known about how the proportional shares of TP storage differ between wetland components under different scenarios (regions, wetland types etc.) and in different zones within wetlands. This research aimed to determine how the proportional shares of TP storage change with enrichment and differ between functional zones (bare sediment, SAV, *Typha*) in the CWC wetlands.

Where does added phosphorus go?

The current conceptual models (Figure 2-4, Figure 2-5, Figure 2-6) do not show which uptake pathways are likely to dominate, that is, which ecosystem components will assimilate most or least added P. These patters may be site specific, depending on types and characteristics of available stores. It may also change over time, due to different uptake rates and capacity of stores. The three studies conducted here collected data to refine the conceptual models by using a mass balance approach (covering inputs, outputs and storage by ecosystem components) to identify the destination of added P in both wetlands and mesocosms. I predict that most added P will be stored in soil, because previous research has showed that the clay soil at CWC (which is high in Al, Fe, and Mn) readily takes up added P (Chambers and McComb 1996).

Ecosystem change

Other potential changes may occur in wetlands to which P is added, such as changes in species composition and relative dominance (Vaithiyanathan and Richardson 1999, Hagerthey *et al.* 2008), or changes in water quality. For example, diurnal fluctuations in primary production and respiration, particularly in algae/SAV dominated ecosystems, may alter water pH and DO (Reddy and DeLaune 2008, Mitsch *et al.* 2015). This research aimed to identify changes like these, caused by the addition of P and where appropriate, incorporate these to the conceptual models.

2.4 Detailed aims and hypotheses

The overarching research questions were broad and more detailed sub-questions were developed to guide data collection and data analysis. These sub-questions aimed to test (sub-question a, e, f) and refine (all sub-questions) the conceptual models presented above, and were linked to the key assumptions and knowledge gaps identified in Sections 2.3.2 and 2.3.4 (respectively). Where sufficient literature was available, hypotheses were constructed to test sub-questions (Table 2-7).

Table 2-7: Summary of research questions, sub-questions, hypotheses (H) and rationale.

Sub-questions	Rationale
Research Question 1 - How do P storage and cycling change as a wetland undergoes nutrient enrichment and how does this differ between functional zones?	
a) How does TP storage of ecosystem components change with enrichment and how does this differ between functional zones?	
H1: TP concentration of ecosystem components will increase with enrichment.	Nutrient enrichment increases TP concentrations of P stores (Howard-Williams 1985).
H2: Mass of ecosystem components associated with primary production will increase with enrichment.	Nutrient enrichment increases primary production (Smith 2003).
H3: TP storage of ecosystem components will increase with enrichment.	Following on from rationale for sub-questions a) and b) above.
H4: Water TP concentration will be higher in unvegetated than vegetated zone(s) during enrichment.	Vegetated systems have more P uptake pathways and higher P storage capacity (Kadlec and Wallace 2009).
b) How does the rate of response to enrichment differ between ecosystem components and how does this differ between functional zones?	
No specific hypothesis	Ecosystem components that extract P directly from water are likely to respond faster than others but there are differences between wetlands. So, no specific hypothesis was made about the order in which ecosystem components may respond.
c) How does the distribution of total TP storage across the ecosystem components (% of total) change with enrichment and how does this differ between functional zones?	
H5: Proportional share of TP storage in soil will reduce with enrichment	Proportional share of total TP storage held in soil was lower in enriched than unenriched systems (Noe *et al.* 2002, Di Luca *et al.* 2015).
d) Where does the added P go?	
H6: Most added P will be taken up by soil.	Clay soil at the CWC is high in Fe and Mn and readily takes up added P (Chambers and McComb 1996).

Sub-questions	Rationale
Research Question 2 - How is nutrient enrichment reflected in ecosystem change in the different functional zones?	
e) How do vegetation characteristics change with enrichment in the different functional zones?	
H7: *Typha* height, biomass and density will increase with enrichment.	Nutrient enrichment has been associated with increased plant height (Newman *et al.* 1996, Rejmánková *et al.* 2008, Macek *et al.* 2010), increased plant biomass (Smith 2003) and increased *Typha* density (Miao and Sklar 1997, Rejmánková 2001, Kadlec and Wallace 2009, Macek *et al.* 2010).
H8: SAV will be lost with enrichment.	Loss of SAVs in response to large increases in turbidity associated with enrichment has been reported (Blindow 1992, Scheffer *et al.* 2001, Kufel and Kufel 2002, Morris *et al.* 2003, Romo *et al.* 2004).
f) How does water quality change with enrichment and how does this differ between the functional zones?	
H9: Algal blooms (chlorophyll α > 30 µg/L) will occur with enrichment.	Algae is quick to respond to increases in nutrient availability (Howard-Williams 1985). P uptake by other ecosystem components may delay and/or reduce the scale of algal production.
H10: Water turbidity and chlorophyll α will be higher in unvegetated than vegetated zones.	Compared to vegetated systems, unvegetated systems have no competition or shading by other plants, providing more productive conditions for growth of suspended algae (Portielje and Van der Molen 1999, Villena and Romo 2007, McCall *et al.* 2017).
H11: Water DO and ORP close to sediment surface will decrease with enrichment.	Treatment wetland can expect DO to decrease down to 1-2 mg/L due to incoming BOD and NOD loads (Kadlec and Wallace 2009). Nutrient enrichment has been associated with decrease in ORP of sediments, likely due to increased accumulation of organic matter resulting in increased consumption of oxygen and other electron acceptors during decomposition (Mortimer 1971, Qualls et al. 2001).

2.5 Research approach

Three studies were developed to answer the research questions and test the hypotheses. Each study was designed to address all six sub-questions but at different temporal scales, thus providing multiple lines of evidence to be synthesised to answer the research questions. Firstly, a nine-month study aimed to capture early-enrichment related changes in the treatment wetland and to provide a seasonal perspective of storage and cycling of P in both the treatment wetland and in two unenriched wetlands. The second study (three-years) aimed to provide a longer-term, multiyear perspective into the changes associated with nutrient enrichment. The third study (three-months) investigated intensive short-term enrichment with a highly controlled *in situ* mesocosm experiment. The mesocosm experiment also investigated enrichment impacts on the SAV zone, which was absent from the treatment

wetland at the time of effluent discharge (enrichment). As ecosystem components were expected to respond to enrichment at different rates, the mesocosm experiment aimed to capture changes in the rapid responders, while the seasonal and multiyear studies were aimed to capture changes in the slower responding stores. The studies were expected to cover all three stages of enrichment: the seasonal and mesocosm studies providing data on the pre-enrichment stage; all three studies covering the functional P uptake stage; the multiyear study likely extending to the hypereutrophic end stage (Table 2-8).

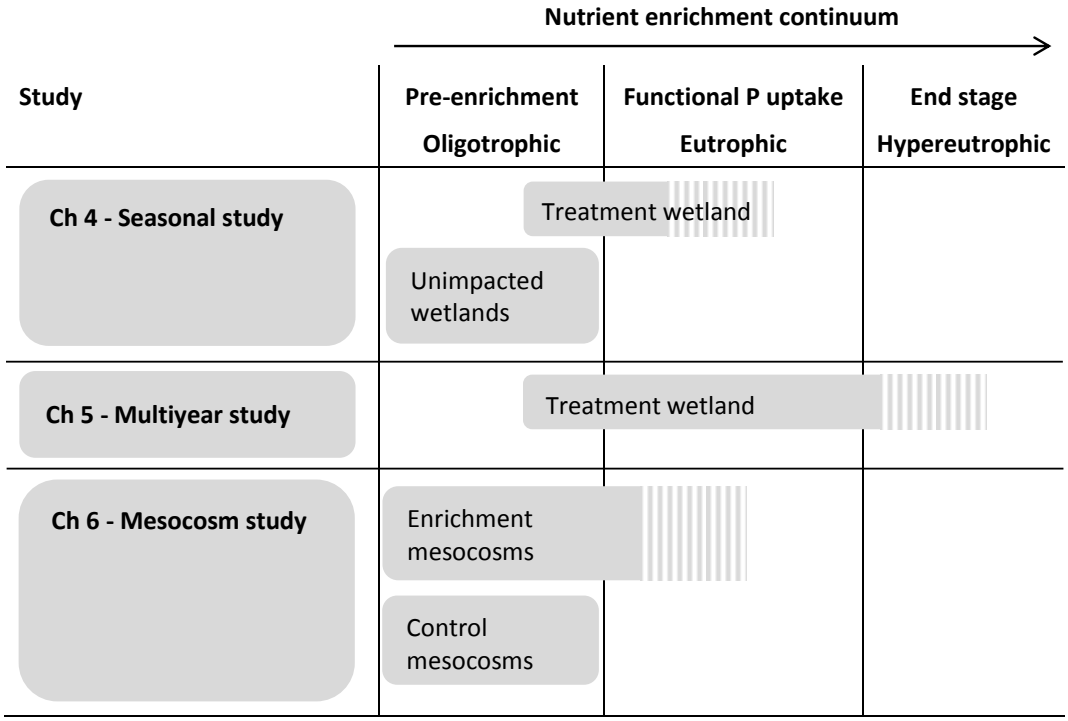

Table 2-8: Coverage of the nutrient enrichment continuum by the three studies.

CHAPTER 3: EXPERIMENTAL DESIGN

3.1 Overview

Where possible, consistency in measures and methods was maintained among the three studies in this thesis. This chapter describes those aspects of the experimental design and methods shared among the three investigations (study area, sampling area, field sampling methods, laboratory analysis and data processing), to avoid unnecessary repetition of methods in Chapters 4, 5 and 6. Detailed methods and data analysis specific to each of the three studies are then described in Chapters 4, 5 and 6.

The seasonal (Chapter 4) and multiyear study (Chapter 5) used virtually identical methods, but differed in sampling frequency, total duration and the number and type of wetlands involved (Table 3-1). The seasonal study collected data from the treatment wetland and two adjacent unimpacted (unenriched) wetlands. Seasonal data from the two unimpacted wetlands provided a control against which to compare the treatment wetland. In contrast, multiyear comparisons were only made using data from the treatment wetland. Functional zones and ecosystem components in the seasonal and multiyear studies were the same (Table 3-1), although some SAV was present seasonally in the bare sediment zone in one unimpacted wetland.

The mesocosm study (Chapter 6) used the same functional zones and ecosystem components as the other studies, with the addition of the SAV zone and the periphyton and SAV components (Table 3-1). It differed from the other studies by the enclosed nature of the mesocosms, and by the application of a higher P loading rate (of FRP only) without the concomitant addition of water, and with more frequent sampling (Table 3-1). The higher loading rate was applied to capture changes in the more rapidly responding variables and to ensure that this short-term experiment would capture significant changes in TP storage of ecosystem components.

Table 3-1: Summary of the key characteristics of the three studies in this thesis.

Characteristic	Seasonal study (Ch4)	Multiyear study (Ch5)	Mesocosm study (Ch6)
Temporal scale	Short-term (9 months)	Medium-term (3 years)	Short-term (13 weeks with 12 weeks of enrichment)
Sampling frequency	Seasonal (spring, summer, autumn, winter)	Annual (during spring)	Fortnightly-monthly
Spatial scale	Different functional zones in small wetlands (1-2ha)		Enclosed 1m^2 *in situ* mesocosms in different functional zones
Systems sampled	One treatment wetland (Swamphen Lake) and two unimpacted wetlands (Island and Peninsula Lakes)	One treatment wetland (Swamphen Lake)	Mesocosms set up in Peninsula Lake
Sampling points	3-5 random points per functional zone per sampling round		12 enrichment and 12 control mesocosms (4 of each per functional zone)
Functional zones available	Bare sediment *Typha*		Bare sediment SAV *Typha*
Ecosystem components available	Water, topsoil, subsoil, floc, litter, *Typha* (SAV in unimpacted wetland)	Water, topsoil, subsoil, floc, litter, *Typha*	Water, topsoil, subsoil, floc, litter, SAV, *Typha*, periphyton
Enrichment type	Enrichment by effluent discharge (associated with large addition of water)		Enrichment by nutrient dosing (no addition of water)
Incoming TP concentration and/or load	Design maximum 2 mg/L. TP load unknown at planning stage.	Design maximum 2 mg/L. TP load unknown at planning stage.	TP concentration of each dose approximately 2 mg/L at 1 m depth. TP load of each dose 2 g/m^2.
P form	Particulate and dissolved, organic and inorganic P in varying ratios.		Dissolved inorganic P

3.2 Study Area

3.2.1 Location

This research was undertaken at the Capel Wetlands Centre (CWC) located on the Swan Coastal Plain in southwest Western Australia, approximately 200 km south of Perth and 5 km south of the town of Capel (Figure 3-1). CWC comprises a series of 15, small (0.5-6.0 ha), shallow, interconnected created wetlands, over a total area of approximately 370 ha. The three northernmost wetlands, called Swamphen, Island and Peninsula Lakes were used in this research (Figure 3-2). For the duration of the project, treated municipal effluent was released

into Swamphen Lake only. All three wetlands were sampled for the seasonal study, whereas only Swamphen Lake (treatment wetland) was sampled for the multiyear study. The mesocosm experiment was carried out in Peninsula Lake.

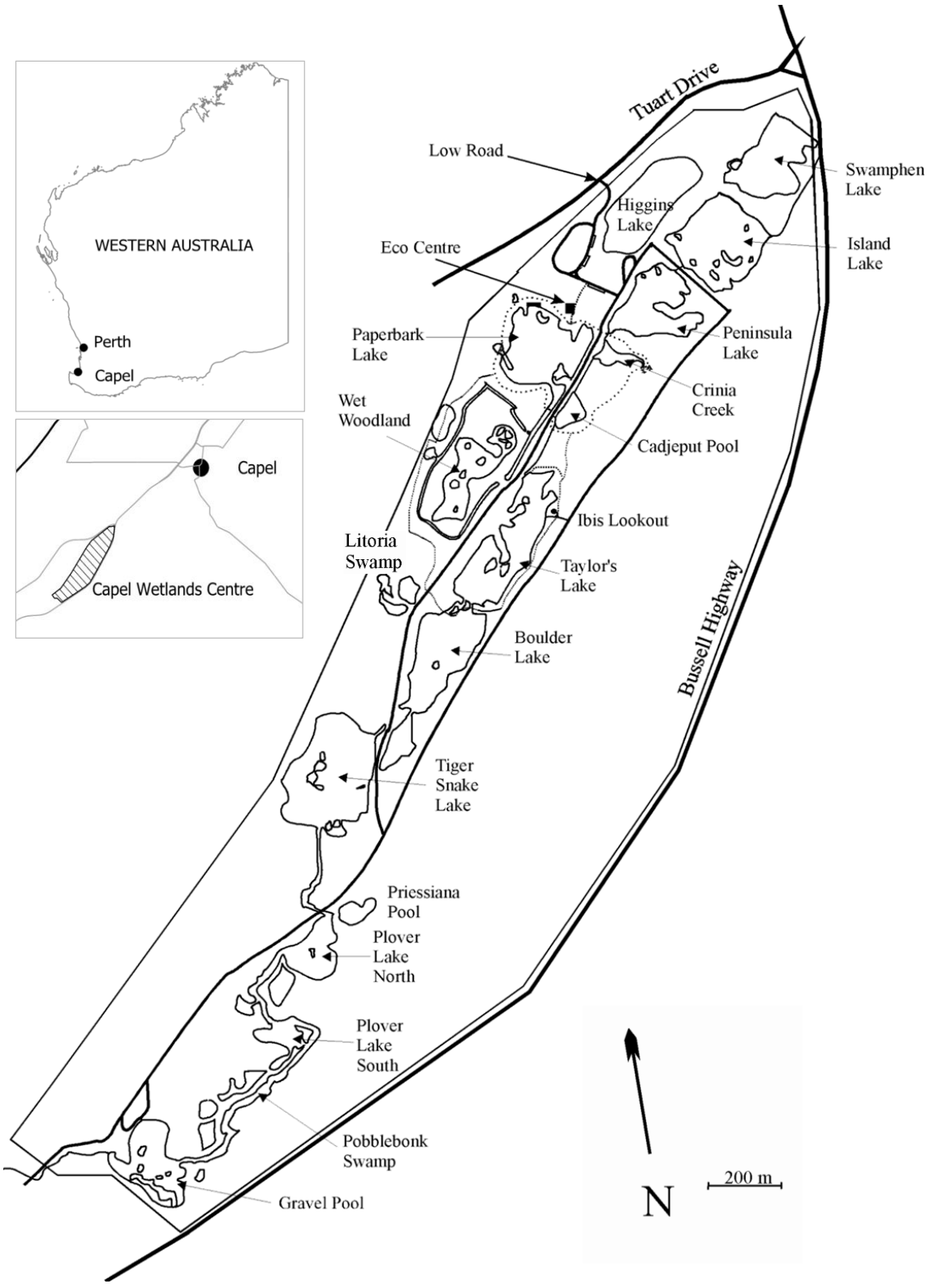

Figure 3-1: Location of Capel Wetland Centre in Western Australia and the layout of the site with the three study wetlands (northern end).

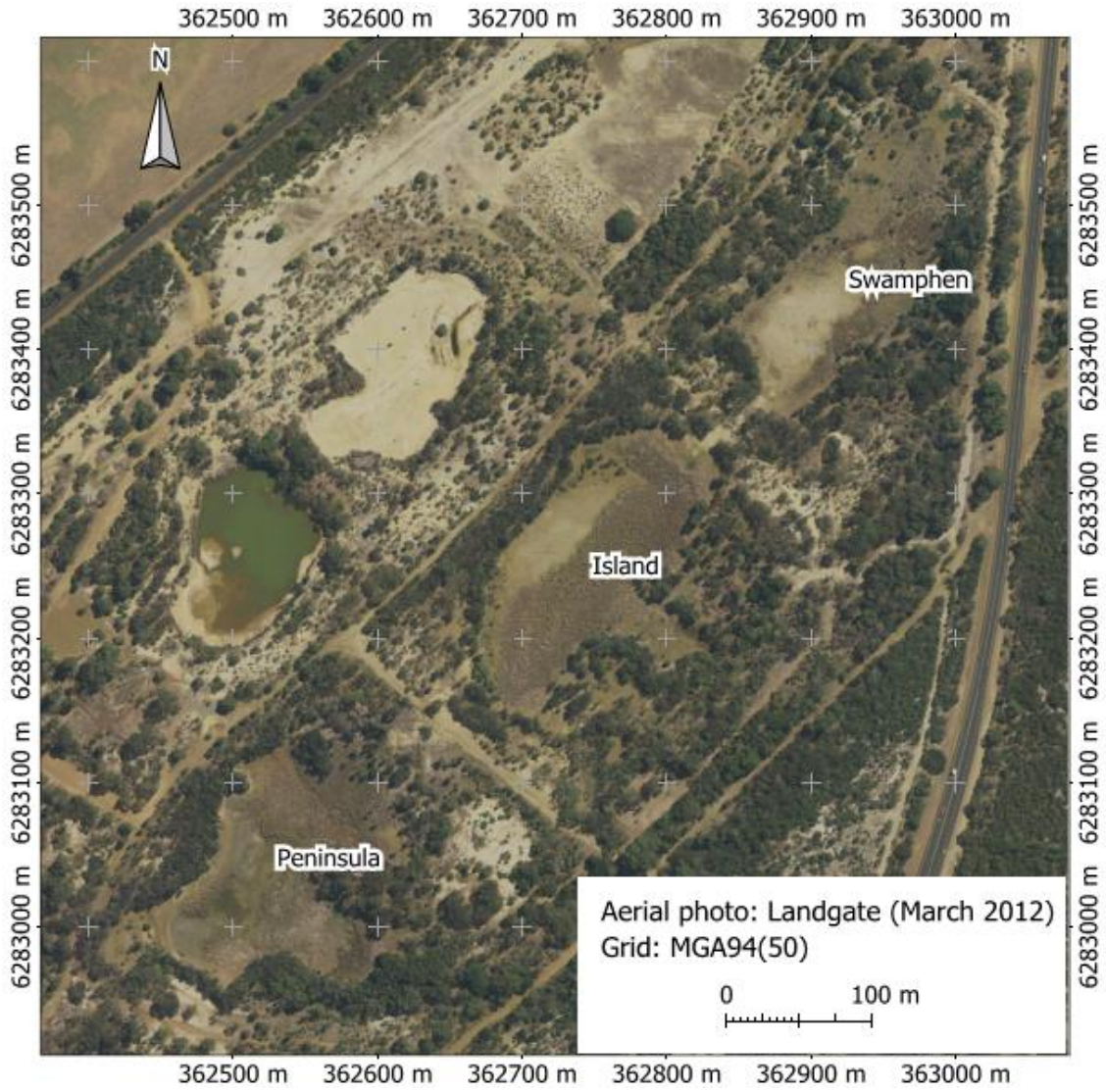

Figure 3-2: Aerial photograph of Swamphen, Island and Peninsula Lakes at the CWC when dry.

3.2.2 Site history

The CWC wetlands were originally formed in the 1970s when mineral sand mining dug below the groundwater table, leaving behind inundated basins. The majority of the basins were partly backfilled with process slimes (silt and clay fractions of processed mineral sands) in order to improve their water holding capacity. Some of the wetlands, including those used in this research, also received mineral sands processing wastewater that initially had very low pH and high concentrations of ammonium chloride, and potentially also sulphate and heavy metals (Gordon and Chambers 1987, Davies 2002, GHD 2004).

As part of mine site rehabilitation between mid-1980s and early 2000s, works were undertaken to develop the basins into functional ecosystems resembling natural wetlands

(Davies 2002). Natural shallow groundwater-fed wetlands occur across the Swan Coastal Plain including the surroundings of the CWC. These natural wetlands sustain high aquatic biodiversity (Pinder 2005, Horwitz *et al.* 2009) and provide a recruitment source of species for the CWC wetlands. Research into the CWC wetlands in the 1980s and 1990s identified P as the factor limiting primary productivity and the development of the wetlands as functional ecosystems (Gordon and Chambers 1987, Chambers and McComb 1996, Chambers, Cale*, et al.* 1998, Chambers, Fletcher*, et al.* 1998). P was added in laboratory microcosms (Chambers and McComb 1996), *in situ* mesocosms (Chambers, Cale*, et al.* 1998) and at the whole wetland scale (one off pulse) (Chambers, Fletcher*, et al.* 1998) and whilst increased biological productivity was observed, there was a rapid decline in available P concentration following application. This was attributed to low water residence time and to high adsorption capacity of suspended and bottom sediments in the CWC wetlands (Chambers, Fletcher*, et al.* 1998).

3.2.3 Climate

Climate in the study area is Mediterranean with warm, dry summers and cool, wet winters. Rainfall is strongly seasonal, with ≈ 85% of annual rainfall occurring during winter (May – August) and spring (September – October) (Figure 3-3). Mean annual rainfall is 811.6 mm (1877-2016), mean maximum temperature 23.1°C and mean minimum temperature 10.3°C (Bureau of Meteorology 2016a), (Figure 3-3). Pan evaporation in the area is approximately 1400 mm/year (based on 1975-2005, Bureau of Meteorology 2006).

Rainfall in the region has been declining (Bates *et al.* 2008), with approximately 15% reduction in winter rainfall recorded since 1970s (Cleugh *et al.* 2011). In the Capel area, rainfall has decreased by ≈ 30 mm/10 years (total decrease of 135.0 mm over 45 years, Bureau of Meteorology 2016b). Reduced rainfall has caused groundwater tables to decline across the region, altering wetland inundation regimes (Boulton *et al.* 2014). Further reductions in rainfall and increases in temperature are expected with climate change in the southwest (Barron *et al.* 2012, Hope *et al.* 2015). Whilst these changes may reduce the external load of nutrients into wetlands, they will also increase water retention times and evapoconcentration of nutrients and decrease water volumes, so are expected to exacerbate eutrophication (Özen *et al.* 2010).

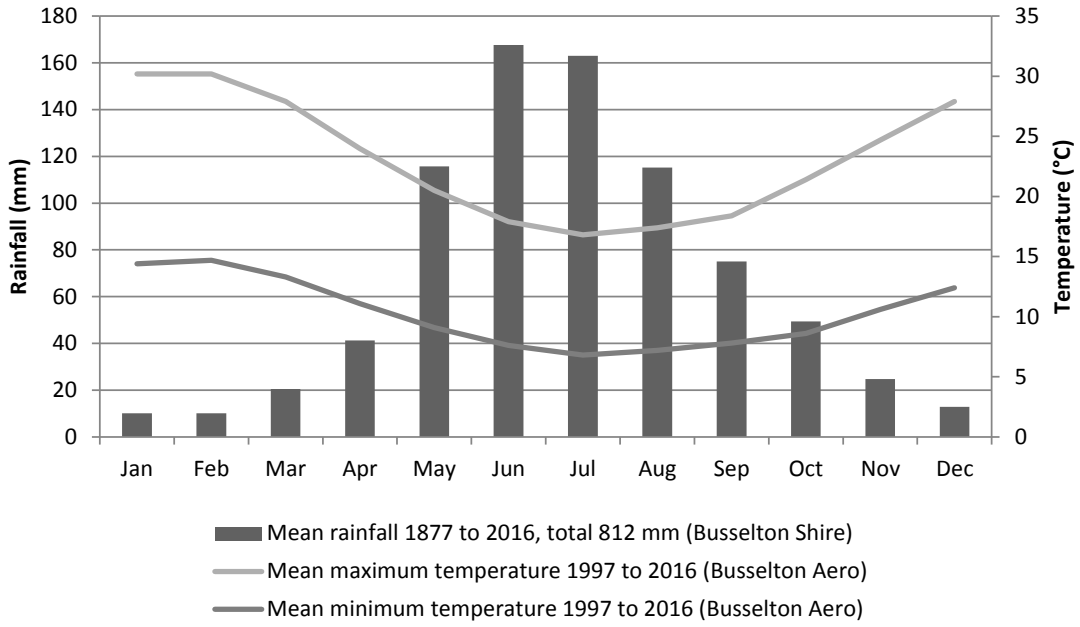

Figure 3-3: Climate Data for Busselton (closest meteorological station, 18 km southwest of CWC, Bureau of Meteorology 2016a).

3.2.4 Geology and hydrology

The CWC is located on the western edge of the Bassendean Dune System, where the natural soils are mostly sandy with minor interbedded clay in some areas. A superficial unconfined aquifer is present in the sand layer and overlies the semi-confined Leederville aquifer (GHD 2004). The backfilling of the wetlands with clay and silt slimes to a depth of several meters (Fowler Surveys 1999) greatly reduced hydraulic connectivity between the aquifers and the CWC wetlands. Hydraulic conductivity of the clay and silt slimes is much lower (0.01-1 m/d) than the sandy superficial aquifer (55 m/d) (GHD 2004). The hydrology of the wetlands has been described as a 'bucket' model, with groundwater interaction being limited to times when groundwater and/or wetland water levels exceed the height of the low permeability slimes, resulting in water flowing either into or out of the 'bucket' over its rim (GHD 2004), (termed 'seepage' in Figure 3-4).

The release of mineral sands processing wastewater into the wetlands ceased in 2000. Water levels subsequently decreased, exacerbated by reduced rainfall in south-western Australia (see Section 3.2.2), and the water regime of many wetlands changed from perennial to seasonal. The seasonal water regime in the lakes comprises a relatively rapid rise in water levels following the onset of winter rains (June or July), with depths peaking in September/October, followed by gradual decline due to evapotranspiration and limited rainfall through the rest of

the year. The three wetlands studied here showed somewhat different seasonal water regimes: from 2006 to the start of effluent release in 2012, Swamphen Lake was mainly dry with short periods of inundation during winter and early spring; Island Lake dried seasonally (i.e. in summer - autumn) in most years; Peninsula Lake was generally perennial (Water Corporation 2006-2017).

Over the study period, Swamphen Lake was operated as an infiltration (mostly seepage over the rim) / evapotranspiration basin with no surface water outflow. Water inputs to the wetland included effluent discharge, direct rainfall over the basin area (approximately 2.3 ha) and potentially groundwater seepage. There was no evidence of surface water runoff into the wetland (Figure 3-4).

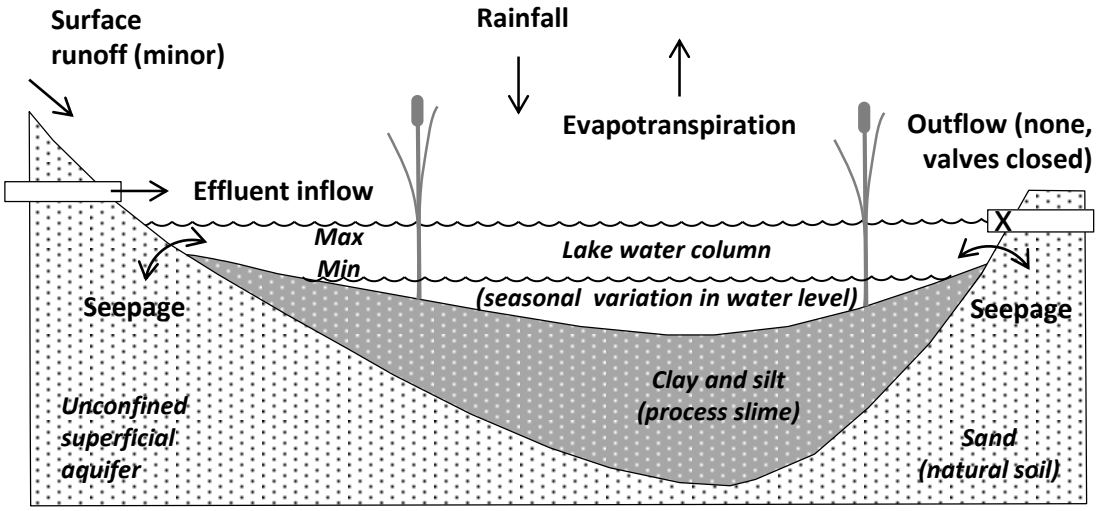

Figure 3-4: Schematic water balance model for Swamphen Lake.

3.2.5 Water quality

Water in Swamphen Lake was very acidic prior to the commencement of domestic effluent discharge (Table 3-2), having undergone a decline in early 2000s after mining wastewater discharge ceased. Island and Peninsula Lakes were more neutral but also showed declining pH (Table 3-2). Reasons for acidification are uncertain, however Capelli *et al.* (2007) concluded that in Swamphen Lake at least, the most likely cause was oxidation of sulphidic material in the soil caused by lower water levels. Electrical conductivity of water in all three wetlands was elevated and varied seasonally due to evapoconcentration over drier months (Table 3-2).

Water TP concentration was generally low in all three wetlands however the ANZECC trigger value (Table 3-2) was occasionally exceeded during drier periods due to evapoconcentration.

Water FRP in all three wetlands remained consistently below the ANZECC trigger value (Table 3-2). Water TN concentration in Swamphen Lake was very high (Table 3-2) with the dominant form being NH_4-N, presumed to originate from the mineral sands processing wastewater discharged to the wetland. Island and Peninsula Lakes had generally much lower TN concentrations and the dominant form was often organic nitrogen. ANZECC trigger values for nitrogen were regularly exceeded in all three wetlands, but particularly in Swamphen Lake.

In all three wetlands relatively high concentrations of dissolved sodium (340 - 560 mg/L), chloride (510 - 840 mg/L), calcium (290-470 mg/L) and sulphate (1000 - 7500 mg/L) were recorded in the past (2001-2002, GHD 2004). Furthermore, elevated concentrations of manganese (5.2-38.0 mg/L, GHD 2004, Capelli *et al.* 2007), iron (13-16 mg/L, Capelli *et al.* 2007) and aluminium (26-31 mg/L, Capelli *et al.* 2007) were recorded in Swamphen Lake and the surrounding groundwater. Values above ecological guidelines for copper and zinc were also recorded in all three wetlands (GHD 2004).

Table 3-2: Water quality of Swamphen, Island and Peninsula Lakes as range of values recorded between July 2006 - January 2012 (FRP data only from October 2010 - January 2012). Comparison is provided against Australian and New Zealand guidelines for water quality for wetlands in south-west Australia (ANZECC and ARMCANZ 2000). Compiled from quarterly data (January, April, July, October) collected by Water Corporation. OL = outlier, shaded values exceed guidelines.

Variable	Unit	ANZECC trigger	Swamphen Lake	Island Lake	Peninsula Lake
pH		<6.5 or >8.5	**2.9 - 3.8**	**3.9** - 7.6	**5.9** - 8.2
EC	µS/cm	<300 or >1500	**1900 - 6500**	**2600 – 8400**	**3750 – 14000**
TP	mg/L	0.06	<0.05 - **0.15** (OL 0.75)	<0.05 - **0.11**	<0.05 - **0.15** (OL 1.30)
FRP	mg/L	0.03	<0.03	<0.03	<0.03
TN	mg/L	1.5	**6.4 - 40.0**	<0.1 - **3.8** (OL 8.8)	0.3 - **4.6**
NH_4-N	mg/L	0.04	**5.3 - 38.0**	<0.05 - **2.6** (OL 7.0)	<0.05 - **1.9** (OL 3.1)
NO_x-N	mg/L	0.1	<0.05 - **1.8**	<0.05 - **1.8**	<0.05 - **0.5**

3.2.6 Sediment quality

Due to the backfilling of the basins with mineral sands process slimes, the bottom sediment of the wetlands comprises clay and silt rather than the natural sandy soil of the surrounding area. The environmental properties of clay and silt are markedly different to sand, not only in their porosity. Sediment quality in the CWC wetlands is variable but high concentrations of iron,

manganese, aluminium and sulphur and at times high concentrations of other metals and contaminants have all been recorded (GHD 2004, 2006, Capelli *et al.* 2007). In 2010, sediment TP concentration (top 20 mm) in Swamphen and Island Lake was approximately 0.30 mg/g and in Peninsula Lake approximately 0.55 mg/g, whilst sediment TN concentration was approximately 3.0 mg/g in Swamphen, 2.0 mg/g in Island and 4.0 mg/g in Peninsula Lake. There are no current sediment quality guidelines for nutrients in Australia (Simpson *et al.* 2013), but these sediment TP concentrations were within the range of those recorded for seven natural wetlands on the Swan Coastal Plain (0.014-1.463 mg/g) (Qiu and McComb 2000). It is important to note that the natural sandy soils of the Swan Coastal Plain retain P very poorly (maximum Langmuir phosphorus sorption 0.5-508 mg/kg, He *et al.* 1998), so generally P that enters natural lakes and is not retained by biota, enters the groundwater table beneath the lakes (Whelan and Barrow 1984). In contrast, sediments in the CWC wetlands have a high P binding capacity, with maximum adsorption capacity of 15 g/m^2 of P in the top 1 mm of the sediment (13100 mg/kg, Chambers and McComb 1996) calculated with Langmuir equation (Syers *et al.* 1973), although the bond was relatively weak.

3.2.7 Vegetation

In 2010-2012, prior to the commencement of municipal effluent discharge, approximately half of Swamphen Lake was covered by *Typha orientalis*. At the southern end of the wetland was an area of bare sediment that, when seasonally inundated, supported filamentous algae. SAV (*Chara* or *Potamogeton* species) recorded in the past at Swamphen Lake (Gordon and Chambers 1987, GHD 2004), were not observed in 2010-2012.

Vegetation in Island Lake was similar to Swamphen Lake with approximately 60-70% of the lakebed covered in *Typha* and the remainder being bare sediment that supported filamentous algae when inundated. Approximately 60-70% of Peninsula Lake was also covered in *Typha*, although unlike the other two wetlands, Peninsula Lake remained wet in most years and in some seasons supported SAV (charophytes; *Chara* and *Nitella* species) in the open water area in addition to bare sediment.

3.2.8 Effluent release into Swamphen Lake

This project studied the effects of P-rich effluent release into Swamphen Lake, which was part of a release of wastewater into the CWC wetlands. The CWC wetlands were designed to act as tertiary treatment wetlands for secondary treated municipal effluent processed through a conventional facultative oxidation pond treatment system at the Capel Wastewater Treatment

Plant (WWTP). Effluent was piped from the WWTP to the CWC and outlets were established in the northern end of Swamphen, Island and Peninsula Lakes. Although for the duration of this research, effluent was discharged into Swamphen Lake only (commencing in February 2012). Pipes allowing water flow between the wetlands were closed and Swamphen Lake was effectively operated as an infiltration/evapotranspiration basin.

According to the treatment design, raw wastewater entering the WWTP was to flow through three facultative ponds and be subject to aeration, alum dosing and chlorination, prior to being pumped into the wetlands. Alum dosing was to be undertaken to reduce concentrations of suspended solids and P in the water. Alum dosing is usually undertaken as part of tertiary treatment and the wetlands would then be considered polishing systems however in this case alum dosing was used as part of secondary treatment to treat excessive P concentrations of the effluent to within treatable levels. Chlorination was to be undertaken to reduce bacterial concentrations to levels suitable for primary contact. Approximately 180 kL of treated effluent per day (65.7 ML/year) was expected to be released from the WWTP to the CWC with volumes increasing over the years. The treated effluent was expected to contain up to 30 mg/L of nitrogen, 2 mg/L of TP, 20 mg/L of BOD and 80 mg/L of suspended solids. Data on quantity and quality of discharged effluent was obtained from Water Corporation.

3.3 Sampling design

3.3.1 Seasonal and multiyear studies

Functional zones present in Swamphen, Island and Peninsula Lakes were mapped using an aerial photo. To minimise spatial variation in P dynamics due to differences in hydroperiod and water depth, a belt (30 m wide) of uniform depth, positioned at the interface of the functional zones was selected for sampling in each wetland in the seasonal and multiyear studies (Figure 3-5). In Swamphen Lake, this sampling area was divided into three or five equal sections (depending on sampling occasion) at increasing distance from the effluent discharge point. This was to account for decreasing P load with distance from source (Figure 3-5) which may have potentially created a gradient in enrichment effects (DeBusk *et al.* 2001, Reddy and DeLaune 2008, Kadlec and Bevis 2009, DeBusk *et al.* 2011). Similar sections were established in Island and Peninsula Lakes although no effluent was released there. This created in total six or ten sub-areas for sampling in each wetland (Figure 3-5, Figure 3-6, Table 3-3).

For the seasonal study, sampling was undertaken in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter). Sampling began eight months after the

commencement of effluent release, once the system had formed a measurable body of surface water as initially the released effluent infiltrated cracks in the dry clay soil close to the inflow point increasing groundwater levels but failing to fill the lake itself. For the multiyear study, samples were taken annually in spring (October) over three consecutive years (2012, 2013 and 2014) following the commencement of effluent release in February (summer) 2012. Spring was chosen as most of the biological ecosystem components were expected to be active and growing then. At each sampling occasion, samples were taken from a randomly located point within each of the sub-areas.

Table 3-3: Number of sampling sub-areas in seasonal and multiyear studies.

Sampling occasion	**Swamphen**	**Island**	**Peninsula**	**Study**
Spring 2012	6 (3 per zone)	6 (3 per zone)	6 (3 per zone)	Seasonal, Multiyear
Summer 2013	10 (5 per zone)	10 (5 per zone)	10 (5 per zone)	Seasonal
Autumn 2013	10 (5 per zone)	10 (5 per zone)	10 (5 per zone)	Seasonal
Winter 2013	10 (5 per zone)	10 (5 per zone)	10 (5 per zone)	Seasonal
Spring 2013	10 (5 per zone)	none	none	Multiyear
Spring 2014	10 (5 per zone)	none	none	Multiyear

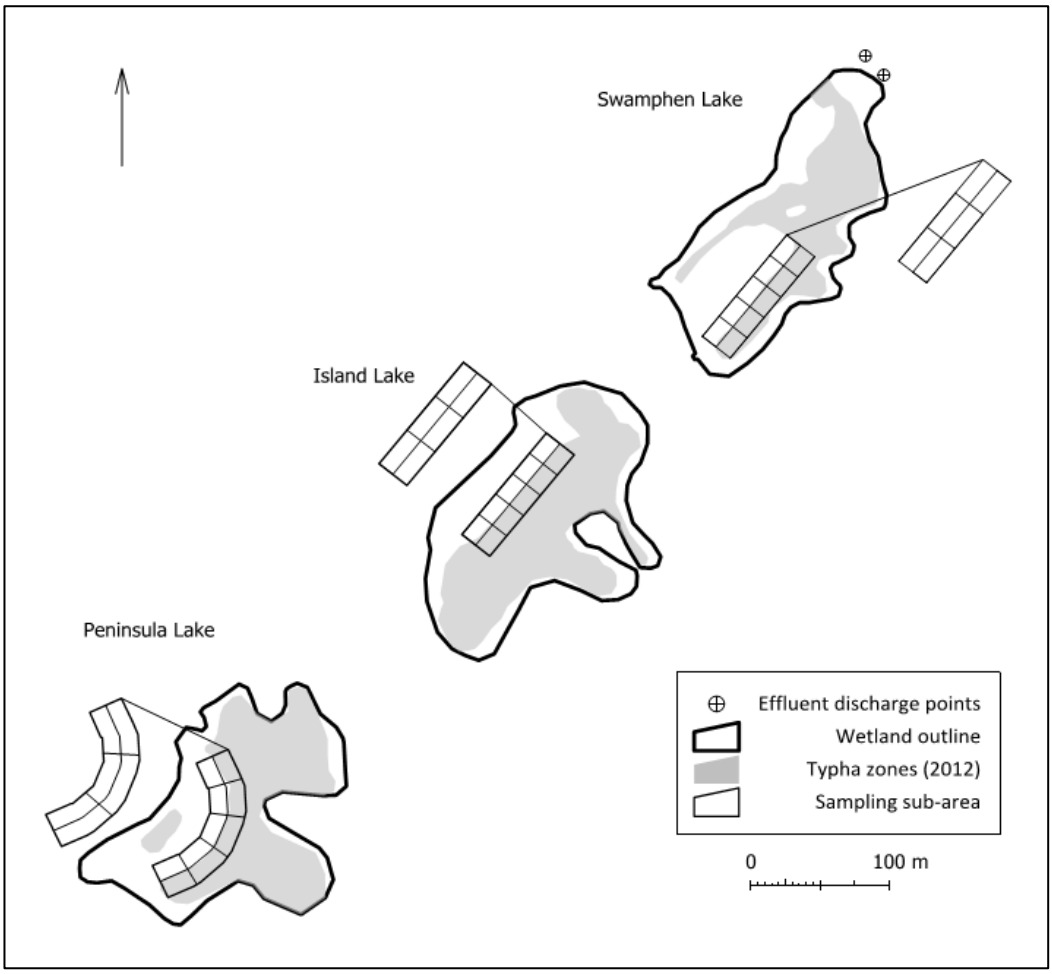

Figure 3-5: Sampling areas in Swamphen, Island and Peninsula Lakes in October 2012 (spring, 6 sub-areas per wetland, shapes shown adjacent to wetlands), January 2013, April 2013 and July 2013 (summer, autumn and winter, 10 sub-areas per wetland, shapes shown within wetlands).

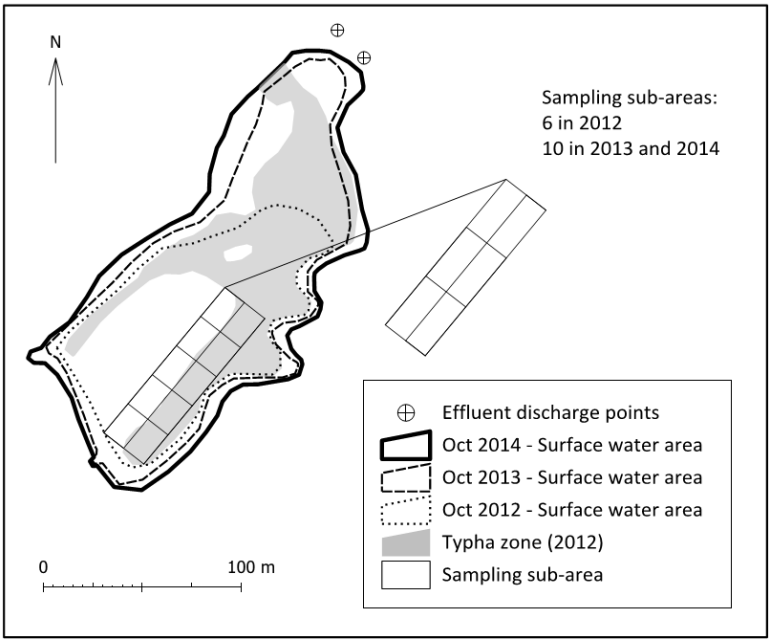

Figure 3-6: Swamphen Lake during multiyear study showing approximate surface water area in each year, initial *Typha* zone, sampling sub-areas and effluent discharge points.

3.3.2 Mesocosm study

Twenty-four mesocosms (1m^2) were placed *in situ* in Peninsula Lake (Figure 3-7), in areas of similar depth. Four enrichment (subject to nutrient enrichment) and four control mesocosms were placed in each of the three functional zones (bare sediment, SAV and *Typha*) in areas of similar depth.

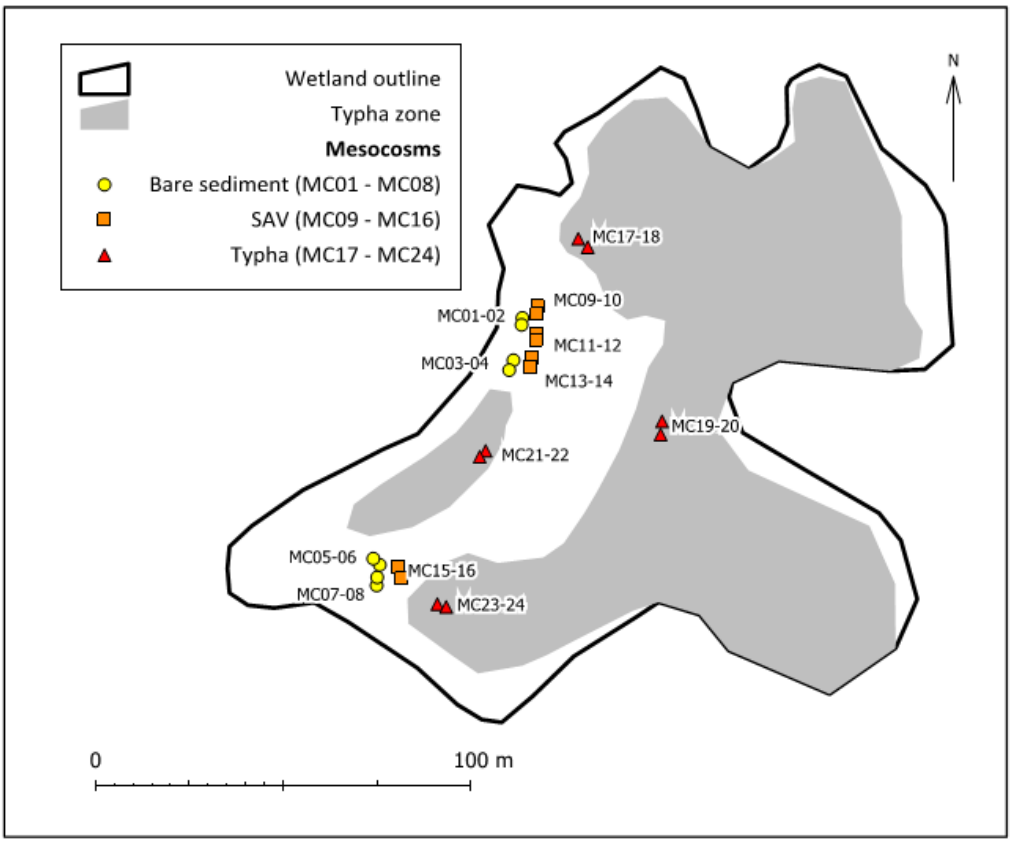

Figure 3-7: Locations of 12 pairs of *in situ* mesocosms in Peninsula Lake during the mesocosm enrichment study. Each pair comprised one control and one treatment mesocosm.

3.4 Field sampling

Field sampling methods were largely the same among the three studies (Table 3-4). Ecosystem components were sampled for TP concentration and mass per unit area to allow calculation of TP storage per unit area. Additionally, water quality and vegetation characteristics were sampled to track ecosystem change and provide context for changes in P storage. In the seasonal and multiyear studies, all available ecosystem components were sampled at each sampling round. In the mesocosm experiment, some stores were only sampled at the start

and end of the experiment due to the destructive nature of the sampling or the disturbance that it caused (described in more detail in Section 6.2.2).

At each sampling location, water temperature, pH, DO, ORP and EC were sampled with a multimeter (YSI 556) and depth was recorded (Table 3-4). Water samples were collected using a PVC tube, to integrate sampling across the depth profile. These samples were subsampled for turbidity, chlorophyll *α*, TP, and FRP. In the mesocosm experiment, TN, NH_4-N and NO_x-N were also subsampled for occasionally. Soil (surface 0-20 mm and deep 20-200 mm) and litter were sampled with a soil corer (40 mm ID). Where present, overlying floc, defined as the portion of the sediment that could be poured out of the corer, was also collected. In the mesocosm experiment, a 40 mm (ID) corer was used for sampling SAV. In the seasonal and mesocosm studies, SAV was sampled with a larger PVC corer (90 mm ID) by combining five random samples into one composite per sampling site. All samples requiring laboratory analysis were kept dark and cool until frozen within 12 hours of collection.

Above-ground parts of five *Typha* culms closest to a randomly selected point (or mesocosm centre) were collected. Samples were separated into live and dead composite samples. Culm heights and distances from the randomly selected point were also recorded. *Typha* sampling used the ordered distance method for estimating density (Pollard 1971, Nielson *et al.* 2004) chosen because it enabled the same method to be used in all three studies. Plot sampling was considered but as there was large variation in *Typha* density between and within the wetlands, choosing a single plot size could result in capture of no *Typha* in one wetland very high densities in another. The plot method would also deliver very high variability in the biomass and density estimates due to the small number of replicates. In contrast, the advantage of the ordered distance method is that balance between accuracy and sampling effort can be achieved by adjusting distance rank choice and the sample size is not dependent on the density being measured (Nielson *et al.* 2004).

Table 3-4: Summary of sampling methods for each variable and the studies and functional zones where these variables were measured. SE = seasonal study, MU = multiyear, ME = mesocosm study, BA = bare sediment zone, SA = SAV zone, TY = *Typha* zone.

Ecosystem component	Variable	Study	Zone	Sampling method
Water	Depth	All	All	Plastic tape measure
	Temperature, pH, EC, DO, ORP.	All	All	Multimeter YSI 556
	All water variables below	All	All	Water sample extracted with PVC tube (0.038 m ID, 1 m long bailer).
	Chlorophyll-*a*	All	All	Filtered onto Whatman GF/C, stored in darkness and frozen within 12h.
	Turbidity	All	All	Hach 2100P turbidity meter
	TP, FRP	All	All	TP and TN (unfiltered), FRP, NH_4-N and NO_x-N (filtered through a 0.45 µm cellulose acetate membrane)
	TN, NH_4-N and NO_x-N	ME	All	
Topsoil (0-20mm)	TP, mass	All	All	Perspex tube soil corer (44 mm ID) pressed across the bottom profile, with the sample being divided into the respective ecosystem components.
Subsoil (20-200mm)	TP, mass	All	All	
Floc	TP, mass	All	BA in SE and MU, SA in ME	
Litter	TP, mass	All	TY only	
SAV (method 1 - small core)	TP, mass	ME	SA only	
SAV (method 2 - large core)	TP, mass	SE Peninsula, ME	SA only	PVC tube (9 cm ID) pressed across SAV into sediment.
Periphyton	TP, mass	ME	All	Polyethylene strips (3cm x 50 cm) suspended vertically in water from start of experiment and collected at desired interval and scraped.
Typha (above-ground; live and dead)	TP, mass, density	All	TY only	Density: ordered distance method (Pollard 1971, Nielson *et al.* 2004) based on the five closest culms. Biomass: composite live and dead biomass of the closest five culms cut at base. TP: Based on subsample of the live and dead composites.
Typha	Height	All	TY only	Plastic tape measure

3.5 Laboratory analysis

Laboratory analysis was undertaken by the Marine and Freshwater Research Laboratory (MAFRL, NATA accreditation No. 10603) at Murdoch University using the following methods. Unfiltered water samples were analysed for TP and selected samples for TN using the method of Valderrama (1981) (autoclave digests with potassium persulphate). Filtered water samples were analysed for FRP (ascorbic acid) and NO_x, (copper-cadmium reduction) using the methods of Johnson and Petty (1983); selected samples were analysed for NH_4 (alkaline phenate) using the method of Switala (1993). Chlorophyll α (on filter papers) was analysed using methods from APHA (2005). Floc, litter, SAV and periphyton (scraped off plastic strips) samples were dried, weighed and analysed for TP using the method of Aspila *et al.* (1976). Soil samples were freeze-dried, weighed, sub-sampled, and analysed for TP (digested in concentrated sulphuric acid in presence of a copper catalyst) (Aspila *et al.* 1976). Live and dead *Typha* samples were oven dried (at 70° Celsius for 72 hours), weighed and then sub-sampled and analysed for TP as above (Aspila *et al.* 1976).

3.6 Data processing

Where necessary, laboratory results for TP concentration were converted to common units (Table 3-5) prior to data analysis. Results below detection limits were substituted with the detection limit. TP storage was then derived from TP concentration and dry store mass per unit area. Total TP storage was calculated as the sum of TP in ecosystem components for each sampling location. Unless otherwise stated, subsoil was excluded from total TP storage because the large size of this store (in comparison to other stores) and its high spatial and temporal variability masked change in other ecosystem components (further described in Section 4.3.4). Mean values throughout the thesis are presented with ± 1 standard error.

Typha density was calculated as follows (Pollard 1971, Nielson et al. 2004):

$$\widehat{D} = (ng - 1) \,/\, \left[\pi \sum (R_{(g)i})^2\right],$$

where *D* is density, *n* is sample size (number of starting points), g is g^{th} nearest individual plant to the starting point (g = 5 in this thesis), $R_{(g)i}$ is distance to the g^{th} nearest plant from the i^{th} random starting point. Two different approaches were used. To calculate *Typha* mass per unit area, n = 1 was used in the formula so that the mass (derived from density and biomass) could be calculated for each sampling site separately. This was necessary to allow subsequent calculation of total TP storage for each sampling site separately. For the purpose of calculating *Typha* density to describe changes in the vegetation community, all the sampling sites (per

wetland or mesocosm type, per sampling occasion) were used together in the equation (n = 3 or n = 5, depending on sampling occasion) to provide one estimate of *Typha* density per wetland or mesocosm type per sampling occasion. Variance for *Typha* density was estimated with associated variance estimator (Nielson et al. 2004):

$$\widehat{var}\left(\widehat{D}\right) = (\widehat{D})^2 \,/\, (ng - 2)$$

Standard deviation was calculated as square root of the variance and further divided by square root of the sample size (*n*) to obtain standard error.

Table 3-5: Data processing for derived variables and exceptions.

Variable	Processing	Units	Exceptions
TP concentration	Unit conversion if necessary	mg/g	Water: µg/L
Mass	Unit conversions from known dry mass per unit area	kg/m^2	Water: depth in cm *Typha*: Calculated from culm density (Pollard 1971, Nielson *et al.* 2004) and associated biomass. Periphyton**:** Mass per mesocosm was used as proxy for mass per m^2. Mass per mesocosm was based on multiplying periphyton mass per sample area (one strip, 0.018m^2) with approximate underwater growth surfaces available including mesocosm walls and strips, totalling 5.0m^2.
TP storage	Derived from TP concentration and known dry mass per unit area for each sampling point	g/m^2	Water: depth converted to litres assuming 1 cm = 10 L in 1m^2 Topsoil: TP storage calculated using average topsoil mass (per wetland) where determined appropriate on the basis of screening the results as explained later. Subsoil: TP storage calculated using average subsoil mass (per wetland) due to subsoil sampling issues explained later.
Total TP storage	Calculated for each sampling point as the sum of TP stored in individual ecosystem components.	g/m^2	Excludes subsoil TP storage unless otherwise stated.

CHAPTER 4: TRACKING WETLAND PHOSPHORUS STORAGE AND CYCLING: A SEASONAL PERSPECTIVE ON PRE-ENRICHMENT AND EARLY-STAGE NUTRIENT ENRICHMENT

4.1 Introduction

Seasonal dynamics in water regime, temperature and vegetation growth patterns affect wetland phosphorus (P) storage and cycling (Howard-Williams 1985, Kadlec and Reddy 2001, Kadlec 2016). In unenriched wetlands, seasonality is likely to be the dominant cause of variation in P storage and cycling. However, in wetlands undergoing nutrient enrichment the incoming P load would be expected to drive changes in the scale and nature of P storage that might eclipse seasonal patterns. Previous research has shown that the quantities of P cycled in oligotrophic systems are lower than in eutrophic systems (e.g. Noe and Childers 2007), but not how P-cycling changes over the enrichment continuum.

Few studies of P enrichment processes have been conducted in Mediterranean-climate wetlands with strong seasonal dynamics (but see Qiu and McComb 1994, Qiu *et al.* 2004, Özen *et al.* 2010, Gilbert *et al.* 2014, Coppens *et al.* 2016). A major review into the performance of treatment wetlands (Land *et al.* 2016) highlighted that further research was needed on the effects of seasonality, particularly in wet/dry climates. The wetlands in this study are located on Swan Coastal Plain in southwestern Australia, where wetland dynamics reflect the strong seasonality of this Mediterranean climate region (Boix *et al.* 2016). Summer-autumn periods are hot and dry with very little rain, in contrast to winter-spring periods that are cooler and when the majority of annual rainfall occurs. Many wetlands on the Swan Coastal Plain are surficial expressions of groundwater, occurring at low points in the landscape (Semeniuk 1988, Semeniuk *et al.* 1990). Water levels in these wetlands reflect a combination of their topographical elevation and the seasonal signature of the Mediterranean climate on groundwater levels.

Prior to the commencement of effluent discharge, Swamphen Lake regularly dried up over the summer-autumn period. Island Lake (unimpacted) also dried out most years, whereas Peninsula Lake (unimpacted) was mostly perennial, although showing seasonal fluctuations in water level. The addition of treated municipal effluent into Swamphen Lake added nutrients but also introduced a supplementary source of water, changing it to a perennial wetland; however, water levels were still expected to fluctuate seasonally. When releases commended,

the wetland was dry, so effluent infiltrated cracks in the clay soil close to the inflow point, increasing groundwater levels but failing to fill the lake itself. Consequently, sampling for this study did not commence until there was a measurable body of surface water in the lake (eight months after the commencement of effluent release).

The conceptual models of P storage and cycling over the nutrient enrichment continuum (Figure 2-4, Figure 2-5, Figure 2-6) described three key stages: a pre-enrichment (unenriched) stage (Stage 1), a functional P uptake stage (Stage 2) and a hypereutrophic end stage (Stage 3). This study aimed to investigate the emergence of Stage 2 in a tertiary treatment wetland (Swamphen Lake) following nutrient input and the dynamics of Stage 1 in two unimpacted wetlands (Island and Peninsula Lakes - no nutrient input) simultaneously. Seasonal sampling occurred over a period of 9 months with a 3-monthly sampling frequency encompassing the four seasons. This design was chosen to capture the gradual emergence of enrichment effects in the treatment wetland (Stage 2) and to determine the scale of seasonal changes in P storage, P cycling and related ecosystem variables (vegetation characteristics and water quality) in both Stages 1 and 2.

Two functional zones, present in all three wetlands, were included in this study: 1) a bare-sediment open-water zone (Figure 2-4) and 2) an emergent macrophyte (*Typha orientalis)* zone (Figure 2-6). Research questions and hypotheses were those presented in Table 2-7, apart from Hypothesis 8 relating to SAV, which was not included here as it was not present in all wetlands. The following sub-questions were adjusted to include patterns related to *seasonality*:

a) How does TP storage of ecosystem components change with enrichment and how does this differ between functional zones and seasons in enriched and unimpacted wetlands?
c) How does the distribution of total TP storage across ecosystem components (% of total) change with enrichment and how does this differ between functional zones and seasons in enriched and unimpacted wetlands?
e) How do vegetation characteristics change with enrichment in the different functional zones and seasons in enriched and unimpacted wetlands?
f) How does water quality change with enrichment and how does this differ between functional zones and seasons; were there seasonal changes in water quality in unimpacted wetlands?

4.2 Methods

4.2.1 Study design

The three study wetlands and the sampling design are described in Chapter 3 (Section 3.2 and Section 3.3 respectively). Sampling areas were positioned to minimise depth variation in the two functional zones, and aligned with increasing distance from the effluent discharge point (Section 3.3.1, Figure 3-5). This stratified the sampling design by distance from effluent discharge point in Swamphen Lake. Within the stratified sub-areas, sampling points were chosen randomly at each sampling time. Samples were taken over four consecutive seasons: October 2012 (spring), January 2013 (summer), April 2013 (autumn), and July 2013 (winter). This design enabled me to address all relevant sub-questions within Research Question 1 and 2 (Table 2-7). Usually, a sampling design focussing on seasonal differences would replicate seasons, to deconfound temporal variation among particular points in time from the effect of season. However, this was not possible in the present study, where changes between seasons were to be compared between a newly enriched wetland and two unenriched wetlands because it was not logically possible to newly enrich the treatment wetland more than once. As seasons in Mediterranean climates show very distinct changes in temperature, precipitation and groundwater levels, we could be sure that, for example, summer would always be much drier than winter, even in a severe year-long drought. Such direct effects of climate on hydrological dynamics, driving large hydrological changes (e.g. in water level), meant that regardless of the particular weather conditions during the study, the results would genuinely reflect the influence of season in Mediterranean-climate wetlands.

4.2.2 Field sampling and laboratory analysis

During each sampling occasion, ecosystem components were sampled for TP concentration and mass per unit area to allow calculation of TP storage per unit area (see Section 3.6). Depth, water quality and vegetation characteristics were sampled to track seasonal and ecosystem changes and to provide context for changes in P storage. Field sampling followed methods described in Section 3.4. Samples were submitted to an accredited laboratory and analysed for nutrients, mass and other characteristics in accordance with methods described in Section 3.5.

4.2.3 Data analysis

Due to stratification of the sampling area by distance from the effluent discharge point (Figure 3-5), results were initially examined for evidence of a gradient by plotting each variable against increasing distance from the discharge point (Appendix 1). In almost all cases, no gradient was

evident. Where a decline with distance was present (Table 4-1, all in the *Typha* zone), it was mostly unlikely to be related to treatment (explained in Table 4-1) but rather due to natural variation, as patterns over distance were not consistent over time. Potential treatment related declines with distance were found in live *Typha* and litter TP concentrations in winter only, however without spatial replication, these trends could also have been due to natural variation. Because distance-related decline was not a consistent or prominent feature of the dataset, the distance variable was excluded from further analyses and data from different distances were used as (spatial) replicates.

Table 4-1: Variables showing potential decline with distance from effluent discharge point. For further detail see Appendix 1.

Variable	Season	Analysis
Topsoil TP concentration (*Typha* zone only)	Autumn	Unlikely to be treatment related as not repeated in winter.
Litter TP concentration	Winter	Potentially treatment related; there is some overlap between winter and other seasons.
Live *Typha* TP concentration	Winter	Potentially treatment related but there is limited overlap between winter and other seasons, so does not affect use of distances as spatial replicates.
Live *Typha* mass	Spring and autumn	Decline in spring unlikely related to treatment as no treatment effect elsewhere at this stage. Autumn also unlikely treatment related as not repeated in winter. Consequently these declines likely natural variation in data.
Dead *Typha* mass	Spring	Decline in spring unlikely related to treatment as no treatment effect elsewhere at this stage.

Means for each variable in each wetland were plotted separately for each functional zone against time. Water TP, FRP and chlorophyll α concentrations and %DO were compared against ANZECC (2000) guidelines; water TP and chlorophyll α concentrations were also compared against trophic status classification (Table 2-6, modified OECD 1982). For each variable and wetland separately, differences between seasons (4 levels = spring, summer, autumn and winter) and (where applicable) functional zones (2 levels = bare sediment, *Typha*) were tested (with SPSS version 22) using two-way analysis of variance (ANOVA) (two factors: season and zone, both fixed) or one-way ANOVA (one factor: season, fixed) to address the research questions. Differences between treatment and unimpacted wetlands were not tested statistically because any treatment effect would have been confounded by pre-existing differences between wetlands in water regime and quality (see Section 3.2). The effect of

treatment (combining the effects of added nutrients and water) was assessed on the basis of changes in the variables over time (treatment wetland only), particularly between the first and last sampling occasions (spring versus winter).

Where there was an interaction between season and zone, simple effects tests were undertaken to identify specific differences among seasons and the interaction was identified as either ordinal or disordinal. In the case of disordinal interactions, the main effects of season and zone were not interpreted (because they were inconsistent between the levels), but only the simple effects tests. For ordinal interactions, both main effects and the interaction were interpreted. When ANOVA identified season as significant, and there was no significant disordinal interaction, post-hoc Tukey's tests were used. In particular, Tukey's tests were used to determine whether the first and last sampling occasions differed in the treatment wetland.

Assumptions of normality and equal variances were checked and where necessary, $\log_{10}$ transformations were used. Where assumptions could not be met after transformation, non-parametric Kruskal-Wallis tests with post-hoc pairwise comparisons were used. For one-way ANOVAs, where the assumption of equal variances was not met after transformation, the more robust Welch test was used with a post-hoc Games-Howell test. Where the assumption of normality or both assumptions of normality and equal variance was not met after transformation, non-parametric Kruskal-Wallis tests with post-hoc pairwise comparisons were used.

Typha density was calculated using the ordered distance method (Pollard 1971, Nielson *et al.* 2004) (see Section 3.6). This method provided one overall density estimate per sampling occasion per wetland and consequently could not be tested statistically. Variance was calculated with an associated estimator (Nielson *et al.* 2004) and converted to standard error (as described in Section 3.6). Sampling occasions and wetlands were considered to differ if their standard errors did not overlap.

Difficulties extracting subsoil samples resulted in variable sample depths. Consequently, sample weights were not comparable, so no valid assessment of differences in mass between seasons and zones could be made. Visual assessment of scatter plots of sample depth versus TP concentration and Pearson's correlation tests showed no linear correlation between these variables (Appendix 2), so no samples were excluded from analysis of subsoil TP concentration. For the purposes of deriving subsoil TP storage, constant subsoil mass, specific to each wetland, was used (Swamphen 155.10 ± 1.91 kg/m^2, n = 26; Island 161.25 ± 3.89 kg/m^2, n = 19;

Peninsula 119.68 ± 3.88 kg/m^2, n = 28). These were calculated from only those samples that reached a depth of 200 mm.

4.3 Results

4.3.1 Hydrology

Over the study period, approximately 52 ML of effluent was discharged into the treatment wetland (Figure 4-1); rainfall (Figure 4-2) contributed approximately 12 ML (assuming 100% contribution from the main basin area of 2.3 ha). There was no surface water outflow from the treatment wetland (Figure 4-3). No data on contributions or losses via groundwater were available. However, as the storage volume of the treatment wetland over the study period peaked at 5.5 ML (based on water depth modelling by GHD 2004), significant losses via groundwater seepage were highly likely. This was supported by water balance calculations in the multiyear study (Section 5.3.1). During the summer sampling occasion, discharged effluent did not reach the surface water directly as overland flow. Rather, it percolated into cracks in the clay lake-bed close to the inflow point (as during the pre-study period), potentially contributing to surface water via underground flow. The unimpacted wetlands received no surface water inputs and showed no surface water outflow. Consequently, water level in those wetlands was dependent on interactions between rainfall and groundwater. Water depths over time in all three wetlands are presented later in Figure 4-5c.

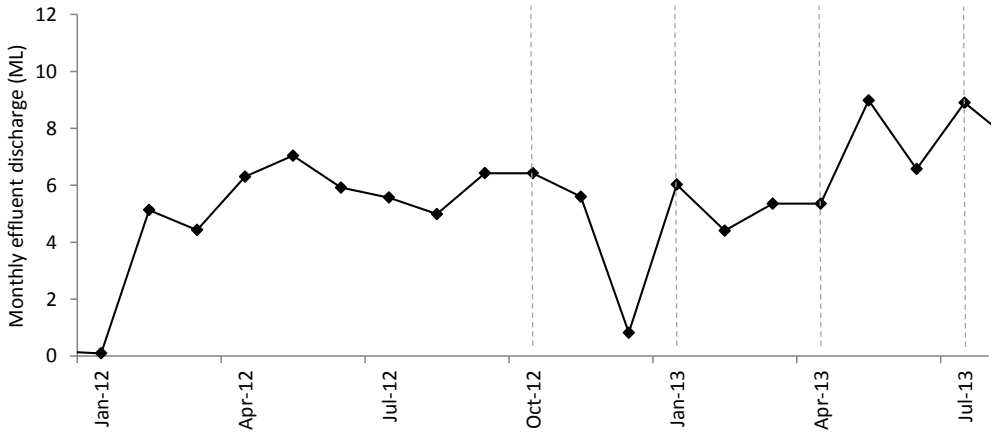

Figure 4-1: Monthly effluent discharge to Swamphen Lake leading up to and during the study period. Dashed lines indicate sampling occasions.

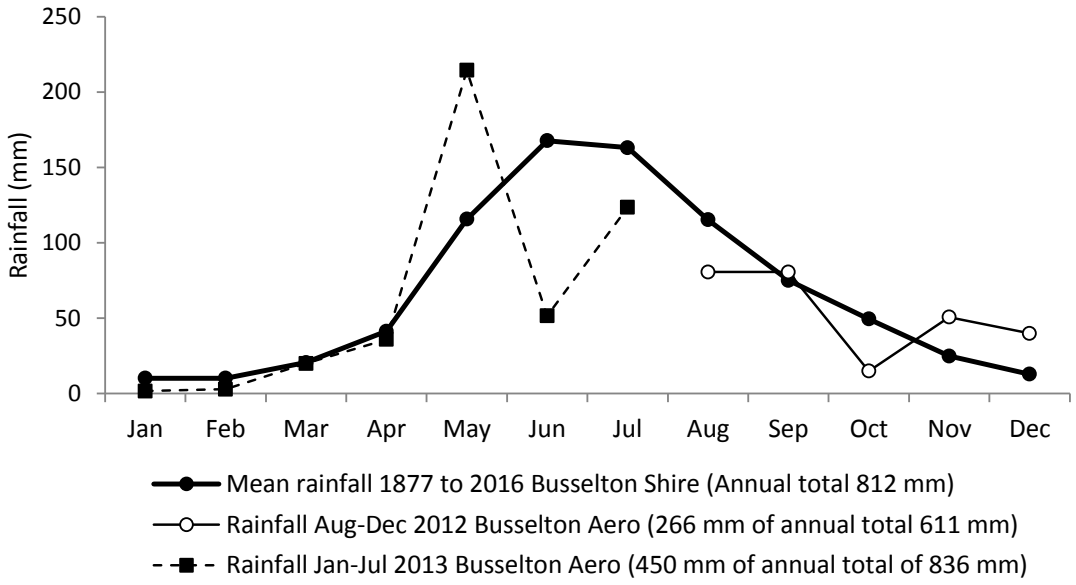

Figure 4-2: Rainfall in Busselton over the study period and long-term (Bureau of Meteorology 2016).

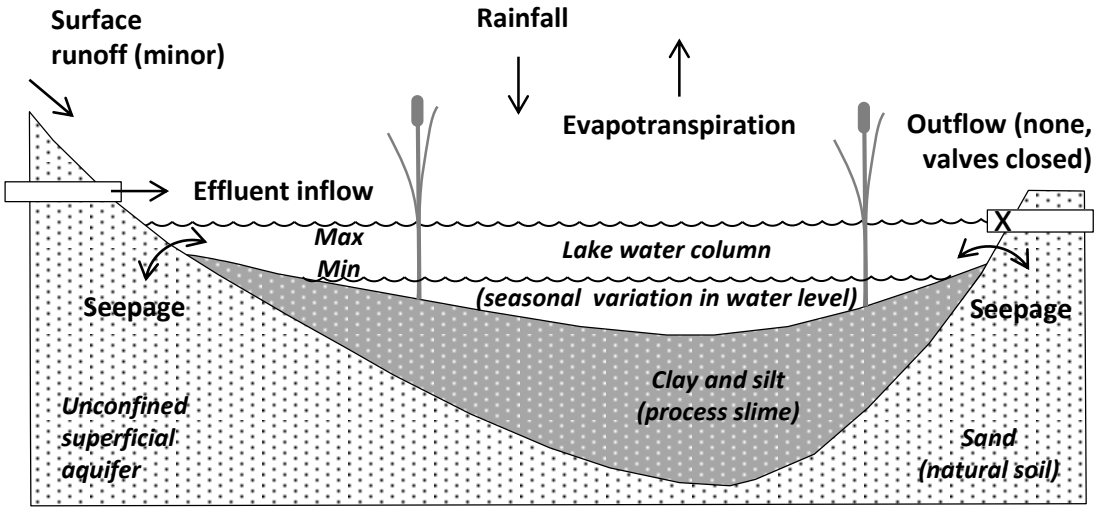

Figure 4-3: Schematic water balance model for Swamphen Lake. The two water levels indicate seasonal maximum (winter-spring) and minimum (summer-autumn).

4.3.2 Effluent quality

The TP concentration of discharged effluent was high (10 mg/L) when effluent release commenced in February 2012, however it fell rapidly as alum dosing (at the water treatment plant) became effective. Between autumn 2012 and end of the study period in winter 2013, effluent TP concentration ranged between 0.48 - 5.00 mg/L, often exceeding the design maximum of 2.00 mg/L (Figure 4-4a). P load increased over the study period and was highest leading up to the last sampling occasion. Between the first (spring) and last (winter) sampling

occasions, approximately 10.18 g/m^2 of P was added to the treatment wetland (in this study, all loads calculated for wetland area of 1.5 ha, being the area of inundation in July (winter) 2013).

TN concentration of effluent ranged between 29-60 mg/L and exceeded the design maximum of 30 mg/L during all but one month (Figure 4-4b). The majority of nitrogen added was ammonium nitrogen (NH_4-N). Between spring and winter, approximately 149.62 g/m^2 of nitrogen was added to the wetland, almost 15 times the P load. The high nitrogen load indicates that the system would have remained P limited.

BOD concentration of effluent ranged between 5-75 mg/L and often exceeded the design maximum of 20 mg/L, particularly between summer and autumn (Figure 4-4c). Between the first and last sampling occasions, a total BOD load of 98.63 g/m^2 was added to the wetland.

Effluent suspended solids (SS) concentration ranged between 10-120 mg/L and exceeded the design maximum of 80 mg/L once between summer and autumn (Figure 4-4d). Between the first and last sampling occasions, a total SS load of 142.49 g/m^2 was added to the wetland.

