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The use of constructed treatment wetlands for water quality amelioration in conservation scenarios

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The Use of Constructed Treatment Wetlands for Water Quality Amelioration in Conservation Scenarios

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PhD Thesis

School of Biological Sciences

Bangor University

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List of Abbreviations

AES – Atomic Emission Spectroscopy
ANOVA – Analysis of Variance
BOD – Biological Oxygen Demand
Ca – Calcium
COD – Chemical Oxygen Demand
CW – Constructed Wetland
Da – Dalton
DIC – Dissolved Inorganic Carbon
DO – Dissolved Oxygen
DOC – Dissolved Organic Carbon
EC – Electrical Conductivity
FCW – Floating Constructed Wetland
FF – Free Floating Macrophyte Wetland
FWS – Free Water Surface
HLR – Hydraulic Loading Rate
HMW – High molecular weight
HPLC – High Performance Liquid Chromatography
HSSF – Horizontal Subsurface Flow
JNCC – Joint Nature Conservation Committee
LMW – Low Molecular Weight
MOL – Moles, Concentration
MW – Molecular Weight
MWCO – Molecular Weight Cut Off
N – Nitrogen
nHRT – Nominal Hydraulic Retention Time
NNR – National Nature Reserve
NRW – Natural Resources Wales
P – Phosphorus
POM – Particulate Organic Matter
ROL – Radial Oxygen Loss
SAC –Special Area of Conservation
SEC – Size Exclusion Chromatography
SPANOVA – Split Plot Analysis of Variance
SS – Suspended Solid
SSF – Subsurface Flow
SSSI – Site of Special Scientific interest
SUVA – Specific Ultra Violet Absorbance
US EPA – United States Environmental Protection Agency
VF – Vertical Subsurface Flow
WFD – Water Framework Directive

Abstract

Agricultural practices have gained intensity over recent decades, which has resulted in enrichment of surface and ground waters. Contamination from livestock wastes; land fill site leachate, road runoff and detergents in wastewater have all contributed to environmental water quality issues and enrichment. Constructed wetlands (CWs) have been increasingly utilised as a method by which nutrient pressures on ecosystems, in the form of nitrogen (N)-containing and phosphorus (P)-containing compounds can be alleviated. This thesis focuses on three main research areas; hybridised floating CWs (FCW) for algal bloom mitigation in standing water bodies combining algaecide release from organic matter with conventional FCW design, installation of CWs on North Wales conservation sites to prevent enrichment and CW system optimisation for calcium (Ca) mobility maintenance.

The process of eutrophication, subsequent algal bloom formation and senescence can have significant negative impacts on the condition and health of a water body. In this study FCW design combined existing technology for N and P removal via rhizosphere processing and plant uptake with the release of anti-algal phenolic compounds from organic matter added to the rhizosphere. These methods were combined in order to assess their potential for enhanced organic matrix material degradation and phenolic release by the inclusion of macrophytes capable of oxygenating the rhizosphere. Combination of these mechanisms resulted in increased algal bloom control. However, control systems lacking wetland macrophytes resulted in an algal density 10 to 20-fold greater than planted systems. However, the research proved that by not adding plants to the organic matter in a FCW, algal growth was significantly accelerated.

As part of the study three CWs were installed on the Llyn and Anglesey Fens project conservation sites in North Wales. Sites of special scientific interest (SSSI), special areas for conservation (SAC) and national nature reserves (NNR) make up the fen conservation sites featured in the project. Here, localised enrichment from surface and ground water inputs has driven plant species assemblage changes within the fen basins. This process has been attributed to the influx of N and P which has led to the invasion of more competitive wetland macrophyte species. CWs located at the site margin were employed to treat eutrophic water flowing into the site. On completion of the systems, average nutrient pollutant removal was

estimated over a 14 month period of operation. Total N removed as a result of the CW installations was approximately equivalent to 1.2 tonnes of inorganic fertilizer. This N would otherwise have continued to enter into the conservation sites.

Further investigation was undertaken into the use of CWs with respect to the conservation targets of the Llyn and Anglesey Fens project. SSSI classification has been applied to the sites on Anglesey due to the presence of Ca-rich, oligotrophic water chemistry supporting a unique vegetation community. Conventionally, CWs for N removal by denitrification require anaerobic conditions in order for N removal to occur. These conditions also result in the precipitation of Ca within the wetland in the form of Ca carbonates. This is undesirable as Ca is required for the development of the rare fen vegetation communities. Experiments were undertaken to examine the potential for Ca mobility maintenance and the prevention of Ca loss due to carbonate flocculation. The potential of dissolved organic carbon (DOC) and phenolic compounds produced in the system to preserve Ca in a dissolved form was investigated. Existing research into the role of DOC in affecting Iron (II) mobility motivated this investigation. Preliminary investigations found that Ca formed an association with with low molecular weight (LMW) (typically <1000 Dalton (Da)) DOC. Ca was also observed to associate with DOC of low phenolic content. These findings were used to inform mesocosm experimental design and hypotheses.

A mesocosm experiment incorporating three treatments was set up in order to assess the impact on Ca mobility of phenolic concentration and DOC molecular weight. Two CW types and two wetland macrophyte species were compared. The experiment showed that horizontal subsurface flow (HSSF) systems reduced total Ca by 28% on average, whilst *Iris pseudacorus* showed a similar result. However, total calcium was reduced by 38% in the *Phragmites australis* mesocosms. Contradictory to preliminary investigations, greater concentrations of phenolics correlated with increased total Ca.

DOC was also characterised in the CW mesocosms using UV-Vis spectral slope ratios. Significant variation in DOC character was observed between plant species. These findings motivated further analysis into the quantification and characterisation of DOC produced as root exudate from the wetland macrophytes. Furthermore, this research also addresses targets as set out by the Llyn and Anglesey fens project with concern to reducing DOC inputs. *Iris pseudacorus* was observed to produce 55% less or 1.59mg DOC/g biomass less DOC than

Phragmites. In addition, this species produced lower MW DOC with higher phenolic content, therefore promoting chelation and solubility maintenance of Ca.

The findings of this research can now be applied to further CWs on the Llyn and Anglesey conservation sites. This research will also be used to inform management best practice at future CW locations where calcium maintenance is required.

Chapter 1 - Constructed Treatment Wetlands for Water Quality Amelioration in Conservation Scenarios Introduction

1.1 Anthropogenic Impacts on Freshwater Ecosystems and Eutrophication Analysis

Freshwater plays a primary role in supporting life on earth including an array of species including mammals, birds, fish, invertebrates and plant life. Consequently has significant economic and biological value. However a mere 0.01% of water on earth is in the form of freshwater, (Dudgeon et al. 2006) covering a surface area as little as 1% of the globe (Wetzel 2001). Freshwaters also directly support

Increasing population, economic growth and industry are bringing about a surge in demand for freshwater (Willoughby 1976) placing strain on a finite resource. Human activity is causing a significant detrimental effect upon the quality of freshwater, especially in developing countries (Markandya 2010). This influence can have a range of effects dependent on scale; however, the primary influencing factor is adjacent land use (McDowell & Wilcock, 2008). Movement of water through catchments affects the mobility of dissolved and particulate pollutants, which become mobilised from the land, enter the pore water and are lost to adjacent surface waters (McDowell & Wilcock, 2008). Overuse of fertilisers in agriculture is one such example.

Nutrient pollutants (e.g.compounds containing Nitrogen (N) and Phosphorus (P)) are observed to cause eutrophic effects (Smith et al. 1999; Pretty et al. 2003; Holman et al. 2008). The addition of allochthonous pollutants (those from outside of the aquatic environment) such as N and P will inevitably cause water quality problems within freshwater ecosystems due to the effects of enrichment (Yang et al. 2008a). In the following studies eutrophication prevention and nutrient sequestration are key targets for the systems utilised and explored.

In both freshwater and marine systems, imbalances in nutrient loading can occur which can be attributed to many different causes (McDowell & Wilcock, 2008). Eutrophication is the process by which pollutants enter a system and have a nutrient enriching effect. Pollutants containing N and P drive increases in primary production. In aquatic systems this often results in the formation of algal blooms whilst in wetland habitats species composition shifts can be observed as trophic levels change. Many studies on lakes and rivers have found a direct correlation between nutrient levels and algal biomass (Smith et al. 1999).

This thesis focusses on these two main detrimental effects of eutrophication. In the case of the former, nutrient enrichment stimulates primary production causing algal blooms form (Manny et al. 1994) which are often associated with the release of toxins (Carmichael 2001;

Yang et al. 2008a). These can include cytotoxins and biotoxins, however these are generally associated with cyanobacteria i.e. “blue-Green” algae (Carmichael 2001). However, decomposition of blooms, irrespective of species in senescence phases by microbial heterotrophs (Pretty et al. 2003) results in hypoxia (<4mg/L Dissolved Oxygen (DO)) which is detrimental to most aquatic fauna (Paerl et al. 2001) and can result in fish kills, loss of biodiversity and further deterioration of water quality (Jöbgen et al. 2004).

Eutrophication within natural wetland systems has been thought to be a driver of plant species change. This has the potential to be ecologically harmful, an example of this is the invasion of *Typha domingensis* into wetlands influenced by phosphorus laden water (Mitsch & Gosselink 2000). Similar observations have been made on the Anglesey Fen case study sites discussed in Chapter 4. Here the ecology of lowland fens is shifting from communities dominated by oligotrophic (low nutrient), calcium dependant vegetation to the rapid colonisation of highly invasive graminoid species. This is occurring to the extent that the conservation status of the sites is now unfavourable.

Several factors influence the degree of eutrophication including presence of flow, retention time and degree of water inundation. Reviews on pollutant concentrations required in order for eutrophic conditions to arise in lentic (standing) and lotic (flowing) systems are discussed by Nürnberg (1996) and Dodds et al. (1998) respectively. Evidence presented in Dodds et al. (1998) indicates clearly that lotic, fast moving water bodies require extremely concentrated N and P inputs in order for eutrophic conditions to prevail. The slow movement of water in lakes, ponds and wetland sites are therefore more susceptible to the effects of enrichment due to the increased water retention time. The addition of dissolved pollutants or insoluble particulate matter is particularly noticeable in the sites studied in this thesis. Fen basin conservation sites located on the Llyn and Anglesey LIFE Fens managed by Natural Resources Wales (NRW) were selected for eutrophication prevention due to their receipt of water from a catchment dominated by agricultural land.

Wetlands are biologically, geologically and chemically unique ecosystems, exhibiting great variability in hydrological status and plant species present (Kadlec & Wallace 2008). Wetlands are regarded as hydrological buffers, stabilizing flow rates and ameliorating flooding and drought by recharging aquifers (Mitsch & Gosselink 2000). The ability of natural wetlands to act as sinks for chemicals has encouraged researchers to investigate the

possibility of treating waste water and water of high nutrient or pollutant content. This is where CW technology evolved from and increasingly diverse applications for wetlands for water treatment are being explored.

1.2 Development of Constructed Wetland Technology

There is growing concern regarding human health and environmental quality with respect to waste disposal. Treatment wetlands have been identified as a possible method for bioremediation of polluted water for over 60 years. Early research undertaken by Seidel (1953), found that polluted drainage and sewage ditches which had been colonised by wetland macrophyes exhibited lower concentrations of measured pollutants. Research by Kickuth (1977) lead to the investigation of basic Horizontal Subsurface Flow (HSSF) wetlands and this became the basis for many CWs now used.

Currently, approaches to water quality improvement by CW include; Free Water Surface (FWS), with either submerged aquatic vegetation (SAV) or emergent macrophytes, Horizontal Sub Surface Flow (HSSF), Vertical Flow (VF) and Free Floating Vegetation (FF) systems. Each has been used with varying success and designs are chosen depending upon treatment requirements and the natural abundance of plant species in the vicinity (Kadlec & Wallace 2008). However, each has features that can cause problems during the treatment process or life span of the system. CW optimisation and process understanding is continually developing. Key research includes minimising risk of system compromises and maximisation of efficiency. There are also additional challenges associated with designing and installing treatment systems within a constrained situation such as conservation of a habitat or protection of a water body, a concern of this thesis.

1. **HSSF wetland** – typical macrophytes used are *Phragmites australis*, these systems are liable to clogging when treating water that has a high level of suspended solid (SS) material or high Biological Oxygen Demand (BOD). Primarily used when reducing anaerobic conditions are required for pollutant biochemical processing (Kadlec & Wallace 2008; Vymazal 2007; Vymazal 2008).

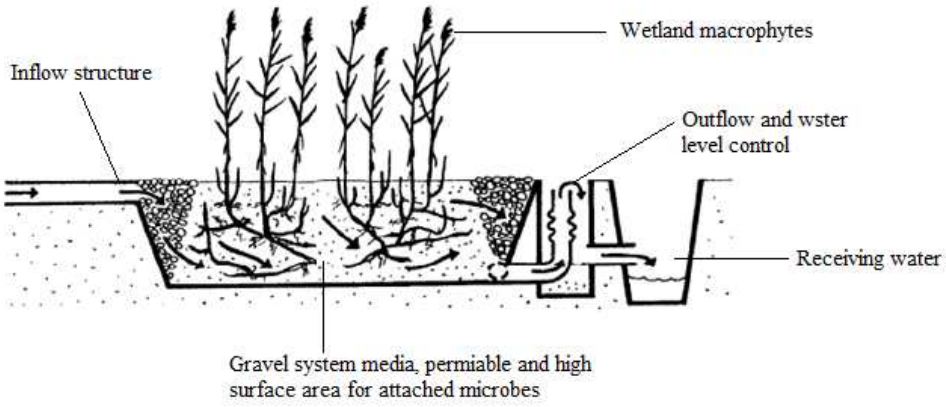


Figure 1.1 Horizontal Subsurface Flow CW, adapted from (Headley et al. 2006).

Water flows into the media and increased porosity media located at the entrance and exit of the system distributes water evenly along the cross section of flow path. Water travels through the media and comes into contact with plant roots and microbial communities responsible for pollutant transformations.

2. **VF Wetland** – These types of system operate in a similar manner to HSSF systems however the water is loaded *via* a distribution system across the surface of the wetland. Water is often pulse loaded to allow percolation downwards through the system and drying of the wetland bed media greatly increases oxygen diffusion, required for biological oxidation of compounds such as ammonium and BOD (Kadlec & Wallace 2008).

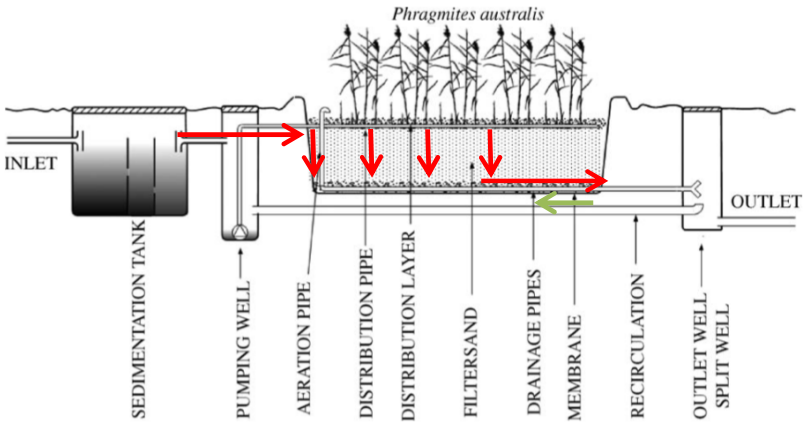


Figure 1.2 Vertical Flow Constructed Wetland, adapted from (Brix & Arias 2005).

Water flows (RED arrows) from the distribution pipe, through the filter sand matrix and into the drainage pipes. The distribution pipe allows a short intense flow of water to flood the surface; it then percolates though the filter media uniformly displacing the air in the system

generating significant oxygen transfer. Partial recirculation (GREEN arrow) allows complete denitrification following a nitrification phase.

3. **Fill and Drain Constructed Wetland–** These wetlands utilise reciprocating filling and draining cycles in order to create oxidising and reducing conditions for nitrification and denitrification respectively.

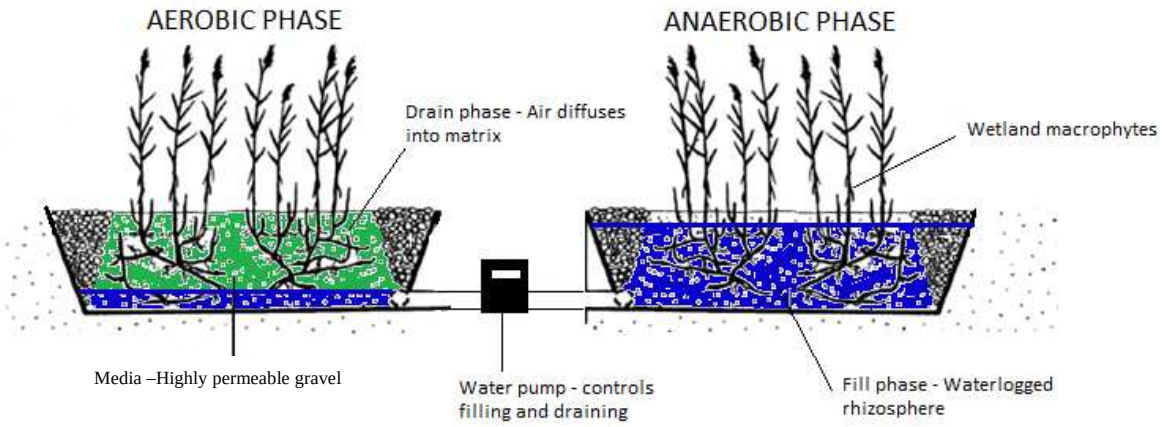


Figure 1.3 Reciprocating fill and drain tidal flow wetland, adapted from (Headley et al. 2006).

GREEN zones denote the diffusion of air into the rhizosphere during drain cycles, producing aerobic conditions for ammonium oxidation. BLUE zones denote filling of effluent into the rhizosphere, producing anaerobic conditions for nitrate reduction. Cycle times, controlled by the water pump, will be modified according to pollutant concentrations. Once the required number of cycles is complete the water is pumped to the outflow.

4. **FWS Wetland** – most of the pollutant uptake and removal is performed by attached microbial biofilms, which grow on the wetted surfaces of the plant and substrates. A small amount diffuses into the soil and is taken up by the plants. These systems can be subject to flooding; submerging the macrophytes for extended periods of time can result in reduction in the observed biomass stand (Mitsch & Gosselink 2000).

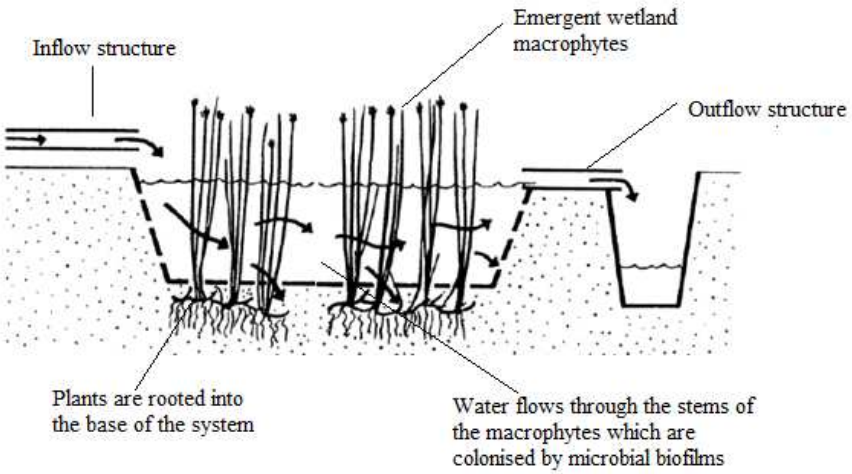


Figure 1.4 Free Water surface CW, adapted from (Headley et al. 2006).

Water flows into the system via an inflow structure. Pollutants are removed by biofilm communities as water passes through the stems of the macrophytes. A percentage of the pollutants diffuse into the rhizosphere where plant uptake occurs.

5. **Free Floating (FF) Vegetation** – Floating vegetation wetlands tend to be used in more tropical climates where high growth rates can be supported. One such example is the use of Water Hyacinth. Management of these systems can be highly labour intensive, due to the constant need to harvest biomass in order to maintain pollutant removal efficiency.

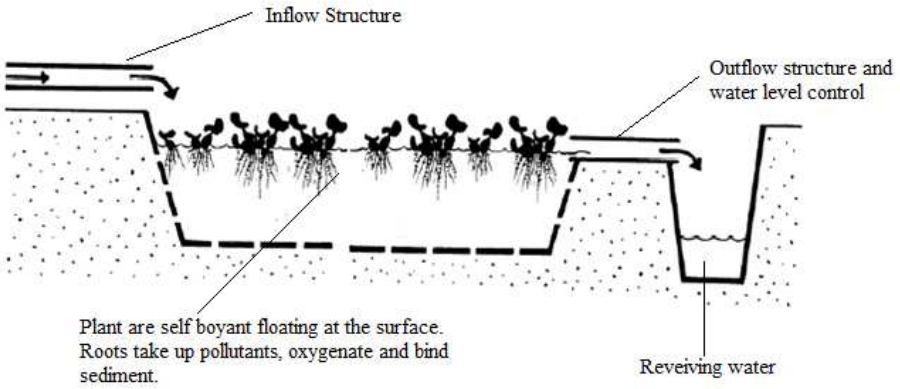


Figure 1.5 Free Floating macrophyte CW (Headley et al. 2006).

These systems rely upon high biomass production and resultant pollutant uptake. Pollutants are taken up directly from the water column into the roots, rhizomes and tissues of the plants.