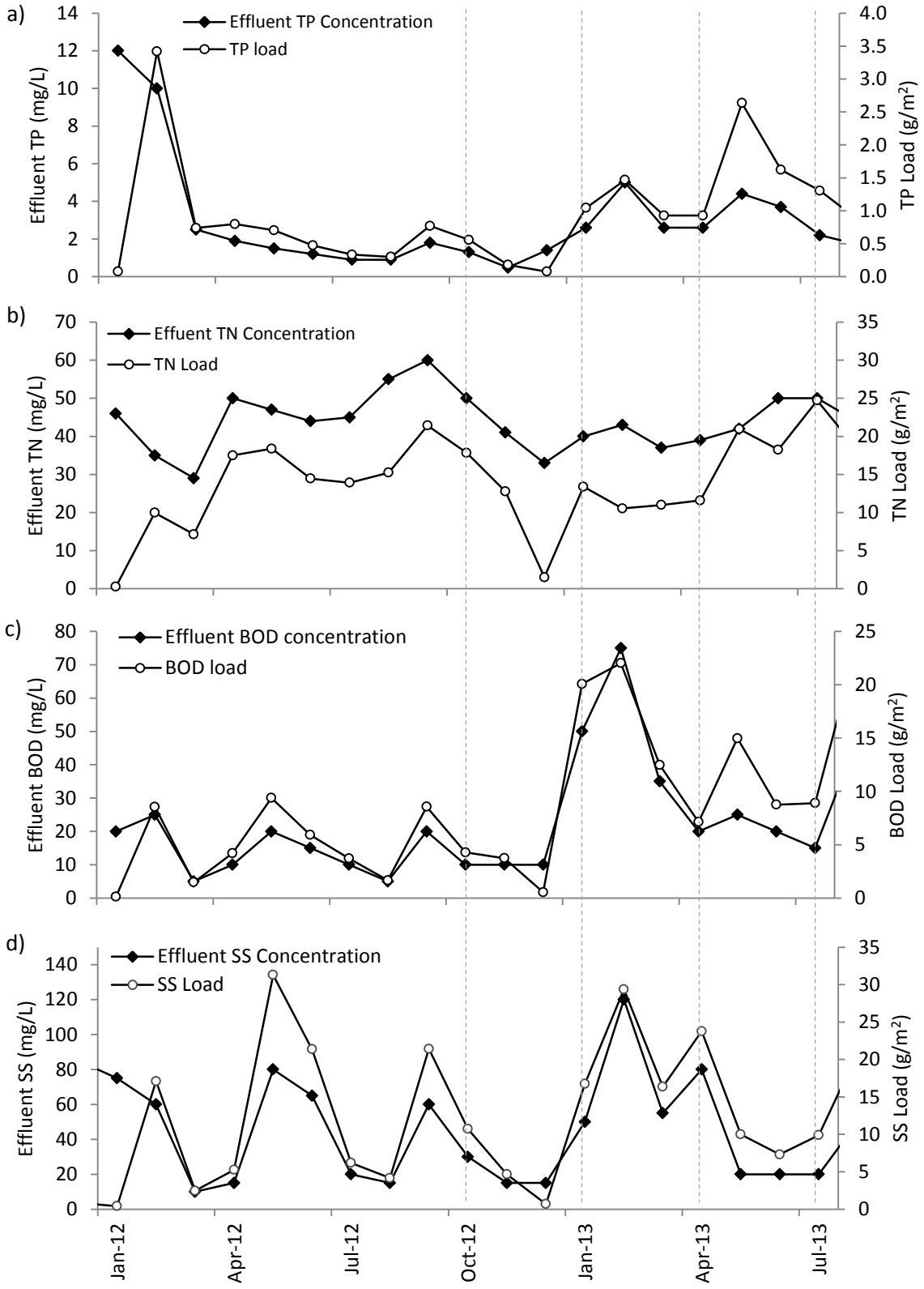

Figure 4-4: Concentration and load of total phosphorus (TP, a), total nitrogen (TN, b), biochemical oxygen demand (BOD, c) and suspended solids (SS, d) of effluent discharged to Swamphen Lake. Load calculated for area of 1.5 ha, being the area of inundation in July 2013. Based on concentration data collected by Water Corporation. Sampling occasions identified with dashed line.

4.3.3 Phosphorus storage of ecosystem components

How does TP storage of ecosystem components change with enrichment and how does this differ between functional zones and seasons in enriched and unimpacted wetlands?

In the treatment wetland, enrichment related increases in TP storage (from first to last sampling occasion) were observed in the water and litter components, but not in topsoil, subsoil or above-ground *Typha* (partly supporting Hypothesis 3, Table 2-7), (Figure 4-5, Figure 4-6, Figure 4-7, Table 4-4). TP storage of floc was highly variable and not suitable for statistical analysis, however it only occurred in the treatment wetland. Significant differences between sampling occasions, likely attributable to seasonal changes rather than enrichment, were recorded in TP storage (and concentration) of water and subsoil, water depth, and TP concentration and mass of above-ground *Typha* in the treatment wetland. In contrast, no significant increases in TP storage occurred in the unimpacted wetlands between the first and last sampling occasions in any ecosystem component. Seasonal changes were found in TP storage (and concentration) of water, topsoil and above-ground *Typha*, water depth and *Typha* mass (Figure 4-5, Figure 4-6, Figure 4-7, Table 4-5, Table 4-6). There were no significant differences in TP storage of the shared ecosystem components (water, topsoil, subsoil) between functional zones in unimpacted or treatment wetlands. Results for each of the ecosystem components are described below in more detail.

Water

Water TP storage in the treatment wetland increased significantly over time (supporting Hypothesis 3, Table 2-7), particularly by the last sampling occasion, overwhelming any minor seasonal differences observed in unimpacted wetlands (Figure 4-5a, Table 4-4). This increase in water TP storage in the treatment wetland was mostly caused by increased TP concentration (supporting Hypothesis 1, Table 2-7) but also supported by increased depth from summer to winter (Figure 4-5b,c). Water TP concentration exceeded the ANZECC trigger value (60 µg/L) only in the treatment wetland during the last two sampling occasions (autumn and winter). While TP concentration in unimpacted wetlands indicated mostly mesotrophic conditions (TP 10-35 µg/L), the treatment wetland moved from mesotrophic/eutrophic into hypereutrophic (TP >100 µg/L) by the end of the study. Seasonal changes in water TP concentration were similar in the two unimpacted wetlands, peaking in spring, whereas the treatment wetland peaked in winter (Figure 4-5b). Water depth in all wetlands was similar and followed seasonal trends, except in autumn (Figure 4-5c) when the unimpacted wetlands were dry but the treatment wetland remained inundated.

Functional zones did not differ in water TP storage in any of the three wetlands (Table 4-4, Table 4-5, Table 4-6). Water TP concentration was significantly higher in *Typha* than in bare sediment zone in Island Lake but similar between zones in the other wetlands. Water TP concentration was therefore not lower in vegetated than unvegetated zones under enrichment (contrary to Hypothesis 4, Table 2-7).

Problems with laboratory analysis resulted in variable detection limits for water FRP and the data was unsuitable for statistical analysis (Table 4-2). The ANZECC trigger value for water FRP concentration (30 µg/L) was exceeded in the treatment wetland and possibly Island Lake in winter and for the latter also in spring. Water FRP concentration in Peninsula Lake remained consistently below or near detection limit.

Table 4-2: Water FRP concentration in Swamphen Lake (treatment wetland) and unimpacted Island and Peninsula Lakes in 2012-2013.

	Water FRP concentration (µg/L)		
Season	**Swamphen**	**Island**	**Peninsula**
Spring 2012	All <10	<10 - 30	All <10
Summer 2012	<4 - 11	<4 - 5	All <4
Autumn 2013	<4 - 5	dry	dry
Winter 2013	52 - 230	All <100	<4 - 5

Benthic stores: Litter in the Typha zone

Litter TP storage in the treatment wetland increased substantially only in winter, at the end of the study period (supporting Hypothesis 3, Table 2-7), (Figure 4-5d, Table 4-4); this was also the only sampling occasion when it was higher in treatment than unimpacted wetlands. Contrary to Hypothesis 1 (Table 2-7), litter TP concentration in the treatment wetland did not change significantly across seasons due to high variability between sampling sites (Figure 4-5, Table 4-4). Litter mass increased over time in the treatment wetland only (supporting Hypothesis 2, Table 2-7), (Figure 4-5f, Table 4-4, Table 4-5, Table 4-6). Unimpacted wetlands showed no temporal change in litter TP storage, concentration or mass (Figure 4-5d-f, Table 4-5, Table 4-6).

Benthic stores: Floc

Floc was only recorded in the bare sediment zone of the treatment wetland during the first (spring) and third (autumn) sampling occasions. At other times, it formed the surficial part of the topsoil and could not be poured from the corer. Contrary to Hypothesis 1 (Table 2-7), TP

concentration of floc remained stable between these two sampling times: 0.83 ± 0.21 mg/g (n = 3) in spring and 0.89 ± 0.04 mg/g (n = 3) in autumn. The amount of floc increased from 182.4 ± 45.2 g/m^2 (n = 3) in spring to 389.6 ± 114.7 g/m^2 (n = 3) in autumn supporting Hypothesis 2 (Table 2-7). Combining TP concentration and mass, TP storage in floc increased from 0.14 ± 0.04 g/m^2 (n = 3) in spring to 0.36 ± 0.12 g/m^2 (n = 3) in autumn supporting Hypothesis 3 (Table 2-7).

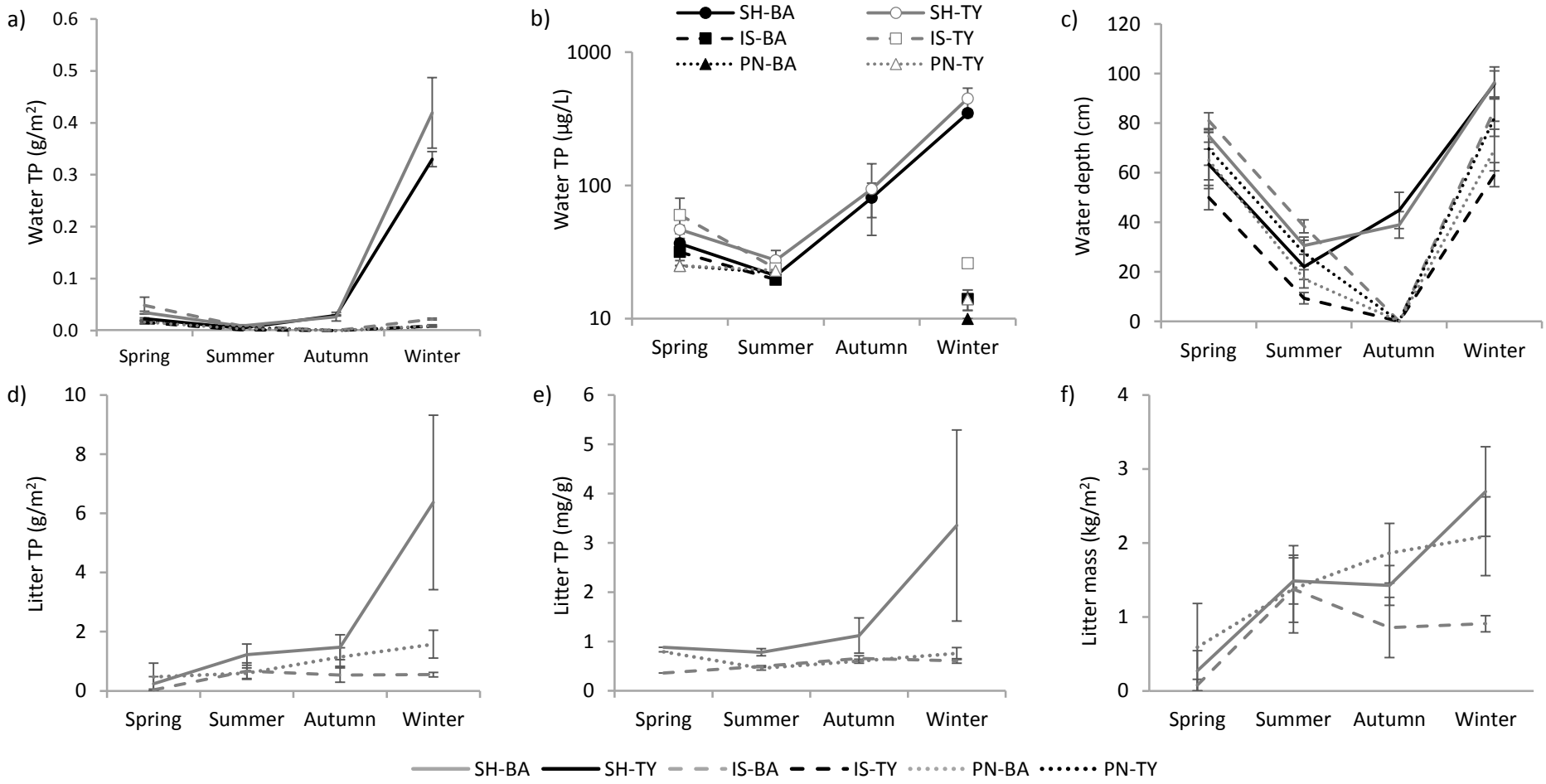

Figure 4-5: Mean water TP storage (a), water TP concentration (b), water depth (c), litter TP storage (d), litter TP concentration (e) and litter mass (f) in bare sediment (BA) and *Typha* (TY) zones of Swamphen (SH), Island (IS) and Peninsula (PN) Lakes in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter). N = 3 for spring, n = 5 for summer, autumn and winter. Error bars are ± 1 standard errors. Island and Peninsula Lakes dry in autumn.

Benthic stores: Topsoil

Contrary to Hypotheses 1 and 3 (Table 2-7), there were no significant differences in topsoil TP storage (or concentration) between seasons in the treatment wetland, although average TP storage increased by 1.5 g/m^2 between the first and last sampling occasions (Figure 4-6a, Table 4-4). In contrast, both unimpacted wetlands exhibited decreasing TP storage from spring to summer, followed by gradual recovery by winter (Figure 4-6a, Table 4-5, Table 4-6). As topsoil mass did not differ between seasons or functional zones in any of the wetlands (Figure 4-6c, Table 4-4, Table 4-5, Table 4-6), average topsoil mass (Swamphen 13.11 ± 0.59 kg/m^2, Island 16.42 ± 0.57 kg/m^2, Peninsula 9.77 ± 0.37 kg/m^2, n = 36 at all wetlands) was used for calculating TP storage. Therefore mass did not contribute to differences in topsoil TP storage over *time*. However, as average mass for the three wetlands differed, mass contributed to differences in TP storage between *wetlands*. Topsoil TP concentration of the treatment wetland was mostly within the range of unimpacted wetlands (Figure 4-6b), whereas topsoil TP storage was higher in the treatment than unimpacted wetlands (from summer onwards, Figure 4-6a) due to soil mass differences between the wetlands. Topsoil TP storage, concentration or mass did not differ between functional zones in any of the wetlands (Table 4-4, Table 4-5, Table 4-6).

Benthic stores: Subsoil

Contrary to Hypotheses 1 and 3 (Table 2-7) subsoil TP storage (and concentration) in the treatment wetland decreased gradually over time, being significantly lower in winter than in spring or summer (Figure 4-6d, Table 4-4). In contrast, no seasonal differences in subsoil TP storage were found in unimpacted wetlands (Figure 4-6d, Table 4-5, Table 4-6). As average subsoil mass (Swamphen 155.10 kg/m^2, ± 1 S.E = 1.91, n = 26; Island 161.25 kg/m^2, ± 1 S.E = 3.89, n = 19; Peninsula 119.68 kg/m^2, ± 1 S.E = 3.88, n = 28) was used for calculating TP storage, mass did not contribute to differences in subsoil TP storage over *time*. However, as average mass for the three wetlands differed, mass contributed to differences in subsoil TP storage between *wetlands*. The treatment wetland had consistently lower subsoil TP concentration than the unimpacted wetlands (Figure 4-6e), but TP storage was lower in treatment than unimpacted wetlands only during the last two sampling rounds (Figure 4-6d). Similarly to topsoil, there were no significant differences in subsoil TP concentration (and thus TP storage) between functional zones in any of the wetlands (Table 4-4, Table 4-5, Table 4-6).

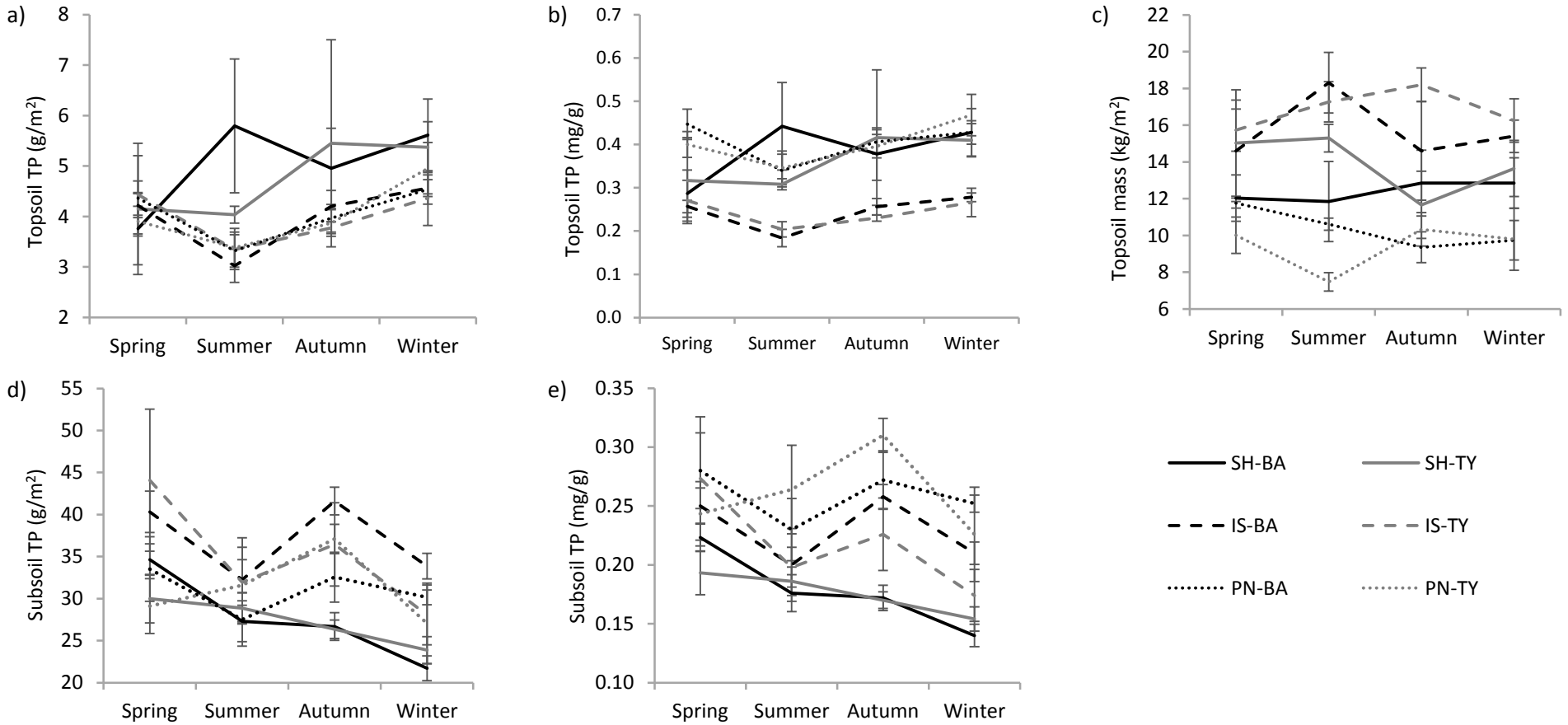

Figure 4-6: Mean topsoil TP storage (a), topsoil TP concentration (b), topsoil mass (c), subsoil TP storage (d) and subsoil TP concentration (e) in bare sediment (BA) and *Typha* (TY) zones of Swamphen (SH), Island (IS) and Peninsula (PN) Lakes in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter). N = 3 for spring, n = 5 for summer, autumn and winter. Error bars are ± 1 standard errors.

Above-ground Typha

Contrary to Hypothesis 3 (Table 2-7), there were no significant differences in live, dead or total *Typha* TP storage between the sampling occasions in the treatment wetland (Figure 4-7, Table 4-4). Furthermore, *Typha* TP storage in the treatment wetland was generally either lower than or similar to *Typha* TP storage in unimpacted wetlands. *Typha* TP storage (live, dead and total) was significantly different between seasons in one unimpacted wetland (Peninsula Lake) but not the other (Table 4-5, Table 4-6). In Peninsula Lake, live *Typha* TP storage was higher in spring than other months, dead *Typha* TP storage higher in winter than summer and total *Typha* TP storage higher in spring than summer or autumn (Table 4-6).

Contrary to Hypothesis 1 (Table 2-7), live *Typha* TP concentration showed no effect of treatment but rather followed a distinct seasonal pattern in all wetlands; it was highest in winter and lowest in summer and autumn (Figure 4-7b, Table 4-4, Table 4-5, Table 4-6). Dead *Typha* TP concentration in unimpacted wetlands followed a seasonal trend, decreasing significantly from spring to summer and then gradually recovering by winter (Figure 4-7e, Table 4-5, Table 4-6). In contrast, and supporting Hypothesis 1 (Table 2-7), dead *Typha* TP concentration was significantly higher in winter than other times in the treatment wetland (Figure 4-7e, Table 4-4), suggesting a treatment-related increase.

Live *Typha* mass in the treatment wetland followed a seasonal pattern: high in summer, low in winter. No significant differences between seasons were found in unimpacted wetlands (Figure 4-7c, Table 4-4, Table 4-5, Table 4-6). Live *Typha* mass was generally similar between the wetlands but was at times (summer, autumn) higher in Island Lake. There were no significant seasonal changes in dead *Typha* mass except in Peninsula Lake (mass in winter higher than in spring or summer, Figure 4-7f, Table 4-4, Table 4-5, Table 4-6). Dead *Typha* mass was similar in all wetlands. Variability in total *Typha* mass was high in all wetlands, so it did not differ between seasons (Figure 4-7h, Table 4-4, Table 4-5, Table 4-6). Contrary to hypothesis 2, total *Typha* mass was similar between the wetlands, although lower in the treatment wetland in winter. Thus, the expected increase in *Typha* biomass as a result of enrichment in Swamphen Lake did not occur (Table 2-7).

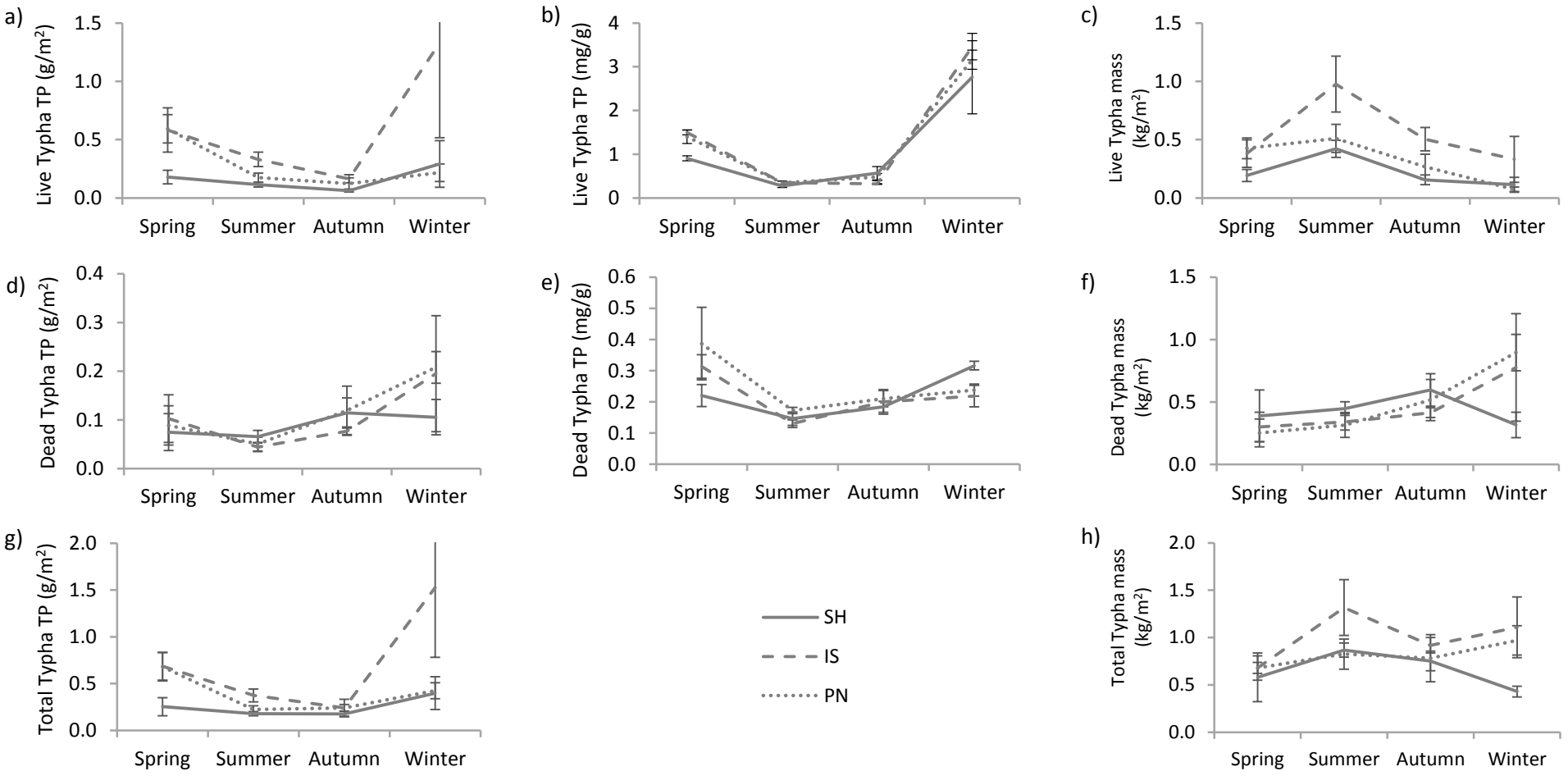

Figure 4-7: Mean live *Typha* TP storage (a), live *Typha* TP concentration (b), live *Typha* mass (c), dead *Typha* TP storage (d), dead *Typha* TP concentration (e), dead *Typha* mass (f), total *Typha* TP storage (g) and total *Typha* mass (h) in the *Typha* zone of Swamphen (SH), Island (IS) and Peninsula (PN) Lakes in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter). N = 3 for spring, n = 5 for summer, autumn and winter. Error bars are ± 1 standard errors.

SAV

SAV was only recorded in the bare sediment zone of Peninsula Lake (unimpacted), so was not subjected to the enrichment treatment. SAV presence was seasonal, recorded in spring and summer and then dying off, so that it was absent in the autumn and winter periods. TP storage, TP concentration and SAV mass were all higher during spring than summer (Table 4-3).

Table 4-3: SAV TP storage, TP concentration and mass in Peninsula Lake in 2012-2013.

	TP storage	**TP concentration**	**Mass**
Season	**g/m^2**	**mg/g**	**g/m^2**
Spring 2012	0.28 ± 0.06 (n = 3)	1.18 ± 0.10 (n = 3)	243.10 ± 60.52 (n = 3)
Summer 2012	0.11 ± 0.05 (n = 5)	0.81 ± 0.08 (n = 5)	131.00 ± 53.84 (n = 5)
Autumn 2013	0	0	0
Winter 2013	0	0	0

Table 4-4: Differences in TP concentration, mass (depth for water) and TP storage of ecosystem components between seasons and zones in Swamphen Lake. Abbreviations include Sp = spring, Su = summer, Au = autumn, Wi = winter, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, na = not applicable, # = non-parametric Kruskal-Wallis. Further details may be found in Appendix 3.

Ecosystem components	Season		Zone	Season*Zone
	Initial test	Post hoc	Initial test	Initial test
TP concentration				
Water	***	Sp/Su ns, Sp/Au ns, Sp/Wi***, Su/Au***, Su/Wi***, Au/Wi***	ns	ns
Topsoil	ns		ns	ns
Subsoil	**	Sp/Su ns, Sp/Au*, Sp/Wi***, Su/Au ns, Su/Wi*, Au/Wi ns	ns	ns
Litter#	ns		na	na
Typha - Live	***	Sp/Su**, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi**, Au/Wi*	na	na
Typha - Dead	***	Sp/Su ns, Sp/Au ns, Sp/Wi*, Su/Au ns, Su/Wi***, Au/Wi**		
Store mass (g/m^2)				
Water depth	***	Sp/Su***, Sp/Au**, Sp/Wi**, Su/Au*, Su/Wi***, Au/Wi***	ns	ns
Topsoil	ns		ns	ns
Litter#	*	Sp/Su ns, Sp/Au ns, Sp/Wi*, Su/Au ns, Su/Wi ns, Au/Wi ns	na	na
Typha - Live	*	Sp/Su ns, Sp/Au ns, Sp/Wi ns, Su/Au*, Su/Wi*, Au/Wi ns	na	na
Typha - Dead	ns		na	na
Typha - Total	ns		na	na
TP storage (g/m^2)				
Water	***	Sp/Su***, Sp/Au ns, Sp/Wi***, Su/Au***, Su/Wi***, Au/Wi***	ns	ns
Topsoil	ns		ns	ns
Subsoil	**	Sp/Su ns, Sp/Au*, Sp/Wi***, Su/Au ns, Su/Wi*, Au/Wi ns	ns	ns
Litter#	**	Sp/Su ns, Sp/Au ns, Sp/Wi*, Su/Au ns, Su/Wi ns, Au/Wi ns	na	na
Typha - Live#	ns		na	na
Typha - Dead	ns		na	na
Typha - Total#	ns		na	na
Total TP	*	Sp/Su ns, Su/Au ns, Sp/Wi*, Su/Au ns, Su/Wi ns, Au/Wi ns	ns	na

Table 4-5: Differences in TP concentration, mass (depth for water) and TP storage of ecosystem components between seasons and zones in Island Lake. Abbreviations include Sp = spring, Su = summer, Au = autumn, Wi = winter, ns = not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, / = versus, na = not applicable, # = non-parametric Kruskal-Wallis. Further details may be found in Appendix 3.

Ecosystem components	Season		Zone	Season*Zone
	Initial test	Post hoc	Initial test	Initial test
TP concentration				
Water TP#	**	Sp/Su*, Sp/Wi**, Su/Wi ns (dry in Au)	*	na
Topsoil	**	Sp/Su*, Sp/Au ns, Sp/Wi ns, Su/Au*, Su/Wi**, Au/Wi ns	ns	ns
Subsoil	*	all ns		
Litter#	ns		na	na
Typha - Live	***	Sp/Su**, Sp/Au**, Sp/Wi***, Su/Au ns, Su/Wi***, Au/Wi***	na	na
Typha - Dead	*	Sp/Su*, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi ns, Au/Wi ns	na	na
Store mass (g/m^2)				
Water depth	***	Sp/Su ns, Sp/Au***, Sp/Wi ns, Su/Au ns, Su/Wi*, Au/Wi***	ns	ns
Topsoil	ns		ns	ns
Litter#	ns		na	na
Typha - Live	ns		na	na
Typha - Dead	ns		na	na
Typha - Total	ns		na	na
TP storage (g/m^2)				
Water#	***	Sp/Su ns, Sp/Au***, Sp/Wi ns, Su/Au ns, Su/Wi ns, Au/Wi***	ns	na
Topsoil	**	Sp/Su*, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi**, Au/Wi ns	ns	ns
Subsoil	*	all ns	ns	ns
Litter#	ns		na	na
Typha - Live	ns		na	na
Typha - Dead	ns		na	na
Typha - Total	ns		na	na
Total TP	**	Sp/Su ns, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi**, Au/Wi ns	**	ns

Table 4-6: Differences in TP concentration, mass (depth for water) and TP storage of ecosystem components between seasons and zones in Peninsula Lake. Abbreviations include Sp = spring, Su = summer, Au = autumn, Wi = winter, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, na = not applicable, # = non-parametric Kruskal-Wallis. Further details may be found in Appendix 3.

Ecosystem components	Season		Zone	Season*Zone
	Initial test	Post hoc	Initial test	Initial test
TP concentration				
Water TP#	***	Sp/Su ns, Sp/Wi***, Su/Wi** (dry in Au)	ns	na
Topsoil	*	Sp/Su ns, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi*, Au/Wi ns	ns	ns
Subsoil	ns		ns	ns
Litter#	ns		na	na
Typha - Live	***	Sp/Su**, Sp/Au**, Sp/Wi***, Su/Au ns, Su/Wi***, Au/Wi***	na	na
Typha - Dead	*	Sp/Su*, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi ns, Au/Wi ns		
Store mass (g/m^2)				
Water depth#	***	Sp/Su ns, Sp/Au***, Sp/Wi ns, Su/Au ns, Su/Wi*, Au/Wi***	ns	ns
Topsoil	ns		ns	ns
Litter#	ns		na	na
Typha - Live	*	All ns	na	na
Typha - Dead	*	Sp/Su ns, Sp/Au ns, Sp/Wi*, Su/Au ns, Su/Wi*, Au/Wi ns	na	na
Typha - Total	ns		na	na
TP storage (g/m^2)				
Water#	***	Sp/Su*, Sp/Au***, Sp/Wi ns, Su/Au ns, Su/Wi ns, Au/Wi***	ns	ns
Topsoil	**	Sp/Su ns, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi **, Au/Wi ns		
Subsoil	ns		ns	ns
Litter#	ns		na	na
Typha - Live	**	Sp/Su**, Sp/Au**, Sp/Wi*, Su/Au ns, Su/Wi ns, Au/Wi ns	na	na
Typha - Dead	*	Sp/Su ns, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi*, Au/Wi ns	na	na
Typha - Total	*	Sp/Su*, Sp/Au*, Sp/Wi ns, Su/Au ns, Su/Wi ns, Au/Wi ns	na	na
Total TP	**	Sp/Su ns, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi**, Au/Wi ns	**	ns

Total TP storage

In the treatment wetland, total TP storage was significantly higher in winter than spring, indicating an enrichment-related increase (Figure 4-8, Table 4-4). In contrast, in the unimpacted wetlands there was no significant difference in total TP storage between spring and winter (start and finish of sampling) but there was a significant seasonal difference between summer and winter (Figure 4-8, Table 4-5, Table 4-6). In the unimpacted wetlands, total TP storage was higher in *Typha* than bare sediment zone whilst no significant difference between zones was found in the treatment wetland due to high variability.

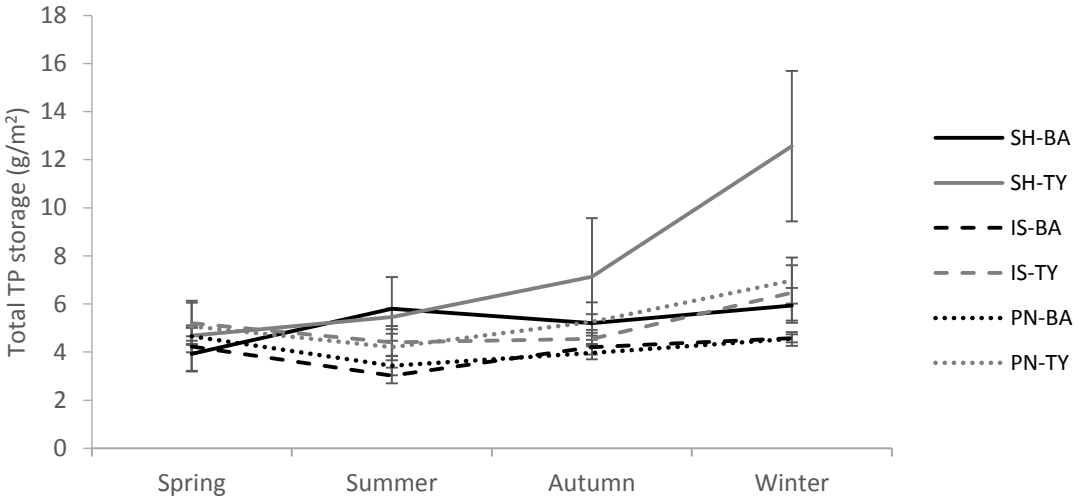

Figure 4-8: Mean total TP storage (± standard error) in bare sediment (BA) and *Typha* (TY) zones in Swamphen (SH) Island (IS) and Peninsula (PN) Lakes in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter). N = 3 for spring, n = 5 for summer, autumn and winter.

4.3.4 Proportional phosphorus storage

How does the distribution of total TP storage across ecosystem components (% of total) change with enrichment and how does this differ between functional zones and seasons in enriched and unimpacted wetlands?

Subsoil was the largest store of P in all wetlands in this study, in all functional zones and across all seasons. Subsoil was not included in the total TP storage calculation because, being much larger than the other stores, and much more variable, its inclusion hid meaningful changes in TP storage of other components. Subsoil is also not in direct contact with other stores except through *Typha* roots, exchange at the topsoil interface or via groundwater movement, rendering changes in subsoil slower and one step removed from other wetland processes. After excluding subsoil, the largest store of P was topsoil in all wetlands, all functional zones

and across all seasons. However, the percentage share of total TP storage held by topsoil varied over time, between functional zones and between wetlands. In the bare sediment zone, topsoil consistently stored over 90% of TP and in the case of Island Lake over 99%. In the *Typha* zone, topsoil TP storage accounted for 70-85% in unimpacted wetlands and between 49-88% in the treatment wetland. In the treatment wetland, the percentage share of TP in topsoil decreased over time particularly in the *Typha* zone, supporting Hypothesis 5 (Table 2-7).

In the bare sediment zone, the second largest store of P differed between wetlands and seasons. In Peninsula Lake, SAV seasonally accounted for up to 6% of TP whereas in Island Lake the only other store was water at <1%. In the treatment wetland, the second largest store in the bare sediment zone was either floc with up to 4% or water with nearly 6% during the last sampling round, although otherwise these components comprised <1%.

In the *Typha* zone, the second largest store was either litter or *Typha*. In Island Lake, litter held <1-13% and *Typha* 5-19% of TP and in Peninsula Lake litter held 9-21% and *Typha* 5-13% of TP. In contrast, in the treatment wetland the percentage share of litter increased over time from 4% in spring to 44% in winter, whereas the percentage share of *Typha* decreased from 6% in spring to 4% in winter. In the *Typha* zone, water accounted for less than 1% of TP in all wetlands and during all seasons, apart from winter in the treatment wetland when water percentage share of TP was nearly 4%. There were no consistent seasonal patterns between wetlands in the distribution of total TP storage across the ecosystem components in either of the functional zones.

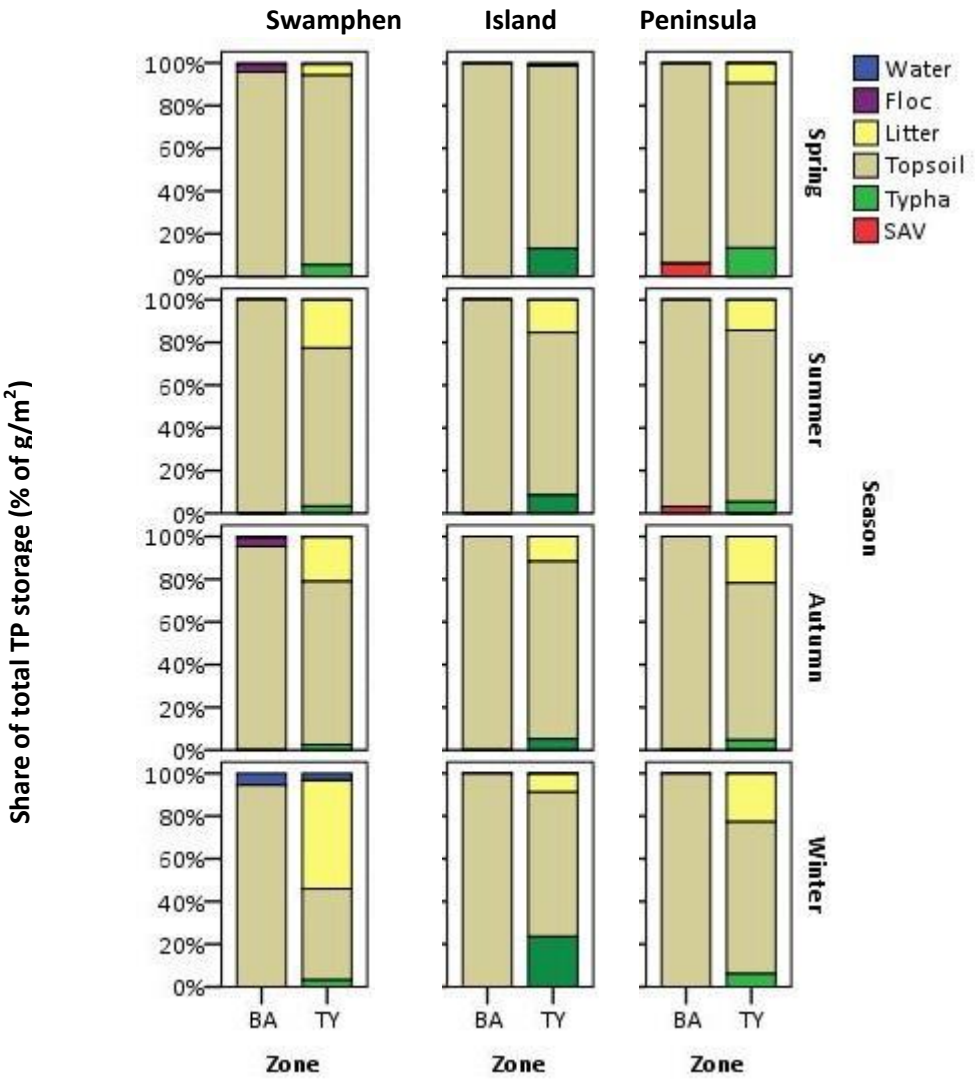

Figure 4-9: Distribution of total TP storage (excluding subsoil) between ecosystem components (% share) in bare sediment (BA) and *Typha* (TY) zones of Swamphen, Island and Peninsula Lakes in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter).

4.3.5 Phosphorus mass balance

Where does the added phosphorus go?

Differences in total TP storage between the first and the last sampling occasions show that in the treatment wetland 2.02 g/m^2 of TP was accumulated in the bare sediment zone and 7.88 g/m^2 in the *Typha* zone (Table 4-7). This accounted for 20% and 77% respectively of the estimated 10.18 g/m^2 of P added to the treatment wetland over the study period. Over the same period, total TP storage in the unimpacted wetlands did not change significantly (Table 4-7, Table 4-5, Table 4-6).

In the treatment wetland, the majority of the accumulated P in the bare sediment zone was stored in topsoil whereas in the *Typha* zone it was mostly stored in litter, with some also stored in topsoil (Table 4-7). However, of these, a significant increase in TP storage was only recorded in litter (Table 4-4) and thus Hypothesis 6 that most added P would be taken up by soil was not supported (Table 2-7).

Table 4-7: Change in the mean values of TP storage by ecosystem components between the first and last sampling occasions. Differences with shading are significant P < 0.05 (based on Tables 4-4, 4-5, 4-6). As spring n = 3 and winter n = 5, only differences between averages could be calculated (rather than average differences).

	TP storage (g/m^2)					
	Bare sediment zone			***Typha* zone**		
Ecosystem component	**Start (spring)**	**Finish (winter)**	**Difference Finish - Start**	**Start (spring)**	**Finish (winter)**	**Difference Finish - Start**
Swamphen						
Water	0.02	0.33	**0.31**	0.04	0.42	**0.38**
Floc	0.14	0.00	-0.14	absent	absent	absent
Litter	absent	absent	absent	0.24	6.37	**6.13**
Topsoil	3.76	5.61	1.85	4.15	5.37	1.22
Typha	absent	absent	absent	0.25	0.40	0.14
Total	*3.92*	*5.94*	*2.02*	*4.68*	*12.56*	*7.88*
Island						
Water	0.02	0.01	-0.01	0.05	0.02	-0.02
Floc	absent	absent	absent	absent	absent	absent
Litter	absent	absent	absent	0.03	0.55	0.52
Topsoil	4.22	4.56	0.35	4.43	4.37	-0.06
Typha	absent	absent	absent	0.68	1.52	0.84
Total	*4.23*	*4.57*	*0.33*	*5.19*	*6.46*	*1.28*
Peninsula						
Water	0.02	0.01	-0.01	0.02	0.01	0.00
Floc	absent	absent	absent	absent	absent	absent
Litter	absent	absent	absent	0.47	1.58	1.11
Topsoil	4.36	4.53	0.17	3.91	4.96	1.05
Typha	absent	absent	absent	0.68	0.42	-0.26
SAV	0.28	0.00	-0.28	absent	absent	absent
Total	*4.66*	*4.54*	*-0.12*	*5.07*	*6.97*	*1.90*

4.3.6 Ecosystem change

How do vegetation characteristics change with enrichment in the different functional zones and seasons in enriched and unenriched wetlands?

Contrary to expectations (Hypothesis 7, Table 2-7), *Typha* height, density and biomass in the treatment wetland did not increase over time (Figure 4-10, Figure 4-7c,f,h, Table 4-8, Table 4-4). However, *Typha* density and height followed different trends in treatment and unimpacted wetlands, which also differed (Figure 4-10, Table 4-8). In the treatment wetland, density was highest in summer and lowest in winter, whereas in unimpacted wetlands, density was lowest in summer and highest in either winter or spring (Figure 4-7a), possibly reflecting optimal water levels. Apart from summer, *Typha* density in the treatment wetland was lower than in unimpacted wetlands. There were no significant seasonal differences in *Typha* height in any of the wetlands. Although average *Typha* height increased 20 cm between the first and last sampling occasions in Swamphen Lake, it was also subject to water level increases over the same period (Figure 4-10b, Table 4-8). Variability in *Typha* height masked any potential treatment effects or seasonal changes. In the treatment wetland, increased epiphyton (filamentous algae) growth was observed (not measured) on *Typha*, particularly on dead *Typha* (Figure 4-11), whereas no such change was observed in the unimpacted wetlands (Kauhanen pers. obs.). No submerged or emergent vegetation or filamentous algae were recorded in the bare sediment zone of the treatment wetland during the study period. In contrast, filamentous and benthic algae were found in Island Lake during winter and spring and SAV was present in Peninsula Lake in spring and summer.

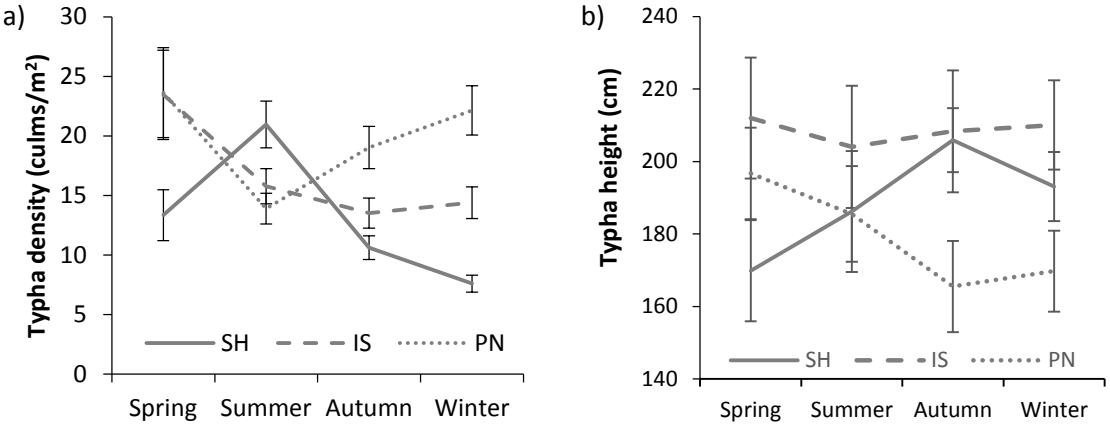

Figure 4-10: *Typha* density (Pollard density estimator ± 1 standard error calculated from associated variance estimator, Pollard 1971, Nielson *et al.* 2004) and mean height (± standard error) in *Typha* zone of Swamphen (SH), Island (IS) and Peninsula (PN) Lakes in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter). N = 3 in spring, n = 5 in summer, autumn, winter.

Figure 4-11: Epiphyton on dead *Typha* in Swamphen Lake winter 2013.

How does water quality change with enrichment and how does this differ between the functional zones and seasons in enriched and unimpacted wetlands?

Water chlorophyll α concentrations in the treatment wetland remained relatively low during spring (mesotrophic) and summer (eutrophic), but increased above the ANZECC trigger value (30 µg/L, hypereutrophic) by autumn (Figure 4-12a). These results support Hypothesis 9 (Table 2-7) that the treatment wetland would sustain algal blooms (defined as chlorophyll α > 30 µg/L). In winter, water chlorophyll α remained high in the *Typha* zone but dropped below the ANZECC trigger value in the bare sediment zone (Figure 4-12a). Chlorophyll α concentration in the unimpacted Peninsula Lake remained consistently lower than in the treatment wetland, mostly oligotrophic and well below the ANZECC trigger value. In contrast, chlorophyll α concentration in the unimpacted Island Lake was mostly eutrophic and exceeded the ANZECC trigger value in the *Typha* zone in spring (Figure 4-12a). In the treatment wetland, chlorophyll α concentrations did not differ between functional zones until winter when chlorophyll α was significantly higher in the *Typha* than the bare sediment zone (Figure 4-12a, Table 4-8). Hypothesis 10 (Table 2-7) that chlorophyll α would be higher in unvegetated than vegetated zones during enrichment was therefore not supported.

In the treatment wetland, turbidity varied greatly, starting at less than 5 NTU but peaking at 21.9 NTU (bare sediment zone) during the second sampling round when no corresponding increase was observed in chlorophyll α (Figure 4-12a,b). Turbidity in the treatment wetland was significantly higher in the last than the first sampling occasion (Table 4-8) but apart from the peak during summer it stayed within the range of values shown by unimpacted wetlands. There were no consistent differences in turbidity between functional zones in any of the wetlands, so Hypothesis 10 (Table 2-7) that turbidity would be higher in unvegetated than vegetated zones during enrichment was not supported.

In the treatment wetland, daytime %DO close to the sediment surface varied significantly over time and zones (14% to 119% in bare sediment, 15% to 89% in *Typha* zone), peaking in autumn and being lowest in spring, with no significant difference between spring and winter (Figure 4-12d, Table 4-8). Therefore the results did not support Hypothesis 11 (Table 2-7) that DO would decrease with enrichment. Apart from bare sediment zone in autumn, %DO in the treatment wetland was below the ANZECC recommended range of 90-120%, and was generally lower than %DO in the unimpacted wetlands (which were dry in autumn). In the unimpacted wetlands, %DO was significantly higher in bare sediment than in *Typha* zones, whereas no consistent difference between zones was found in the treatment wetland (Table 4-8).

In the treatment wetland, bottom water ORP varied between 101 mV and 394 mV, peaking in summer (Figure 4-12e). There was no significant difference between spring and winter (Table 4-8) and the results did not support Hypothesis 11 (Table 2-7) that ORP would decrease with enrichment. ORP was lower in treatment than in unimpacted wetlands during spring and winter but within the range of unimpacted wetlands during summer. ORP had no consistent seasonal trend in unimpacted wetlands. There were no significant differences in ORP between functional zones in any of the wetlands (Figure 4-12e, Table 4-8).

In the treatment wetland, pH was stable (slightly acidic, 6.2 - 6.6), except in summer, when pH dropped significantly to approximately 4.0 (Figure 4-12c, Table 4-8). In unimpacted wetlands, pH varied seasonally, being highest in spring and lowest in winter, however variation was small compared to the large drop in the treatment wetland. Island Lake was consistently highly acidic (pH 3.0 - 4.0) and Peninsula Lake consistently slightly acidic (pH 5.7 - 7.1) (Figure 4-12c). There were no consistent differences in pH between functional zones in any of the wetlands.

Electrical conductivity (EC) of the bottom water followed a similar seasonal trend in all of the wetlands, increasing from spring to peak in summer and then decreasing to winter (Figure 4-12g, Table 4-8), presumably due to concentration and dilution. The three wetlands differed in EC: treatment wetland relatively fresh (0.77-1.76 mS/cm), Island Lake brackish (2.23-4.16 mS/cm) and Peninsula Lake saline (3.99-9.23 mS/cm). There were no significant differences in EC between functional zones in any of the wetlands (Figure 4-12g, Table 4-8).

As expected, water temperature followed seasonal trends in all wetlands: highest in summer (20.1-25.1 °C) and lowest in winter (11.6-14.4 °C) (Figure 4-12f, Table 4-8). There were no significant differences in water temperature between functional zones.

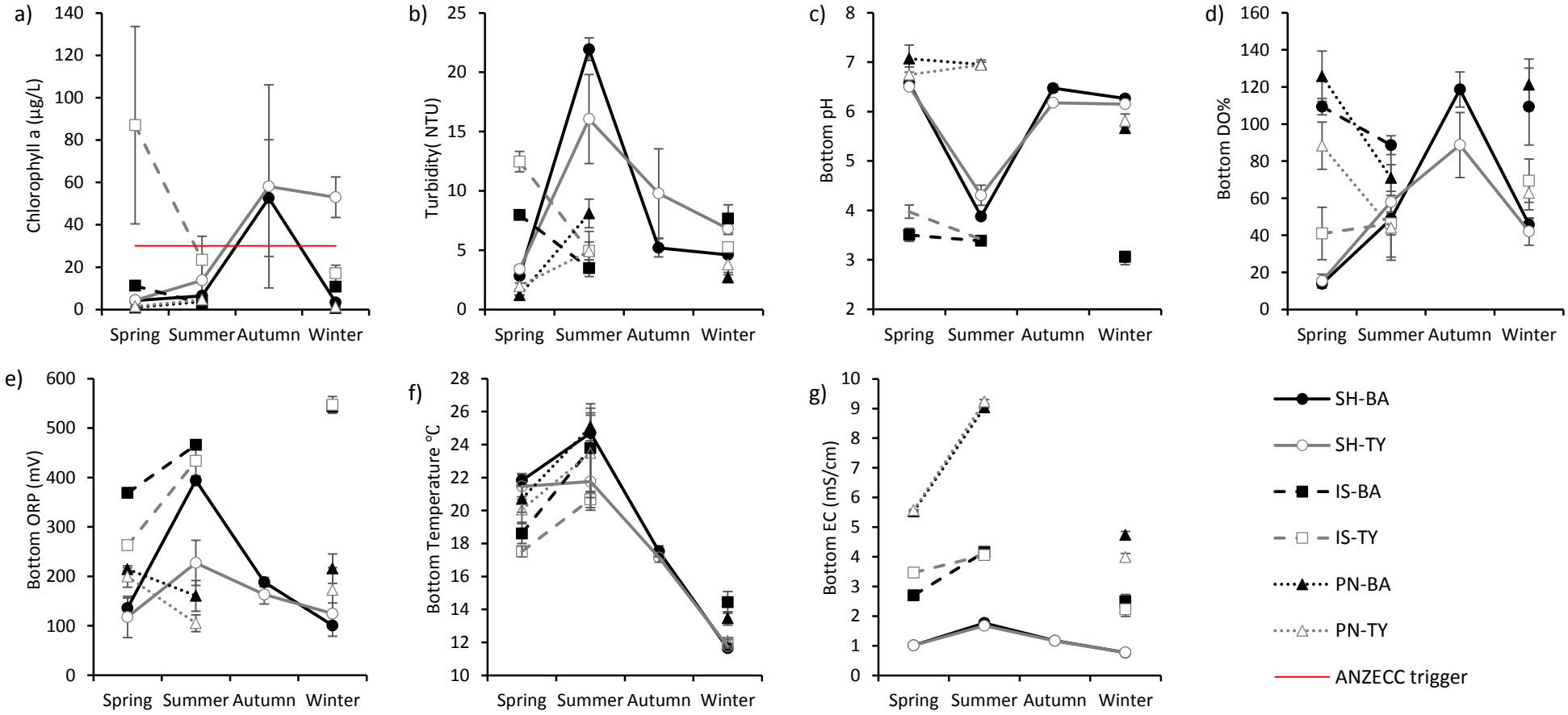

Figure 4-12: Mean water chlorophyll α (a), turbidity (b), bottom water pH (c), dissolved oxygen % (DO%) (d), oxidation-reduction potential (ORP) (e), temperature (f) and electrical conductivity (EC) (g) in bare sediment (BA) and *Typha* (TY) zones Swamphen (SH), Island (IS) and Peninsula (PN) Lakes in October 2012 (spring), January 2013 (summer), April 2013 (autumn) and July 2013 (winter). N = 3 for spring, n = 5 for summer, autumn and winter. Error bars are ± 1 standard errors. Island and Peninsula Lakes dry in autumn.

Table 4-8: Differences in water quality variables and *Typha* height between seasons and zones in Swamphen, Island and Peninsula Lakes. Abbreviations: Sp = spring, Su = summer, Au = autumn, Wi = winter, ns = not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, / = versus, na = not applicable, do = disordinal, # = non-parametric Kruskal-Wallis. Parentheses indicate a significant disordinal interaction between treatment and zone. Further details may be found in Appendix 3.

Ecosystem components	**Season**		**Zone**	**Season*Zone**		
	Initial test	**Post hoc**	**Initial test**	**Initial test**	**Simple effects - Season**	**Simple effects - Zone**
Swamphen						
Chlorophyll α	(**)	(Sp/Su ns, Sp/Au**, Sp/Wi ns, Su/Au**, Su/Wi ns, Au/Wi ns)	ns	** do	BA: Sp/Su ns, SpAu**, Sp/Wi ns, Su/Au**, Su/Wi ns, Au/Wi*** TY: Sp/Su ns, Sp/Au*, Sp/Wi**, Su/Au*, Su/Wi**, Au/Wi ns	Sp ns, Su ns, Au ns, Wi***
Turbidity	(***)	(Sp/Su***, Sp/Au**, Sp/Wi*, Su/Au***, Su/Wi***, Au/Wi ns)	ns	* do	BA: Sp/Su***, Sp/Au*, Sp/Wi ns, Su/Au***, Su/Wi***, Au/Wi ns TY: Sp/Su***, Sp/Au**, Sp/Wi*, Su/Au*, Su/Wi**, Au/Wi ns	All ns
Bottom Temperature #	***	Sp/Su ns, Sp/Au ns, Sp/Wi**, Su/Au*, Su/Wi***, Au/Wi ns	ns	na		
Bottom pH	***	Sp/Su***, Sp/Au ns, Sp/Wi ns, Su/Au**, Su/Wi*, Au/Wi ns	ns	na		
Bottom EC #	***	Sp/Su**, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi***, Au/Wi***	ns	na		
Bottom DO % #	***	Sp/Su ns, Sp/Au***, Sp/Wi ns, Su/Au*, Su/Wi ns, Au/Wi*	ns	na		
Bottom ORP #	**	Sp/Su*, Sp/Au ns, Sp/Wi ns, Su/Au ns, Su/Wi**, Au/Wi ns	ns	na		
Typha height	ns		na	na		

Ecosystem components	Season		Zone	Season*Zone		
	Initial test	Post hoc	Initial test	Initial test	Simple effects - Season	Simple effects - Zone
Island						
Chlorophyll α	**	Sp/Su**, Sp/Wi*, Su/Wi ns	***	*	BA: Sp/Su**, Sp/Wi ns, Su/Wi** TY: Sp/Su**, Sp/Wi**, Su/Wi ns	Sp**, Su***, Wi ns
Turbidity	(***)	(Sp/Su***, Sp/Wi*, Su/Wi**)	ns	** do	BA: Sp/Su**, Sp/Wi ns, Su/Wi** TY: Sp/Su***, Sp/Wi***, Su/Wi ns	Sp ***, Su ns, Wi*
Bottom Temperature #	***	Sp/Su ns, Sp/Wi ns, Su/Wi***	ns	na		
Bottom pH	***	Sp/Su*, Sp/Wi***, Su/Wi**	ns	ns		
Bottom EC #	***	Sp/Su ns, Sp/Wi ns, Su/Wi***	ns	na		
Bottom DO%	ns		***	ns		
Bottom ORP #	***	Sp/Su ns, Sp/Wi***, Su/Wi*	ns	na		
Typha height	ns		na	na		
Peninsula						
Chlorophyll α	***	Sp/Su***, Sp/Wi ns, Su/Wi***	**	ns		
Turbidity	(***)	(Sp/Su***, Sp/Wi*, Su/Wi*)	ns	* do	BA: Sp/Su***, Sp/Wi*, Su/Wi** TY: Sp/Su*, Sp/Wi ns, Su/Wi ns	Sp ns, Su*, Wi ns
Bottom Temperature #	***	Sp/Su ns, Sp/Wi ns, Su/Wi***	ns	na		
Bottom pH	***	Sp/Su ns, Sp/Wi***, Su/Wi***	ns	ns		
Bottom EC #	***	Sp/Su ns, Sp/Wi ns, Su/Wi***	ns	na		
Bottom DO %	**	Sp/Su**, Sp/Wi ns, Su/Wi*	**	ns		
Bottom ORP #	ns		ns	na		
Typha height	ns		na	na		

4.4 Discussion

4.4.1 Overview

Pre-enrichment stage – unimpacted wetlands

The results from the unimpacted wetlands supported the conceptual models developed from the literature (Reddy and DeLaune 2008, Kadlec and Wallace 2009, Boulton *et al.* 2014, Mitsch and Gosselink 2015). They showed that while some seasonal changes in TP storage of ecosystem components occur at the pre-enrichment stage, these changes reflect internal cycling between ecosystem components rather than net accumulation, and the quantities of P involved are trivial compared to quantities present during enrichment (Table 4-9, Figure 4-13, Figure 4-14).

Functional P uptake stage - treatment wetland

It was hypothesised (3, Table 2-7) that TP storage of ecosystem components in the treatment wetland would increase over time due to enrichment associated with effluent discharge (Howard-Williams 1985). By the end of the study period, significant increases in TP storage were found in water and litter components, as expected. However, TP storage under nutrient enrichment showed some interesting and unexpected patterns: *Typha* was expected to take up additional nutrients (i.e. luxury uptake, Reddy and DeLaune 2008) as enrichment progressed, but this was not observed; topsoil and subsoil were expected to be enriched, but instead subsoil lost P and topsoil showed no significant change (Table 4-9, Figure 4-13, Figure 4-14). As this study was short and covered only the initial stages of enrichment, it was potentially too early to see changes in *Typha*, topsoil or subsoil – although the loss of P from subsoil remains difficult to explain. Lastly, floc was only observed in the treatment wetland, but did not appear to play a large part in P storage at this stage of enrichment. At this early stage of enrichment, major P uptake from water occurred only in the *Typha* zone (through litter), with minor uptake by suspended algae in the bare sediment zone (Figure 4-13, Figure 4-14).

Table 4-9: Summary of changes to conceptual model made using results of this study (P = phosphorus).

Functional zone	Pre-enrichment stage	Functional phosphorus uptake stage
Bare sediment zone	• None	• Floc - store added but change in P storage uncertain. • Topsoil - expected increase in P storage insignificant. • Subsoil - significant decrease rather than the expected increase in P storage.
Typha zone	• None	• Topsoil - expected increase in P storage insignificant. • Subsoil - significant decrease rather than the expected increase in P storage. • *Typha* - expected increase in TP storage not observed.
All	• Pie charts added showing distribution of total TP storage between ecosystem components (excluding subsoil). • Pie charts added showing where the added P went as % of total load.	

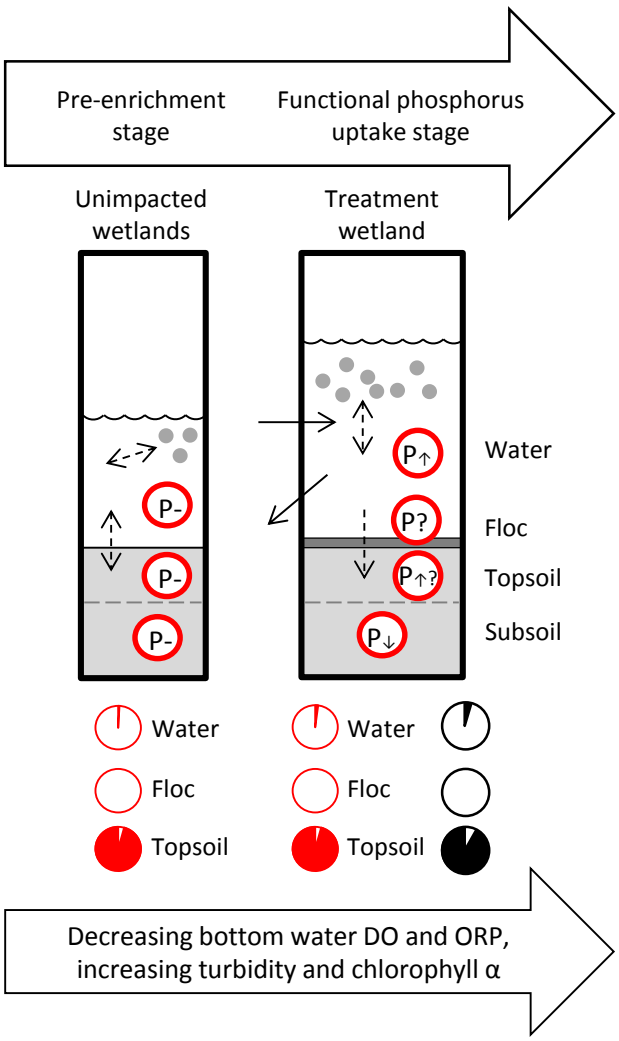

Figure 4-13: Conceptual models of phosphorus storage and cycling in the bare sediment zone in the seasonal study. Solid line arrows indicate definite phosphorus cycling pathways and dashed lines likely pathways. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached. Grey dots show algal bloom intensity. Red pie charts indicate proportional share of total phosphorus (excluding subsoil) held by each store. Black pie charts indicate where the added phosphorus went based on mass balance, excluding subsoil and pathways not sampled (e.g. groundwater seepage).

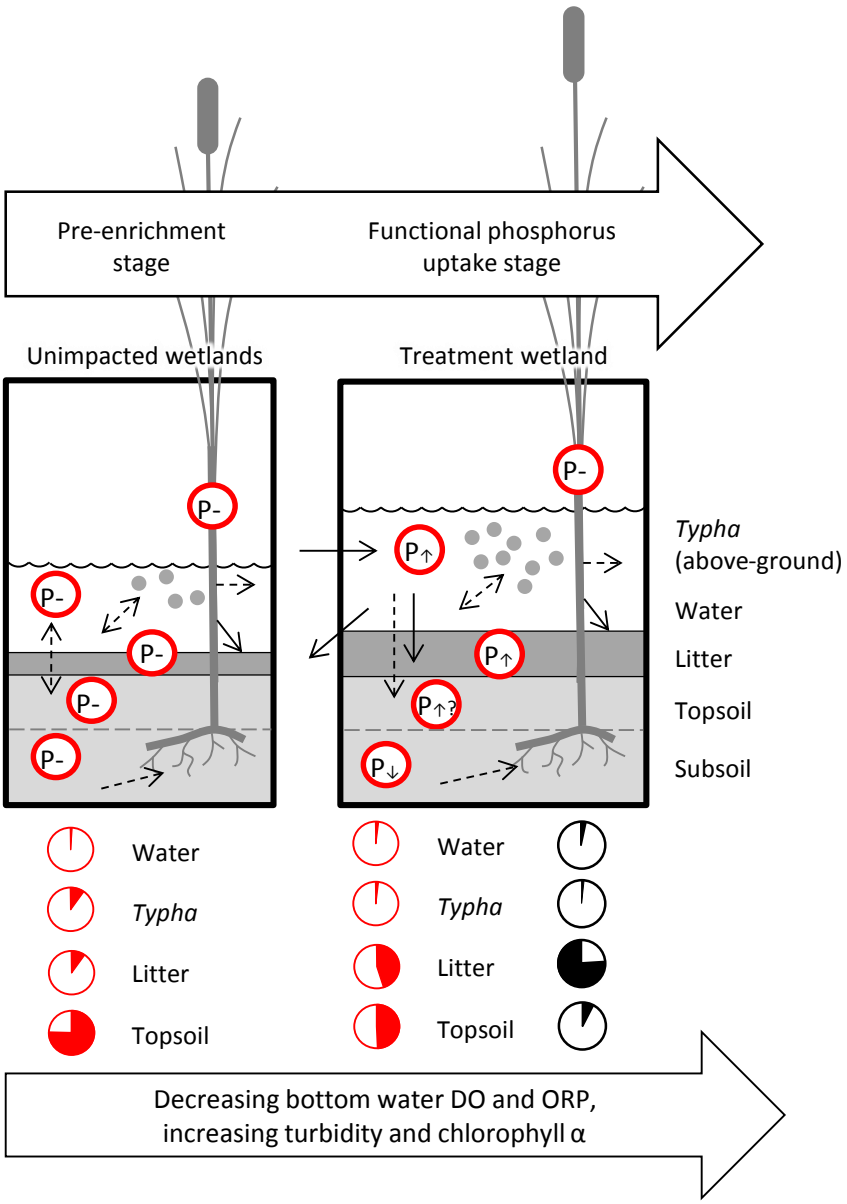

Figure 4-14: Conceptual models of phosphorus storage and cycling in the *Typha* zone in the seasonal study. Solid line arrows indicate definite phosphorus cycling pathways and dashed lines likely pathways. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached. Grey dots show algal bloom intensity. Red pie charts indicate proportional share of total phosphorus (excluding subsoil) held by each store. Black pie charts indicate where the added phosphorus went based on mass balance, excluding subsoil and pathways not sampled (e.g. groundwater seepage).

4.4.2 Phosphorus storage and cycling response

Research Question 1 - How do P storage and cycling change as a wetland undergoes nutrient enrichment and how does this differ between functional zones?

Delay in treatment response

Despite effluent discharge having commenced eight months prior to the first sampling occasion, the first significant increases in TP storage of ecosystem components were only observed during the last sampling round (17 months following the commencement of discharge). Effluent was initially lost into cracks in the clay soil (presumed seepage into groundwater) close to the inflow point, meaning that nutrients in the incoming effluent did not reach the sampling area. This also occurred during the summer sampling occasion. Further, when the effluent did reach to the sampling area as surface water flow, nutrients were taken up by ecosystem components present in the 100 m distance between the effluent inflow point and the sampling area, so lower quantities of nutrients reached the sampling area. This uptake would have gradually decreased through time (White *et al.* 2000, Reddy and DeLaune 2008) resulting in increased nutrient inputs to the sampling area. Even then, a large share of incoming P would have continued to be lost via groundwater seepage along the edges of the wetland (as discussed further in Chapter 5). Fringing vegetation (trees) may have also removed some of the incoming P if their roots under laid the lake bed (approximately 5-50 kg/ha/year, Mander *et al.* 1997, Hoffmann *et al.* 2009). The delay in detecting a treatment response therefore reflected site specific factors; a faster response could be expected in systems where incoming nutrients immediately entered the sampling area.

Rapid responders

As water and litter were the only stores whose TP storage responded to enrichment during this study (supporting Hypothesis 3, Table 2-7, Figure 4-13, Figure 4-14), they were considered rapid responders. Increase in water TP storage reflected increases in both water TP concentration (supporting Hypothesis 1, Table 2-7) and water depth, both directly influenced by the incoming effluent. The rapid response in water TP concentration indicated that P uptake by stores other than suspended algae was not matching the rate of incoming P. In previous research involving low P loads, water was the last of the stores to respond (Gaiser *et al.* 2005), in contrast to the findings of this study. This suggests the P load was likely high in relation to the (unknown) sustainable P assimilation capacity of the wetland and that the findings of this study are most relevant to systems receiving high P loads.

Increased litter TP storage reflected increases in both litter TP concentration and mass (supporting Hypotheses 1 and 2, Table 2-7, Figure 4-14). Litter is known to sustain a microbial community capable of responding quickly to P enrichment (Qualls and Richardson 2000, Grace *et al.* 2008), which likely explained the increased litter TP concentration. The observed increased litter mass did not appear connected to increased primary productivity, because *Typha* biomass did not increase, suggesting that this pattern was merely due to the natural seasonal cycle of *Typha* growth, death and litter fall (Davis and van der Valk 1983). Also, the lower water DO concentrations in treatment than unenriched wetlands may have decreased the rate of breakdown of litter, thus supporting greater litter accumulation (Ryder and Horwitz 1995). Litter TP storage and concentration had high variation during the last sampling occasion but were in the upper ranges of the previously recorded values (Table 2-3, Table 2-4), suggesting this store was near saturation.

The two rapid responders greatly differed in the scale of P uptake. Litter took up most of the P added to the wetland (sub-question d, Table 2-7); 16 times as much P as taken up by water. Consequently, the percentage share of total TP stored in water hardly changed but increased rapidly for litter (Figure 4-13, Figure 4-14). This is a critical finding for management, because the accumulation of P in water potentially drives short-term adverse ecosystem outcomes (e.g. algal blooms, shift from clear to turbid water, DO depletion due to oxygen demand of decomposing algae, release of sediment bound P, Verhoeven *et al.* 2006, Boulton *et al.* 2014) whereas P stored in litter is less available to drive such processes.

There was also a significant increase in TP concentration of above-ground standing dead *Typha* supporting Hypothesis 1 (Table 2-7). This was not related to uptake by *Typha* itself as live *Typha* TP concentration did not change in contrast to Hypothesis 1 (Table 2-7), but rather likely a reflection of microbial uptake in the decaying tissue similar to that described above for litter. This provided another P-uptake pathway, but as the mass of dead *Typha* did not change in contrast to Hypothesis 2 (Table 2-7), there was no net change in TP storage by dead *Typha* mass over time in contrast to Hypothesis 3 (Table 2-7).

Lack of treatment response in other stores

Assimilation of P by topsoil was expected due to the high P uptake capacity of the clay soil (containing Fe, Al and Mn, Chambers and McComb 1996), however no significant increase was detected in topsoil TP concentration or storage (in contrast to Hypotheses 1 and 3, Table 2-7), despite the mass balance indicating substantial P accumulation in this store (Figure 4-13, Figure 4-14). This may have been due to the lag in P reaching the sampling area as explained

earlier; a more significant increase in TP storage by soil may have been observed over a longer study period. The slower response to enrichment in soil than in litter supports previous research describing soil related P uptake processes (precipitation and sorption) as fast to moderate and microbial processes (in this study likely associated with litter) as very fast (Table 2-2) (Richardson and Vaithiyanathan 2009).

The reason for the significant decrease in subsoil TP storage (and concentration) in the treatment wetland in contrast to Hypotheses 1 and 3 (Table 2-7) is uncertain (Figure 4-13, Figure 4-14). Subsoil TP concentration was highly variable in all wetlands throughout this study, so the decrease detected here may not be meaningful. The multiyear study that followed the seasonal study showed that the decrease in subsoil TP storage was reversed within three months. As the lack of anticipated increase in TP storage of subsoil was consistent with the lack of increase in TP storage of the topsoil layer above, it could be related to the short duration of the study.

Above-ground *Typha* did not respond to enrichment in contrast to Hypotheses 1, 2 and 3 (Table 2-7, Figure 4-13, Figure 4-14). There was no change in TP storage or mass of *Typha* over time and changes recorded in TP concentration of live *Typha* were similar to seasonal changes observed in the unimpacted wetlands. Above-ground *Typha* cannot take up P directly from water; it needs P to enter via roots in the sediment. There was no increase in TP storage in the root zone soil (topsoil or subsoil) and while adventitious roots may have been present in the soil/litter interface (Rejmánková and Snyder 2008), significant increase in litter TP storage was only observed at the end of the study. Even if below-ground *Typha* may have taken up some P, there was little time for this to be reflected in above-ground *Typha*. Response times of *Typha* and other macrophytes vary widely in previous research, so the findings of this study are not unusual. Weng *et al.* (2006) reported increases in both *Typha* TP concentration and biomass following approximately 40 days of enrichment in a laboratory scale (microcosm) experiment. However, their experiment used higher inflowing TP concentrations (10 mg/L or 4 mg/L versus Swamphen Lake 2 mg/L) and the substrate was coarse gravel with much higher porosity than the clay in Swamphen Lake. This highlights the role of substrate type in nutrient dynamics. In contrast, a field study of low-level enrichment by Gaiser *et al.* (2005) found that macrophytes did not respond until the third year of enrichment, later than periphyton, floc and soil stores.

Differences between functional zones

There were no significant differences in TP storage of the shared ecosystem components (water, topsoil, subsoil) between functional zones at the pre-enrichment stage. No differences between functional zones were found in water TP storage (or concentration) during enrichment either in contrast to Hypothesis 4 (Table 2-7), although the water store responded to enrichment rapidly. This was in agreement with a similar lack of differences in water TP concentration between macrophyte meadows and open water controls observed in an eutrophic, impounded river also in southwestern Australia (Paice *et al.* 2016).

Differential effects of enrichment on topsoil and subsoil TP storage between zones could not be assessed, as no treatment effect occurred in these stores within the time frame of the study in contrast to Hypotheses 3 and 6 (Table 2-7). The lack of differences in water TP storage between zones at both Stage 1 and Stage 2 could have been partly due to free movement of water between the zones masking potential differences.

Total TP storage was higher in *Typha* than bare sediment zones in unimpacted wetlands, likely because the vegetated *Typha* zone had more P-storage components and uptake capacity (i.e. *Typha* and litter), as observed in previous research (e.g. Menon 2011). The lack of similar difference between zones in the treatment wetland appeared to be due to higher variation in P storage in components following enrichment.

Seasonal differences

Consistent and significant seasonal changes in the unimpacted wetlands were observed in TP storage of water, topsoil and *Typha*. These appeared to reflect expected changes in water level (water, topsoil), water temperature (water, topsoil) and plant growth patterns (*Typha*) (Howard-Williams 1985, Kadlec and Reddy 2001, Kadlec 2016). However, it is noted that this study did not involve replication of seasons over time and thus the results are representative only of the study period; seasonal cycling would be expected to vary between years due to annual climatic variability. As expected, the scale of changes associated with nutrient enrichment (observed in water and litter) was clearly much larger the than scale of seasonal changes associated with these stores. Seasonality can affect the capacity of stores to take up P. For example, algal and microbial growth and associated P uptake is stimulated by warmer temperatures, solubility of soil bound P increases with increasing temperature, and capacity of *Typha* to take up P is expected to be highest during the active spring growth phase rather than during autumn senescence (Reddy and DeLaune 2008). None of these processes appear to have had major impact on the results of this study, perhaps in part due to the mild climate.

Increases in TP storage of water and litter were observed in winter when temperatures were low (not stimulating algal or microbial P uptake). Lack of increases in TP storage of topsoil, subsoil and above-ground *Typha* were likely related to the slow response of these stores rather than solubility changes in P (soil, topsoil) or seasonal growth patterns in *Typha*. Overall, managers need to be aware of the seasonal context of site data and understand how it might be expected to change over time.

Proportional TP storage

At the pre-enrichment stage, the majority of total TP was stored in soil, as expected, especially in the bare sediment zone. Percentage share of total TP held in topsoil decreased with enrichment as anticipated (Hypothesis 5, Table 2-7) coinciding with findings by Noe et al (2002) and DiLuca et al (2015). This occurs because the other components (in this study litter and water) take up proportionally more P than soil; it is usually not an indication of soil losing P.

While the percentage distribution of total TP is a good way to visualise the differences in the scale of TP storage between the ecosystem component and any changes in this over time, it provides no indication of the potential importance of this P for the eutrophication process. While the majority of P is stored in soil, only a small portion is commonly readily bioavailable; the majority of soil P is in the very slowly and slowly available forms (mineral phosphates, recent precipitates, refractory organic P) (Reddy and DeLaune 2008). This means that the majority of soil-bound P will not contribute to increased primary production and thus will not drive eutrophication. In contrast, water remained a very small store of P even with enrichment, but this small portion of total TP was much more labile and capable of driving adverse ecosystem changes as discussed earlier. Overall, understanding both the scale of P storage by different stores and the potential bioavailability of this store is important to predict ecosystem outcomes along the enrichment continuum and direct management effort.

4.4.3 Ecosystem changes

Research Question 2 - How is nutrient enrichment reflected in ecosystem change in the different functional zones in enriched and unimpacted wetlands?

Limited vegetation changes

Although it was hypothesised that *Typha* height, density and biomass in the treatment wetland would increase with enrichment (Hypothesis 7, Table 2-7), the duration of the study was likely

too short to detect such ecosystem changes. No SAV or filamentous algae were recorded in the bare sediment zone over the study period, however both were recorded in unimpacted wetlands. The lack of filamentous algae, previously common at Swamphen Lake (K.Kauhanen, unpublished data), may have been enrichment-related, potentially due to increased turbidity and poorer light penetration. The treatment wetland had not sustained SAV prior to the study and so the absence of SAV following effluent release was not a factor of enrichment.

Chlorophyll a and turbidity

Algal blooms developed in Swamphen Lake supporting Hypothesis 9 (Table 2-7), despite substantial P removal from water by litter and losses via groundwater seepage. Algae take up P rapidly (Kadlec and Wallace 2009) and may thus outcompete other components, at least in the short-term (until P is released from algae during decomposition). In the treatment wetland, turbidity fluctuated differently to chlorophyll α over time, peaking in summer whereas chlorophyll α peaked in autumn, suggesting that at least during summer turbidity was related to other processes, potentially sediment resuspension as water levels were low. Apart from summer, turbidity in the treatment wetland was within range of turbidity in unimpacted wetlands and thus appeared not impacted by enrichment at least in the early stages of the enrichment continuum. In contrast to both Hypothesis 10 (Table 2-7) and results of a nearby lowland river study (Paice *et al.* 2016), neither chlorophyll α or turbidity was higher in unvegetated than vegetated zones during enrichment and reflected similar lack of difference in water TP concentration between zones discussed earlier.

DO and ORP

Dissolved oxygen % in treatment wetland peaked during autumn, likely due to the algal bloom present and increasing daytime DO production (Reddy and DeLaune 2008). Apart from autumn, %DO in the treatment wetland was lower than in the unimpacted wetlands (supporting Hypothesis 11, Table 2-7, Figure 4-13, Figure 4-14), likely due to the incoming effluent having higher biochemical oxygen demand than water in the unimpacted wetlands. Although ORP did not decline over time in the treatment wetland, ORP was mostly lower in the treatment than unimpacted wetlands and below 200 mV, indicating enrichment may have lowered ORP (and supporting Hypothesis 11, Table 2-7, Figure 4-13, Figure 4-14).

Differences between functional zones

Chlorophyll α was probably higher in *Typha* than in bare sediment zone because better conditions for phytoplankton growth occurred among the emergent macrophytes than in open water (e.g. less turbulent during winter, Madsen *et al.* 2001, Horppila *et al.* 2013, Gebrehiwot,

Kifle, Stiers*, et al.* 2017) (Hypothesis 10, Table 2-7). Bottom %DO was the only other water quality variable that differed between functional zones. Decomposition processes in the *Typha* zone may have consumed more oxygen than areas of bare sediment.

Differences between seasons

There were strong seasonal changes in water quality variables at the pre-enrichment stage as well as during enrichment. Seasonal changes in most water quality variables differed between the two unimpacted wetlands (potentially due to seasonal presence of SAV only in Peninsula Lake) such that no 'generic' seasonal changes could be established for the pre-enrichment stage. This made it harder to determine whether the changes over time in the treatment wetland were seasonal changes or treatment related.

4.5 Conclusion

In this study, the pre-enrichment stage was characterised by small-scale, seasonal cycling of P between ecosystem components. In contrast, enrichment caused P to accumulate in the ecosystem components in quantities that far exceeded quantities of P involved in cycling at the pre-enrichment stage. It also overwhelmed any seasonal P dynamics in water and litter stores (no treatment response observed in other stores). Early stages of enrichment were typified by the majority of added P being taken up by litter in the *Typha* zone, whereas uptake in the bare sediment zone was limited to suspended algae in water. Contrary to expectations, no significant increases in TP storage of topsoil, subsoil or above-ground *Typha* were recorded, likely due to the short duration of the study. No significant differences in TP storage of ecosystem components between functional zones were found in the treatment wetland, and the only consistent difference in the unimpacted wetlands was higher total TP storage in *Typha* than bare sediment zone. This is consistent with the higher number of stores and uptake pathways present in the *Typha* zone. Water quality (excluding nutrients) showed little consistent evidence of deterioration due to enrichment aside from higher chlorophyll α, however DO and ORP in bottom water were generally lower in the treatment than unimpacted wetlands. Overall this study provides a rare holistic picture of seasonal P storage and cycling at the pre-enrichment stage and changes associated with the initial emergence of nutrient enrichment effects, most relevant for systems subject to addition of both water and nutrients (high P load).

CHAPTER 5: TRACKING PHOSPHORUS STORAGE AND CYCLING IN A SHALLOW LAKE OVER THREE YEARS OF WASTEWATER RELEASE

5.1 Introduction

While natural low-level nutrient enrichment may result in eutrophication over thousands of years, cultural eutrophication generally results in major ecosystem changes within decades (Havens *et al.* 1996, Gustafsson *et al.* 2012, Sand-Jensen *et al.* 2017) and more quickly where systems receive high nutrient loads (e.g. treatment wetlands). The rate of change will depend on the incoming nutrient load and the capacity of the receiving system to assimilate it (Richardson and Vaithiyanathan 2009). Phosphorus (P) assimilation capacity between wetlands varies greatly on the basis of a range of physical, chemical and biological wetland characteristics (Reddy *et al.* 1999, Reddy and DeLaune 2008, Kadlec and Wallace 2009, Land *et al.* 2016). For example, vegetated wetlands would be expected to have higher P assimilation capacity than unvegetated wetlands due to additional P uptake and storage pathways (Sollie and Verhoeven 2008, Menon and Holland 2013, Di Luca *et al.* 2015). Consequently, some wetlands are prone to become more quickly saturated in P than others (Richardson and Vaithiyanathan 2009).

While multiyear and longer datasets reporting wetland eutrophication and the performance of treatment wetlands are relatively common, they are usually limited to individual ecosystem components or processes (e.g. Greenway and Woolley 2001, Childers *et al.* 2003, Kagalou *et al.* 2008, Dunne *et al.* 2015). In contrast, this study tracked P storage and cycling across all ecosystem components and in different functional zones simultaneously, identifying subsequent ecosystem changes. This provides a more holistic view of the internal processes by which eutrophication proceeds in a treatment wetland directly underpin observed treatment performance. As argued by Smith and Schindler (2009), we need to better understand the interactions that occur between nutrient enrichment and key physical, chemical and biological characteristics of receiving waters, to understand how treatment wetlands perform.

This study investigated P storage and cycling in a tertiary treatment wetland (Swamphen Lake) over three consecutive spring periods following commencement of effluent discharge, providing a temporal contrast to the shorter seasonal study (Chapter 4). Rates of change in phosphorus (P) storage and cycling processes vary from a matter of hours in controlled micro- and mesocosms (Richardson and Marshall 1986, Noe *et al.* 2003) to months to several years to

emerge, especially under low nutrient load (Gaiser *et al.* 2005). While three years is not a long time in the context of eutrophication, major changes to P storage and cycling and other ecosystem characteristics were expected because of the relatively high P load entering a small wetland.

Changes in P storage and cycling were investigated in two functional zones: 1) a bare sediment open water zone (Figure 2-4) and 2) an emergent macrophyte (*Typha orientalis)* zone (Figure 2-6). The conceptual models for these two zones described three key stages of enrichment: a pre-enrichment stage, a functional P uptake stage and a hypereutrophic end stage. This study focused on the second stage, potentially reaching the third stage. As enrichment was caused by release of treated municipal effluent, the treatment effect was a combination of nutrient and water additions. Research questions and hypotheses for this study were those presented in Table 2-7, excluding Hypothesis 8 relating to SAV (which was not present in Swamphen Lake). One additional sub-question was added for this study to provide another perspective of differences between years: which ecosystem components account for most of the variation in TP storage between years and how does this differ between functional zones?

5.2 Methods

5.2.1 Study Design

The seasonal study (Chapter 4) identified consistent, significant seasonal changes in TP storage in the unimpacted wetlands that reflected expected changes in hydrology, temperature and plant growth patterns. However, the magnitude of changes in TP associated with enrichment in Swamphen Lake was much greater than the seasonal changes observed in the unimpacted wetlands, indicating enrichment in this system was a stronger driver of P dynamics than natural seasonal changes. Therefore, rather than a spatial comparison of treatment versus control wetlands (already provided by the seasonal study in Chapter 4), this study focussed on examining accumulation of P over longer (inter-annual) time scales in the wetland receiving effluent, Swamphen Lake (described in Chapter 3, Section 3.2).

For inter-annual comparison, sampling was undertaken during spring when the biological components of the ecosystem (including microbes) would be most active (Garver *et al.* 1988, Verhoeven and Meuleman 1999, Kadlec and Wallace 2009, Eid *et al.* 2012, Rodrigo *et al.* 2013) and thus P storage and cycling across all elements would occur. New shoots of *Typha* emerge over the winter period and are approaching maximum biomass in spring, providing an

indication of annual uptake required to support growth. In these wetlands, seasonal SAV (charophytes) emerge in winter and die back over summer (Annan 2008) and filamentous algae occur in winter and spring (K.Kauhanen pers. obs.). In summer and autumn, Swamphen Lake is affected by low rainfall and high evapoconcentration, whereas in winter, heavy rainfall dilutes nutrients and cold temperatures restrict plant growth (and nutrient uptake). In contrast, spring has few weather extremes, making it an optimal time for making interannual comparisons.

Sampling of P stores was undertaken annually in spring (October) over three consecutive years (2012, 2013, 2014) following the commencement of effluent release in February 2012. Sampling areas were chosen as described in Section 3.3.1. At each sampling point, key ecosystem components (P stores) were sampled for TP concentration and mass per unit area (or depth in case of water) to allow calculation of TP storage as g/m^2. Ecosystem components sampled in the bare sediment zone included water, topsoil (0-20 mm), subsoil (20-200 mm) and floc. Ecosystem components sampled in the *Typha* zone included water, topsoil (0-20 mm), subsoil (20-200 mm), litter, and above-ground *Typha* (live and dead leaves). Water quality variables (temperature, pH, DO, ORP, EC, turbidity, chlorophyll α, FRP) were also sampled to provide context for the P storage samples and to identify other ecosystem changes associated with nutrient enrichment. *Typha* height and density (and biomass as per above) were recorded and any other changes in vegetation or other ecosystem characteristics were described to further identify changes associated with nutrient enrichment. Data on quantity and quality of discharged effluent was obtained from Water Corporation. Rainfall data for Busselton (18 km southwest of CWC) was obtained from Bureau of Meteorology.

5.2.2 Field sampling and laboratory analysis

Field sampling followed methods described in Section 3.4. Samples were submitted to an accredited laboratory and analysed for nutrients, mass and other characteristics in accordance with methods described in Section 3.5.

5.2.3 Data analysis

Data processing followed methods described in Section 3.6. Due to stratification of the sampling area by distance from effluent discharge point (see Section 3.3.1), results were initially screened for the impact of this distance by plotting all data against increasing distance (Appendix 4). In most cases, no distance-related trend was present and when it was present,

the range of values for different years mostly did not overlap. Consequently, the distance variable was excluded from further analysis and data from different distances were used as replicates in subsequent analyses.

Water TP, FRP and chlorophyll α concentrations were compared against ANZECC (2000) guidelines and water TP and chlorophyll α concentrations against trophic status classification (Table 2-6, modified OECD 1982). Issues with extracting subsoil samples resulted in variable sample depths. Visual assessment of a scatter plot of sample depth versus TP concentration and a Pearson's correlation test (Appendix 5) showed no linear correlation between these variables, so no samples were excluded from analysis of subsoil TP concentration. Variable subsoil sample depths also resulted in sample mass not being comparable between sites. Consequently subsoil TP storage was derived using constant average subsoil mass (155.10 ± 1.91 kg/m^2, n = 26), calculated on the basis of only those samples reaching the depth of 200 mm, including samples collected in Swamphen Lake for other thesis chapters between October 2012 and October 2014. For each variable, differences between years (3 levels = 2012, 2013, 2014) and, where applicable, difference between functional zones (2 levels = bare sediment, *Typha*) were tested using two-way ANOVA (factors year and zone, both fixed) or one-way ANOVA (factor year, fixed), using SPSS version 22. Assumptions of ANOVA were tested and transformations used when required, as described in Chapter 4 (Section 4.2.3). *Typha* density was calculated using the ordered distance method (Pollard 1971, Nielson *et al.* 2004) (see Section 3.6). This method provided one overall density estimate per year and was consequently not suitable for statistical testing. Variance was calculated with an associated estimator (Nielson *et al.* 2004) and converted to standard error (as described in Section 3.6). Sampling occasions were considered to differ if their standard errors did not overlap.

When two-way ANOVA identified significant interactions between year and zone ($P < 0.05$), simple effects tests were undertaken to further identify significant differences. Following the procedure described in Chapter 4, when disordinal interactions were identified, the main effects of year and zone were not interpreted, but rather only the simple effects tests. For ordinal interactions both main effects and the interaction were interpreted. Post-hoc Tukey's tests were used unless there was a significant disordinal interaction. Principal components analysis (PCA, with Euclidean distance) of P stores was used (Primer version 6 by PRIMER-E Ltd) to identify which ecosystem components accounted for most variation in TP storage between years and functional zones by illustrating differences in TP storage between years for each functional zone. All data was normalised prior to PCA.

Approximate water balance for Swamphen Lake for the study period was calculated to support interpretation of the P mass balance. In accordance with the schematic water balance model (Figure 3-4), potential water inputs included effluent inflow, rainfall, surface runoff and upward seepage, and water outputs evapotranspiration, downward seepage and lake overflow. Rainfall and runoff contribution to wetland water balance was calculated assuming 100% rainfall contribution from an area of 2.3 ha (approximate area of the main basin). It was assumed that wetland evapotranspiration was approximately 80% of the average annual Class A pan evaporation (Kadlec and Wallace 2009) of 1400 mm in the study area (based on 1975-2005, Bureau of Meteorology 2006). Evaporation was calculated based on the inundated wetland area: 1.0 ha in 2012, 1.5 ha in 2013 and 1.8 ha in 2014. Surface overflow from the wetland was zero during the study period. Wetland storage volume increased with increasing water level from 1.5 ML in 2012, to 7.0 ML in 2013 to 11.0 ML in 2014 (based on modelling by GHD 2004). Net seepage was estimated as the balance of other inputs and outputs.

Hydraulic loading rate (HLR) and nominal hydraulic retention time (HRT) were calculated on the basis of effluent discharge rate (averaged for the study period), wetland area (estimated at 1.8 ha in 2014) and wetland volume (estimated at 11 ML in 2014) as follows (Kadlec and Wallace 2009) to assist comparison of the results against treatment wetland literature:

$$q = \frac{Q}{A}$$

where

q = hydraulic loading rate (HLR)

A = wetland area, m_2

Q = water flow rate, m^3/d

$$\tau_n = \frac{V_{nominal}}{Q}$$

where

τ_n = nominal hydraulic retention time (HRT)

$V_{nominal}$ = estimated wetland volume, m^3

Q = water flow rate, m^3/day

5.3 Results

5.3.1 Water balance

An approximate water balance for Swamphen Lake (Table 5-1) indicates that the majority of water entered the wetland via effluent inflow and exited via groundwater seepage. Effluent volume discharged to Swamphen Lake increased over the study period (Figure 5-1) but remained within design expectations. Rainfall over the study period was variable (Figure 5-2) with over 200 mm more rainfall received in 2013, than in 2012 or 2014 (Bureau of Meteorology 2016). However, this difference in rainfall volume was small compared to the volume of incoming effluent (Table 5-1), so it had no noticeable effects on other results.

Hydraulic loading rate (HLR) was 0.012 m/d and nominal hydraulic retention time (HRT) 49.9 days.

Table 5-1: Estimated water balance for Swamphen Lake

Time period	Inputs (ML)		Outputs (ML)			Storage (ML)
	Effluent inflow	Rainfall	Evapo-transpiration	Seepage	Overflow	Volume increase
Nov 2011 - Oct 2012	53	13	11	53	0	2
Nov 2012 - Oct 2013	77	20	17	76	0	6
Nov 2013 - Oct 2014	84	14	20	79	0	4
Total	213	48	48	208	0	11

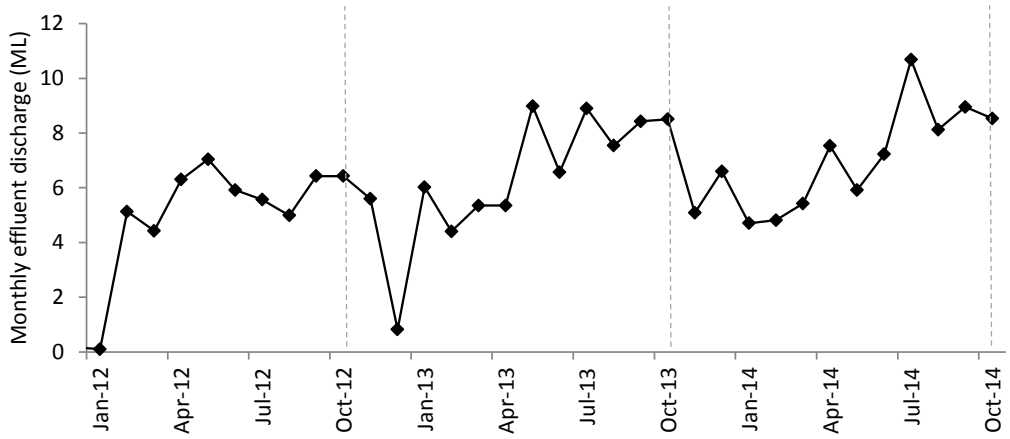

Figure 5-1: Monthly effluent discharge to Swamphen Lake. Sampling occasions identified with dashed lines.

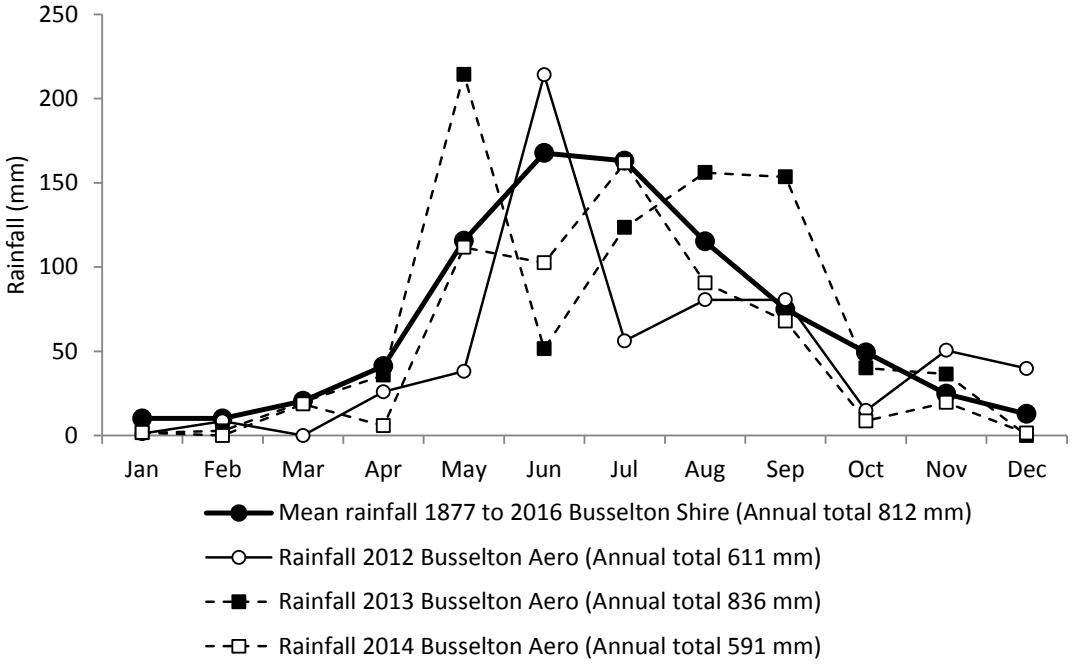

Figure 5-2: Rainfall in Busselton over the study period and long-term (Bureau of Meteorology 2016).

5.3.2 Effluent quality

Between March 2012 and October 2014, effluent TP concentration ranged between 0.48 - 5.00 mg/L, often exceeding the design maximum of 2.00 mg/L (Figure 5-3a). Annual TP load increased from approximately 7.0 g/m^2/year (November 2011 to October 2012), to 10.5 g/m^2/year (November 2012 to October 2013) and then decreased to 7.7 g/m^2/year (November 2013 to October 2014). Between the first and last rounds of sampling (October 2012 to October 2014), approximately 18.2 g/m^2 of P was added to the wetland equalling approximately 9.1 g/m^2/year (all loads calculated for wetland area of 1.8 ha, being the area of inundation in 2014 (n.b. differs from area used in Chapter 4).

TN concentration of effluent ranged between 29-60 mg/L and exceeded the design maximum of 30 mg/L during all but one month (Figure 5-3b). Annual TN load increased steadily over the years, from 137 g/m^2/year (November 2011 to October 2012) to 233 g/m^2/year (November 2013 to October 2014), being 18-30 times the P load. Majority of the TN added was ammonium nitrogen (NH_4-N). The high nitrogen load indicates that the system would have remained P limited.

BOD concentration of effluent ranged between 5-75 mg/L and regularly exceeded the design maximum of 20 mg/L in 2013 and 2014 (Figure 5-3c). This was reflected in annual BOD load that increased from 41 g/m^2 (November 2011 to October 2012), to 113 g/m^2 (November 2012 to October 2013) and then slightly decreased to 96 g/m^2 (November 2013 to October 2014).

SS concentration of effluent was highly variable between sampling occasions, ranging between 10-120 mg/L, but exceeded the design maximum of 80 mg/L only during one month (Figure 5-3d). Annual SS load increased steadily over the years, from 122 g/m^2/year (November 2011 to October 2012) to 207 g/m^2/year (November 2013 to October 2014).

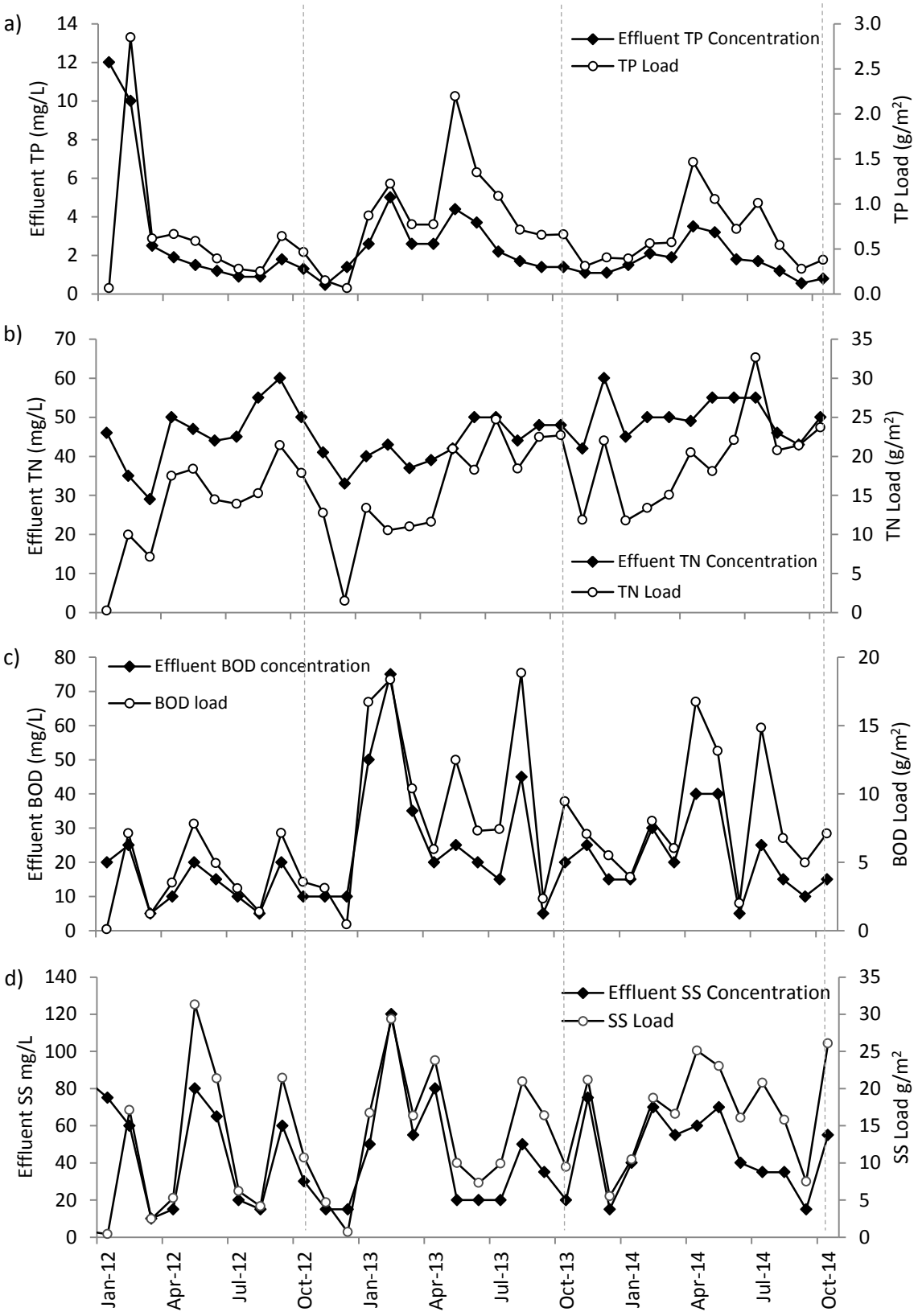

Figure 5-3: Concentration and load of total phosphorus (TP, a), total nitrogen (TN, b), biochemical oxygen demand (BOD, c) and suspended solids (SS, d) of effluent discharged to Swamphen Lake. Sampling occasions identified with dashed lines. Loads were calculated for an area of 1.8 ha, being the area of inundation in 2014. Concentration data collected by Water Corporation.

5.3.3 Phosphorus storage of ecosystem components

How does TP storage of ecosystem components change with enrichment and how does this differ between functional zones?

It was hypothesised that TP storage of ecosystem components would increase over time with enrichment (Hypothesis 3, Table 2-7) and this was supported by the results for water, topsoil (bare sediment zone), floc and litter. However, no significant increase over time was found in subsoil or *Typha* zone topsoil, and above-ground *Typha* TP storage decreased (Figure 5-4, Figure 5-5, Figure 5-10a, Table 5-3). The stores that recorded increases in TP storage had variable temporal trends. TP storage of water and topsoil (bare sediment zone only) increased significantly year to year whereas TP storage of floc and litter increased significantly from 2012 to 2013 but then remained stable in 2014.

Water

Significant increases in TP storage reflected significant increases in both water depth (Figure 5-4a-c, Table 5-3) and TP concentration (supporting Hypothesis 1, Table 2-7) over the study period. Water TP storage was significantly higher in *Typha* than bare sediment zone in 2012 but the opposite was true in 2014. Water TP concentration was significantly higher in *Typha* than bare sediment zone in 2012 in contrast to Hypothesis 4 (Table 2-7). Water depth did not differ between the zones. Comparison of incoming effluent TP concentration to the average lake water TP concentration showed a decreasing trend in wetland P removal capacity over time (reduction %, Table 5-2).

In 2012, water TP concentration was lower in the bare sediment than the *Typha* zone while FRP was below detection limit in both zones. However, as effluent discharge continued, Swamphen Lake became hypereutrophic: ANZECC trigger value for TP concentration (60 µg/L) was exceeded in 2013 and 2014 in both the bare sediment and *Typha* zones (Figure 5-4b, Table 2-6). However, the ANZECC trigger value for FRP concentration (30 µg/L) was exceeded only in 2013 in all bare sediment and some *Typha* sampling sites (Figure 5-6). It was hypothesised (4, Table 2-7) that water TP concentration would be higher in bare sediment than in the *Typha* zone due to higher P uptake capacity. However, this hypothesis was not supported, potentially due to low power in the statistical tests due to low numbers of replicates (Figure 5-4a-c, Figure 5-6, Table 5-3).

Table 5-2: TP concentration of effluent versus lake water.

Sampling occasion	Effluent TP	Lake TP	Reduction	Long-term average effluent TP	Reduction against long-term average effluent TP
	mg/L	mg/L	%	mg/L	%
October 2012	1.30	0.04	97%	2.05 across the three years	98%
October 2013	1.40	0.29	79%		86%
October 2014	0.80	0.53	34%		74%

Benthic stores: topsoil and subsoil

For the topsoil store, changes in TP storage mainly reflected changes in TP concentration but were also influenced by mass, which was stable between 2012 and 2013, but then decreased significantly into 2014 (Figure 5-4d-f, Table 5-3). There were significant differences between zones in both TP concentration and mass, with TP concentration being higher in bare sediment zone (2013, 2014) and mass being higher in the *Typha* zone. In contrast to Hypothesis 1 (Table 2-7), TP concentration of topsoil in the *Typha* zone appeared completely unaffected by enrichment whereas topsoil in the bare sediment zone increased (supporting Hypothesis 1, Table 2-7, Figure 5-4e). The lack of change in subsoil TP storage was a direct reflection of lack of change in TP concentration (in contrast to Hypotheses 1 and 3, Table 2-7) because mass was kept constant (155.10 ± 1.91 kg/m^2, n = 26) (Figure 5-5, Table 5-3).

Benthic stores: floc and litter

Significant increase in TP storage in floc 2012-2013 (supporting Hypothesis 3, Table 2-7), was followed by no change into 2014 (Figure 5-4g, Figure 5-10a, Table 5-3). Patterns in both floc TP concentration and mass were similar (Figure 5-4h-i, Table 5-3). For litter, the increase in TP storage 2012-2013 (supporting Hypothesis 3, Table 2-7), followed by no change into 2014 (Figure 5-4j, Figure 5-10a, Table 5-3), was a combination of different trends in litter TP concentration and mass. In contrast to Hypothesis 1 (Table 2-7), TP concentration of litter did not change over time and was highly variable (plus litter was only recorded at one sampling point in 2012, Figure 5-4k), whereas litter mass increased from 2012-2014 (supporting Hypothesis 2, Table 2-7, Figure 5-4l, Table 5-3).

Above-ground Typha

Decline in total above-ground *Typha* TP storage (in contrast to Hypothesis 3, Table 2-7) reflected opposing trends in *Typha* TP concentration and mass. TP concentration of both live and dead *Typha* increased significantly over time (supporting Hypothesis 1, Table 2-7), whereas mass of both live and dead *Typha* decreased significantly (in contrast to Hypothesis 2,

Table 2-7, Figure 5-4m-o, Table 5-3). It was hypothesised that mass of ecosystem components associated with primary production (floc, litter, *Typha*) would increase with enrichment (Hypothesis 2, Table 2-7) and this was supported by litter and floc, but not by *Typha* (Figure 5-4, Table 5-3).

Total TP storage

As expected, total TP storage (excluding subsoil) increased over time (2012 – 2013) due to enrichment however, from 2013 to 2014 total TP storage remained stable despite increasing cumulative P load. Total TP storage was expected to be higher in *Typha* than in bare sediment zone due to higher P uptake capacity in the former, but the zones did not differ (Figure 5-7, Table 5-3).

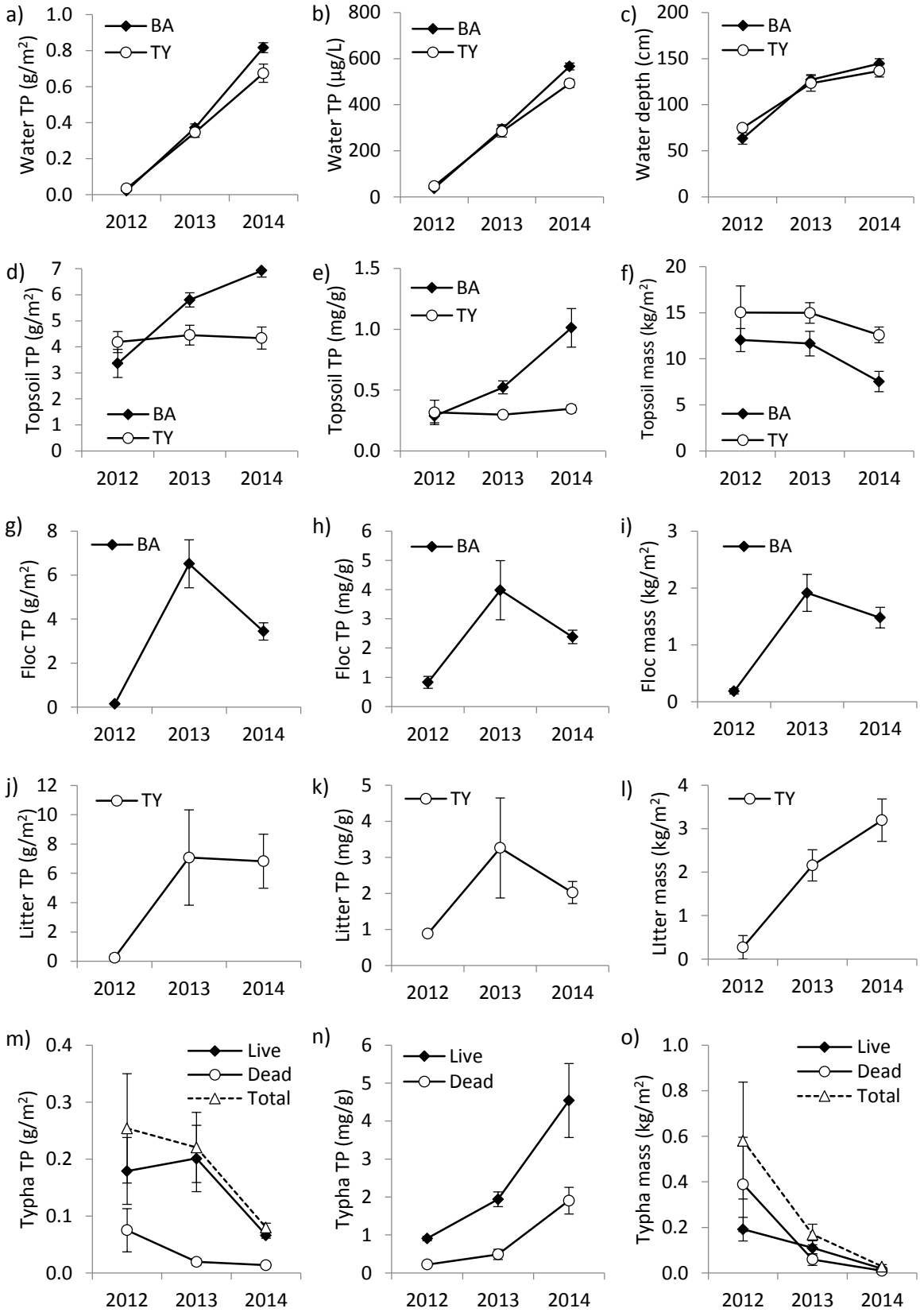

Figure 5-4: Mean TP storage, TP concentration and mass (depth for water) of ecosystem components (± 1 standard error) in bare sediment (BA) and *Typha* (TY) zones: water (a, b, c), topsoil (d, e, f), floc (g, h, i), litter (j, k, l) and above-ground *Typha* (m, n, o) with n = 3 in 2012 (apart from n = 1 for litter TP concentration) and n = 5 in 2013 and 2014.

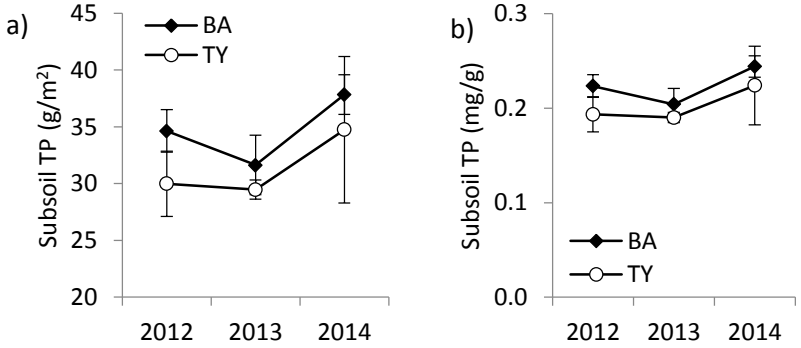

Figure 5-5: Mean TP storage (a) and TP concentration (b) of subsoil (± 1 standard error) in bare sediment (BA) and *Typha* (TY) zones (n = 3 in 2012 and n = 5 in 2013 and 2014).

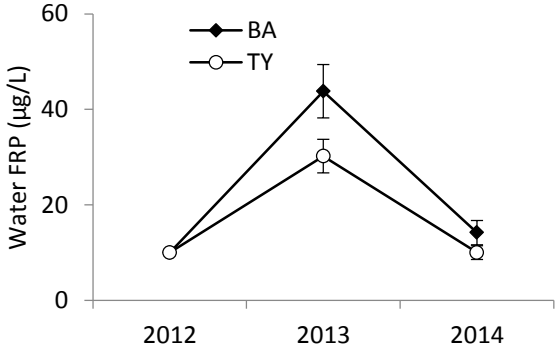

Figure 5-6: Mean FRP concentration of water (± 1 standard error) in bare sediment (BA) and *Typha* (TY) zones of Swamphen Lake (n = 3 in 2012 and n = 5 in 2013 and 2014). In 2012 all sites below detection limit 10 µg/L.

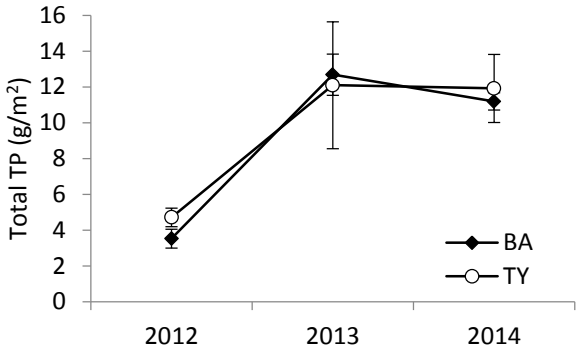

Figure 5-7: Mean total TP storage (± 1 standard error) in bare sediment (BA) and *Typha* (TY) zones of Swamphen Lake in October 2012, 2013 and 2014 (n = 3 in 2012, n = 5 in 2013 and 2014).

Table 5-3: Differences in TP concentration (and water FRP), mass (depth for water) and TP storage of ecosystem components between years and zones. Abbreviations include 12 = 2012, 13 = 2013, 14 = 2014, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, na = not applicable, do = disordinal, # = non-parametric test. Parentheses indicate a significant disordinal interaction between year and zone. Further details in Appendix 6.

Ecosystem components	Year		Zone	Year*Zone		
	Initial test	Post hoc	Initial test	Initial test	Simple effects - Year	Simple effects - Zone
TP concentration						
Water	(***)	na	(ns)	* do	BA and TY: 12/13***, 13/14***, 12/14***	12*, 13^{ns}, 14^{ns}
Water FRP#	***	12/13**, 13/14**, 12/14^{ns}	ns	na	na	na
Topsoil	(**)	na	(***)	* do	BA 12/13*, 13/14**, 12/14***, TY all ns	12^{ns}, 13*, 14***
Subsoil	ns	na	ns	ns	na	na
Floc	**	12/13**, 13/14^{ns}, 12/14*	na	na	na	na
Litter#	ns	na	na	na	na	na
Typha - Live	**	12/13*, 13/14^{ns}, 12/14*	na	na	na	na
Typha - Dead	**	12/13^{ns}, 13/14*, 12/14*	na	na	na	na
Store mass (g/m^2)						
Water depth	***	12/13***, 13/14^{ns}, 12/14***	ns	ns	na	na
Topsoil	*	12/13^{ns}, 13/14*, 12/14^{ns}	**	ns	na	na
Floc	**	12/13**, 13/14^{ns}, 12/14*	na	na	na	na
Litter#	*	12/13^{ns}, 13/14^{ns}, 12/14*	na	na	na	na
Typha - Live	**	12/13^{ns}, 13/14**, 12/14**	na	na	na	na
Typha - Dead	**	12/13^{ns}, 13/14^{ns}, 12/14**	na	na	na	na
Typha - Total	**	12/13^{ns}, 13/14*, 12/14**	na	na	na	na
TP storage (g/m^2)						
Water	(***)		(ns)	** do	all ***	12**, 13^{ns}, 14*
Topsoil	(**)		(**)	** do	BA 12/13***, 13/14*, 12/14***, TY all ns	12^{ns}, 13*, 14***
Subsoil	ns		ns	ns	na	na
Floc	***	12/13***, 13/14ns, 12/14***	na	na	na	na
Litter#	*	12/13ns, 13/14ns, 12/14*	na	na	na	na
Typha - Live	*	all ns	na	na	na	na
Typha - Dead	ns	na	na	na	na	na
Typha - Total	*	all ns	na	na	na	na
Total TP	***	12/13***, 13/14ns, 12/14***	ns	ns	na	na

How does the rate of response to enrichment differ between ecosystem components and how does this differ between functional zones?

TP concentration of most ecosystem components responded to enrichment between 2012-2013 with significant increases in water, topsoil (bare sediment zone only), floc and live *Typha*. By 2014, additional responses were recorded in dead *Typha* (increase in TP concentration) and litter (increase in TP storage). Topsoil TP concentration in the *Typha* zone and subsoil TP concentration in both zones did not respond to enrichment during the study period.

What ecosystem components account for most of the variation in TP storage between years and how does this differ between functional zones?

In the bare sediment zone, the first two principal components explained 96.4% of total variation in TP stores. Axis 1 explained most of the variation and clearly divided the samples into the three years, showing that patterns of TP storage changed over time (Figure 5-8). Axis 2 divided the samples into those where more TP was stored in floc compared to those where most was stored in water or topsoil.

Variation in TP storage of ecosystem components in *Typha* zone was high within years, but the first two principal components of the PCA still explained 79.6% of total variation. Differences between years were still evident on axis 1, but were not as large (Figure 5-9). Variation on axis 2 explained most of the variation within the years for 2013 and 2014 and was mainly influenced by TP storage in topsoil.

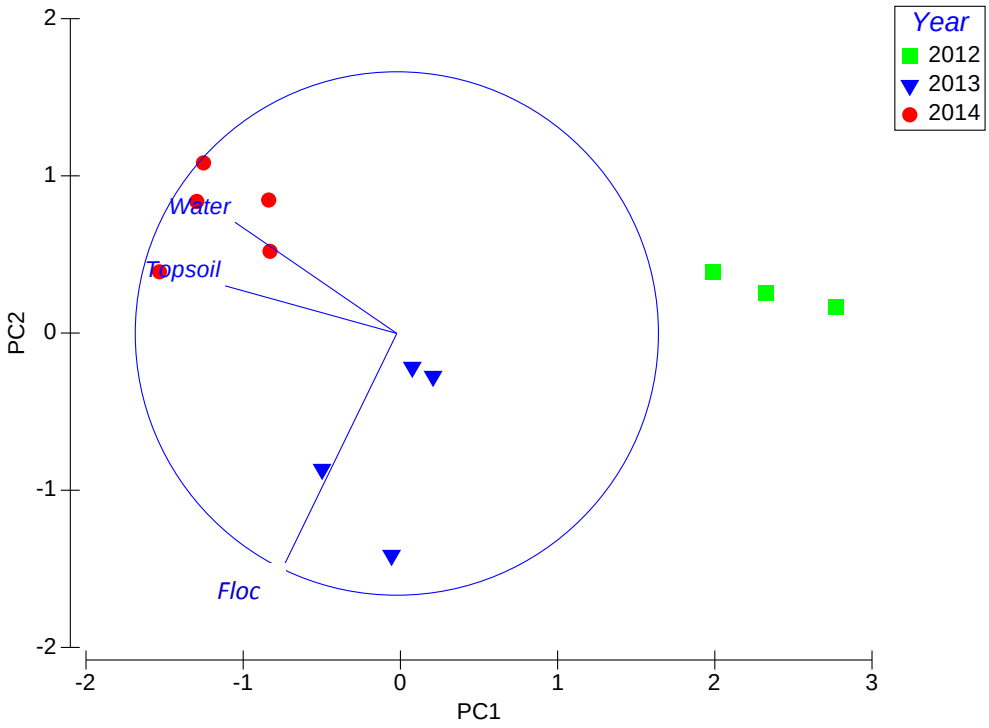

Figure 5-8: PCA ordination plot showing variation in TP storage of ecosystem components in bare sediment zone in 2012, 2013 and 2014 (n=3 in 2012, n=5 in 2013 and 2014). PC1 explained 70.9% of the variation and PC2 explained 25.5% of the variation in the data.

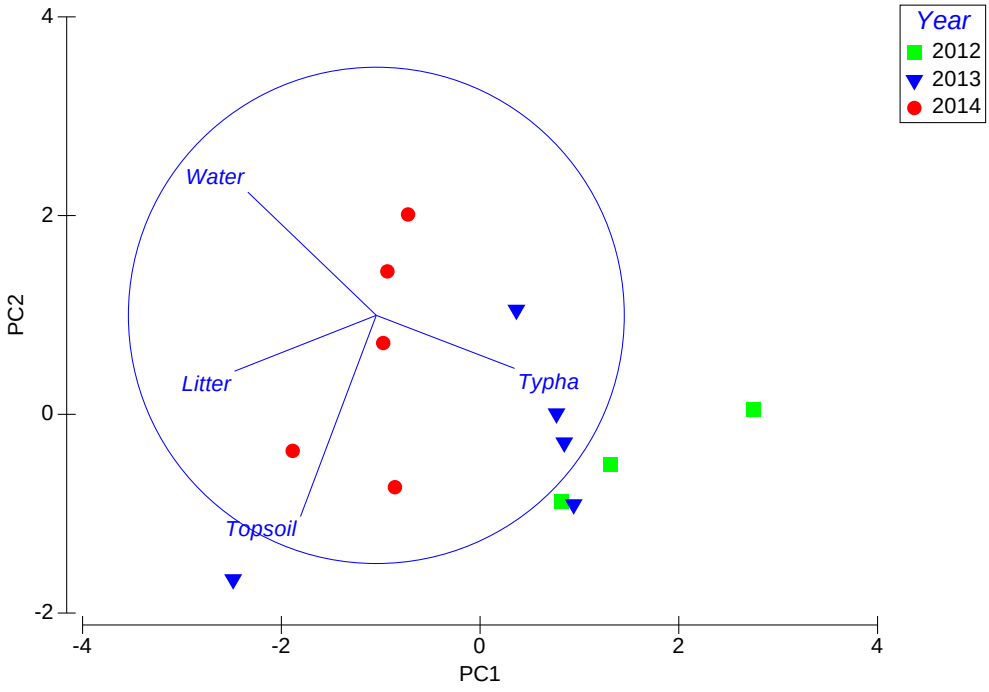

Figure 5-9: PCA ordination plot showing variation in TP storage of ecosystem components in *Typha* zone in 2012, 2013 and 2014 (n=3 in 2012, n=5 in 2013 and 2014). PC1 explained 52.3% of the variation and PC2 explained 27.3% of the variation in the data.

5.3.4 Proportional phosphorus storage

How does the distribution of total TP storage across the ecosystem components (% of total) change with enrichment and how does this differ between functional zones?

Subsoil was the largest store of TP in this study in all functional zones and years, and did not change over time. As explained earlier, subsoil was not included in the total TP storage calculation and the following results on the distribution of total TP storage across the stores also do not include subsoil.

In the early stages of enrichment in 2012, the majority of TP in the ecosystem was stored in topsoil in both the bare sediment and *Typha* zones (95% and 89% respectively, Figure 5-10a, b). As enrichment progressed, the proportion of total TP stored in topsoil decreased (supporting Hypothesis 5, Table 2-7, Figure 5-10b, Table 5-4), in both the *Typha* zone (44% in 2013 and 39% in 2014) and the bare sediment zone (47% in 2013 and 62% in 2014). Topsoil was replaced as the largest store by floc in bare sediment and by litter in the *Typha* zone (Figure 5-10a, b). However, uptake by floc ceased while uptake by topsoil continued and by 2014, floc was once again the second largest store (30%) after topsoil in the bare sediment zone. Litter remained the largest store in the *Typha* zone in 2014. In the *Typha* zone, *Typha* was the second largest store in 2012 (6%) but became the smallest store in 2013 (2%) and 2014 (1%). Although water remained a small store in both zones, its proportional storage of total TP increased from <1% in 2012 to 7% (bare sediment) and 6% (*Typha*) in 2014.

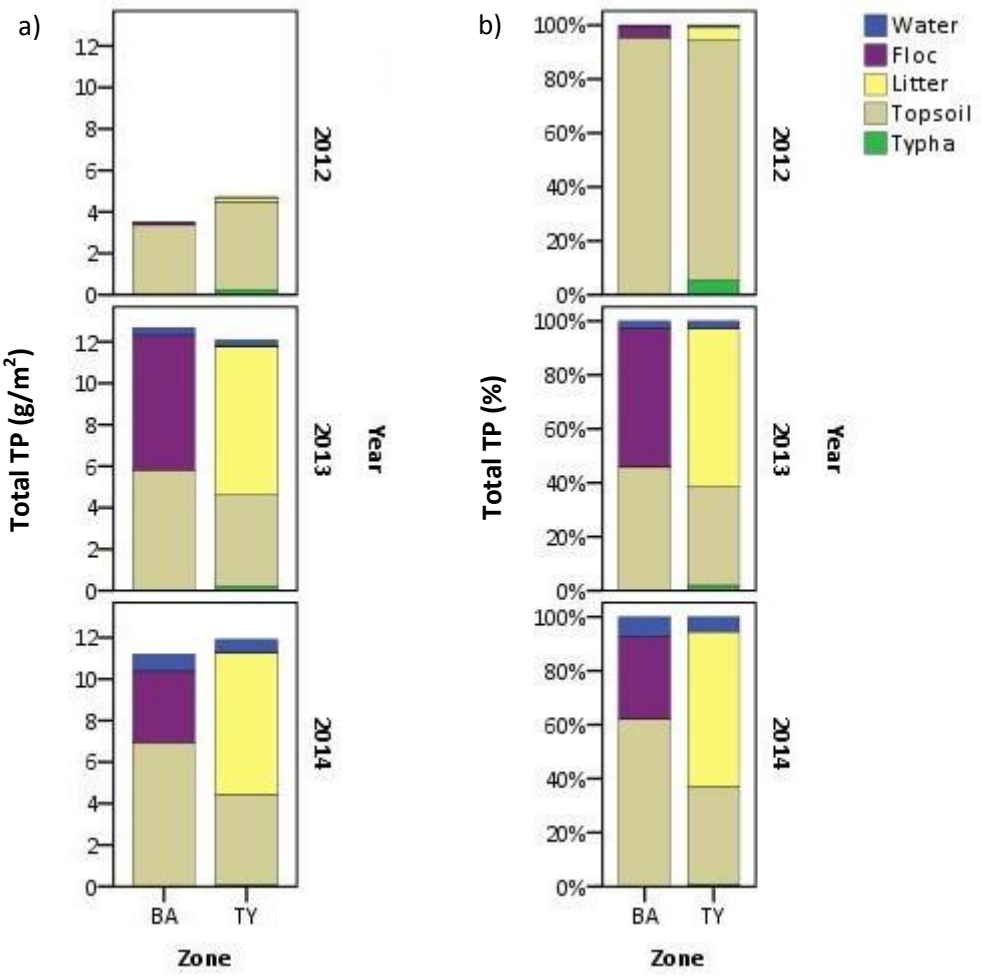

Figure 5-10: Breakdown on total TP storage between ecosystem components as g/m^2 (a) and % share (b) in bare sediment (BA) and *Typha* (TY) zones of Swamphen Lake in October of 2012, 2013 and 2014.

Table 5-4: Differences in percentage share of total TP storage of ecosystem components between years and zones. Abbreviations include 12 = 2012, 13 = 2013, 14 = 2014, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, na = not applicable, # = non-parametric Kruskal-Wallis. Further details may be found in Appendix 6.

Ecosystem component	Year		Zone	Year*Zone
	Initial test	Post hoc	Initial test	Initial test
Water	***	12/13**, 13/14***, 12/14***	ns	ns
Topsoil	***	12/13***, 13/14ns, 12/14***	*	ns
Floc	***	12/13**, 13/14*, 12/14***	na	na
Litter#	*	12/13ns, 13/14ns, 12/14*	na	na
Typha	*	12/13na, 13/14ns, 12/14*	na	na

5.3.5 Phosphorus mass balance

Where does the added P go?

Between October 2012 and October 2014, 7.66 g/m^2 of P accumulated in the bare sediment zone while 7.20 g/m^2 accumulated in the *Typha* zone (both excluding subsoil, Table 5-5). In bare sediment zone the majority of the accumulated P was taken up by floc and topsoil (partly supporting Hypothesis 6, Table 2-7) whereas in the *Typha* zone nearly all of the accumulated P was taken up by litter (Table 5-5). Extrapolating the average P accumulated in both zones (7.43 g/m^2) to the whole wetland area (1.8 ha) results in a total of 133.7 kg of P being accumulated in the wetland ecosystem components between 2012 and 2014. This equals approximately 41% of the 327.6 kg of P (18.2 g/m^2) added to the wetland through effluent discharge over that period. The majority, if not all of the remaining P load is anticipated to have been lost through groundwater seepage estimated at 155 ML between October 2012 and October 2014 (Section 5.3.1). The TP concentration of the seepage is unknown but it would have varied, being highest closest to the discharge point and increasing over time as water TP concentration in the wetland increased. Estimating TP concentration of seepage in the range of 0.3 - 1.5 mg/L results in P loss through seepage of between 46.5 - 232.5 kg, equalling 14-71% of the total P added to the wetland.

Table 5-5: Change in TP storage between October 2012 and October 2014. Differences are differences between mean values. As 2012 n = 3 and 2014 n = 5, only differences between averages could be calculated (rather than average differences). Significant differences as per Table 5-3 are shaded.

	TP storage (g/m^2)					
	Bare sediment			***Typha***		
Ecosystem component	**2012**	**2014**	**Difference 2014-2012**	**2012**	**2014**	**Difference 2014-2012**
Water	0.02	0.82	**0.79**	0.03	0.67	**0.64**
Floc	0.14	3.44	**3.30**	absent	absent	absent
Litter	absent	absent	absent	0.24	6.83	**6.59**
Topsoil	3.36	6.93	**3.57**	4.18	4.33	0.15
Typha	absent	absent	absent	0.25	0.08	-0.17
Total	3.52	11.18	7.66	4.71	11.91	7.20

5.3.6 Ecosystem change

How do vegetation characteristics change with enrichment in the different functional zones?

Major changes were recorded in the vegetation characteristics of the *Typha* zone. In partial support of Hypothesis 7 (Table 2-7), *Typha* height increased significantly from 2012 to 2013 and then remained stable into 2014 (Figure 5-11b, Table 5-6). In contrast to Hypothesis 7, *Typha* density and biomass decreased from 2012 to 2013 and again in 2014 (Figure 5-11a, Figure 5-4o, Table 5-6, Table 5-3). Over the study period, I observed that *Typha* rhizomes and root-balls became smaller and were positioned closer to the sediment surface. In 2014, it was possible to pull out an entire *Typha* culm with the rhizome and most roots attached whereas in 2012 the culms were firmly embedded in the sediment. Some *Lemna minor* was recorded amongst *Typha* in 2013 and 2014, and green filamentous algae in 2013, whereas neither of these species were present in 2012. A major bloom of *L. minor* (100% cover of water surface) was present outside the sampling areas, close to the effluent discharge point both in 2013 and 2014.

In the bare sediment zone, no submerged or emergent vegetation or filamentous algae was recorded during the study period. Few individuals of floating *L. minor* were recorded in 2013 and 2014 but these were being blown across the open water area into *Typha* zone and their interaction with the bare sediment zone was fleeting.

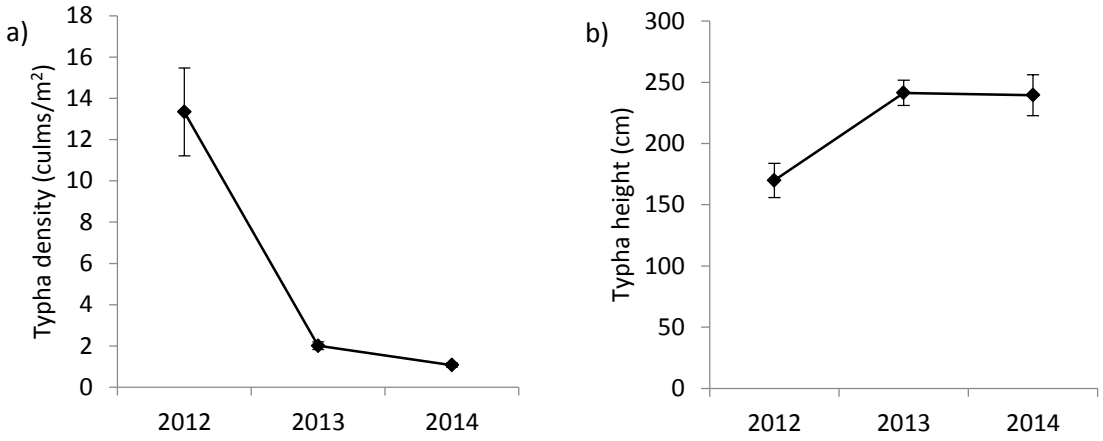

Figure 5-11: *Typha* density (Pollard density estimator ± 1 standard error as converted from associated variance estimator) (a) and mean height (b) (± standard error) in *Typha* zone of Swamphen Lake in October of 2012, 2013 and 2014 (n = 3 in 2012, n = 5 in 2013 and 2014).

How does water quality change with enrichment and how does this differ between functional zones?

In both bare sediment and *Typha* zones, there were significant changes in water quality over time. In support of Hypothesis 9 (Table 2-7), turbidity and chlorophyll α, that were low in 2012, increased significantly from 2012 to 2013 and further into 2014 (Figure 5-12a,b, Table 5-6). However, Hypothesis 10 (Table 2-7) was not supported, as there were no significant differences between zones in turbidity or chlorophyll α. In 2013, chlorophyll α remained just below the ANZECC trigger value of 30 µg/L but by 2014 chlorophyll α was tenfold the trigger value. Based on chlorophyll α there was a shift from mesotrophic conditions in 2012 into hypereutrophic conditions by 2014 (see scale in Table 2-6). Site observations recorded a shift from relatively clear water in 2012 to turbid water state in 2014. Effluent discharged in 2014 was greenish and was importing an algal bloom from the wastewater treatment plant (Kauhanen pers. obs.).

Surface water pH was significantly more alkaline in 2014 than other years when it was neutral, whereas bottom water pH was significantly lower in 2013 (slightly acidic) than in other years (neutral) (Figure 5-12c,d, Table 5-6) and neither variable differed between zones. Daytime surface water DO increased significantly each year and was supersaturated in 2013 and 2014, exceeding the ANZECC upper trigger value of 120% (Figure 5-12e). Daytime bottom water DO remained low and well below the ANZECC lower trigger value of 90% throughout the study, but was slightly higher in 2013 than other years (Figure 5-12f). Daytime bottom DO was significantly lower in bare sediment than *Typha* in 2013 and 2014 (Table 5-6). Hypothesis 11 (Table 2-7) was supported in 2014 when eight out of 10 sites sampled had bottom water DO levels <1 mg/L, meaning that they were essentially anoxic and unable to support aerobic organisms. Similarly, surface and bottom water ORP were stable between 2012 and 2013 but decreased significantly from 2013 to 2014 (supporting Hypothesis 11, Table 2-7) and the zones did not differ (Figure 5-12g,h, Table 5-6). In 2014, bottom water ORP was much lower than surface ORP and the conditions close to the bottom were highly reducing (Figure 5-12g,h). Surface water temperature did not change between years but bottom water temperature and surface and bottom water EC decreased with increasing water depth and dilution of original lake water with discharged effluent (Figure 5-13, Table 5-6).

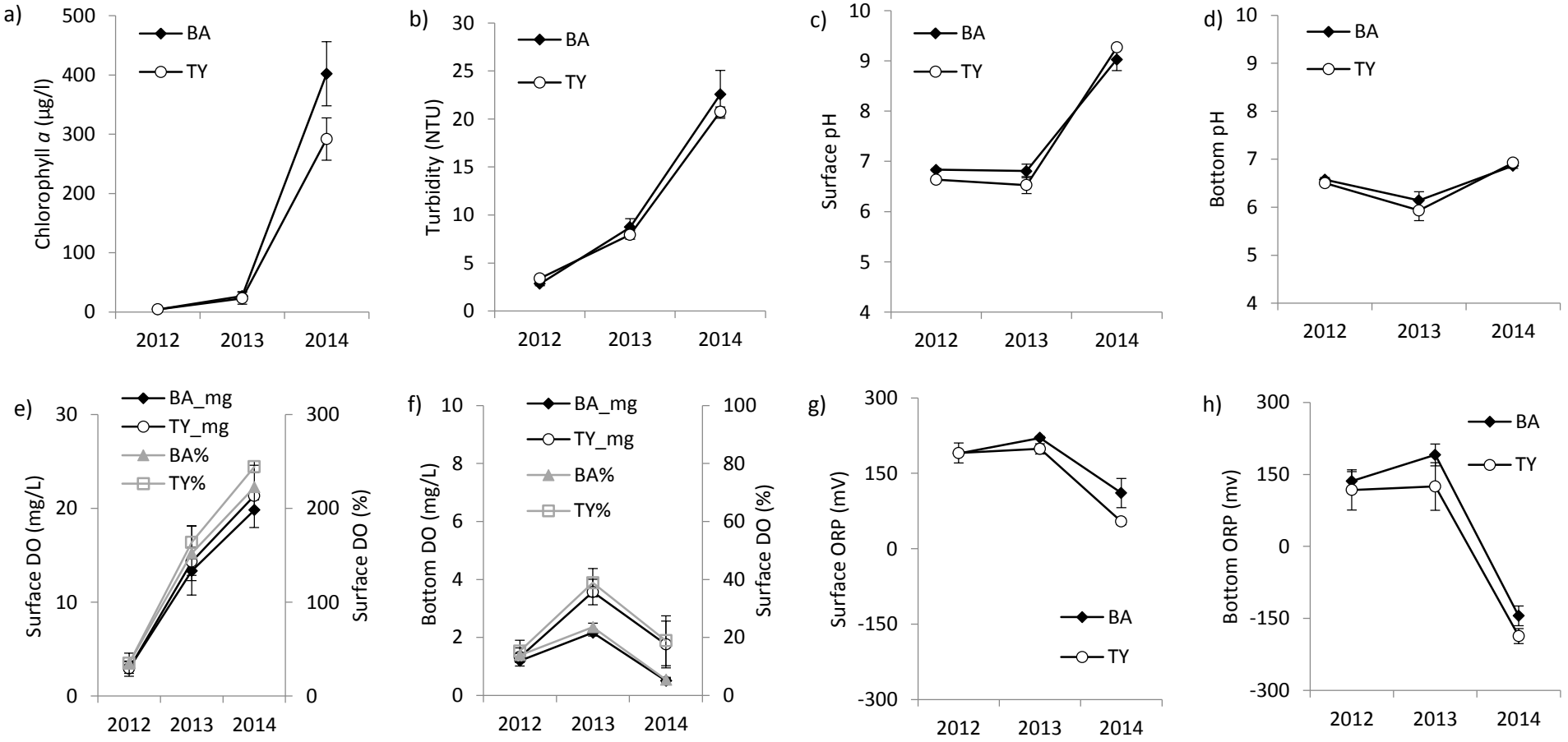

Figure 5-12: Mean chlorophyll α (a), turbidity (b), surface pH (c), bottom pH (d), surface DO (e), bottom DO (f), surface ORP (g) and bottom ORP (h) (± 1 standard error) in bare sediment (BA) and *Typha* (TY) zones of Swamphen Lake in October 2012, 2013 and 2014 (n = 3 in 2012, n = 5 in 2013 and 2014).

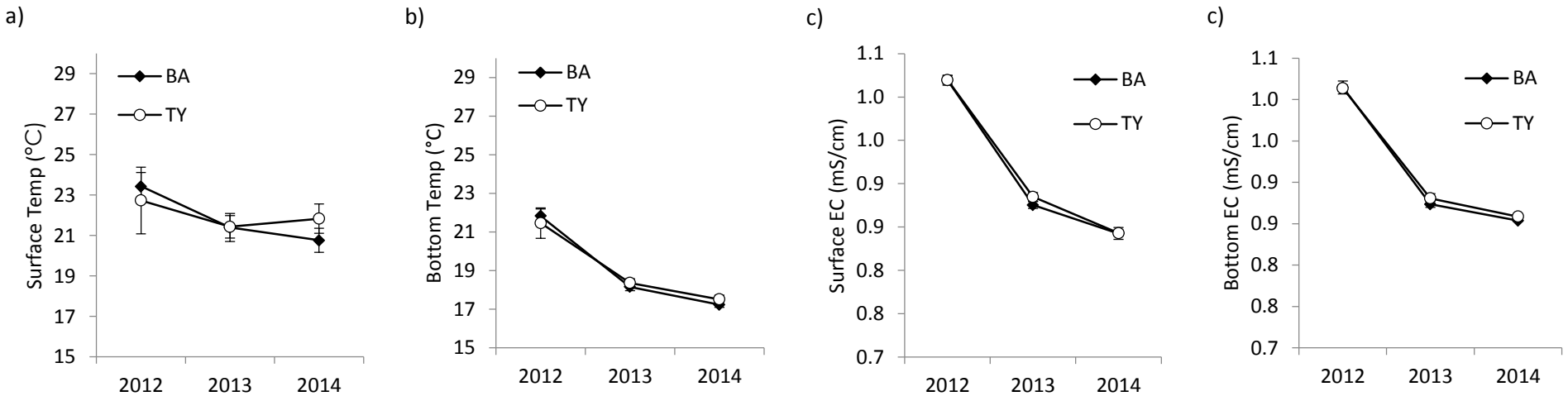

Figure 5-13: Mean surface temperature (a), bottom temperature (b), surface EC (c) and bottom EC (d) of water (± 1 standard error) in bare sediment (BA) and *Typha* (TY) zones of Swamphen Lake in October 2012, 2013 and 2014 (n = 3 in 2012, n = 5 in 2013 and 2014).

Table 5-6: Differences in water quality variables and *Typha* height between years and zones. Abbreviations: 12 = 2012, 13 = 2013, 14 = 2014, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, na = not applicable, # = non-parametric Kruskal-Wallis. Further details may be found in Appendix 6.

Variable	**Year**		**Zone**	**Year*Zone**
	Initial test	**Post hoc**	**Initial test**	**Initial test**
Chlorophyll α	***	2012/2013***, 2013/2014***, 2012/2014***	ns	ns
Turbidity	***	2012/2013***, 2013/2014***, 2012/2014***	ns	ns
Surface Temperature	ns	na	ns	ns
Bottom Temperature	***	2012/2013***, 2013/2014**, 2012/2014***	ns	ns
Surface pH	***	2012/2013 ns, 2013/2014***, 2012/2014***	ns	ns
Bottom pH	***	2012/2013*, 2013/2014***, 2012/2014 ns	ns	ns
Surface EC	***	2012/2013***, 2013/2014***, 2012/2014***	ns	ns
Bottom EC	***	2012/2013***, 2013/2014***, 2012/2014***	ns	ns
Surface DO	***	2012/2013***, 2013/2014**, 2012/2014***	ns	ns
Bottom DO	**	2012/2013*, 2013/2014**, 2012/2014 ns	*	ns
Surface ORP	***	2012/2013 ns, 2013/2014***, 2012/2014***	ns	ns
Bottom ORP	***	2012/2013 ns, 2013/2014***, 2012/2014***	ns	ns
Typha height	**	2012/2013**, 2013/2014 ns, 2012/2014**	na	na

5.4 Discussion

5.4.1 Overview and conceptual models

Over the three year study period, the treatment wetland transformed from a mesotrophic to eutrophic and finally to a hypereutrophic system. For the first two years, Swamphen Lake was in the second stage of the nutrient enrichment continuum, where P added to the system (via effluent) was being actively taken up by ecosystem components. However, by the end of the study, some ecosystem components were potentially reaching their P uptake capacity (floc, litter, *Typha*), as total TP storage (excluding subsoil) was no longer increasing, water TP concentration was greatly elevated (hypereutrophic) and algal blooms were present (Figure 5-4, Figure 5-7, Figure 5-12). The hypereutrophic end-stage was evident, despite some ecosystem components (topsoil, subsoil) not having reached their full P uptake capacity (Figure 5-14, Figure 5-15). The original description of the hypereutrophic end-stage (Table 2-1) and the conceptual models (Figure 2-4, Figure 2-6) had predicted that all components would be at capacity at this stage, so these models needed to be modified to accommodate this finding (Table 5-7).

Water depth doubled from 2012 to 2014 (0.7 m to 1.4 m Figure 5-14, Figure 5-15) due to the volume of discharged effluent. This increase in water depth contributed to the increase in water TP storage, but also to other ecosystem changes such as *Typha* decline and reduction in bottom water DO and ORP (see section 5.4.3) that affected P storage and cycling.

The phosphorus load (approximately 9.1 g/m^2/year) and hydraulic loading rate (HLR, 0.012 m/d) were midrange in the context of other treatment wetlands (typical ranges: P load <0.1 - >1000 g/m^2/year, HLR <0.01 - >0.10 m/d, Kadlec and Wallace 2009) but the nominal hydraulic retention time (nHRT 49.9 d) was high (1 - 21 d, Kadlec and Wallace 2009). The high nHRT meant that incoming nutrients had more time to interact with ecosystem components, likely resulting in higher removal of P than in wetlands with lower nHRT (Tanner *et al.* 1995, Toet, Van Logtestijn*, et al.* 2005). Consequently, the system may have proceeded faster along the enrichment continuum than another system with similar P load but lower nHRT. Also while the P load was midrange for a treatment wetland, it was high in comparison to estimated sustainable phosphorus assimilation loads in both natural wetlands 0.5 - 1.0 g/m^2/year (Richardson and Qian 1999, Vymazal 2007, Hefting *et al.* 2012) and constructed wetlands 0.5-5.0 g/m^2/year (Mitsch *et al.* 2000, Hefting *et al.* 2012).

Table 5-7: Summary of study changes to conceptual model.

Functional zone	Functional phosphorus uptake stage	Hypereutrophic end-stage
Bare sediment zone	• Floc - store added to model, increase in TP storage recorded. • Subsoil - expected increase in P storage not observed.	• Floc - store added to model, TP storage capacity exhausted with potential loss of P. • Subsoil - expected increase in P storage not observed.
Typha zone	• Topsoil - expected increase in TP storage not observed. • Subsoil - - expected increase in P storage not observed. • *Typha* - expected increase in TP storage not observed.	• Topsoil - expected increase in TP storage not observed. • Subsoil - expected increase in P storage not observed. • *Typha* - significant decrease rather than the expected increase in P storage.
All	• Pie charts added showing distribution of TP between ecosystem components (excluding subsoil). • Pie charts added showing where the added P went as % of total load.	

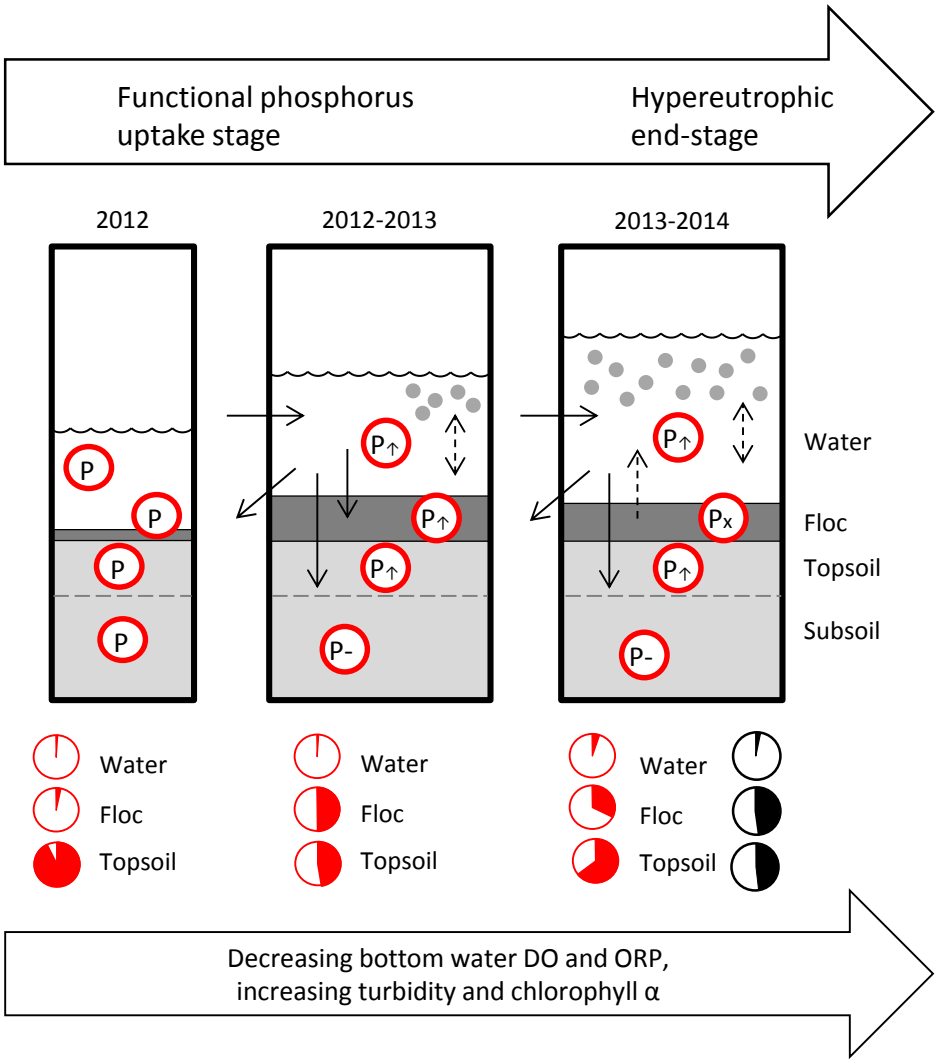

Figure 5-14: Conceptual models of phosphorus storage and cycling in the bare sediment zone between 2012 and 2014. Solid line arrows indicate definite phosphorus cycling pathways and dashed lines likely pathways. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached. Grey dots show algal bloom intensity. Red pie charts indicate proportional share of total phosphorus (excluding subsoil) held by each store. Black pie charts indicate where the added phosphorus went based on mass balance (difference in TP storage between 2014 and 2012, Table 5-5), excluding subsoil and pathways not sampled (e.g. groundwater seepage).