The use of floating constructed wetlands (FCW), as an alternative to FF systems, where buoyancy is engineered within the design is a possible solution to the problem of eutrophic water bodies. This system combines mechanisms of water quality improvements highlighted in the FF systems with the flexibility of greater species selection. FCWs investigated in chapters 2 and 3 allow for the use of temperate macrophyte species suspended in the water column in a hydroponic manner. The system allows for the roots of the macrophytes to be suspended in the water column resulting in direct uptake of nutrients. This may be a more suitable option than FF when treating naturally occurring or culturally driven eutrophic water bodies. The FCW can be constructed and designed in order to deal with varying amounts of pollutant loading and the ability of the floating systems to track the water table is also advantageous. Additionally the flotation system allows macrophytes that typically grow rooted into benthic sediments to have greater nutrient uptake rates due to the difference in uptake pathway. In FWS systems nutrients must diffuse into the benthic sediments of the system prior to uptake by the macrophytes. In FCW this process does not need to occur. This function allows the wetland to continue treatment where other systems would become submerged preventing the removal of pollutant chemicals (Headley et al. 2006).

Currently, the number of treatment wetlands in the UK is estimated at 1000 (Cooper 2009), however, numbers are likely to increase as the technology progresses. Similarly, the use of treatment wetlands may gain popularity due to the ever expanding range of applications being developed. For example, water reuse is of high priority in arid countries where water is scarce and wetlands have been used as tertiary polishing systems for coliform bacteria removal preparing water for reuse in irrigation (Greenway 2005). Similarly, diversification and wetland optimisation has been achieved, for example forced bed aeration in order to treat de-icing glycols compounds from airports where biological oxygen demand (BOD) reaches extreme concentrations (Higgins et al. 2011). Intensive systems have also been implemented at municipal waste water treatment plants in order to achieve sludge dewatering (Nielsen 2013). Here pollutant removal was not paramount, instead utilisation of wetland macrophyte evapotranspiration was used to remove the water while allowing for nutrient assimilation and reuse in agriculture. At the other end of the scale, wetlands have been used to treat small volumes of household wastewater by means of Compact Vertical Flow (CVF) systems utilising granular media planted with macrophytes in a hydroponic set up (Brix & Arias 2005).

A range of biochemical techniques are being developed in the CW and water quality domain and relevant areas are discussed below.

1.3 Nutrient Pollutant Removal Mechanisms in Constructed Wetlands

Nitrate is the dominant form of N-based pollutants in spring waters supplying the conservation sites on Anglesey, and many locations where agricultural practices affect water quality. In order to assess the way in which to maximise weathering of the pollutant the nitrogen cycle must be considered. Complete removal of nitrate can be undertaken by microbial dentitrification (Shapleigh 2013). This process breaks down nitrate to nitrogen gas using a number of intermediate phases. Denitrification is facilitated via microbial communities by the production of reductase enzymes (Knowles 1982). In many cases N_2O (nitrous oxide) a potent greenhouse gas is be emitted from the CW before complete transformation to N_2 (Kadlec & Wallace 2008).

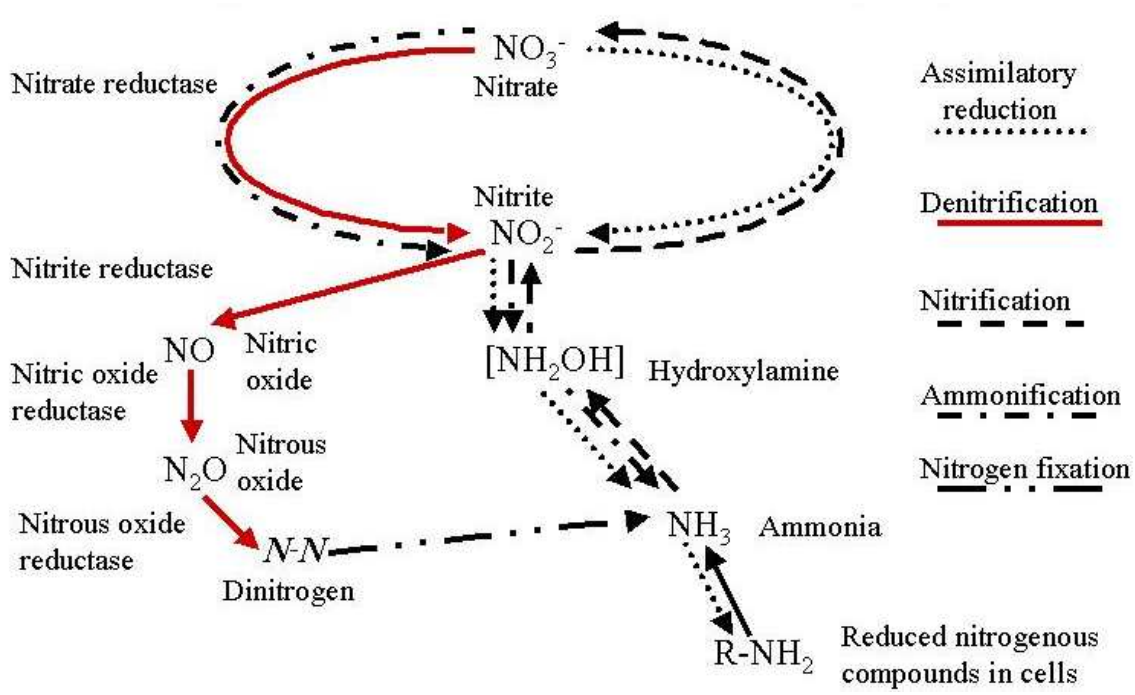


Figure 1.6 Substrates, processes and products of the nitrogen cycle (Shapleigh 2013)

The presence of nitrate and anoxic conditions are a prerequisite for the reaction to be catalysed by nitrate reductase (Van Cleemput et al. 2006; Knowles 1982). These conditions are generally found in HSSF systems where oxygen diffusion in limited by the reduced air-water interfaces. Although these systems are generally anoxic, wetland plants are able to

oxygenate the rhizosphere (Sorrell et al. 2000) causing localised areas of oxygenated conditions. Denitrification occurs by nitrate acting as a terminal electron acceptor in the reaction (Kadlec & Wallace 2008). Although FWS wetlands have increased oxygen diffusion rates through the water surface, anaerobic zones are still present in the system. Locations within the FWS system where anaerobic zones occur are primarily; soil substrates (into which the macrophytes are rooted), and within polysaccharide matrices formed by biofilm communities. These occur on all wetted surfaces within the system. Stratified anaerobic and aerobic zones are observed within biofilms, where acceptance and donation of electrons occur respectively (Amann & Kühl 1998; Terada et al. 2007).

A secondary mandate for denitrification to occur is the presence of a labile carbon source (Narkis et al. 1979; Terada et al. 2007). The bulk of denitrifiers are microbial heterotrophs and rely heavily upon organic carbon to act as a source of electrons, which in turn controls microbial activity (Knowles 1982) and limits activity when lacking. This situation occurs during nitrogen removal in sewage treatment plants. Here much of the carbon is lost during primary sedimentation stages of treatment. Narkis et al., (1979) discuss the need for carbon source addition during dentitrification stages at water treatment works, however in CWs plants have been shown to contribute significantly to denitrification carbon demand by root exudate production (Lin et al. 2002).

As mentioned, HSSF systems tend to be used for dentitrification stages. However these systems are compromised when high suspended solid (SS) content is observed in the inflowing water. A HSSF system can become rapidly blocked causing overland flow when SS are high. In this case FWS systems may be employed. Although efficiency is marginally reduced by decreased anoxia, system maintenance and SS removal is possible.

Ammonium is a N-containing pollutant species which can be readily treated with the use of CWs. In order to remove this nitrogenous compound from the water column, a very different approach may be needed. This is due to the steps that transform ammonium to nitrate. H atoms from the NH_4^+ molecule need to be replaced by oxygen in order to form nitrate NO_3^-, which can then be denitrified. Again the process follows a series of intermediate compounds, namely Hydroxylamine (NH_2OH) followed by nitrite and finally nitrate (Shapleigh 2013)

The reactions described here require oxygen rich conditions in order to be completed. Suitable CW types must be selected in order to achieve this. Increased oxygen demand can be

met using a number of mechanisms. One such method utilised pulse flow VF CWs where the wetland is intermittently loaded with influent and is allowed to displace the air in the rhizosphere. Between pulses oxygen diffuses back into the rhizosphere resetting the system for oxygenation (Kadlec & Wallace 2008). These systems may be employed in order to deal with ammonium in the water supplying the fens in the study. However, complexity and lack of passive design in the latter system may add increased need for maintenance and monitoring, and also increased system set up costs.

Phosphorus is a significant nutrient pollutant driving water quality degradation and eutrophication effects that are manifested in aquatic environments in the form of algal blooms. Phosphorus, like the nitrogenous compounds, may be removed in CWs through various pathways including sorption, biomass storage and cycling, microbial phosphorus in flocs, soils and accretion (Kadlec & Wallace 2008). Similarly to nitrogen, phosphorus may change state. However, valency changes do not occur. Primary methods of removal in conservation site CWs will be precipitation with other compounds, dissolution resulting in sedimentation and peat accretion (Vymazal 2007). Plant-bound phosphorus will cycle in the wetland from plants to soils to microbes, some of which will be lost from the system during biomass degradation.

1.4 Conclusion - Scenario Specific Utilisation of Constructed Wetland Technology and Synthesis of Knowledge

The research undertaken in this study aims to deal directly with mitigation of poor water quality. The systems explored here have immediate effects on local conservation projects and ecology of the water bodies suffering eutrophic effects.

Chapters 2 and 3 focus on the potential for water quality amelioration in freshwater bodies exhibiting signs of eutrophication. Although the use of Floating Constructed Wetlands (FCWs) has been researched extensively (Headley et al. 2006) in this study, hybridisation between FCW technology and research undertaken by Wingfield et al (1985), Welch et al (1990) and Pillinger et al (1994) into biological anti-algal compounds released from organic matter are combined. Wingfield et al (1985), Welch et al (1990) and Pillinger et al (2004) analysed the effect of different forms of Dissolved Organic Carbon (DOC) released from organic matter, such as barley straw, and monitored their effect upon algal blooms. The research presented in Chapters 2 and 3 combines aspects of pollutant removal and

transformations observed in conventional FCW (as described in Headley et al. 2006), with the presence of an anti-algal compound releasing substrate. This research also draws upon areas such as nutrient biochemical cycling, plant uptake and additionally, how the macrophytes planted in the FCW's interact in with organic matter added to the FCW.

Increased anti-algal compound production is potentially mediated by phenol oxidase enzymes. This group of enzymes are capable of breaking down lingo-cellulose based material, and primarily break down recalcitrant phenolic compounds (Freeman et al. 2001; Freeman et al. 2004). Low molecular weight phenolic compounds were found to have more significant anti-algal effect (Pillinger et al. 1994). However, phenol oxidase is limited in anaerobic conditions and requires bi-molecular oxygen in order to function (Freeman et al. 2001). Therefore the effect of plant addition and particularly the function of radial oxygen loss (ROL) from the plant roots was examined. The success of the systems was measured by the presence and density of pelagic green algae observed in the water column as compared to control systems.

Chapters 4 to 7 are directly linked to the Natural Resources Wales (NRW) Anglesey and Llyn Fens Project. NRW set out to restore 751 hectares of fen to good or recovering status. After many years of mismanagement and neglect the sites have become severely degraded. The Anglesey and Llyn Fens project aims to improve many aspects of the Fen sites that range in category from RAMSAR, SSSI, Special Area for Conservation and National Nature Reserves. Issues involving water quality at the sites are addressed by Chapters 4, 5, 6 and 7. Chapter 4 details a number of case studies where the application of constructed wetlands significantly improved the water quality of the streams, springs and other inflows directly supplying the fen.

The NRW fens project funding was granted due to the rare type of fen habitat present on both Anglesey and the Llyn fen sites which are classed as alkaline fen. Secondly, particularly on the Anglesey sites, there are large areas of typically acidic peat directly adjacent to calcitic muds and marl (Gilman & Newson 1982). At these locations rare calcium dependent fen sub communities exist. These communities are an indicator for good quality fen and are characterised by the presence of *Shoenus nigricans*, shallow, slow flowing water and most crucially water that is highly oligotrophic and with a high concentration of calcium (Elkington et al. 2001). The project involved the installation of CWs two main aims; to protect the existing areas of high quality fen from nutrient enrichment and to create an

oligotrophic water environment allowing areas that had been selected for peat re-profiling to potentially flourish.

Throughout all areas of CW design and installation, optimisation of the systems was required. Laboratory mesocosms were investigated in order to guide continual improvements to field systems and allow modifications to be made in the future. Calcium mobility was the focus of Chapters 5 to 7. The mechanisms explored are based on observations in metal mobility studies whereby phenolic compounds in solution prevent metal precipitation (White et al. 2011). More specifically each chapter deals with the following points.

- Chapter 2 focusses on the potential for combined use of of anti-algal phenolic compounds release aided by the presence of wetland macrophytes, and act as a system feasibility study to guide later experiments for holistic analysis. This chapter sets out to provide a method by which the FCWs can be implemented and tested.
- Chapter 3 represents an in depth study into phenolic break down optimisation by the inclusion of wetland macrophytes and linked plant uptake of nutrients. The results from this mesocosm FCW experiment may inform future design, optimal set up and macrophyte selection for the installation of full-scale systems for algal bloom mitigation.
- Chapter 4 details CWs used directly in conservation scenarios and aims to illustrate the possible use in large scale conservation projects, where water quality is a serious issue. The chapter aims are to highlight successes and challenges involved in CW use whilst providing an immediate tool by which NRW can achieve targets for enrichment mitigation.
- Chapter 5 provides a new insight into maintaining higher calcium concentrations in water supplying rare fen systems to maximise conservation of NRW SSSI sites. The aims being to understand if the wetland environment can be modified in such a way that calcium concentration is maintained.
- Chapter 6 is based upon the findings of Chapter 5 and aims to test if methods for calcium maintenance developed at *In-vitro* scale apply in a more natural wetland environment.
- Chapter 7 develops further the research on processes involved in the production of suitable conditions in the wetland environment for the maintenance of high calcium

levels. Here *in vitro* experiments aim to elucidate the effects of plant root exudates and DOC production on chelation of calcium.

Chapter 2 - Preliminary Feasibility Study for the Use of Floating Constructed Wetlands for Algal Bloom Mitigation

2.1 Abstract - Rational for the Use of Floating Constructed Wetlands

The ecological problems associated with eutrophic water bodies and subsequent algal bloom formation has been discussed in Chapter 1. Combining research on the effect of DOC releasing organic matter which exhibits anti-algal properties with FCW design was undertaken in order to maximise efficiency of eutrophication and algal bloom prevention.

Chapter 2 draws on two key areas of research; nutrient mitigation in freshwater bodies and algal bloom reduction by anti-algal DOC compounds. This chapter aims to confirm the anti-algal effect of DOC compounds reported by Pillinger, Cooper, & Ridge, (1994); Welch, Barrett, Gibson, & Ridge, (1990); Wingfield, Greaves, Bebb, & Seager, (1985), whilst combining this with a macrophyte dominated Floating Constructed Wetland (FCW) as outlined in Headley et al. (2006), in order to ascertain feasibility for implementation in real world scenarios. The findings in Chapter 2 were a prerequisite for further research into mechanistic analysis of hybrid system processes detailed in Chapter 3.

2.2 Introduction - Eutrophication and Water Pollution Negative Impacts of Algal Blooms

Eutrophication and water pollution are an increasing phenomenon, especially in lakes and ponds located in fertile lowland regions (Wetzel 2001). Biogeochemical cycling has been dramatically affected over the past few centuries due to the growth of the human population, industry and waste disposal (Willoughby 1976). Dramatic changes have been observed in carbon, nitrogen and phosphorus cycles (Schlesinger 1997; Vitousek et al. 1997; Smith et al. 1999).

The term eutrophication has become synonymous with the accelerated growth of phytoplankton and other forms of algae, particularly *Anabaena, Microcystis, Nodularia,* and *Oscillatoria* species (Herath 1997). Occurring due to the increase in availability of dissolved nutrient chemicals (Manny et al. 1994). Enrichment from nitrogen (N) and phosphorus (P) containing compounds is most commonly the driver. However, P based compounds are most regularly the limiting factor due to natural availability. When both of these compounds are in excess, nutrient compounds such as potassium can become limiting to plant growth (Herath 1997).

Concentrations of nutrient pollutants exhibited in a water body which is suffering eutrophication vary. One review widely accepted is Smith et al. (1999) which examines

concentrations of nitrate and phosphate required for bloom formation. Concentrations in lentic and lotic water bodies are addressed separately and defined by Nürnberg (1996) and Dodds et al. (1998). It is well recognised that under eutrophic or indeed hypertrophic conditions, the likelihood of the formation of ecologically harmful algal blooms are substantially increased. As explained in Chapter 1 such blooms are not only ecologically harmful, but economically costly. Economic costs are primarily linked to water treatment plants, where algal blooms cause filter blockages and changes in water chemistry which complicate treatment (Mason et al. 2003; Pretty et al. 2003).

Examples of N and P containing sources to a water body include farmland runoff from fields, carrying with it excess nutrient pollutants that are not absorbed by crop plants and other micro pollutants such as heavy metals (Poe et al. 2003). In locations where surface water runoff is high, increased levels of suspended solids (SS) will also be found in the water body. Sources of phosphorous include sewage treatment plants, detergents from wastewater and land fill sites and phosphorous release directly from livestock wastes. These sources are the main causes of eutrophication across much of Europe (Herath 1997).

Significant ecologically harmful effects associated with algal blooms occur during bloom senescence. Reduction in available dissolved oxygen (DO) in the water column and toxin production are a direct result of heterotrophic decomposition of the algal bloom (Yang et al. 2008a). Although losses in ecosystem services and value are difficult to quantify, they can be measured by the effect that is observed on the biota of the ecosystem (Pretty et al. 2003).

CWs are increasingly being considered as a naturalistic, cost effective and passive means of treating water containing high nutrient concentrations; therefore, reducing the risk of eutrophic conditions and algal bloom formation.

2.3 System Selection, Installation Scenarios and Primary Pollutant Removal Mechanisms in Floating Constructed Wetlands

FCWs have been shown to be highly successful at dealing with fluctuating water levels and high particulate content in water treatment. HSSF or VF wetlands become ineffective when particulate matter or SS levels are high. This is due to the bed media or substrates becoming clogged (Kadlec & Wallace 2008). However in floating systems, SSs are caught up and

contained within biofilms that cover the root surface or settle in the base of the water body (Headley et al. 2006) and do not significantly affect pollutant removal.

Chapters 2 and 3 focus primarily on FCW system installation into water bodies suffering eutrophic effects. However, it is suggested that if nutrient pollutants can be tackled before entering water courses, the use conventional CWs may be more appropriate. FCWs therefore provide unique a solution in scenarios where eutrophication is being exhibited and pollutants have already entered the water body.

Primary pollutant removal mechanisms utilised within the FCW include nitrification, denitrification and plant uptake. Microbial nitrogen removal in the rhizosphere and biofilms has been explained in Chapter 1. Mitsch & Gosselink (2000) discuss the possibility of biomass harvesting and state that wetlands exhibiting eutrophic water conditions readily show an annual primary production of 1000-4000 g m^{-2} y^{-1}. Harvesting will not occur during the experiments carried out in Chapters 2 and 3 to prevent disruption to the systems. FCW biomass production involves the removal of pollutants from the water column and direct incorporation into plant tissues (Kadlec & Wallace 2008), effectively removing the pollutant from the water column. Vymazal (2005) in Kadlec & Wallace (2008) discuss how pollutant removal by harvesting varies greatly between treatment wetland type, scale and level of treatment. Assimilation rate of pollutants may also vary in accordance with species, explored further in the following chapters.

2.4 Potential Anti-algal Activity of DOC Compounds

Wingfield et al. (1985) hypothesised that organic matter such as barley straw or hay, which is comprised of lignin and cellulose, may provide microbial communities and biofilms with a substrate to attach to and labile carbon source for denitrification. Wingfield et al. (1985) also hypothesised that the microbial communities growing on the barley straw would be able to immobilise phosphates. This hypothesis was further tested by Welch et al. (1990) who discovered there were negligible reductions in nutrient pollutant concentrations. Instead, they observed the release of low molecular weight (LMW) DOC compounds which were inhibitory to the growth of algae. However, these compounds were shown to be short lived, which is very true of low molecular weight phenolics.

Phenolics contribute to total DOC (White et al. 2011) because they consist of aromatic compounds characterised by the presence of a six carbon ring with attached functional groups, namely carboxylic acid group. Although LMW DOC was found to have an inhibitory effect on algal blooms, DOC characterisation may inform FCW design to improve anti-algal compounds formation. One such method may be characterisation by Specific Ultra-violet Absorbance (SUVA) analysis. This method elucidates towards the general chemical characteristics of the DOC (Weishaar et al. 2003) values for which are discussed later.

Pillinger et al. (1994) further discusses anti-algal properties of lingo-cellulose based DOC compounds, outlining the role of phenolic compounds in the anti-algal effect of Barley straw. Evidence suggests that of DOC types analysed oxidised phenols and quinones showed strong anti-algal properties. However the rate at which algae was suppressed was shown to have increased correlation with aeration rates and total dissolved oxygen. Pillinger et al. (1994) concluded that it was lignin and cellulose breakdown products which provided the source of phenols from the barley straw.