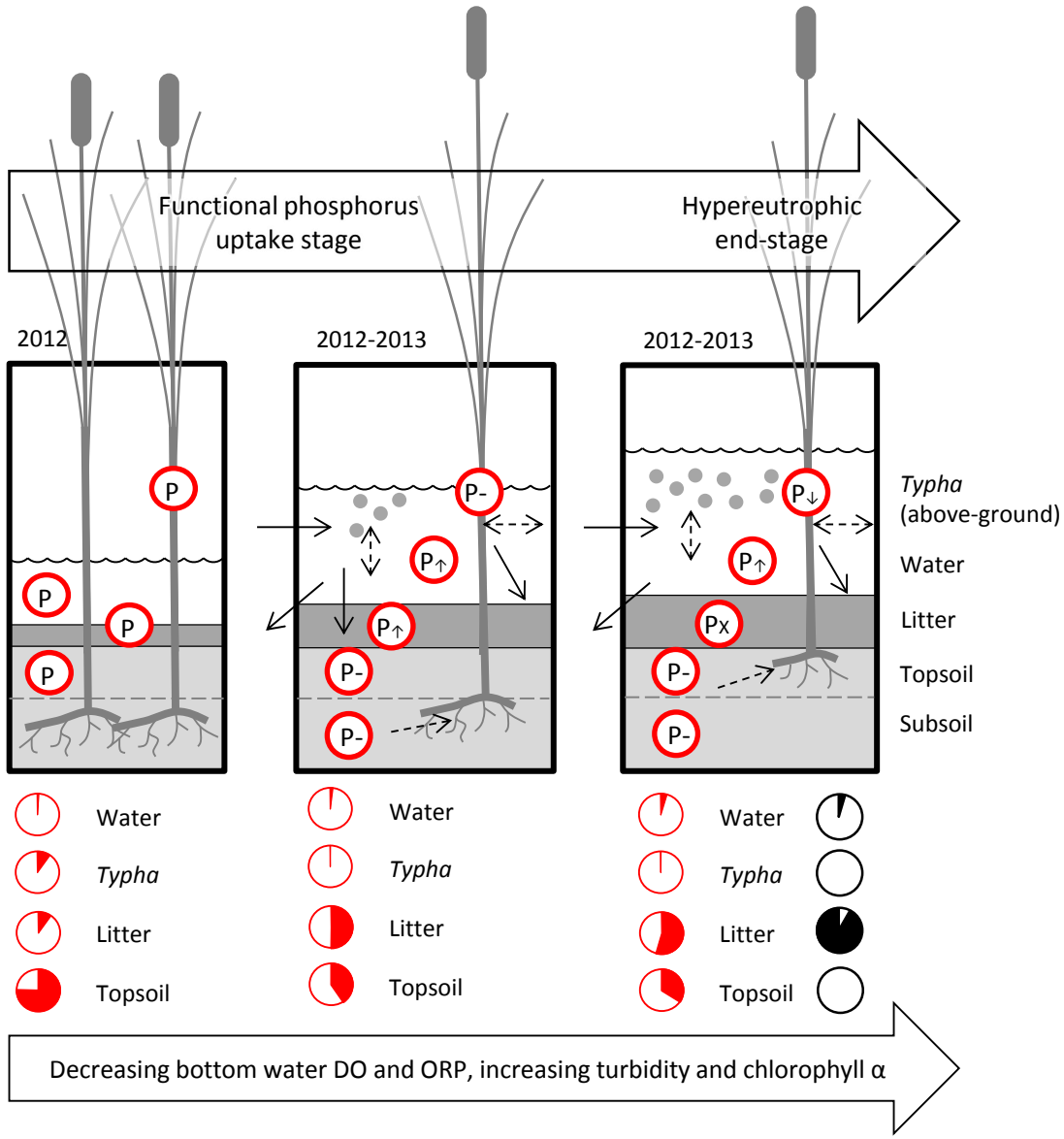

Figure 5-15: Conceptual models of phosphorus storage and cycling in the *Typha* zone between 2012 and 2014. Solid line arrows indicate definite phosphorus cycling pathways and dashed lines likely pathways. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached. Grey dots show algal bloom intensity. Red pie charts indicate proportional share of total phosphorus (excluding subsoil) held by each store. Black pie charts indicate where the added phosphorus went based on mass balance (difference in TP storage between 2014 and 2012, Table 5-5), excluding subsoil and pathways not sampled (e.g. groundwater seepage).

5.4.2 Phosphorus storage of ecosystem components

Research Question 1 - How do P storage and cycling change as a wetland undergoes nutrient enrichment and how does this differ between functional zones?

Rate of response to enrichment

Most ecosystem components (water, topsoil in bare sediment zone, floc, litter, live *Typha*) responded to enrichment between October 2012 and October 2013 (following commencement of effluent discharge in February 2012). Standing dead *Typha* responded by October 2014, but *Typha* zone topsoil and subsoil in both zones, did not respond over the study period. The high incoming P load (approximately 9.1 g/m^2/year) and long nHRT are responsible for the rapid responses seen in this study; systems subject to lower load and/or shorter retention time would be expected to show a slower response (e.g. Gaiser *et al.* 2005). The relatively rapid emergence of treatment response across ecosystem components and the large magnitude of the associated changes indicate that the treatment wetland had progressed quickly along the nutrient enrichment continuum.

Temporal patterns in P uptake pathways

Active phosphorus uptake pathways varied over time. Water (including suspended algae) and topsoil (bare sediment zone only) took up P throughout the study 2012-2014 whereas floc and litter took up P only between 2012 and 2013 (all supporting Hypothesis 3, Table 2-7). The P uptake capacity of floc and litter may have been reached, as their TP concentrations (floc 4.0 mg/g, litter 3.3 mg/g) were at or near the maximum values recorded in literature (Table 2-3). The findings are in agreement with previous research indicating that microbial and litter pathways are important at the early stages of enrichment (1-2 years) (Richardson and Marshall 1986) whereas soil uptake pathways remain active longer (Wood *et al.* 2008, Mustafa and Scholz 2011) (although eventually they too will be exhausted except through soil accretion). Determining active P uptake pathways for a wetland at a particular point in time could thus potentially be used to gauge how far along the nutrient enrichment continuum the system is. However, assessing active P uptake can be difficult. Even stores that have reached their uptake capacity, undergo seasonal and lifecycle related cycling (Reddy *et al.* 1999), although without net P accumulation over time as shown in Chapter 4. Consequently, careful interpretation of time series data that considers potential temporal dynamics in P stores will enable the progress of nutrient enrichment to be assessed correctly.

Magnitude of P uptake and potential release

The magnitude (scale) of P uptake varied between ecosystem components. Topsoil P uptake was 4.5 times that of water. Water TP storage reflected accumulation of P in water, but also uptake by suspended algae. Although algae provide a rapid uptake pathway, storage capacity (algal biomass) is limited, and is expected to regularly recycle stored P (Richardson and Vaithiyanathan 2009). Uptake by floc and topsoil were similar, but uptake by litter was nearly twice that of floc. High P uptake by topsoil was expected as the clay soil containing Fe, Al and Mn was known to have a high maximum P absorption capacity (15 g/m^2 of in top 1 mm) (Chambers and McComb 1996). However, the recorded maximum in this study (6.9 g/m^2 in top 20 mm) was much lower than the maximum absorption capacity by Chambers and McComb (1996). Topsoil bound P would be expected to be stored in the long-term but reductions in DO and ORP could result in P release (Palmer-Felgate *et al.* 2011). In contrast to topsoil, increases in TP storage of floc and litter were due to increases in both TP concentration and mass (supporting Hypotheses 1, 2 and 3 Table 2-7). Litter is likely to have contained a large microbial flora capable of rapidly assimilating P but also subject to regular recycling (Qualls and Richardson 2000, Grace *et al.* 2008); it is also sensitive to water quality deterioration (Section 5.4.3). The detrital component of floc may also have shown microbial P uptake. However, subsequent analysis indicated that the floc was mainly inorganic in composition (77% in 2013 and 2017) (K. Kauhanen unpublished data), unlike the predominantly organic floc described elsewhere (Sommer 2006, Turner *et al.* 2006). The inorganic component of the floc, likely the result of mixing with the topsoil layer (wind and bioturbation), would have had a high capacity for P sorption as indicated by P fractionation of floc (for 2013 and 2017) which showed that NaOH alkali extractable P (Fe and Al bound P) was the largest component of P (K. Kauhanen unpublished data). Floc P could potentially also be made re-available through the processes described above for litter and topsoil.

The scale and nature of P uptake over enrichment continuum has implications for system management. While large P uptake can be positive in the short-term, potentially maintaining low water TP concentration and clear water conditions (although not in this study), the accumulated P poses a risk of becoming a future internal source (Scharf 1999, Søndergaard *et al.* 2003, Song and Burgin 2017). This depends on how readily the stored P can be converted back into bioavailable P and could also be driven by declining water quality (e.g. lower DO and ORP as discussed in Section 5.4.3).

As topsoil TP storage appeared to be much lower than the estimated maximum absorption capacity, there should still be capacity for further assimilation by soil in the future. However,

this depends on water and soil conditions (e.g. ORP) and the degree of interaction between water, floc/litter and soil (Reddy and DeLaune 2008). Litter TP concentration was high and the peak litter mass 3195g/m^2 (2014) was among the maximum values recorded in enriched systems (Table 2-5). High litter mass was an outcome of widespread *Typha* death, and unlikely to increase in the future due to the decline in standing *Typha* biomass. Consequently, P uptake by litter is unlikely to increase substantially in the future. While floc TP concentration was unlikely to increase further, floc mass could continue to increase through detrital inputs within the wetland (e.g. algal blooms) and through effluent. However, the accumulation of floc mass also depends on the rate of consolidation and incorporation into new soil (Turner *et al.* 2006).

The consistent year-to-year increases in TP concentration of water (and associated decrease in treatment efficiency) indicate decreasing removal of P from water by other ecosystem components. This is a common finding in newly established treatment wetlands as the initially high uptake capacity of stores is gradually exhausted (e.g. Nichols 1983, Richardson 1985, Mitsch *et al.* 2012, Dzakpasu *et al.* 2015, Griffiths and Mitsch 2017). The rate at which treatment capacity decreases is dependent on P load into the wetland and its capacity to assimilate this P (Richardson and Vaithiyanathan 2009). The relatively rapid decrease in the treatment efficiency of Swamphen Lake indicates that the P load was high in proportion to the assimilation capacity of the wetland (discussed further in Chapter 7).

Typha P response

TP concentration of live and dead *Typha* increased over time as expected (e.g.Reddy and DeLaune 2008, Kadlec and Wallace 2009) supporting Hypothesis 1 (Table 2-7) and live *Typha* TP concentration at the end of the study (4.5 mg/g) was in the upper range of reported concentrations (commonly 1-5 mg/g, Table 2-3). Consequently, *Typha* provided a P uptake pathway throughout the study period. However, P uptake was exceeded by P loss via decrease in *Typha* biomass. While previous research has found above-ground biomass of *Typha* in the range of several thousand g/m^2 (Table 2-5), in this study it was close to 600 g/m^2 in 2012 and only 28 g/m^2 in 2014 in contrast to Hypothesis 2 (Table 2-7). Clearly, overall TP storage in above-ground *Typha* decreased over time in contrast to Hypothesis 3 (Table 2-7). This illustrates the importance of sampling both TP concentration and store mass in order to determine the overall response of a store to enrichment (see also Vymazal 2016a). Loss of *Typha* results in the loss of P cycling pathways characteristic of the *Typha* zone (such as uptake of P from the root zone, temporary storage in biomass, release into water and contributions to detritus) (Davis and van der Valk 1983, White *et al.* 2006) and reduces the total P removal

capacity of the zone. P uptake by plants (including *Typha*) can be substantial, as both tissue TP concentration and biomass increase to new equilibria. For example, Kadlec and Bevis (2009) found plant tissue TP concentration was three times higher and live plant biomass 2.5 times higher in enriched than unenriched areas of a wastewater treatment wetland.

Lack of response in subsoil

The heavy clay soil in Swamphen Lake had very low hydraulic conductivity (0.01-1 m/day, GHD 2004) and high P uptake capacity (Chambers and McComb 1996), meaning slow movement of water down the profile and high likelihood of P uptake by topsoil prior to water reaching the subsoil. The loss of P via groundwater seepage would have only occurred over the edges of the clay bucket into higher porosity sand surrounding the wetland (Figure 3-4) and would not have resulted in movement of P into subsoil in the sampling area. These reasons explain why little change in TP storage and concentration of subsoil was observed in contrast to Hypotheses 1 and 3 (Table 2-7). However, had the study continued longer, P uptake in subsoil may have been recorded. Even if P uptake by subsoil occurred in the future, it would not make a difference to the overall degree of eutrophication of the system because the uptake processes involved would be too slow to prevent the accumulation of P in water and subsequent adverse ecosystem outcomes. In wetlands with higher hydraulic conductivity of soil, changes in subsoil TP storage could be observed more quickly.

Differences between functional zones

Overall, the bare sediment and *Typha* zones took up a similar total amount of P, but through different pathways. In the bare sediment zone, floc and topsoil took up roughly equal amounts (partly supporting Hypothesis 6, Table 2-7) whereas in the *Typha* zone the only substantial uptake pathway, litter, took up nearly as much P as floc and topsoil combined in the bare sediment zone. However, as litter ceased to take up further P after 2013, the *Typha* zone effectively removed no further P from the water column. In contrast, P uptake in the bare sediment zone continued until the end of the study, because topsoil continued to accumulate P in this zone. This shows how enrichment progresses along different pathways in the two functional zones and also that the timing of P uptake may differ between functional zones. These findings are not typical, with previous research indicating vegetated systems take up more P from water than unvegetated systems (Sollie and Verhoeven 2008, Menon and Holland 2013, Di Luca *et al.* 2015). The difference in this study was no doubt a function of *Typha* decline.

Water TP storage (and concentration) was higher in *Typha* than bare sediment zone in 2012 whereas by 2014 there was no difference in concentration (in contrast to Hypothesis 4, Table 2-7). In 2012, the *Typha* zone may have had more P available (e.g. leaching from *Typha*) than the bare sediment zone, where P was tightly bound in topsoil. In 2014, the higher TP storage in bare sediment could have been increased by leaching from floc, although TP storage and concentration in floc did not decline significantly. At that time, declining *Typha* and litter did not take up P, so the original prediction that the *Typha* zone would have lower water TP due to uptake by *Typha* and associated stores (Table 2-7) was unlikely.

The complete absence of any increase in topsoil TP storage (and TP concentration) in the *Typha* zone was unexpected, in contrast to Hypotheses 1 and 3 (Table 2-7). While differences in topsoil P uptake between vegetated and unvegetated systems have been have recorded in other studies, findings have been conflicting, with some reporting higher uptake by topsoil in unvegetated than vegetated systems (Di Luca *et al.* 2015) and others the opposite (Reina *et al.* 2006, Menon and Holland 2013). The results of this study agree with those of Di Luca *et al.* (2015): topsoil P uptake was lower in vegetated (*Typha*) than unvegetated (bare sediment) areas. The lack of change in the *Typha* zone may have been associated with the overlying litter layer reducing contact between the water column and the soil. The litter layer assimilated large amounts of P and this may have resulted in little P reaching the soil beneath. In contrast, the topsoil of the bare sediment zone was overlain by floc, which consists of much smaller, more easily disturbed particles than litter. More interaction (e.g. wind mixing, bioturbation) between topsoil, the floc layer and the water column is likely than in *Typha* beds. Incorporation of floc into topsoil through soil accretion may have also contributed to the observed increase in topsoil TP storage and concentration over time. A longer study period may have seen increased TP storage and concentration of topsoil in the *Typha* zone, especially if litter uptake was saturated.

Proportional TP storage

Most P in wetlands is stored in soils and sediments (Kadlec and Wallace 2009), and this study was no exception. As eutrophication progresses, the proportion of P stored in topsoil tends to decline (Hypothesis 5, Table 2-7) while other stores, such as floc and litter, develop (Noe *et al.* 2002, Di Luca *et al.* 2015) and this was supported by this study. However, as floc and litter only removed significant amounts of P during the early-middle stages of this study, the dominance of topsoil storage would be expected to increase again later in the enrichment continuum (had the study continued). Indeed over long time periods, the great majority of P added to the

wetland would be expected to find its way to the sediments, gradually contributing to soil accretion (Richardson and Vaithiyanathan 2009, Mustafa and Scholz 2011, Kadlec 2016).

Low proportional TP storage by *Typha* has been recorded in other studies (e.g. Di Luca *et al.* (2015). However, studies of *Typha* generally record increases rather than decreases in the share of P held by macrophytes following enrichment (Noe *et al.* 2002, Noe and Childers 2007). This study was an unusual example of *Typha* in decline, and clearly shows how a sparse *Typha* bed makes minimal contributions to total TP storage, holding less P than the water column in hypereutrophic conditions.

The proportional TP storage of water increased over time (from <1% to 7%), but water remained a comparatively small store in both zones, in agreement with other research (Noe *et al.* 2002, Noe and Childers 2007, Kadlec and Wallace 2009). However, despite having a small proportion of TP storage, the corresponding TP concentrations in water make a big difference to ecosystems because they drive algal blooms.

Phosphorus not captured in the sampling area

The sampled ecosystem components accumulated approximately 41% of the P load (approximately 9.1 g/m^2/year) into the wetland over the study period. The majority of the remaining 59% was likely lost via seepage, as supported by the water balance calculations. Fringing vegetation outside the sampling area may have also taken up P (approximately 5-50 kg/ha/year, Mander *et al.* 1997, Hoffmann *et al.* 2009). Also, ecosystem components close to the effluent inflow point likely took up more P than those in the sampling area due to higher TP concentrations in water close to the inflow point (e.g. Craft and Richardson 1993). The review by Land *et al.* (2016) notes that existing literature commonly contains limited data on groundwater losses and gains, or even hydrology related changes overall, probably because these can be difficult to measure. Consequently, phosphorus mass balances prepared in the absence of this knowledge necessarily involve higher error margins, complicating both transfer of knowledge to other sites and also prediction of future changes within sites.

5.4.3 Ecosystem change

Research Question 2 - How is nutrient enrichment reflected in ecosystem change in the different functional zones?

Chlorophyll α and turbidity

Both bare sediment and *Typha* zones supported algal blooms (supporting Hypothesis 9, Table 2-7) with large associated increases in turbidity, but not until the third year. This bloom may have been partly imported from the wastewater treatment plant. FRP was uncharacteristically low, and TP very high, in 2014 suggesting that FRP was consumed by the algal bloom and thereby contributed to the water TP concentration as organic P. The algal bloom was the biggest visual change in ecosystem condition following commencement of nutrient enrichment, despite being fuelled by only a small share of total P added to the system.

DO and ORP

Daytime bottom DO in 2014 was at concentrations critically limiting for many animals (Boulton *et al.* 2014) and well below concentrations in the two unimpacted wetlands during the seasonal study (Chapter 4), supporting Hypothesis 11 (Table 2-7). This was despite supersaturated daytime surface water DO peaking in 2014, likely due to increased photosynthesis associated with the concurrent algal bloom (Reddy and DeLaune 2008). Depletion of oxygen is a well-recognised result of eutrophication (Smith 2003) as microbial decomposition of increased quantities of detritus consumes more oxygen (Reddy and DeLaune 2008). Surface and bottom water ORP were lowest in 2014 also supporting Hypothesis 11, likely associated with reducing conditions deeper in the sediments.

Low ORP and DO conditions have been shown to reduce the amount of microbial P uptake (McLatchey and Reddy 1998, Grace *et al.* 2008) and anaerobic conditions increase the solubility of P bound with Al and Fe (Reddy and DeLaune 2008, Wu *et al.* 2014). Both of these may result in release of P from stores in the benthos. While no significant losses of P from benthic stores were recorded in this study, the lack of increase in TP storage of floc and litter into 2014 could have potentially been partly related to the deterioration in DO and ORP concentration.

Vegetation changes

Typha height, culm density and biomass were expected to increase in this study (Miao and Sklar 1997, Rejmánková 2001, Kadlec and Wallace 2009, Macek *et al.* 2010) (Hypothesis 7, Table 2-7). Although *Typha* height increased, culm density, biomass, root-ball size and root

depth decreased. Such changes have all been associated with increased water depth over time (Chen *et al.* 2013, Chen and Vaughan 2014) and with reduced water quality in wastewater treatment (Kadlec and Wallace 2009). A key driver of decline is reduced oxygen availability for plant roots (Kadlec and Wallace 2009) and deeper water and wastewater addition both reduce oxygen availability, the latter through increased biological oxygen demand. The observed reduction in deep roots and increased number of surficial adventitious roots in the treatment wetland over this study support this being a key driver of *Typha* decline.

The frequency and duration of flooding is also an important consideration for plant survival as local populations may have previously experienced highly variable water levels, in situations where maximum water levels are only sustained for short periods (Boers *et al.* 2007). Prior to effluent discharge, Swamphen Lake was only inundated for short periods to a depth of approximately 0.5 m. Once releases commenced, depths in Swamphen Lake doubled and seasonal fluctuations were minimised. Depth change of this scale would contribute to *Typha* decline, but is unlikely to be the sole cause. *Typha orientalis* is considered a deep water species (Brix *et al.* 1992, Matsui and Tsuchiya 2006) and neighbouring Peninsula Lake sustained *Typha* beds in areas of water at least 1.5 m deep over the winter-spring period, decreasing to 0.5 m over the drier seasons. This suggests that, in addition to water depth change, it was the deterioration in water quality that drove *Typha* decline in Swamphen Lake. Following completion of the study, water depth in Swamphen Lake was reduced and maintained at 0.5 - 0.6 m. By June 2017, *Typha* was only present along the edges of the wetland where the water depth was ≤ 0.2 m, and was absent from the area formerly sampled, again suggesting that deteriorating water quality was the primary factor in *Typha* decline.

Sediment quality in the CWC wetlands has historically been variable but high concentrations of iron, manganese, aluminium and sulphur and at times high concentrations of other metals and contaminants have been recorded (GHD 2004, 2006, Capelli *et al.* 2007). Consequently, the decline in ORP and DO concentrations observed in Swamphen Lake would have increased the availability of toxic metals and also promoted formation of toxic hydrogen sulphide. Toxic metals and hydrogen sulphide are known to adversely impact on aquatic plants (Koch *et al.* 1990, Reddy and DeLaune 2008, Lamers *et al.* 2013) and as such would have contributed to the *Typha* decline as part of the overall deterioration of water quality. However, the concentrations of metals and hydrogen sulphide were not directly measured. Another potentially deleterious factor, ammonium toxicity (Britto and Kronzucker 2002) is unlikely to have contributed to *Typha* decline due to pre-existing high ammonium levels in the system prior to effluent discharge commencing. Toxicity relating to chlorination (Watkins and

Hammerschlag 1984) of incoming wastewater was also unlikely to have played a part due to the long transfer distance of the effluent after chlorination, significant aeration at discharge and mixing with the lake water body.

Typha is commonly used in treatment wetlands (Vymazal 2013) and adverse impacts on it are not often recorded. However, this study clearly illustrated that migration or loss of *Typha* beds may occur due to increased water depth, decreased water quality and/or synergistic effects of the two. Deterioration and loss of *Typha* equals more than just the loss of one species and its associated P storage capacity and cycling pathways. Rather, it represents the loss of a wide variety of functions associated with the emergent macrophyte zone, such as: stabilisation of sediment, reduced turbulence, physical filtration, surface area for microbial growth, oxygen input into sediments, production of organic matter, and provision of a range of habitats (in and above water, live and dead, litter layer) (Brix 1994, Rejmankova 2011, Gopal 2016, Gebrehiwot, Kifle, and Triest 2017).

Enrichment did not result in macrophyte growth in the bare sediment zone. The accumulation of floc would have made it difficult for rooted macrophytes to germinate and establish (Hilt *et al.* 2006, Paice *et al.* 2016) and high turbidity would have also limited light availability needed for their establishment (Scheffer *et al.* 1993, Hilt *et al.* 2006, Verhofstad, Alirangues*, et al.* 2017).

Differences between functional zones

Overall, water quality differed little between functional zones; the only significant difference was higher bottom %DO in *Typha* than in bare sediment zone. This could have been a reflection of differences in the biochemical oxygen demand of the floc and litter layers. The hypothesis that chlorophyll α and turbidity would be higher in the unvegetated than the vegetated zone (10, Table 2-7) was not supported as they were similar. This may have been due to free movement of water between the zones, resulting in mixing. This is one benefit of conducting whole-lake studies: natural connectivity is maintained between different parts of wetlands, so natural processes such as mixing occur and are included when interpreting results.

5.5 Conclusion

Over the first two and a half years of effluent discharge to Swamphen Lake, P storage increased in most ecosystem components, but P storage and cycling differed between

functional zones. In particular, soil uptake of P was only observed in the bare sediment zone, despite the known high P uptake capacity of the soil. The extent of soil P uptake may thus depend on the nature of the overlying layers (e.g. floc in bare sediment versus litter layer in the *Typha* zone) that determine the level of interaction between the nutrient rich water column and the soil. These results emphasise the importance of floc and litter as early P uptake pathways, however, they also suggest that these stores may potentially become P sources within a few years, especially if water quality shifts to highly reducing conditions. Overall, despite substantial P uptake by ecosystem components and equally large losses through groundwater seepage, TP concentration of water increased over the study period, the system became hypereutrophic, and algal blooms occurred in both zones by the last sampling occasion. The last sampling occasion was also characterised by high turbidity, low DO and low ORP close to the sediment surface. Increased water depth and decreased water quality, both commonly associated with wastewater discharge, adversely affected *Typha* resulting in decreases in biomass and TP storage rather than the increases generally expected with nutrient enrichment. The study thus demonstrates the potential risks with using wastewater to stimulate biological activity in created wetlands. It indicated that where nutrient load and changes to water depth are excessive, degradation can be rapid. Further research tracking P storage and cycling over the long-term would be valuable to provide more detail on the hypereutrophic end-stage, in particular to determine whether P-stores unresponsive here (subsoil and *Typha* zone topsoil) subsequently increased in P storage over longer time periods. Questions remain about the quantity of P that these unresponsive stores can assimilate, how TP storage by floc and litter respond in the long-term and whether *Typha* re-establishes over time in a different area of the wetland and becomes a substantial P-store.

CHAPTER 6: TRACKING WETLAND PHOSPHORUS STORAGE AND CYCLING: RAPID RESPONSES TO EARLY NUTRIENT ENRICHMENT IN *IN SITU* MESOCOSMS

6.1 Introduction

Changes in some phosphorus (P) storage and cycling processes can be detected in a matter of hours in controlled micro- and mesocosms (Richardson and Marshall 1986, Noe *et al.* 2003), while others can take months to several years to emerge, especially under low nutrient load (Gaiser *et al.* 2005). The seasonal and multiyear studies, which captured changes in functional zones across wetlands, could not identify P uptake and cycling processes at smaller spatial and temporal scales. This third study targeted this gap by investigating the impacts of intensive short-term nutrient enrichment on wetland P storage and cycling through a controlled *in situ* mesocosm experiment. It tested and refined conceptual models of P storage and cycling over nutrient enrichment continuum developed for the three functional zones present at the study wetland: 1) bare sediment open water zone (Figure 2-4), 2) submerged aquatic vegetation (SAV) zone with macroalgae *Chara* and *Nitella* spp. (Figure 2-5) and 3) emergent macrophyte *Typha orientalis* zone (Figure 2-6). This study focussed on the transition from the first (pre-enrichment stage) to the second (functional P uptake) stage of nutrient enrichment at small spatial and temporal scales. Research questions and hypotheses were those outlined in Table 2-7, together with an additional sub-question to provide another perspective and further differentiate between control and enriched mesocosms: Which ecosystem components account for most of the difference in TP storage between enriched and unenriched systems and how does this differ between functional zones?

Mesocosms are well established as means to identify processes in aquatic ecosystems (Grice and Reeve 1982, Odum 1984, Ahn and Mitsch 2002, Stewart *et al.* 2013, Sagarin *et al.* 2016) and are commonly used in nutrient enrichment experiments (e.g.Qualls and Richardson 2000, Romo *et al.* 2004, Liston *et al.* 2008, Butzler and Chase 2009, Ozkan *et al.* 2010, Davidson *et al.* 2015, Mitsch *et al.* 2015, Olsen *et al.* 2015, Ferriol *et al.* 2016). *In situ* mesocosms have the benefit of being more representative of natural conditions than *ex situ* containers. Mesocosms enable the creation of controlled and replicable units, unlike the whole wetland enrichment experiments described in Chapters 4 and 5. While they offer many benefits, such as realism and the ability to control variables, mesocosms also have weaknesses. For example, the smaller scale and enclosed nature of mesocosms gives them lower biological complexity, lower

spatial heterogeneity and limited connectivity between habitat types compared to whole wetland studies (Ahn and Mitsch 2002). The enclosed nature of mesocosms may also enhance differences in water quality and biological productivity between mesocosms when compared to whole wetlands (Ahn and Mitsch 2002). Care must therefore be taken when extrapolating mesocosm study results to larger, open wetland scales (Carpenter 1996, Schindler 1998, Ahn and Mitsch 2002). Ideally, studies combining these two scales (mesocosm and whole-wetland studies), provide a more robust estimate of wetland processes. This is the approach taken in the present study, where whole-wetland observations provided context for this mesocosm study.

Previous nutrient enrichment studies using mesocosms have commonly focused on one or few ecosystem components and associated cycling pathways (e.g. Qualls and Richardson 2000, Romo *et al.* 2004, Liston *et al.* 2008, Olsen *et al.* 2015). In contrast, this study aimed to provide a holistic view of P storage and cycling and by tracking changes in TP storage of all ecosystem components simultaneously. This approach provides a much better understanding of the context in which changes occur and should thus improve the transferability of results to other wetland ecosystems. This study aimed to capture changes in TP storage and cycling that occur more rapidly and would thus not have been detected in the other studies, by sampling at fortnightly to monthly intervals compared to the three and 12 month intervals of the other two studies (Chapters 4 and 5 respectively).

6.2 Methods

6.2.1 Experimental Design

The experiment was undertaken in Peninsula Lake (described in Section 3.2). It comprised 24 *in situ* mesocosms: four subject to nutrient enrichment and four control mesocosms in each of three functional zones (bare sediment, SAV and *Typha*). The presence of SAV in Peninsula Lake enabled its response to enrichment to be assessed, alongside bare sediment and *Typha* zones covered in the seasonal and multiyear studies. The experiment ran for 13 weeks (12 week enrichment period) during spring - early summer, when all the biological P stores were likely to be active and growing. It was terminated after 12 weeks of enrichment to minimise enclosure effects, as the main changes likely to be observed had already occurred (Ahn and Mitsch 2002). Nutrients were added eight times during the experiment, so the manipulation examined the effect of continuing enrichment (as opposed to a single dose of nutrients at the start of the experiment). Mesocosms were sampled twice before enrichment commenced

(baseline) and five times during enrichment (Figure 6-1). The design was therefore a standard MBACI design (multiple before-after, control-impact design, Downes 2002).

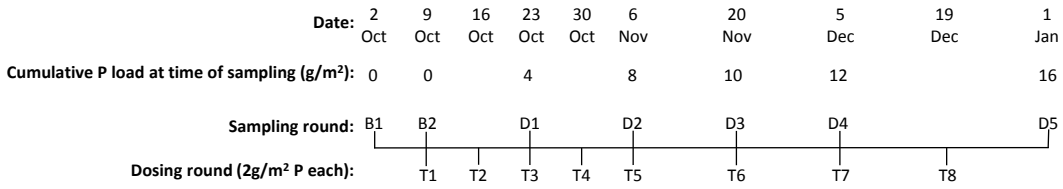

Figure 6-1: Timeline of sampling occasions (pre-enrichment B1 - B2; during treatment D1 - D5) and dosing (T1 - T8, 2 g/m^2 of P per time) for the mesocosm experiment at Peninsula Lake in 2014/2015.

To reduce variability, mesocosms were created at similar depth (x = 1.3 m at the start of the experiment) and enrichment and control mesocosms placed in pairs (Figure 3-7) randomly within the appropriate functional and depth zones. Bare sediment areas that initially contained some SAV (charophytes) were manually cleared prior to mesocosm construction.

The area (1m^2) and number (24) of mesocosms was determined on the basis of previous studies on nutrient enrichment (e.g. up to a few square metres in Chambers, Cale*, et al.* 1998, Havens *et al.* 2004, Romo *et al.* 2004, Collins 2005, Ozkan *et al.* 2010, Olsen *et al.* 2015, Ferriol *et al.* 2016) and the size of suitable area available in the study wetland. Mesocosms comprised steel posts (240 cm tall, with black bituminous coating) in each corner, a PVC pipe frame for support, with walls of clear plastic polyethylene sheeting (200 µm thick, heat-bonded to create water-tight seams) extending 0.2 m above the initial water level (Figure 6-2). Metal chain, slotted through a sealed fold in the bottom of the plastic was used to weigh down the walls and was pressed approximately 5 cm into the sediment to create a sealed enclosure, 13 days prior to baseline sampling. The integrity of the enclosures was inspected during each sampling occasion.

Ten polyethylene strips (3 cm x 50 cm) commonly used for periphyton sampling (e.g.Liboriussen and Jeppesen 2006, Lambert and Davy 2011, Tóth 2013), were weighted with galvanised steel nails and hung from a wire strung diagonally across each mesocosm. The strips were installed on 25 September 2014, one week prior to baseline sampling commencing. These strips allowed estimation of potentially significant periphyton growth on container walls (Chen *et al.* 1997) and associated P uptake.

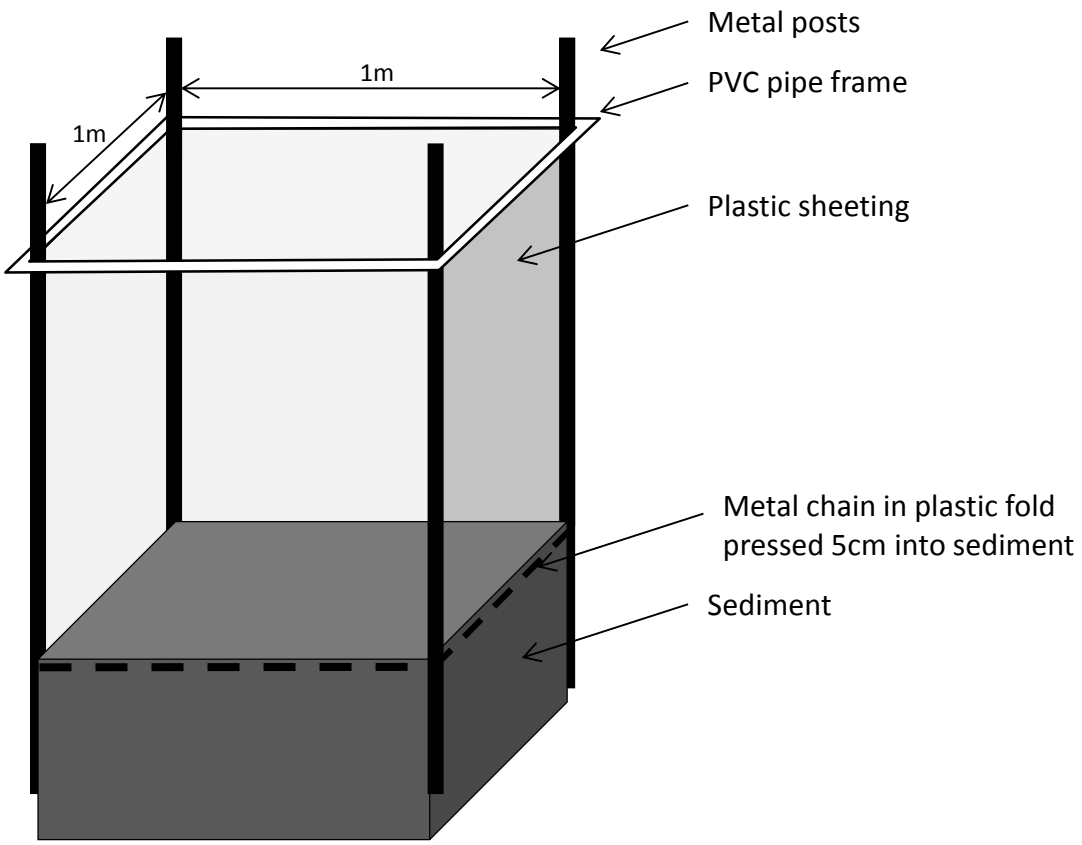

Figure 6-2: Mesocosm design

Gradual nutrient enrichment was simulated in enrichment mesocosms by pulse dosing (using a watering can for even distribution) with water soluble forms of P (K_2HPO_4) and nitrogen (NH_4NO_3), first weekly (five times) and then fortnightly (three times) following sampling (Figure 6-1). Dosing frequency was reduced during the experiment in an effort to delay the loss of SAV in the enrichment mesocosms.

Each dose of P equalled 2 g/m^2 accumulating to total load of 16 g/m^2 over the course of the experiment (12 weeks of enrichment). This equated to 19.8 mg/L at the end of the experiment when average depth was 0.8 m. Considering the short duration of the experiment, this P loading rate was higher than most experimental P loading rates quoted in available literature (e.g. 0.4 - 12.8 g/m^2/year in McCormick *et al.* (2001), Liston *et al.* (2008), Rejmánková *et al.* (2008)) but was chosen based on previous research at adjacent lakes which indicated P additions of up to 10 mg/L resulted in low water P concentrations due to rapid uptake by the sediment (Chambers and McComb 1996, Chambers, Cale*, et al.* 1998, Chambers, Fletcher*, et al.* 1998).

Nitrogen was added to enrichment mesocosms to avoid it becoming limiting and thereby affecting P uptake responses. The dose of nitrogen was ten times that of P (based on mass), accumulating to total load of 160 g/m^2 over the course of the experiment. With this N loading rate and P being readily taken up by the sediment, the N:P ratio in the water following the nutrient addition was expected to exceed the molecular Redfield ratio of 16:1 avoiding N limitation (Reddy and DeLaune 2008).

Water, topsoil, floc, litter and SAV were sampled for TP concentration and mass per unit area (or depth in the case of water) on each sampling occasion, to allow calculation of TP storage as g/m^2 (Table 6-1). Periphyton on the polyethylene strips was also sampled for TP concentration and mass during each round of enrichment sampling but not during baseline sampling due to negligible periphyton growth at this stage (Table 6-1). Subsoil and above-ground *Typha* were sampled only during the first and last sampling rounds due to the likely slow response to enrichment (Table 6-1). A pilot study had showed that it was not possible to extract representative samples of below-ground *Typha* biomass in the heavy clay soil. SAV mass was sampled using two methods: with a small corer (40 mm ID) as part of sediment sampling each round, and with large corer (90 mm ID) during the first and last sampling occasions (Table 6-1). The large corer method was expected to be more accurate but was more destructive and thus could be not be undertaken during each round. For the purpose of estimating total TP storage (B1 and D5 only), the large core mass was combined with the small core TP concentration.

Water quality variables (water FRP, temperature, pH, DO, ORP, EC, turbidity and chlorophyll α) were measured at each sampling round (Table 6-1). Water TN, NH_4 and NO_x were sampled during B1, D3 and D5 to check for nitrogen limitation. *Typha* height was sampled during first and last rounds as an indicator of enrichment (Table 6-1). In addition to the mesocosms, 12 in-lake sites located adjacent to every control mesocosm were sampled for water quality variables and three of them (the first in each functional zone) for TP concentration and store mass to estimate enclosure effects.

Table 6-1: Sampling schedule (ORP and pH not sampled B1 due to probe failure).

Store*	Variables	Zone	Sampling Round						
			B1	**B2**	**D1**	**D2**	**D3**	**D4**	**D5**
Water	Depth, temperature, pH, EC, DO, ORP, turbidity, chloro-*a*	All	x	x	x	x	x	x	x
Water	TP, FRP	All	x	x	x	x	x	x	x
Water	TN, NH4-N and NOx-N	All	x				x		x
Topsoil (0-20mm)	TP, weight	All	x	x	x	x	x	x	x
Subsoil (20-200mm)	TP, weight	All	x						x
Floc	TP, weight	SAV only	x	x	x	x	x	x	x
Litter	TP, weight	*Typha* only	x	x	x	x	x	x	x
SAV (method 1 - small core)	TP, weight	SAV only	x	x	x	x	x	x	x
SAV (method 2 - large core)	Weight	SAV only	x						x
Periphyton	TP, weight	All			x	x	x	x	x
Typha (above-ground; live and dead)	TP, weight	*Typha* only	x						x
Typha	Height, density	*Typha* only	x						x

* when present

6.2.2 Field sampling and laboratory analysis

On each sampling occasion, mesocosms were sampled over a period of two days, with all variables completed in one mesocosm prior to moving to the next. In-lake samples were taken immediately after sampling the adjacent control mesocosm. Field sampling followed methods described in Section 3.4. Samples were submitted to an accredited laboratory and analysed for nutrients, mass and other characteristics in accordance with methods described in Section 3.5.

6.2.3 Data analysis

Data processing followed methods described in Section 3.6. Total TP storage (and proportional distribution of total TP storage between ecosystem components) was calculated for the first and last sampling rounds only, which encompassed data from all ecosystem components. Two SAV mesocosms (one control and one treatment) on a single occasion (B1), recorded

extremely high TP concentrations, so these values were removed as outliers. Birds destroyed a large portion of the *Typha* culms in one pair of control and enrichment mesocosms; these were removed from the dataset prior to analysis, reducing the number of replicates for the *Typha* zone from four to three.

Water TP, FRP and chlorophyll α concentrations were compared against ANZECC (2000) guidelines and water TP and chlorophyll α concentrations against trophic status classification (Table 2-6, modified OECD 1982). A two factor ANOVA (treatment, fixed factor, 2 levels = enrichment, control; functional zone, fixed factor, 3 levels = bare sediment, SAV, *Typha*) was used (with SPSS version 22) to analyse all variables apart from topsoil and subsoil mass, from the first and last sampling rounds (B1 and D5 all variables), and the round preceding the change in dosing frequency (D2, for those variables where data was available). These sampling rounds were chosen because they were expected to best characterise changes over the enrichment continuum. When the zone factor was not applicable (e.g. litter not present in a zone), a single factor ANOVA (treatment, fixed factor, 2 levels = enrichment, control) was used. For three variables (water nitrogen concentration, oxidation-reduction potential, pH), data was analysed using the same two-factor ANOVA design but different rounds were used (nitrogen: D3 instead of D2; redox, pH: B2 instead of B1) due to data availability.

When the two-factor ANOVA identified a significant interaction between treatment and zone ($p<0.05$), simple effects tests were undertaken to further identify significant differences. Where interactions were disordinal, the main effects of treatment and zone were not interpreted, but only the simple effects tests. Where interactions were ordinal, both main effects and the interaction were interpreted. Post-hoc Tukey's tests were used for the zone variable unless there was significant disordinal interaction. As described in Chapter 4, assumptions of ANOVA were tested and transformations used when required, and when assumptions could not be met, alternative non-parametric tests were undertaken.

The effect of time (sampling round) was not tested for variables other than topsoil mass because the research questions were best answered by comparing differences between the treatment and control mesocosms. Furthermore, testing for the effect of time would have resulted in complex disordinal three-way interactions between time, treatment and zone that would have required a range of overlapping follow-up tests, which would have further increased the Family wise Type I error (Quinn and Keough 2002). Changes over time were described using graphs where relevant.

Topsoil mass was tested with repeated measures ANOVA (within-subjects factor: time with seven levels; between-subjects factors: treatment with two levels and zone with three levels). Repeated measures analyses identify whether temporal trends differ between factor levels, so it showed whether it would be appropriate to use constant mass (averaged over time) for the TP storage calculation (in order to minimise topsoil mass related variation in total TP storage). The assumption of normality was assessed with residual plots, the assumption of equal variances with Levene's test and the assumption of sphericity with Mauchly's test. No transformations were necessary.

Difficulties extracting subsoil samples resulted in variable sample depths. Consequently the sample weights were not comparable and no assessment of differences in mass between sampling occasions and zones could validly be made. Visual assessment of a scatter plot (Appendix 7) of sample depth versus TP concentration and Pearson correlation tests indicated that subsoil TP concentration increased with depth during B1 but not during D5 (Appendix 7). However, sample depth did not differ significantly between treatments or functional zones either during B1 or D5 (based on two-factor ANOVA: treatment, fixed factor, 2 levels = enrichment, control; functional zone, fixed factor, 3 levels = bare sediment, SAV, *Typha*) and consequently should not have had significant influence on differences in subsoil TP concentration between treatments and functional zones. For the purposes of deriving subsoil TP storage, constant subsoil mass was used (119.68 ± 3.88 kg/m^2, n = 28), calculated using only those samples reaching 200 mm depth, including samples collected in Peninsula Lake for other parts of the thesis between October 2012 and July 2013.

Principal components analysis (PCA, with Euclidean distance) on ecosystem components was used (Primer version 6 by PRIMER-E Ltd) to illustrate differences in TP storage between enriched (D5 enrichment mesocosms) and unenriched (all B1 mesocosms and D5 control mesocosms) mesocosms for each functional zone. PCA was used to explore the enclosure effect by illustrating differences between control mesocosms and adjacent in-lake sites (for rounds B2-D5 for each functional zone separately) based on water quality variables and depth. All data were normalised prior to undertaking PCA. The enclosure effect was also explored by comparing TP storage data from control mesocosms and in-lake sites based on presence/absence of overlap in the recorded range of values.

6.3 Results

6.3.1 Enclosure effect

Temporal change (seasonal changes of decreasing water depth and increasing temperature and EC) explained a larger proportion of the variation in the datasets than did sample type (controls and in-lake sites) in any of the functional zones (Figure 6-3, Figure 6-4, Figure 6-5). Together, these three variables (depth, temperature, EC) explained between 30-45% of the variation captured by PC1 and PC2 in all zones. In the bare sediment and *Typha* zones, there were no consistent differences between control mesocosms and in-lake sites. In contrast, in the SAV zone, the control mesocosms generally had higher bottom water pH ($\bar{x}$ = 9.01 vs 7.33), higher %DO ($\bar{x}$ = 143% vs 97%) and lower ORP ($\bar{x}$ = 168 mV vs 210 mV) than the in-lake sites. Comparison of TP concentration and store mass data between control mesocosms and in-lake sites showed no consistent differences indicative of an enclosure effect (Table 6-2).

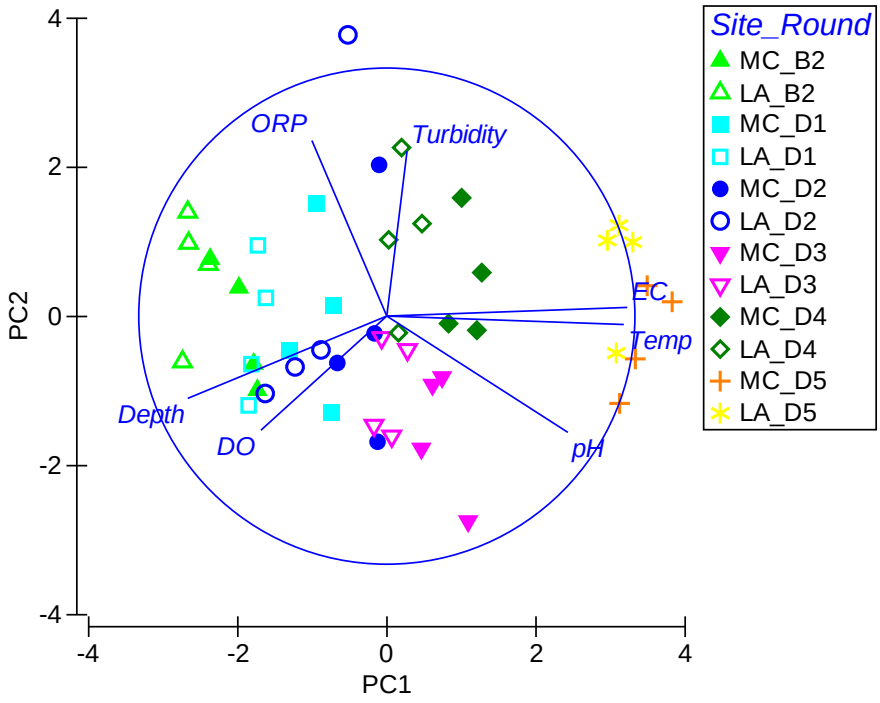

Figure 6-3: PCA ordination plot showing variation in water quality variables of control mesocosm (MC) and in-lake sites (LA) of the bare sediment zone during pre-enrichment (B2) and enrichment (D1-D5) sampling rounds. PC1 explained 48.39% of the variation and PC2 21.2% of the variation in the data.

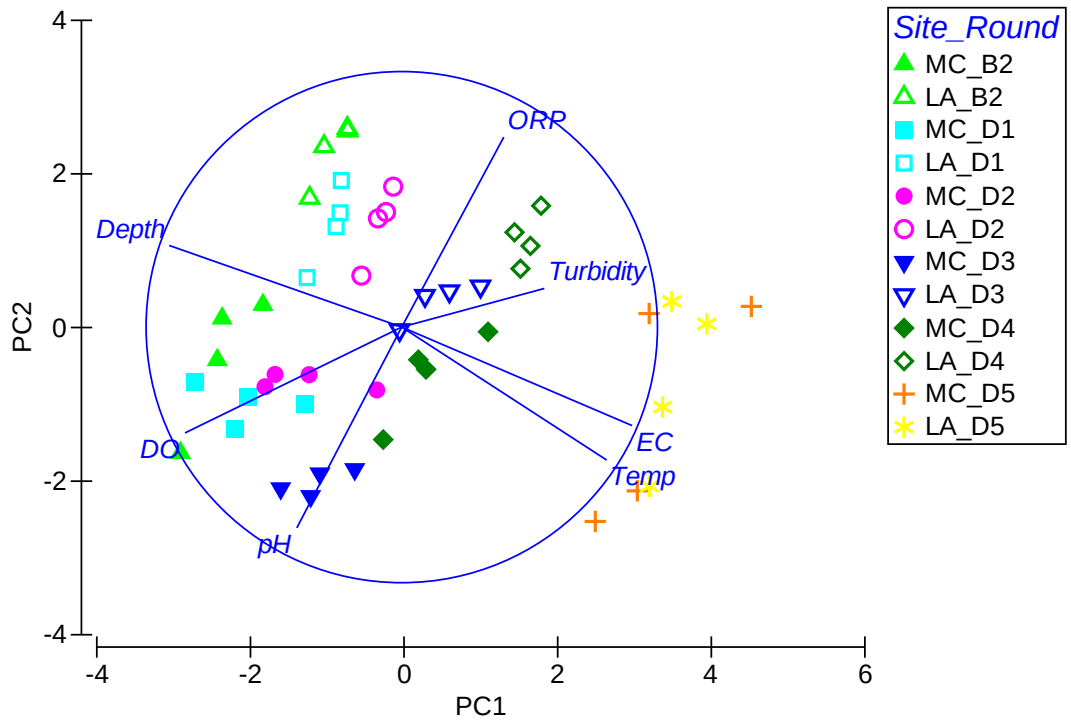

Figure 6-4: PCA ordination plot showing variation in water quality variables of control mesocosm (MC) and in-lake sites (LA) of the SAV zone during pre-enrichment (B2) and enrichment (D1-D5) sampling rounds. PC1 explained 52.0% of the variation and PC2 26.9% of the variation in the data.

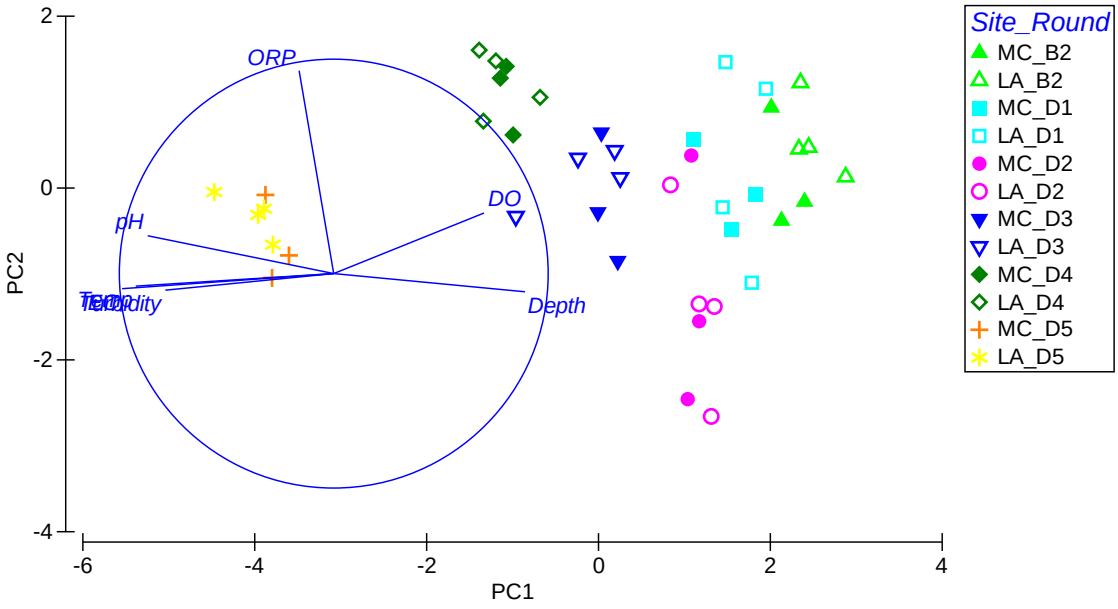

Figure 6-5: PCA ordination plot showing variation in water quality variables of control mesocosm (MC) and in-lake sites (LA) of the *Typha* zone during pre-enrichment (B2) and enrichment (D1-D5) sampling rounds. PC1 explained 64.4% of the variation and PC2 14.7% of the variation in the data.

Table 6-2: Comparison of TP concentration and store mass data between control mesocosms (4 per each zone) and in-lake sites (1 per each zone). The comparison determined whether the value of each variable in the in-lake site was within the range of values of the respective control mesocosms. When it was not, the values are provided in format: 'in-lake value (range of values in control mesocosms)'. IR = in range, ND = no data and NA = not applicable (when variable not present in the zone).

Variable	Bare sediment			SAV			*Typha*		
	B1	**D2**	**D5**	**B1**	**D2**	**D5**	**B1**	**D2**	**D5**
Water TP concentration (µg/L)	IR	7 (<5-6)	15 (9-10)	IR	IR	IR	IR	IR	IR
Periphyton	Not sampled in lake.								
Topsoil TP concentration (mg/g)	IR	IR	0.20 (0.21-0.37)	0.21 (0.25-0.44)	IR	IR	IR	IR	0.21 (0.26-0.40)
Subsoil TP concentration (mg/g)	IR	ND	0.15 (0.19-0.29)	0.13 (0.17-0.34)	ND	IR	IR	ND	IR
Floc	No floc in lake or control mesocosms								
Litter TP concentration (mg/g)	NA	NA	NA	NA	NA	NA	0.45 (0.49-0.99)	IR	ND
Litter mass (g/m^2)	NA	NA	NA	NA	NA	NA	IR	IR	ND
SAV TP concentration (mg/g)	NA	NA	NA	IR	IR	IR	NA	NA	NA
SAV mass (g/m^2)	NA	NA	NA	IR	IR	138.1 (171.0-677.4)	NA	NA	NA
Live *Typha* TP concentration (mg/g)	NA	NA	NA	NA	NA	NA	NA	ND	IR
Dead *Typha* TP concentration (mg/g)	NA	NA	NA	NA	NA	NA	NA	ND	IR
Live *Typha* mass (g/m^2)	NA	NA	NA	NA	NA	NA	NA	ND	IR
Dead *Typha* mass (g/m^2)	NA	NA	NA	NA	NA	NA	NA	ND	IR
Total *Typha* mass (g/m^2)	NA	NA	NA	NA	NA	NA	NA	ND	IR

6.3.2 Phosphorus storage of ecosystem components

How does TP storage of ecosystem components change with enrichment and how does this differ between functional zones?

Prior to enrichment, TP storage, TP concentration and mass (depth for water) of ecosystem components did not differ between enrichment and control mesocosms (Figure 6-6 to Figure 6-13, Table 6-4 to Table 6-6), but differences emerged once enrichment started (Figure 6-6 to Figure 6-13).

Water

Rapid, step-wise increases in TP storage and concentration of water occurred after enrichment commenced (supporting Hypotheses 1 and 3, Table 2-7) (Figure 6-6a,b), with increases (between B2 and D1) over 50 times higher in the bare sediment and SAV zones than in *Typha*. TP storage and concentration in the bare sediment zone peaked during the first enrichment sampling round (D1, 1.58 g/m^2, 1375 µg/L) and in SAV during the second enrichment sampling round (D2, 3.00 g/m^2, 2450 µg/L) but then gradually declined to 130 µg/L and 115 µg/L respectively, as the dosing frequency decreased, despite increase in cumulative P load. TP storage and concentration remained higher in the enrichment than control mesocosms until the end of the experiment (Figure 6-6a,b, Table 6-4, Table 6-5). Water FRP concentration responded similarly except for a complete lack of treatment response in the *Typha* zone, where FRP remained below the detection limit of 2 µg/L (Figure 6-6d). Approximately 77% and 71% of the peak water TP concentration was FRP in bare sediment (D1) and SAV (D2) zones respectively, compared to 3% and 7% during the last sampling round.

The results partly support Hypothesis 4 (Table 2-7) that water TP concentration would be lower in vegetated (*Typha* and SAV) than unvegetated (bare sediment) zones during enrichment. While water TP and FRP concentrations were lower in the *Typha* zone (below ANZECC TP and FRP trigger values - 60 and 30 µg/L respectively) than in bare sediment and SAV zones, there were no differences between bare sediment and SAV zones (both exceeding ANZECC trigger values) (Figure 6-6b,d, Table 6-5).

Water depth showed a gradual seasonal decrease (of approximately 0.45 m) in all mesocosms (Figure 6-6c) from 1.17 - 1.35 m at the start of the experiment to 0.73-0.89 m at the end (Figure 6-6c). Natural variation in the bottom topography resulted in mesocosms in the SAV zone being by approximately 0.16 m deeper than the bare sediment zone, whereas *Typha* zone

depth did not differ from other zones (Table 6-6). Water TP storage strongly reflected water TP concentration rather than changes in depth. While TP storage of water was higher in enrichment than control mesocosms, only an increasingly small portion of the cumulative P load was retained in the water column (Table 6-3), particularly in the *Typha* zone, inferring that other ecosystem components removed most of the added P from water within the 1 - 2 week period between dosing and the subsequent sampling.

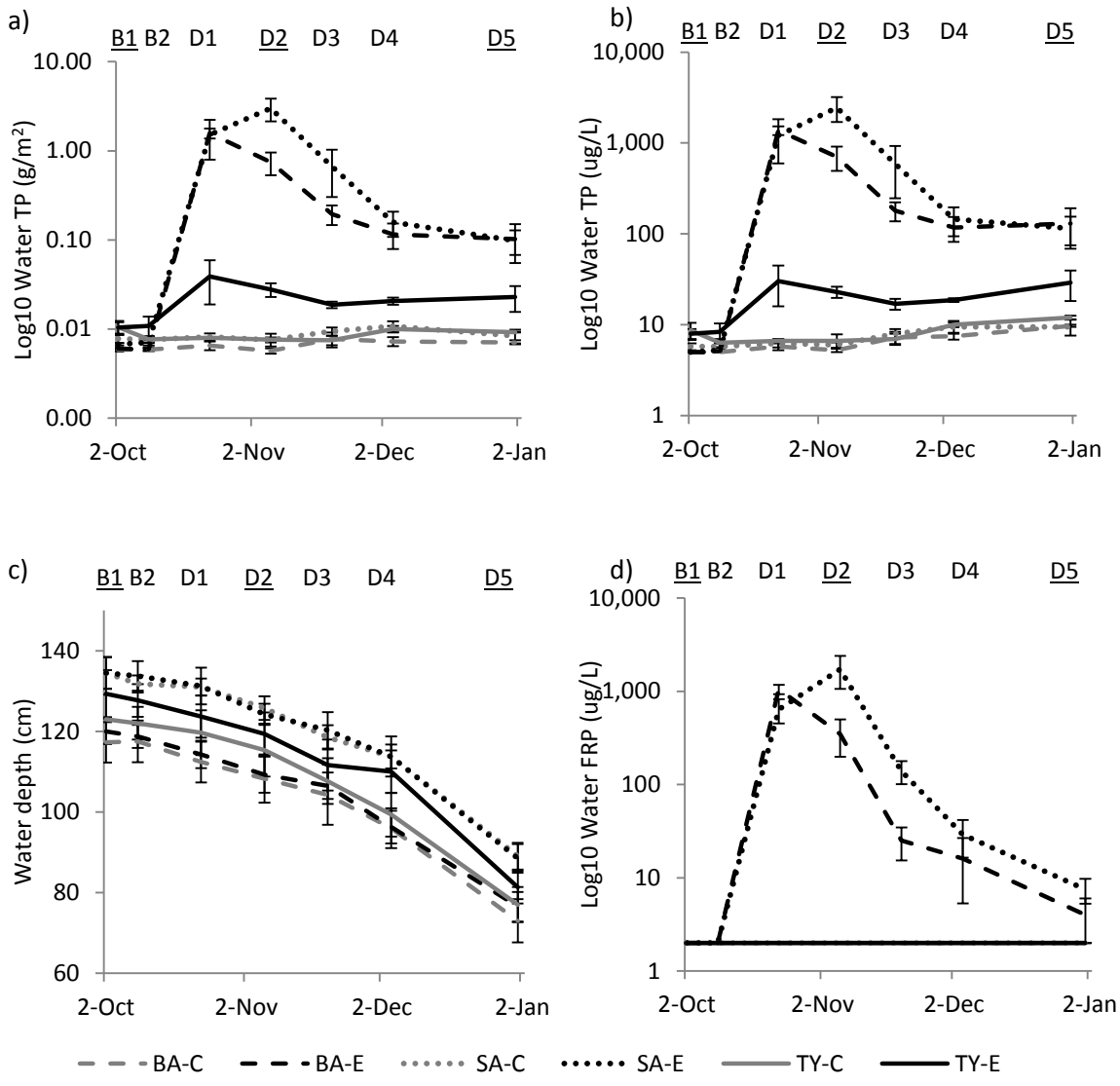

Figure 6-6: Water TP storage (a), TP concentration (b), depth (c) and FRP concentration (d) (±1 standard error) in the bare sediment (BA), SAV (SA) and *Typha* (TY) zones (n = 4 in BA and SAV, n = 3 in TY per treatment). E = enrichment, C = control mesocosms.

Table 6-3: Proportion of phosphorus load remaining in the water store in bare sediment, SAV and *Typha* zones across sampling occasions.

Round	Cumulative load (g/m^2)	Portion of load remaining in water store (%)		
		Bare sediment	SAV	*Typha*
D1	4.00	39.5%	37.9%	1.0%
D2	8.00	9.3%	37.5%	0.3%
D3	10.00	1.9%	6.7%	0.2%
D4	12.00	1.0%	1.3%	0.2%
D5	16.00	0.6%	0.6%	0.1%

Periphyton

While no baseline data for periphyton TP storage (concentration or mass) was available, the large difference in TP concentration between enrichment and control mesocosms during D1 suggests that a rapid step-wise increase had occurred in response to enrichment (supporting Hypothesis 1, Table 2-7). Periphyton was one of the early P uptake pathways (Figure 6-7, Table 6-4, Table 6-5). Periphyton TP storage was significantly higher in enrichment than control mesocosms both at D2 and D5 (supporting Hypothesis 3, Table 2-7), however there were also differences between functional zones (Table 6-4). Periphyton in the SAV zone took up more P than in bare sediment zone, and had minimal uptake in *Typha* zone.

TP concentration of periphyton in all enrichment mesocosms decreased (Figure 6-7b), as the dosing frequency decreased, despite increasing cumulative P load. In contrast, periphyton mass gradually increased over time in most mesocosms (enrichment and control, Figure 6-7c), but remained stable in the *Typha* enrichment mesocosms. Only in the SAV zone was periphyton mass was significantly higher in enrichment than control mesocosms (Table 6-6) and the difference was not consistent over time (i.e. significant at D2 but not D5, Figure 6-7c; partly supporting Hypothesis 2, Table 2-7).

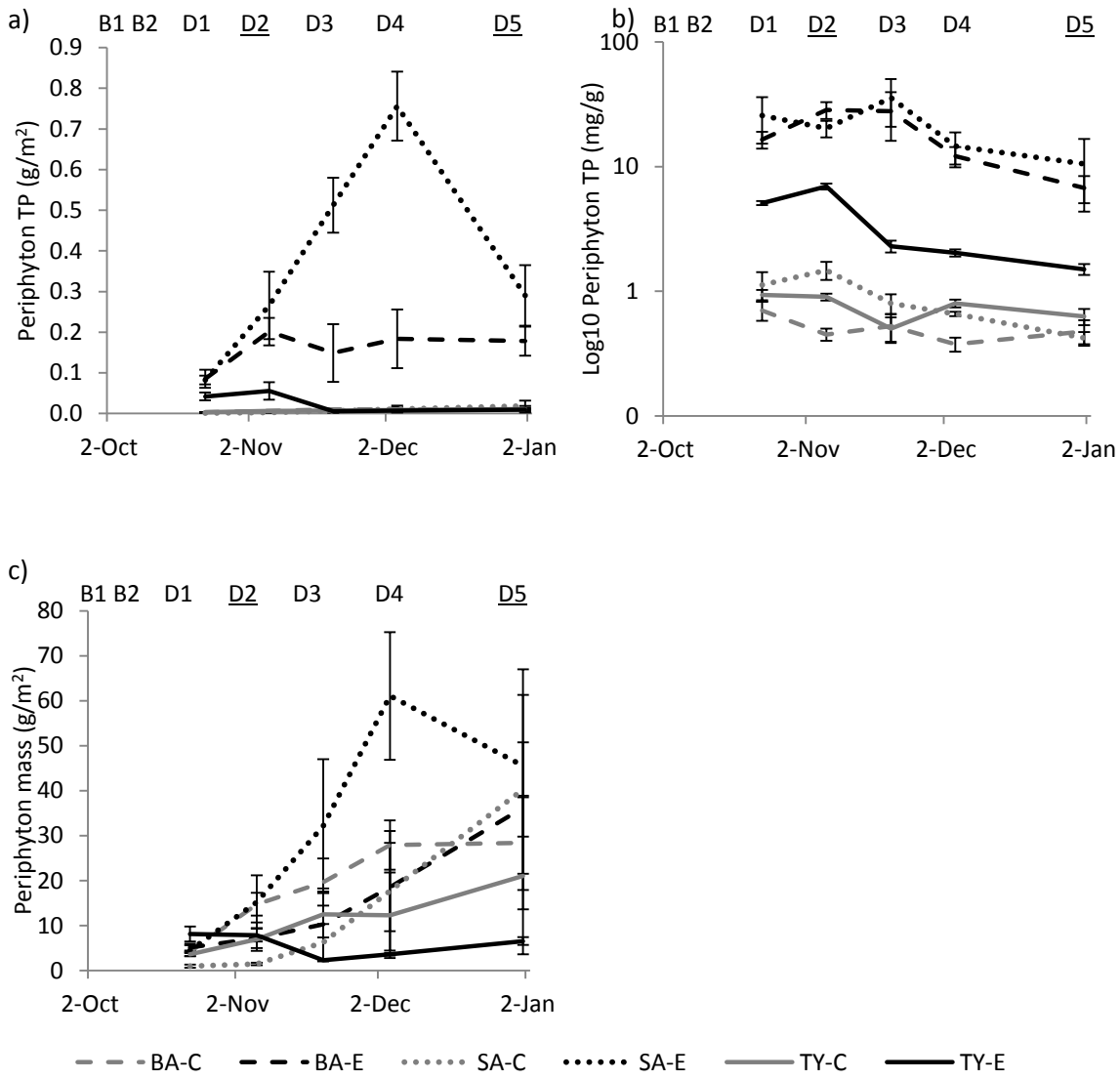

Figure 6-7: Periphyton TP storage (a), TP concentration (b) and mass (c) (±1 standard error) in the bare sediment (BA), SAV (SA) and *Typha* (TY) zones (n = 4 in BA and SAV, n = 3 in TY per treatment). E = enrichment, C = control mesocosms.

SAV

SAV was another store that responded rapidly to enrichment, with large, step-wise increases in TP storage and concentration (supporting Hypotheses 1 and 3, Table 2-7; Figure 6-8a,b). However, SAV mass did not increase (in contrast to Hypothesis 2, Table 2-7) and was gradually lost in the enrichment mesocosms so that by D4, no SAV was left (supporting Hypothesis 8, Table 2-7). In contrast, the control mesocosms continued to sustain SAV throughout the study (Figure 6-8c). Sampling with the large corer (B1 and D5 only) resulted in higher SAV mass estimates per unit area than with the small corer (Figure 6-8c,d).

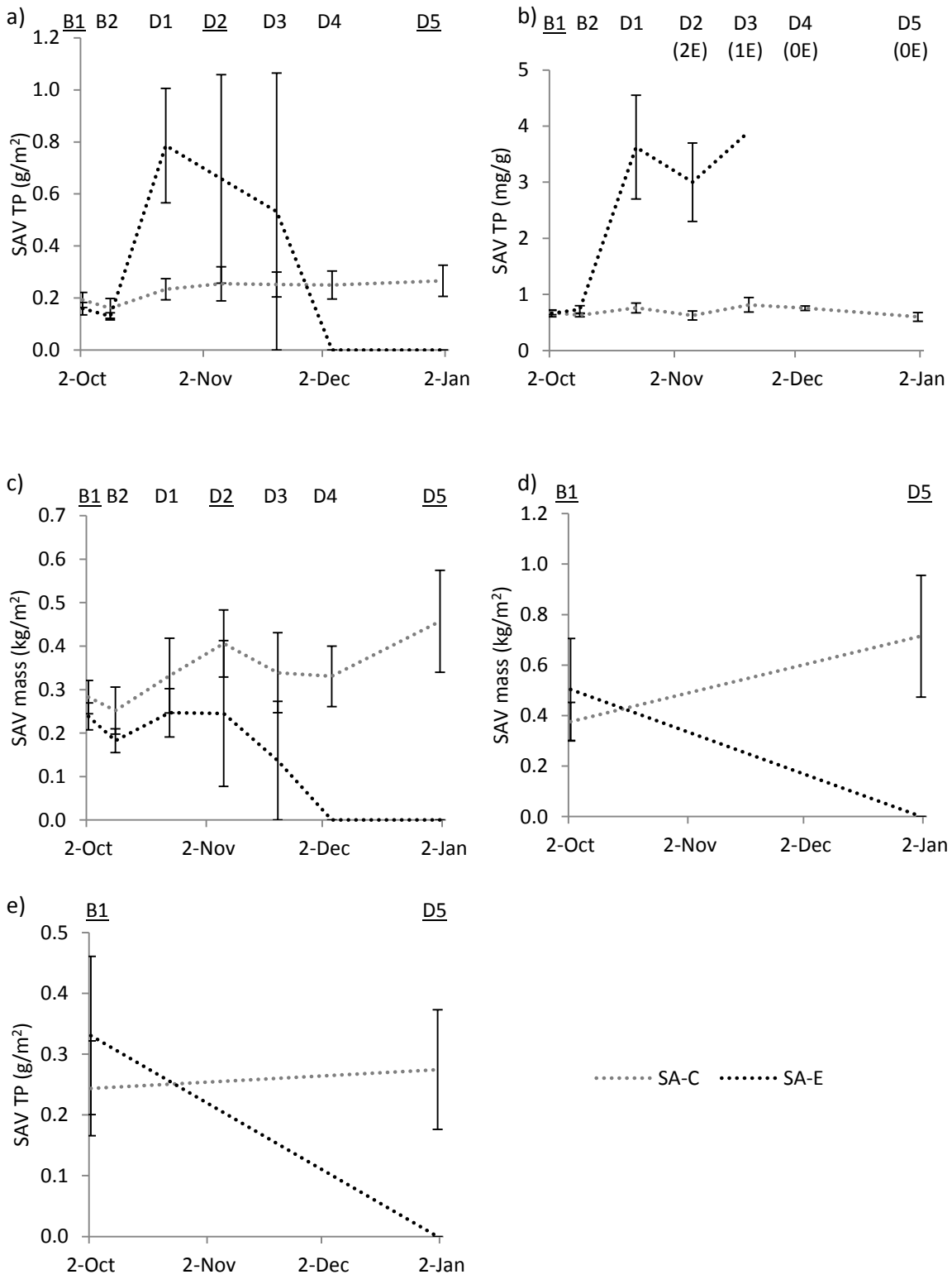

Figure 6-8: SAV TP storage (a), TP concentration (b) and mass (c) for small corer, SAV mass for large corer (d) and SAV TP storage with small corer TP concentration and larger corer mass (e) (±1 standard error) in the SAV (SA) zone (n = 4 per treatment unless otherwise indicated in brackets under sampling round). E = enrichment, C = control mesocosms.

Benthic stores: floc

Floc was only present in a few SAV enrichment mesocosms and thus statistical comparison against control mesocosms could not be made. However, average TP concentration and floc mass (and derived TP storage) increased over time in enrichment mesocosms (supporting Hypotheses 1 and 2, Table 2-7) whereas floc was never observed in control mesocosms (Figure 6-9).

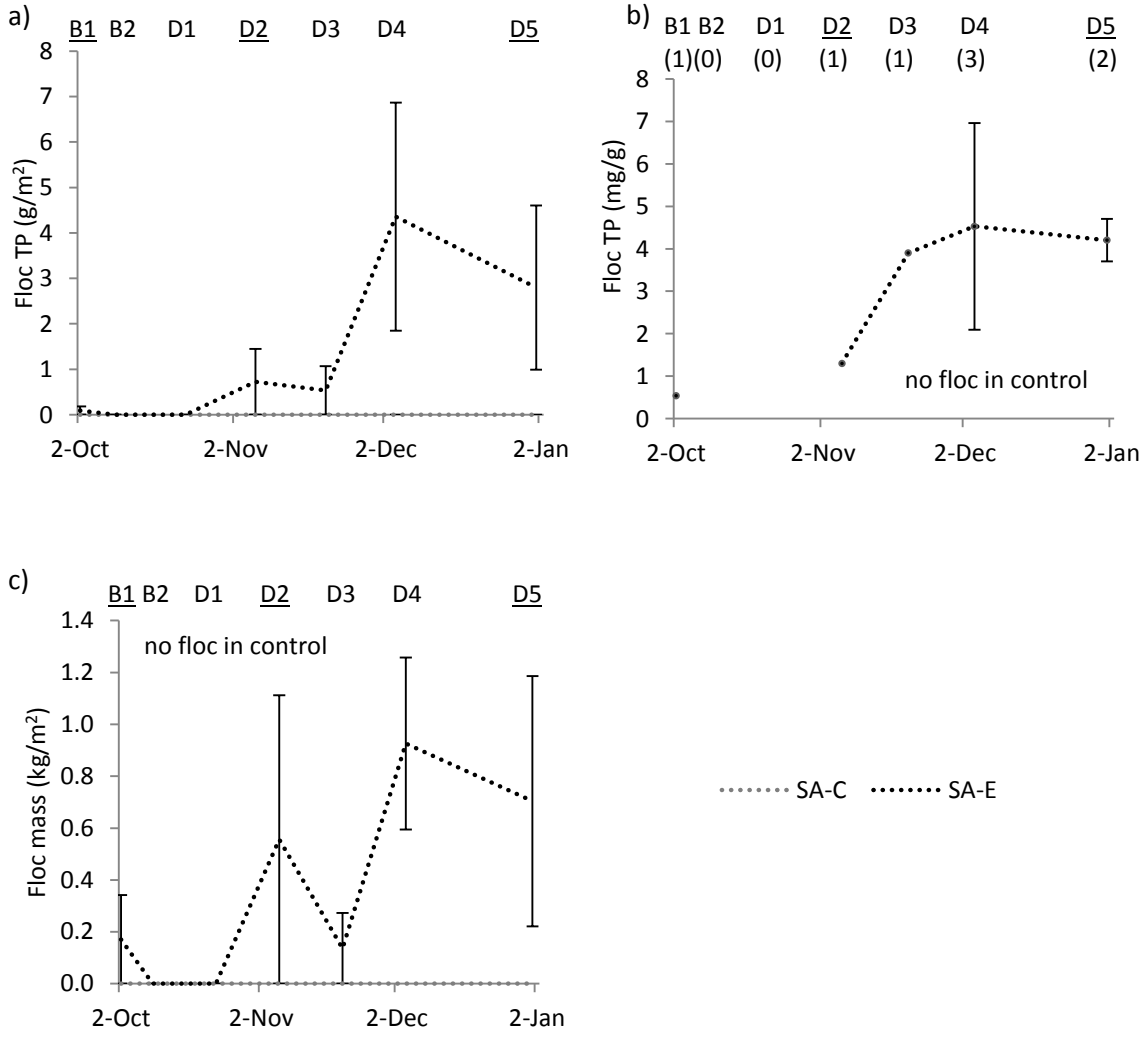

Figure 6-9: Floc TP storage (a), TP concentration (b) and mass (c) (±1 standard error) in the SAV (SA) zone (n = 4 per treatment unless otherwise indicated in brackets under sampling round). E = enrichment, C = control mesocosms.

Benthic stores: litter in the Typha zone

TP storage and concentration of litter increased gradually in response to enrichment (supporting Hypotheses 1 and 3, Table 2-7; Figure 6-10a). Significant difference in litter TP storage (and concentration) between enrichment and control mesocosms was only recorded at the end of the experiment in D5 (not D2) (Figure 6-10, Table 6-4, Table 6-5). Litter TP concentration continued to increase even when water TP concentration was declining. Litter mass had high variation and remained similar between enrichment and control mesocosms until the last sampling round when it was significantly higher in enrichment than control mesocosms (supporting Hypothesis 2, Table 2-7; Figure 6-10c, Table 6-6).

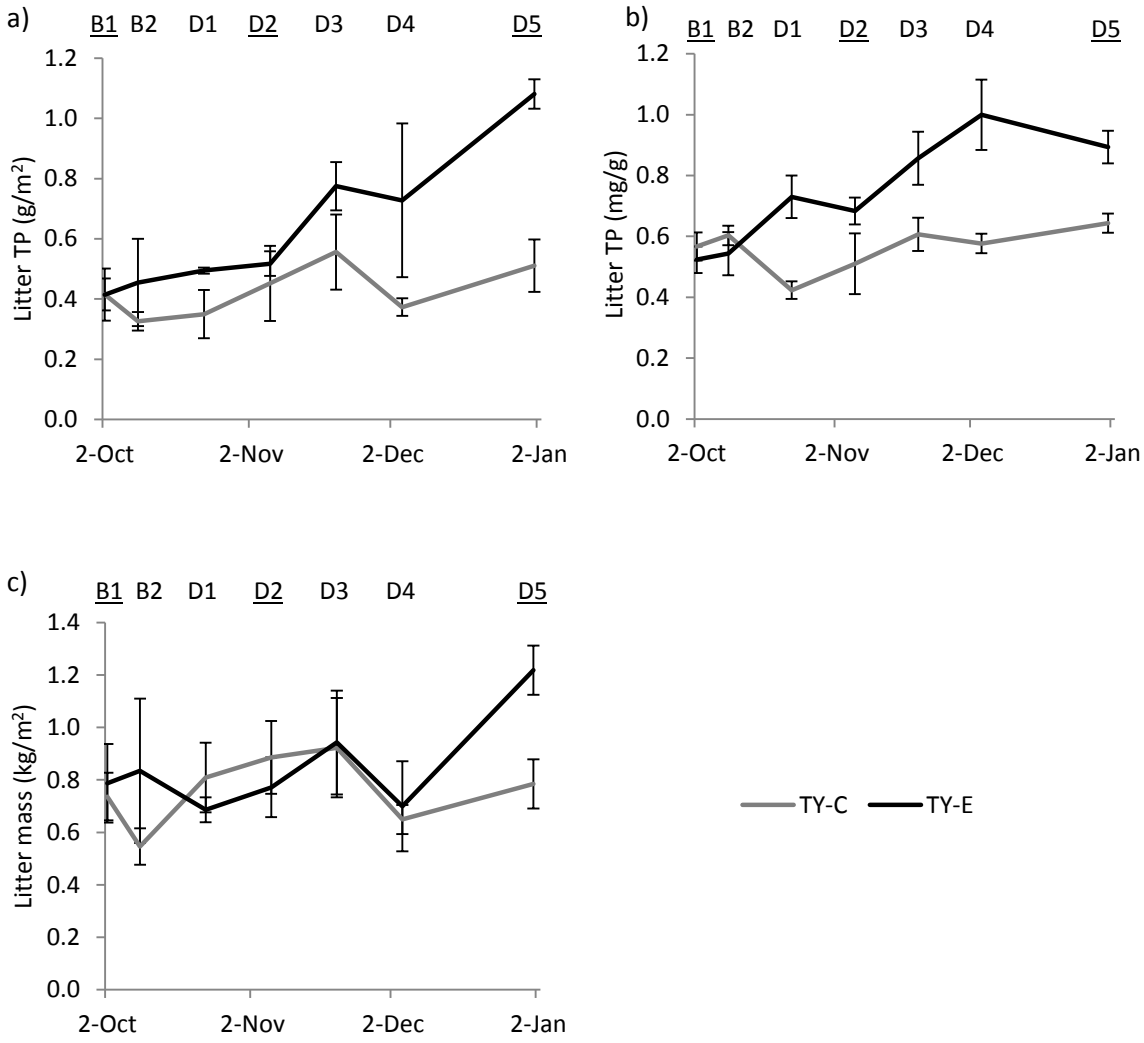

Figure 6-10: Litter TP storage (a), TP concentration (b) and mass (c) (±1 standard error) in the *Typha* (TY) zone (n = 3 per treatment). E = enrichment, C = control mesocosms.

Benthic stores: topsoil

TP storage and concentration of topsoil increased gradually in response to enrichment (supporting Hypothesis 1 and 3, Table 2-7; Figure 6-11a, b). Both were significantly higher in enrichment than control mesocosms in D2 and D5 (Table 6-4, Table 6-5). Topsoil TP concentration continued to increase even when water TP concentration was declining. As with water and periphyton stores, topsoil TP storage and concentration were much higher in bare sediment and SAV zones than in the *Typha* zone. Analysis of topsoil mass found no treatment effect but sampling round (time) and zone had significant effects (Figure 6-11c, Table 6-7). The sampling time effect was due to different temporal trends in each treatment combination (treatment x zone), however these changes were variable and overlapped one another (Figure 6-11c), so no pattern was apparent. As topsoil mass was significantly higher in the bare sediment zone than the other zones (Figure 6-11d), mean topsoil mass for each zone was used in further analyses to calculate topsoil TP storage (bare sediment 13.89 ± 0.42 kg/m^2, n = 56; SAV 11.89 ± 0.42 kg/m^2, n = 56; *Typha* 12.61 ± 0.48 kg/m^2, n = 42). As topsoil mass within each zone remained constant over time, topsoil TP storage directly reflected topsoil TP concentration for each zone.

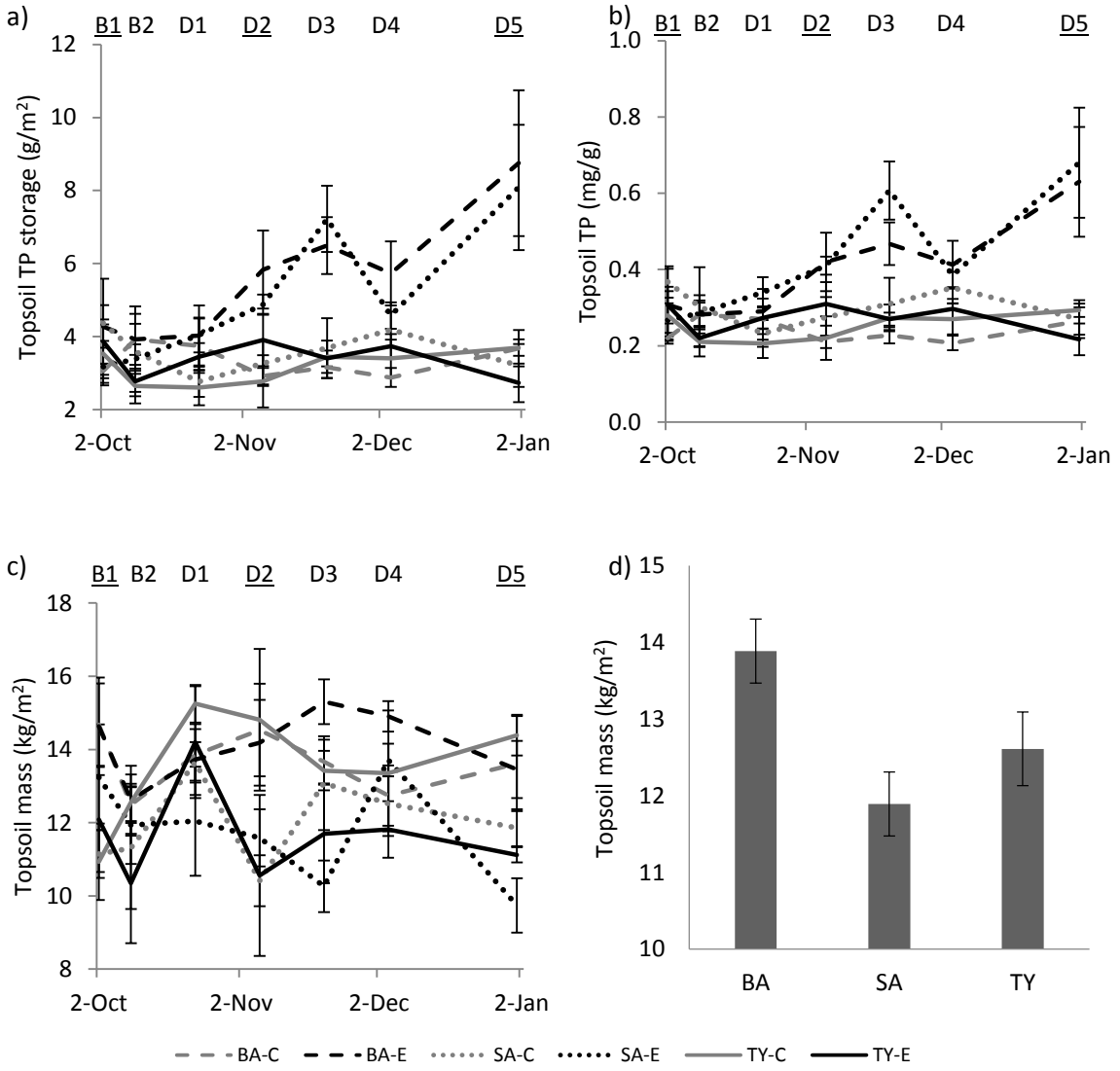

Figure 6-11: Topsoil TP storage (a), TP concentration (b) and mass (c) and constant mass for TP storage calculation (d) (±1 standard error) in the bare sediment (BA), SAV (SA) and *Typha* (TY) zones (for a-c: n = 4 in BA and SAV, n = 3 in TY per treatment; for d: n = 56 in BA and SAV, and 42 in TY). E = enrichment, C = control mesocosms. Constant mass (d) based on least-squares means and standard error from ANOVA.

Benthic stores: subsoil

Subsoil mass was kept constant (mass 155.10 ± 1.91 kg/m^2, n = 26) for the calculation of subsoil TP storage (due to sampling issues described in Section 6.2.3), which therefore directly reflected changes in TP concentration. There were no significant differences in subsoil TP storage (or concentration) between enrichment and control mesocosms or between zones (in contrast to Hypotheses 1 and 3, Table 2-7; Figure 6-12, Table 6-5).

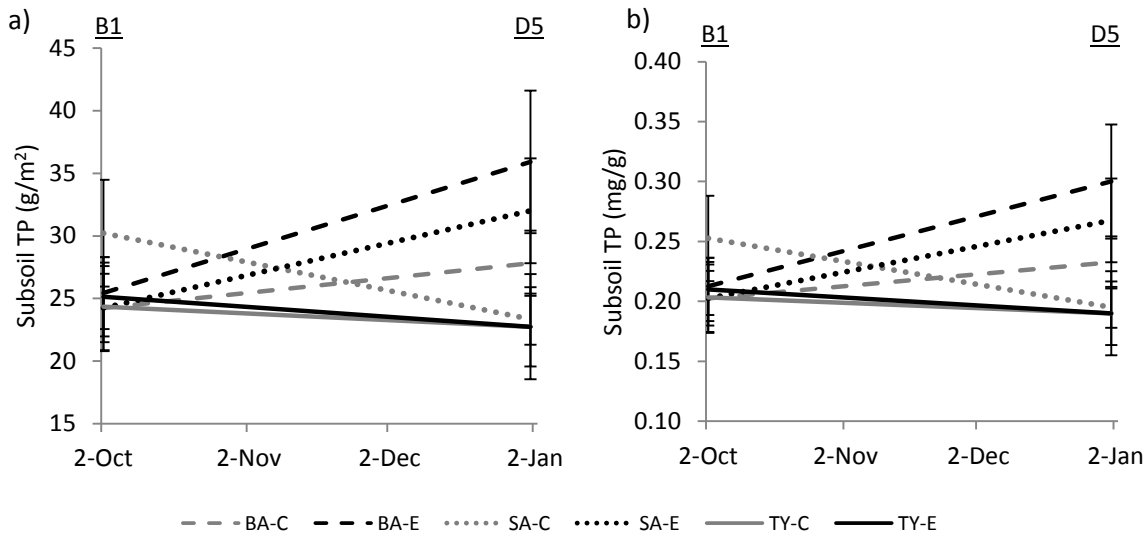

Figure 6-12: Subsoil TP storage (a) and TP concentration (b) (±1 standard error) in the bare sediment (BA), SAV (SA) and *Typha* (TY) zones (n = 4 in BA and SAV, n = 3 in TY per treatment). E = enrichment, C = control mesocosms.

Typha

Above-ground *Typha* showed little evidence of treatment effect (Figure 6-13). While TP concentration of dead *Typha* was significantly higher in enrichment than control mesocosms during D5 (supporting Hypothesis 1, Table 2-7; Figure 6-13b, Table 6-5), no such difference was found in live *Typha* TP concentration, *Typha* mass (live or dead) or the derived TP storage (in contrast to Hypotheses 1, 2 and 3, Table 2-7; Figure 6-13a-c, Table 6-4, Table 6-5, Table 6-6).

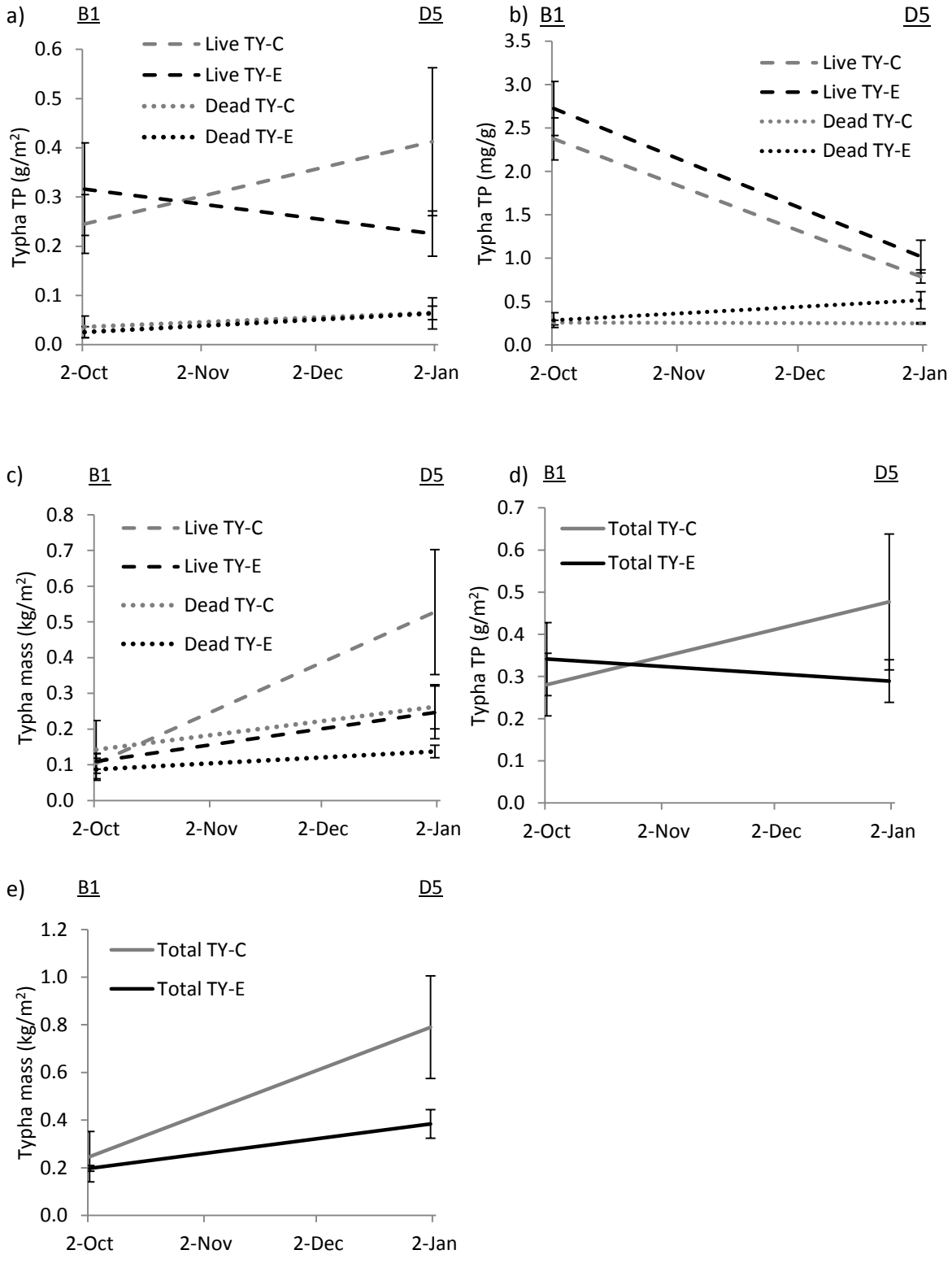

Figure 6-13: Live and dead *Typha* TP storage (a), TP concentration (b) and mass (c) and Total *Typha* TP storage (d) and mass (e) (±1 standard error) in the *Typha* (TY) zone (n = 3 per treatment). E = enrichment, C = control mesocosms.

Total TP storage

Total TP storage (excluding subsoil) of all mesocosms was similar at B1 (Figure 6-14, Table 6-4). The experimental P load (16 g/m^2) added to the enrichment mesocosms was nearly four times this initial storage. By the end of the experiment (D5), total TP storage was significantly higher in enrichment than control mesocosms in bare sediment and SAV zones, but not in *Typha* which did not change (Figure 6-14, Table 6-4). Total TP storage was significantly lower in the *Typha* zone compared with bare sediment and SAV zones (Figure 6-14, Table 6-4).

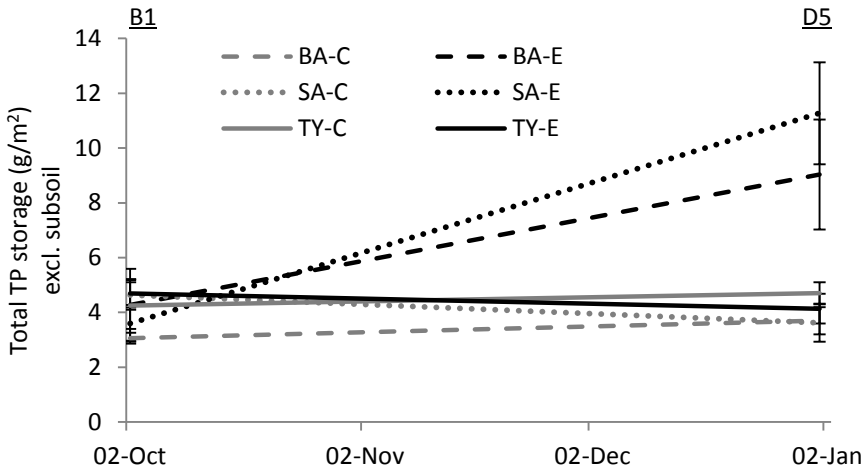

Figure 6-14: Mean total TP storage (±1 standard error) in bare sediment (BA), SAV (SA) and *Typha* (TY) mesocosms excluding subsoil (n = 4 for BA and SA, n = 3 for TY per treatment) during first (B1, pre-enrichment) and last (D5, end of enrichment) sampling rounds.
E = enrichment and C = control.

Table 6-4: Differences in TP storage of ecosystem components between treatments and zones. Abbreviations include BA = bare sediment, SA = SAV, TY = *Typha*, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, do = disordinal, # = non-parametric test. Parentheses indicate a significant disordinal interaction between treatment and zone. Further details in Appendix 8.

Ecosystem component	Round	Treatment	Zone		Treatment * Zone		
		Initial test	Initial test	Post hoc	Initial test	Simple effects - Treatment	Simple effects -Zone
Water	B1	ns	**	BA/SA ns, BA/TY ***, SA/TY **	ns		
	D2	(***)	(***)		*** do	BA***, SA***, TY **	Control ns, Enrichment BA/SA **, BA/TY ***, SA/TY ***
	D5	***	ns		ns		
Periphyton	B1	no data	no data		no data		
	D2	***	ns		ns		
	D5	(***)	ns		** do	BA**, SA***, TY ns	Control ns, Enrichment BA/SA *, BA/TY **, SA/TY ***
Topsoil	B1	ns	ns		ns		
	D2	**	ns		ns		
	D5	(**)	(*)		** do	BA**, SA**, TY ns	Control ns, Enrichment BA/SA ns, BA/TY ***, SA/TY ***
Subsoil	B1	ns	ns		ns		
	D5	ns	ns		ns		
Litter	B1	ns					
	D2	ns					
	D5	**					
SAV (large core)	B1	ns					
	D5#	*					
Typha Live	B1	ns					
	D5	ns					
Typha Dead	B1	ns					
	D5	ns					
Typha Total	B1	ns					
	D5	ns					
Total TP	B1	ns	ns		ns		
	D5	(**)	(ns)		* do	BA **, SA **, TY ns	Control ns, Treatment BA/SA ns, BA/TY *, SA/TY **

Table 6-5: Differences in TP concentration of ecosystem components between treatments and zones. Abbreviations include BA = bare sediment, SA = SAV, TY = *Typha*, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, do = disordinal, # = non-parametric test. Parentheses indicate a significant disordinal interaction between treatment and zone. Further details in Appendix 8.

Ecosystem component	Round	Treatment	Zone		Treatment * Zone		
		Initial test	Initial test	Post hoc	Initial test	Simple effects - Treatment	Simple effects - Zone
Water	B1#	ns	**	BA/SA ns, BA/TY **, SA/TY *	none#		
	D2	(***)	(***)		*** do	BA ***, SA ***, TY *	Control ns Enrichment BA/SA **, BA/TY ***, SA/TY ***
	D5	***	ns		ns		
Periphyton	B1	no data	no data		no data		
	D2	(***)	(***)		*** do	BA ***, SA ***, TY ***	Control BA/SA***, BA/TY **, SA/TY * Enrichment BA/SA ns, BA/TY ***, SA/TY ***
	D5	(***)	(ns)		** do	BA ***, SA ***, TY ns	Control ns Enrichment BA/SA ns, BA/TY **, SA/TY **
Topsoil	B1	ns	ns		ns		
	D2	**	ns		ns		
	D5	(**)	(*)		** do	BA **, SA **, TY ns	Control ns Enrichment BA/SA ns, BA/TY **, SA/TY **
Subsoil	B1	ns	ns		ns		
	D5	ns	ns		ns		
Litter	B1	ns					
	D2	ns					
	D5	*					
SAV (small core)	B1	ns					
	D2	na					
	D5	na					
Typha Live	B1	ns					
	D5	ns					
Typha Dead	B1	ns					
	D5#	*					

Table 6-6: Differences in mass of ecosystem components between treatments and zones. Abbreviations include BA = bare sediment, SA = SAV, TY = *Typha*, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, do = disordinal, # = non-parametric test. Parentheses indicate a significant disordinal interaction between treatment and zone. Further details in Appendix 8.

Ecosystem component	Round	Treatment	Zone		Treatment * Zone		
		Initial test	Initial test	Post hoc	Initial test	Simple effects - Treatment	Simple effects - Zone
Water (depth)	B1	ns	**	BA/SA**, BA/TY ns, SA/TY ns	ns		
	D2	ns	**	BA/SA**, BA/TY ns, SA/TY ns	ns		
	D5	ns	**	BA/SA**, BA/TY ns, SA/TY ns	ns		
Periphyton	B1	no data	no data		no data		
	D2	(ns)	(ns)		* do	BA ns, SA **, TY ns	Control BA/SA**, BA/TY ns, SA/TY ns Enrichment ns
	D5	ns	ns		ns		
Litter	B1	ns					
	D2	ns					
	D5	*					
SAV (small core)	B1	ns					
	D2	ns					
	D5[#]	*					
SAV (large core)	B1	ns					
	D5[#]	*					
Typha Live	B1	ns					
	D5	ns					
Typha Dead	B1	ns					
	D5	ns					
Typha Total	B1	ns					
	D5	ns					

Table 6-7: Differences in topsoil mass between sampling rounds, treatments and zones. Abbreviations include BA = bare sediment, SA = SAV, TY = *Typha*, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus. Further details in Appendix 8.

Variable	**Time**	**Treatment**	**Zone**		**Factor interactions**
	Initial test	**Initial test**	**Initial test**	**Post hoc**	**Initial test**
Topsoil	*	ns	*	BA/SA*, BA/TY ns, SA/TY ns	**ns**

Which ecosystem components account for most of the difference in TP storage between enriched and unenriched systems and how does this differ between functional zones?

The PCA of TP storage in the bare sediment zone (Figure 6-15) clearly separated enriched mesocosms (at the end of the experiment: D5) from both the unenriched (control) mesocosms (at D5) and from all mesocosms prior to enrichment (B1). The first two principal components explained most of the variation in the dataset (89.0%). The pre-enrichment (B1) mesocosms and D5 control mesocosms were tightly clustered showing that their patterns of TP storage were similar. In contrast, the D5 enriched mesocosms were widely spread across the ordination plot, indicating variability in storage patterns between the mesocosms along both axes. Separation along PC1 was associated with TP storage in water, topsoil, and subsoil and along PC2 spread was mainly associated with TP storage in periphyton.

In the SAV zone, the PCA again explained most of the variation in the data (82.3% by the first two principal components). Once again, all pre-enrichment (B1) and D5 control mesocosms were clustered showing that their patterns of TP storage were similar, whereas the D5 enriched mesocosms were widely spread across the ordination plot (Figure 6-16). Most of the variation in D5 enriched mesocosms was explained by PC1, associated with TP storage in water, periphyton and floc. One D5 enriched mesocosm differed because it had higher TP storage in topsoil and subsoil. Importantly, it was again clear that enrichment not only increased the total amount of TP stored, but also increased the variation in storage patterns between the mesocosms.

In the *Typha* zone, the ordination plot explained a lower (but still satisfactory) proportion of the total variation in the dataset (64.0% by the first two principal components). The D5 enriched mesocosms were still clearly separated from the pre-enrichment (B1) and D5 control mesocosms: two along PC1, associated mostly with TP storage in topsoil, subsoil and litter, whereas the third was separated primarily along PC2 associated with TP storage in water. Variance among mesocosms was higher in the *Typha* zone (Figure 6-17) than the other zones (Figure 6-15, Figure 6-16).

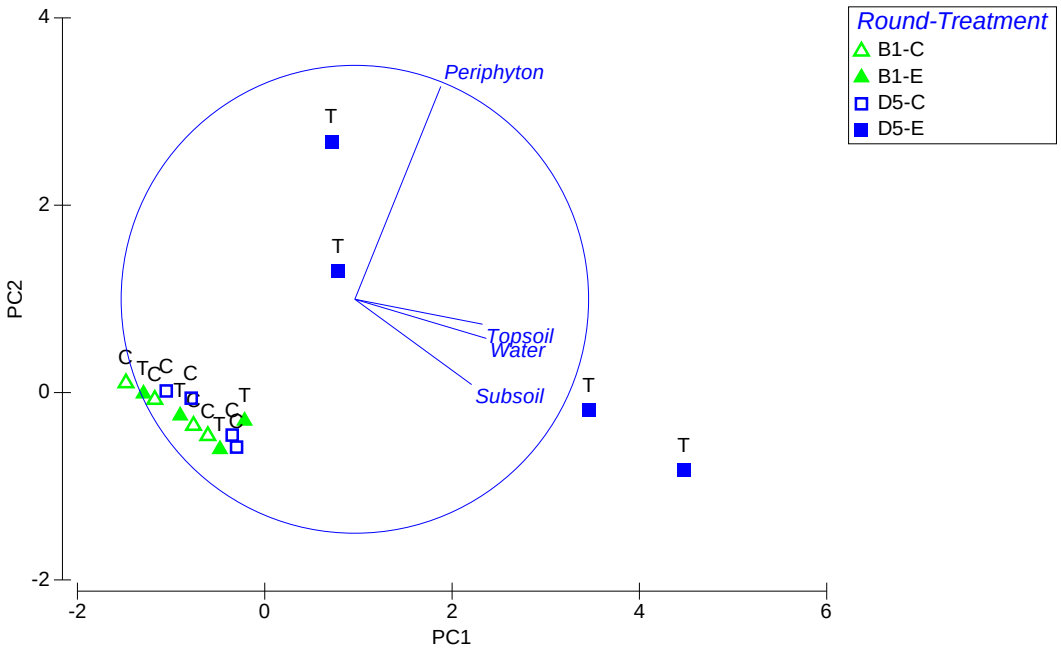

Figure 6-15: PCA ordination plot of TP storage data for bare sediment zone showing variations in TP storage of different ecosystem components with combinations of sampling round (B1 or D5) and treatment (E = enrichment, C = control). PC1 explained 70.7% of the variation and PC2 18.3% of the variation in the data.

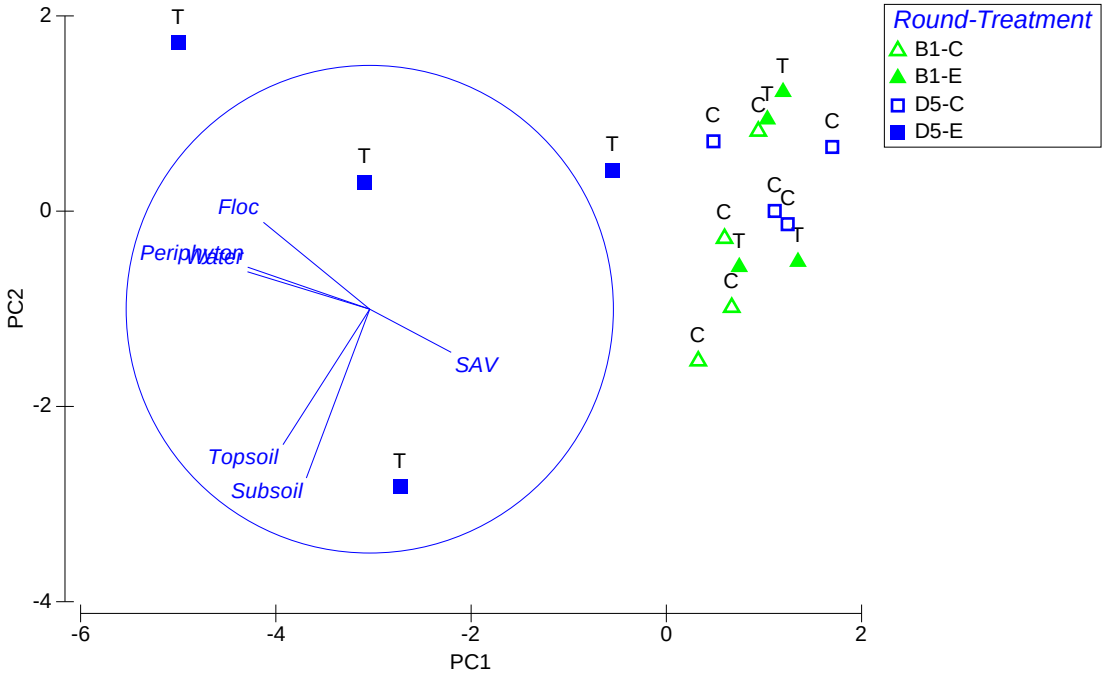

Figure 6-16: PCA ordination plot of TP storage data for SAV zone showing variations in TP storage of different ecosystem components with combinations of sampling round (B1 or D5) and treatment (E = enrichment, C = control). PC1 explained 61.0% of the variation and PC2 21.3% of the variation in the data.

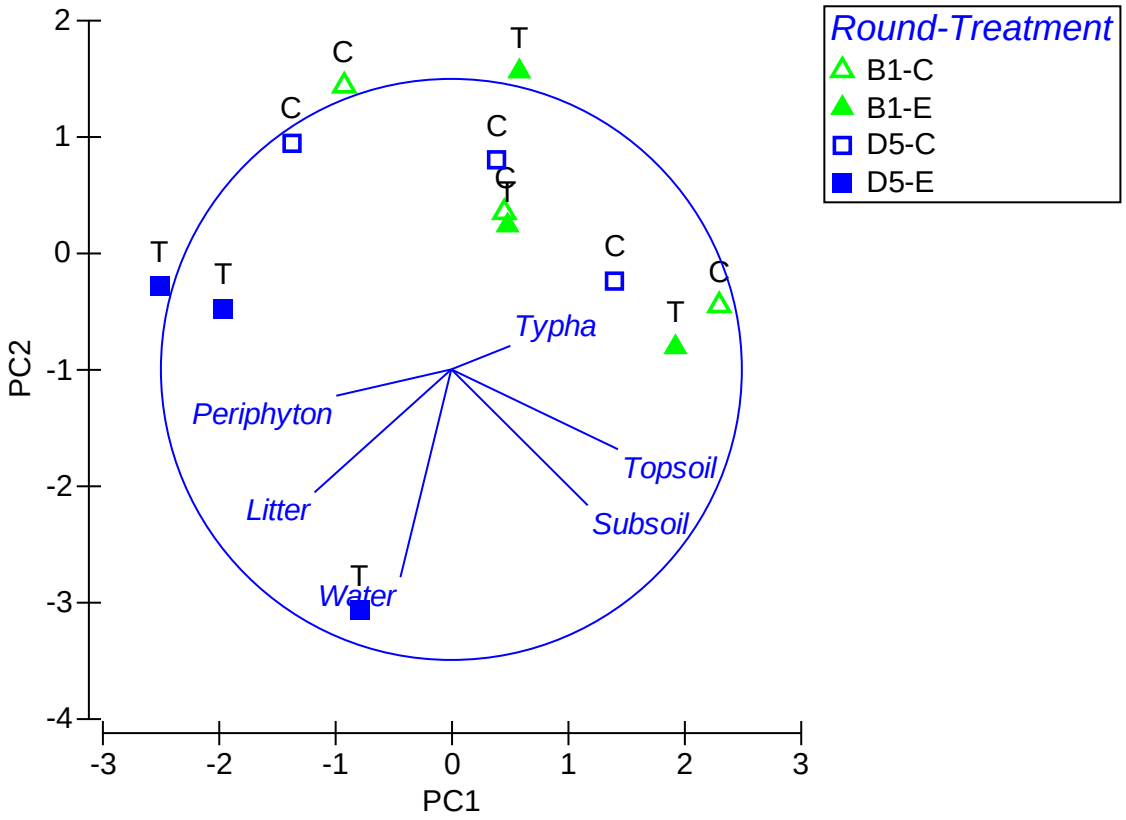

Figure 6-17: PCA ordination plot of TP storage data for *Typha* zone showing variations in TP storage of different ecosystem components with combinations of sampling round (B1 or D5) and treatment (E = enrichment, C = control).). N.b. – n = 3 per treatment in the *Typha* zone due to the loss of one pair of mesocosms from the design. PC1 explained 38.4% of the variation and PC2 25.5% of the variation in the data.

6.3.3 Proportional phosphorus storage

How does the distribution of total TP storage across the ecosystem components (% of total) change with enrichment and how does this differ between functional zones?

Subsoil was the largest store of P in all treatments and functional zones both at the beginning (B1) and at the end (D5) of the experiment. Subsoil was not included in the total (or proportional) TP storage calculations as it was much larger than the other stores (7-9 times the combined TP storage of all other stores) and had relatively high variability (± 1 S.E. up to 5.7 g/m^2, compared to combined TP storage of all other stores around 4 g/m^2) that hid meaningful changes in TP storage in both it and other components.

Excluding subsoil, topsoil was the largest store of P across treatments and zones, both at the start (B1) and the end (D5) (Figure 6-18). Prior to enrichment, nearly 100% of TP in the bare sediment zone was stored in topsoil, with slightly smaller share in the SAV zone (≈ 90%) and

smaller again in the *Typha* zone (≈ 70%). Enrichment reduced the share of total TP stored in topsoil (supporting Hypothesis 5, Table 2-7), particularly in the SAV and *Typha* zones where there were alternative potential stores. In the SAV zone, SAV was the second largest store in all mesocosms before enrichment (B1) and in all control mesocosms at the end of the experiment (D5). The loss of SAV in the enrichment mesocosms resulted in floc becoming the second largest store by D5. In the *Typha* zone, litter was the second largest store in all mesocosms both before enrichment and at the end of the experiment. However, the percentage share of litter was higher in enrichment than control mesocosms by the end of the experiment. *Typha* was the third largest store both before enrichment and at the end of the experiment with a stable percentage share.

Water was the smallest TP store (mostly < 1%) across all treatments and zones, both at the start (B1) and the end (D5). Periphyton (on plastic strips and walls) held more P than water at the end of the experiment, with percentage shares up to 3% in enrichment mesocosms (Figure 6-18). In the enrichment mesocosms, the percentage share of periphyton was higher in bare sediment and SAV zones (≈ 2.5 - 2.9%) than in *Typha* (≈ 0.5%).

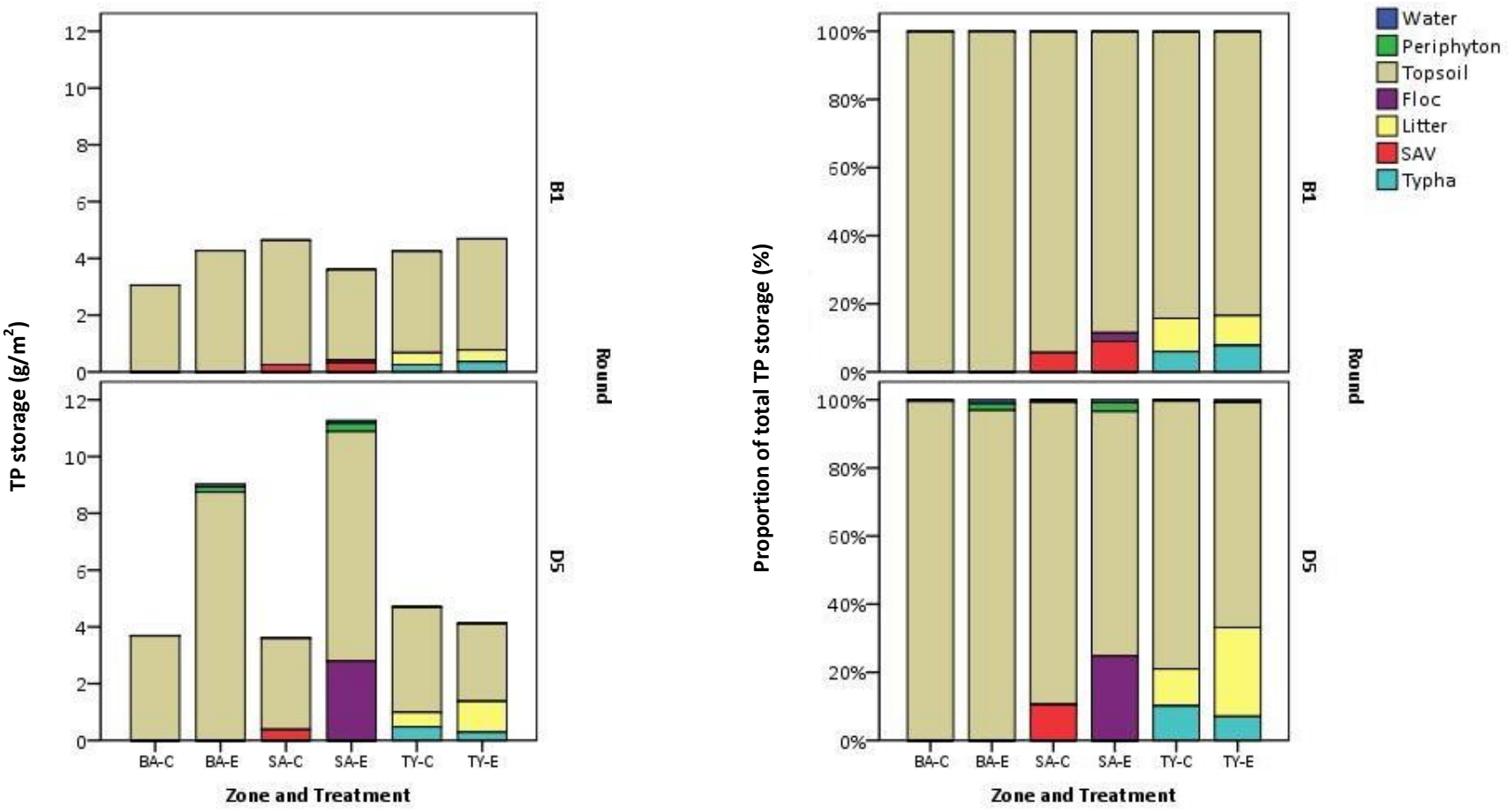

Figure 6-18: Breakdown of total TP storage between ecosystem components in g/m2 (a) and as percentage of total (b) for bare sediment (BA), SAV (SA) and *Typha* (TY) zone mesocosms (n=4 for BA and SA, n=3 for TY per treatment) during the first (B1, pre-enrichment) and last (D5, last enrichment) sampling rounds. E = enrichment and C = control.

6.3.4 Phosphorus mass balance

Where does the added P go?

Bare sediment and SAV zones captured majority of the P added to enrichment mesocosms (16 g/m^2), with most of it accumulating in subsoil and topsoil (supporting Hypothesis 6, Table 2-7) as well as in floc (SAV zone only) (Table 6-8). However, significant differences in TP storage were not evident between enrichment and control mesocosms for subsoil and floc. Little accumulation of P in litter and water occurred in the *Typha* zone (in contrast to Hypothesis 6, Table 2-7). Differences in TP storage of control mesocosms occurred between the first and last sampling rounds as well but these were smaller than in the enrichment mesocosms and within error margins (± S.E.).

While bare sediment and SAV zones included all the key P stores, sampling in the *Typha* zone did not include below-ground *Typha* or thatch-type dead *Typha* that had separated from the standing dead *Typha* (thus not captured by sampling of dead *Typha*) but remained suspended between *Typha* culms (thus not captured by sampling of benthic litter). On the basis of photos taken during sampling, thatch mass was potentially higher than litter mass (based on litter layer being 0.5 - 2.5 cm thick). The photos also showed that thatch in the enrichment mesocosms started supporting extensive periphyton growth during enrichment (Figure 6-19) while no such growth was observed in the control mesocosms. More periphyton was observed growing on the thatch than on the plastic periphyton strips.

Table 6-8: Average difference in TP storage of ecosystem components between last enrichment (D5) and pre-enrichment (B1) sampling rounds (± 1 standard error in brackets) for each functional zone (bare sediment, SAV, *Typha*) and treatment (control and enrichment). When significant treatment effect in TP storage (difference between control and enrichment) was found in D5 (Table 6-4), the enrichment value is shaded. For periphyton presumes B1 = 0. The enrichment mesocosms received 16 g/m^2 of P between B1 and D5.

	Difference in TP storage between first and last sampling rounds (g/m^2)					
Variable	**Bare sediment**		**SAV**		***Typha***	
	Control	**Enrichment**	**Control**	**Enrichment**	**Control**	**Enrichment**
Water	0.0012 (±0.0002)	**0.0963** (±0.0474)	0.0006 (±0.0020)	**0.0911** (±0.0302)	-0.0013 (±0.0020)	**0.0125** (±0.0067)
Periphyton	0.0105 (±0.0035)	**0.1781** (±0.0358)	0.0182 (±0.0134)	**0.2906** (±0.0747)	0.0106 (±0.0081)	0.0097 (±0.0012)
Topsoil	0.62 (±0.67)	**4.48** (±1.20)	-1.16 (±0.61)	**4.90** (±2.10)	0.13 (±0.91)	-1.18 (±0.90)
Floc	na	na	na	2.70 (1.85)	na	na
Litter	na	na	na	na	0.10 (±0.13)	**0.67** (±0.14)
SAV	na	na	0.119 (±0.153)	-0.32 (±0.12)	na	na
Typha	na	na	na	na	0.22 (0.12)	-0.07 (0.13)
Total (excl. subsoil)	0.64 (±0.67)	**4.75** (±1.21)	-1.02 (±0.73)	**7.67** (±2.42)	0.45 (±0.86)	-0.56 (±0.83)
Subsoil	3.59 (±3.42)	10.47 (±4.59)	-6.88 (±3.54)	7.78 (±6.67)	-1.60 (±7.09)	-2.39 (±5.98)
Total (incl. subsoil)	4.23 (±3.88)	15.22 (±5.70)	-7.90 (±3.90)	15.43 (±8.65)	-1.14 (±7.92)	-2.96 (±6.78)

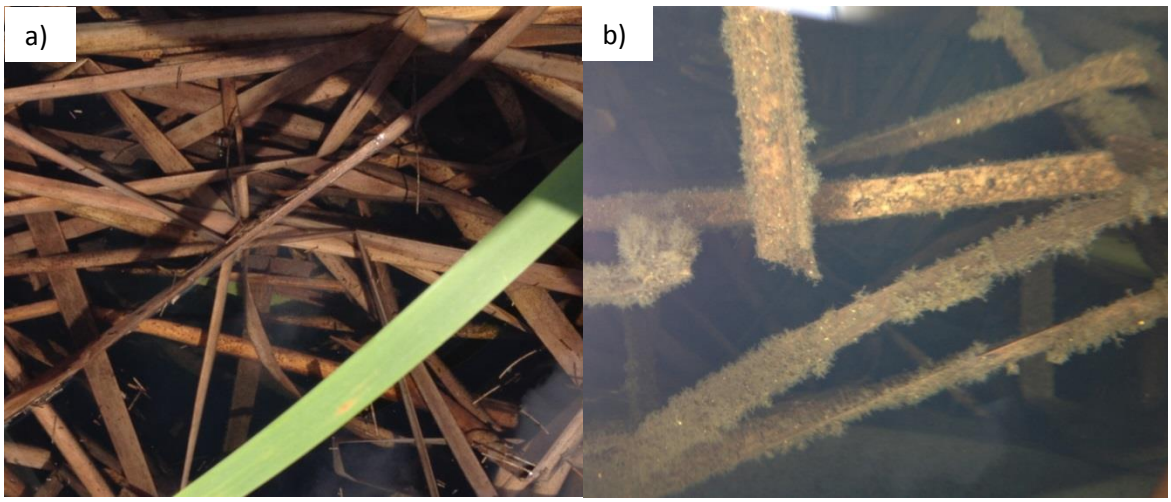

Figure 6-19: Loose dead *Typha* thatch in treatment mesocosms (a) prior to enrichment (B1) with minimal periphyton growth and (b) during the last enrichment sampling round (D5) with substantial periphyton growth.

6.3.5 Ecosystem change

How do vegetation characteristics change with enrichment in the different functional zones?

Bare sediment mesocosms sustained no vascular plants or macroalgae throughout the experiment, however suspended algae increased with enrichment. A complete loss of charophytes (SAV) occurred in the SAV enrichment mesocosms by the second last sampling round (supporting Hypothesis 8, Table 2-7), whereas control mesocosms sustained a healthy SAV bed until the end of the experiment. Epiphytic algal growth on charophytes was visually observed to increase with enrichment. The loss of SAV removed a key nutrient store and the mesocosms became similar to the bare sediment enrichment mesocosms. In the *Typha* zone, enrichment had no significant effect on *Typha* culm density, height (Figure 6-20) or biomass (Figure 6-13c, Table 6-6) in contrast to Hypothesis 7 (Table 2-7). *Lemna minor* occurred at low density in the *Typha* enrichment mesocosms from round D2 onwards; none were recorded in control mesocosms.

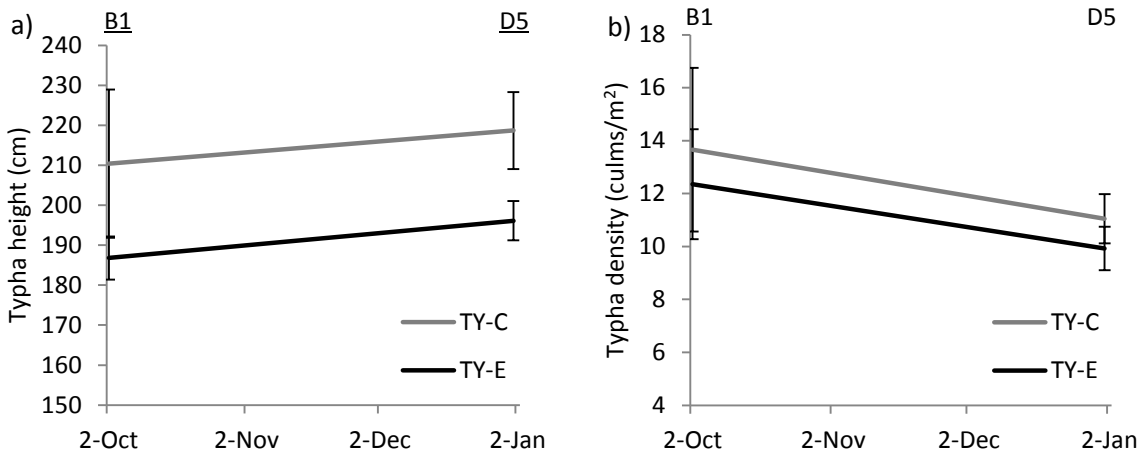

Figure 6-20: Mean *Typha* height (±1 standard error) (a) and density (Pollard density estimator ± 1 standard error as converted from associated variance estimator) (b) in *Typha* zone mesocosms (n=3 per treatment) during pre-enrichment (B1) and last enrichment (D5) sampling rounds. E = enrichment and C = control.

How does water quality change with enrichment and how does this differ between the functional zones?

Prior to enrichment, water chlorophyll α and turbidity were similar in all mesocosms (Figure 6-21a,b). During enrichment chlorophyll α and turbidity were higher in enrichment than control mesocosms (Figure 6-21a,b, Table 6-9) but with a stronger response in the bare sediment and SAV zones than in *Typha*. Chlorophyll α exceeded the ANZECC trigger value (30 µg/L) in bare sediment and SAV enrichment mesocosms but not in *Typha* enrichment mesocosms and all control mesocosms. Thus, Hypothesis 9 (Table 2-7) that enrichment would result in algal blooms was supported in bare sediment and SAV zones but not in *Typha*. It was also hypothesised that chlorophyll α and turbidity would be higher in unvegetated (bare sediment) than vegetated (*Typha*, SAV) zones during enrichment (Hypothesis 10, Table 2-7). This was true for the *Typha* zone (Figure 6-21a,b), but chlorophyll α and turbidity were not significantly different in the SAV zone compared to the bare sediment zone.

DO and ORP were not consistently lower in enrichment than control mesocosms and there was no decreasing trend over time (Figure 6-21c,d, Table 6-9, in contrast to Hypothesis 11, Table 2-7). Water quality in bare sediment and SAV zones responded differently to enrichment (Figure 6-21, Figure 6-22). In bare sediment, both pH and daytime DO increased in response to enrichment and remained higher in enrichment than control mesocosms until the end of the experiment (Figure 6-22c, Figure 6-21c). In the SAV zone, both pH and DO decreased in response to enrichment but pH partly recovered on their death and since pH and DO in the

control mesocosms decreased over time, both variables were higher in the enrichment than control mesocosms by the end of the experiment (Figure 6-22c, Figure 6-21c). In the *Typha* zone, DO and pH did not differ between enrichment and control mesocosm. Both DO and pH were lower in *Typha* than bare sediment and SAV zones during the entire experiment.

As expected, variation in water temperature and EC were unrelated to treatment (Figure 6-22a,b, Table 6-9). Seasonal increases in water temperature over the course of the experiment were recorded in all mesocosms: from ≈20°C at the start to ≈25°C at the end. Occasionally temperature was ≈1°C lower in *Typha* than other zones but the difference was not consistently significant. EC increased in all mesocosms over time reflecting seasonal evapoconcentration. Treatment did not have an effect on EC in bare sediment or *Typha* zones but in the SAV zone EC was at times significantly higher in enrichment than control mesocosms (Figure 6-22b). There were no consistent differences in EC between zones.

Prior to enrichment, water TN concentration was similar between enrichment and control mesocosms (Figure 6-22d, Table 6-9). In contrast, during enrichment TN concentration was significantly higher in enrichment than control mesocosms in bare sediment and SAV zones but not in *Typha* (Figure 6-22d, Table 6-9). Trends in water NH_4 and NO_x concentrations were the same as for TN.

Prior to enrichment, all treatments and functional zones had high water N:P ratio (average molar ratios between 104:1 and 153:1 at B1), indicating P limitation. During enrichment the average water N:P ratio in control mesocosms ranged from 95:1 and 155:1 and in enrichment mesocosms from 31:1 to 72:1. Consequently, all treatments and zones remained P limited throughout the experiment.

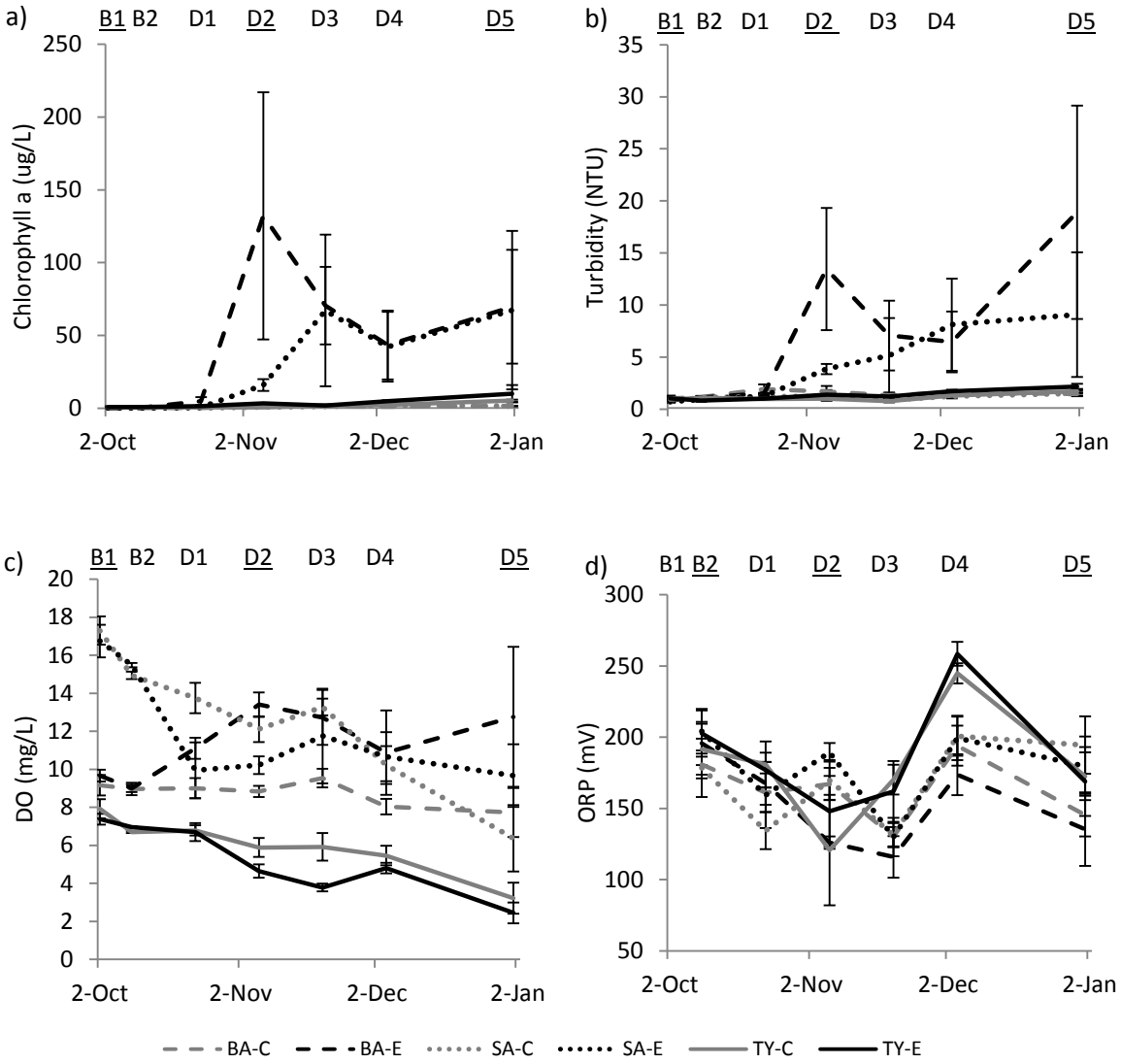

Figure 6-21: Mean chlorophyll α (a), turbidity (b), DO (c) and ORP (d) (±1 standard error) in bare sediment (BA), SAV (SA) and *Typha* (TY) mesocosms (n = 4 for BA and SA, n = 3 for TY treatments) over pre-enrichment (B1, B2) and enrichment (D1, D2, D3, D4, D5) sampling rounds. E = enrichment and C = control.

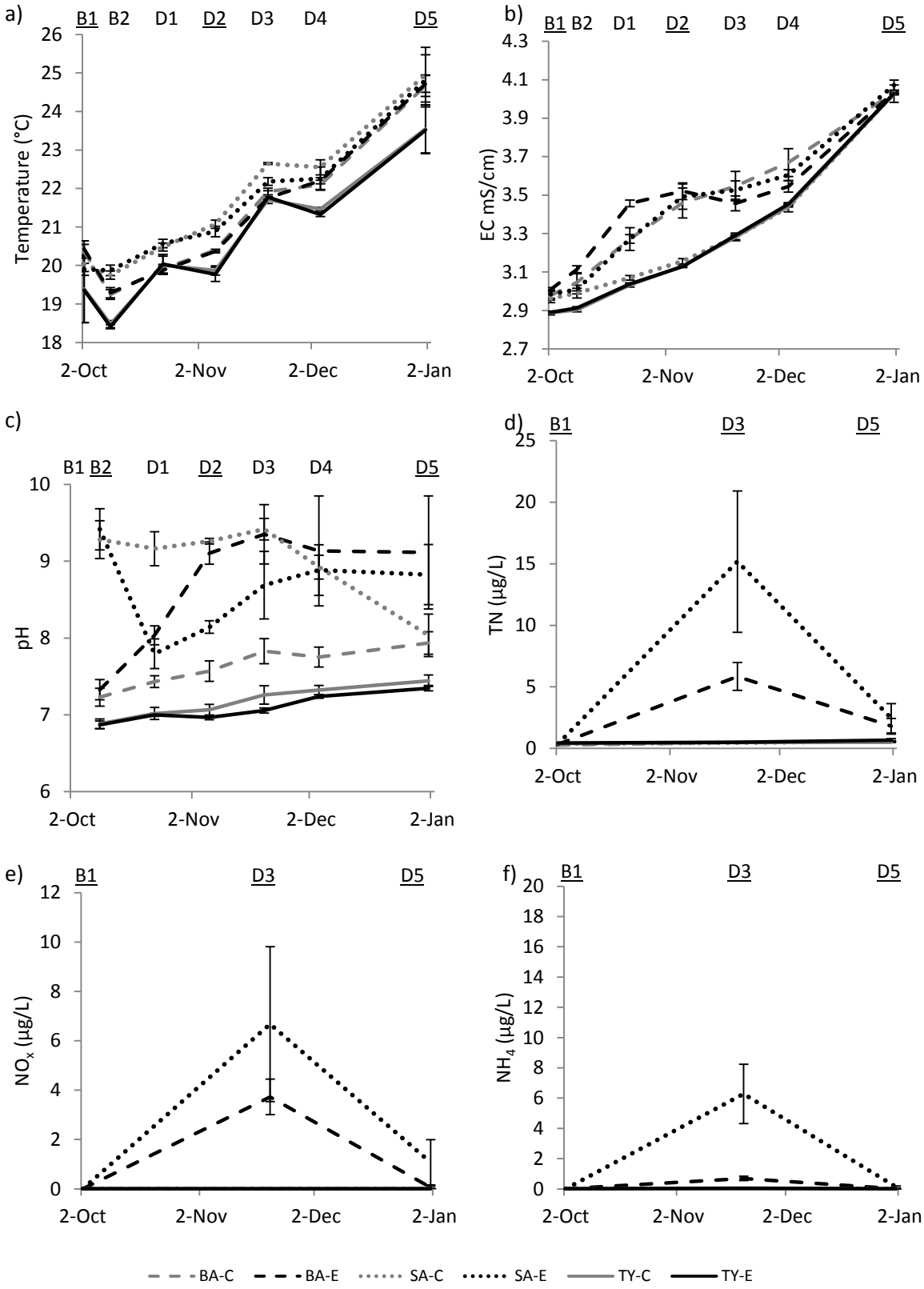

Figure 6-22: Mean temperature (a), electrical conductivity (b), pH (c), TN (d), NO_X (e) and NH_4 (f) (±1 standard error) in bare sediment (BA), SAV (SA) and *Typha* (TY) mesocosms (n = 4 for BA and SA, n = 3 for TY treatments) over pre-enrichment (B1, B2) and enrichment (D1, D2, D3, D4, D5) sampling rounds. E = enrichment and C = control.

Table 6-9: Differences in water quality variables and *Typha* height between treatments and zones. Abbreviations include BA = bare sediment, SA = SAV, TY = *Typha*, ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001, / = versus, do = disordinal, # = non-parametric test. Parentheses indicate a significant disordinal interaction between treatment and zone. Further details in Appendix 8.

Variable	Round	Treatment	Zone		Treatment * Zone interaction		
		Initial test	Initial test	Post hoc Tukey's test	Initial test	Simple effects - Treatment	Simple effects - Zone
Chlorophyll α	B1	no test, all values below detection limit					
	D2	***	ns		ns		
	D5	**	ns		ns		
Turbidity	B1	ns	ns		ns		
	D2	***	**	BA/SA ns, BA/TY **, SA/TY ns	ns		
	D5	*	ns		ns		
Temperature	B1	ns	ns		ns		
	D2	ns	***	BA/SA ***, BA/TY ***, SA/TY ***	ns		
	D5	ns	ns		ns		
pH	B1	ns	***	BA/SA ***, BA/TY *, SA/TY ***	ns		
	D2	(ns)	(***)		*** do	BA ***, SA ***, TY ns	Control BA/SA ***, BA/TY **, SA/TY *** Treatment BA/SA ***, BA/TY ***, SA/TY ***
	D5	ns	*	BA/SA ns, BA/TY *, SA/TY ns	ns		
Electrical	B1	ns	***	BA/SA ns, BA/TY **, SA/TY ***	ns		
conductivity	D2	(**)	(***)		** do	BA ns, SA ***, TY ns	Control BA/SA ***, BA/TY ***, SA/TY ns Treatment BA/SA ns, BA/TY ***, SA/TY ***
	D5	ns	ns		ns		
Dissolved oxygen	B1	ns	***	BA/SA ***, BA/TY ns, SA/TY ***	ns		
	D2	(ns)	(***)		*** do	BA ***, SA *, TY ns	Control BA/SA ***, BA/TY **, SA/TY *** Treatment BA/SA ***, BA/TY ***, SA/TY ***
	D5	ns	**	BA/SA ns, BA/TY **, SA/TY **	ns		
Oxidation	B1	ns	ns		ns		
reduction	D2	ns	*	all ns	ns		
potential	D5	ns	ns		ns		
TN	B1	ns	**	BA/SA**, BA/TY*, SA/TY**	ns		
	D3	(***)	(***)		*** do	BA ***, SA ***, TY ns	Control ns; Treatment BA/SA*, BA/TY***, SA/TY***
	D5	*	ns		ns		
Typha height	B1	ns					
	D5	ns					

6.4 Discussion

6.4.1 Overview

The high nutrient load (16 g/m^2 of P) drove rapid nutrient enrichment in treatment mesocosms over the 13 week experiment, but the treatment response varied between functional zones. Bare sediment and SAV mesocosms changed from oligotrophic to hypereutrophic, resulting in loss of SAV, whereas *Typha* mesocosms only shifted from oligotrophic to mesotrophic. In contrast, control mesocosms in all zones showed no net accumulation of P (apart from biomass on periphyton strips) (Figure 6-23, Figure 6-24, Figure 6-25, Table 6-10) and remained mostly oligotrophic. Hypereutrophic conditions developed very rapidly in water in bare sediment and SAV zones even while ecosystem components were still actively taking up P (Figure 6-23, Figure 6-24). Although the enrichment period was short, significant treatment effects were recorded in TP storage in most ecosystem components (water, periphyton, SAV, litter, topsoil) supporting Hypothesis 3 (Table 2-7). Increased TP concentration in standing dead *Typha* was likely a reflection of microbial P uptake in dead *Typha* tissue (Qualls and Richardson 2000), otherwise above-ground *Typha* (TP concentration or mass) did not respond. Another treatment effect was the floc that formed only in SAV enrichment mesocosms. The rate of response to enrichment varied between ecosystem components: water, periphyton and SAV responded with rapid step-wise increases, whereas more gradual and sustained increases were observed in topsoil and litter and to some extent, floc. The functional P uptake stage of the conceptual models was consequently split into two parts to illustrate early and later responders (Figure 6-23, Figure 6-24, Figure 6-25, Table 6-10).

The decreased dosing frequency after the second enrichment sampling round (from weekly to fortnightly; effectively halving P load) resulted in an order magnitude decrease in water TP concentrations in bare sediment and SAV zones (from peaks at 1375 µg/L and 2450 µg/L respectively to just above 100 µg/L by the end of the experiment). This supported previous research showing better retention efficiency (higher uptake by other components) with lower load (Richardson and Vaithiyanathan 2009).

Table 6-10: Summary of study changes to conceptual models.

Functional zone	Pre-enrichment stage	Functional phosphorus uptake stage
Bare sediment zone	• None	• Substantial algal blooms observed early in enrichment. • Subsoil - increase in P storage was insignificant.
SAV zone	• None	• Floc - store added to the model with increasing TP storage. • SAV - lost already at this stage (rather than the hypereutrophic end-stage). • Subsoil - increase in P storage was insignificant.
Typha zone	• None	• Topsoil - expected increase in P storage not observed. • Subsoil - expected increase in P storage not observed. • *Typha* - expected increase in TP storage not observed.
All	• P uptake by periphyton on plastic frame and strips included.	• Stage split to differentiate between early and later responders. • P uptake by periphyton on plastic frame and strips included.
All	• Pie charts added showing distribution of TP between ecosystem components (excluding subsoil). • Pie charts added showing where the added P went as % of total load.	

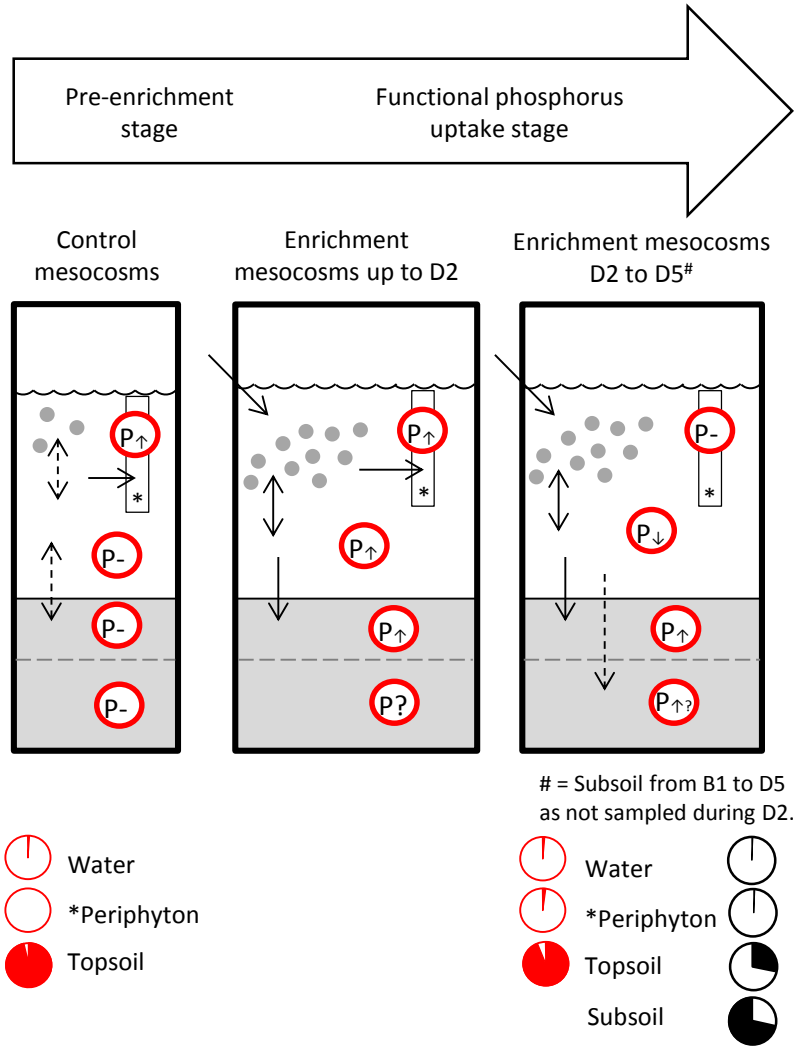

Figure 6-23: Conceptual model of phosphorus storage and cycling in the bare sediment zone during three month mesocosm experiment. Solid line arrows indicate definite phosphorus cycling pathways and dashed lines likely pathways present. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached, ? = uncertain. Grey dots show algal bloom intensity. Red pie charts indicate proportional share of total phosphorus (excluding subsoil) held by each store (no data for D2 as some stores not sampled during this round). Black pie charts indicate where the added phosphorus went based on mass balance (difference in TP storage between start and end, Table 6-8).

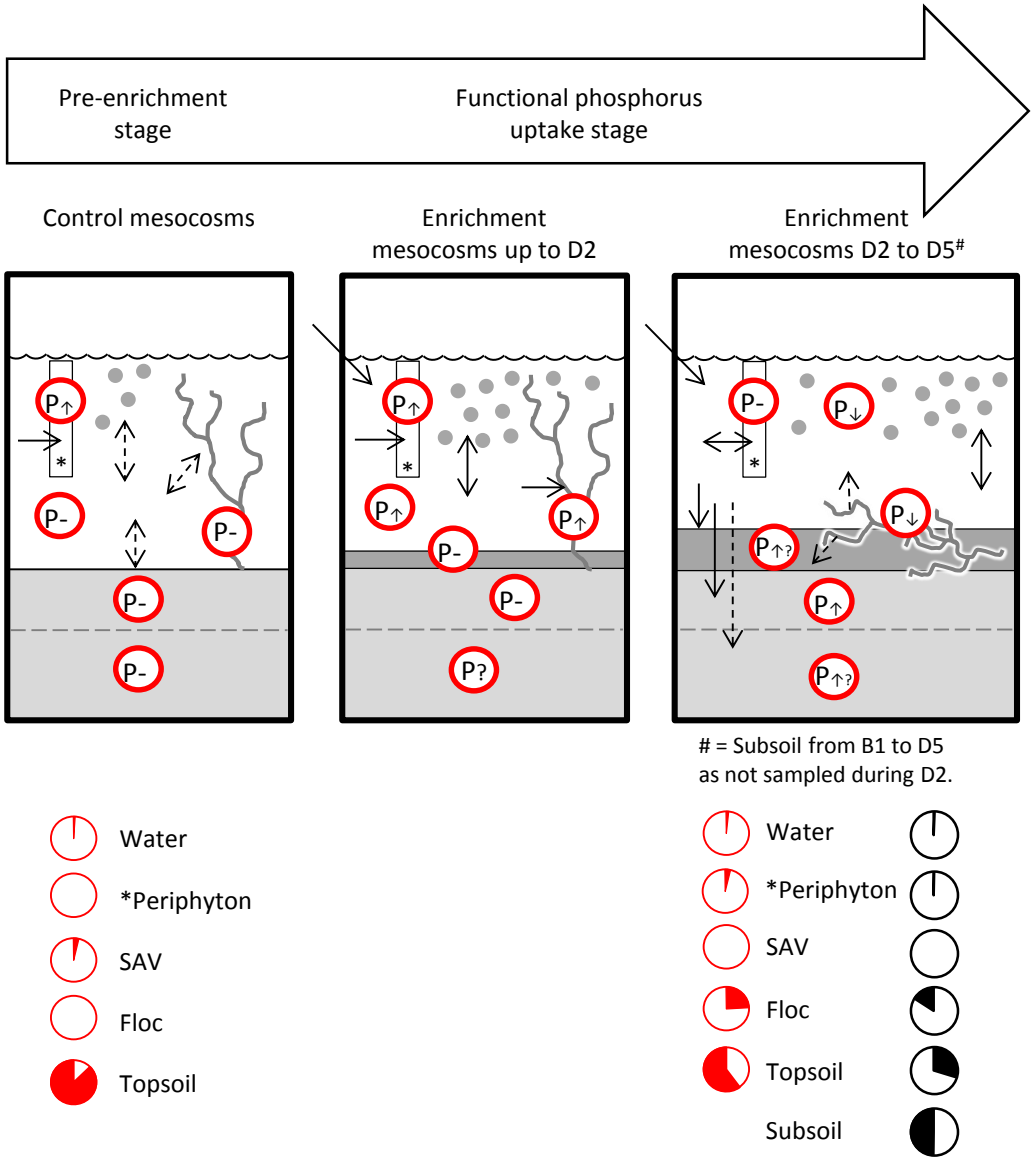

Figure 6-24: Conceptual model of phosphorus storage and cycling in the SAV zone during three month mesocosm experiment. Solid line arrows indicate definite phosphorus cycling pathways and dashed lines likely pathways present. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached, ? = uncertain. Grey dots show algal bloom intensity. Red pie charts indicate proportional share of total phosphorus (excluding subsoil) held by each store (no data for D2 as some stores not sampled during this round). Black pie charts indicate where the added phosphorus went based on mass balance (difference in TP storage between start and end, Table 6-8).