Given that break down of lignin results in anti-algal phenolics produced. Facilitation of this breakdown may allow for FCW optimisation and increased anti algal effect. Moreover, studies of phenolic sub categories by Nakai et al. (2001) suggests that caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, syringic acid, vanillic acid, catechol and hydroquinone, protocatechuic acid quinic acid, shikimic acid, phenol, resorcinol, hydroxy hydroquinone, and phloroglucinol are potentially responsible for algal inhibition. The greatest algal inhibition was observed with caffeic acid and hydroxy hydroquinone.

2.5 Exploitation of Peatland Enzymic Latch Mechanisms

Phenol oxidases are extracellular enzymes present within wetland soils which are responsible for the decomposition of phenolic compounds. Phenol oxidase, which requires bimolecular oxygen to work efficiently, breaks down polyphenolic compounds that are released into the soils by microbial breakdown of lignin and cellulosic structural components of plants (Wetzel 1992; Freeman et al. 2001; Freeman et al. 2004). Microbes within the soils release polyphenolic compounds and organic acids as a by-product of their metabolic processes (Dickinson 1983). In senescence and biological breakdown, plants also release organic acids as lignin present in the cell walls begins to degrade. The polyphenols released as a result of

the breakdown of lignin and cellulose are tightly related to plant species and their decomposition conditions (Wetzel 1992).

It follows that colonisation of microbial communities capable of producing extra cellular phenol oxidase may result in more efficient breakdown of organic matter. If phenol oxidase is observed in the rhizosphere of the FCW, organic matter such as barley straw or heather is added to the system may be broken down more efficiently. This may result in greater concentrations of LMW phenolic groups leading to greater anti-algal effect.

2.6 Linking Enzyme Activity to Rhizosphere Oxygenation by Radial Oxygen Loss

Wetland rhizospheres are spatially diverse with respect to redox potential, allowing the formation of aerobic and anaerobic zones for simultaneous oxidation and reduction. Oxygen transport into the rhizosphere is essential for the removal of N-containing substances (Brix 1993). This is shown in the Nitrogen cycle by the transformation of NH_4-N by nitrifying bacteria such as *Nitrosomas* and *Nitrobacter* to NO_3^-, then denitrification by denitrifying bacteria to atmospheric nitrogen (N_2) and plant uptake (Sprent 1987). It would also be expected to observe differences in rhizosphere DO based upon species morphological adaptations. Radial oxygen loss from vascular macrophyte roots may be a candidate for optimizing enzyme activity within the rhizosphere of the FCWs. Wetland plants have internal structures which help guarantee their survival in highly waterlogged conditions present in wetland rhizospheres (Wiessner & Kuschk 2006). Internal gas transport into the rhizosphere by means of a morphological adaptation is crucial for plant survival due to O_2 diffusion being around 10,000 times slower in water than air (Colmer 2003). It is the presence of the arenchyma tissue structures that prevent asphyxiation of the waterlogged, below ground parts of the plant. Oxygen supply is also particularly important to metabolically active and growing sections of the roots especially when the surrounding soil they are penetrating is highly anaerobic (Jackson & Armstrong 1999). The arenchyma represents a means of long distance, apoplastic gas transport pathways, supplying tissues that are photosynthetically inactive and require an oxygen supply (Jackson & Armstrong 1999).

Sorrell et al. (2000) highlighted the differences in species aeration capacities. This may be due to the degree of adaptation expressed specifically by the individual species as a result of water logging and depth tolerance. (Armstrong 1980) also found that intracellular gas spaces vary greatly among species and therefore the total radial oxygen loss (ROL) to the

rhizosphere. Root arenchyma tissues exhibit 30-40% loss of oxygen transported into the roots to the surrounding soil, altering the chemical environment of the rhizosphere, influencing microbial communities and therefore extracellular enzymes (Armstrong 1980).

The growth of plants in anoxic soils is undoubtedly influenced by the presence of arenchymal tissues and the resulting ROL, however total oxygen loss and rhizosphere oxygenation are limited by a number of factors. These factors include the path length of the gas space continuum from shoot tip to root tip, causing gas flow resistance and the diameter and density of the arenchyma pathway (Jackson & Armstrong 1999).

From the above it follows that oxygen loss into the rhizosphere may be correlated to phenol oxidase activity, leading to the increased breakdown in aerobic conditions increasing the anti-algal effect (Wiessner & Kuschk 2006).

2.7 Hypotheses

The following hypotheses presented are produced in order to confirm mechanisms proposed in the literature and act as prerequisite for FCW research in Chapter 3. Hypotheses also inform test system design and aim to shed light on organic material and plant species selection.

1. different types of Organic matter introduced into the water column result in different observed rates of release and resultant concentration
2. Organic matter introduced into the water column broken down by phenol oxidase results in increased concentration of phenolics observed.
3. Phenolics within the DOC pool exhibit an anti-algal effect.
4. The use of FCWs containing macrophytes significantly reduces the effects of eutrophication in small water bodies.

2.8 Methods

Testing of hypotheses was undertaken at a number of scales. This included bench scale, 200ml *in-vitro* analysis prior to 80L mesocosm tests for organic matter selection and full scale 480L mesocosms testing FCW design and feasibility. Pilot testing in Chapter 2 would inform the method for hypothesis testing Chapter 3.

2.8.1 Experiment Setup for Organic Matter Compound Release, Anti-algal Effect and FCW Design Analysis

2.8.1.1 200ml *in vitro* - DOC Release

Twenty five grams of organic Barley straw (as used in Welch et al. (1990)) and Heather were chopped to 3-4cm lengths and added to a sealable container. Five replicate containers containing each material were used. Material was not subjected to desiccation prior to weighing in order to replicate use in the FCW. To each container, 200ml of ultrapure water was added and lid sealed. The containers were left to stand for a period of 1 week in full natural light. Samples were taken from each container and passed through 0.45µm syringe filters and stored at 4°C prior to analysis.

2.8.1.2 80L mesocosms – Anti-algal effect of DOC/Phenolics Released from Barley Straw

Pilot testing at 80L level aimed to confirm anti-algal effect of DOC released by organic matter observed by Wingfield et al. (1985), Welch et al. (1990) and Pillinger et al. (1994). Prior to testing, an 80L, open topped container was filled with tap water and allowed to colonise naturally with pelagic green algae for a period of 4 weeks. The algae present in the container were sampled and cultured in order to create a dense pelagic colony. Mesh bags were filled with 300g of barley straw and heather (again not subjected to desiccation to replicate use in FCW systems), 3 replicated of each material were used. A 100ml floatation device (capped centrifuge vial) was added to each of the bags to suspend the material at the water surface.

Mesocosms were set up in a greenhouse of the roof of the Memorial Building, Bangor University, Wales, using 80L containers filled with 70L of tap water and allowed to settle for 24 hours. Systems were filled with tap water rather than water sources from a natural water body in order to create uniform water chemistry between all replicates The tap water was vigorously stirred to “drive off” excess chlorine added at local water treatment plants. A volume of 300ml of algal culture was added to each container prior to the addition of organic matter contained in the mesh bags. Each container was vigorously stirred to mix the algal culture through the container. This was repeated prior to each sample taken from the water column. Samples were taken every 3-5 days over a 3.5 week period (08/10/2010 – 03/11/2010) passed through 0.45µm syringe filters and stored at 4°C prior to analysis.

2.8.1.3 480L Mesocosms – FCW Feasibility

Testing at a pond scale was undertaken in order to assess the viability of the systems prior to analysis of the mechanisms involved in eutrophication mitigation, studies in Chapter 3. Small water bodies were replicated using 480L tanks located on the roof of the Brambell Building of the University of Wales, Bangor. The small pond mesocosms were filled with 480 litres of tap water and vigorously mixed as before. Two FCW replicates were used; ponds without FCWs were used as controls (N=2). FCWs were constructed using wire cages supported by a floatation device around the circumference. A rooting media mixture of equal measures of peat, Coir (coconut fibre) and finely chopped heather were used to replicate organic matter added to the water column as demonstrated in Welch et al. (1990). Porous liners were used in order to prevent loss of the organic media into the water column. Into each FCW equal numbers of *Juncus effusus* plants were added (figure 2.1). Plants used were 1 year old, FCWs were constructed 2 months prior to use in the experiment.

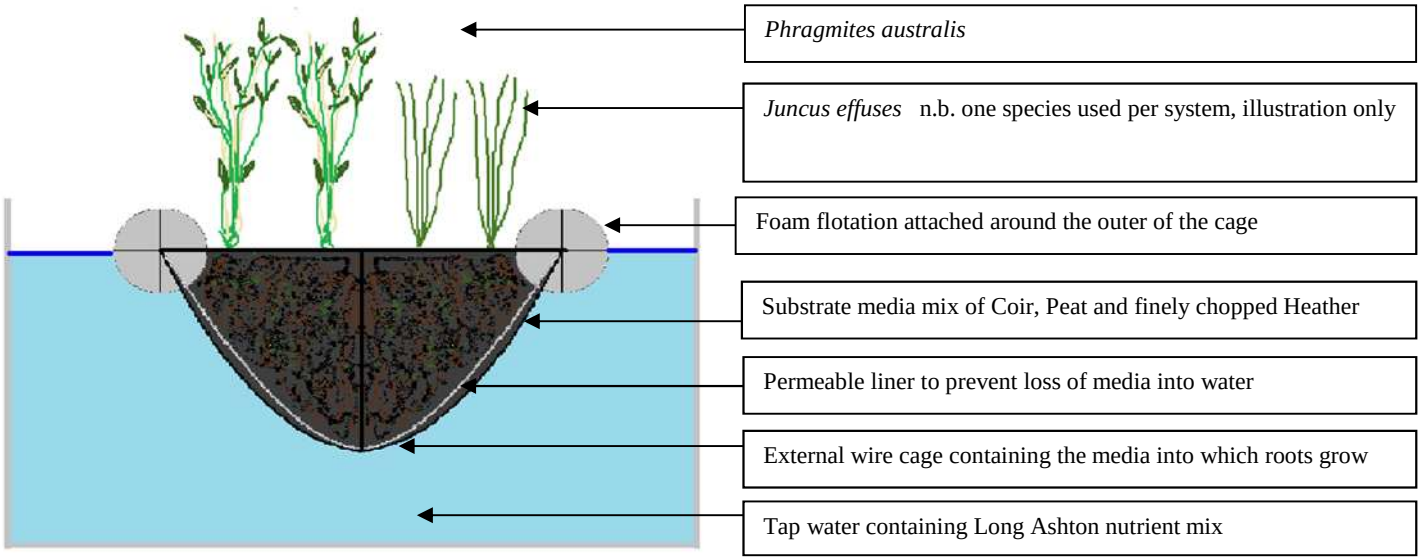


Figure 2.1 Diagram of FCW set up.

Diagram indicates position of the FCW in the water body. Water is able to freely diffuse from the water column into the rhizosphere. DOC is also able to leach out of the organic matter media into the water column allowing interaction with algal blooms.

High nutrient concentration solution was also added to the water to induce eutrophic water chemistry and stimulate algal growth. This solution is a modified Long Ashton solution, designed to provide plants with all macro and micro nutrients required by plants for healthy

growth. The solution was added every 3–4 days over 2 weeks in order to replicate the build-up of nutrients in small eutrophic ponds before FCWs were added. Water samples were taken 6 times over a 4 week period. Two additional samples in the following were taken for Chlorophyll –a analysis.

Separate nutrient solution stocks were made up as 1 molar solution. Compounds used were:

1. KNO_3, $Ca(NO_3)_2.4H_2O$,
2. $NaH_2PO_4.2H_2O$,
3. $MgSO_4.7H_2O$,
4. EDTA.FeIII.Na,
5. Micro nutrient multi stock - $MnSO_4.4H_2O$, $CuSO_4.5H_2O$, $ZnSO_4$. $7H_2O$, H_3BO_3, $Na2MoO_4$. $2H_2O$,NaCl
6. Na2SiO3. 5H2O

Total stock volume added (ml)

1. 61.74
2. 5.4
3. 0.78
4. 123.42
5. 61.74
6. 61.74
7. 12.36

2.8.2 Barley Straw Boom Addition

For the final 2 sampling dates, one FCW was replaced with a barley straw boom. This was undertaken in order to replicate organic matter addition as undertaken in Welch et al. (1990). This replacement aimed to assess chemical release at a pond mesocosm scale.

2.8.3 Water Quality Parameters and Analytical Methods

In order to quantify DOC/phenolic release from organic matter, confirm DOC/phenolic anti-algal effect and suitability of FCW design a number of water quality parameters were measured at each level of testing. *In-vitro* 200ml level quantified water column phenolics alone; 80L mesocosms quantified release of DOC by organic matter, phenolic concentration,

$SUVA_{254}$ and chlorophyll-a concentration as a proxy for algal density. The FCW feasibility study included algal density parameters and additionally ion chromatography to quantify phosphate, nitrite and nitrate concentrations.

2.8.4 Phenolic Analysis Method

Phenolic concentrations were determined using a method adapted from Box (1983). 250 µl of sample was added to a clear micro plate well. 12.5 µl of Folin-Ciocalteau reagent was added to each sample followed by 37.5 µl of Na_2CO_3 (200 g L-1). After 1.5 hours the absorbance was measured at 750nm on a Molecular Devices M2e Spectramax plate-reader. Phenolic concentrations were then derived from the preparation of a standard curve using tannic acid phenol standards of known concentration (0, 1, 2, 4, 6, 8, 10, 15, 20 mg L^{-1}).

2.8.5 DOC Quantification

DOC concentration was measured using an Analytical Sciences Thermalox TOC/TN analyser. In order to measure DOC the samples were acidified to between pH2 and 3 and sparged with oxygen for 2 minutes in order to remove inorganic carbon compounds. The instrument was calibrated using potassium hydrogen phthalate standards of known concentration (0, 5, 10, 15, 20, 30 and 40 mg L-1).

Machine replicates using the Thermalox are unnecessary due to multiple sample injections during analysis. For this analysis the multiple injection number was 5 injections.

2.8.6 DOC Chemical Composition as SUVA 254nm Analysis

$SUVA_{254}$ elucidates the general chemical characteristic of DOC. This is achieved by measuring absorbance of the sample at 254nm as a function of DOC concentration. 300µl of each sample was placed into a clear 96 well micro plate which was scanned at 1nm intervals from 200nm to 800nm. Three wells of Ultrapure water was used as a blank, machine used was a Molecular Devices M2e Spectramax plate-reader.

$SUVA_{254}$ values show strong correlations with aromatic and humic content of the DOC. Values generated also infer molecular weight and degree to which the DOC is labile or recalcitrant (Weishaar et al. 2003). Values <1 are generally LMW, highly aromatic and linked to plant DOC release, whereas >4 generally indicates highly humic substances originating from more recalcitrant organic matter. Values between these ranges express a transition

between DOC which is aromatic, LMW which is highly labile towards more humic, higher MW and more recalcitrant forms of DOC

SUVA is calculated by dividing light absorbance by the concentration of DOC. The path length of the micro plate is 1cm therefore a multiplication of 100 is required to convert the value to $L^{-1}mg^{-1}$ DOC / m^{-1}.

2.8.7 Algal Biomass quantified as Chlorophyll-a Content

Algal content is assumed to be directly proportional to the concentration of chlorophyll-a present in solution. The chlorophyll-a content was determined by taking forty millilitres of each water sample passed though Whatman Glass Fibre grade A filter paper. Samples were wrapped in foil and frozen until all samples had been collected. Once all water samples had been filtered, the filter paper was defrosted at $4^{o}C$ for 12 hours. Each filter paper containing algal cells was placed into a 15 ml centrifuge tube with a lid. To the tube a 90% acetone solution was added (1 ml milli Q grade water/9ml acetone). Tubes were left in a $4^{o}C$ incubator in the dark for 20 hours, and inverted occasionally. 2.5 ml of sample were then transferred to 3ml centrifuge tubes and spun at 3200 rpm for 10 minutes. Spectrophotometry absorbances are taken at 665 and 750 nm. These absorbances were then applied to the formula outlined in Golterman & Clymo (1971).

$$TotalChlorophyll = 11.0\,(Abs_{665} - Abs_{750})\frac{v}{Vp}$$

(V) Volume filtered, (v) volume of extract (ml), (p) is the path length (cm) and 11.0 as the specific absorbance coefficient of 90% acetone.

2.8.8 Nutrient Pollutant Concentration Analysis by Ion Chromatography

A suite of ions were analysed using ion chromatography using an 850 Professional IC and 858 auto sampler, Thermo Fisher AS14A anion column and Metrohm C4 cation column. A range of standards were used separately for anions and cations using Fluka Multi-ion standard. Although multiple ion concentration values are generated, only nitrogen and phosphorus containing ions are discussed.

2.8.9 Statistical Analysis Used to Assess Phenolic Release, Anti-algal Effect of Organic Matter and FCW Feasibility

In order to ascertain where significant differences in mean water quality parameters lay, analysis of variance (ANOVA) was used. This method compares the mean value of the parameter of each treatment (group) in question. In order for ANOVA analysis to be valid, homogeneity of variance around the means of each group is required. Levene's statistic clarifies if the assumption of equal variances had been violated.

SPSS version 19 was used for all statistical analysis. The confidence level for significant effect detection was set at $p \leq 0.05$.

2.9 Results

2.9.1 *In-Vitro* 200ml Phenolic Release

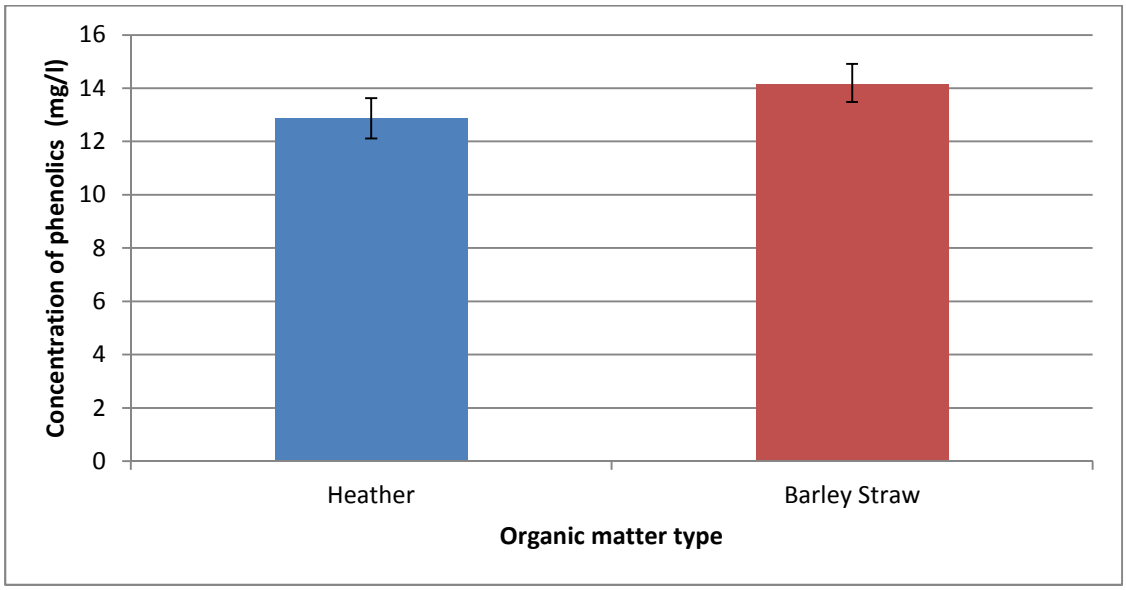


Figure 2.2 –Concentration of phenolics observed as a component of total DOC released from two forms of organic matter; heather and barley straw. Error bars represent standard error of the data.

Barley straw is observed to release slightly greater quantities of phenolics into the water column of the container compared to Heather, 14.16 and 12.87 mg/L, respectively. ANOVA analysis indicates a statistically significant difference between treatments ($p \leq 0.040$).

2.9.2 80L Mesocosms Anti-algal Study.

Figure 2.3 - 80L mesocosms testing pictures. Barley Straw and Heather are shown, Left and Right, respectively.

2.9.3 Analysis of Phenol Release from Barley Straw and Heather

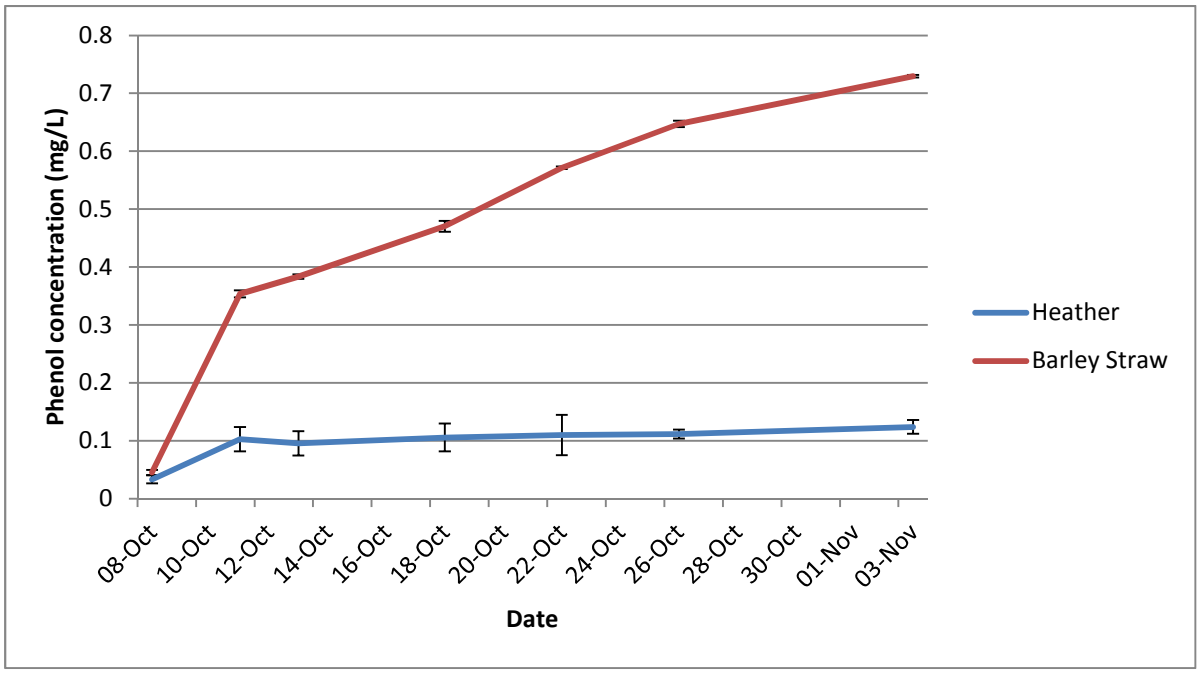


Figure 2.4 –phenolic concentration observed leaching into the water column surrounding organic matter containing bags in and 80L mesocosm, observations over a period of 3.5 weeks.