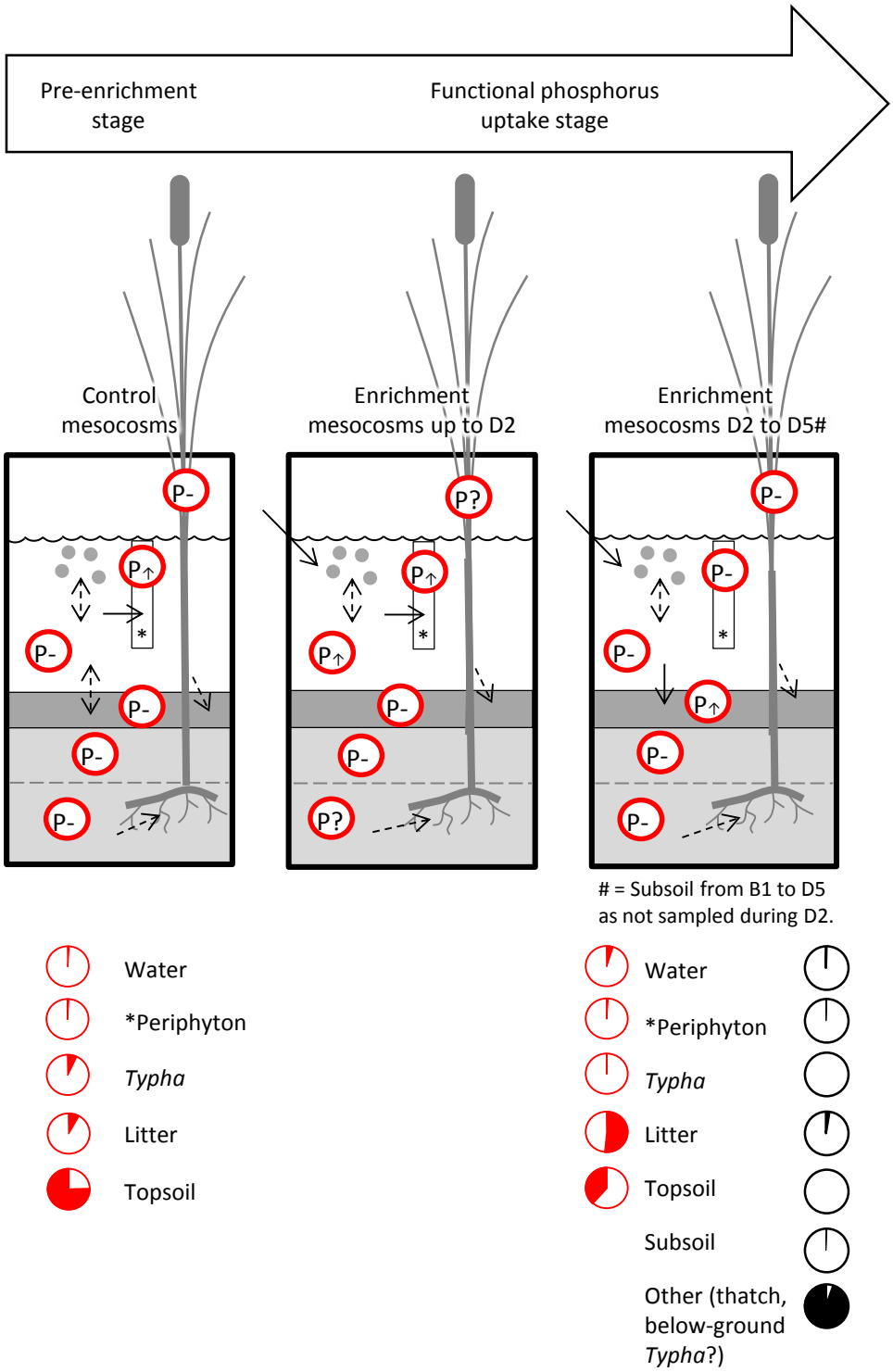

Figure 6-25: Conceptual model of phosphorus storage and cycling in the *Typha* zone during three month mesocosm experiment. Solid line arrows indicate definite phosphorus cycling pathways and dashed lines likely pathways present. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached, ? = uncertain. Grey dots show algal bloom intensity. Red pie charts indicate proportional share of total phosphorus (excluding subsoil) held by each store (no data for D2 as some stores not sampled during this round). Black pie charts indicate where the added phosphorus went based on mass balance (difference in TP storage between start and end, Table 6-8).

6.4.2 Procedural versus treatment effects

Enclosure effect

Enclosure effects were small, much smaller than the treatment effects, existing mainly in the SAV zone. In the SAV zone, photosynthesis and respiration by SAV directly interacted with water; these processes were absent from the other two zones. Only minor enclosure effects were detected here, but in general, it is important to sample natural conditions alongside those in the mesocosms to enable evaluation of procedural effects caused by the mesocosm enclosures themselves.

Similarity of mesocosms prior to enrichment

Prior to enrichment, no significant differences between enrichment and control mesocosms were found in any of the sampled or derived variables. This demonstrates that any differences between treatment and control mesocosms observed during the enrichment were caused by enrichment.

Type of P load

The results of this study reflect the P load being 100% dissolved inorganic P that is readily bioavailable. Systems subject to similar P load but comprising mainly organic and or particulate P would be expected to respond differently because transformations would be necessary to make the P bioavailable and particulate P would likely be removed through settling on the bottom rather than through biological or chemical pathways.

Periphyton P uptake

The plastic strips were useful for calculating the contribution of periphyton on the mesocosm walls to the P mass balance. However, periphyton growth on the strips was likely not representative of periphyton growth on natural surfaces (e.g. plant matter or bottom sediment) over the same time period due to differences in substrate characteristics and light availability (Cronk and Mitsch 1994, Guariento *et al.* 2009, Yang and Flower 2012) and potentially substrate orientation (vertical plastic strips versus some horizontal *Typha* leaves). Consequently, periphyton results from this study should not be used to guide management decision on periphyton growing on other substrates. In this study, periphyton growing on natural surfaces was included in the TP storage of that growth surface (e.g. topsoil or *Typha*).

Typha zone stores

Sampling methods for the three studies in this thesis were kept as similar as possible to ensure valid comparisons could be made between the studies. The methods were effective in

capturing changes in P storage and cycling in the seasonal and multiyear studies, and in bare sediment and SAV zones in the mesocosm study. However, the methods were less suitable for detecting rapidly occurring changes in the *Typha* zone in the mesocosm experiment because the loose *Typha* thatch suspended between the culms was not sampled. Later, during data analysis, it was noted that this component provided an ideal substrate for microbiota and periphyton responsible for the rapid P uptake observed in the *Typha* zone. Thatch was not an issue in earlier studies because *Typha* density in Swamphen Lake was lower than in Peninsula Lake, so any loose dead *Typha* fell to the bottom (as litter) rather than remaining suspended between the culms as thatch. Any P taken up by underground *Typha* had more time to contribute to above-ground *Typha* in the seasonal and multiyear studies than in the mesocosm study; the latter being too short for the uptake by underground *Typha* to become apparent. Overall, the results of this mesocosm study in the *Typha* zone are valid for the stores sampled, although they do not represent the entire picture of rapid P uptake processes in the zone.

6.4.3 Phosphorus storage of ecosystem components

How does TP storage of ecosystem components change with enrichment and how does this differ between functional zones?

Rapid responders

Water (including suspended algae), periphyton and SAV (charophytes) were rapid responders to enrichment, taking up significant amounts of P by the first post-enrichment sampling round (supporting Hypotheses 1 and 3, Table 2-7). These stores involve algae, phytoplankton and microbiota that are able to take up P rapidly (Havens *et al.* 1999, Noe *et al.* 2003, Kadlec and Wallace 2009, Kobayashi *et al.* 2009),directly from the water column (Reddy and DeLaune 2008) where it was added, giving them a competitive advantage in the short-term when compared to other stores. Algae and microbiota are also capable of increasing their biomass more rapidly than higher plants (Cuellar-Bermudez *et al.* 2017) (partly supporting Hypothesis 2, Table 2-7). Consequently, these organisms would be expected to be involved in initial rapid P uptake in any wetland ecosystem undergoing nutrient enrichment, so they have potential to be used as early indicators of enrichment (Gaiser *et al.* 2004, Corstanje and Reddy 2006).

Overall, suspended algae in water and periphyton on plastic surfaces took up only a small portion of added P (<1% and <2% respectively of the total P load of 16 g/m^2) and their proportional share of total TP storage also remained small (<1% for water, up to 3% for periphyton) broadly supporting previous literature (Reddy and DeLaune 2008, Kadlec and

Wallace 2009). However as noted earlier, the periphyton growth on plastic was likely an underestimate of growth on natural surfaces over the same period (Cronk and Mitsch 1994, Guariento *et al.* 2009, Yang and Flower 2012). Many rapid responders (algae and microbiota) are found attached and incorporated into the substrate of other stores (soil, plants, litter) (Qualls and Richardson 2000, Huang *et al.* 2011, dos Santos *et al.* 2013, Kuehn *et al.* 2014) and would have contributed to the observed increases in TP concentration of litter and dead *Typha* and potentially loose *Typha* thatch (not sampled).

Despite suspended algae taking up <1% of total P load and having low proportional TP storage (<1%), this store played a key role in modifying the environmental conditions experienced by all ecosystem components. Algal blooms developed within a few weeks of enrichment and although water TP concentration (in bare sediment and SAV zones) decreased over an order of magnitude to just above 100 μg/L by the end of the study, treatment mesocosms remained hypereutrophic with algal blooms, consistent with previous research (Carlson 1977, OECD 1982). These algal blooms increased turbidity, potentially contributing to loss of SAV through shading (discussed in Section 6.4.4). Over time, decomposing algal blooms would reduce DO and ORP in water and sediment (Reddy and DeLaune 2008, Paerl and Otten 2013, Wang *et al.* 2016) with implications for survival of organisms and nutrient cycling (Boulton *et al.* 2014). This highlights how sensitive wetlands are to nutrient enrichment especially under high nutrient loads.

Compared to the other rapid responders, SAV (charophytes) showed greater capacity to take up P, with a large step-wise increase between the baseline and the first sampling round post-enrichment (0.16 g/m^2 to 0.79 g/m^2); storing more P than *Typha* (0.35 g/m^2 across sampling occasions). However, TP storage in SAV was temporary in enrichment mesocosms, because SAV was subsequently completely lost, resulting in net release of P from SAV. Loss of SAV resulted in loss of P storage and cycling pathways characteristic of the SAV zone and reduced the overall P storage capacity of the zone. SAV can remove P efficiently even at low concentrations (Gumbricht 1993, Dierberg *et al.* 2002, Knight *et al.* 2003, DeBusk *et al.* 2011). However, loss of SAV (particularly sensitive charophytes) has also been recorded when enrichment significantly increases turbidity (Blindow 1992, Scheffer *et al.* 2001, Kufel and Kufel 2002, Morris *et al.* 2003, Romo *et al.* 2004, Bakker *et al.* 2010); as seen here (supporting Hypothesis 8, Table 2-7; changes in environmental conditions discussed further in Section 6.4.4). Furthermore, when P load is high, loss of SAV (charophytes) can occur very rapidly (within weeks), more rapidly than the more gradual decline in *Typha* observed in the multiyear study.

TP concentration also increased in dead *Typha*, likely due to uptake by periphyton and microbiota present in the decaying tissue (similar to microbial uptake in litter) (Qualls and Richardson 2000). This uptake would have been rapid, but as *Typha* was only sampled at the start and end of the experiment, the rate of response could not be verified. This uptake pathway did not result in net change in standing dead *Typha* TP storage as associated mass did not change.

Slower responders

The benthic stores litter, topsoil and floc were slow responders when compared to water, periphyton and SAV, likely a reflection of the different P uptake pathways involved. While the rapid responders used fast, direct biological uptake pathways, benthic stores used a mixture of physical (e.g. settling of particulate P, Coveney *et al.* 2002, Maynard *et al.* 2009), chemical (sorption and precipitation of P, Vymazal 2007) and biological P uptake processes (uptake by attached algae and microbiota, Qualls and Richardson 2000, Grace *et al.* 2008). Also, benthic stores received P through indirect pathways, for example, through uptake by rapid responders (e.g. SAV or algae) that then died and contributed P to the benthos.

The slower responding benthic stores took up more P (litter in *Typha* zone 0.67 g/m^2; floc in SAV zone 2.7 g/m^2; topsoil in bare sediment and SAV zones ≈ 4 g/m^2) than the rapid responders (water and periphyton combined <0.4 g/m^2 in bare sediment and SAV zones and <0.04 g/m^2 in *Typha* zone), supporting Hypothesis 6 (Table 2-7). Previous literature (Chambers and McComb 1996) indicated that most added P would be taken up by soil, and this was supported in bare sediment and SAV zones. The proportion of total TP storage in topsoil decreased with enrichment as hypothesised (Hypothesis 5 Table 2-7) due to competing uptake by other stores. Topsoil P uptake in this short study was slightly higher than in the multiyear study (bare sediment zone 3.57 g/m^2), despite water TP concentration at the end being four times higher in the multiyear study. Lower bottom water DO and ORP in the multiyear study than in the mesocosm study may have reduced soil P uptake (Reddy and DeLaune 2008, Wang *et al.* 2016). Litter TP storage and concentration at the end of the mesocosm study were several times lower than in the multiyear study (3.26 mg/g) or previous literature (Table 2-3) and litter took up only about 4% of the total TP load of 16 g/m^2. This suggests that litter does not provide a key P uptake pathway amongst *Typha* in the first few months of enrichment (even intensive enrichment), despite being a major sink in the first year or two of the multiyear study and in research elsewhere (Richardson and Marshall 1986).

In contrast to the increases in mass observed in rapid responders (suspended algae and periphyton), no increases in mass (related to primary production) were recorded in slow responders (in contrast to Hypothesis 2, Table 2-7). While mass of litter increased, this was unrelated to primary production (as *Typha* mass remained stable). Instead, it appeared to result from faster transfer of dead *Typha* into the litter layer, possibly due to increased, nutrient-stimulated decomposition of standing dead biomass (Corstanje and Reddy 2006). Logically, stores whose mass relies on the growth and death of organisms will be slower to respond in mass than fast metabolising primary producers. For these slower responding stores, TP concentration is a better early indicator of nutrient enrichment than mass.

Detecting subsoil response

Phosphorus mass balance indicated that the majority of added P went to subsoil in bare sediment and SAV zones, however at the end of the experiment, treatments and controls did not differ because variation among mesocosms was high. As noted previously, the heavy clay soil had very low hydraulic conductivity (0.01-1 m/day, GHD 2004) and high P uptake capacity (Chambers and McComb 1996), resulting in slow movement of P through topsoil to subsoil. As no subsoil treatment response was found in the longer-term studies, it was logical that none was recorded in this study either. Subsoil remained by far the largest store of P throughout the study, (as in the seasonal and multiyear studies and existing literature, Kadlec and Wallace 2009), but was also likely the least bioavailable.

Typha zone response

Above-ground *Typha* showed little response to enrichment apart from increased TP concentration in dead *Typha*. The experiment was possibly too short for detectable effects on live *Typha* TP concentration and *Typha* mass. Previous research identifies highly variable response times for *Typha* (see Section 4.4.2), and as such the findings of this study are not unusual. Furthermore, even if P was taken up by *Typha* roots, it may have been transported to the rhizome (which was not sampled) rather than to live above-ground tissue. Bayly and O'Neill (1972) found that TP concentration of *Typha* leaves peaked early in the growing season followed by steady decline, whereas rhizome TP concentration stayed low during the early season and then increased rapidly, staying high for the remainder of the growing season. Here, TP concentration of live *Typha* decreased during the experiment, indicating that the early growing season was over and that instead rhizome TP concentration would have been expected to increase. Overall, although *Typha* may have taken up P during this short-term study, understanding P dynamics of *Typha* and their effect on the nutrient enrichment

continuum requires a longer-term, preferably seasonal, dataset that samples all parts (above and below-ground) of *Typha*.

The phosphorus mass balance indicated that the majority of P added to the *Typha* enrichment mesocosms was not captured by sampling, yet regular inspections of mesocosm integrity indicated leakage was highly unlikely. Some P may not have been captured due to the low sensitivity of the sampling in an environment of high spatial variation and may be reflected in the variation shown by the large topsoil and subsoil stores. However, the majority of the missing P is likely to have been taken up by ecosystem components not sampled here, including: below-ground parts of *Typha,* loose dead *Typha* thatch and to a lesser extent sporadically occurring *Lemna minor* and SAV (charophytes and *Potamogeton* sp.). Although TP storage of soil did not change, *Typha* could have accessed the added P via adventitious roots in the litter layer (Rejmánková and Snyder 2008). In high P-load systems, below-ground P storage by macrophytes (mixed species) increased from 1.6 g/m^2 to 15.1 g/m^2 in four months (Dolan *et al.* 1981) and 6-7 fold increase was observed in below-ground tissue TP concentration of *Typha domingensis* in 30 days (Di Luca *et al.* 2015). No other research on TP storage by *Typha* thatch was found, but being similar to *Typha* litter, uptake of P by thatch probably comprised uptake by periphyton and microbial components. However, thatch uptake may have been higher than litter, as thatch was more exposed to light and incoming nutrients (due to higher vertical position), supported by the observed emergence of substantial periphyton on thatch. As the multiyear study recorded litter TP storage to be on average up to 7.07 g/m^2, with some sites containing up to 19.95 g/m^2, underground *Typha* (roots, rhizomes) and loose thatch together could account for all the missing P added to *Typha* enrichment mesocosms.

Functional zones

Differences between functional zones reflected the number and type of stores available and interactions between them, which influenced the way zones progressed along the nutrient enrichment continuum. In the bare sediment zone, enrichment was evident in two processes: blooms of suspended algae in water and accumulation of P in benthos (specifically topsoil). In the SAV zone, enrichment initially progressed with biological P uptake by SAV, however subsequent rapid loss of SAV made P dynamics more similar to those seen in the bare sediment zone, as algal blooms and benthos-focussed enrichment developed over time. The dead SAV did have a legacy though: P and carbon released from SAV, unavailable in the bare sediment zone, resulted in the development of a nutrient-rich floc layer and increased periphyton growth (on plastic surfaces), modifying nutrient storage and environmental

conditions alike. Enriched mesocosms in both bare sediment and SAV quickly became hypereutrophic.

Enrichment in the *Typha* zone progressed very differently and more slowly, as those mesocosms did not become hypereutrophic. Unlike the other two zones, there was no accumulation of P in topsoil, likely due to the blanketing layer of litter that overlaid the topsoil and took up P instead (Qualls and Richardson 2000, Di Luca *et al.* 2015). Consequently, the proportion of TP storage in topsoil was only 70% in the *Typha* zone compared to nearly 100% in bare sediment zone. FRP remained below the detection limit and there were no algal blooms, indicating that added P was taken up rapidly by other pathways, likely biological (especially algal and microbial) pathways (Richardson and Marshall 1986, Noe *et al.* 2003). The presence of live and dead *Typha* and associated litter and thatch provided a structurally more complex environment containing a range of microhabitats for a variety of organisms (Rejmankova 2011, Gopal 2016), that was clearly more complex than the bare sediment zone (including SAV zone following loss of SAV). At least in the short-term, the *Typha* zone was much more effective in assimilating P than the other two zones, supporting Hypothesis 4 (Table 2-7) and previous research (Tanner *et al.* 1995, Huett *et al.* 2005, Greenway and Lucas 2008, Menon and Holland 2013). In contrast, the longer seasonal and multiyear studies did not show higher P uptake in the vegetated *Typha* zone. Although this difference may be attributable to the use of mesocosms, it is more likely that the decline of *Typha* in Swamphen Lake underestimated the potential for P uptake of the *Typha* zone. Persistent aquatic vegetation appears beneficial for wetlands undergoing nutrient enrichment: even if plants do not directly prevent adverse impacts of eutrophication in the long-term, they delay them in the short-term.

Which ecosystem components account for most of the difference in TP storage between enriched and unenriched systems and how does this differ between functional zones?

As outlined above, the ecosystem components that showed the greatest response to enrichment differed between functional zones in the wetland. What is interesting is that additional nutrients not only increased amounts of P stored in enriched mesocosms, but also increased variation in the relative proportions of nutrients held in different stores compared with control mesocosms. Variance among enriched mesocosms was also higher in the *Typha* zone than the other zones, potentially due to larger numbers of alternative P stores. No discussion of these types of increases in variance associated with enrichment was found in literature. However, they have significant implications for management as increased variance will make it more difficult to detect, predict and manage ecosystem response. For example,

the spatial intensity of sampling of TP stores should increase with enrichment to maintain statistical power (Quinn and Keough 2002).

6.4.4 Ecosystem change

Research Question 2 - How is nutrient enrichment reflected in ecosystem change in the different functional zones?

Vegetation changes, chlorophyll α and turbidity

In contrast to Hypothesis 7 (Table 2-7), the *Typha* zone underwent limited ecosystem change, with no changes in density, height or biomass of *Typha.* This may have been because the experiment was too short for above-ground responses by *Typha* to enrichment. Contrary to Hypothesis 9 (Table 2-7), no algal blooms developed in the *Typha* zone, despite blooms in other zones. The absence of these phenomena is likely to be another reflection of the larger number of P uptake pathways and storage capacity in the *Typha* zone, slowing the enrichment process when compared to other zones.

In contrast, the SAV zone underwent major ecosystem changes as enrichment caused increased turbidity (due to increased phytoplankton production) resulting in light limitation and the death of SAV (Blindow 1992, Scheffer *et al.* 2001, Kufel and Kufel 2002, Romo *et al.* 2004). Nutrient enrichment appeared to increase epiphytic algal growth on SAV potentially contributing further to their loss through smothering the plants (Phillips *et al.* 1978, Asaeda *et al.* 2004, Phillips *et al.* 2016). The presence of flourishing charophyte beds in controls adjacent to enriched mesocosms, showed categorical proof that high nutrient load (total 16 g/m^2 P in 12 weeks) causes rapid SAV loss within weeks (< 8 weeks). SAV provides diverse ecological niches and food sources for other organisms (Jeppesen *et al.* 1998, Mitsch *et al.* 2005, Bolduc *et al.* 2016, Kuczyńska-Kippen and Joniak 2016), promotes sedimentation, reduces resuspension (Kufel and Kufel 2002, van Donk and van de Bund 2002), and influences water chemistry through primary production and respiration (Reddy and DeLaune 2008). Its loss therefore causes profound changes to ecosystems.

In the bare sediment zone, the main ecosystem change with enrichment was the rapid development of algal blooms causing the loss of clear water conditions. The rate of development of bloom conditions was faster in bare sediment and in SAZ zone because there were fewer P uptake pathways and lower uptake capacity there. The hypothesis that chlorophyll α and turbidity would be higher in unvegetated than vegetated systems during

enrichment (Hypothesis 10, Table 2-7) was supported when bare sediment and *Typha* zones were compared. Unvegetated systems have no competition for nutrients with other plants or shading by them, thereby providing more productive conditions for phytoplankton growth. Unvegetated systems also lack collision and trapping surfaces (e.g. plant stems) that reduce turbidity (Portielje and Van der Molen 1999, Villena and Romo 2007, McCall *et al.* 2017), further enhancing conditions for phytoplankton growth.

DO, ORP and pH

Hypothesis 11 (Table 2-7) was not supported because DO and ORP of water close to sediment surfaces did not decrease with enrichment, potentially due to the short duration of the experiment. Changes in pH and daytime DO in bare sediment and SAV zones with enrichment reflected changes in rapid responding primary producers (SAV and phytoplankton). Increased turbidity limiting light and thus photosynthetic oxygen release from SAV would have initially reduced pH and DO (Phillips *et al.* 2016). In contrast, initial increases in pH and DO in the bare sediment zone could be explained by increased phytoplankton photosynthesis (Reddy and DeLaune 2008). Towards the end of the experiment, pH and DO became similar in bare sediment and SAV zone enrichment mesocosms, reflecting the loss of SAV and the dominance of phytoplankton. The absence of a treatment effect on pH and DO in the *Typha* zone was consistent with the absence of treatment effects plants (above-ground) in the *Typha* zone. Had the mesocosm experiment ran for longer, reductions in DO and ORP might have been expected as biomass production and associated breakdown occurred (Smith 2003).

6.5 Conclusion

Short-term nutrient enrichment (12 weeks) increased TP storage by most (water, periphyton, SAV, litter, topsoil, and possibly floc and subsoil) but not all (*Typha*) wetland ecosystem components and resulted in a loss of sensitive ecosystem components (SAV) due to changes in environmental conditions. The rate and magnitude of response to nutrient enrichment varied between ecosystem components: water, periphyton and SAV responded with rapid, step-wise but generally small increases in P storage, whereas litter, topsoil and (to some extent) floc responded with more gradual but more substantial P storage. Functional zones differed, with all shared stores (water, periphyton, topsoil, subsoil) consistently showing larger treatment effects in bare sediment and SAV zones than the *Typha* zone. Increases in chlorophyll α and turbidity and changes in pH and DO were observed only in bare sediment and SAV zones. The greater number and type of ecosystem components in the *Typha* zone created more P uptake

pathways and storage capacity, dividing the P load and slowing the progress of enrichment compared to the bare sediment and SAV zones.

The majority of added P was accounted for in the bare sediment and SAV zones with most accumulating in topsoil and possibly in subsoil and floc (SAV zone only). In contrast, in the *Typha* zone the majority of P was not captured and, despite the contribution of litter to P uptake, it was assumed that most P was taken up by un-sampled ecosystem components. Further research tracking P storage in the *Typha* zone is necessary.

Out of the three studies, this mesocosm experiment best detected ecosystem components that responded rapidly to enrichment. It allowed understanding of the functional P uptake stage, and particularly the performance of SAV, to be refined in the conceptual models. The enclosed nature of the mesocosms facilitated better detection of differences in the progress of nutrient enrichment between the functional zones. Specifically, it highlighted the value of *Typha* zones in slowing the progress of enrichment, and the rapidity with which vulnerable SAV could be lost, in the early stages of the enrichment continuum.

CHAPTER 7: GENERAL DISCUSSION

7.1 Overview

Nutrient enrichment of wetlands and other aquatic ecosystems remains a common environmental concern across the world (Davis and Koop 2006, Verhoeven *et al.* 2006, Smith and Schindler 2009, Dodds and Smith 2016, Schindler *et al.* 2016). Understanding the dynamics of phosphorus (P) in wetland environments is critical to effectively manage wetlands undergoing nutrient enrichment. The aim of this thesis was to provide a holistic account of simultaneous changes in P storage of different ecosystem components and ecosystem characteristics in different functional zones as a wetland undergoes the process of nutrient enrichment. The overarching research questions were:

- How does P storage and cycling change as a wetland undergoes nutrient enrichment and how does this differ between functional zones?
- How is nutrient enrichment reflected in ecosystem change in the different functional zones?

Using the literature, conceptual models of P storage and cycling over the eutrophication continuum were constructed for three functional zones (submerged bare sediment, submerged aquatic vegetation (SAV), emergent *Typha*) across three stages: pre-enrichment, functional P uptake and hypereutrophy (end-stage). Six sub-questions and 11 hypotheses (Table 2-7) were developed to address the two research questions (above) based on knowledge gaps identified during development of the conceptual models. To refine the conceptual models, three studies at different temporal scales explored these questions and tested these hypotheses: seasonal (9 months) and annual (3 years) whole-wetland studies and a within-wetland mesocosm experiment (3 months). The studies were conducted in semi-natural shallow wetlands of mining origin, characterised by the high seasonal variability of the Mediterranean climate, fine clay-silt sediment with high metal content (Al, Fe, Mn), low water TP but high TN concentration, lack of surface water outflow (during study period), and presence of areas of open water, stands of emergent *Typha* and (at times) SAV meadows. Enrichment in the two whole-wetland studies occurred via addition of treated municipal effluent, so the treatment comprised the combined effects of nutrient and water addition.

The P load in the three studies was high (seasonal 10.2 g/m^2 over nine months equalling 13.6 g/m^2/year, multiyear 18.2 g/ m^2 over 24 months equalling 9.1 g/m^2/year, mesocosm 16.0 g/m^2 over 12 weeks equalling 69.3 g/m^2/year) in the context of natural wetlands, but was midrange

in the context of wastewater treatment wetlands (Figure 7-1). The P load exceeded estimates of the sustainable P assimilation capacity of wetlands: 0.5 g/m^2/year (Vymazal 2007, Hefting *et al.* 2012), 1 g/m^2/year (Richardson and Qian 1999, Hefting *et al.* 2012), 0.5-5.0 g/m^2/year in constructed wetlands (Mitsch *et al.* 2000). As such, ecosystem changes and changes to P storage and cycling as a result of nutrient enrichment were considered inevitable and indeed occurred during the three studies.

The research findings supported most of the hypotheses rising from the conceptual models (Table 7-1), although often only partially. The rate and scale of response to enrichment varied between ecosystem components and between functional zones. Water, periphyton and SAV responded quickly, however the long-term scale of storage in these components was low compared to the more gradually responding floc, litter and topsoil. Litter and floc emerged as significant stores of P at least in the early stages of enrichment rivalling the anticipated high uptake by topsoil. Contrary to expectations, topsoil did not take up any P in the *Typha* zone. SAV was adversely impacted by enrichment and lost within weeks. The effect of wastewater input (synergistic effects of increased water depth and decreased water quality) also adversely affected the usually robust *Typha,* which was lost over a period of several years. Overall, the P load resulted in relatively rapid eutrophication of the treatment wetland within a few years, with a range of adverse ecosystem outcomes. One key difference between functional zones was the slower progress of eutrophication in the *Typha* zone compared to bare sediment and SAV zones in the short-term mesocosm experiment, however this was not replicated in the whole-wetland scale multiyear study.

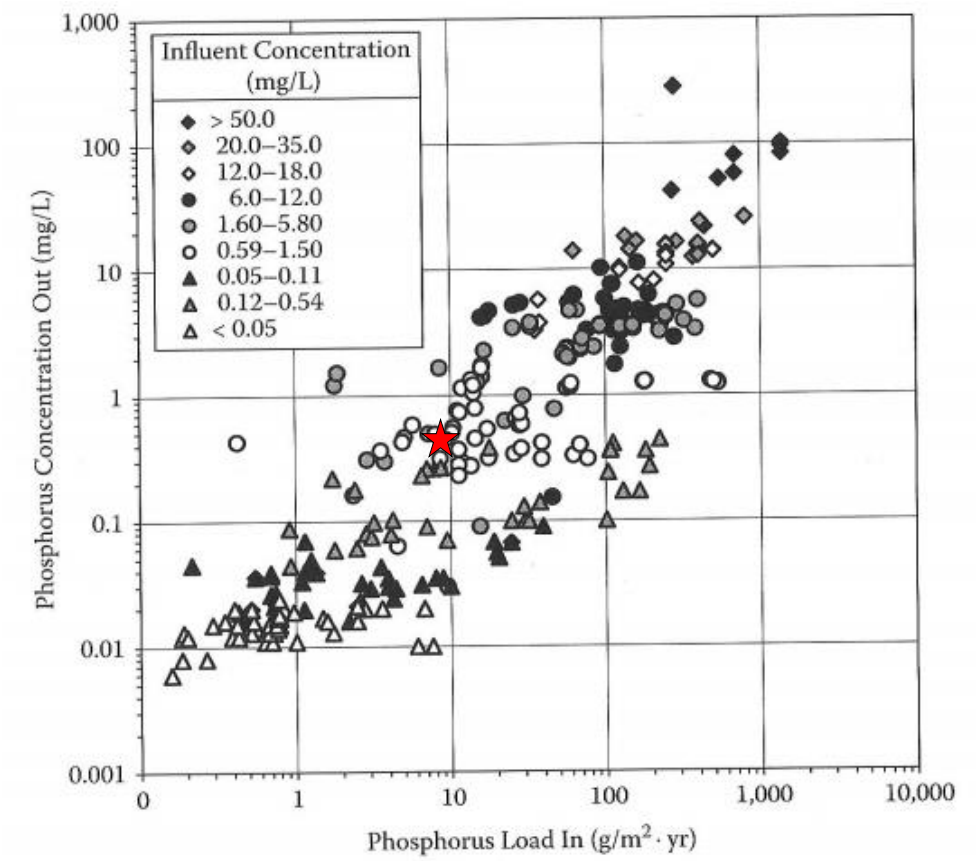

Figure 7-1: Phosphorus (load, input and outflow concentrations) in 282 free water surface treatment marshes, with data groups coded in nine influent concentration ranges (Kadlec and Wallace 2009, p.376). Modified to add the CWC wetland trial on the basis of the multiyear study results (red star, mean effluent TP concentration 2 mg/L).

Table 7-1: Summary of research findings in relation to the overarching research questions, sub-questions and hypotheses.

Sub-questions	Summary findings
Research Question 1 - How do P storage and cycling change as a wetland undergoes nutrient enrichment and how does this differ between functional zones?	
a) How does TP storage of ecosystem components change with enrichment and how does this differ between functional zones?	
H1: TP concentration of ecosystem components will increase with enrichment.	Mostly supported. The rate of response to enrichment differed between ecosystem components and no significant increases in TP concentration were observed in the *Typha* zone topsoil or in the subsoil of any zone.
H2: Mass of ecosystem components associated with primary production will increase with enrichment.	Poorly supported. Litter was the only component that showed consistent increases in mass with enrichment and these were likely not related to increased primary production as *Typha* biomass did not increase. Periphyton, SAV, *Typha* and floc had variable results.
H3: TP storage of ecosystem components will increase with enrichment.	Mostly supported (increases in TP storage of water, floc, litter, topsoil, SAV, periphyton) but the rate of response to enrichment differed between ecosystem components and functional zones. No significant increases in TP storage were observed in the *Typha* zone topsoil or in the subsoil of any zone. SAV initially increased in TP storage but was then lost. *Typha* TP storage decreased in the long-term, despite increases in TP concentration. In the mesocosm experiment, TP storage in bare sediment and SAV zones was greater in all shared stores (water, periphyton, topsoil, subsoil) compared to the *Typha* zone.
H4: Water TP concentration will be higher in unvegetated than vegetated zone(s) during enrichment.	Poorly supported. In the seasonal and multiyear studies, water TP concentration was not significantly higher in unvegetated than vegetated zones. In the mesocosm experiment, water TP concentration was higher in the bare sediment zone (unvegetated) than the *Typha* zone (vegetated) but similar between bare sediment and vegetated SAV zones.
b) How does the rate of response to enrichment differ between ecosystem components and how does this differ between functional zones?	
No specific hypothesis	Water, periphyton and SAV were the early responders to enrichment, followed by floc, litter and topsoil. Above-ground *Typha* and then subsoil were the slowest to respond. Topsoil was slower to respond in the *Typha* zone than in the bare sediment zone.
c) How does the distribution of total TP storage across the ecosystem components (% of total) change with enrichment and how does this differ between functional zones?	
H5: Proportional share of TP storage in soil will reduce.	Fully supported by all three studies (calculations excluded subsoil). Other findings: Topsoil was the largest store of P in all functional zones, with its share of TP highest in the bare sediment zone and lowest in the *Typha* zone. With enrichment, the proportional storage of TP increased substantially in floc and litter (from 4% to 50% and 51% respectively in the multiyear study). At the same time, the proportional share of topsoil decreased. The smallest share of TP was generally held by water, however decline in *Typha* in the multiyear study resulted in *Typha* becoming the smallest store.

Sub-questions	Summary findings
d) Where does the added P go?	
H6: Most added P will be taken up by soil.	Partly supported. In the bare sediment and SAV zones most of the added P was taken up by soil and then floc. In the *Typha* zone, most of the P in the long-term was taken up by litter, with soil showing no response.
Research Question 2 - How is nutrient enrichment reflected in ecosystem change in the different functional zones?	
e) How do vegetation characteristics change with enrichment in the different functional zones?	
H7: *Typha* height, biomass and density will increase with enrichment.	Poorly supported. Significant increase in height but significant decrease in biomass was recorded in the multiyear study. Density appeared to decrease in both seasonal and multiyear studies. No significant differences were found in the mesocosm study, likely due its short duration.
H8: SAV will be lost with enrichment.	Supported by the mesocosm experiment, not tested in other studies. The loss of SAV was preceded by occurrence of algal blooms with high turbidity and build-up of epiphytic algae on SAV.
f) How does water quality change with enrichment and how does this differ between the functional zones?	
H9: Algal bloom conditions (chlorophyll *a* > 30 µg/L) will occur with enrichment.	Well supported. Algal bloom conditions associated with enrichment were recorded in all studies and in all functional zones, with the exception of the *Typha* zone in the mesocosm study that suggested that algal blooms were slower to develop in the *Typha* zone.
H10: Water turbidity and chlorophyll *a* will be higher in unvegetated than vegetated zones.	Partly supported. Significant differences in support of the hypothesis were found in the mesocosm study, where turbidity and chlorophyll α were higher in the bare sediment zone than the *Typha* zone but differences between the bare sediment and SAV zones were not consistent, likely due to the loss of SAV. The multiyear study found no significant differences in chlorophyll α or turbidity between zones and the only significant difference in the seasonal study was opposite to the hypothesis (chlorophyll α higher in *Typha* than bare sediment zone).
H11: Water DO and ORP close to sediment surface will decrease with enrichment.	Partly supported in the multiyear study, where ORP decreased, while DO remained low (<1 - 4 mg/L). Supported in the seasonal study, where DO and ORP were often lower in the treatment than unimpacted wetlands. No significant differences in support of the hypothesis were found in the mesocosm study probably due to its short duration.

7.2 Refined conceptual models

This research refined the conceptual models developed through review of the scientific literature. The three studies best covered the first and second stages (pre-enrichment stage, functional P uptake stage), with the multiyear study extending into the third stage (hypereutrophic end-stage). The second and third stages were divided into two parts (Table 7-2) to better show how ecosystem components respond to enrichment at different rates and how characteristics of the hypereutrophic end-stage could be evident, even when the P

storage of some ecosystem components was not saturated. Changes made to the refined conceptual models (Figure 7-2, Figure 7-3, Figure 7-4) are summarised in Table 7-3. Periphyton was not included in the original conceptual models and was also excluded from the refined conceptual models (Figure 7-2, Figure 7-3, Figure 7-4) because while the sampling method was suitable for estimating accumulation of P in the mesocosm walls, the data collected was not sufficient for informing the conceptual models that involved different scales (open wetland versus mesocosm), different growth media (natural surfaces versus plastic) and different time scales (12 weeks versus several years) to the mesocosm experiment.

Table 7-2: Three key stages along nutrient enrichment continuum in regards to phosphorus (P) storage and cycling, refined on the basis of research findings.

Nutrient enrichment continuum →

Pre-enrichment stage (Oligotrophic -Mesotrophic)	**Functional P uptake stage (Eutrophic)**	**Hypereutrophic end stage**
• Low water P concentration with rapid uptake by other ecosystem components. • Low water chlorophyll α concentration (low suspended algae), resulting in clear water. • P mostly bound in soil. • Some seasonal P cycling (e.g. related to changes in temperature, water levels, biota lifecycles) but little overall accumulation apart from litter layer in the *Typha* zone.	• Incoming P readily taken up by ecosystem components that have not yet reached full storage capacity. • Early responders to enrichment likely to include water, periphyton and SAV and any other algae, followed by floc, litter and topsoil (latter in bare sediment zone only), with *Typha* and subsoil responding slowest. • Water P concentration may start increasing as the capacity of other components to remove P gradually decreases, or if the rate of P addition exceeds the rate of P uptake by the ecosystem components. • Decline and loss of sensitive or less competitive organisms starts.	• P uptake capacity of most ecosystem components exhausted. • P accumulates more rapidly in water resulting in more persistent algal blooms. • Some slower responding stores (e.g. subsoil) may have further P uptake capacity but due to the slow uptake rate, this will not prevent increases in water P. • Eventually long-term permanent removal of P will be limited to soil accretion. • Potential release of P to water from other ecosystem components (e.g. litter) if water and sediment quality decrease. • Only organisms suited for hypereutrophic conditions will survive.

Table 7-3: Summary of changes to conceptual models.

Functional zone	Pre-enrichment stage	Functional P uptake stage	Hypereutrophic end stage
Bare sediment zone	• None	• Floc - store added to model, increase in TP storage recorded. • Subsoil - expected increase in P storage was insignificant.	• Floc - store added to model, TP storage capacity potentially exhausted with loss of P. • Subsoil - expected increase in P storage insignificant. • Exhaustion of topsoil and subsoil capacity not observed (thus noted with '?' mark).
SAV zone	• None	• Floc - store added to the model with increasing TP storage. • SAV - Lost already at this stage (rather than the hypereutrophic end stage). • Subsoil - expected increase in P storage was insignificant.	• Floc - store added to model (no data for this stage in this zone) • Exhaustion of floc, topsoil and subsoil capacity not observed (thus noted with '?' mark).
Typha zone	• None	• Topsoil - expected increase in P storage not observed. • Subsoil - expected increase in P storage not observed. • *Typha* - expected increase in above-ground TP storage not observed in these studies due to short-time scale (mesocosm study) or synergistic impacts of nutrient enrichment with water level increase (multiyear and potentially seasonal study) however for the purposes of the model increase in TP storage is assumed due to wide support in literature.	• Topsoil - expected increase in TP storage not observed. • Subsoil - expected increase in TP storage not observed. • *Typha* - expected increase in above-ground TP storage changed to ultimate decrease as the studies showed *Typha* can be adversely impacted by enrichment (especially when combined with water level increase).
All	• None	• Stage split to differentiate between early and later responders.	• Stage split to show trophic status turning hypereutrophic despite some ecosystem components P storage not saturated.

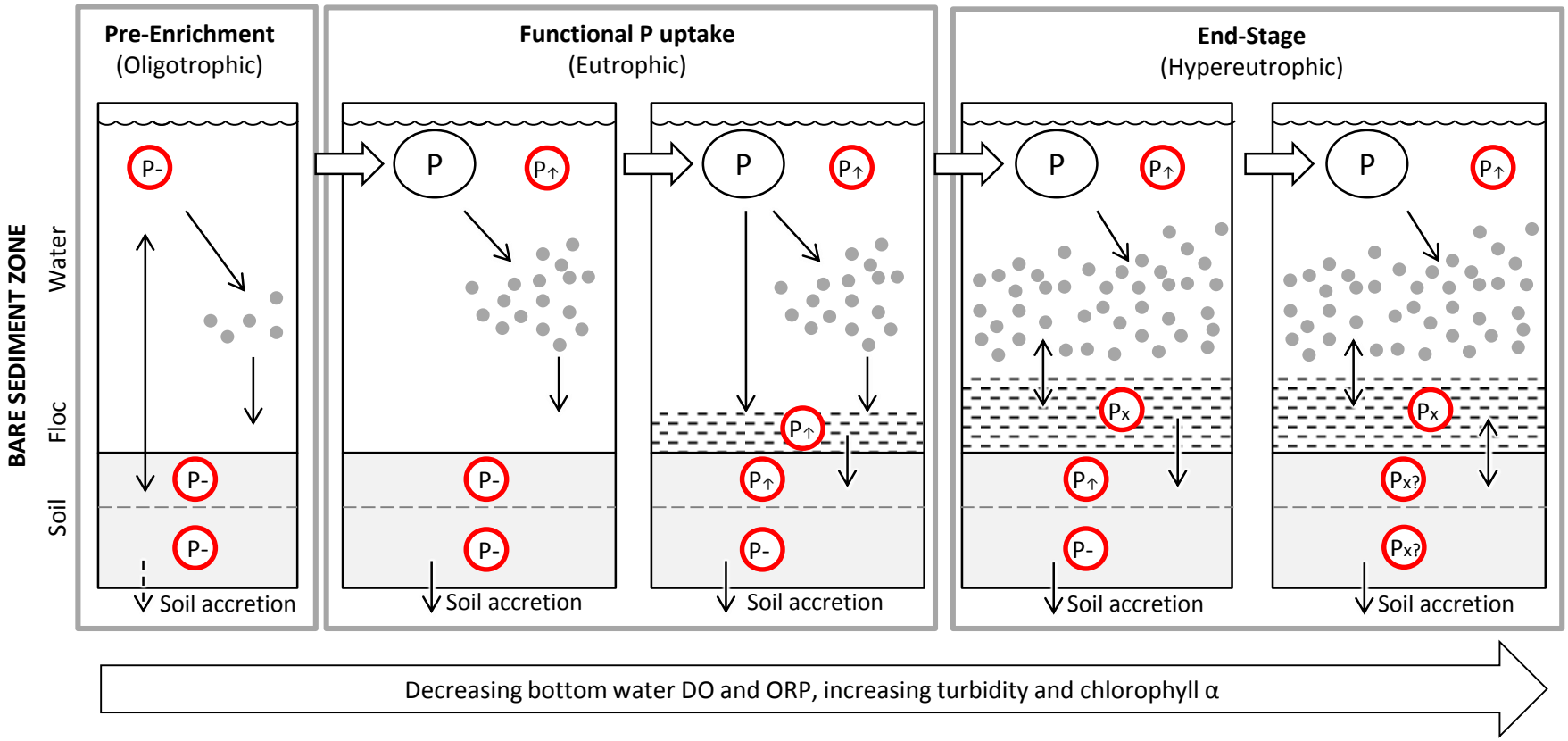

Figure 7-2: Conceptual models of phosphorus storage and cycling in the bare sediment zone based on research findings and/or literature. Arrows indicate phosphorus cycling pathways. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached, ? = uncertain. Dots indicate relative size of algal bloom.

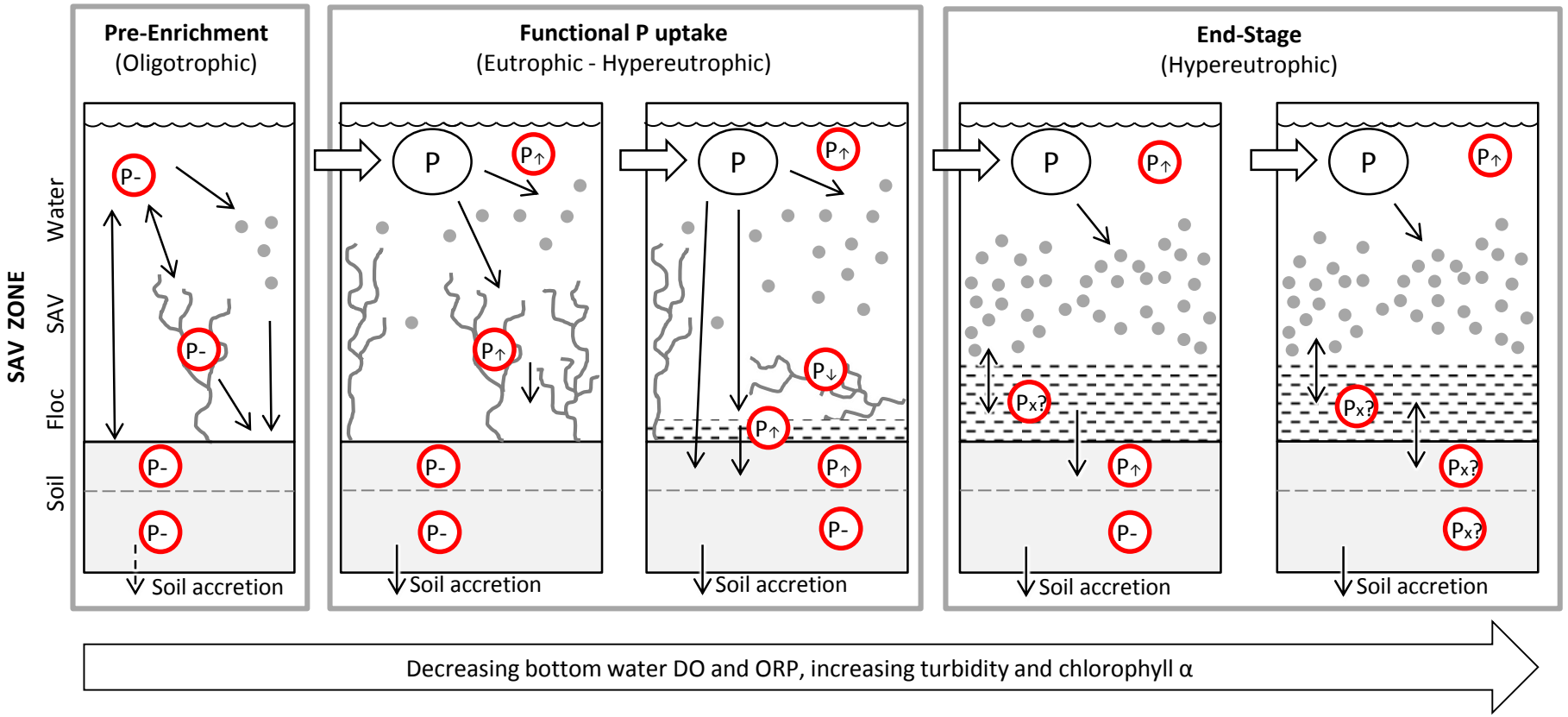

Figure 7-3: Conceptual models of phosphorus storage and cycling in the SAV zone based on research findings and/or literature. Arrows indicate phosphorus cycling pathways. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached, ? = uncertain. Dots indicate relative size of algal bloom.

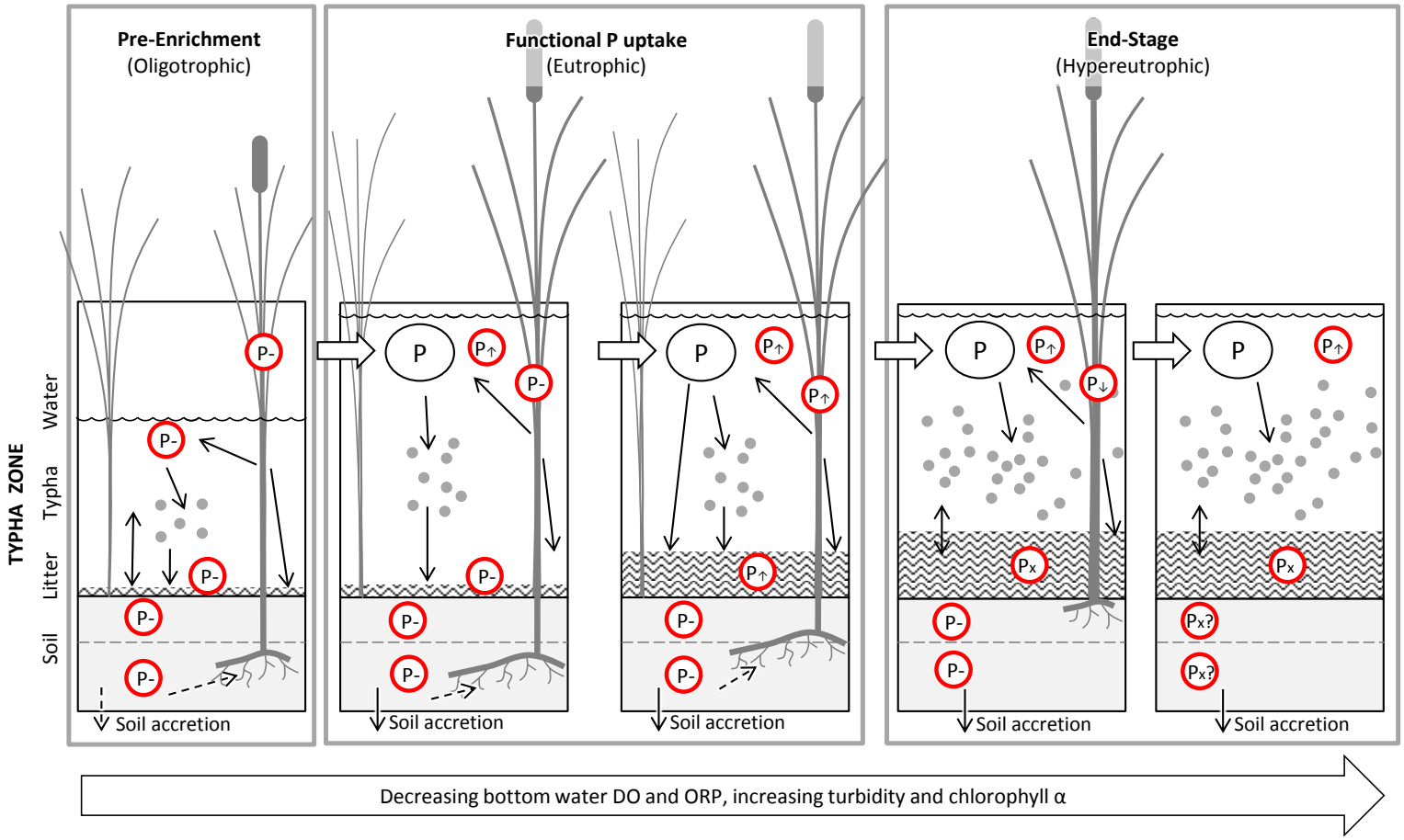

Figure 7-4: Conceptual models of phosphorus storage and cycling in the *Typha* zone based on research findings and/or literature. Arrows indicate phosphorus cycling pathways. Change in TP storage is indicated by ↑ = increase, ↓ = decrease, - = no change, x = P storage capacity reached, ? = uncertain. Dots indicate relative size of algal bloom.

7.3 What does this tell us about the eutrophication process?

7.3.1 Pre-enrichment stage

Processes in the unimpacted wetlands (Chapter 4) and control mesocosms (Chapter 6) concurred with the conceptual models developed for the pre-enrichment stage based on the literature. Cycling of P between ecosystem components occurred over time, however the quantities of P involved were trivial compared to quantities during enrichment. As anticipated, the studies showed no significant accumulation of P in the ecosystem components due to the lack of P inputs. Changes in P storage and concentration of components (especially water, topsoil, *Typha*) were dominated by seasonal variation that appeared largely controlled by seasonal dynamics in water regime, temperature and vegetation growth patterns as shown by others (Howard-Williams 1985, Kadlec and Reddy 2001, Kadlec 2016).

7.3.2 High nutrient loads may modify the eutrophication pathway

Eutrophication associated with the medium-high P load was expected to follow a gradual pathway with the P storage capacity of the various ecosystem components being saturated prior to significant accumulation of P in the water and emergence of algal blooms. Instead, many of the key characteristics of the hypereutrophic end-stage were reached before P storage capacity of some ecosystem components was exhausted. On that basis, I propose that excessive P loads (higher than observed in this thesis) might follow a different eutrophication pathway (Figure 7-5, Table 7-4). Removal of P from water by different ecosystem components occurs at different rates. Under excessive P loads, uptake by some stores will be too slow to prevent rapid accumulation of P in the water column and associated emergence of algal blooms. This means that under excessive P loads the wetland would effectively bypass the gradual functional P uptake stage and jump straight into hypereutrophic end-stage. P uptake by ecosystem components would continue, however such removal would not make a significant difference in terms of the trophic state of the system as the rate and scale of such removal would not match the rate and scale of incoming P. Furthermore, the water quality modification might prevent effective uptake by slower reacting stores e.g. high water TP concentration and algal blooms would drive deterioration of water and sediment quality (decrease in DO and ORP), and consequently the actual P uptake capacity of soil and litter would be lower than under the more gradual enrichment pathway. Additionally, water quality and sediment changes would cause more rapid loss of macrophytes (SAV and potentially also

more robust species like *Typha*) and this would further reduce P uptake and storage capacity of the wetland compared to the more gradual enrichment pathway.

The impact of P load on the speed and type of eutrophication pathway is summarised in Table 7-4 and Figure 7-5. When attempting to manage a wetland undergoing eutrophication it is vital to understand the magnitude of the P load in context of the assimilative capacity of the system as the P load may directly affect the ecosystem response. Reducing incoming P concentration and loading should not just be a long-term goal. If nutrient load can be reduced in the short-term (by some form of intervention) in the early stage of eutrophication, the ecosystem components will have more time to respond and in the longer term will be able to assimilate more nutrients without drastic changes to ecosystem character, while also retaining better ecosystem resilience.

How was this concept illustrated in the three studies?

The P load in the three studies relative to the unknown P assimilation capacity of the wetlands appeared high, potentially bordering excessive (using the terminology in Figure 7-5). While gradual P uptake was observed in some ecosystem components (floc, litter, topsoil in bare sediment zone), others were not observed to respond at all (topsoil in *Typha* zone, subsoil both zones) or were adversely affected by the synergistic effect of nutrient and water addition without significant uptake (*Typha*). The mesocosm experiment showed instantaneous emergence of algal blooms and high water TP concentration in the bare sediment and SAV zones following commencement of treatment, whereas no such change was observed in the *Typha* zone. This suggested that the P assimilation capacity of the *Typha* zone was higher and illustrated how the same P load can result in different eutrophication pathways in different systems (differences between functional zones discussed further in Section 7.3.4). Reduction in the P dosing frequency (reducing load over time) in the mesocosm experiment, resulted in decreased water TP concentration (in bare sediment and SAV zones) despite increasing cumulative P load. This shows how the ecosystem components were better able to remove P from water under the lower P load, potentially resulting in the systems following a more gradual eutrophication pathway.

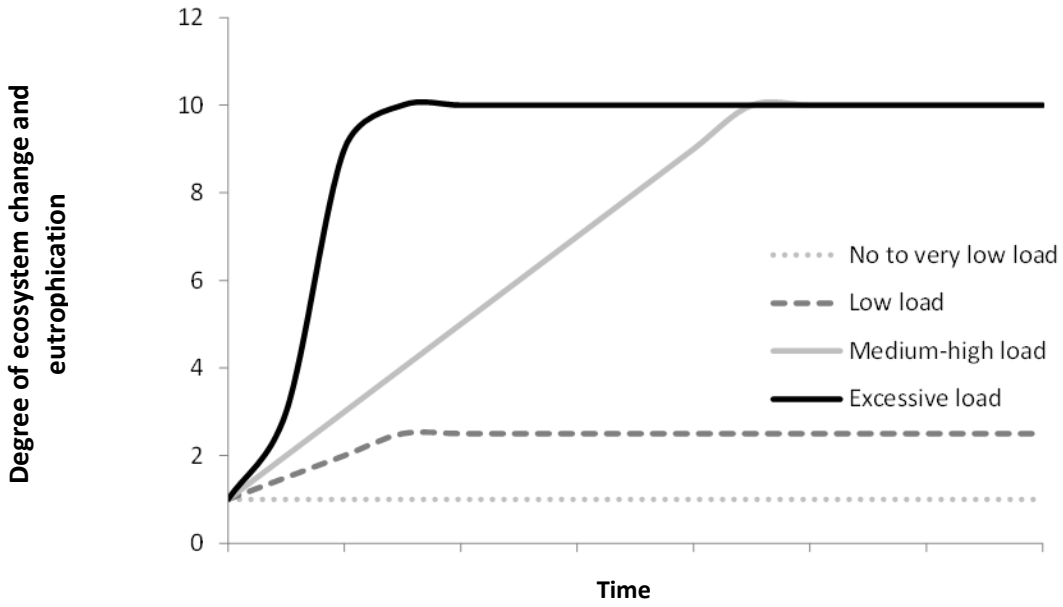

Figure 7-5: Conceptual model of the influence of phosphorus load (relative to the phosphorus assimilation capacity of the wetland) on the degree of ecosystem change and eutrophication over time.

Table 7-4: Influence of phosphorus (P) load on the speed and type of eutrophication pathway. Assumes the load remains relatively stable over time (press rather than seasonal pulse type load).

P load	Sustainability	P storage and cycling	Ecosystem change	Outflow P concentration change
None to very low	Load remains within sustainable P removal capacity	Minor changes in quantity of P stored and cycled but no changes to P stores or cycling pathways. The added P can be assimilated by the existing processes.	No ecosystem change	No change to outflow P concentration
Low	Load remains within adjusted sustainable P removal capacity	Changes in biological stores (e.g. increase in biomass, emergence of alternative species) creating new storage pathways and increasing sustainable P removal capacity.	Some ecosystem changes followed by stabilisation (e.g. changes in species composition)	No change or small increase followed by stabilisation
Medium-High	Load above sustainable P removal capacity	Changes to biological stores and cycling pathways as above. Gradual exhaustion of P storage capacity of ecosystem components, eventually resulting in P removal being limited to soil accretion, accumulation of P in water and P cycling pathways associated with algal blooms becoming more dominant. Potential P release from sediment and microbial stores as water and sediment quality deteriorate.	Significant ecosystem changes over time (e.g. loss of sensitive species, permanent shift from macrophyte dominated to phytoplankton dominated). Gradual reductions in bottom water and sediment DO and ORP.	Significant increase as the storage capacity of ecosystem components exhausted
Excessive	Load greatly exceeds sustainable P removal capacity	Rate of P addition so high that it exceeds the speed of P uptake by ecosystem components and rapidly accumulates in water. Algal blooms develop quickly and water and sediment quality deteriorate quickly, resulting in early loss of macrophytes. The full P storage capacity is never utilised, unlike above, and rather storage capacity decreases rapidly via loss of macrophytes and changes in sediment quality (lower DO and ORP meaning lower P storage capacity). Long-term P removal limited to soil accretion.	Above mentioned ecosystem changes occur rapidly.	Rapid increase in outflow P concentration.

7.3.3 Temporal responses to eutrophication and their scale

The scale (magnitude of uptake/release) and timing of response to nutrient enrichment differed between ecosystem components. This means that the availability of different P uptake pathways and their importance to the overall P uptake changes over time across the enrichment continuum. These differences in scale and timing of response between ecosystem components could not be effectively presented in the refined conceptual models (Figure 7-2, Figure 7-3, Figure 7-4) and thus a different set of models were created focusing on this aspect (Figure 7-6, Figure 7-7).

Figure 7-6 and Figure 7-7 show the scale of net P uptake or release by the various ecosystem components over time, relative to the overall P storage. Additional pie charts show the proportional share of total TP storage by the ecosystem components (excluding subsoil). The figures are based on interpretation of the results from the three studies (enriched systems only) and predictions from literature. The models focus on the functional P uptake stage and early stages of hypereutrophy. Creating a multiyear model for SAV (that was only studied in the short-term mesocosm experiment) involved extrapolating results from the mesocosm study and as well as applying findings from the bare sediment zone. The *Typha* zone model is based on the case of declining *Typha* that was observed in the multiyear study due to the synergistic effects of water depth increase and water quality decrease. As discussed later this is not a commonly recorded outcome of enrichment in *Typha* systems.

In Figure 7-6 and Figure 7-7 net P uptake means increase in TP storage of the component over time, net P release a decrease in TP storage. 'Stable' means lack of significant change in TP storage over time and excludes potentially significant seasonal cycling that was not incorporated into these models. The models do not include losses from the system e.g. via groundwater seepage, as this was not directly measured. Periphyton was also excluded from the models, as the short-term sampling undertaken was not suitable to inform the development of long-term conceptual models at ecosystem scale (see reasons described at the end of Section 7.2).

Only one similar example of illustration of the scale and timing of P uptake pathways was found in literature (Figure 7-8, by Richardson and Marshall 1986), however it relates more to the importance of different pathways to P cycling whereas my models are more specific to P uptake and release. The P load used for Figure 7-8 was approximately 60 kg/ha/year (6 g/m^2/year) and thus lower than the loads in this thesis. The following paragraphs discuss

the scale and timing of responses by the different ecosystem components in the order of response rate (from fast to slow responders) and also address some of the similarities and differences between my models (Figure 7-6, Figure 7-7) and Figure 7-8.

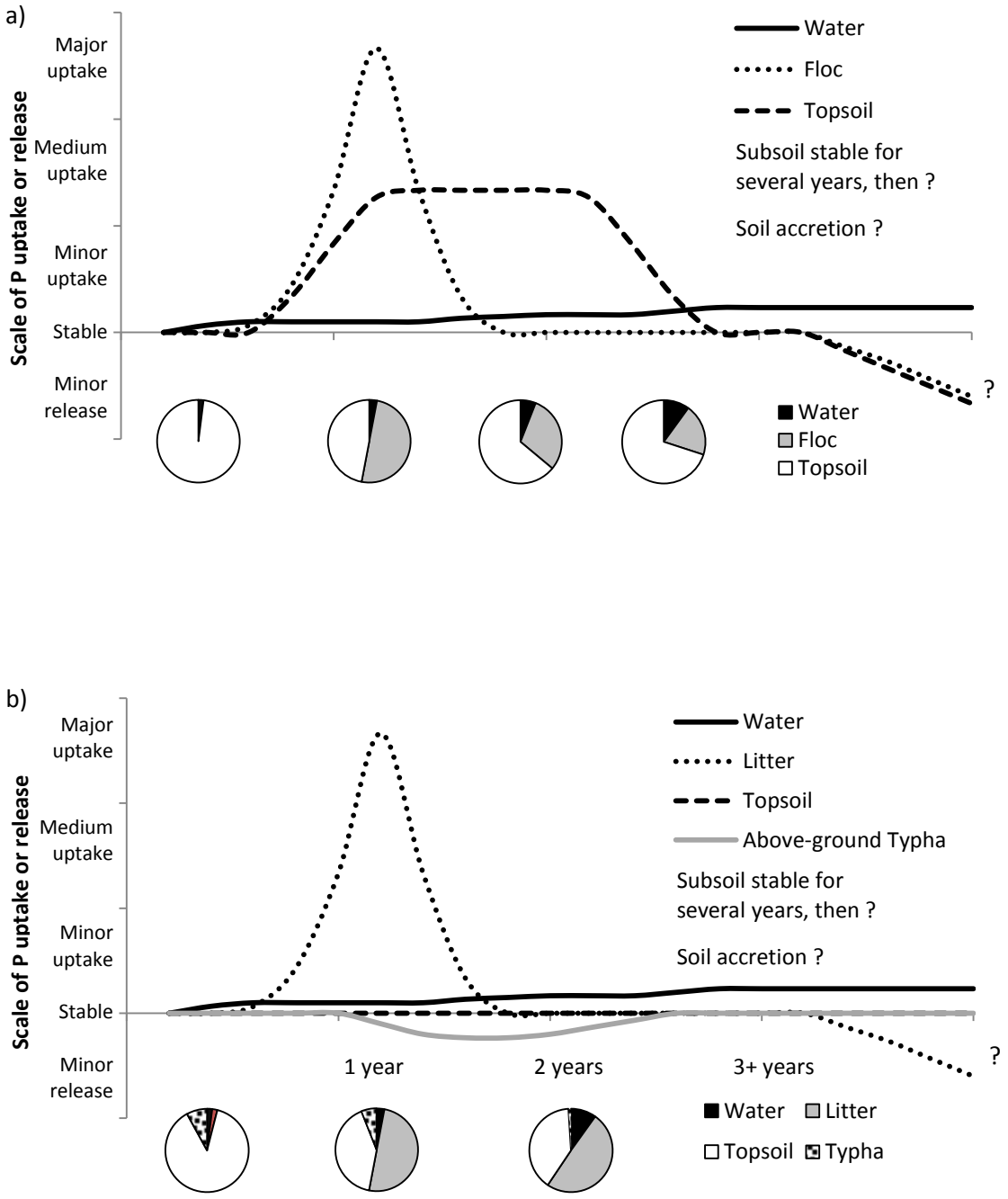

Figure 7-6: Conceptual model of scale (y-axis) and timing (x-axis) of phosphorus uptake and/or release by ecosystem components relative to overall P storage in bare sediment (a) and *Typha* (b) zones of the CWC treatment wetland receiving approximately 9.1 g/m^2/year of P. Pie charts illustrate the proportional share of total TP storage by the ecosystem components (excluding subsoil). Based on research findings and/or predictions from literature. The *Typha* zone model presents the case of declining *Typha* due to combined effects of water level increase and water quality decrease. Seasonal variation not included.

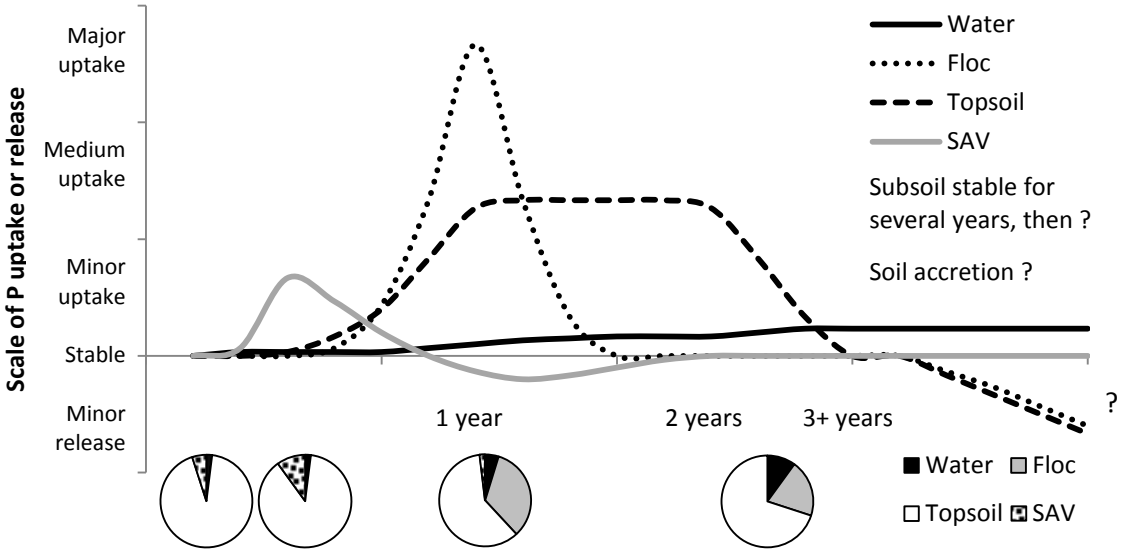

Figure 7-7: Conceptual model of scale (y-axis) and timing (x-axis) of phosphorus uptake and/or release by ecosystem components relative to overall P storage in SAV zones of the CWC treatment wetland receiving approximately 9.1 g/m^2/year of P. Pie charts illustrate the proportional share of total TP storage by the ecosystem components (excluding subsoil). Based on research findings and/or predictions from literature. Seasonal variation not included.

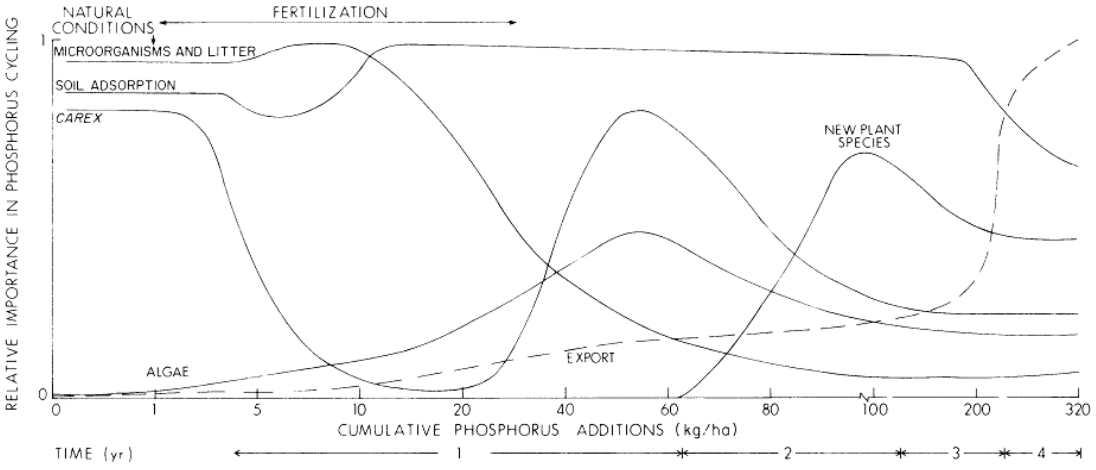

Figure 7-8: Conceptual model by Richardson and Marshall (1986) of the relative importance of biotic and abiotic components controlling new additions of phosphorus in the Michigan fen. The trend lines (y-axis) are not to scale and only reflect the proportional amount of P influenced at each level of addition. The x-axis has a dual legend that denotes inputs of P and time. Seasonal variations are not included. The local introduction of new species and the export of P increase significantly after year 1 and 3, respectively.

Rapid responders

Rapid responders to enrichment were best detected in the short-term mesocosm study. Within two weeks of initial nutrient dosing, significant P uptake was observed in water (including suspended algae), periphyton and SAV (charophytes). Previous research found that some stores like microbiota, phytoplankton and algae are able to respond to enrichment immediately (Havens *et al.* 1999, Noe *et al.* 2003, Kadlec and Wallace 2009, Kobayashi *et al.* 2009) and this was broadly supported because all of the early responders included forms of algae and likely forms of microbiota and phytoplankton.

The total P uptake by the rapid responders was small in these studies. The initial uptake by the water component was so small in scale that was is difficult to differentiate from 'stable' when presented in the context of later more substantial P uptake pathways (Figure 7-6, Figure 7-7). P uptake by water continued over time in the multiyear study (also illustrated in Figure 7-6, Figure 7-7) but the uptake remained minor in scale compared to other stores (0.7 g/m^2, or approximately 7% of the total TP storage per m^2 excluding subsoil). Although the scale of uptake was small in comparison to other stores, it corresponded an increase of over 3000% in the water TP storage over time (in multiyear study). This increase was capable of fuelling algal blooms and thus driving major changes in ecosystem character (e.g. a shift from clear to turbid water, loss of SAV, DO depletion due to oxygen demand of decomposing algae, release of sediment bound P) (Smith 2003, Verhoeven *et al.* 2006, Boulton *et al.* 2014). The mesocosm experiment also indicated that algal blooms could develop very rapidly at the very start of the enrichment continuum when the load was high and other ecosystem components were unable to remove P sufficiently quickly. Overall, the changes in water TP and algal production in these studies highlight how wetlands are highly sensitive to nutrient enrichment. While wetlands are able to remove P from water and store it in large quantities in other ecosystem components (e.g. sustainable retention of 0.5 - 5.0 g/m^2/year in constructed wetlands suggested by Mitsch *et al.* 2000), only a small amount of excess P in water can rapidly drive ecosystem change through the algal pathways. For example, hypereutrophic conditions with algal blooms are associated with water TP concentrations of more than 100 µg/L (OECD 1982), which corresponds to only 0.1 g/m^2 in a 1 m deep wetland (0.6% of the total TP load of 16 g/m^2 added to the enrichment mesocosms in the 13 week study).

TP storage of SAV increased five-fold immediately following commencement of enrichment (mesocosm experiment) due to increase in TP concentration. SAV provided a substantial initial P uptake pathway, storing more P than litter and *Typha* at that stage. However, because SAV was adversely impacted by enrichment and soon lost from the enrichment mesocosms, most

of this stored P was released. The uptake and release of P by SAV was included in the model for the SAV zone (Figure 7-7). Although SAV was not studied in the multiyear setting it was concluded that the conditions observed in the bare sediment and *Typha* zones early in the enrichment would have likely resulted in P uptake by SAV similar to that observed in the mesocosm experiment. Subsequent deterioration in water quality (increased turbidity and suspended algal growth) in the bare sediment and *Typha* zones, would likely have resulted in loss of SAV, without reoccurrence. Previous research has indicated that SAV can be effective in removal of P in lightly loaded systems (Gumbricht 1993, Dierberg *et al.* 2002, Knight *et al.* 2003, DeBusk *et al.* 2011) but is commonly lost with higher loads due to increases in turbidity (Blindow 1992, Scheffer *et al.* 2001, Kufel and Kufel 2002, Romo *et al.* 2004) and epiphyte growth (Phillips *et al.* 1978, Asaeda *et al.* 2004, Phillips *et al.* 2016) and this was supported by these studies. Thus the model for the SAV zone (Figure 7-7) is applicable to heavily loaded systems only (approximate P load in multiyear study 9.1 g/m^2/year). In systems where P load is lower than in these studies (e.g. 1.75 g/m^2/year in DeBusk *et al*. 2011), the uptake by SAV (and other components) would be expected to maintain clear water conditions and thus SAV for much longer. In that case, the model would show a longer period of P uptake and storage by SAV and delayed release of P from SAV.

The short lifecycles of the organisms involved in early response (algae, microbiota, macroalgal SAV) mean that the great majority of P stored in biomass is regularly released and recycled, adding to the complexity of temporal patterns over the nutrient enrichment continuum. Such seasonal and shorter time scale cycling was not illustrated in the models.

Slower responders

Overall, floc, litter and topsoil responded slowly. In contrast to the rapid responders, they took up to an order of magnitude more P (as illustrated in Figure 7-6 and Figure 7-7) and stored it for a longer term (years). These three components stored the majority of P accumulated in the treatment wetland and in the mesocosm experiment. They also contributed to differences between zones in all studies because floc was only present in bare sediment or SAV zones, litter only in *Typha* zone and topsoil only took up P in the bare sediment and SAV zones (as illustrated in Figure 7-6 and Figure 7-7).

Substantial P uptake by floc has been recorded in the past, for example, Noe *et al.* (2002) found increases in the proportional TP storage held by floc in a six-month study. However, floc remains poorly researched. The short-term mesocosm experiment showed that accumulation of fine detrital organic matter from dying SAV resulted in the creation of floc. No floc

developed in the bare sediment mesocosms that lacked such organic matter. In the multiyear study, the accumulation of fine detrital organic matter from algal blooms, dying *Typha* and potentially incoming effluent similarly stimulated floc production, however this time in the bare sediment zone. The difference in presence/absence of floc in the bare sediment zone between the studies was likely due to the short-duration and enclosed nature of the mesocosm study, that allowed less time for internal production of organic matter (e.g. algae) and prevented inflows of organic matter. The mostly inorganic composition of floc in the treatment wetland (77%, K. Kauhanen unpublished data) suggests that over time floc formation involved mixing of organic matter with underlying clay and silt slimes (e.g. through bioturbation and wind mixing).