Barley straw indicates a strong increase in phenolic release over time, reaching a 7 fold increase compared to heather by 3.5 weeks. Heather indicates limited increase in phenolic concentration for the duration of the experiment, with exception of the first 3 days. Phenolic concentration observed at the end of the experiment was statistically examined by ANOVA, which indicated significant differences in phenolic release between treatments (<0.001). This

data concurs with figure 2.3 indicating greater concentration of phenolics released by barley straw compared to heather.

2.9.4 DOC Release from Barley Straw and Heather

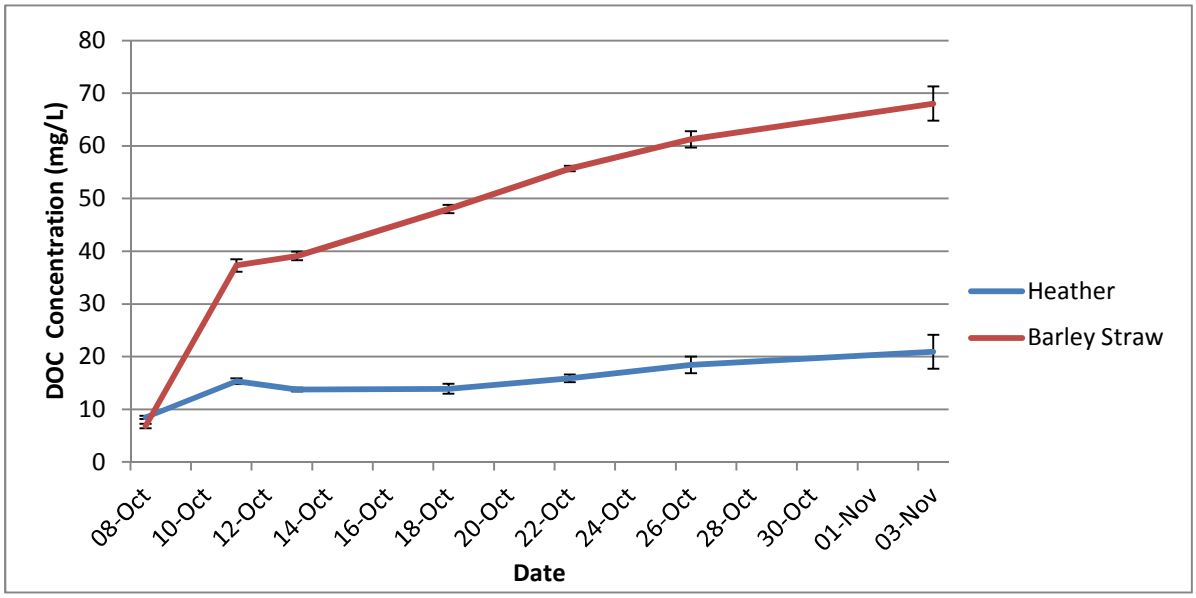


Figure 2.5 - Graph to indicate DOC leaching into the water column surrounding organic matter containing bags in an 80L mesocosm, observations over a period of 3.5 weeks.

DOC concentrations mimics figure 2.4 showing phenol concentrations. Although DOC production indicated a similar relationship to phenolics, concentrations are an order of magnitude greater.

2.9.5 SUVA Absorbance Values of DOC leached from Barley Straw and Heather

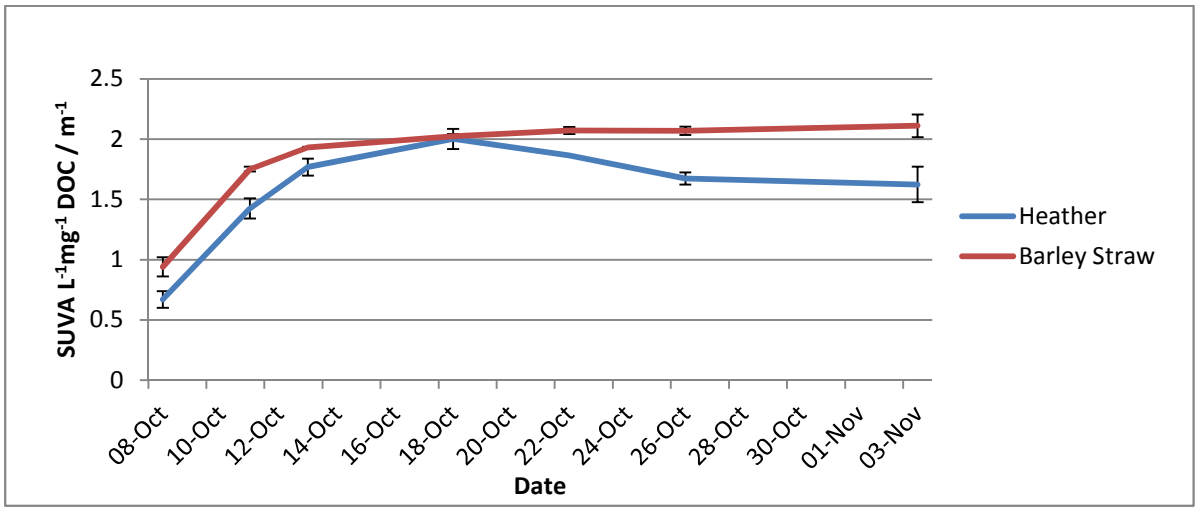


Figure 2.6 – Graph to indicate change in $SUVA_{254}$ over time for Barley Straw and Heather treatments in 80L mesocosm.

Rapid increases are observed in both of the treatments rising rapidly from below 1 up to 1.8-2 within 5 days. SUVA values remain reasonably stable for both treatments for the duration of the experiment.

2.9.6 Effect of Phenol Release on Algal Density

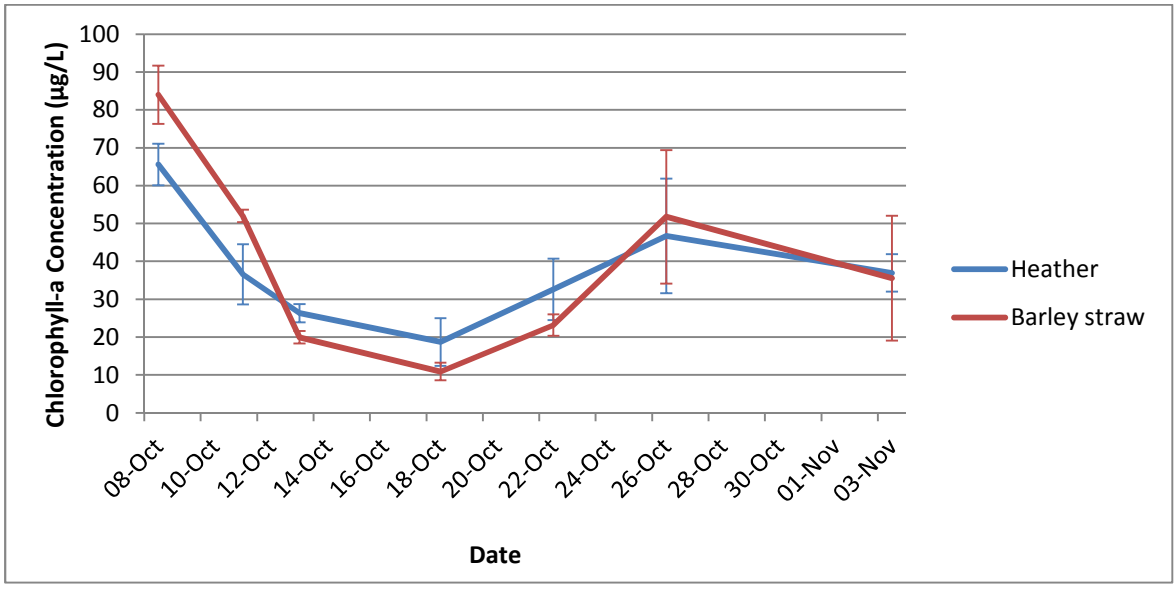


Figure 2.7 –Water column Chlorophyll-a concentration in 80L mesocoms analysing the effect of organic matter addition.

Densities use as a proxy for algal density. Both treatments indicate an initial rapid drop in chlorophyll-a concentration in the first 10 days of the experiment following the addition of phenolic and DOC releasing material, however the reduction in algal density is short lived. Both treatments show increases in Chlorophyll-a concentration in the latter stages. The large error bars in the final two samples are associated with barley straw Chlorophyll density whilst error bars for Heather remain similar throughout.

2.10 Pond Scale (480L) Testing, Nutrient Uptake, Algal Assays, DOC and Phenol Release

2.10.1 Water Column Phosphate Concentration

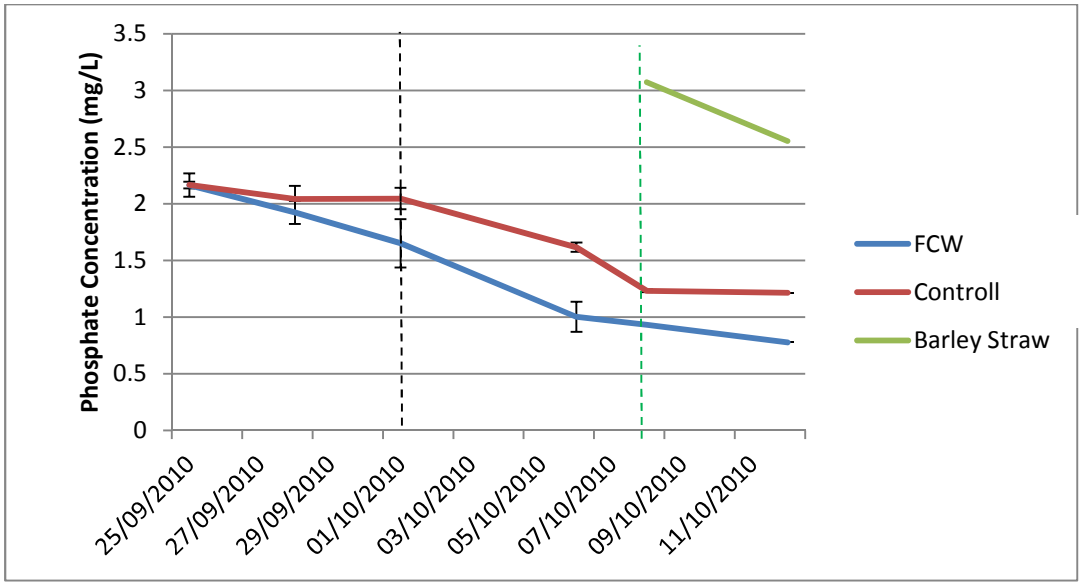


Figure 2.8 – Graph to indicate phosphate dynamics in the water column of 480L mesocosms. GREEN dashed line indicates removal of one FCW replicate and replacement of a barley containing mesh bag. BLACK dashed line represents the stage at which nutrient addition is halted.

Phosphate concentrations in the 480L mesocosm FCW treatments show an initial decrease, however limited change in concentration is observed between 15/10/201 and 22/10/2010. The control shows stable phosphate concentration until 07/10/2010 before concentration of phosphate is observed to decrease. At the Barley straw addition stage, 200% higher phosphate is detected in the barley straw treatment compared to the FCW or the control treatments.

2.10.2 Water Column Nitrate Concentration

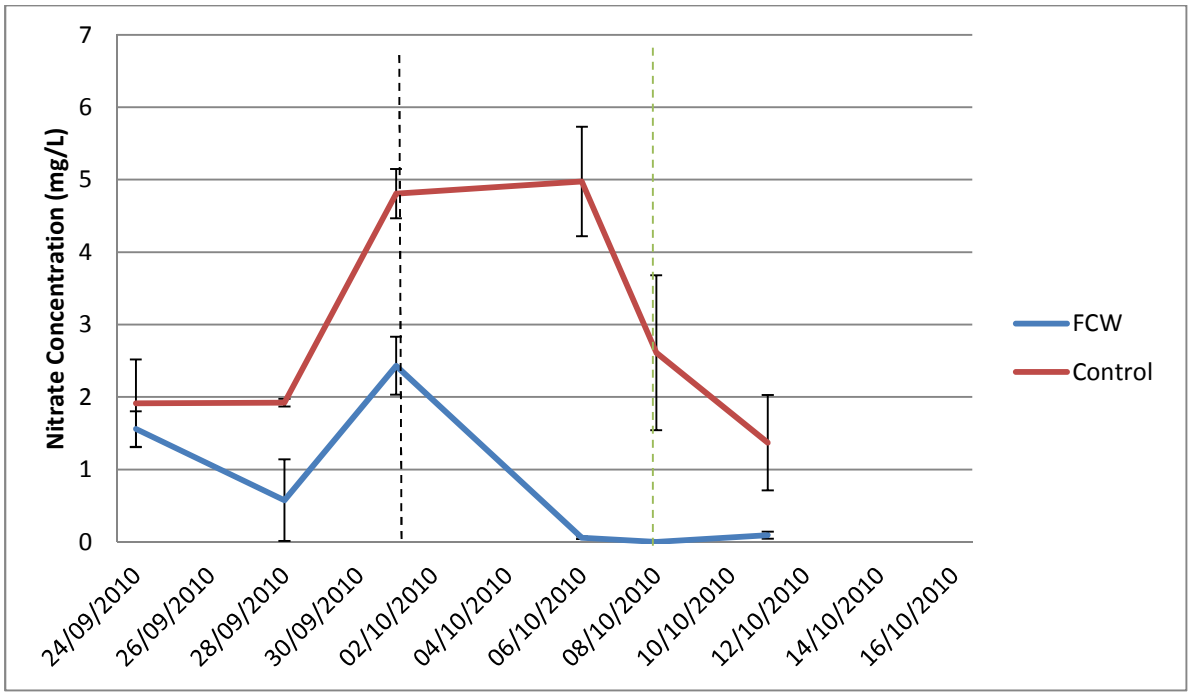


Figure 2.9 – Graph to indicate nitrate concentrations observed in the water column of 480L pond mesocosms. BLACK dashed line represents the stage at which nutrient addition is halted.

The control shows an increase in nitrate up to time 07/10/2010 of the experiment, whilst nitrate concentration in the FCW treatment remain lower throughout. The treatments containing FCWs show a lesser increase in nutrient concentration during nutrient addition. Nitrate concentrations in the FCW treatment quickly reduced to near zero once nutrient addition is halted.

2.10.3 Water Column Nitrite Concentration

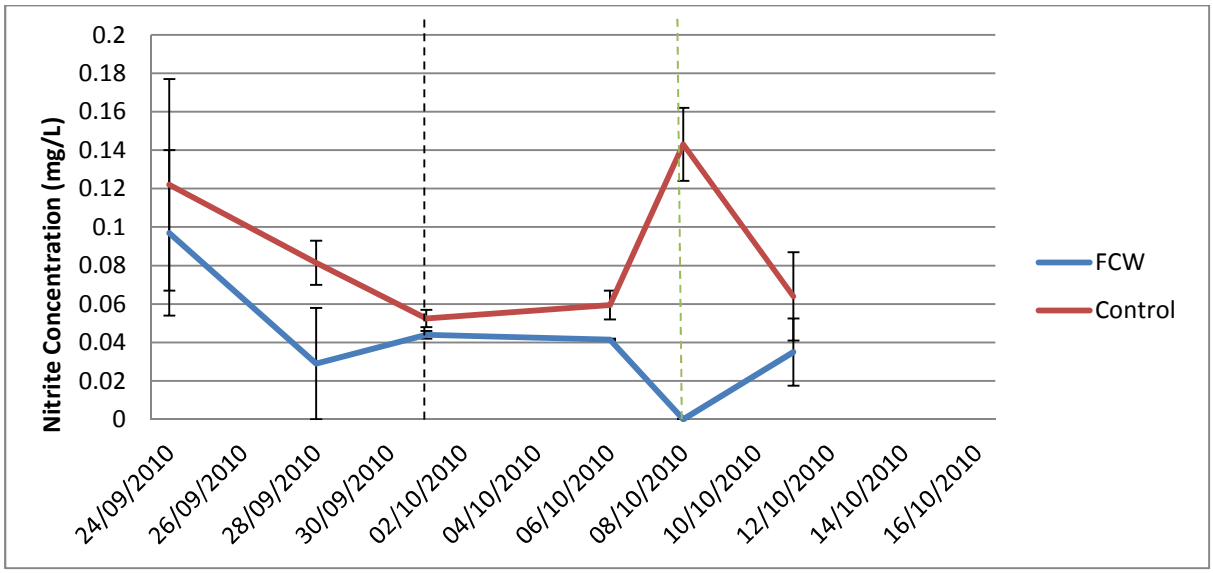


Figure 2.10 – Graph to indicate nitrite concentration observed in the water column of 480L pond mesocosms. BLACK dashed line represents the stage at which nutrient addition is halted.

Nitrite concentrations are observed to be very low for the duration of the experiment. However, nitrite concentrations remain lower in the FCW ponds than in control treatments at all times.

2.10.40Chlorophyll Density Analysis

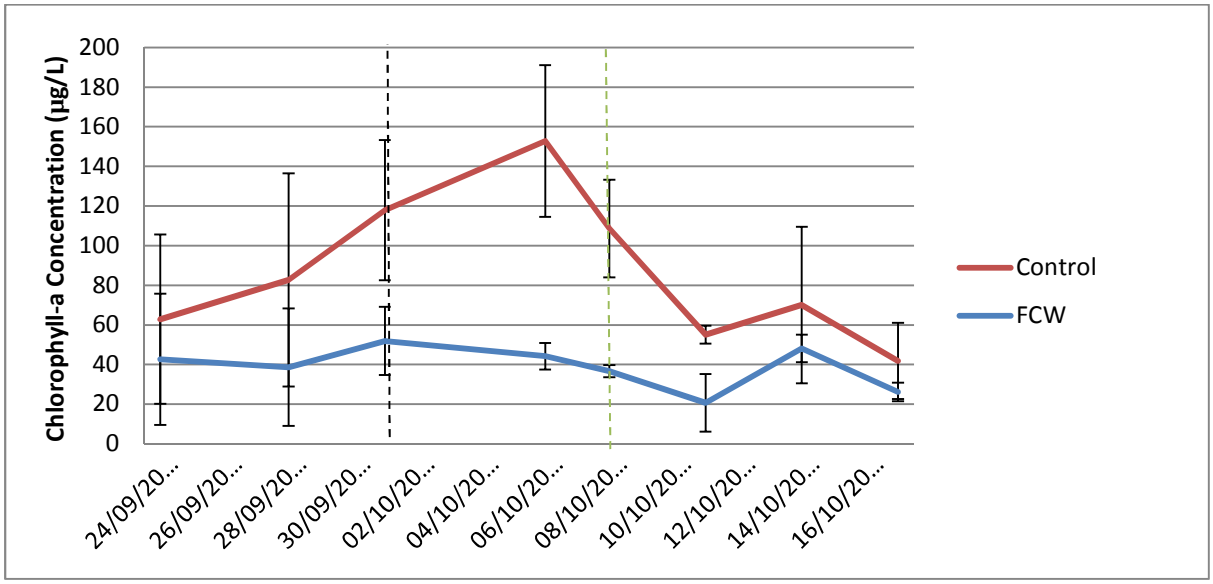


Figure 2.11 – Graph to indicate Chlorophyll-a concentration in the water column of 480L pond mesocosms. Chlorophyll-a concentration is used as a proxy for total algal biomass. BLACK dashed line represents the stage at which nutrient addition is halted.

Chlorophyll-a concentrations observed in the FCW treatments are observed to be a minimum of 35% less concentrated that the control treatments for the duration of the experiment. FCW treatments also do not indicate a significant increase in concentration as observed in the controls.

2.11 Discussion - Small Scale Testing

Barley Straw released greater mean concentration of phenolics. This allows us to deduce that water treated with Barley Straw would show lower chlorophyll densities in relation to a lower amount of algae in the water column.

Although significant differences were detected between the concentration of phenolics released by the two organic compounds, this difference may not be significant biologically. Further testing at 80L which replicates a more representative system scale may confirm only effect on algal density with respect to phenolic and DOC release from the material.

2.12 Discussion – 80L FCW Matrix Material and Algal Density

The release of phenolics had a profound effect upon the chlorophyll-a density, but not in the manner expected. Despite the higher concentrations of phenolics shown in figure 2.4, algal density remains similar for both treatments (Figure 2.7). Regarding figure 2.4, phenolics released from barley straw increased in concentration throughout the experiment. Evidence presented in Wingfield *et al* (1985), Welch *et al* (1990), Pillinger *et al* (2004) indicated that the barley straw would have an increased anti-algal effect and decrease the chlorophyll density to a greater degree. In fact, the relationship of algal densities over time is much the same. This may shed some light upon the type of phenolics released by each substrate material. In that they may not only vary in terms of composition of phenolics but also in terms of potency in their anti-algal effect, although basic chemical composition (indicated by $SUVA_{254}$, figure 2.6) seems to be similar.

Initially chlorophyll density is rapidly reduced by both substrate types from approximately 65-85 ppm to between 10 and 20 ppm over a period of 12 days. However, all of the test containers experienced a secondary bloom in the final days of the testing. Algae will strongly bloom when conditions are optimal correlating with high light levels and warmer conditions, as seen in natural situations during the summer months. The test containers were located in a glass house on the roof of the Memorial building of Biological Sciences and Bangor

University. The glass house is heated in order to maintain a minimum temperature of 12 ^{o}C and automated venting allows a small amount of maximum temperature control.

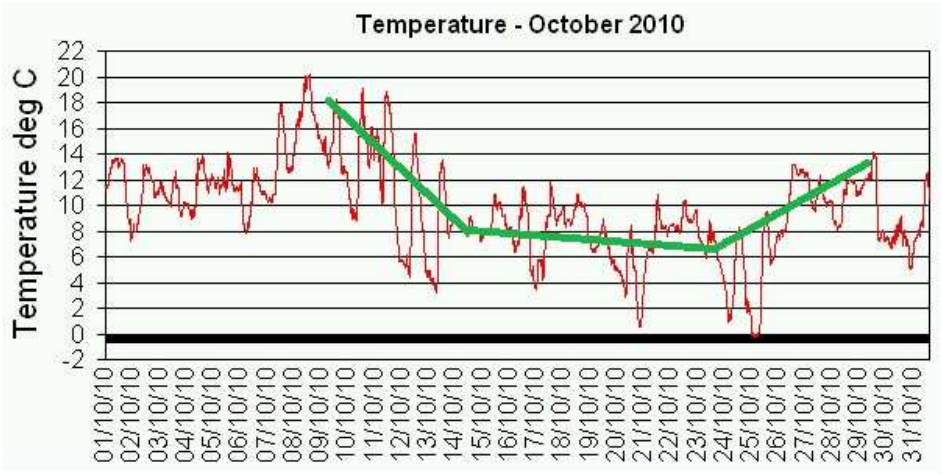


Figure 2.12 - Local temperature (Mynydd Llandigai Weather station, North Wales)

Figure 2.12 indicates temperature from a local weather station from the 8th of October to 29th of October. A slight rise in temperature is shown in the final weeks of the experiment, this, along with the possibility of high light levels could have provided for optimum algal growing conditions. Potentially the secondary rise in chlorophyll density could be attributed to temperature and light, as opposed to senescence as a result of anti-algal DOC compounds. However, the 80L mesocosms targeted anti algal effect on an established bloom alone, anti-algal DOC might still prove to prevent the growth of algal blooms rather than acting as an algaecide.

Figure 2.6 alludes to changes in the source of DOC throughout the experiment. Early values indicate lower SUVA values that correspond with plant (algal) exudates are the source of DOC whereas higher values indicate a release from organic material such as heather or straw. Indicating the DOC released is changing the light absorption properties of the water. The increase in SUVA value over time is matched to the pattern seen in the progressive release of DOC and phenolics released by the organic matter.

2.13 Discussion of Pond Scale Testing

2.13.1 Nutrient Pollutant Analysis, N and P Compounds

Phosphate was seen to decrease more rapidly in the FCW treatment than the control (figure 2.8). This may be attributed to plant biomass present during the initial phases of the experiment. Faster rates of phosphate removal may be achieved by FCW treatment because the plants are already established; whilst in the control the algal density may not have been sufficient to dramatically reduce available phosphate. Control system algal density in figure 2.11 indicates a slow increase until the point where algal biomass is significantly large as to have an effect on phosphate concentration.

When analysing the phosphorus cycle in more detail it can be seen that mechanisms of phosphorus removal, although they include plant uptake, are also heavily influenced by precipitation with other compounds and also binding to sediments and flocculation (Vymazal, 2007). It is proposed that the addition of the FCW may have released a degree of sediments into the pond mesocosm or acted as a method by which phosphate became bound to the soils in the system.

The latter part of the experiment a FCW was replaced with a barley straw boom. At this point in time the phosphate concentration associated with this treatment increased rapidly. It is proposed that as the barley straw broke down large amounts of phosphate were lost from the boom (DOC and phenolics also released) and may explain why Wingfield *et al* (1985) observed little effect on algal density.

Figure 2.13 – Pond scale 480L mesocosm showing a barley straw boom, which replaced the FCW

Although a slight improvement in phosphorus removal rate was observed in FCW treatments, unpublished data from West (2010, Unpublished) provides an explanation into the limited rate at which phosphate is accumulated into the FCW. The research was undertaken in order to establish if there were differences in nutrient chemical removal rate in relation to species present in the FCW. The results of this experiment showed that *Juncus effusus* was a species that performed poorly at phosphate uptake in comparison to 4 other species of wetland macrophytes from temperate climates. Species selection may therefore implicate the rate at which phosphorus removed from the water column and converted to biomass.

Nitrate was observed to increase over time in the control pond mesocosms. This was as expected due to the gradual increase in nitrogen addition of the Long Ashton Nutrient solution. Again chlorophyll-a concentration builds as nitrate increases and nitrate is not seen to be depleted until algal density becomes sufficiently great and nutrient addition is halted.

Similarly, a lesser rate of nitrate concentration increase was observed in the treatment systems. This again may be attributed to the presence of plants taking up nutrient chemicals instantly. Secondly, due to the set-up of the FCW prior to the pond scale mesocosm test it is likely that the systems will have microbial communities, possibly containing denitrifyers, developing within the rhizospheres. This would aid removal of nitrate to gaseous forms removing it from the water column (Shapleigh, 2013)

2.13.2 Characterisation of Algal Bloom Density and Formation

The preceding parameters all indicate reductions in nutrient content of the water column as a result of the installation of the FCW systems. Chlorophyll-a concentrations remained below the concentrations exhibited in the control systems at all times. This result emphasises the feasibility of installing FCWs into ponds exhibiting eutrophic water conditions in order to prevent and mitigate against algal blooms.

Figure 2.14 and 2.15 – Images of the control and FCW mesocosm, respectively.

Control shows the density of the algae colonising the water column of the mesocosm whilst visibly lower algal density is observed in the FCW treatment. Control pond exhibiting chlorophyll-a concentration of approximately 150µg/L, as opposed to under 50µg/L in the FCW treatment.

Algal density was observed to decrease shortly after nutrient addition was halted. This process initiated the senescence phase of the algal bloom showing decreasing concentrations of chlorophyll-a in the water column. This process would initiate water quality degradation as heterotrophs decompose the dead algal bloom. By installing the FCW, bloom formation was prevented leading to uncompromised water quality due to the lack of heterotrophic respiration as a result of organic breakdown of the algal bloom.

2.14 Conclusions

All the systems containing an FCW system or indeed a lignocellulose releasing substrate were able to reduce or suppress algal growth under conditions of nutrient enrichment, this combined with data on species specific nutrient dynamics (West 2010, unpublished) progresses research into the mechanisms involved in water quality mitigation.

With reference to the original hypotheses of Chapter 2;

1. Organic matter of different types introduced into the water column was found release of DOC into the water column, at different rates. Although barley straw released greater concentrations of DOC, heather indicated similar anti-algal effect. Although chlorophyll-a concentrations observed may have been an artefact of light and temperature, heather was selected as the organic matter to be used in Chapter 3.

2. Water sampling confirmed that organic matter introduced into the water column broken down by phenol oxidase or otherwise resulted in increased concentrations of phenolics.
3. Released DOC and phenolics exhibiting an anti-algal effect is as yet unconfirmed, further mechanistic analysis and experimental design modifications in chapter 3 should indicate anti algal effect of DOC and phenolics.
4. The use of FCWs containing macrophytes was found to significantly reduce the effects of eutrophication in small water bodies. Mesocosms where FCW were not utilised resulted in a 3 fold increase in algal density.

Formation of hypotheses based upon species specific nutrient dynamics informed research proposals into the optimisation of FCWs and development of understanding of bloom mitigation mechanisms. Development of knowledge gained into the use of lignocellulose releasing substrates in conjunction with nutrient removal mechanisms of a FCW may now be explored based on the information gained in this research.

Chapter 3 - Investigation into Floating Constructed Wetlands for Algal Bloom Mitigation

3.1 Introduction - Species Dependent Nutrient Dynamics, Algal Bloom Mitigation in Floating Constructed Wetlands

Following experiments confirming DOC release from organic matter and subsequent anti-algal effect inline with Pillinger, Cooper, & Ridge, (1994); Welch, Barrett, Gibson, & Ridge, (1990) and Wingfield, Greaves, Bebb, & Seager, (1985), and investigation into the hybrid use of Floating Constructed Wetlands (FCWs), Chapter 3 focusses on mechanistic analysis of the processes involved in eutrophication and bloom mitigation.

This chapter utilised a number of developments in experimental design. More specifically, a series of 3 plant species are tested, including planted and unplanted systems and the use of two trophic levels were investigated in 80L mesocosms.

A number of hypotheses were proposed;

5. There are significant differences in nutrient sequestration characteristics between wetland plant species
6. Morphological differences between species results in variation in radial oxygen loss (ROL) from roots and rhizomes
7. ROL directly affect phenol oxidase activity
8. Phenolic degradation by phenol oxidase within the FCW is enhanced by the presence of wetland plants

3.2 Method

3.2.1 Experimental Mesocosm Set-up

Thirty 80 litre containers were set up on the roof top area of the Brambell Building, Biological Sciences department at Bangor University, Wales, UK. Each tank was filled with 70 L of tap water and allowed to settle. Following a settling period of approximately 1 hour the water in the tanks was vigorously mixed in order to “drive off” any excess dissolved chlorine added to the water at the local water treatment plant and then allowed to rest for a 2 day period.

The experimental design employed the random assignment of trophic state and species/treatment type with respect to location. The testing tanks were positioned in 3 rows of 10 tanks. Although randomly assigned, in order to achieve robust experimental design each

trophic state and treatment type occurred in each of the 3 rows. Each treatment type was then randomly positioned within the row in order to reduce environmental effects.

The experiment was divided into two trophic levels

- H = Hypertrophic nutrient balance
- M = Mesotrophic nutrient balance

Each trophic level used 3 replicates of 5 sub-treatments.

- P = treatment FCW planted with *Phragmites australis*
- J = treatment FCW planted with *Juncus effusus*
- I = treatment FCW planted with *Iris pseudacorus*
- F = FCW unplanted
- C = Control system – No Plants or FCW present
- 1, 2, 3 = Replicate number

Example - MP2 = Mesotrophic Phragmites replicate 2

HC1	**HI1**	**MI1**	**HF1**	**MC1**	**MJ1**	**MP1**	**HJ1**	**MF1**	**HP1**
HP2	**MJ2**	**MF2**	**MP2**	**HJ2**	**HI2**	**MC2**	**MI2**	**HF2**	**HC2**
MP3	**MC3**	**HJ3**	**MI3**	**HC3**	**HF3**	**HP3**	**MJ3**	**MF3**	**HI3**

Figure 3.1 – Randomised block of treatment locations within the experiment

3.2.2 Trophic State and Nutrient Compound Concentration Manipulation

A nutrient solution similar to the experimental phase (chapter 3) as in the preliminary testing (chapter 2) was used in the 80L mesocosms. The Long Ashton Nutrient solution was manipulated in such a way as to achieve two trophic levels (Figure 3.2). These trophic levels were based upon analysis carried out by (Wetzel 2001; Smith et al. 1999; Nürnberg 1996; Dodds et al. 1998) on freshwater bodies. The nutrient solution was added at the start of the experiment (7th June 2011), a secondary nutrient replenishment of nitrogen and phosphorus containing solutions was performed on the 1st of August 2010. For both hypertrophic and mesotrophic treatments micronutrient concentrations were identical, only solutions containing nitrogen and phosphorus were adjusted to create specific trophic conditions (fig 3.2).

The nutrient pollutants KNO_3, $Ca(NO_3)_2.4H_2O$ and NaH_2PO_4. $2H_2O$ were manipulated in order to achieve the following concentrations in the mesocosm study.

Nutrient type/trophic state	Hyper trophic	Mesotrophic
Nitrate concentration (mg/L)	25	2.5
Phosphate concentration (mg/L)	2	1

Figure 3.2 - Concentration of nutrients achieved in the water column of the 70L mesocosms

3.2.4 FCW Design and Addition to Mesocosms Systems

The FCW systems were exact replicas of the systems used in the 480L pond scale testing in chapter 2. Again a 1:1:1 ratio of peat, coir and shredded heather was used as organic material within a semi spherical cage and liner suspended at the surface of the water column with an inert foam floatation ring.

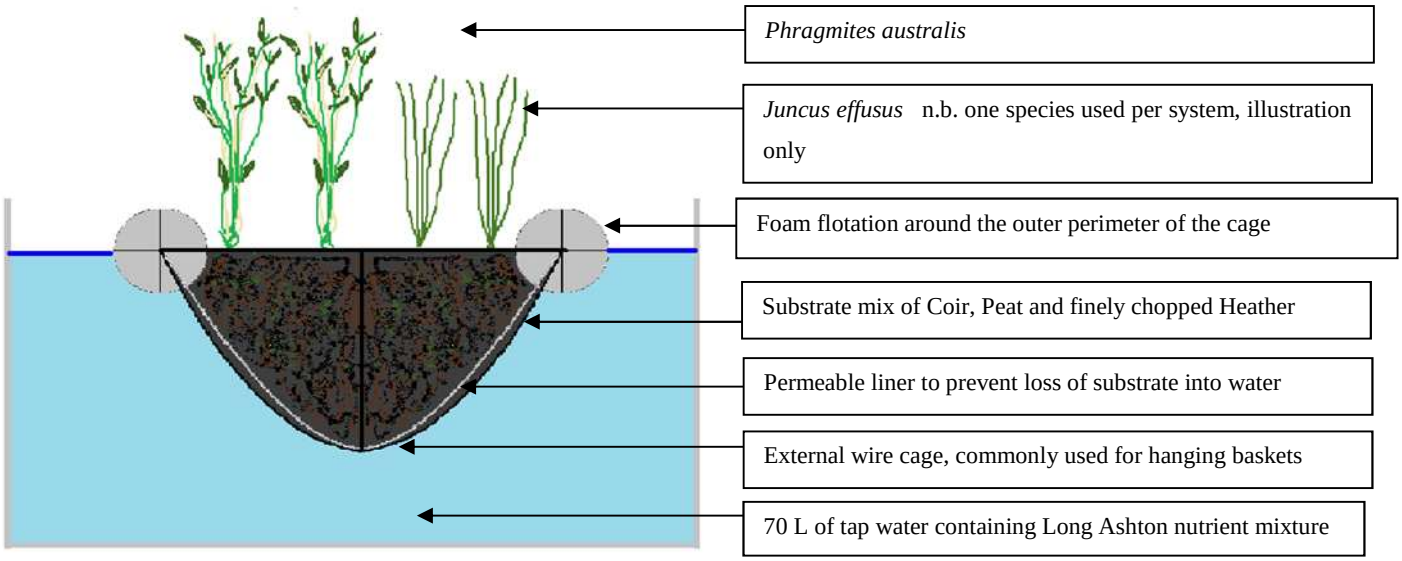


Figure 3.3 - Diagram of FCW set up.

Diagram indicates position of the FCW in the water body. Water is able to freely diffuse from the water column into the rhizosphere. DOC is also able to leach out of the organic matter media into the water column allowing interaction with algal blooms.

Once the nutrients within the test containers had been manipulated to provide a Hypertrophic and Mesotrophic nutrient solution the FCWs were added immediately.

Systems were planted with equal quantities of plants by biomass. *Phragmites australis, Juncus effusus* and b *Iris pseudacorus* species were used. Three replicates of each species were present in each trophic level. When planting out the new FCWs, attached material was washed from the root zone of the plants in order to avoid contamination.

FCWs containing *Juncus effusus* were planted in advance to the testing period. These systems were constructed 9 months prior to the main testing stage. This was done in order to achieve a pseudo-control which aimed to highlight how performance changes with established systems. It was proposed that DOC and phenolic release from the organic matter would reduce over time.

Once fully planted each system was rinsed with tap 50 L of water in order to remove any residual nutrient within the organic material added to the substrate material. This allowed for more accurate plant nutrient assimilation quantification.

3.2.5 Method for Sample Collection and Preparation and In Situ Analyses

Sampling was carried out on a weekly basis; thorough mixing the water within the test container was undertaken prior to and taking a 50ml sample from the water column. All water chemistry parameters were measured from the water column samples rather than the pore water unless otherwise stated. The pH and electrical conductivity (EC) were taken from raw unfiltered samples. Dissolved oxygen (DO) was measured *in situ* using a simple DO probe, calibrated prior to each sample run. Both the pore water of the FCWs and a water column sample were measured. In order to sample pore water successfully a 10cm porous tube was inserted into the system rhizosphere and sealed with a cap, this allowed weekly *in-situ* DO measurements. DO was measured weekly following each water sampling event.

It is well documented that pelagic planktonic algal bloom regularly exhibit pH values of around pH 9-11. One example of pH studies in relation to algal blooms is research carried out by Seitsinger (1991) who recorded pH values of 9.5 to 10.5 in an estuarine algal bloom. Therefore pH may act as simple indication of water chemistry as a result of algal bloom formation.

The samples were subjected to filtration through GF/C 1.2 µm filter paper in order to extract an algal sample as previously described in the methods of Chapter 2. Secondary filtration of the same samples though 0.45µm membrane filters was performed in order to stabilise the sample and prevent degradation. Samples were stored at 4°C until analysis.

DOC, SUVA and ion chromatography were carried out per the methods detailed in chapter 2. As described the specific ultraviolet absorbance analysis is used to describe the nature of the DOC observed in water samples. There is much conflict and debate over the use of SUVA analysis due the analysis acting as a qualitative assessment of DOC. Due to this SUVA provides a guide to the degree of aromaticity or humic content of the DOC (Traina et al. 1990). In general values above 2.0 indicate the presence of humic carbon compounds (Weishaar et al. 2003) rather than fulvic carbon compounds. Although SUVA provides relatively little information about the actual functionality of individual carbon compounds, within a mixture it may be more applicable and provide and average measure of the DOC (Weishaar et al. 2003; Miller 1999).

3.2.6 Chlorophyll Extraction for the Quantification of Algal Bloom Density

Following analysis of algal bloom development in the 480L mesocosm study, algal colonies were not added to the mesocosms, rather algal blooms developed naturally. Chlorophyll analysis was modified from the method used in chapter 2 which followed the method of Golterman & Clymo (1971). Sample preparation here was identical, however the solvent was changed from 90% acetone solution to 100% methanol solvent. Secondly the incubation period was modified from 20 hours at 4ºC to 10 minutes in a 60°C water bath. This modified protocol is outlined in (Jespersen & Christoffersen 1987) who statistically compared a number of solvents, incubation times and temperatures. No significant difference in chlorophyll concentrations were found between 90% acetone at 4ºC for 20 hours and the alternative method using a in a 60º water bath for 10 minutes. Additionally the absorption coefficient was adjusted to 13.9 to reflect the absorption coefficient of 100% methanol.

3.2.9 Water column and rhizosphere Enzyme Activity Analysis

The enzymes studied in the experiment were divided into two sub groups; phenol oxidase and hydrolases. Only hydrolase phosphate was studied, based on preliminary findings where only this enzyme was found to indicate any activity in the FCW system.

Freeman *et al,* (2003) discuss the potential for greater concentrations of phenolic breakdown products in oxygenated conditions. In the FCW experiment Phenol oxidase activity was measured and correlated against rhizosphere and water column DO concentrations. As stated in the hypotheses, it was expected that greater oxygen availability should correlate with greater phenol oxidase activity in the rhizosphere. This should therefore result in phenolic compounds and resultant suppression of algal growth.

To study the level of phenol oxidase activity, a protocol was modified from that of Pind et al. (1994). Rather than sampling rhizosphere organic matter, which is destructive to the FCW, pore water samples were taken from the rhizospheres of the FCWs. These samples were taken from the 10cm porous tube. A final destructive phenol oxidase activity assay, using substrate from the FCW systems, was performed in the final week of testing.

Three replicate samples of pore water from each treatment were used. Three blank and 3 treatment centrifuge vials were used for each sample taken from the FCWs. A volume of 0.75ml of sample was added to 1.5 ml centrifuge tubes. To which 0.75 ml of Mili-Q grade water was added in order to achieve a blank value. In the treatment centrifuge vials 0.75 ml of L-dihydroxy phenylalanine (L-DOPA) was added. The centrifuge vials were incubated at field temperature for 9 minutes and inverted occasionally before centrifugation at 300rpm for 1 minute to terminate the reaction. A volume of 300µl of each sample was transferred to a clear microplate and measured for absorbance at a wavelength of 460nm using a Molecular Devices M2e Spectramax plate-reader.