In the multiyear study, floc TP concentration and mass both increased initially but then stabilised, indicating that storage capacity may have been reached, owing to the gradual incorporation of floc into topsoil through accretion and the low ORP and DO conditions reducing additional P uptake by microbiota (McLatchey and Reddy 1998, Grace *et al.* 2008). Anaerobic conditions increase the solubility of P by weakening bonds with Al and Fe (Reddy and DeLaune 2008), probably also limiting further increase in TP concentration of floc. Overall, the three studies highlight the capacity of floc to take up substantial amounts of P relatively rapidly early in the enrichment process but also indicate that this storage capacity may become exhausted equally rapidly. Consequently, the P sink could turn into a P source, if water and sediment quality deteriorates (Figure 7-6, Figure 7-7).

Substantial P uptake by litter has been recorded in the past (Richardson and Marshall 1986, Kadlec and Bevis 2009) associated with the microbial component of this store (Qualls and Richardson 2000, Grace *et al.* 2008). However, Richardson and Marshall (1986) noted that the litter and microbial P uptake pathways appeared to decrease in importance following the early stages of enrichment (Figure 7-8). Similarly, in the multiyear study, litter TP storage increased substantially but then stabilised, reflecting similar stabilisation in TP concentration despite continued increase in litter mass across the study. Increased mass was likely due to ongoing litter fall from dying *Typha.* Whereas, the stabilised TP concentration could have arisen from the microbiota reaching their uptake limit (i.e. maximum litter TP concentration) or the deterioration in water and sediment quality (described above) preventing further increase (see Sections 5.4.2 and 5.4.3). As observed for floc, the three studies highlighted the potential of litter as a store of P early in the enrichment process, but also showed that the uptake pathway may be rapidly exhausted and the P sink could turn into a P source if conditions change (Figure 7-6).

While the first response by topsoil TP storage to enrichment was detected around the same time as in floc and litter, the overall pattern in quantity and timing of P uptake was different. Floc and litter took up a very large quantity of P rapidly and then stopped taking up further P whereas topsoil P uptake rate was lower but uptake lasted much longer (Figure 7-6, Figure 7-7). The models assume that the P uptake capacity of topsoil will eventually be exhausted even though this was not observed in the multiyear study (it was likely too short). The composition of topsoil in Swamphen Lake gave it a high P sorption capacity (15 g/m2 of P in the top 1 mm of the sediment based on Langmuir equation, Chambers and McComb 1996). Yet, the highest mean TP concentration (1.0 mg/g) and storage (6.9 g/m^2) recorded for topsoil in the multiyear study were low in comparison to previously recorded values at other sites (Table 2-3, Table 2-4). Thus, P uptake by topsoil is expected to continue in Swamphen Lake for some time, as predicted by the conceptual model by Richardson and Marshall (1986) (Figure 7-8). However, as discussed above with floc, reductions in water and sediment quality (reduced DO and ORP) could potentially limit soil uptake and even result in the P sink turning into a P source; potential P release from soil is illustrated in the models (Figure 7-6, Figure 7-7). Due to the large amount of P stored in soil by the end of the multiyear study (3 years), a substantial quantity of P may be released from sediment in the future. This is particularly problematic for restoration of eutrophic wetlands, as even after drastic reductions in external P inputs, the internal release of P from sediment can continue to maintain eutrophic conditions with algal blooms long into the future (Scharf 1999, Søndergaard *et al.* 2003, Jeppesen *et al.* 2005, Song and Burgin 2017).

Whether topsoil in the *Typha* zone would contribute to P removal at a later stage is difficult to predict, thus the scale and timing of such uptake has not been included in the models (Figure 7-6, Figure 7-7). The poor exposure of *Typha* zone topsoil to nutrient enriched water due to the litter layer may also limit P uptake in the future. However, gradual movement of P into these deeper layers could also be seen over longer periods of time. If *Typha* zone topsoil does not contribute to P removal, then a major potential sink of P has been lost in this zone, reducing the overall P retention capacity of the wetland. However, this is unlikely to make a practical difference, as the uptake process would be too slow to ameliorate the hypereutrophic state of the wetland.

Slowest responders

Subsoil TP storage did not increase significantly in any of the studies, possibly because the degree of accumulation could not be detected against background of high initial storage and

variability between sampling occasions and sites. Subsoil TP storage decreased over time in the seasonal study, but the multiyear study that followed showed that levels recovered within 3 months (thus potentially showing seasonal cycling); no further change was observed in the multiyear study. For the purpose of the models, subsoil TP storage was assumed to remain stable (thus not illustrated in Figure 7-6, Figure 7-7), although potential long-term changes are uncertain. It is possible that even the multiyear study was too short to observe significant changes in subsoil TP storage. The heavy clay soil had very low hydraulic conductivity, meaning slow movement of water down the profile, together with high likelihood of P uptake by topsoil (Chambers and McComb 1996) prior to reaching subsoil. Even if P uptake by subsoil did occur in the future, it would not change the overall degree of wetland eutrophication because the uptake processes involved would be too slow to prevent P accumulation in water and subsequent adverse ecosystem outcomes. In wetlands with higher hydraulic conductivity of soil, changes in subsoil TP storage could be observed more quickly and subsoil could potentially play a more important role in preventing P accumulation in water (until saturated).

Above-ground *Typha* was slow to respond to nutrient enrichment; no enrichment-related changes in above-ground *Typha* biomass or live *Typha* TP concentration were observed in the two shorter studies (mesocosm and seasonal studies). Others have also found emergent macrophytes to respond slowly (Richardson and Marshall 1986, Gaiser *et al.* 2005). *Typha* did ultimately respond to nutrient enrichment in the multiyear study, showing significant increases in live *Typha* TP concentration. However, decreased above-ground *Typha* mass (caused by deteriorating water quality and increased water depth) resulted in decreased TP storage in *Typha* over the same time period. Thus, the *Typha* zone model (Figure 7-6b) shows no P uptake but rather only release by above-ground *Typha*. Wetlands with lower water depths and/or lower nutrient load, would likely see P uptake by *Typha*, followed by stabilisation (Kadlec and Wallace 2009), and with some future potential for P release if decreased oxygen availability caused *Typha* decline (as illustrated in Figure 7-4).

The three studies did not sample below-ground parts of *Typha,* as a pilot study was unable to develop reliable methods for extracting reproducible samples of below-ground *Typha* in the wet, heavy clay soil. However, mass balance calculations for the enclosed mesocosm enrichment experiment indicated that below-ground *Typha* (and/or suspended *Typha* thatch) potentially took up some of the added P (see Section 6.4.3). Although some research has shown substantial P uptake by below-ground parts of *Typha* (Dolan *et al.* 1981, Di Luca *et al.* 2015), the information provided was insufficient in regards to the scale or timing of the

response, or the proportional storage of total P, to satisfactorily inform the conceptual models (Figure 7-4, Figure 7-6b).

Rate of response to enrichment differs between TP concentration and mass

In the stores related to biological productivity, TP concentration and mass responded to enrichment at different rates. Changes in TP concentration happened more quickly than changes in mass. Maximum TP concentrations could be reached relatively quickly whereas mass can potentially increase for long periods. Existing biomass has capacity to take up additional P (i.e. luxury uptake, Reddy and DeLaune 2008, Rejmánková *et al.* 2008, Brown and Shilton 2014) but increase in biomass is subject to the use of added P for growth. For some stores, growth and turnover in mass could be rapid (e.g. algae, microbiota) (Cuellar-Bermudez *et al.* 2017) but for others (e.g. emergent macrophytes) it could take years to reach maximum biomass under enrichment conditions (e.g. emergent macrophytes) (Kadlec and Wallace 2009). Overall, the quantity and timing of P uptake reflects the combined effect of initial increase in TP concentration followed by longer-term increases in mass. In order to determine whether net TP storage of an ecosystem component is increasing, decreasing or stable at any particular point in time, wetland managers will need to sample both TP concentration and (the more labour intensive) mass, as TP concentration alone is a poor indicator of whether net uptake is occurring.

Detection of enrichment responses

Detection of significant enrichment responses was easier in some stores than others due to differences in the quantity of TP stored and its variation between stores. For example, TP storage in water was small and spatial variation within sampling occasions was low, whereas soil TP storage was much larger and spatial variation within sampling occasions was also higher. This meant that a significant increase in TP storage of 0.5 g/m^2 was detected for water (representing an increase of several thousand %) whereas the same magnitude of change (representing an increase of 15%) could not be detected for topsoil or subsoil (representing an increase of only 1%).

7.3.4 Differences in response between functional zones

The mesocosm experiment highlighted differences between functional zones that became apparent early in the enrichment process. All of the shared stores (water, periphyton, topsoil and subsoil) consistently showed larger treatment effects (increase in P storage) in the bare sediment and SAV zones than in the *Typha* zone. This difference between zones was likely due

to the *Typha* zone having more alternative P uptake pathways and greater storage capacity (effectively higher sustainable P assimilation capacity) than bare sediment and SAV zones, especially once SAV was lost. Vegetated wetlands remove P more effectively from water than unvegetated wetlands, thus maintaining better water quality (Tanner *et al.* 1995, Huett *et al.* 2005, Greenway and Lucas 2008, Menon and Holland 2013). Eutrophication may progress more slowly in vegetated wetlands and also in wetlands that have large vegetated zones in addition to bare sediment (open water) areas. This could not be examined in my whole-wetland studies because they all included several different zones. The whole wetland studies did not find evidence of eutrophication progressing more slowly in the *Typha* than the bare sediment zone, but this may have been due to the decline in *Typha* in the treatment wetland as healthy *Typha* in the mesocosms did effectively slow the eutrophication process.

SAV provided a rapid uptake pathway for P that was missing from the bare sediment zone and which resulted in lower phytoplankton production in the SAV zone in the early stages of enrichment. As SAV was lost, the dead SAV had legacy effects. Firstly, the release of nutrients from dead and dying SAV back into the water increased its TP concentration such that it was higher than in the bare sediment zone. It may have thereby contributed to the higher treatment effect observed in periphyton in the SAV zone compared to other zones. Furthermore, the SAV zone was the only zone in the mesocosm experiment to develop floc, and dead SAV may have contributed to the floc store (although due to high variability among mesocosms an increase in floc mass or TP storage was not detected). The maximum amount of P stored in SAV was only 0.8 g/m^2, which compared to the total P load over the course of the study (16 g/m^2), was unlikely to cause major differences in the progress of nutrient enrichment in these wetlands.

In the seasonal study, no differences in TP concentration, mass or TP storage of shared ecosystem components (water, topsoil, subsoil) was found between functional zones in the treatment wetland, potentially because the effects of enrichment were only just emerging towards the end of the experiment. However, litter present only in the *Typha* zone took up the majority of added P there, indicating that the *Typha* zone was more actively removing P from water than the bare sediment zone, at least during this early stage of enrichment. The multiyear study showed strong initial P uptake by floc in the bare sediment zone and by litter in the *Typha* zone, however these pathways did not continue to take up P past the second year. The only other large P uptake pathway was topsoil in the bare sediment zone, which continued to sustain its P uptake rate over the multiyear study period. Following the large early uptake of P by litter, the *Typha* zone failed to provide further pathways for continued P

removal. In contrast, P removal in bare sediment continued, following early rapid uptake by floc, through the topsoil uptake pathway. The lack of P removal pathways in the *Typha* zone partly reflected the decline in *Typha* and consequent release of P by dead plant matter. Wetlands with healthy *Typha* would be expected to see continued P uptake by growing *Typha* biomass for several years (Macek *et al.* 2010), as well as microbial P uptake associated with the growing standing stock of dead litter biomass (Richardson and Marshall 1986) and P uptake by periphyton on live and dead plant tissue (Ray *et al.* 2014).

Overall, the three studies showed that the functional zones responded differently to enrichment, partly due to the presence of different P uptake pathways and storage capacity but also partly due to interactions between stores that may affect the timing and quantity of uptake (discussed further in next section). For wetland managers this means that even if P uptake by an ecosystem component (e.g. topsoil) is recorded in one zone, this does not mean that the same component (e.g. topsoil) is simultaneously taking up similar quantities of P in all zones. In fact, that component might never take up P in the other zones. Also, even if the uptake capacity of a component (e.g. topsoil) has been reached in one zone, that does not mean that its capacity has been reached in all zones.

7.3.5 Interactions between ecosystem components

Interactions between ecosystem components were a critical element in the progress of eutrophication. Although topsoil was known to have a high P uptake capacity, the availability of this pathway (or at least the rate of uptake) was dependent on the nature of the overlying ecosystem component. Whether topsoil was bare or covered by floc, it actively took up P. However, when overlaid with litter, topsoil did not provide an active P uptake pathway. Unlike the conceptual model by Richardson and Vaithiyanathan (2009) (Figure 2-7) that shows the different stores as buckets to be filled, the results from this study indicate that the stores are more like bottles with openings that vary in size depending on whether other stores are present to create a bottle neck. Bottle-necks were found in other stores too. For example, subsoil (root zone) P uptake relied on P first migrating down the soil profile. Due to the low hydraulic conductivity of the clay soil, the topsoil layer created a bottle-neck that prevented the P reaching the subsoil. In other wetlands with more porous soils, this might not be the case. A blanketing layer of litter may also affect the movement of P to the roots of emergent macrophytes like *Typha* and thus create a bottle-neck that slows down the quantity and timing of P uptake by *Typha*.

Another kind of interaction was competition between uptake pathways. For example, during the early stages of enrichment, where SAV was present it was able to compete for P with phytoplankton (in terms of uptake speed) reducing their growth rate amongst SAV compared to the bare sediment zone. Once SAV died, phytoplankton biomass was more similar between the bare sediment and SAV zones. In general, faster uptake pathways can be expected to dominate over slower ones until they are exhausted.

Another form of interaction was the sequential contribution of stores to other stores. For example, changes in live *Typha* TP concentration and mass were reflected in litter (e.g. increased litter mass with *Typha* decline). In the mesocosm experiment, the occurrence of floc appeared dependent on the occurrence and particularly the death of SAV as enrichment continued. Overall, the internal cycling of P following initial uptake pathways results in P from different stores (particularly the living stores) gradually findings its way to the sediments and being removed from cycling through soil accretion. No matter what uptake pathways dominate initially, over the enrichment continuum, sediments become an increasingly dominant store of P. The availability of this stored P for recycling depends on how easily it can be transformed into bioavailable forms and this is in turn partly affected by earlier P pathways. For example, P uptake pathways that result in more recalcitrant deposits of P into sediments (e.g. fibrous, bulky *Typha* debris) would result in less potential release than deposits from finer material, such as smaller algae or microbiota. Understanding how initial P uptake pathways influence later P cycling and storage by other ecosystem components is important in proactive management of wetland eutrophication, because it enables either early intervention or preparation for later control of problematic P flows.

These interactions highlight that the quantity and timing of P uptake/release by an ecosystem component is not only dependent on the characteristics of that store but also on characteristics of stores around it. This is why holistic datasets covering a range of ecosystem components are necessary to understand the interplay between ecosystem components that changes over time (Schindler 2012). This also makes findings from holistic studies more transferable to other sites - they provide the wider context of the processes involved which allows managers to better determine what might happen at their particular site.

7.3.6 Seasonal cycling versus treatment effect

Seasonal changes in TP storage by ecosystem components occurred in both treatment and unimpacted wetlands. Because the amount of P entering the ecosystem during enrichment

was much higher than P cycled through seasonal processes, nutrient enrichment had the potential to mask seasonal changes, especially if a store was actively involved in P uptake at the time. However, *Typha* and other macrophytes would undergo significant and distinct seasonal changes even with enrichment, due to their lifecycle. Understanding how seasonality may impact observed changes in TP storage by ecosystem components is needed to understand how eutrophication proceeds. Sampling once per year at the same time of year is only sufficient when one understands what the results mean in the seasonal context and what type of variation in the results could be expected to occur across seasons.

7.4 Ecosystem change

Research question 2: How is nutrient enrichment reflected in ecosystem change in the different functional zones?

Vegetation changes: Bare sediment zone

Seasonal occurrence of filamentous algae and SAV observed in the bare sediment zone of unimpacted wetlands at the pre-enrichment stage did not occur during enrichment in the treatment wetland or mesocosms; extensive growth of phytoplankton occurred instead. The turbidity and accumulation of floc associated with phytoplankton blooms in the treatment wetland would have hindered the establishment and growth of plants (other than floating plants, see below). Also, increased water depth in the treatment wetland was associated with effluent discharge, and would have further reduced light availability at the sediment surface. Overall, enrichment (at least at the high P loads used in this research) promoted conditions that maintained bare sediment across the enrichment continuum. Promotion of plant growth in the bare sediment zone would require a lower P load that allowed clear water conditions to be maintained.

Vegetation changes: SAV zone

As expected, enrichment resulted in loss of SAV in the mesocosm experiment, due to increases in turbidity (Blindow 1992, Scheffer *et al.* 2001, Kufel and Kufel 2002, Romo *et al.* 2004). Opportunistic observations indicated that nutrient enrichment also increased epiphytic algal growth on SAV, potentially contributing to its loss through smothering (Phillips *et al.* 1978, Asaeda *et al.* 2004, Phillips *et al.* 2016). The accumulation of fine, easily resuspended floc might make it more difficult for SAV to anchor and remain established (Schutten *et al.* 2005, Sand-Jensen and Møller 2014). In addition to the loss of P storage and cycling pathways, the loss of SAV has wider implications for wetland condition as it provides diverse habitat and food

sources for other organisms (Jeppesen *et al.* 1998, Bolduc *et al.* 2016), promotes sedimentation and reduces resuspension (Kufel and Kufel 2002, van Donk and van de Bund 2002), and influences water chemistry through primary production and respiration (Reddy and DeLaune 2008). Consequently, it is desirable to avoid the loss of SAV from wetlands undergoing enrichment. To achieve this, incoming P loads must be kept at a level that can be sustainably assimilated by SAV and other ecosystem components; a level that prevents accumulation of P in the water column and thereby prevents algal blooms and increased turbidity. SAV can be sustained in treatment wetlands if nutrient loads are kept low (Gumbricht 1993, Dierberg *et al.* 2002, Knight *et al.* 2003, DeBusk *et al.* 2011).

Vegetation changes: Typha zone

It was hypothesised that nutrient enrichment would increase *Typha* height (Newman *et al.* 1996, Rejmánková *et al.* 2008, Macek *et al.* 2010), biomass and density (Miao and Sklar 1997, Rejmánková 2001, Smith 2003, Kadlec and Wallace 2009, Macek *et al.* 2010) (Hypothesis 7, **Table 2-7**). While *Typha* height increased in the multiyear study, the effect of nutrient enrichment could not be separated from the effect of increasing water depth that also forces *Typha* to elongate leaves and stems (Grace 1989). In contrast to the hypothesis, *Typha* biomass and density decreased with enrichment over a period of several years; more gradually than the rapid loss of SAV (within weeks). *Typha* growth appeared compromised by increased water depth and decreased water quality. This may seem an unusual finding as *Typha* generally thrives in enrichment scenarios (see Chapter 5), but it is likely the consequence of the simultaneous increase in water depth with enrichment (i.e. wastewater treatment). Reductions in P load or water depth would both be expected to decrease adverse impacts on *Typha*. As illustrated in the refined conceptual model, it is predicted that even in systems where *Typha* initially responds with increased growth, decline may occur towards the end stage of the nutrient enrichment continuum, because hypereutrophic conditions decrease oxygen availability. *Typha* may not be completely lost from the wetland, but instead may be limited to the shallows where sufficient oxygen is available. As emergent macrophyte provide benefits such as: stabilisation of sediment, reduced turbulence, physical filtration, surface area for microbial growth, oxygen input into sediments, production of organic matter, range of microhabitats (in and above water, live and dead, litter layer) (Brix 1994, Rejmankova 2011, Gopal 2016, Gebrehiwot, Kifle, and Triest 2017), its loss represents the loss of a wide variety of ecosystem functions. Consequently, it is highly desirable to maintain healthy zones of macrophytes. Because the decline in *Typha* occurred more slowly than the decline in SAV, there may be more time for managers to detect potential problems with emergent plants (e.g.

loss in density) and address them (e.g. with reductions in water level or improvements in water quality).

Changes in water quality: chlorophyll α and turbidity

At the levels of incoming P recorded here (seasonal 13.6 g/m^2/year, multiyear 9.1 g/m^2/year, mesocosm 16.0 g/m^2 over 12 weeks) the development of algal blooms was nearly inevitable and of course algal blooms are generally associated with eutrophication (e.g.Smith 2003). Only in the *Typha* zone during the short-term mesocosm experiment were algal blooms not observed. Algal blooms could develop rapidly in the early stages of enrichment (within few weeks) when added P was not removed sufficiently rapidly from water. Under high P load conditions, algal blooms are not just an end-result of enrichment, but rather a constant feature across the enrichment continuum.

The lack of algal blooms in the *Typha* zone of the mesocosm experiment occurred because the *Typha* zone was able to remove more P from the water column through uptake pathways not available in other zones (i.e. *Typha*, associated biofilms and litter). This lowered water TP concentrations that delayed the onset of algal blooms. Shading by *Typha* may also have decreased algal production through light limitation (Yeh *et al.* 2011, Halliday *et al.* 2016, McCall *et al.* 2017) and *Typha* may have provided more refuges for grazing zooplankton and other herbivores (Choi *et al.* 2014) than the other zones. Also, *Typha* may have inhibited algal growth through allelopathy (Jarchow and Cook 2009, Chicalote-Castillo *et al.* 2017) and enclosed mesocosms may have amplified allelopathic effects (Lombardo *et al.* 2013). These results were not replicated in the open wetland studies, where algal blooms developed in all zones. *Typha* was declining in the whole-wetland studies, and was much less dense there. A healthy *Typha* bed is likely to take up more P from water and thus inhibit phytoplankton growth.

Increased turbidity was nearly always associated with high phytoplankton densities, so to control turbidity it is generally necessary to control phytoplankton productivity. However, other processes may also drive turbidity. The seasonal study exhibited a peak in turbidity not linked to phytoplankton production, indicating that other processes may be driving turbidity, such as sediment resuspension via wind or fauna activity, or increases in incoming loads of suspended sediments. Such events also need to be controlled to prevent them from exacerbating the impacts of nutrient enrichment.

Changes in water quality: pH, DO, ORP

Surface water pH and DO increased with enrichment in the multiyear study (pH: 6.7 to 9.1; %DO: 35% to 233%) likely due to increased photosynthesis associated with the algal bloom (Reddy and DeLaune 2008). However, changes in surface water were not reflected in bottom water, where pH remained stable and %DO remained low (<40%, down to 5.4% or 0.5 mg/L). Surface water ORP was also higher than bottom water ORP and both decreased with enrichment. This shows the need to monitor water quality across the depth profile to accurately characterise water quality and its potential implications for P storage and cycling.

The hypothesis that bottom water DO and ORP would decrease with enrichment (Mortimer 1971, Qualls *et al.* 2001, Kadlec and Wallace 2009) was partly supported. As DO in the treatment wetland was lower than in the unimpacted wetlands when sampling commenced, DO appeared to have already declined prior to sampling (effluent release commenced eight months prior to sampling). Decreases in DO and ORP have been associated with reduced microbial P storage (McLatchey and Reddy 1998) and release of sediment bound P (Reddy and DeLaune 2008). Not only do such changes reduce the P storage and uptake capacity of the system, they can also drive further eutrophication. This is particularly important in wetlands (such as the CWC treatment wetland) where the floc, litter and soil layers have taken up a large amount of P. Even small releases from these P stores due to reduced DO and ORP could drive significant internal eutrophication processes through rapid phytoplankton uptake pathways. Whilst these studies did not look into ways of improving water DO and ORP, reducing biochemical and nitrogenous oxygen demand of incoming water would help, particularly in the case of municipal wastewater (Kadlec and Wallace 2009).

7.5 Wastewater treatment performance

Since 2012 the CWC wetlands have provided tertiary treatment for municipal wastewater from the CWWTP. Release of effluent into the CWC was the preferred short- to medium-term (approximately 5 years) strategy designed to provide the dual benefit of removing excess nutrients from the effluent prior to release into the surrounding environment and utilising these nutrients to accelerate development of the CWC wetland ecosystems. During the PhD study period, the nutrients stimulated biological productivity of some of the wetlands as desired and also provided a secure source of water in the drying climate. However, the adverse outcomes of nutrient enrichment also became apparent in Swamphen Lake.

While this research did not focus on assessing the wastewater treatment performance of Swamphen Lake, the results of the multiyear study provide some indication of the

performance of the system. The P load (9.1 g/m^2/year), incoming effluent TP concentration (0.48 - 5.00 mg/L) and lake water TP concentration (0.53 mg/L by third year) were all midrange in the context of wastewater treatment wetlands (Figure 7-1), however it is noted that the previously recorded range in all of these parameters is vast (several scales of magnitude) and wetlands with similar load and incoming effluent TP concentration could produce much lower or higher outgoing TP concentrations. This is a reflection of the diversity of wetlands and the range of characteristics that influence treatment performance (Reddy *et al.* 1999, Reddy and DeLaune 2008, Kadlec and Wallace 2009, Land *et al.* 2016) (see Section 2.3.1).

Nutrient load in this system was too high to enable sustainable uptake and ecological health of the wetlands. Over the three year study period, lake water TP concentration in Swamphen Lake increased significantly and the system became hypereutrophic. This indicated that the treatment performance of the wetland deteriorated relatively rapidly compared to more sustainable P removal recorded in other studies (e.g. Mustafa et al. 2009, Mitsch et al. 2014, Dzakpasu et al. 2015), especially if compared to wetlands receiving low P loads (e.g. Chen et al. 2015). The wetlands successfully removed nutrients from the wastewater since 2012 but efficacy declined. Adverse outcomes of nutrient enrichment (e.g. algal blooms, high turbidity, oxygen depletion) became apparent in Swamphen Lake. The rapid rate of change in Swamphen Lake was at least partly a reflection of the following special characteristics of the wetland: 1) the incoming effluent was the main source of water and there was limited mixing/dilution other sources of water e.g. precipitation (see water balance in **Table 5-1**); 2) the hydrology of the system differed to that expected, and together with the lack of outflow this resulted in potentially higher than anticipated releases of nutrients to the surrounding environment via groundwater flow. As Swamphen Lake had no surface water outflow during the study period, it acted as an infiltration-evaporation basin. A wetland with higher throughflow is likely to have responded differently.

While lake water TP concentration in Swamphen Lake increased, it remained lower than the incoming effluent TP concentration. This indicated that the system continued to remove P from water. As the study area was no longer recording significant increases in total TP storage by the end of the study, the removal of P from water may have been occurring via settling and trapping of sediments near the effluent discharge point (outside the study area). Sedimentation and other forms of soil accretion (e.g. burial of organic matter) have been shown to be the long-term, sustainable ways to remove P (Vaithiyanathan and Richardson 1999), however additional research would be needed to quantify the long-term P removal capacity of this pathway in Swamphen Lake.

Over the three year study period, the various P stores within Swamphen Lake took up and retained approximately 41 % of P added to the wetland via effluent discharge. This falls close to the median TP removal efficiency of 46% found by Land *et al.* (2016) in a systematic review of existing literature. However, the long-term P removal efficiency at Swamphen Lake is likely to be substantially lower due to the initial major P uptake pathways being exhausted.

The P that was not retained in the wetland likely leached into the surrounding environment via groundwater seepage. Considering the surrounding sandy soils have limited P binding capacity (He *et al.* 1998), this release of P has potential to enrich surrounding groundwater dependent ecosystems. As the treatment performance of the wetland (in terms of P removal) is likely to continue to decrease over time, the amount of P released via groundwater seepage would also increase. This would be further compounded by expected increase in effluent volume due to growth of the nearby town. Consequently, the continuing use of Swamphen Lake for treatment purposes needs to be reviewed, balancing potential long-term water treatment benefits with the potential adverse outcomes on the health of surrounding ecosystems and Swamphen Lake itself. As stated in this thesis, balancing the incoming nutrient load with the assimilative capacity of the wetland is essential for successful wetland treatment systems. Excessive load, as in the CWC wetlands results in a short life expectancy of the system, rapidly declining efficacies and undesirable ecological outscomes.

7.6 Limitations, applications and further research

The findings of this thesis are most relevant to wetlands enriched with relatively high nutrient loads (such as tertiary treatment wetlands), involving additional supply of water together with the nutrients. Wetlands subject to substantially lower or higher loads may respond to enrichment differently over time. Further, the findings of this thesis are most relevant to wetlands in a Mediterranean climate. For example, wetlands with periods of low rainfall and high evaporation result in seasonal concentration of nutrients in water that would be less influential in wetlands of a more temperate climate. Application of the findings to other sites should also consider the hydrology of the studied system (absence of surface water outflow and relatively small through-flow via groundwater). For example, compared to a high flow-through system with surface outflow, the studied system is likely to have experienced faster accumulation of P due to lack of flushing.

One limitation of research into P storage and cycling has been high variability that reduces transferability of research findings (e.g. particularly predictive mathematical modelling, Kadlec

2016). This reflects the wide range of regulating factors that affect P dynamics, however, the holistic approach used here should improve the transferability of these findings. Studies that focus on individual components or processes or fail to provide sufficient background context are more difficult to apply to other situations than holistic studies, that present the findings in the wider context. Also, this research developed conceptual models that focused on explaining ecosystem function with relation to nutrients, which is likely to be more easily transferable than more specific numerical findings.

Further research at the CWC following similar methodology is necessary to identify truly long-term changes in P storage and cycling and associated ecosystem changes. More broadly, similar holistic research tracking P storage and cycling over the enrichment continuum in a variety of different types of wetlands (and vegetation types) would be valuable, especially if combined with the development of conceptual models. This would make it possible to determine more comprehensively the similarities and differences in eutrophication processes between wetlands.

7.7 Management implications for wetlands undergoing nutrient enrichment

Nutrient enrichment and associated eutrophication should be viewed as multitude of interrelated reactions by different ecosystem components over different temporal scales. Temporal patterns are important because the studies showed that: ecosystem components responded at different rates; some components take up P quickly but are then exhausted while others sustained substantial P uptake for longer; some components stored P only for a short period of time before recycling it while others provided longer-term storage; TP concentration of stores responded faster than their mass; seasonal changes occurred in P storage and cycling reflecting seasonal wetland dynamics. These temporal patterns mean that different P storage and cycling pathways dominate at different points in time both seasonally but also over the longer term as enrichment progresses. Temporal patterns also influence the timing of associated ecosystem changes (e.g. changes in vegetation characteristics and water quality). Overall, this means that managers interpreting wetland monitoring data need to take into account the temporal context of the data when interpreting the progress of enrichment in the wetland and how to best manage it.

The research illustrated the benefits of tracking P storage and cycling of all (or most) ecosystem components simultaneously; particularly in understanding how nutrient enrichment in a wetland progressed. The data provided a holistic view of what quantities of P

were held in what stores at different points in time, and whether the P storage of these components was increasing, decreasing or stable over time. Interpretation of causes and potential future implications of changes in P storage and cycling of any one store was much easier with data on surrounding stores than without it. Also, this type of dataset allowed identification of various potential interactions between ecosystem components (e.g. bottle-neck effect, competition, sequential contribution) improving predictions of future change. Consequently, collection of holistic datasets is recommended in order to improve understanding and management of wetlands undergoing nutrient enrichment. This will cost more than collecting data on only a few variables, however it enables development of more targeted management measures, thereby saving money by avoiding ineffective actions.

As P storage and cycling differed between functional zones, management should aim to maintain zones of healthy vegetation for as long as possible over the enrichment continuum, to provide a range of benefits. Zones of vegetation have more P uptake pathways and storage capacity than bare sediment zones and this could effectively slow the progress of nutrient enrichment in vegetated zones, if vegetation can be sustained. Vegetation also increases sedimentation, stabilises sediments and provides a range of habitat for other organisms that not only take up additional P but also contribute to wetland biodiversity. SAV, particularly charophytes, is sensitive to turbidity and so it is likely better suited for low P load situations where clear water conditions can be maintained for longer. *Typha* would generally be expected to thrive in wetlands undergoing nutrient enrichment, although it appears sensitive to reduction in oxygen availability. In order to maintain healthy beds of *Typha* and other emergent plant species, water depth and DO and ORP of bottom water and sediment need to be monitored in addition to macrophyte density and biomass so that adverse changes can be detected early and mitigated as far as possible.

While nutrient enrichment and associated eutrophication generally follows a gradual pathway from pre-enrichment, through a functional P uptake stage to the hypereutrophic end-stage, excessive P loads could result in rapid emergence of the hypereutrophic end-stage without the P uptake capacity of other stores being exhausted. The gradual pathway takes advantage of the P storage capacity of different ecosystem components, maintaining clear water conditions and a fully-functional ecosystem for longer. In contrast, the rapid change pathway can result in decreased overall P storage capacity before it has even been used (e.g. through loss of vegetative stores and through decreased water and sediment quality decreasing soil and microbial uptake capacity). Consequently, it is recommended that as far as possible the P load is reduced to levels that will allow gradual change to occur and avoid rapid accumulation of P

in water. Ideally, P load should be kept below the sustainable P assimilation capacity of the wetland, likely below 0.5 g/m^2/year, in which case eutrophication of the system could be avoided altogether.

REFERENCES

Ahn, C., and Mitsch, W. J. 2002. Scaling considerations of mesocosm wetlands in simulating large created freshwater marshes. *Ecological Engineering* 18 (3):327-342.

Aldous, A., McCormick, P., Ferguson, C., Graham, S., and Craft, C. 2005. Hydrologic Regime Controls Soil Phosphorus Fluxes in Restoration and Undisturbed Wetlands. *Restoration Ecology* 13 (2):341-347.

Aldridge, K. T., and Ganf, G. G. 2003. Modification of sediment redox potential by three contrasting macrophytes: implications for phosphorus adsorption/desorption. *Marine and Freshwater Research* 54 (1):87-94.

Anderson, C. J., Mitsch, W. J., and Nairn, R. W. 2005. Temporal and spatial development of surface soil conditions at two created riverine marshes. *Journal of environmental quality* 34 (6):2072-2081.

Annan, I. 2008. *Nitella congesta - a charophyte as a tool for the rehabilitation of sand mine-void wetlands at Capel, Western Australia.* PhD Thesis, Department of Environmental and Aquatic Sciences, Curtin University of Technology.

ANZECC and ARMCANZ. 2000. National Water Quality Management Strategy, Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand.

APHA. 2005. Phosphorus sample preparation. In *Standard methods for the examination of water & wastewater*, edited by A. E. Greenberg, L. S. Clesceri and A. D. Eaton. Washington, DC: American Public Health Association.

Asaeda, T., Sultana, M., Manatunge, J., and Fujino, T. 2004. The effect of epiphytic algae on the growth and production of *Potamogeton perfoliatus* L. in two light conditions. *Environmental and Experimental Botany* 52 (3):225-238.

Aspila, K. I., Agemian, H., and Chau, A. S. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *The Analyst* 101 (1200):187-197.

Bakker, E. S., Van Donk, E., Declerck, S. A. J., Helmsing, N. R., Hidding, B., and Nolet, B. A. 2010. Effect of macrophyte community composition and nutrient enrichment on plant biomass and algal blooms. *Basic and Applied Ecology* 11 (5):432-439.

Barron, O., Silberstein, R., Ali, R., Donohue, R., McFarlane, D. J., Davies, P., Hodgson, G., Smart, N., and Donn, M. 2012. Climate change effects on water-dependent ecosystems in south-western Australia. *Journal of Hydrology* 434-435:95-109.

Bates, B. C., Hope, P., Ryan, B., Smith, I., and Charles, S. 2008. Key findings from the Indian Ocean Climate Initiative and their impact on policy development in Australia. *Climatic Change* 89 (3):339-354.

Bayly, I. L., and O'Neill, T. A. 1972. Seasonal ionic fluctuations in a "*Typha glauca*" community. *Ecology* 53 (4):714-719.

Bentzen, E., Taylor, W. D., and Millard, E. S. 1992. The Importance of Dissolved Organic Phosphorus to Phosphorus Uptake by Limnetic Plankton. *Limnology and Oceanography* 37 (2):217-231.

Blindow, I. 1992. Decline of charophytes during eutrophication: comparison with angiosperms. *Freshwater Biology* 28 (1):9-14.

Boers, A. M., Veltman, R. L. D., and Zedler, J. B. 2007. *Typha* × glauca dominance and extended hydroperiod constrain restoration of wetland diversity. *Ecological Engineering* 29 (3):232-244.

Boix, D., Kneitel, J., Robson, B. J., Duchet, C., Zúñiga, L., Day, J., Gascón, S., Sala, J., Quintana, X. D., and Blaustein, L. 2016. Invertebrates of Freshwater Temporary Ponds in Mediterranean Climates. In *Invertebrates in freshwater wetlands: an international perspective on their ecology*, edited by D. P. Batzer and D. Boix, 141-190. Cham, Switzerland: Springer International Publishing.

Bolduc, P., Bertolo, A., and Pinel-Alloul, B. 2016. Does submerged aquatic vegetation shape zooplankton community structure and functional diversity? A test with a shallow fluvial lake system. *Hydrobiologia* 778 (1):151-165.

Boon, P. I., Pollard, P. C., and Ryder, D. S. 2014. Wetland microbial ecology and biogeochemistry. In *Ecology of freshwater and estuarine wetlands*, edited by D. P. Batzer and R. R. Sharitz. Oakland, California: University of California Press.

Boulton, A., Brock, M., Robson, B., and Chambers, J. 2014. *Australian Freshwater Ecology: Processes and Management*. Somerset, UK: Wiley.

Boyd, C. E. 1970. Production, Mineral Accumulation and Pigment Concentrations in *Typha latifolia* and *Scirpus americanus*. *Ecology* 51 (2):285-290.

Braskerud, B. C., Tonderski, K. S., Wedding, B., Bakke, R., Blankenberg, A. G. B., Ulén, B., Koskiaho, J., and Sveriges, l. 2005. Can constructed wetlands reduce the diffuse phosphorus loads to eutrophic water in cold temperate regions? *Journal of environmental quality* 34 (6):2145.

Britto, D. T., and Kronzucker, H. J. 2002. NH4+ toxicity in higher plants: a critical review. *Journal of Plant Physiology* 159 (6):567-584.

Brix, H. 1994. Functions of Macrophytes in Constructed Wetlands. *Water Science and Technology* 29 (4):71-78.

Brix, H., Sorrell, B. K., and Orr, P. T. 1992. Internal Pressurization and Convective Gas Flow in Some Emergent Freshwater Macrophytes. *Limnology and Oceanography* 37 (7):1420-1433.

Brown, N., and Shilton, A. 2014. Luxury uptake of phosphorus by microalgae in waste stabilisation ponds: current understanding and future direction. *Reviews in Environmental Science and Bio/Technology* 13 (3):321-328.

Bureau of Meteorology. *Average annual, monthly and seasonal evaporation*. Commonwealth of Australia 2006 [cited July 2017. Available from http://www.bom.gov.au/watl/evaporation/.

Bureau of Meteorology. *Climate Data Online*. Commonwealth of Australia 2016a [cited July 2017. Available from http://www.bom.gov.au/climate/data/.

Bureau of Meteorology. *Trend in Total Rainfall 1970-2015*. Commonweatlh of Australia 2016b [cited October 2016. Available from http://www.bom.gov.au/climate/change/index.shtml#tabs=Tracker&tracker=trend-maps.

Butzler, J. M., and Chase, J. M. 2009. The effects of variable nutrient additions on a pond mesocosm community. *Hydrobiologia* 617 (1):65-73.

Capelli, L., Jones, L., and John, J. 2007. Acidification of Capel Wetland Centre Lakes and the Identification of Acid Producing Species. Technical Report No. 58. Perth, Western Australia: Curtin University of Technology.

Carlson, R. E. 1977. A Trophic State Index for Lakes. *Limnology and Oceanography* 22 (2):361-369.

Carpenter, S. R. 1996. Microcosm Experiments have Limited Relevance for Community and Ecosystem Ecology. *Ecology* 77 (3):677-680.

Chambers, J. M., Cale, D. J., McComb, A. J., and Edward, D. H. 1998. The effect of added phosphorus on algal and macroinvertebrate communities in experimental enclosures in Taylor's Lake, Capel, Western Australia. Capel Wetlands Centre Technical Report No. 42. Murdoch, Western Australia: Murdoch University.

Chambers, J. M., Fletcher, N. L., and McComb, A. J. 1998. The effect of phosphorus addition on the algal productivity of Cadjeput Pool, Capel Wetlands Centre, Western Australia. Capel Wetlands Centre Technical Report No. 43. Murdoch, Western Australia: Murdoch University.

Chambers, J. M., and McComb, A. J. 1996. Investigations into improving water quality and phytoplankton growth in lakes at Capel, Western Australia. Capel Wetlands Centre Technical Report No. 28. Murdoch, Western Australia: Murdoch University.

Cheesman, A. W., Turner, B. L., Inglett, P. W., and Reddy, K. R. 2010. Phosphorus transformations during decomposition of wetland macrophytes. *Environmental science & technology* 44 (24):9265-9271.

Chen, C.-C., Petersen, J. E., and Kemp, W. M. 1997. Spatial and temporal scaling of periphyton growth on walls of estuarine mesocosms. *Marine Ecology Progress Series* 155:1-15.

Chen, H., Ivanoff, D., and Pietro, K. 2015. Long-term phosphorus removal in the Everglades stormwater treatment areas of South Florida in the United States. *Ecological Engineering* 79:158-168.

Chen, H., and Vaughan, K. 2014. Influence of Inundation Depth on *Typha domingensis* and its Implication for Phosphorus Removal in the Everglades Stormwater Treatment Area. *Wetlands* 34 (2):325-334.

Chen, H., Zamorano, M. F., and Ivanoff, D. 2013. Effect of deep flooding on nutrients and non-structural carbohydrates of mature *Typha domingensis* and its post-flooding recovery. *Ecological Engineering* 53:267-274.

Chicalote-Castillo, D., Ramírez-García, P., and Macías-Rubalcava, M. L. 2017. Allelopathic effects among selected species of phytoplankton and macrophytes. *Journal of Environmental Biology* 38 (6):1221-1227.

Childers, D. L., Doren, R. F., Jones, R., Noe, G. B., Rugge, M., and Scinto, L. J. 2003. Decadal change in vegetation and soil phosphorus pattern across the Everglades landscape. *Journal of environmental quality* 32 (1):344-362.

Chimney, M. J., and Pietro, K. C. 2006. Decomposition of macrophyte litter in a subtropical constructed wetland in south Florida (USA). *Ecological Engineering* 27 (4):301-321.

Choi, J.-Y., Jeong, K.-S., Kim, S.-K., La, G.-H., Chang, K.-H., and Joo, G.-J. 2014. Role of macrophytes as microhabitats for zooplankton community in lentic freshwater ecosystems of South Korea. *Ecological Informatics* 24:177-185.

Christensen, J. R., Crumpton, W. G., and Arnold, G. v. d. V. 2009. Estimating the Breakdown and Accumulation of Emergent Macrophyte Litter: A Mass-Balance Approach. *Wetlands* 29 (1):204-214.

Cleugh, H., Stafford Smith, M., Battaglia, M., and Graham, P., eds. 2011. *Climate Change: Science and Solutions for Australia*. Australia: CSIRO Publishing.

Collins, S. D. 2005. *Utilizing in-situ benthic mesocosms to quantify phosphorus and nitrogen fluxes in south Florida agricultural canals*. Masters Thesis, Engineering, University of Florida, Gainesville, Florida.

Coppens, J., Özen, A., Tavşanoğlu, Ü. N., Erdoğan, Ş., Levi, E. E., Yozgatlıgil, C., Jeppesen, E., and Beklioğlu, M. 2016. Impact of alternating wet and dry periods on long-term seasonal phosphorus and nitrogen budgets of two shallow Mediterranean lakes. *Science of the Total Environment* 563-564:456-467.

Correll, D. L. 1998. The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review. *Journal of Environment Quality* 27 (2):261-266.

Corstanje, R., and Reddy, K. R. 2006. Microbial indicators of nutrient enrichment: a mesocosm study. *Soil Science Society of America Journal* 70 (5):1652-1661.

Coveney, M. F., Stites, D. L., Lowe, E. F., Battoe, L. E., and Conrow, R. 2002. Nutrient removal from eutrophic lake water by wetland filtration. *Ecological Engineering* 19 (2):141-159.

Craft, C. B., and Richardson, C. J. 1993. Peat Accretion and N, P, and Organic C Accumulation in Nutrient-Enriched and Unenriched Everglades Peatlands. *Ecological Applications* 3 (3):446-458.

Cronk, J. K., and Mitsch, W. J. 1994. Periphyton productivity on artificial and natural surfaces in constructed freshwater wetlands under different hydrologic regimes. *Aquatic Botany* 48 (3):325-341.

Cuellar-Bermudez, S. P., Aleman-Nava, G. S., Chandra, R., Garcia-Perez, J. S., Contreras-Angulo, J. R., Markou, G., Muylaert, K., Rittmann, B. E., and Parra-Saldivar, R. 2017. Nutrients utilization and contaminants removal. A review of two approaches of algae and cyanobacteria in wastewater. *Algal Research* 24:438-449.

Davidson, T. A., Audet, J., Svenning, J. C., Lauridsen, T. L., Søndergaard, M., Landkildehus, F., Larsen, S. E., Jeppesen, E., and Sveriges, l. 2015. Eutrophication effects on greenhouse gas fluxes from shallow-lake mesocosms override those of climate warming. *Global Change Biology* 21 (12):4449-4463.

Davies, S. J. 2002. The Capel Wetlands Centre: A Survey of its Historical Developments, Significance and Research Results. School of Environmental Biology Bulletin No 22. . Bentley, Western Australia: Curtin University of Technology.

Davis, C. B., and van der Valk, A. G. 1983. Uptake and release of nutrients by living and decomposing *Typha glauca* godr. tissues at Eagle Lake, Iowa. *Aquatic Botany* 16 (1):75-89.

Davis, J. R., and Koop, K. 2006. Eutrophication in Australian Rivers, Reservoirs and Estuaries – A Southern Hemisphere Perspective on the Science and its Implications. *Hydrobiologia* 559 (1):23-76.

Davis, S. M. 1991. Growth, decomposition, and nutrient retention of *Cladium jamaicense* Crantz and *Typha domingensis* Pers. in the Florida Everglades. *Aquatic Botany* 40 (3):203-224.

DeBusk, T. A., Grace, K. A., Dierberg, F. E., Jackson, S. D., Chimney, M. J., and Gu, B. 2004. An investigation of the limits of phosphorus removal in wetlands: a mesocosm study of a shallow periphyton-dominated treatment system. *Ecological Engineering* 23 (1):1-14.

DeBusk, T. A., Kharbanda, M., Jackson, S. D., Grace, K. A., Hileman, K., and Dierberg, F. E. 2011. Water, vegetation and sediment gradients in submerged aquatic vegetation mesocosms used for low-level phosphorus removal. *Science of The Total Environment* 409 (23):5046-5056.

DeBusk, W. F., Newman, S., and Reddy, K. R. 2001. Spatio-temporal patterns of soil phosphorus enrichment in Everglades water conservation area 2A. *Journal of environmental quality* 30 (4):1438-1446.

Debusk, W. F., and Reddy, K. R. 2005. Litter Decomposition and Nutrient Dynamics in a Phosphorus Enriched Everglades Marsh. *Biogeochemistry* 75 (2):217-240.

Di Luca, G. A., Maine, M. A., Mufarrege, M. M., Hadad, H. R., and Bonetto, C. A. 2015. Influence of *Typha domingensis* in the removal of high P concentrations from water. *Chemosphere* 138:405-411.

Dierberg, F. E., DeBusk, T. A., Henry, J. L., Jackson, S. D., Galloway, S., and Gabriel, M. C. 2012. Temporal and spatial patterns of internal phosphorus recycling in a South Florida (USA) stormwater treatment area. *Journal of environmental quality* 41 (5):1661-1673.

Dierberg, F. E., DeBusk, T. A., Jackson, S. D., Chimney, M. J., and Pietro, K. 2002. Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: response to hydraulic and nutrient loading. *Water Research* 36 (6):1409-1422.

Dodds, W. K., and Smith, V. H. 2016. Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters* 6 (2):155-164.

Dolan, T. J., Bayley, S. E., Zoltek, J., and Hermann, A. J. 1981. Phosphorus Dynamics of a Florida Freshwater Marsh Receiving Treated Wastewater. *Journal of Applied Ecology* 18 (1):205-219.

dos Santos, T. R., Ferragut, C., and de Mattos Bicudo, C. E. 2013. Does macrophyte architecture influence periphyton? Relationships among Utricularia foliosa, periphyton assemblage structure and its nutrient (C, N, P) status. *Hydrobiologia* 714 (1):71-83.

Douglas, G. B., Hamilton, D. P., Robb, M. S., Pan, G., Spears, B. M., and Lurling, M. 2016. Guiding principles for the development and application of solid-phase phosphorus adsorbents for freshwater ecosystems. *Aquatic Ecology* 50 (3):385-405.

Downes, B. J. 2002. *Monitoring ecological impacts: concepts and practice in flowing waters*. New York, NY: Cambridge University Press.

Droppo, I. G. 2001. Rethinking what constitutes suspended sediment. *Hydrological Processes* 15 (9):1551-1564.

Dunck, B., Lima-Fernandes, E., Cássio, F., Cunha, A., Rodrigues, L., and Pascoal, C. 2015. Responses of primary production, leaf litter decomposition and associated communities to stream eutrophication. *Environmental Pollution* 202:32-40.

Dunne, E. J., Coveney, M. F., Hoge, V. R., Conrow, R., Naleway, R., Lowe, E. F., Battoe, L. E., and Wang, Y. 2015. Phosphorus removal performance of a large-scale constructed treatment wetland receiving eutrophic lake water. *Ecological Engineering* 79:132-142.

Dzakpasu, M., Scholz, M., McCarthy, V., and Jordan, S. N. 2015. Assessment of long-term phosphorus retention in an integrated constructed wetland treating domestic wastewater. *Environmental Science and Pollution Research* 22 (1):305-313.

Eid, E. M., Shaltout, K. H., and Asaeda, T. 2012. Modeling growth dynamics of *Typha domingensis* (Pers.) Poir. ex Steud. in Lake Burullus, Egypt. *Ecological Modelling* 243:63-72.

Emsens, W. J., Aggenbach, C. J. S., Grootjans, A. P., Nfor, E. E., Schoelynck, J., Struyf, E., and Diggelen, R. 2016. Eutrophication triggers contrasting multilevel feedbacks on litter accumulation and decomposition in fens. *Ecology* 97 (10):2680-2690.

Erler, D. V., Tait, D., Eyre, B. D., and Bingham, M. 2011. Observations of nitrogen and phosphorus biogeochemistry in a surface flow constructed wetland. *Science of the Total Environment* 409 (24):5359-5367.

Ferriol, C., Miracle, M. R., and Vicente, E. 2016. Effects of nutrient addition, recovery thereafter and the role of macrophytes in nutrient dynamics of a Mediterranean shallow lake: a mesocosm experiment. *Marine and Freshwater Research* 68 (3):506-518.

Fowler Surveys. 1999. Capel Wetlands Centre Lake Surveys June/July 1999. Prepared for Murdoch University.

Gaiser, E. E., Scinto, L. J., Richards, J. H., Jayachandran, K., Childers, D. L., Trexler, J. C., and Jones, R. D. 2004. Phosphorus in periphyton mats provides the best metric for

detecting low-level P enrichment in an oligotrophic wetland. *Water Research* 38 (3):507-516.

Gaiser, E. E., Trexler, J. C., Richards, J. H., Childers, D. L., Lee, D., Edwards, A. L., Scinto, L. J., Jayachandran, K., Noe, G. B., and Jones, R. D. 2005. Cascading ecological effects of low-level phosphorus enrichment in the Florida Everglades. *Journal of Environmental Quality* 34 (2):717-723.

Garver, E. G., Dubbe, D. R., and Pratt, D. C. 1988. Seasonal patterns in accumulation and partitioning of biomass and macronutrients in *Typha* spp. *Aquatic Botany* 32 (1):115-127.

Gebrehiwot, M., Kifle, D., Stiers, I., and Triest, L. 2017. Phytoplankton functional dynamics in a shallow polymictic tropical lake: the influence of emergent macrophytes. *Hydrobiologia* 797 (1):69-86.

Gebrehiwot, M., Kifle, D., and Triest, L. 2017. Emergent Macrophytes Support Zooplankton in a Shallow Tropical Lake: A Basis for Wetland Conservation. *Environmental Management* 60 (6):1127-1138.

Gentile, J. H., Harwell, M. A., Harwell, C. C., Cropper Jr, W., DeAngelis, D., Davis, S., Ogden, J. C., and Lirman, D. 2001. Ecological conceptual models: a framework and case study on ecosystem management for South Florida sustainability. *Science of the Total Environment* 274 (1):231-253.

GHD. 2004. Capel Wastewater Treatment Plant, Proposed Wetland Enhancement Program, Referral Document. Prepared for Water Corporation. Perth, Western Australia: GHD.

GHD. 2006. Report for Capel Wastewater Treatment Plant Treated Effluent Management System Upgrade, Condition Assessment of Capel Wetlands. Prepared for Water Corporation. Perth, Western Australia: GHD.

Gilbert, J. D., Guerrero, F., and de Vicente, I. 2014. Sediment desiccation as a driver of phosphate availability in the water column of Mediterranean wetlands. *The Science of the total environment* 466-467:965-975.

Gopal, B. 2016. Should 'wetlands' cover all aquatic ecosystems and do macrophytes make a difference to their ecosystem services? *Folia Geobotanica* 51 (3):209-226.

Gopal, B., and Sharma, K. P. 1984. Seasonal changes in concentration of major nutrient elements in the rhizomes and leaves of *Typha elephantina* roxb. *Aquatic Botany* 20 (1):65-73.

Gordon, D. M., and Chambers, J. M. 1987. Water Ecology and Vegetation of the Capel-Ludlow Wetlands, Western Australia. Capel Wetlands Centre Technical Report No. 5. Prepared for Associated Minerals Consolidated Limited. Nedlands, Western Australia: University of Western Australia.

Grace, J. B. 1989. Effects of Water Depth on *Typha latifolia* and *Typha domingensis*. *American Journal of Botany* 76 (5):762-768.

Grace, K. A., Dierberg, F. E., DeBusk, T. A., and White, J. R. 2008. Phosphorus Uptake by *Typha* Leaf Litter as Affected by Oxygen Availability. *Wetlands* 28 (3):817-826.

Greenway, M. 2005. The role of constructed wetlands in secondary effluent treatment and water reuse in subtropical and arid Australia. *Ecological Engineering* 25 (5):501-509.

Greenway, M., and Lucas, W. C. 2008. A Study of Nutrient Retention Dynamics in Vegetated and Non-Vegetated Bioretention Mesocosms. In *Low impact development : new and continuing applications : proceedings of the second National Low Impact Development Conference, March 12-14, 2007, Wilmington, North Carolina*, 140-158. American Society of Civil Engineers.

Greenway, M., and Woolley, A. 2001. Changes in plant biomass and nutrient removal over 3 years in a constructed wetland in Cairns, Australia. *Water science and technology : a journal of the International Association on Water Pollution Research* 44 (11-12):303-310.

Grice, G. D., and Reeve, M. R. 1982. *Marine Mesocosms : Biological and Chemical Research in Experimental Ecosystems*. 1 ed. New York, NY: Springer.

Griffiths, L. N., and Mitsch, W. J. 2017. Removal of nutrients from urban stormwater runoff by storm-pulsed and seasonally pulsed created wetlands in the subtropics. *Ecological Engineering* 108 (Part B):414-424.

Guariento, R. D., Caliman, A., Esteves, F. A., Bozelli, R. L., Enrich-Prast, A., and Farjalla, V. F. 2009. Substrate influence and temporal changes on periphytic biomass accrual and metabolism in a tropical humic lagoon. *Limnologica* 39 (3):209-218.

Gumbricht, T. 1993. Nutrient removal processes in freshwater submersed macrophyte systems. *Ecological Engineering* 2 (1):1-30.

Gustafsson, B. G., Schenk, F., Blenckner, T., Eilola, K., Meier, H. E. M., Müller-Karulis, B., Neumann, T., Ruoho-Airola, T., Savchuk, O. P., Zorita, E., Stockholms, u., Stockholm Resilience, C., and Baltic Nest, I. 2012. Reconstructing the Development of Baltic Sea Eutrophication 1850-2006. *Ambio* 41 (6):534-548.

Hagerthey, S. E., Newman, S., Rutchey, K., Smith, E. P., and Godin, J. 2008. Multiple Regime Shifts in a Subtropical Peatland: Community-Specific Thresholds to Eutrophication. *Ecological Monographs* 78 (4):547-565.

Halliday, S. J., Skeffington, R. A., Wade, A. J., Bowes, M. J., Read, D. S., Jarvie, H. P., and Loewenthal, M. 2016. Riparian shading controls instream spring phytoplankton and benthic algal growth. *Environmental science. Processes & impacts* 18 (6):677-689.

Hansson, L., Bronmark, C., Nilsson, A., and Abjornsson, K. 2005. Conflicting demands on wetland ecosystem services: nutrient retention, biodiversity or both? *Freshwater biology* 50 (4):705-714.

Havens, K. E., Aumen, N. G., James, R. T., and Smith, V. H. 1996. Rapid Ecological Changes in a Large Subtropical Lake Undergoing Cultural Eutrophication. *Ambio* 25 (3):150-155.

Havens, K. E., East, T. L., J. Rodusky, A., and Sharfstein, B. 1999. Littoral periphyton responses to nitrogen and phosphorus: an experimental study in a subtropical lake. *Aquatic Botany* 63 (3):267-290.

Havens, K. E., Sharfstein, B., Rodusky, A. J., and East, T. L. 2004. Phosphorus accumulation in the littoral zone of a subtropical lake. *Hydrobiologia* 517 (1):15-24.

He, J. Z., Gilkes, R. J., and Dimmock, G. M. 1998. Mineralogical properties of sandy podzols on the Swan Coastal Plain, south-west Australia, and the effects of drying on their phosphate sorption characteristics. *Soil Research* 36 (3):395-410.

Hefting, M. M., van den Heuvel, R. N., and Verhoeven, J. T. A. 2012. Wetlands in agricultural landscapes for nitrogen attenuation and biodiversity enhancement: Opportunities and limitations. *Ecological Engineering* 56:5-13.

Hernández-Crespo, C., Oliver, N., Bixquert, J., Gargallo, S., and Martín, M. 2016. Comparison of three plants in a surface flow constructed wetland treating eutrophic water in a Mediterranean climate. *Hydrobiologia* 774 (1):183-192.

Hill, B. H. 1987. Typha productivity in a Texas pond: Implications for energy and nutrient dynamics in freshwater wetlands. *Aquatic Botany* 27 (4):385-394.

Hilt, S., Gross, E. M., Hupfer, M., Morscheid, H., Mählmann, J., Melzer, A., Poltz, J., Sandrock, S., Scharf, E.-M., Schneider, S., and van de Weyer, K. 2006. Restoration of submerged vegetation in shallow eutrophic lakes – A guideline and state of the art in Germany. *Limnologica* 36 (3):155-171.

Hoffmann, C. C., Kjaergaard, C., Uusi-Kamppa, J., Hansen, H. C. B., and Kronvang, B. 2009. Phosphorus Retention in Riparian Buffers: Review of Their Efficiency. *Journal of Environmental Quality* 38 (5):1942-1955.

Hogan, D. M., Jordan, T. E., and Walbridge, M. R. 2004. Phosphorus retention and soil organic carbon in restored and natural freshwater wetlands. *Wetlands* 24 (3):573-585.

Hogan, D. M., and Walbridge, M. R. 2009. Recent Land Cover History and Nutrient Retention in Riparian Wetlands. *Environmental Management* 44 (1):62-72.

Hope, P., Abbs, D., Bhend, J., Chiew, F., Church, J., Ekström, M., Kirono, D., Lenton, A., Lucas, C., McInnes, K., Moise, A., Monselesan, D., Mpelasoka, F., Timbal, B., Webb, L., and Whetton, P. 2015. Southern and South-Western Flatlands Cluster Report. In *Climate Change in Australia Projections for Australia's Natural Resource Management Regions: Cluster Reports*, edited by M. Ekström, P. Whetton, C. Gerbing, M. Grose, L. Webb and J. Risbey. Australia: CSIRO and Bureau of Meteorology.

Horppila, J., Kaitaranta, J., Joensuu, L., and Nurminen, L. 2013. Influence of emergent macrophyte (Phragmites australis) density on water turbulence and erosion of organic-rich sediment. *Journal of Hydrodynamics* 25 (2):288-293.

Horwitz, P., Rogan, R., Halse, S., Davis, J., and Sommer, B. 2009. Wetland invertebrate richness and endemism on the Swan Coastal Plain, Western Australia. *Marine and Freshwater Research* 60 (10):1006-1020.

Howard-Williams, C. 1985. Cycling and retention of nitrogen and phosphorus in wetlands: a theoretical and applied perspective. *Freshwater Biology* 15 (4):391-431.

Huang, L., Du, S., Fan, L., Lin, X., Wang, H., and Zhang, Y. 2011. Microbial activity facilitates phosphorus adsorption to shallow lake sediment. *Journal of Soils and Sediments* 11 (1):185-193.

Huett, D. O., Morris, S. G., Smith, G., and Hunt, N. 2005. Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands. *Water Research* 39 (14):3259-3272.

Jarchow, M. E., and Cook, B. J. 2009. Allelopathy as a Mechanism for the Invasion of *Typha angustifolia*. *Plant Ecology* 204 (1):113-124.

Jeppesen, E., Kangur, K., Kohler, J., Willen, E., Carvalho, L., Tatrai, I., Gerdeaux, D., Coveney, M., Schelske, C., Peder Jensen, J., Sondergaard, M., Foy, B., Gunnar Persson, P., Dokulil, M., Hampton, S., Winder, M., Romo, S., Lammens, E., Havens, K., Portielje, R., Straile, D., Phillips, G., Hilt, S., Deneke, R., and Anneville, O. 2005. Lake responses to reduced nutrient loading - an analysis of contemporary long-term data from 35 case studies. *Freshwater biology* 50 (10):1747-1771.

Jeppesen, E., Søndergaard, M., Søndergaard, M., and Christoffersen, K., eds. 1998. *The Structuring Role of Submerged Macrophytes in Lakes*. New York, NY: Springer-Verlag.

Johnson, K. S., and Petty, R. L. 1983. Determination of Nitrate and Nitrite in Seawater by Flow Injection Analysis. *Limnology and Oceanography* 28 (6):1260-1266.

Kadlec, R. H. 2005. Phosphorus Removal in Emergent Free Surface Wetlands. *Journal of Environmental Science and Health, Part A* 40 (6-7):1293-1306.

Kadlec, R. H. 2009a. Wastewater treatment at the Houghton Lake wetland: Hydrology and water quality. *Ecological Engineering* 35 (9):1287-1311.

Kadlec, R. H. 2009b. Wastewater treatment at the Houghton lake wetland: Soils and sediments. *Ecological Engineering* 35 (9):1333-1348.

Kadlec, R. H. 2016. Large Constructed Wetlands for Phosphorus Control: A Review. *Water* 8 (6):243.

Kadlec, R. H., and Bevis, F. B. 2009. Wastewater treatment at the Houghton Lake wetland: Vegetation response. *Ecological Engineering* 35 (9):1312-1332.

Kadlec, R. H., Pries, J., and Lee, K. 2012. The Brighton treatment wetlands. *Ecological Engineering* 47:56-70.

Kadlec, R. H., and Reddy, K. R. 2001. Temperature Effects in Treatment Wetlands. *Water Environment Research* 73 (5):543-557.

Kadlec, R. H., and Wallace, S. D. 2009. *Treatment wetlands*. 2nd ed. Boca Raton, Florida: CRC Press.

Kagalou, I., Papastergiadou, E., and Leonardos, I. 2008. Long term changes in the eutrophication process in a shallow Mediterranean lake ecosystem of W. Greece: Response after the reduction of external load. *Journal of Environmental Management* 87 (3):497-506.

Kim, S. Y., and Geary, P. M. 2001. The impact of biomass harvesting on phosphorus uptake by wetland plants. *Water science and technology: a journal of the International Association on Water Pollution Research* 44 (11-12):61.

Knight, R. L., Gu, B., Clarke, R. A., and Newman, J. M. 2003. Long-term phosphorus removal in Florida aquatic systems dominated by submerged aquatic vegetation. *Ecological Engineering* 20 (1):45-63.

Kobayashi, T., Ryder, D. S., Gordon, G., Shannon, I., Ingleton, T., Carpenter, M., and Jacobs, S. J. 2009. Short-term response of nutrients, carbon and planktonic microbial communities to floodplain wetland inundation. *Aquatic Ecology* 43 (4):843-858.

Koch, M. S., Mendelssohn, I. A., and McKee, K. L. 1990. Mechanism for the Hydrogen Sulfide-Induced Growth Limitation in Wetland Macrophytes. *Limnology and Oceanography* 35 (2):399-408.

Kröger, R., Holland, M. M., Moore, M. T., and Cooper, C. M. 2007. Plant senescence: A mechanism for nutrient release in temperate agricultural wetlands. *Environmental Pollution* 146 (1):114-119.

Kuczyńska-Kippen, N., and Joniak, T. 2016. Zooplankton diversity and macrophyte biometry in shallow water bodies of various trophic state. *Hydrobiologia* 774 (1):39-51.

Kuehn, K. A., Francoeur, S. N., Findlay, R. H., and Neely, R. K. 2014. Priming in the microbial landscape: periphytic algal stimulation of litter-associated microbial decomposers. *Ecology* 95 (3):749-762.

Kufel, L., and Kufel, I. 2002. *Chara* beds acting as nutrient sinks in shallow lakes—a review. *Aquatic Botany* 72 (3–4):249-260.

Lambert, S. J., and Davy, A. J. 2011. Water quality as a threat to aquatic plants: discriminating between the effects of nitrate, phosphate, boron and heavy metals on charophytes. *New Phytologist* 189 (4):1051-1059.

Lamers, L. P., Govers, L. L., Janssen, I. C., Geurts, J., Welle, M. E. v. d., Katwijk, M. M. v., Heide, T. v. d., Roelofs, J. G., and Smolders, A. J. 2013. Sulfide as a soil phytotoxin-a review. *Frontiers in Plant Science* 4:268.

Land, M., Grannli, W., Grimvall, A., Hoffmann, C. C., Mitsch, W. J., Tonderski, K. S., and Verhoeven, J. T. A. 2016. How effective are created or restored freshwater wetlands for nitrogen and phosphorus removal? A systematic review. *Environmental Evidence* 5:9.

Li, X., and Nan, R. 2017. A bibliometric analysis of eutrophication literatures: an expanding and shifting focus. *Environmental Science and Pollution Research* 24 (20):17103-17115.

Liboriussen, L., and Jeppesen, E. 2006. Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations. *Freshwater Biology* 51 (1):95-109.

Liston, S. E., Newman, S., and Trexler, J. C. 2008. Macroinvertebrate Community Response to Eutrophication in an Oligotrophic Wetland: An in situ Mesocosm Experiment. *Wetlands* 28 (3):686-694.

Lombardo, P., Mjelde, M., Källqvist, T., and Brettum, P. 2013. Seasonal and scale-dependent variability in nutrient- and allelopathy-mediated macrophyte–phytoplankton interactions. *Knowledge and Management of Aquatic Ecosystems* (409):10.

Lu, S. Y., Wu, F. C., Lu, Y. F., Xiang, C. S., Zhang, P. Y., and Jin, C. X. 2009. Phosphorus removal from agricultural runoff by constructed wetland. *Ecological Engineering* 35 (3):402-409.

Macek, P., Rejmánková, E., and Lepš, J. 2010. Dynamics of *Typha domingensis* spread in Eleocharis dominated oligotrophic tropical wetlands following nutrient enrichment. *Evolutionary Ecology* 24 (6):1505-1519.

Maddison, M., Mauring, T., Remm, K., Lesta, M., and Mander, Ü. 2009. Dynamics of *Typha latifolia* L. populations in treatment wetlands in Estonia. *Ecological Engineering* 35 (2):258-264.

Madsen, J. D., Chambers, P. A., James, W. F., Koch, E. W., and Westlake, D. F. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia* 444 (1):71-84.

Mahdy, A., Hilt, S., Filiz, N., Beklioğlu, M., Hejzlar, J., Özkundakci, D., Papastergiadou, E., Scharfenberger, U., Šorf, M., Stefanidis, K., Tuvikene, L., Zingel, P., Søndergaard, M., Jeppesen, E., and Adrian, R. 2015. Effects of water temperature on summer periphyton biomass in shallow lakes: a pan-European mesocosm experiment. *Aquatic Sciences* 77 (3):499-510.

Mander, Ü., Kuusemets, V., Lõhmus, K., and Mauring, T. 1997. Efficiency and dimensioning of riparian buffer zones in agricultural catchments. *Ecological Engineering* 8 (4):299-324.

Matsui, T., and Tsuchiya, T. 2006. Root aerobic respiration and growth characteristics of three *Typha* species in response to hypoxia. *Ecological Research* 21 (3):470-475.

Maynard, J. J., O'Geen, A. T., and Dahlgren, R. A. 2009. Bioavailability and fate of phosphorus in constructed wetlands receiving agricultural runoff in the San Joaquin Valley, California. *Journal of environmental quality* 38 (1):360-372.

McCall, S. J., Hale, M. S., Smith, J. T., Read, D. S., and Bowes, M. J. 2017. Impacts of phosphorus concentration and light intensity on river periphyton biomass and community structure. *Hydrobiologia* 792 (1):315-330.

McCormick, P. V., and Laing, J. A. 2003. Effects of increased phosphorus loading on dissolved oxygen in a subtropical wetland, the Florida Everglades. *Wetlands Ecology and Management* 11 (3):199-216.

McCormick, P. V., O'Dell, M. B., Shuford, R. B. E., Backus, J. G., and Kennedy, W. C. 2001. Periphyton responses to experimental phosphorus enrichment in a subtropical wetland. *Aquatic Botany* 71 (2):119-139.

McLatchey, G. P., and Reddy, K. R. 1998. Regulation of Organic Matter Decomposition and Nutrient Release in a Wetland Soil. *Journal of Environment Quality* 27 (5):1268-1274.

Menon, R. 2011. *Influence of vegetation on sediment bacterial community structure, microbial enzyme activities and phosphorus retention in constructed freshwater wetlands*. PhD Thesis, Department of Biology, University of Mississippi.

Menon, R., and Holland, M. M. 2013. Phosphorus Retention in Constructed Wetlands Vegetated with Juncus effusus, Carex lurida, and Dichanthelium acuminatum var. acuminatum. *Water, Air, & Soil Pollution* 224 (7):1-11.

Menon, R., and Holland, M. M. 2014. Phosphorus Release due to Decomposition of Wetland Plants. *Wetlands* 34 (6):1191-1196.

Miao, S. L., and Sklar, F. H. 1997. Biomass and nutrient allocation of sawgrass and cattail along a nutrient gradient in the Florida Everglades. *Wetlands Ecology and Management* 5 (4):245-264.

Mitsch, W. J., Cronk, J. K., Wu, X., Nairn, R. W., and Hey, D. L. 1995. Phosphorus Retention in Constructed Freshwater Riparian Marshes. *Ecological Applications* 5 (3):830-845.

Mitsch, W. J., Dorge, C. L., and Wiemhoff, J. R. 1979. Ecosystem Dynamics and a Phosphorus Budget of an Alluvial Cypress Swamp in Southern Illinois. *Ecology* 60 (6):1116-1124.

Mitsch, W. J., and Gosselink, J. G. 2015. *Wetlands*. Fifth;5; ed. Hoboken, New Jersey: Wiley.

Mitsch, W. J., Horne, A. J., and Nairn, R. W. 2000. Nitrogen and phosphorus retention in wetlands - ecological approaches to solving excess nutrient problems (Editorial). *Ecological Engineering* 14 (1):1-7.

Mitsch, W. J., Zhang, L., Anderson, C. J., Altor, A. E., and Hernández, M. E. 2005. Creating riverine wetlands: Ecological succession, nutrient retention, and pulsing effects. *Ecological Engineering* 25 (5):510-527.

Mitsch, W. J., Zhang, L., Marois, D., and Song, K. 2015. Protecting the Florida Everglades wetlands with wetlands: Can stormwater phosphorus be reduced to oligotrophic conditions? *Ecological Engineering* 80:8-19.

Mitsch, W. J., Zhang, L., Stefanik, K. C., Nahlik, A. M., Anderson, C. J., Bernal, B., Hernandez, M., and Song, K. 2012. Creating Wetlands: Primary Succession, Water Quality Changes and Self-Design over 15 Years. *BioScience* 62 (3):237-250.

Mitsch, W. J., Zhang, L., Waletzko, E., and Bernal, B. 2014. Validation of the ecosystem services of created wetlands: Two decades of plant succession, nutrient retention, and carbon sequestration in experimental riverine marshes. *Ecological Engineering* 72:11-24.

Morris, K., Bailey, P. C., Boon, P. I., and Hughes, L. 2003. Alternative stable states in the aquatic vegetation of shallow urban lakes. II. Catastrophic loss of aquatic plants consequent to nutrient enrichment. *Marine & Freshwater Research* 54 (3):201-215.

Mortimer, C. H. 1971. Chemical Exchanges Between Sediments and Water in the Great Lakes-Speculations on Probable Regulatory Mechanisms. *Limnology and Oceanography* 16 (2):387-404.

Murphy, S. M., Wimp, G. M., Lewis, D., and Denno, R. F. 2012. Nutrient Presses and Pulses Differentially Impact Plants, Herbivores, Detritivores and Their Natural Enemies. *PLOS ONE* 7 (8):e43929.

Mustafa, A., and Scholz, M. 2011. Nutrient Accumulation in *Typha latifolia* L. and Sediment of a Representative Integrated Constructed Wetland. *Water, Air, & Soil Pollution* 219 (1):329-341.

Mustafa, A., Scholz, M., Harrington, R., and Carroll, P. 2009. Long-term performance of a representative integrated constructed wetland treating farmyard runoff. *Ecological Engineering* 35 (5):779-790.

Neto, R. R., Mead, R. N., Louda, J. W., Jaff, xe, and Rudolf. 2006. Organic Biogeochemistry of Detrital Flocculent Material (Floc) in a Subtropical, Coastal Wetland. *Biogeochemistry* 77 (3):283-304.

Newman, S., Grace, J. B., and Koebel, J. W. 1996. Effects of Nutrients and Hydroperiod on *Typha*, *Cladium*, and *Eleocharis*: Implications for Everglades Restoration. *Ecological Applications* 6 (3):774-783.

Nichols, D. S. 1983. Capacity of Natural Wetlands to Remove Nutrients from Wastewater. *Journal (Water Pollution Control Federation)* 55 (5):495-505.

Nielson, R. M., Sugihara, R. T., Boardman, T. J., and Engeman, R. M. 2004. Optimization of ordered distance sampling. *Environmetrics* 15 (2):119-128.

Noe, G. B., and Childers, D. L. 2007. Phosphorus budgets in Everglades wetland ecosystems: the effects of hydrology and nutrient enrichment. *Wetlands Ecology and Management* 15 (3):189-205.

Noe, G. B., Childers, D. L., Edwards, A. L., Gaiser, E., Jayachandran, K., Lee, D., Meeder, J., Richards, J., Scinto, L. J., Trexler, J. C., and Jones, R. D. 2002. Short-Term Changes in Phosphorus Storage in an Oligotrophic Everglades Wetland Ecosystem Receiving Experimental Nutrient Enrichment. *Biogeochemistry* 59 (3):239-267.

Noe, G. B., Scinto, L. J., Taylor, J., Childers, D. L., and Jones, R. D. 2003. Phosphorus cycling and partitioning in an oligotrophic Everglades wetland ecosystem: A radioisotope tracing study. *Freshwater Biology* 48 (11):1993-2003.

Nolte and Associates. 1997. Sacramento Regional Wastewater Treatment Plant Demonstration Wetlands Project, 1996 Annual Report to Sacramento Regional County Sanitation District. Sacramento, California: Nolte and Associates.

Odum, E. P. 1984. The Mesocosm. *BioScience* 34 (9):558-562.

Odum, H. T. 1983. *Systems ecology; An introduction*. USA.