Activities were calculated by considering activity per ml of pore water. The calculations used the molar absorbance coefficient of 3-dihydroindole-5,6-quinone-2-carboxylate; 37000. Results are presented as mM 2, 3-dihydroindole-5,6-quinone-2-carboxylate/ min/ ml (Pind et al. 1994).

3.2.11 Statistical Analyses

Statistical analysis was undertaken using general linear model analysis with statistical package SPSS version 19. Testing by means of a mixed between-within subjects ANOVA, alternatively named Split Pilot ANOVA (SPANOVA) allowed statistical analysis of differences in the relationships of the treatments, and also if rates of change were statistically different with respect to time. Two factors were analysed; between subjects analysed treatment effect whilst within subjects confirmed effect over time. ANOVA analysis was also undertaken.

3.3. Results for Hypertrophic and Mesotrophic FCW Experiment

3.3.1 Water column pH

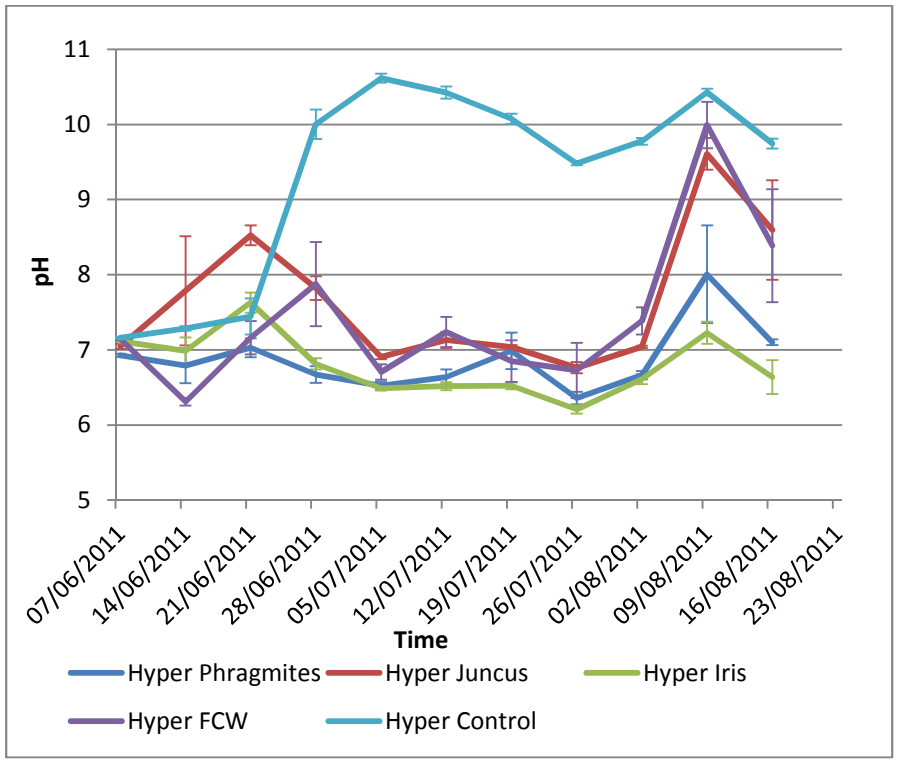


Figure 3.4 –pH over time under hypertrophic concentration.

Error bars represent standard error of the data. Hypertrophic control system can be seen to increase in pH dramatically on the 28th of June. This is linked to a rapid increase in algal density observed at the same period of time (Fig. 3.22). The graph also shows a secondary rise in pH for all treatments following nutrient replenishment on the 1st of August. A smaller increase in pH is observed in Phragmites and Iris treatments. SPANOVA analysis showed statistical difference ($p<0.05$) between pH values of the varying treatments over time.

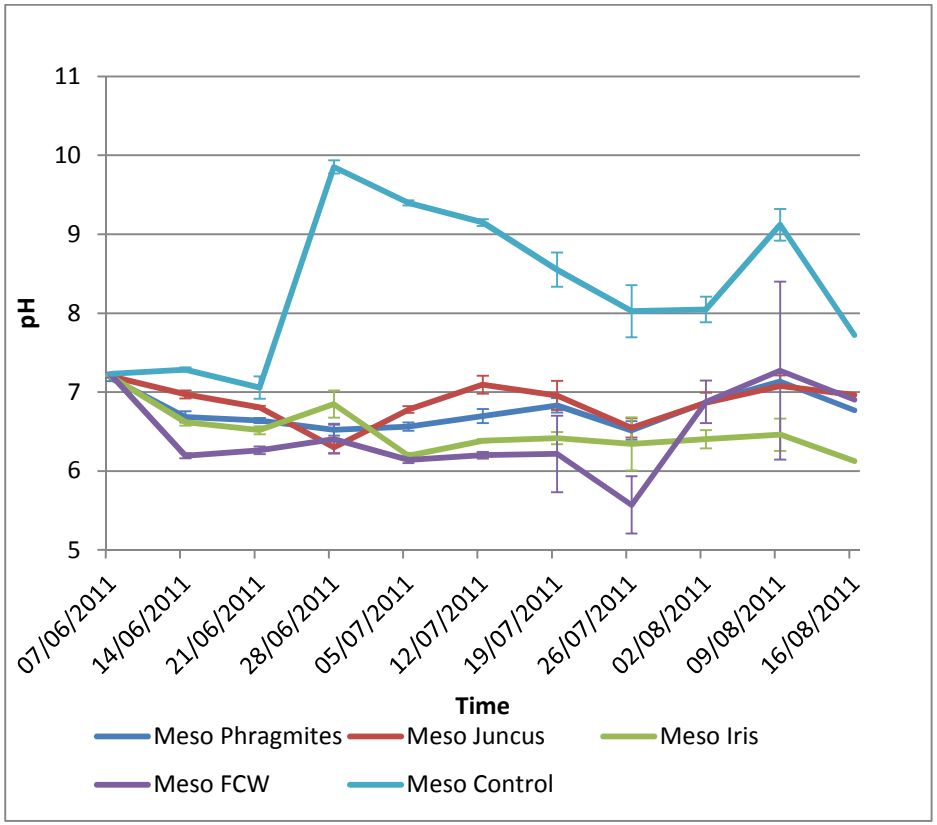


Figure 3.5 –pH over time under mesotrophic concentration.

Error bars represent standard error of the data. The control system peaks at a similar time to the hypertrophic nutrient solution. The planted FCW's also show a small amount of change in pH, but remain slightly acidic throughout the test. SPANOVA analysis for mesotrophic pH values indicating significant differences between the pH values of the different treatments over time.

3.3.2 Electrical Conductivity

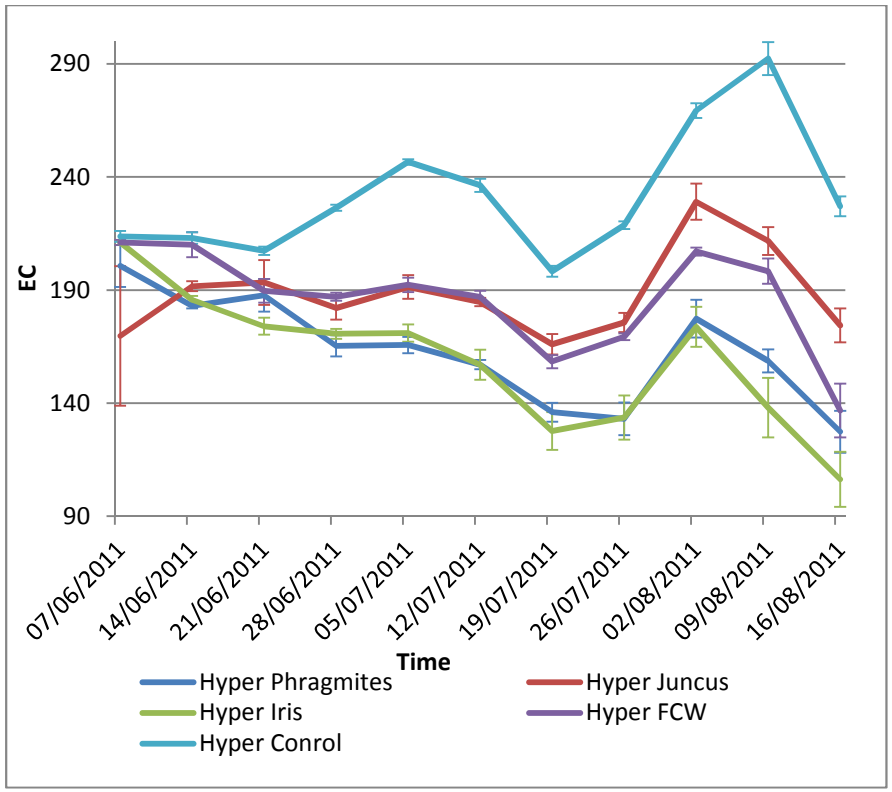


Figure 3.6 – Electrical conductivity over time under hypertrophic concentrations.

Error bars represent standard error of the data. Electrical conductivity shows a slow decrease in over time, except in the control, where electrical conductivity increases. Control treatment indicates greatest EC throughout the experimental procedure. A secondary increase in EC occurs in the time after the 1st of August following nutrient replenishment
No significant difference was shown over time response curves for SPANOVA analysis. However significant difference between treatments was detected. Visually *Phragmites* and *Iris* treatments may a form homogenous group.

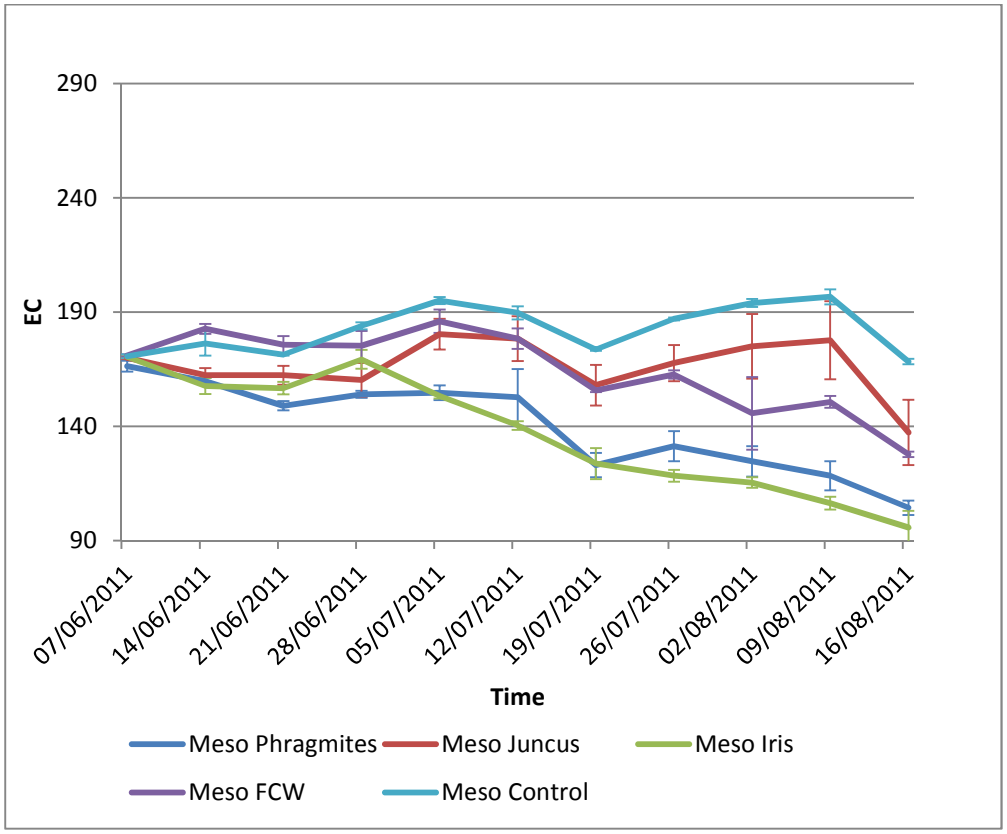


Figure 3.7 –Electrical conductivity over time under mesotrophic concentrations.

Error bars represent standard error of the data. Containers showed 30 to 40 units lower values than hypertrophic EC. Lowest EC is recorded in *Iris* treatments, decreasing from 165 to 96 µ Sieverts. Following nutrient replenishment a rise in EC is observed in control, FCW and *Juncus* treatments. SPANOVA analysis for mesotrophic EC showed no significant difference within subjects over time. However, significant difference between treatments was detected; treatments are observed in the same order as hypertrophic treatments.

3.3.3 Phenolic Compound Concentrations

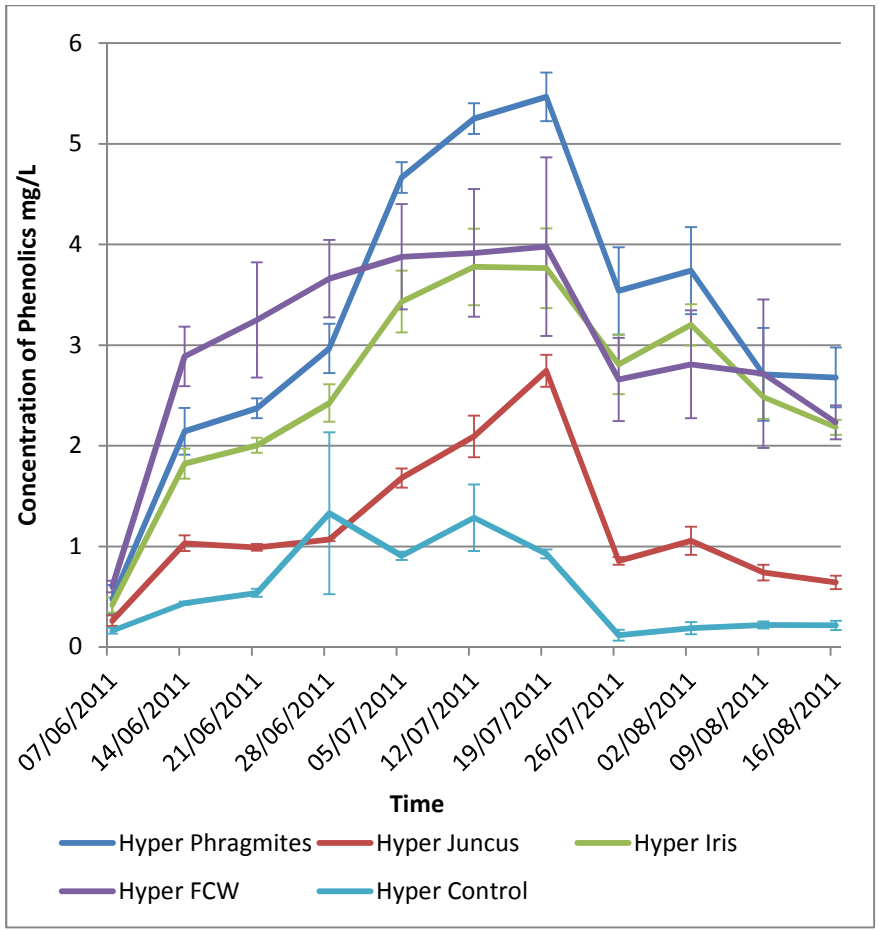


Figure 3.8 – Phenolics over time under hypertrophic concentration.

Error bars represent standard error of the data. Greater concentrations of phenolics were detected in *Iris*, *Phragmites* and the FCW control system. Lower concentrations of phenolics were observed in the *Juncus*. Yet phenolics presence is detected in control treatments. SPANOVA indicated significant differences over time and also between treatments.

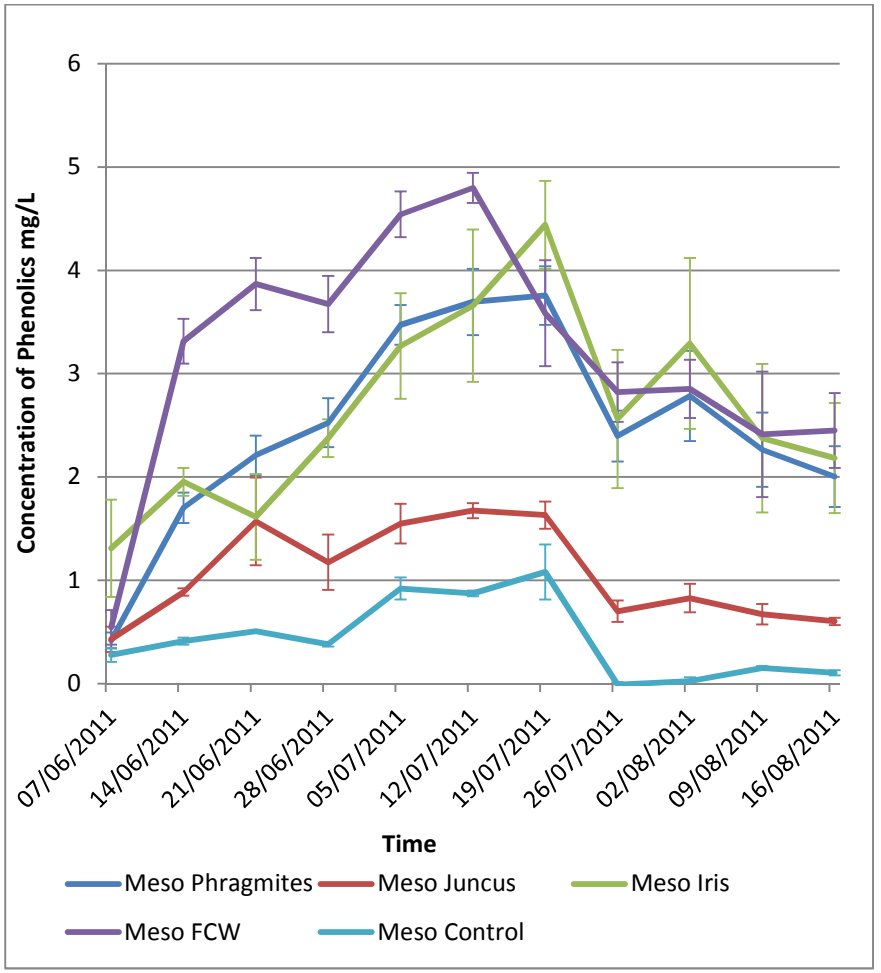


Figure 3.9 – Phenolics over time under mesotrophic concentration.

Error bars represent standard error of the data. Phenolics in all treatments indicate similar concentrations as hypertrophic mesocosms. FCW control released the greatest concentration of phenolics during the primary stages of the experiment. Lowest phenolic concentration was detected in control, similar to figure 3.8. SPANOVA analysis indicates significant differences between treatments over time, ($p=0.018$). Significant difference between treatment types was also detected.

3.3.4 Dissolved Organic Carbon

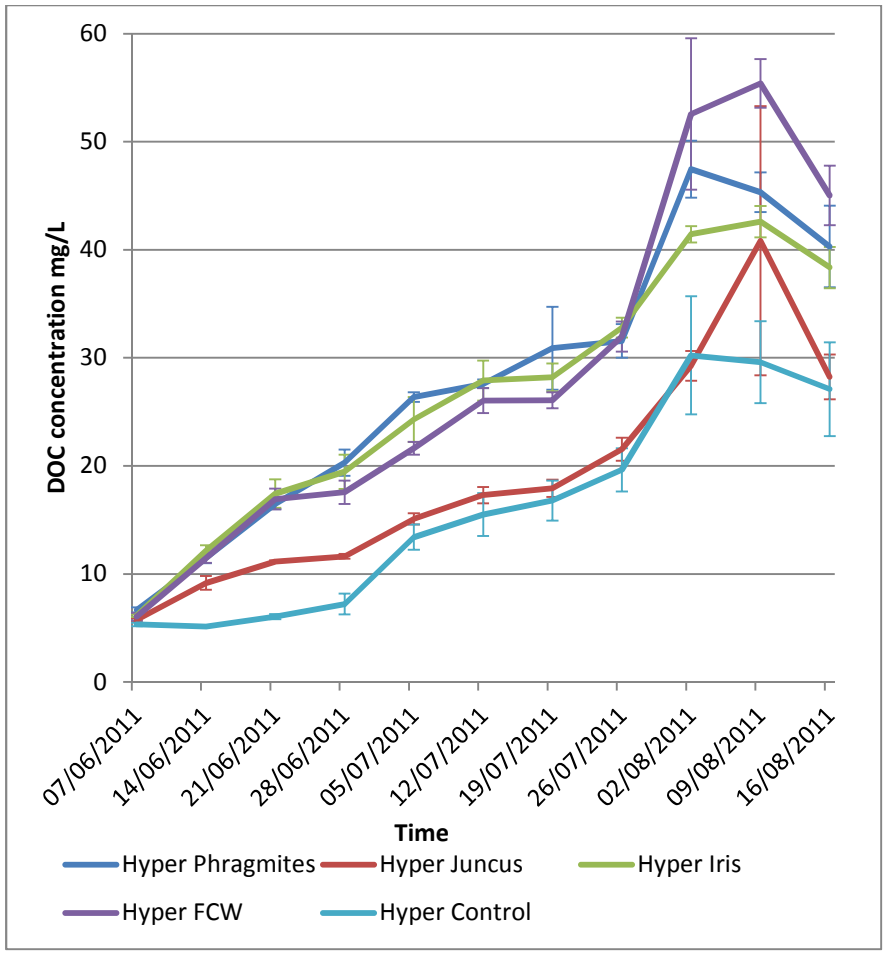


Figure 3.10 – DOC over time under hypertrophic concentration.

Error bars represent standard error of the data. The graph indicates an increase in DOC over time. Control and Juncus treatments indicate very similar and consistently lower concentrations of DOC throughout the experiment. SPANOVA analysis indicated statistically significant between treatments ($p<0.001$).

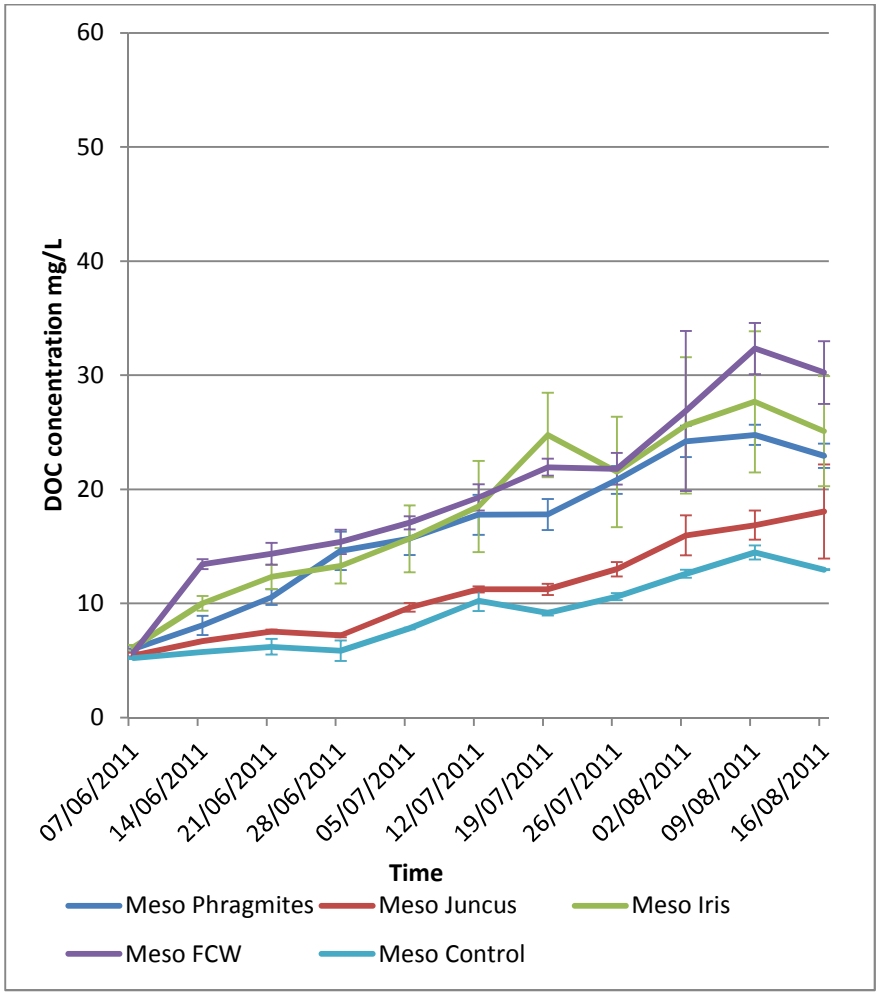


Figure 3.11 – DOC over time under mesotrophic concentration.

Error bars represent standard error of the data. Similar relationships are observed in DOC release over time between mesotrophic and hypertrophic systems. However, the concentrations of DOC observed in the mesotrophic systems are only 50% of that observed in the hypertrophic systems. SPANOVA analysis indicated significant differences over time and also between treatments.

3.3.5 SUVA 254nm Analysis

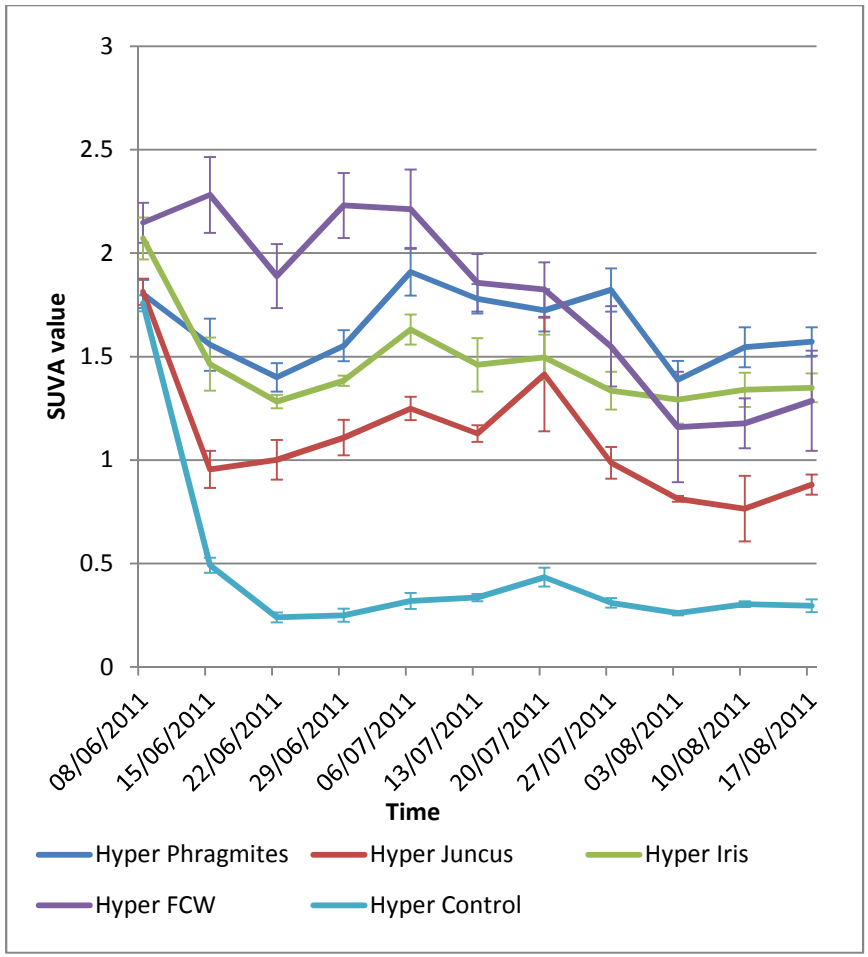


Figure 3.12 - SUVA 254 over time under hypertrophic concentration.

Error bars represent standard error of the data. All treatments rapidly stabilise by 22/06/2011. All systems except the control exhibit similar SUVA values. Control treatment mesocosms exhibited SUVA of <0.5 throughout the experiment

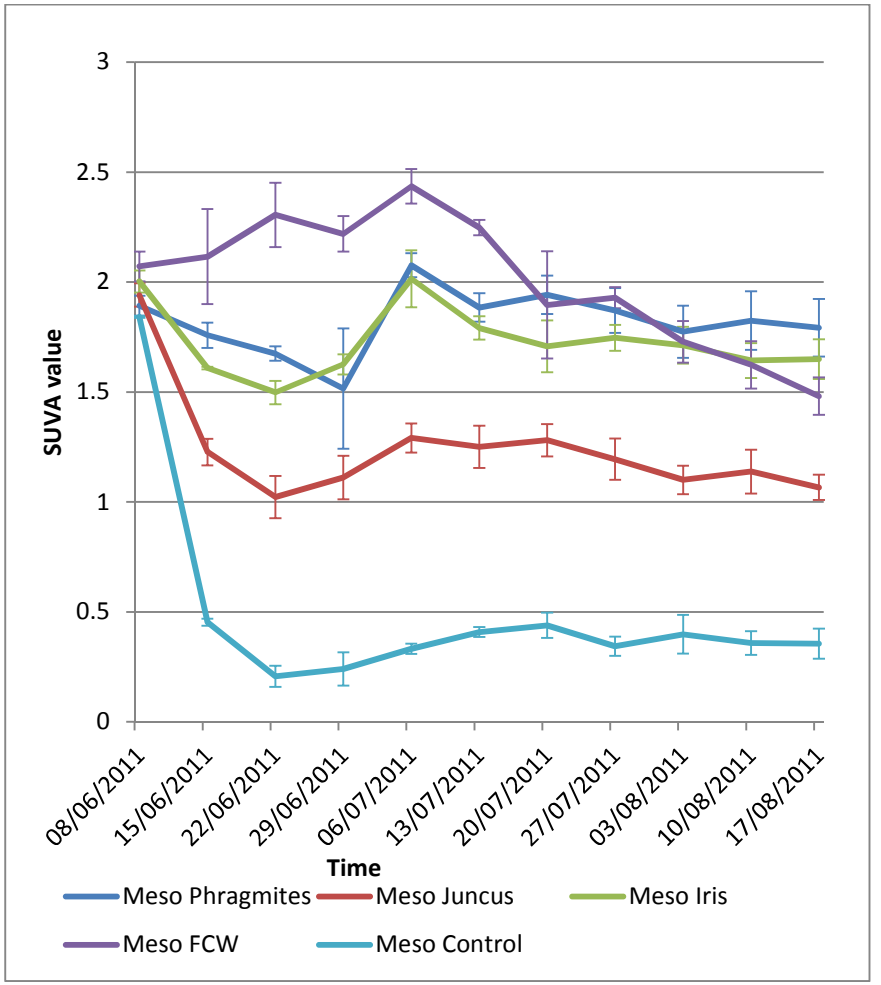


Figure 3.13 - SUVA 254 over time under mesotrophic concentration.

Error bars represent standard error of the data. Similar relationship in SUVA is shown over time. Stabilisation occurs rapidly, Phragmites and Iris indicate slightly greater SUVA values as compared to Figure 3.12. Differences between Juncus and the other treatments is more pronounced in the mesotrophic mesocosms as compared to hypertrophy

3.3.6.1 Phosphate

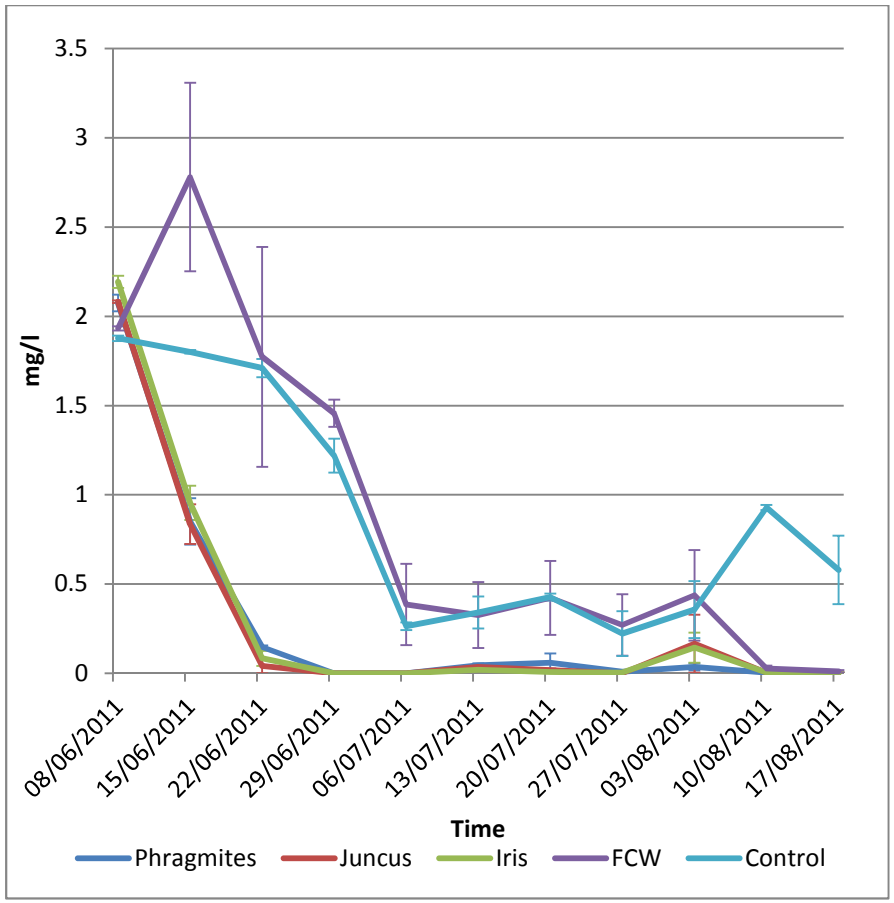


Figure 3.14 – Phosphate over time under hypertrophic concentration.

Error bars represent standard error of the data. Planted treatments show a rapid reduction in concentration to virtually nil within two weeks of start-up. The control treatments both indicate a lesser reduction gradient. SPANOVA analysis shows significance in phosphorus for the treatments over time and between treatments. The effect size between the treatments was large; with a strong level of significance found between the treatments. ANOVA analysis in week 3 indicates that both control types form homogenous sub sets separate from any of the planted treatment.

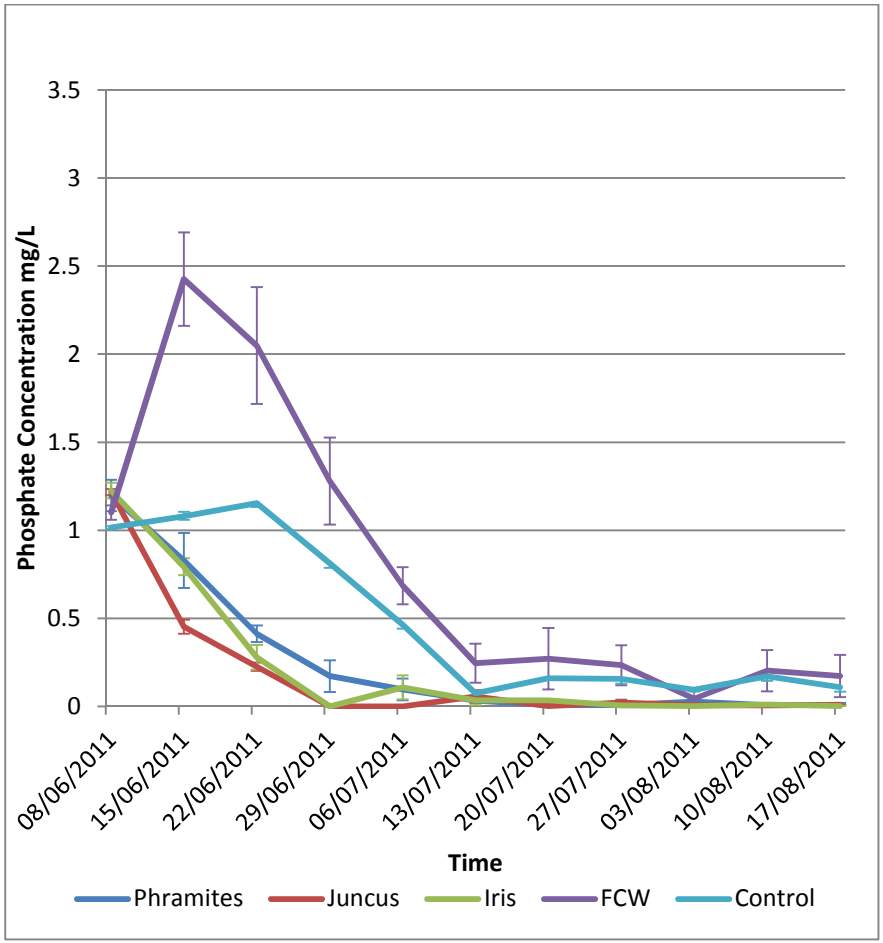


Figure 3.15 – Phosphate over time under mesotrophic concentration.

Error bars represent standard error of the data. Concentration of phosphate at start-up indicate 50% lower concentrations, however similar concentration dynamics occur. The FCW control rapidly becomes a source of phosphorus, which then declines. SPANOVA analysis does not indicate statistical significance over time however significance between treatments is detected.

3.3.6.2.1Nitrite

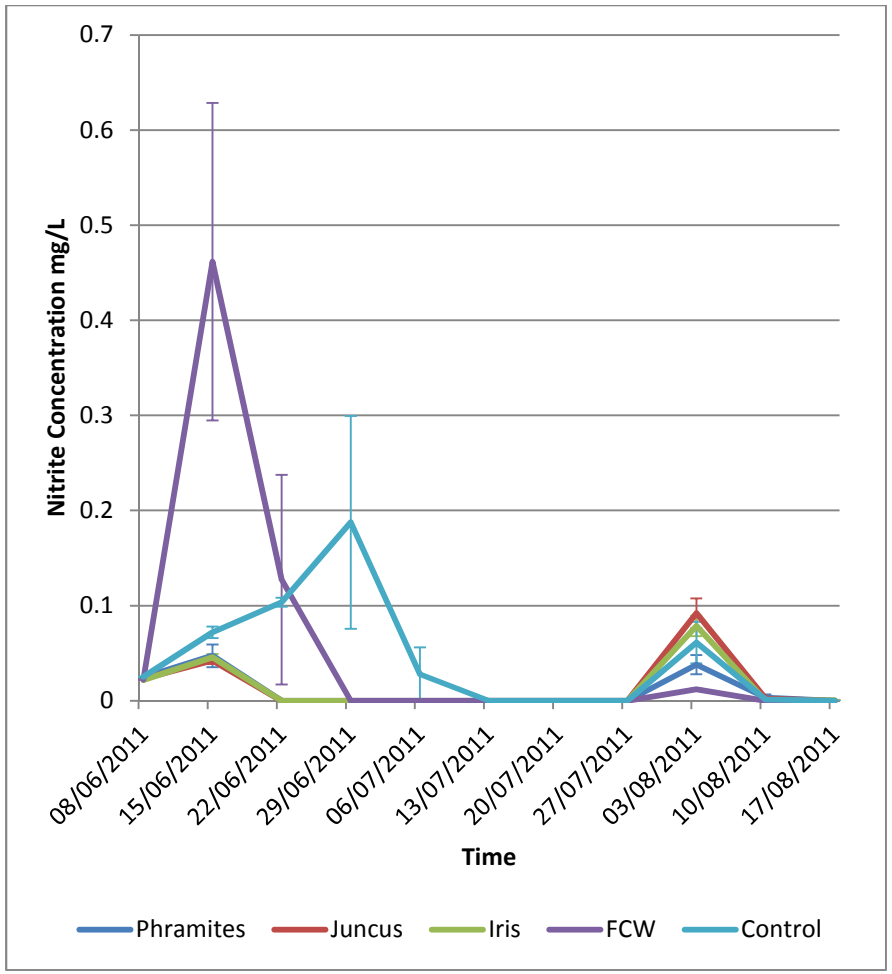


Figure 3.16 – Nitrite over time under hypertrophic concentration.

Error bars represent standard error of the data. Initial peaks in nitrite are observed in both the controls, particularly in the unplanted FCW system. Following nutrient replenishment a pulse of nitrite is observed in all treatments indicating Juncus and FCW as greatest and least effect, respectively. SPANOVA analysis indicated significance between treatments and treatments over time

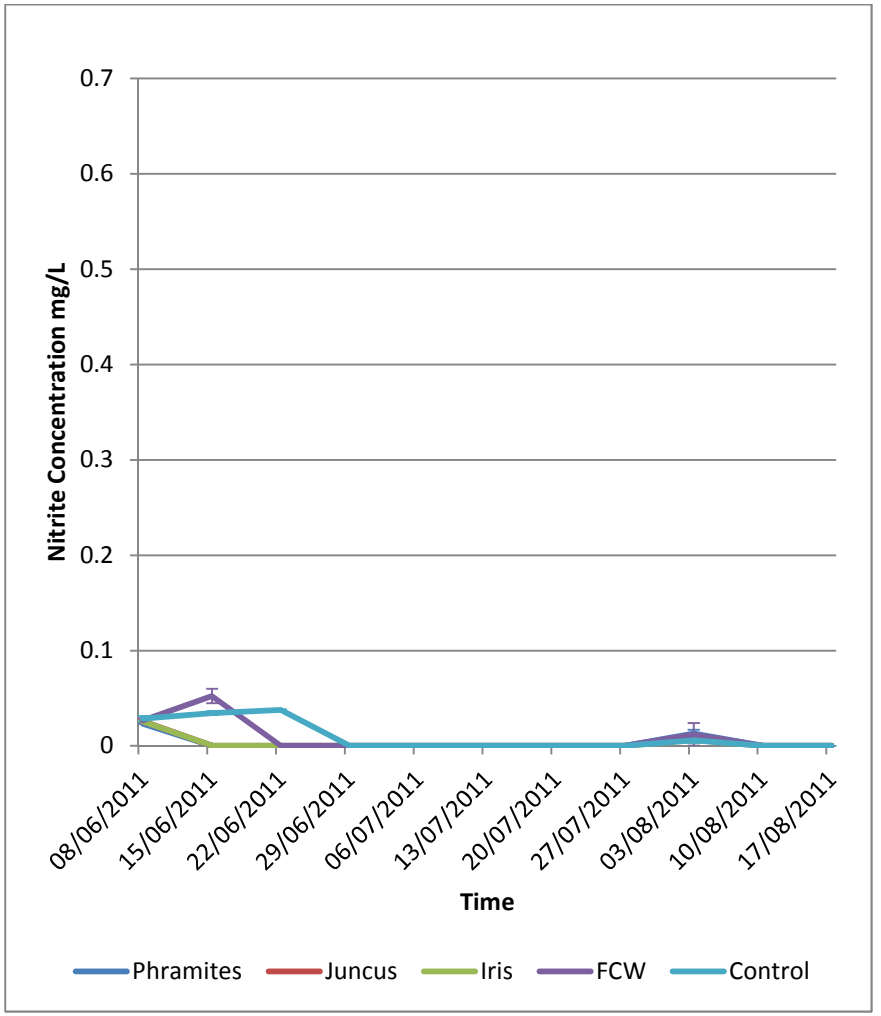


Figure 3.17 – Nitrite over time under mesotrophic concentration.