OECD. 1982. *Eutrophication of waters: monitoring, assessment and control*. Edited by O. f. E. C.-o. a. Development. Paris, France: Organisation for Economic Co-operation and Development.

Ogden, J. C., Davis, S. M., Jacobs, K. J., Barnes, T., and Fling, H. E. 2005. The use of conceptual ecological models to guide ecosystem restoration in South Florida. *Wetlands* 25 (4):795-809.

Olsen, S., Chan, F., Li, W., Zhao, S., Søndergaard, M., and Jeppesen, E. 2015. Strong impact of nitrogen loading on submerged macrophytes and algae: a long-term mesocosm experiment in a shallow Chinese lake. *Freshwater Biology* 60 (8):1525-1536.

Orth, R. J., Williams, M. R., Marion, S. R., Wilcox, D. J., Tim, J. B. C., Moore, K. A., Kemp, W. M., Dennison, W. C., Rybicki, N., Bergstrom, P., and Batiuk, R. A. 2010. Long-Term Trends in Submersed Aquatic Vegetation (SAV) in Chesapeake Bay, USA, Related to Water Quality. *Estuaries and Coasts* 33 (5):1144-1163.

Özen, A., Karapınar, B., Kucuk, İ., Jeppesen, E., and Beklioglu, M. 2010. Drought-induced changes in nutrient concentrations and retention in two shallow Mediterranean lakes subjected to different degrees of management. *Hydrobiologia* 646 (1):61-72.

Ozkan, K., Jeppesen, E., Johansson, L. S., and Beklioglu, M. 2010. The response of periphyton and submerged macrophytes to nitrogen and phosphorus loading in shallow warm lakes: a mesocosm experiment. *Freshwater Biology* 55 (2):463-475.

Paerl, H. W., and Otten, T. G. 2013. Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology* 65 (4):995-1010.

Paice, R. L., Chambers, J. M., and Robson, B. J. 2016. Outcomes of submerged macrophyte restoration in a shallow impounded, eutrophic river. *Hydrobiologia* 778 (1):179-192.

Palmer-Felgate, E. J., Mortimer, R. J. G., Krom, M. D., Jarvie, H. P., Williams, R. J., Spraggs, R. E., and Stratford, C. J. 2011. Internal loading of phosphorus in a sedimentation pond of a treatment wetland: Effect of a phytoplankton crash. *Science of the Total Environment* 409 (11):2222-2232.

Pezeshki, S. R., and DeLaune, R. D. 2012. Soil oxidation-reduction in wetlands and its impact on plant functioning. *Biology* 1 (2):196-221.

Pezeshki, S. R., DeLaune, R. D., Kludze, H. K., and Choi, H. S. 1996. Photosynthetic and growth responses of cattail (*Typha domingensis*) and sawgrass (*Cladium jamaicense*) to soil redox conditions. *Aquatic Botany* 54 (1):25-35.

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

Phillips, G. L., Eminson, D., and Moss, B. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. *Aquatic Botany* 4:103-126.

Pinder, A. M. 2005. A review of biodiversity in wetlands with organic sediments on the Swan Coastal Plain, Western Australia, with an emphasis on aquatic invertebrates. *Journal of the Royal Society of Western Australia* 88 (3):129-132.

Pollard, J. H. 1971. On Distance Estimators of Density in Randomly Distributed Forests. *Biometrics* 27 (4):991-1002.

Portielje, R., and Van der Molen, D. T. 1999. Relationships between eutrophication variables: from nutrient loading to transparency. *Hydrobiologia* 408:375-387.

Qiu, S., and McComb, A. 1994. Effects of oxygen concentration on phosphorus release from reflooded air-dried wetland sediments. *Marine and Freshwater Research* 45 (7):1319-1328.

Qiu, S., and McComb, A. 2000. Properties of sediment phosphorus in seven wetlands of the Swan Coastal Plain, South-Western Australia. *Wetlands* 20 (2):267-279.

Qiu, S., McComb, A. J., Bell, R. W., and Davis, J. A. 2004. Partitioning processes controlling water column phosphorus concentrations in a shallow wetland. *Freshwater Biology* 49 (5):563-575.

Qualls, R. G., and Richardson, C. J. 2000. Phosphorus Enrichment Affects Litter Decomposition, Immobilization, and Soil Microbial Phosphorus in Wetland Mesocosms. *Soil Science Society of America Journal* 64 (2):799-808.

Qualls, R. G., Richardson, C. J., and Sherwood, L. J. 2001. Soil reduction-oxidation potential along a nutrient enrichment gradient in the Everglades. *Wetlands* 21 (3):403-411.

Quinn, G. P., and Keough, M. J. 2002. *Experimental design and data analysis for biologists*. Cambridge, U.K; New York: Cambridge University Press.

Ray, A. M., Mebane, C. A., Raben, F., Irvine, K. M., and Marcarelli, A. M. 2014. Evaluation of a combined macrophyte–epiphyte bioassay for assessing nutrient enrichment in the Portneuf River, Idaho, USA. *Environmental Monitoring and Assessment* 186 (7):4081-4096.

Reddy, K. R., and DeLaune, R. D. 2008. *Biogeochemistry of wetlands : science and applications*. Boca Raton, Florida: CRC.

Reddy, K. R., Kadlec, R. H., Flaig, E., and Gale, P. M. 1999. Phosphorus Retention in Streams and Wetlands: A Review. *Critical Reviews in Environmental Science and Technology* 29 (1):83-146.

Reina, M., Espinar, J. L., and Serrano, L. 2006. Sediment phosphate composition in relation to emergent macrophytes in the Doñana Marshes (SW Spain). *Water Research* 40 (6):1185-1190.

Rejmankova, E. 2011. The role of macrophytes in wetland ecosystems. *Journal of Ecology and Environment* 34 (4):333-345.

Rejmánková, E. 2001. Effect of experimental phosphorus enrichment on oligotrophic tropical marshes in Belize, Central America. *Plant and Soil* 236 (1):33-53.

Rejmánková, E., Macek, P., and Epps, K. 2008. Wetland Ecosystem Changes After Three Years Of Phosphorus Addition. *Wetlands* 28 (4):914-927.

Rejmánková, E., and Snyder, J. M. 2008. Emergent macrophytes in phosphorus limited marshes: do phosphorus usage strategies change after nutrient addition? *Plant and Soil* 313 (1/2):141-153.

Richardson, C. J. 1985. Mechanisms Controlling Phosphorus Retention Capacity in Freshwater Wetlands. *Science* 228 (4706):1424-1427.

Richardson, C. J., and Marshall, P. E. 1986. Processes Controlling Movement, Storage, and Export of Phosphorus in a Fen Peatland. *Ecological Monographs* 56 (4):280-302.

Richardson, C. J., and Qian, S. S. 1999. Long-Term Phosphorus Assimilative Capacity in Freshwater Wetlands: A New Paradigm for Sustaining Ecosystem Structure and Function. *Environmental Science & Technology* 33 (10):1545-1551.

Richardson, C. J., and Vaithiyanathan, P. 2009. Biogeochemical Dynamics II: Cycling and Storage of Phosphorus in Wetlands. In *The Wetlands Handbook*, edited by E. Maltby and T. Barker, 228-248. Oxford, UK: Wiley-Blackwell.

Rodrigo, M. A., Rojo, C., Alonso-Guillén, J. L., and Vera, P. 2013. Restoration of two small Mediterranean lagoons: The dynamics of submerged macrophytes and factors that affect the success of revegetation. *Ecological Engineering* 54:1-15.

Romo, S., Miracle, M. R., Villena, M. J., Rueda, J., Ferriol, C., and Vicente, E. 2004. Mesocosm experiments on nutrient and fish effects on shallow lake food webs in a Mediterranean climate. *Freshwater Biology* 49 (12):1593-1607.

Ryder, D. S., and Horwitz, P. 1995. Seasonal water regimes and leaf litter processing in a wetland on the Swan Coastal Plain, Western Australia. *Marine & Freshwater Research* 46 (7):1077-1084.

Sagarin, R. D., Adams, J., Blanchette, C. A., Brusca, R. C., Chorover, J., Cole, J. E., Micheli, F., Munguia-Vega, A., Rochman, C. M., Bonine, K., Haren, J., and Troch, P. A. 2016. Between control and complexity: opportunities and challenges for marine mesocosms. *Frontiers in Ecology and the Environment* 14 (7):389-396.

Sand-Jensen, K., Bruun, H. H., Baastrup-Spohr, L., and Lee, J. 2017. Decade-long time delays in nutrient and plant species dynamics during eutrophication and re-oligotrophication of Lake Fure 1900–2015. *Journal of Ecology* 105 (3):690-700.

Sand-Jensen, K., and Møller, C. L. 2014. Reduced root anchorage of freshwater plants in sandy sediments enriched with fine organic matter. *Freshwater Biology* 59 (3):427-437.

Sayer, C. D., Davidson, T. A., and Jones, J. I. 2010. Seasonal dynamics of macrophytes and phytoplankton in shallow lakes: a eutrophication-driven pathway from plants to plankton? *Freshwater Biology* 55 (3):500-513.

Schaafsma, J. A., H. Baldwin, A., and Streb, C. A. 1999. An evaluation of a constructed wetland to treat wastewater from a dairy farm in Maryland, USA. *Ecological Engineering* 14 (1):199-206.

Scharf, W. 1999. Restoration of the highly eutrophic Lingese Reservoir. *Hydrobiologia* 416:85-96.

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., and Walker, B. 2001. Catastrophic shifts in ecosystems. *Nature* 413 (6856):591-596.

Scheffer, M., and Carpenter, S. R. 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends in Ecology & Evolution* 18 (12):648-656.

Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., and Jeppesen, E. 1993. Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution* 8 (Generic):275-279.

Schindler, D. W. 1998. Whole-Ecosystem Experiments: Replication Versus Realism: The Need for Ecosystem-Scale Experiments. *Ecosystems* 1 (4):323-334.

Schindler, D. W. 2006. Recent Advances in the Understanding and Management of Eutrophication. *Limnology and Oceanography* 51 (1):356-363.

Schindler, D. W. 2012. The dilemma of controlling cultural eutrophication of lakes. *Proceedings of the Royal Society, Biological Sciences* 279 (1746):4322-4333.

Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., and Orihel, D. M. 2016. Reducing Phosphorus to Curb Lake Eutrophication is a Success. *Environmental science & technology* 50 (17):8923-8929.

Schindler, D. W., and Vallentyne, J. R. 2008. *The algal bowl: overfertilization of the world's freshwaters and estuaries*. London, UK: Earthscan.

Schutten, J., Dainty, J., and Davy, A. J. 2005. Root Anchorage and Its Significance for Submerged Plants in Shallow Lakes. *Journal of Ecology* 93 (3):556-571.

Semeniuk, C. A. 1988. Consanguineous wetlands and their distribution in the Darling System, southwestern Australia. *Journal of the Royal Society of Western Australia* 70 (3):69-87.

Semeniuk, V., Semeniuk, C. A., Cresswell, I. D., and Marchant, N. G. 1990. Wetland of the Darling System, southwestern Australia: a descriptive classification using vegetation pattern and form. *Journal of the Royal Society of Western Australia* 72 (4):109-121.

Sethi, S. A., and Hollmen, T. 2015. Conceptual Models for Marine and Freshwater Systems in Alaska: Flexible Tools for Research Planning, Prioritization and Communication. *Arctic* 68 (4):422-434.

Simpson, S. L., Batley, G. B., and Chariton, A. A. 2013. Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines. CSIRO Land and Water Science Report 08/07. Australia: CSIRO.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environmental Science and Pollution Research* 10 (2):126-139.

Smith, V. H., Joye, S. B., and Howarth, R. W. 2006. Eutrophication of Freshwater and Marine Ecosystems. *Limnology and Oceanography* 51 (1):351-355.

Smith, V. H., and Schindler, D. W. 2009. Eutrophication science: where do we go from here? *Trends in Ecology & Evolution* 24 (4):201-207.

Smith, V. H., Tilman, G. D., and Nekola, J. C. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* 100 (1):179-196.

Sollie, S., and Verhoeven, J. T. A. 2008. Nutrient Cycling and Retention Along a Littoral Gradient in a Dutch Shallow Lake in Relation to Water Level Regime. *Water, Air, and Soil Pollution* 193 (1):107-121.

Sommer, B. 2006. *Drying and re-wetting of organic wetland sediments: biogeochemistry and implications for wetland management*. PhD Thesis, Faculty of Computing, Health and Science, Edith Cowan University.

Søndergaard, M., Jensen, J. P., and Jeppesen, E. 2003. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* 506 (1):135-145.

Song, K., and Burgin, A. J. 2017. Perpetual Phosphorus Cycling: Eutrophication Amplifies Biological Control on Internal Phosphorus Loading in Agricultural Reservoirs. *Ecosystems* 20 (8):1483-1493.

Stewart, R. I. A., Dossena, M., Bohan, D. A., Jeppesen, E., Kordas, R. L., Ledger, M. E., Meerhoff, M., Moss, B., Mulder, C., Shurin, J. B., Suttle, B., Thompson, R., Trimmer, M., and Woodward, G. 2013. Mesocosm Experiments as a Tool for Ecological Climate-Change Research: Chapter Two. *Advances in Ecological Research* 48:71-181.

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., Müller, R. A., and Moormann, H. 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. *Biotechnology Advances* 22 (1):93-117.

Switala, K. 1993. Determination of ammonia by flow injection analysis colorimetry (dialysis). Milwaukee, USA: Lachat Instruments.

Syers, J. K., Browman, M. G., Smillie, G. W., and Corey, R. B. 1973. Phosphate Sorption by Soils Evaluated by the Langmuir Adsorption Equation1. *Soil Science Society of America Journal* 37 (3):358-363.

Tanner, C. C., Clayton, J. S., and Upsdell, M. P. 1995. Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands—II. Removal of nitrogen and phosphorus. *Water Research* 29 (1):27-34.

Tercero, M. d. C., Álvarez-Rogel, J., Conesa, H. M., Párraga-Aguado, I., and González-Alcaraz, M. N. 2017. Phosphorus retention and fractionation in an eutrophic wetland: A one-year mesocosms experiment under fluctuating flooding conditions. *Journal of Environmental Management* 190:197-207.

Toet, S., Bouwman, M., Cevaal, A., and Verhoeven, J. T. A. 2005. Nutrient Removal Through Autumn Harvest of Phragmites australis and Thypha latifolia Shoots in Relation to Nutrient Loading in a Wetland System Used for Polishing Sewage Treatment Plant Effluent. *Journal of Environmental Science and Health, Part A* 40 (6-7):1133-1156.

Toet, S., Van Logtestijn, R. S. P., Kampf, R., Schreijer, M., and Verhoeven, J., T. A. . 2005. The effect of hydraulic retention time on the removal of pollutants from sewage treatment plant effluent in a surface-flow wetland system. *Wetlands* 25 (2):375-391.

Tóth, V. R. 2013. The effect of periphyton on the light environment and production of *Potamogeton perfoliatus* L. in the mesotrophic basin of Lake Balaton. *Aquatic Sciences* 75 (4):523-534.

Turner, B. L., Newman, S., and Newman, J. M. 2006. Organic phosphorus sequestration in subtropical treatment wetlands. *Environmental science & technology* 40 (3):727-733.

Tuya, F., Betancor, S., Viera-Rodríguez, M. A., Guedes, R., Riera, R., Haroun, R., and Espino, F. 2015. Effect of Chronic Versus Pulse Perturbations on a Marine Ecosystem: Integration of Functional Responses Across Organization Levels. *Ecosystems* 18 (8):1455-1471.

Vaithiyanathan, P., and Richardson, C. J. 1999. Macrophyte Species Changes in the Everglades: Examination along a Eutrophication Gradient. *Journal of Environment Quality* 28 (4):1347-1358.

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry* 10 (2):109-122.

van Donk, E., and van de Bund, W. J. 2002. Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. *Aquatic Botany* 72 (3):261-274.

Verhoeven, J. T. A. 1986. Nutrient dynamics in minerotrophic peat mires. *Aquatic Botany* 25:117-137.

Verhoeven, J. T. A., Arheimer, B., Yin, C., and Hefting, M. M. 2006. Regional and global concerns over wetlands and water quality. *Trends in Ecology & Evolution* 21 (2):96-103.

Verhoeven, J. T. A., and Meuleman, A. F. M. 1999. Wetlands for wastewater treatment: Opportunities and limitations. *Ecological Engineering* 12 (1):5-12.

Verhofstad, M. J. J. M., Alirangues, M. M., Reichman, E., van Donk, E., Lamers, L. P. M., and Bakker, E. S. 2017. Mass development of monospecific submerged macrophyte vegetation after the restoration of shallow lakes: roles of light, sediment nutrient levels, and propagule density. *Aquatic Botany* 141 (July):29-38.

Verhofstad, M. J. J. M., Poelen, M. D. M., Van Kempen, M. M. L., Bakker, E. S., and Smolders, A. J. P. 2017. Finding the harvesting frequency to maximize nutrient removal in a constructed wetland dominated by submerged aquatic plants. *Ecological Engineering* 106, Part A:423-430.

Villena, M.-J., and Romo, S. 2007. Effects of Nutrients, Fish, Charophytes and Algal Sediment Recruitment on the Phytoplankton Ecology of a Shallow Lake. *International Review of Hydrobiology* 92 (6):626-639.

Vymazal, J. 1995. *Algae and element cycling in wetlands*. Boca Raton, Florida: Lewis Publishers.

Vymazal, J. 2007. Removal of nutrients in various types of constructed wetlands. *The Science of the total environment* 380 (1-3):48-65.

Vymazal, J. 2011. Constructed wetlands for wastewater treatment: five decades of experience. *Environmental science & technology* 45 (1):61.

Vymazal, J. 2013. Emergent plants used in free water surface constructed wetlands: A review. *Ecological Engineering* 61:582-592.

Vymazal, J. 2016a. Concentration is not enough to evaluate accumulation of heavy metals and nutrients in plants. *The Science of the total environment* 544:495-498.

Vymazal, J., ed. 2016b. *Natural and constructed wetlands: nutrients, heavy metals and energy cycling, and flow*. Switzerland: Springer International Publishing.

Vymazal, J., and Richardson, C. J. 2003. The relationships between soil, water nutrients and biomass of *Cladium jamaicense* and *Typha domingensis* in the Northern Everglades. In *Wetlands - nutrients, metals and mass cycling*, edited by J. Vymazal, 107-127. Leiden, Netherlands: Backhuys Publishers.

Walker, W. W., and Kadlec, R. H. 2011. Modeling Phosphorus Dynamics in Everglades Wetlands and Stormwater Treatment Areas. *Critical Reviews in Environmental Science and Technology* 41 (sup1):430-446.

Wang, J., Jiang, X., Zheng, B., Chen, C., Kang, X., Zhang, C., Song, Z., Wang, K., Wang, W., and Wang, S. 2016. Effect of algal bloom on phosphorus exchange at the sediment–water interface in Meiliang Bay of Taihu Lake, China. *Environmental Earth Sciences* 75 (1):1-9.

Wang, L., Liu, Q., Hu, C., Liang, R., Qiu, J., and Wang, Y. 2018. Phosphorus release during decomposition of the submerged macrophyte *Potamogeton crispus*. *Limnology*:1-12.

Wang, N., and Mitsch, W. J. 2000. A detailed ecosystem model of phosphorus dynamics in created riparian wetlands. *Ecological Modelling* 126 (2):101-130.

Water Corporation. 2006-2017. Quarterly Capel Wetlands Monitoring Data. edited by Water Corporation.

Watkins, C. H., and Hammerschlag, R. S. 1984. The toxicity of chlorine to a common vascular aquatic plant. *Water Research* 18 (8):1037-1043.

Weng, S., Putz, G., and Kells, J. A. 2006. Phosphorus uptake by cattail plants in a laboratory-scale experiment related to constructed treatment wetlands. *Journal of Environmental Engineering and Science* 5 (4):295-308.

Wentzell, B. M., Boylen, C. W., and Nierzwicki-Bauer, S. A. 2016. Wetland ecosystem comparison using a suite of plant assessment measures. *Ecological Indicators* 67:283-291.

Whelan, B., and Barrow, N. 1984. The movement of septic tank effluent through sandy soils near Perth. II. Movement of phosphorus. *Soil Research* 22 (3):293-302.

White, J. R., Reddy, K. R., and Majer-Newman, J. 2006. Hydrologic and Vegetation Effects on Water Column Phosphorus in Wetland Mesocosms. *Soil Science Society of America Journal* 70 (4):1242.

White, J. S., Bayley, S. E., and Curtis, P. J. 2000. Sediment storage of phosphorus in a northern prairie wetland receiving municipal and agro-industrial wastewater. *Ecological Engineering* 14 (1):127-138.

Wood, J. D., Gordon, R., Madani, A., and Stratton, G. W. 2008. A Long Term Assessment of Phosphorus Treatment by a Constructed Wetland Receiving Dairy Wastewater. *Wetlands* 28 (3):715-723.

Wu, H., Zhang, J., Li, C., Fan, J., and Zou, Y. 2013. Mass Balance Study on Phosphorus Removal in Constructed Wetland Microcosms Treating Polluted River Water. *CLEAN – Soil, Air, Water* 41 (9):844-850.

Wu, Y., Wen, Y., Zhou, J., and Wu, Y. 2014. Phosphorus release from lake sediments: Effects of pH, temperature and dissolved oxygen. *KSCE Journal of Civil Engineering* 18 (1):323-329.

Yang, H., and Flower, R. J. 2012. Effects of light and substrate on the benthic diatoms in an oligotrophic lake: A comparison between natural and artificial substrates. *Journal of Phycology* 48 (5):1166-1177.

Yeh, T. Y., Ke, T. Y., and Lin, Y. L. 2011. Algal Growth Control Within Natural Water Purification Systems: Macrophyte Light Shading Effects. *Water, Air, & Soil Pollution* 214 (1):575-586.

Zemanová, K., Picek, T., Dušek, J., Edwards, K., and Šantrůčková, H. 2010. Carbon, Nitrogen and Phosphorus Tranformations are Related to Age of a Constructe Wetland. *Water, Air, and Soil Pollution* 207 (1):39-48.

APPENDICES

APPENDIX 1: EFFECT OF DISTANCE FROM EFFLUENT DISCHARGE POINT IN SWAMPHEN LAKE (CHAPTER 4)

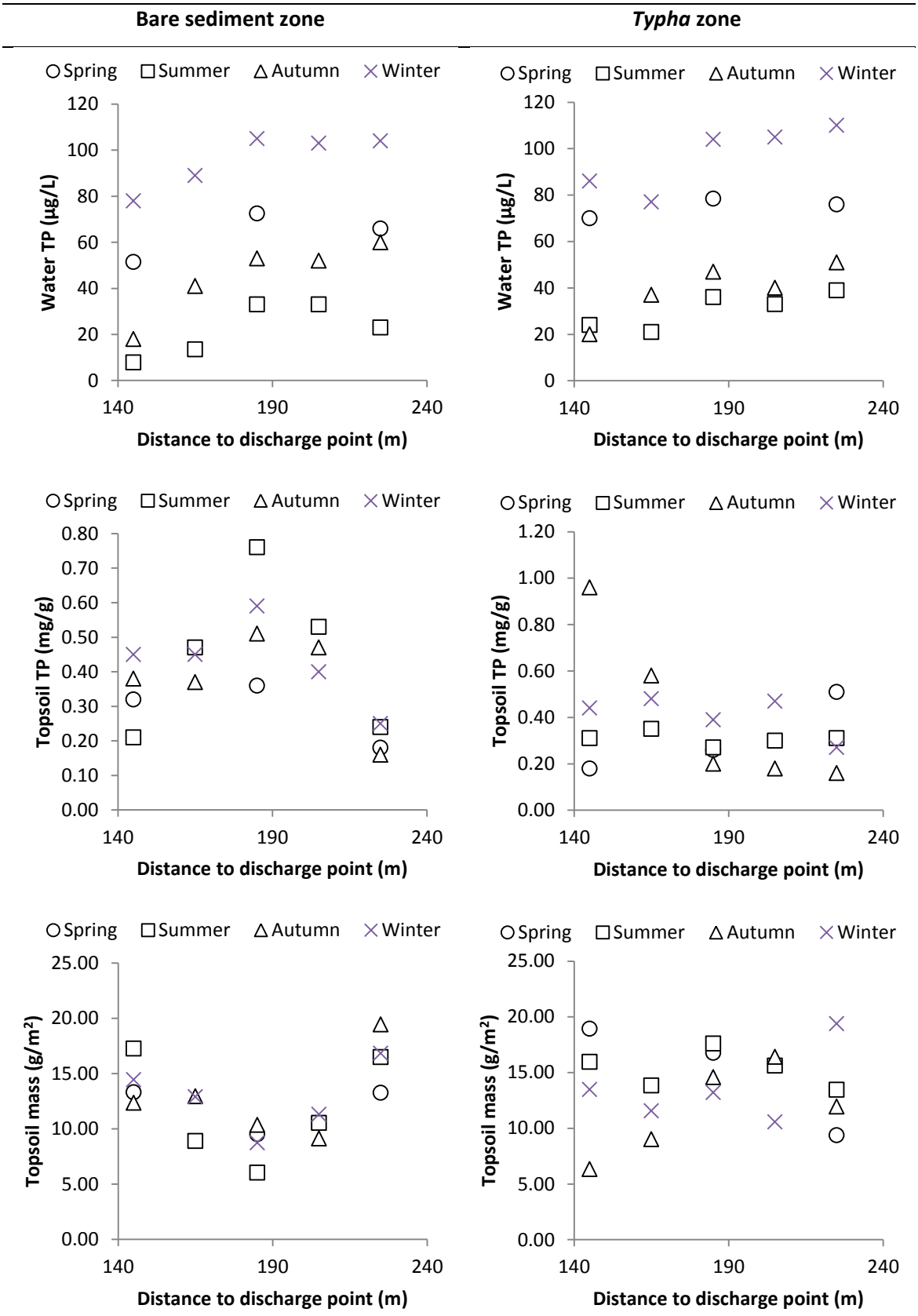

Figure A1-1: Phosphorus concentration and mass of ecosystem components against distance to effluent discharge point in bare sediment and *Typha* zones of Swamphen Lake sampled in spring 2012 and summer, autumn winter 2013.

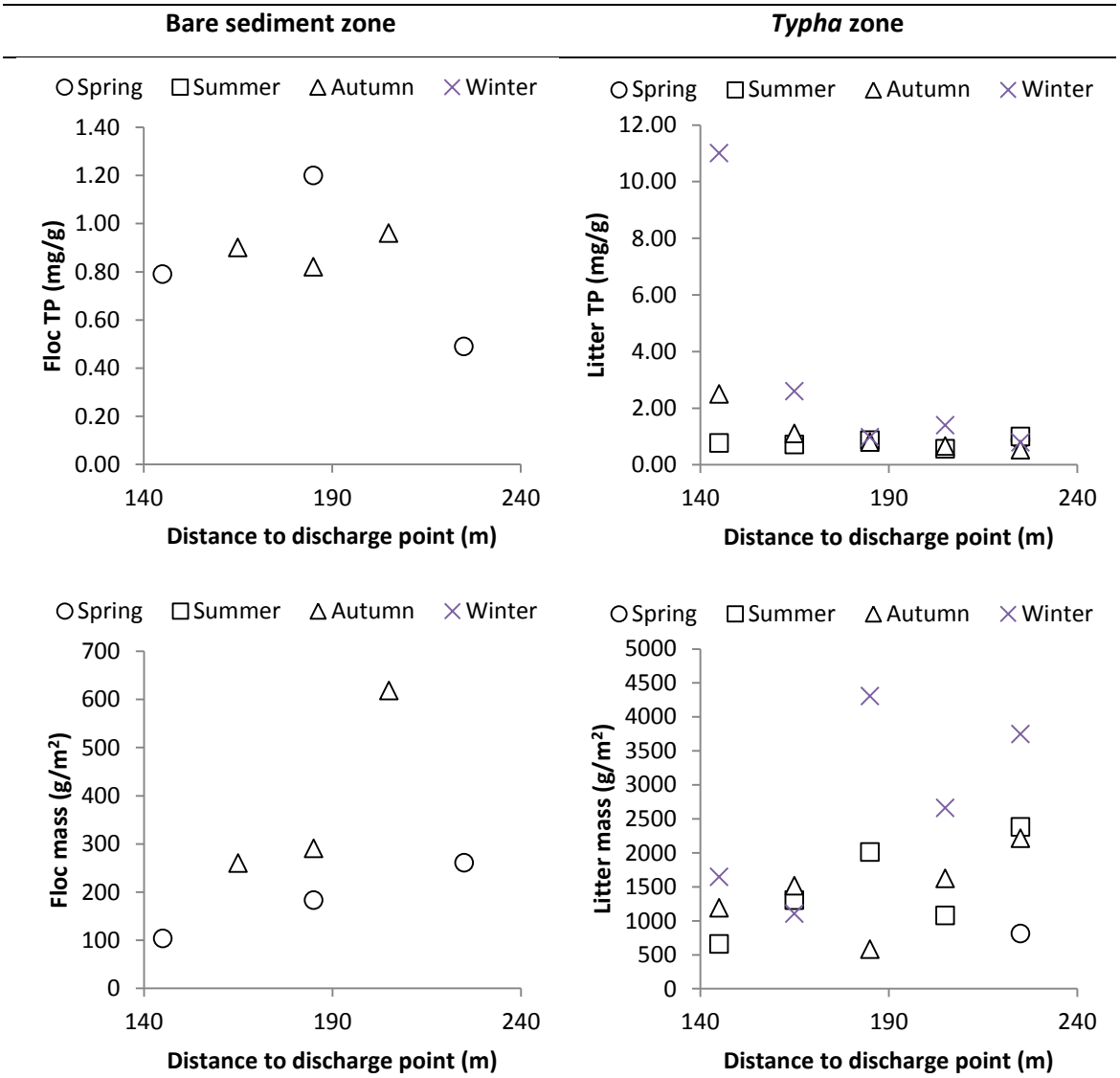

Figure A1-1: continued.

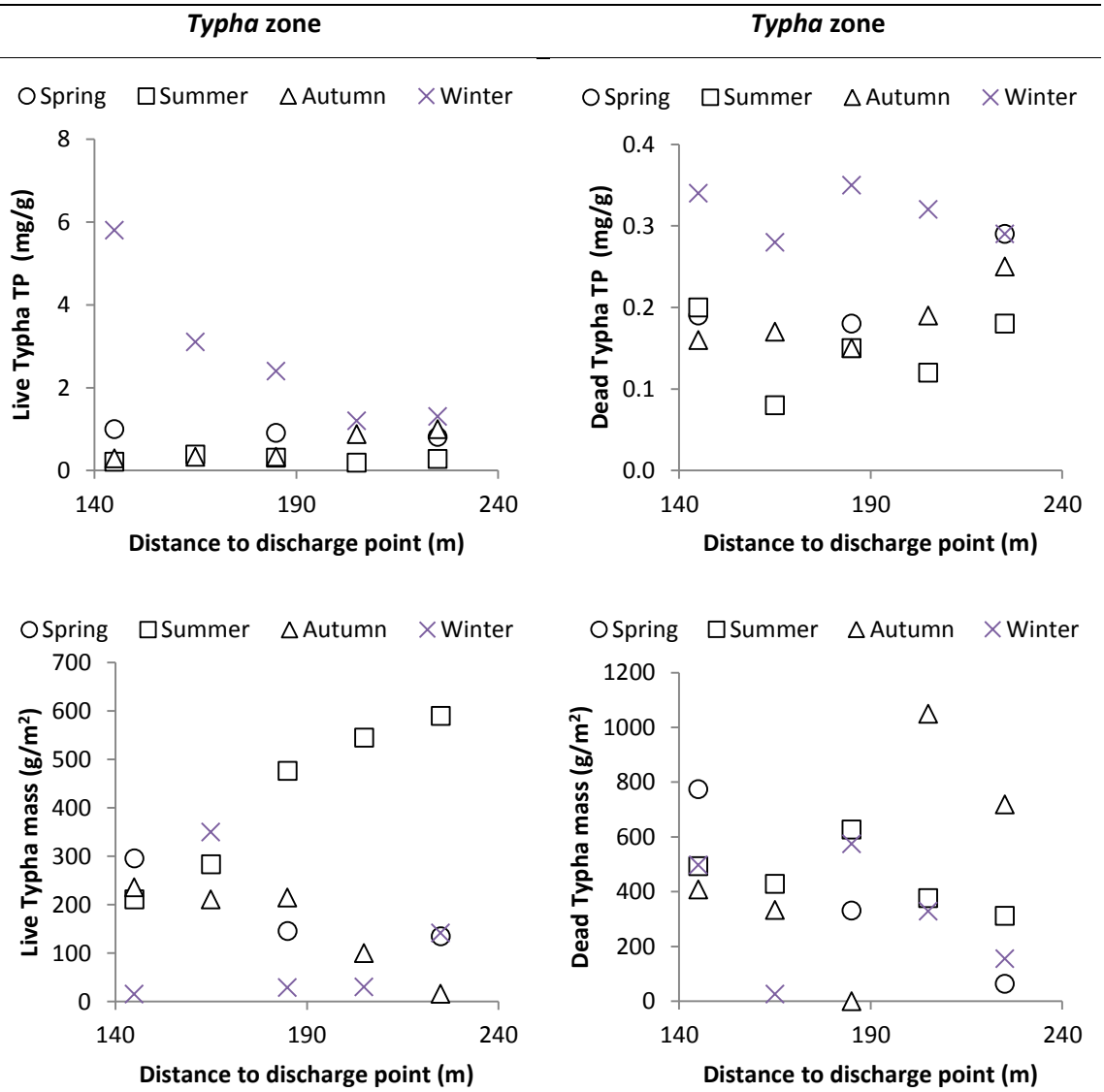

Figure A1-1: continued.

APPENDIX 2: SUBSOIL TP CONCENTRATION VERSUS DEPTH (CHAPTER 4)

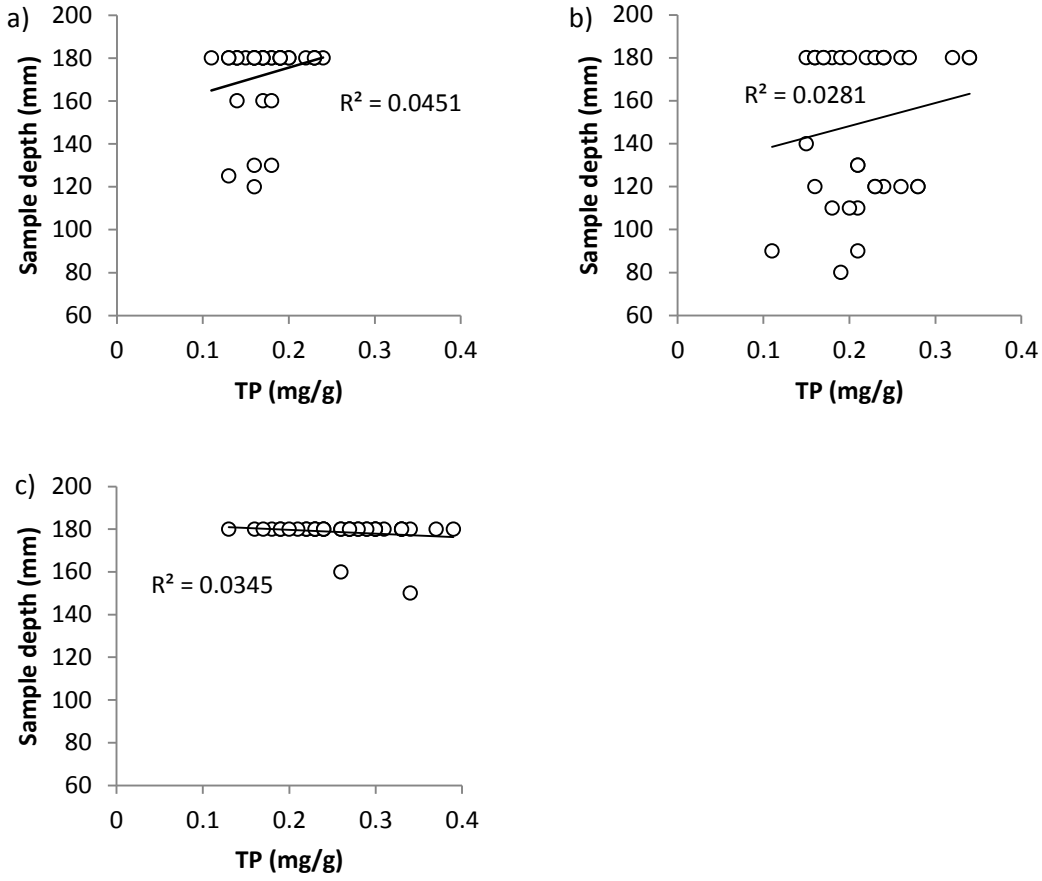

Figure A2-1: Scatter plots of subsoil TP concentration and sample depth (max 180 mm = 200 mm - 20mm) for Swamphen (a), Island (b)and Peninsula (c) Lakes representing combined data for October 2012, January 2013, April 2013 and July 2013 sampling occasions.

Table A2-1: Pearson's correlation test results for subsoil TP concentration versus sample depth for Swamphen, Island and Peninsula Lakes combining data for October 2012, January 2013, April 2013 and July 2013.

Wetland	**r**	***p***	**n**
Swamphen	0.212	0.213	36
Island	0.199	0.244	36
Peninsula	-0.193	0.259	36

APPENDIX 3: STATISTICAL ANALYSIS RESULTS FOR SEASONAL STUDY (CHAPTER 4)

Table A3-1: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in TP concentration, mass (depth for water) and TP storage of ecosystem components between seasons and zones in Swamphen Lake in the seasonal study.

Ecosystem component	Season effect			Zone effect			Season*Zone interaction			Ordinal or disordinal
	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
TP concentration										
Water TP	3, 28	68.667	**<0.001**	1, 28	0.675	0.418	3, 28	0.332	0.802	na
Water FRP#										
Topsoil	3, 28	0.814	0.497	1, 28	0.274	0.605	3,28	0.156	0.925	na
Subsoil	3, 28	7.751	**0.001**	1, 28	0.053	0.819	3, 28	1.085	0.372	na
Litter#	3, 16	4.359	0.225	na			na			
Typha - Live	3, 7.513	26.54	**<0.001**	na			na			
Typha - Dead	3, 14	13.998	**<0.001**	na			na			
Mass/depth										
Water depth	3, 28	64.146	**<0.001**	1, 28	0.815	0.374	3, 28	0.905	0.451	na
Topsoil	3, 28	0.262	0.852	1, 28	1.440	0.240	3, 28	0.782	0.514	na
Litter#	3, 18	8.494	**0.037**	na			na			
Typha - Live	3, 14	5.440	**0.011**	na			na			
Typha - Dead	3, 14	1.119	0.375	na			na			
Typha - Total	3, 14	3.221	0.055	na			na			
TP storage										
Water	3, 28	180.962	**<0.001**	1, 28	3.350	0.078	3, 28	2.040	0.131	
Topsoil	3, 28	0.816	0.496	1, 28	0.275	0.604	3, 28	0.157	0.924	na
Subsoil	3, 28	7.751	**0.001**	1, 28	0.053	0.819	3, 28	1.085	0.372	na
Litter#	3, 18	11.538	**0.009**	na			na			
Typha - Live#	3, 18	5.309	0.151	na			na			
Typha - Dead	3, 14	0.632	0.606	na			na			
Typha - Total#	3, 18	2.703	0.440	na			na			
Total TP	3, 28	3.123	**0.042**	1, 28	2.569	0.120	3, 28	0.914	0.447	na

Table A3-2: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in TP concentration, mass (depth for water) and TP storage of ecosystem components between seasons and zones in Island Lake in the seasonal study.

Ecosystem component	Season effect			Zone effect			Season*Zone interaction			Ordinal or disordinal
	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
TP concentration										
Water TP#	2, 26	10.360	**0.006**	1, 26	5.886	**0.015**	na			
Water FRP#										
Topsoil	3, 28	6.608	**0.002**	1, 28	0.015	0.904	3, 28	0.652	0.589	na
Subsoil	3, 28	3.419	**0.031**	1, 28	0.435	0.515	3, 28	0.553	0.650	ns
Litter#	3, 12	7.421	0.060	na			na			
Typha - Live	3, 14	80.026	**<0.001**	na			na			
Typha - Dead	3,14	3.959	0.031	na			na			
Mass/depth										
Water depth#	3, 36	30.790	**<0.001**	1, 36	2.118	0.146	na			
Topsoil	3, 28	0.881	0.463	1, 28	0.925	0.344	3, 28	0.755	0.529	na
Litter#	3, 18	3.532	0.317	na			na			
Typha - Live	3, 6.227	2.996	0.113	na			na			
Typha - Dead	3, 5.913	0.627	0.624	na			na			
Typha - Total	3, 14	1.025	0.411	na			na			
TP storage										
Water#	3, 36	28.304	**<0.001**	1, 36	3.621	0.057	na			
Topsoil	3, 28	5.298	**0.005**	1, 28	0.007	0.936	3, 28	0.492	0.691	na
Subsoil	3, 28	3.419	**0.031**	1, 28	0.435	0.515	3, 28	0.553	0.650	na
Litter#	3, 18	3.299	0.348	na			na			
Typha - Live#	3, 18	3.629	0.304	na			na			
Typha - Dead	3, 5.791	2.339	0.176	na			na			
Typha - Total	3, 5.688	3.583	0.090	na			na			
Total TP	3, 28	5.007	**0.007**	1, 28	8.622	**0.007**	3, 28	0.704	0.558	na

Table A3-3: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in TP concentration, mass (depth for water) and TP storage of ecosystem components between seasons and zones in Peninsula Lake in the seasonal study.

Ecosystem component	Season effect			Zone effect			Season*Zone interaction			Ordinal or disordinal
	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
TP concentration										
Water TP#	3, 36	30.478	**<0.001**				na			
Water FRP#										
Topsoil	3, 28	3.366	**0.033**	1, 28	0.010	0.919	3, 28	0.434	0.731	na
Subsoil	3, 28	1.429	0.255	1, 28	0.013	0.911	3, 28	0.892	0.458	na
Litter#	3, 15	6.813	0.078	na			na			
Typha - Live	3, 13	85.814	**<0.001**	na			na			
Typha - Dead	3, 14	4.176	**0.026**	na			na			
Mass/depth										
Water depth#	3, 36	31.281	**<0.001**	1, 36	0.230	0.631	na			na
Topsoil	3, 28	1.101	0.365	2, 28	2.409	0.132	3, 28	2.077	0.126	ns
Litter#	3, 18	2.728	0.435	na			na			
Typha - Live	3, 5.345	7.746	**0.022**	na			na			
Typha - Dead	3, 14	4.261	**0.025**	na			na			
Typha - Total	3, 14	0.360	0.783	na			na			
TP storage										
Water#	2, 26	16.599	**<0.001**	1, 26	0.318	0.573	na			
Topsoil	3, 28	5.592	**0.004**	1, 28	0.005	0.947	3, 28	0.440	0.726	na
Subsoil	3, 28	1.429	**0.255**	1, 28	0.013	0.911	3, 28	0.892	0.458	na
Litter#	3, 18	4.217	0.239	na			na			
Typha - Live	3, 14	7.670	**0.003**	na			na			
Typha - Dead	3, 14	3.631	**0.040**	na			na			
Typha - Total	3, 14	4.724	**0.018**	na			na			
Total TP	3, 28	5.382	**0.005**	1, 28	9.190	**0.005**	3, 28	0.673	0.576	na

Table A3-4: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in water quality variables and *Typha* height between seasons and zones in Swamphen, Island and Peninsula Lakes in the seasonal study.

Ecosystem component	Season effect			Zone effect			Season*Zone interaction			Ordinal or disordinal
	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
Swamphen										
Chlorophyll α	3, 28	6.778	**0.001**	1, 28	2.898	**0.100**	3, 28	6.698	**0.002**	do
Turbidity	3, 28	31.480	**<0.001**	1, 28	1.270	0.269	3, 28	3.024	**0.046**	do
Surface Temperature										
Bottom Temperature #	3, 36	28.733	**<0.001**	1, 36	0.225	0.635	na			
Surface pH										
Bottom pH #	3, 36	26.461	**<0.001**	1, 36	1.725	0.189	na			
Surface EC										
Bottom EC #	3, 36	32.617	**<0.001**	1, 36	0.121	0.728	na			
Surface DO										
Bottom DO % #	3, 36	22.849	**<0.001**	1, 36	0.484	0.486	na			
Surface ORP										
Bottom ORP #	3, 31	16.197	**0.001**	1, 31	0.977	0.323	na			
Typha height	3, 90	5.142	0.162	na			na			
Island										
Chlorophyll α	2, 20	10.370	**0.001**	1, 20	32.374	**<0.001**	2, 20	4.119	**0.032**	or
Turbidity	2, 20	24.429	**<0.001**	1, 20	3.095	0.094	2, 20	8.509	**0.002**	do
Surface Temperature										
Bottom Temperature #	2, 26	19.788	**<0.001**	1, 26	0.716	0.397	na			
Surface pH										
Bottom pH	2, 20	22.236	**<0.001**	1, 20	3.382	0.081	2, 20	3.167	0.064	na
Surface EC										
Bottom EC #	2, 26	20.189	**<0.001**	1, 26	0.190	0.663	na			
Surface DO										
Bottom DO %	2, 20	1.761	0.197	1, 20	22.223	**<0.001**	2, 20	0.626	0.545	na
Surface ORP										
Bottom ORP#	2, 26	21.101	**<0.001**	1, 26	0.111	0.739	na			
Typha height	3, 90	0.256	0.968	na			na			

Ecosystem component	Season effect			Zone effect			Season*Zone interaction			Ordinal or disordinal
	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
Peninsula										
Chlorophyll α	2, 20	70.037	**<0.001**	1, 20	11.222	**0.003**	2, 20	2.183	0.139	na
Turbidity	2, 20	15.747	**<0.001**	1, 20	0.041	0.842	2, 20	3.991	**0.035**	do
Surface Temperature										
Bottom Temperature #	2, 26	19.095	**<0.001**	1, 26	1.579	0.209	na			
Surface pH										
Bottom pH	2, 20	68.105	**<0.001**	1, 20	0.371	0.549	2, 20	1.630	0.221	na
Surface EC										
Bottom EC #	2, 26	21.880	**<0.001**	1, 26	0.001	0.980	na			
Surface DO										
Bottom DO %	2, 20	7.361	**0.004**	1, 20	14.049	**0.001**	2, 20	0.867	0.435	na
Surface ORP										
Bottom ORP #	2, 26	5.912	**0.052**	1, 26	1.989	0.158	na			
Typha height	3, 90	3.819	0.282	na			na			

APPENDIX 4: EFFECT OF DISTANCE FROM EFFLUENT DISCHARGE POINT IN SWAMPHEN LAKE (CHAPTER 5)

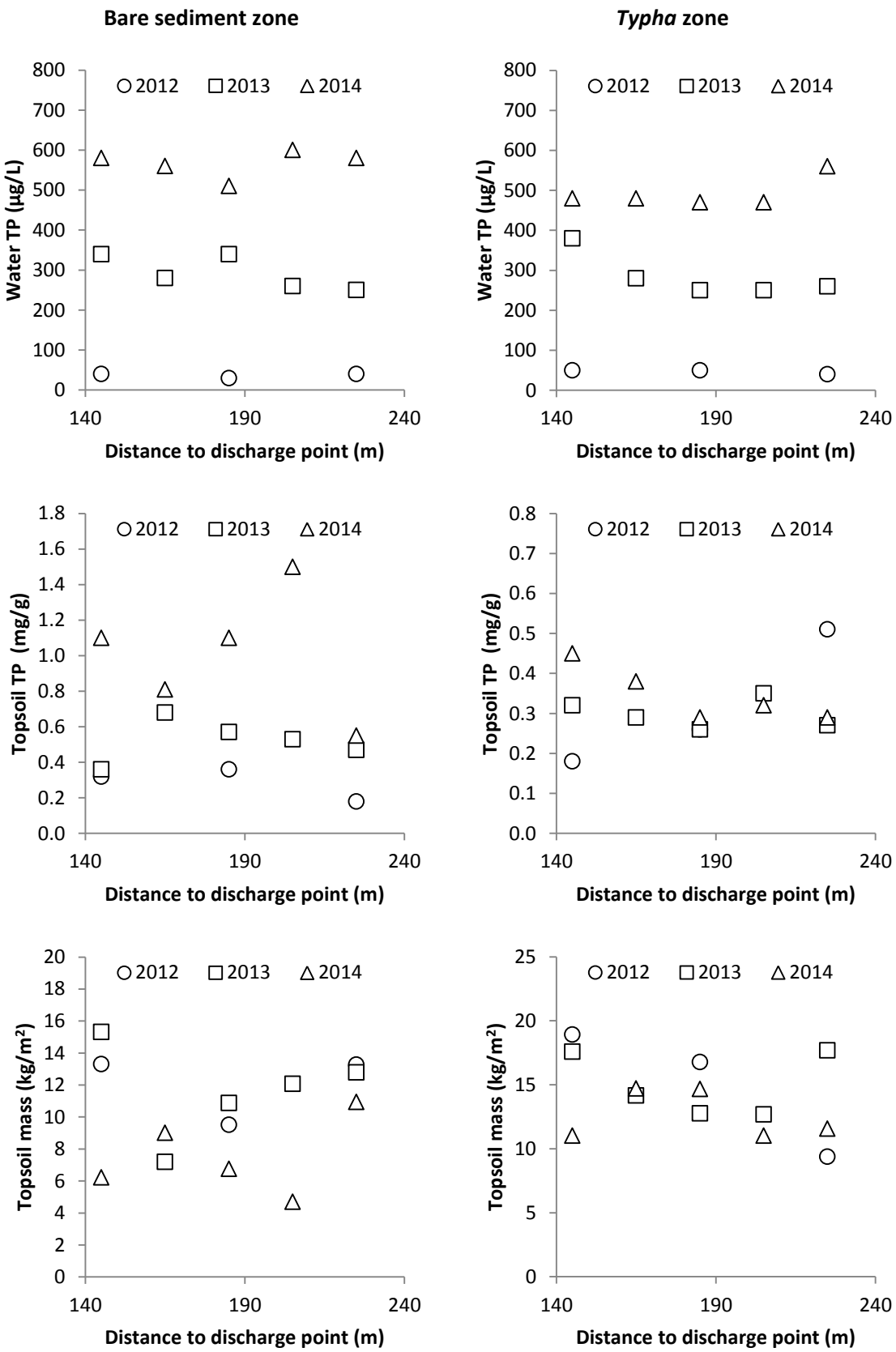

Figure A4-1: Phosphorus concentration and mass of ecosystem components against distance to effluent discharge point in bare sediment and *Typha* zones of Swamphen Lake sampled in 2012, 2013 and 2014.

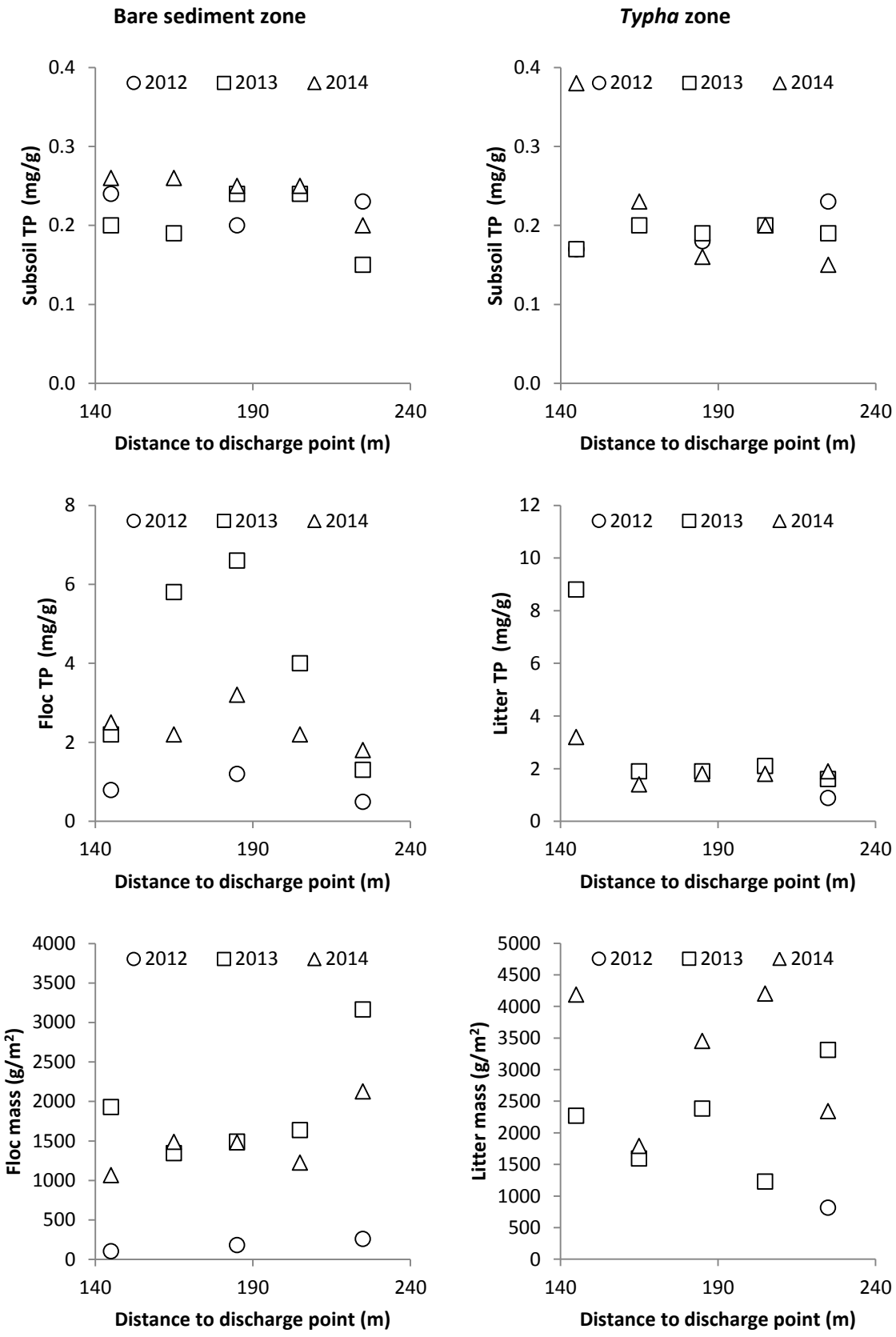

Figure A4-1: continued.

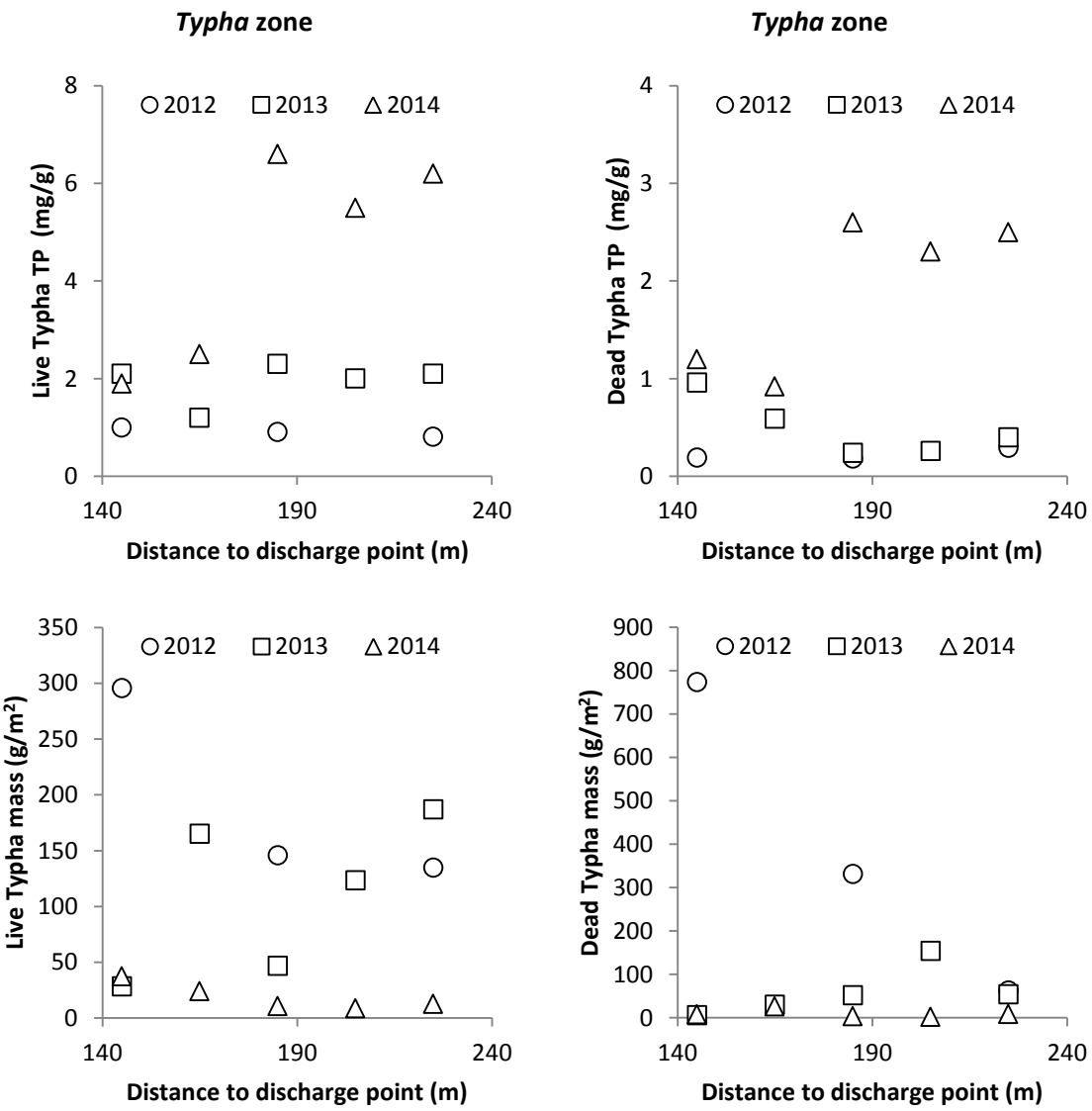

Figure A4-1: continued.

APPENDIX 5: SUBSOIL TP CONCENTRATION VERSUS DEPTH (CHAPTER 5)

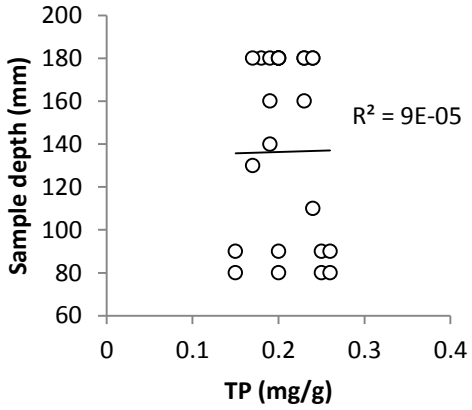

Figure A5-1: Scatter plot of subsoil TP concentration and sample depth (max 180 mm = 200 mm - 20mm) for Swamphen Lake representing combined data for October 2012, October 2013 and October 2014 sampling occasions.

Table A5-1: Pearson's correlation test results for subsoil TP concentration versus sample depth for Swamphen Lake combining data from October 2012, October 2013 and October 2014.

Wetland	**r**	***p***	**n**
Swamphen	0.009	0.965	25

APPENDIX 6: STATISTICAL ANALYSIS RESULTS FOR MULTIYEAR STUDY (CHAPTER 5)

Table A6-1: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in TP concentration, mass (depth for water) and TP storage of ecosystem components between years and zones in Swamphen Lake in the multiyear study.

Ecosystem component	Year effect			Zone effect			Year*Zone interaction			Ordinal or disordinal
	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
TP concentration										
Water TP	2, 20	748.0	**<0.000**	1, 20	0.2	0.685	2, 20	4.2	**0.029**	do
Water FRP#	2, 26	17.959	**<0.001**	1, 26	0.775	0.379	na			na
Topsoil	2, 20	10.4	**0.001**	1, 20	18.1	**<0.001**	2, 20	5.7	**0.011**	do
Subsoil	2, 20	1.3	0.287	1, 20	2.0	0.175	2, 20	0.1	0.874	na
Floc	2, 10	8.5	**0.007**							
Litter#	2, 11	3.312	0.191							
Typha - Live	2, 5.733	17.998	**0.003**							
Typha - Dead	2, 5.711	11.736	**0.009**							
Mass										
Water depth	2, 20	52.0	**<0.001**	1, 20	0.0	0.986	2, 20	1.0	0.397	na
Topsoil	2, 20	4.326	**0.027**	1, 20	11.135	**0.003**	2, 20	0.343	0.714	na
Floc	2, 10	10.264	**0.004**							
Litter#	2, 13	7.934	**0.019**							
Typha - Live	2, 10	14.0	**0.001**							
Typha - Dead	2, 10	10.1	**0.004**							
Typha - Total	2, 10	14.3	**0.001**							
TP storage										
Water	2, 20	1080.4	**<0.001**	1, 20	0.7	0.399	2, 20	9.9	**0.001**	do
Topsoil	2, 20	11.064	**0.001**	1, 20	11.120	**0.003**	2, 20	9.182	**0.001**	do
Subsoil	2, 20	1.5	0.241	1, 20	1.2	0.285	2, 20	0.1	0.950	na
Floc	2, 10	114.351	**<0.001**							
Litter#	2, 13	6.506	**0.039**							
Typha - Live	2, 10	4.6	**0.039**							
Typha - Dead	2, 10	4.0	0.054							
Typha - Total	2, 10	4.2	**0.047**							
Total TP	2, 20	26.476	**<0.001**	1, 20	0.198	0.661	2, 20	1.048	0.369	na

Table A6-2: ANOVA test results for differences in proportional share of total TP storage by ecosystem components between years and zones in Swamphen Lake in the multiyear study.

Ecosystem component	Year effect			Zone effect			Year*Zone interaction			Ordinal or disordinal
	df	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	
Water	2, 20	49.820	**<0.001**	1, 20	0.207	0.654	2, 20	1.106	0.350	na
Topsoil	2, 20	41.172	**<0.000**	1, 20	6.464	**0.019**	2, 20	2.416	0.115	na
Floc	2, 10	6.431	**<0.001**							
Litter#	2, 13	6.612	**0.037**							
Typha	2, 10	4.845	**0.034**							

Table A6-3: ANOVA test results for water quality variables and *Typha* height between years and zones in Swamphen Lake in the multiyear study.

Ecosystem component	Year effect			Zone effect			Year*Zone interaction			Ordinal or disordinal
	df	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	
Chlorophyll α	2, 20	120.830	**<0.001**	1, 20	0.838	0.371	2, 20	0.374	0.693	na
Turbidity	2, 20	249.884	**<0.001**	1, 20	0.025	0.875	2, 20	1.227	0.314	na
Surface Temperature	2, 20	2.632	0.097	1, 20	0.041	0.842	2, 20	0.599	0.559	na
Bottom Temperature	2, 20	100.546	**<0.001**	1, 20	0.065	0.802	2, 20	0.618	0.549	na
Surface pH	2, 20	188.565	**<0.001**	1, 20	0.398	0.535	2, 20	1.878	0.179	na
Bottom pH	2, 20	21.268	**<0.001**	1, 20	0.359	0.556	2, 20	0.531	0.596	na
Surface EC	2, 20	520.086	**<0.001**	1, 20	0.596	0.449	2, 20	0.533	0.595	na
Bottom EC	2, 20	680.446	**<0.001**	1, 20	1.133	0.300	2, 20	0.425	0.659	na
Surface DO	2, 20	584.380	**<0.001**	1, 20	0.345	0.563	2, 20	0.089	0.915	na
Bottom DO	2, 20	9.780	**0.001**	1, 20	5.924	**0.024**	2, 20	0.935	0.409	na
Surface ORP	2, 20	39.459	**<0.001**	1, 20	3.623	0.072	2, 20	1.482	0.251	na
Bottom ORP	2, 20	70.304	**<0.001**	1, 20	2.526	0.128	2, 20	0.254	0.778	na
Typha height	2, 62	6.539	**0.003**							

APPENDIX 7: SUBSOIL TP CONCENTRATION VERSUS DEPTH (CHAPTER 6)

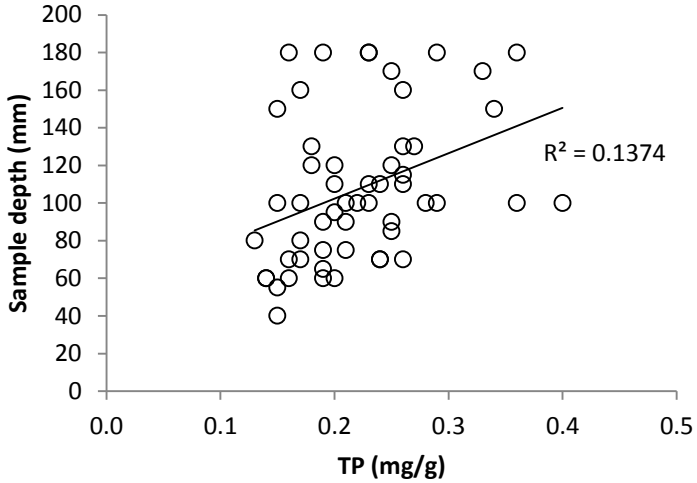

Figure A7-1: Scatter plot of subsoil TP concentration and sample depth (max 180 mm = 200 mm - 20mm) for all mesocosm experiment data from Peninsula Lake.

Table A7-1: Pearson's correlation test results for subsoil TP concentration versus sample depth for B1 (pre-enrichment) and D5 (last enrichment) sampling rounds of the mesocosm experiment.

Sampling occasion	**r**	***p***	**n**
B1	0.542	**0.006**	24
D5	0.209	0.327	24

Table A7-2: Two-way ANOVA results for differences in subsoil sample depth between functional zones and treatments for B1 (pre-enrichment) and D5 (last enrichment) sampling rounds of the mesocosm experiment.

Sampling occasion	**Zone effect**			**Treatment effect**			**Zone*Treatment interaction**		
	df	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
B1	2, 18	2.942	0.075	1, 18	0.529	0.476	2, 18	0.081	0.922
D5	2, 18	1.014	0.383	1, 18	2.158	0.159	2, 18	0.153	0.859

APPENDIX 8: STATISTICAL ANALYSIS RESULTS FOR MESOCOSM STUDY (CHAPTER 6)

Table A8-1: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in TP storage of ecosystem components between treatments and zones for B1 (pre-enrichment), D2 (second enrichment) and D5 (last enrichment) sampling rounds of the mesocosm experiment.

Ecosystem component		Treatment effect			Zone effect			Treatment*Zone interaction			Ordinal or disordinal
	Round	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
Water	B1	1, 16	0.192	0.667	2, 16	12.627	**0.001**	2, 16	0.287	0.754	na
	D2	1, 16	317.973	**<0.001**	2, 16	34.560	**<0.001**	2, 16	35.597	**<0.001**	do
	D5	1, 16	44.036	**<0.001**	2, 16	1.822	0.194	2, 16	3.056	0.075	na
Periphyton	B1										
	D2	1, 16	24.985	**<0.001**	2, 16	3.040	0.076	2, 16	3.232	0.066	na
	D5	1, 16	21.775	**<0.001**	2, 16	6.712	**0.008**	2, 16	6.046	**0.011**	do
Topsoil	B1	1, 16	0.038	0.848	2, 16	0.012	0.988	2, 16	1.414	0.272	na
	D2	1, 16	13.384	**0.002**	2, 16	1.180	0.332	2, 16	0.376	0.692	na
	D5	1, 16	9.902	**0.006**	2, 16	4.722	**0.024**	2, 16	6.710	**0.008**	do
Subsoil	B1	1, 16	0.225	0.642	2, 16	0.357	0.705	2, 16	0.739	0.493	na
	D5	1, 16	3.003	0.102	2, 16	2.549	0.109	2, 16	0.686	0.518	na
Litter	B1	1, 4	0.000	0.995							
	D2	1, 4	0.250	0.643							
	D5	1, 4	32.948	**0.005**							
SAV	B1	1, 6	0.213	0.661							
	D5#	1, 8	6.054	**0.014**							
Typha live	B1	1, 6	0.407	0.547							
	D5	1, 6	1.414	0.300							
Typha dead	B1	1, 6	0.176	0.689							
	D5	1, 6	0.001	0.978							
Typha total	B1	1, 6	0.283	0.614							
	D5	1, 6	1.237	0.328							
Total TP storage	B1	1, 16	0.103	0.752	2, 16	0.507	0.612	2, 16	1.153	0.340	na
	D5	1, 16	14.622	**0.001**	2, 16	2.496	0.114	2, 16	4.731	**0.024**	do

Table A8-2: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in TP concentration of ecosystem components between treatments and zones for B1 (pre-enrichment), D2 (second enrichment) and D5 (last enrichment) sampling rounds of the mesocosm experiment.

Ecosystem component		Treatment effect			Zone effect			Treatment*Zone interaction			Ordinal or disordinal
	Round	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
Water	B1#	1, 22	0.309	0.578	2, 22	11.938	**0.003**				
	D2	1, 16	283.725	**<0.001**	2, 16	29.582	**<0.001**	2, 16	32.578	**<0.001**	do
	D5	1, 16	42.337	**<0.001**	2, 16	1.450	0.264	2, 16	3.213	0.067	na
Periphyton	B1	Not sampled									
	D2	1, 16	754.904	**<0.001**	2, 16	16.033	**<0.001**	2, 16	34.150	**<0.001**	do
	D5	1, 16	83.798	**<0.001**	2, 16	2.379	0.125	2, 16	6.472	**0.009**	do
Topsoil	B1	1, 16	0.002	0.967	2, 16	0.858	0.443	2, 16	1.532	0.246	na
	D2	1, 16	13.384	**0.002**	2, 16	1.310	0.297	2, 16	0.376	0.692	na
	D5	1, 16	9.902	**0.006**	2, 16	4.221	**0.034**	2, 16	6.710	**0.008**	do
Subsoil	B1	1, 16	0.225	0.642	2, 16	0.357	0.705	2, 16	0.739	0.493	na
	D5	1, 16	3.003	0.102	2, 16	2.549	0.109	2, 16	0.686	0.518	na
Litter	B1	1, 4	0.467	0.532							
	D2	1, 16	2.508	0.188							
	D5	1, 4	16.071	**0.016**							
SAV	B1	1, 6	0.029	0.871							
	D2										
	D5										
Typha live	B1	1, 6	0.784	0.410							
	D5	1, 4	1.277	0.322							
Typha dead	B1	1, 6	0.116	0.748							
	D5#	1,6	3.971	**0.046**							

Table A8-3: Results of ANOVA and non-parametric Kruskal-Wallis (indicated with #) tests for differences in mass (depth for water) of ecosystem components between treatments and zones for B1 (pre-enrichment), D2 (second enrichment) and D5 (last enrichment) sampling rounds of the mesocosm experiment.

Ecosystem component		Treatment effect			Zone effect			Treatment*Zone interaction			Ordinal or disordinal
	Round	df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
Water	B1	1, 16	0.650	0.432	2, 16	6.618	**0.008**	2, 16	0.226	0.801	
	D2	1, 16	0.092	0.766	2, 16	6.585	**0.008**	2, 16	0.162	0.852	
	D5	1, 16	0.515	0.483	2, 16	6.488	**0.009**	2, 16	0.198	0.823	
Periphyton	B1	no data									
	D2	1, 16	0.815	0.380	2, 16	0.664	0.528	2, 16	6.256	**0.010**	do
	D5	1, 16	0.001	0.973	2, 16	1.356	0.286	2, 16	0.226	0.800	
Litter	B1	1, 4	0.083	0.788							
	D2	1, 4	0.402	0.561							
	D5	1, 4	10.601	**0.031**							
SAV (small core)	B1	1, 6	0.808	0.403							
	D2	1, 6	0.763	0.416							
	D5#	1, 8	6.054	**0.014**							
SAV (large core)	B1	1, 6	0.350	0.576							
	D5	1	6.054	**0.014**							
Typha live	B1	1, 6	0.024	0.883							
	D5	1, 6	2.188	0.213							
Typha dead	B1	1, 6	0.395	0.553							
	D5	1, 6	3.777	0.124							
Typha total	B1	1, 6	0.215	0.659							
	D5	1, 6	3.292	0.144							

Table A8-4: Repeated measures ANOVA results for differences in topsoil mass between treatments, zones and sampling occasions in the mesocosm experiment.

Time effect			**Treatment effect**			**Zone effect**			**Time*Treatment**			**Time*Zone**			**Treatment*Zone**			**Time*Treatment*Zone**		
df	**F**	**p**	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
6, 96	2.49	**0.028**	1, 16	1.08	0.315	2, 16	5.84	**0.012**	6, 96	1.80	0.107	12, 96	1.55	0.120	2, 16	1.68	0.217	12, 96	1.32	0.223

Table A8-5: ANOVA test results for differences in water quality variables and *Typha* height between treatments and zones for B1 (pre-enrichment), D2 (second enrichment) and D5 (last enrichment) sampling rounds of the mesocosm experiment.

Ecosystem component	**Round**	**Treatment effect**			**Zone effect**			**Year*Zone interaction**			**Ordinal or disordinal**
		df	**F (or H)**	***p***	**df**	**F (or H)**	***p***	**df**	**F**	***p***	
Water chlorophyll α	B1	no test, all values below detection limit									
	D2	1, 16	36.526	**<0.001**	2, 16	2.089	0.156	2, 16	2.767	0.093	na
	D5	1, 16	11.928	**0.003**	2, 16	0.050	0.952	2, 16	1.847	0.190	na
Water turbidity	B1	1, 16	0.003	0.958	2, 16	2.746	0.094	2, 16	0.614	0.553	na
	D2	1, 16	20.025	**<0.001**	2, 16	7.416	**0.005**	2, 16	3.386	0.059	na
	D5	1, 16	6.409	**0.022**	2, 16	0.559	0.583	2, 16	0.954	0.406	na
Water temperature	B1	1, 16	0.013	0.911	2, 16	2.690	0.098	2, 16	0.032	0.968	na
	D2	1, 16	1.384	0.257	2, 16	54.135	**0.000**	2, 16	0.298	0.746	na
	D5	1, 16	0.008	0.928	2, 16	3.286	0.064	2, 16	0.016	0.984	na
Water pH	B2	1, 16	0.246	0.626	2, 16	122.604	**<0.001**	2, 16	0.084	0.920	na
	D2	1, 16	1.642	0.218	2, 16	138.659	**<0.001**	2, 16	95.578	**<0.001**	do
	D5	1, 16	3.531	0.079	2, 16	4.317	**0.032**	2, 16	1.198	0.328	na
Water EC	B1	1, 16	1.526	0.234	2, 16	15.727	**<0.001**	2, 16	0.256	0.777	na
	D2	1, 16	10.198	**0.006**	2, 16	24.034	**<0.001**	2, 16	6.404	**0.009**	do
	D5	1, 16	0.634	0.438	2, 16	0.422	0.663	2, 16	0.527	0.600	na
Water DO	B1	1, 16	0.152	0.702	2, 16	132.169	**<0.001**	2, 16	0.499	0.616	na
	D2	1, 16	1.203	0.289	2, 16	73.796	**<0.001**	2, 16	23.969	**<0.001**	do
	D5	1, 16	0.857	0.368	2, 16	10.146	**0.001**	2, 16	1.008	0.387	na
Water ORP	B1	1, 16	1.649	0.217	2, 16	0.137	0.873	2, 16	0.111	0.895	na
	D2	1, 16	0.016	0.900	2, 16	3.660	**0.049**	2, 16	2.417	0.121	na
	D5	1, 16	0.303	0.590	2, 16	2.768	0.093	2, 16	0.032	0.969	na

Ecosystem component	Round	Treatment effect			Zone effect			Year*Zone interaction			Ordinal or disordinal
		df	F (or H)	*p*	df	F (or H)	*p*	df	F	*p*	
Water TN	B1	1, 16	2.365	0.146	2, 16	6.682	**0.009**	2, 16	0.316	0.734	na
	D3	1, 16	95.078	**<0.001**	2, 16	21.391	**<0.001**	2, 16	23.113	**<0.001**	do
Typha height	B1	1, 28	2.888	0.100							
	D5	1, 28	0.570	0.457							