Error bars represent standard error of the data. Concentrations are initially one tenth of the concentrations observed in hypertrophic testing. Similarly, the nitrite shows a comparable removal pattern, where both control treatments lag. SPANOVA analysis indicated significance between treatments and treatments over time

3.3.6.3 Nitrate

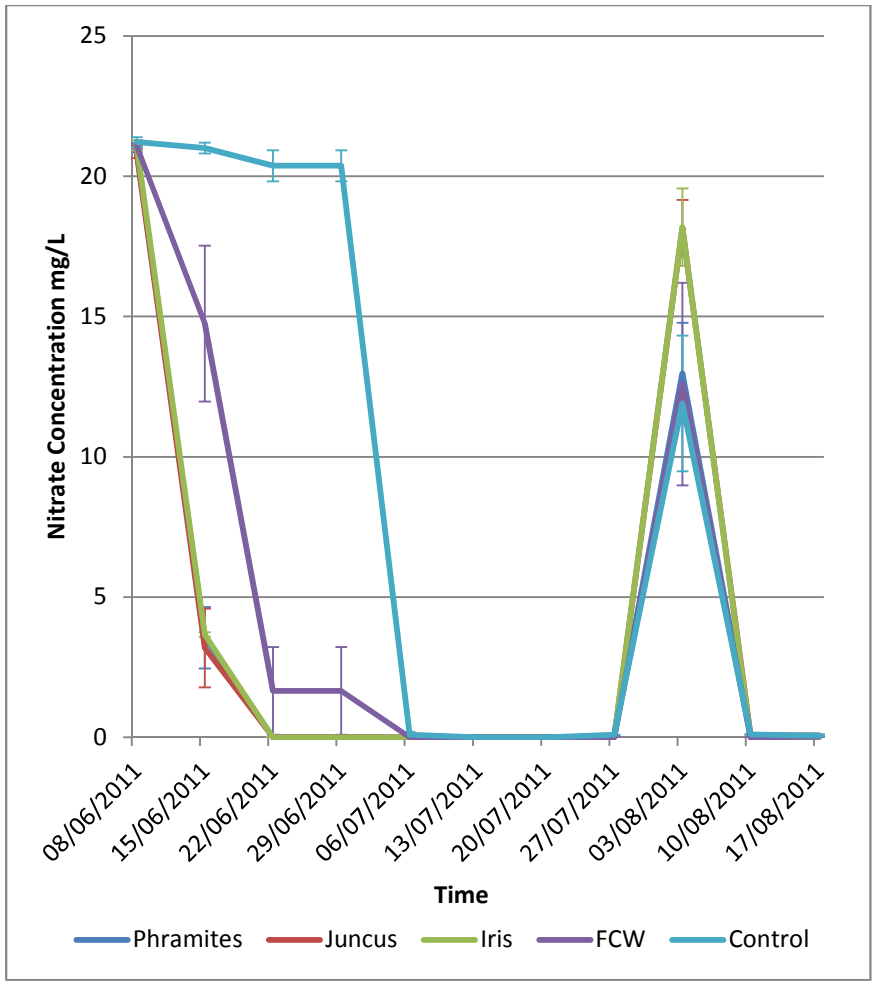


Figure 3.18 – Nitrate over time under hypertrophic concentration.

Error bars represent standard error of the data. The graph indicates varying rates concentration decrease with treatment type. Rapid removal is observed in the planted treatments. FCW and control treatments achieve similar low concentration 2 weeks after planted treatments. SPANOVA analysis indicated significant differences between treatments and over time.

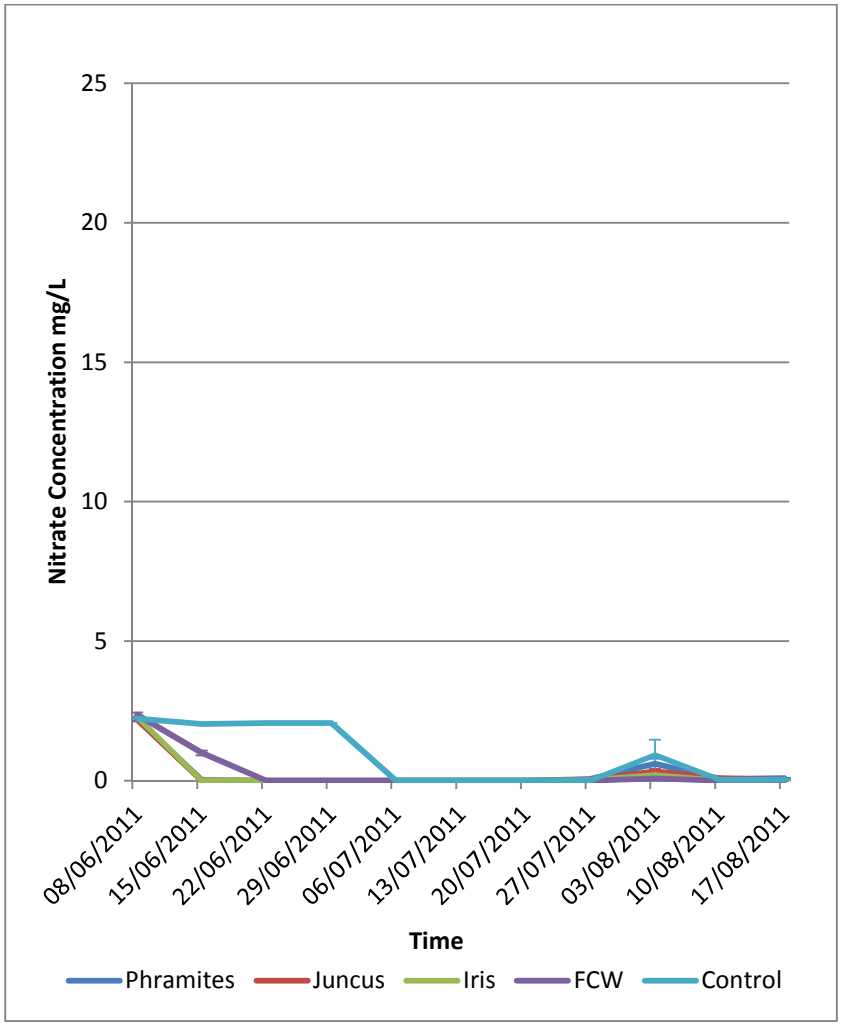


Figure 3.19 - Nitrate over time under mesotrophic concentration.

Error bars represent standard error of the data. A very similar relationship between treatment type and nitrate removal is observed. Notably, the concentrations recorded are one tenth of the concentration observed in the hypertrophic systems. SPANOVA analysis indicated significance between treatments and over time.

3.3.7 Dissolved Oxygen

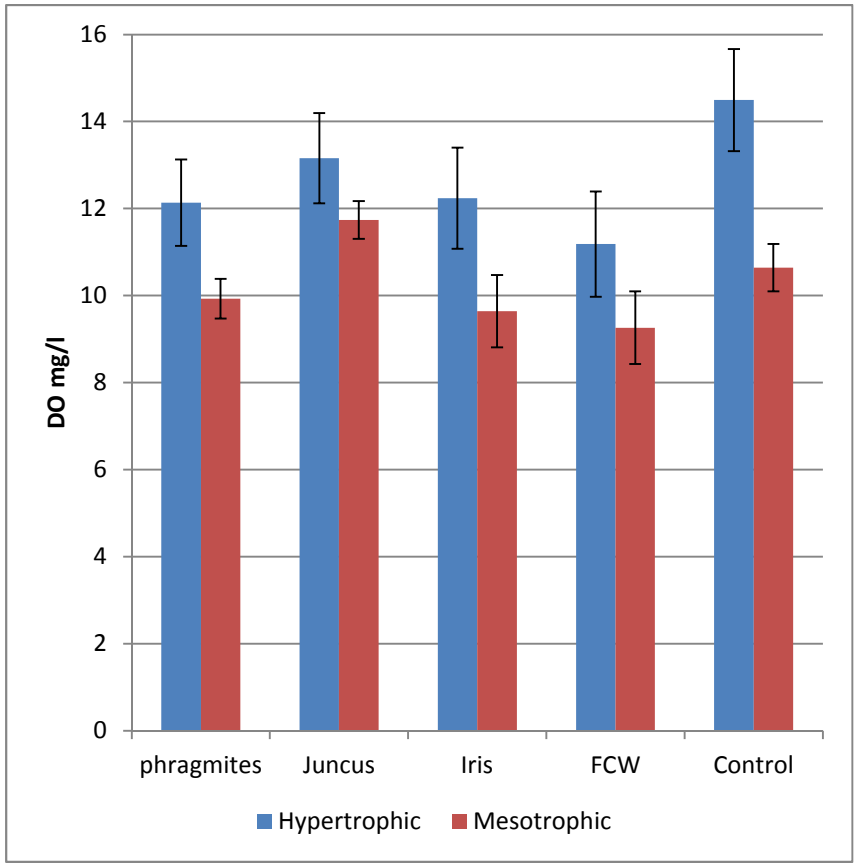


Figure 3.20 - Water column DO, treatments averaged for the duration of the experiment.

Error bars represent standard error of the data. The bar graph indicates that DO concentrations are consistently higher in hypertrophic treatments. ANOVA indicated significant difference in DO between trophic level treatments. Analysing the trophic level treatments separately showed that a significant difference also lies with the FCW treatment exhibiting significantly lower DO.

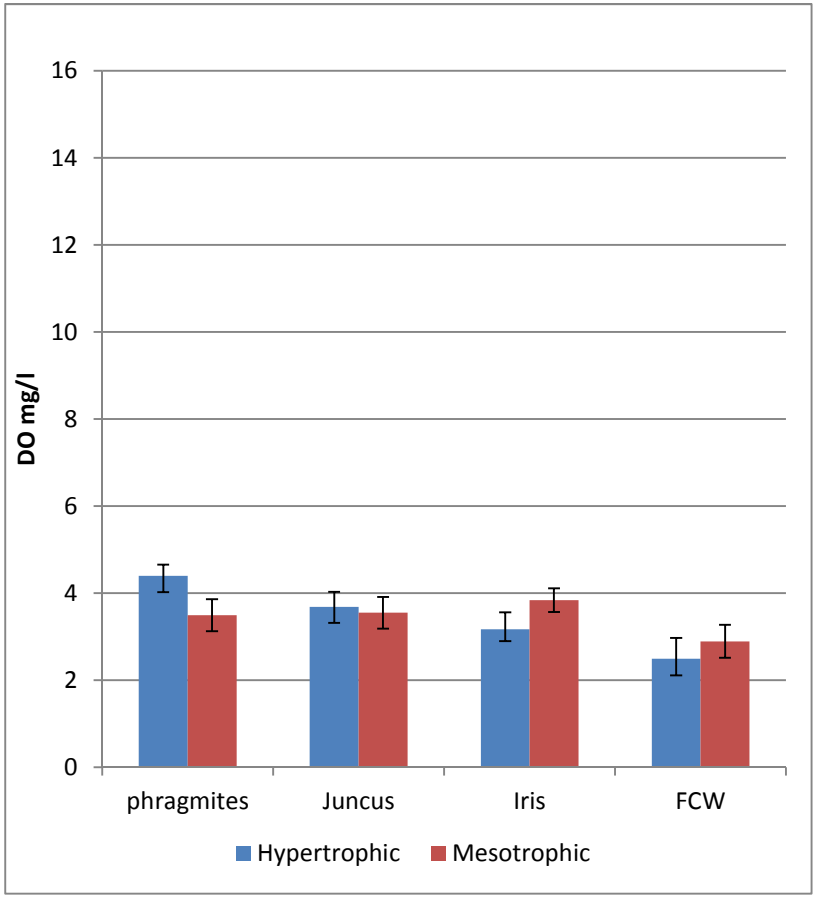


Figure 3.21 - Pore Water DO, treatments averaged for the duration of the experiment.

Error bars represent standard error of the data. Hypertrophic pore water dissolved oxygen was shown to have a statistically significant difference between treatments. Conversely, no significant difference was observed in pore water DO concentration between treatments at the mesotrophic nutrient level. Note – No pore water sample for Control

3.3.8 Chlorophyll-a Concentration Inferring Algal Bloom Density

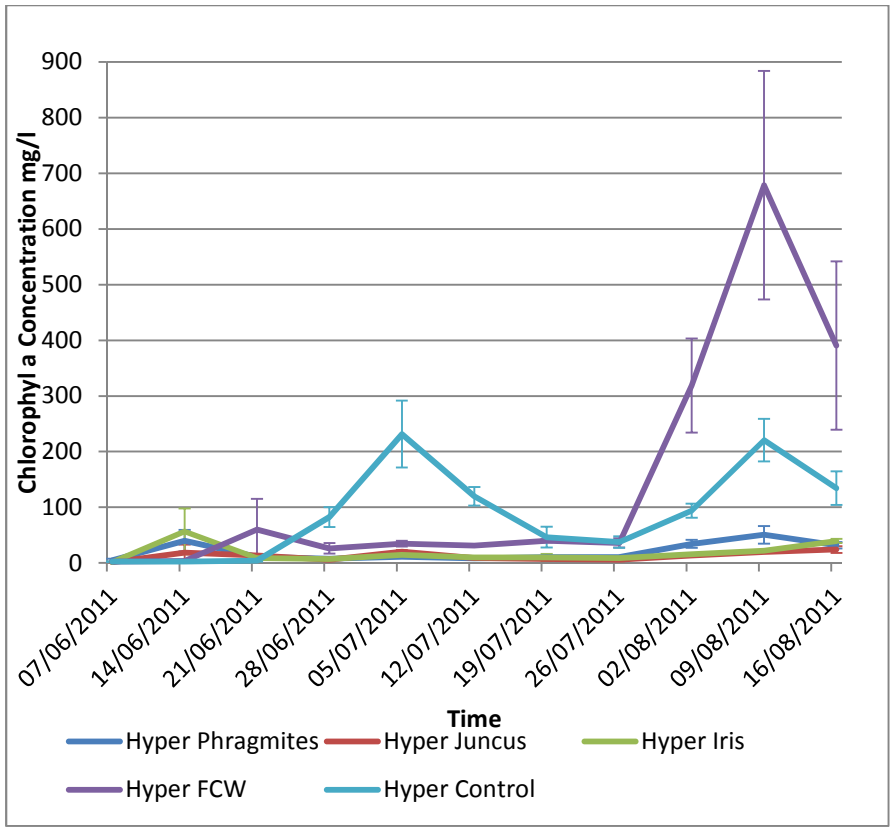


Figure 3.22 – Chlorophyll-a over time under hypertrophic concentration.

Error bars represent standard error of the data. Both the FCW and control show peaks where chlorophyll A is above 200 mg/l. The control shows a peak at 05/07/2011 and second at 09/08/2011 following nutrient replenishment. Following both of these peaks senescence of algae is observed indicated by the decrease in chlorophyll a concentration. The FCW control however only shows one large increase in chlorophyll-a concentration following the second nutrient addition reaching a maximum approximately 700 µg/l. All of the treatment systems containing planted macrophytes maintained low concentrations of Chlorophyll A throughout the duration of the experiment, never increasing above 45 µg/l. SPANOVA analysis showed significance over time and between treatments

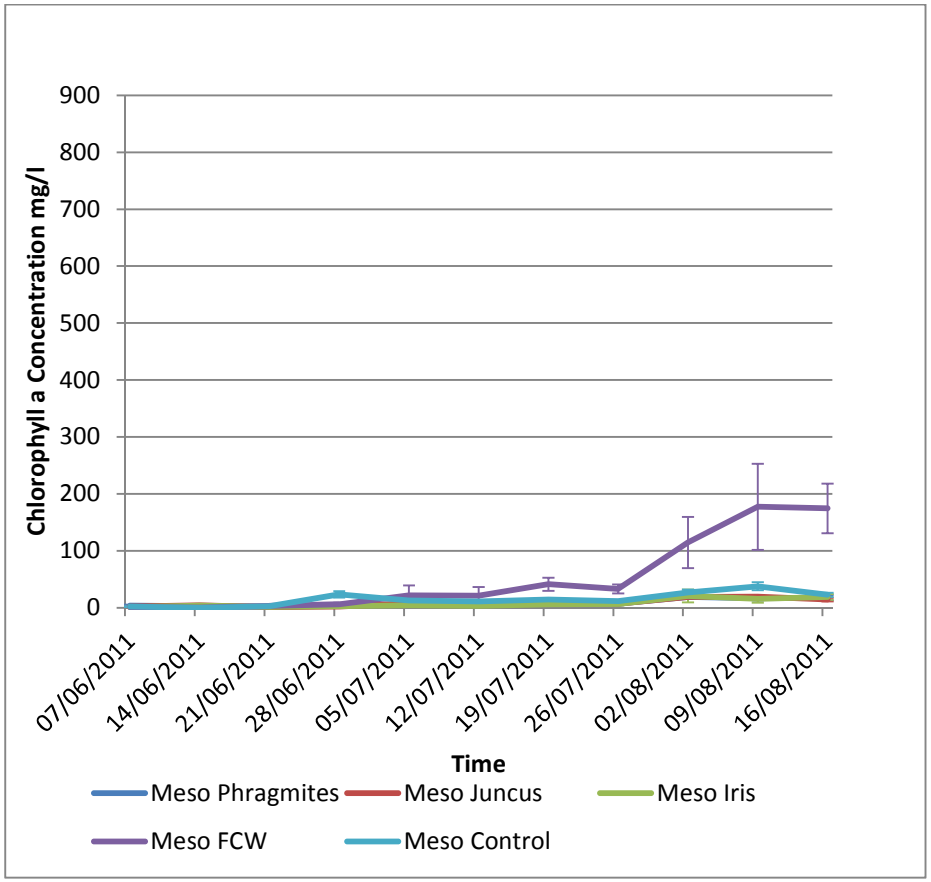


Figure 3.23 – Chlorophyll-a over time under mesotrophic concentration.

Error bars represent standard error of the data. Similar to the hypertrophic systems the FCW treatment showed a distinct rise in Chlorophyll-a concentration on 26/07/2011 following nutrient replenishment, whilst relatively low concentrations were observed in the planted systems throughout the test. SPANOVA analysis indicated significance between treatments

3.3.9 Phenol Oxidase Activity

Time	Enzyme activity µM / min/ ml	Correlation with DO Pore water (R^2)	Correlation with DO water column (R^2)
week 0	0.88	0.096	0.0159
week 5	1.29	0.0017	0.0021
week 11	2.37	0.0158	0.0052
week 11 soil	5.63	0.0128	0.0072

Figure 3.24 – phenol oxidase activity recorded in the pore water of the FCWs.

Week 11 soil indicated destructive test on FCW organic matter.

3.4. Discussion

3.4.1 Basic Hydrochemistry – pH, EC

Hypertrophic pH values followed trends as described by Seitzinger (1991). pH rose dramatically in the control treatment peaking at around 10.7. Seitzinger (1991) observed values of 9.5 to 10.5 in an algal bloom; however this may have been related to the benthic phosphorus release and associated high pH values rather than the algal bloom causing the pH rise; however benthos was used in the experiment. Phytoplanktonic species grow by assimilating carbon into their biomass (Schippers et al. 2004). This process is an important component in all plant life, including pelagic algae associated with eutrophication. As algae assimilates carbon and is converted to biomass, the concentration of CO_2 in the water column decreases. Dissolved CO_2 contributes to the acidic balance of water chemistry (Willoughby 1976), as this is removed and bound within the algal cells, pH increases. This is likely to be the main driver for pH increases in the FCW mesocosm experiment. Although nutrient concentrations in the mesotrophic experiment were lower, similar results were found, typically between 9.5 and 10. However, planted treatments pH levels remained relatively stable around 6.5. This baseline pH was not observed to the same degree in the hypertrophic treatments, here greater fluctuations were observed. Secondly, following the nutrient replenishment on the 1st of August, pH dramatically rose to similar levels as the control in both the *Phragmites* and the *Juncus* tests. This may indicate the presence of a CO_2 consuming algal bloom formation in the later stages. Although there were variations in pH for all of the tests, the statistical analysis confirms that when plants or anti-algal phenolic releasing substances are not present, pH can be observed to dramatically increase, as explained above.

EC provides an indication of total dissolved ions. EC values exhibited in the hypertrophic and mesotrophic treatments are initially around 200 and 170 µs respectively (Fig 3.6 and 3.7).

This indicated that the hypertrophic system did indeed have a greater charge carrying capability suggesting higher concentrations of nutrient pollutants in solution. This is as expected, due to the dissolved nutrient concentration manipulation. If uptake or removal of nutrient pollutants such as nitrogen and phosphorus based compounds was occurring, it would be expected that EC declines over time. Therefore the rate at which EC is depleted may provide indications into the rate at which the nutrient ions are being taken up or removed from the water column. The EC of the control treatment was distinct compared with other treatments used. A significant increase in EC was observed following the addition of nutrient solution on the 1st of August however a general decrease in EC was observed in both hypertrophic and mesotrophic systems.

Nutrient pollutants mobility in the mesocosms was unrestricted between the water column and the organic material rhizosphere of the FCW. Further experimentation would need to be undertaken to confirm the fate of nutrients in the system, in particular biomass accumulation. Sasser et al. (1996) (in Headley and Tanner, 2006) found higher pollutant concentrations in natural floating wetland root and sediment mats than in the surrounding water column. Pollutants were also more concentrated in floating mat root zones than in natural marginal marshes, where the macrophytes were rooted into the sediments. Headley and Tanner (2006) suggested that this could be due to the amount of interaction the roots of the macrophytes in the FCW have with the water. In other treatment wetlands, especially free water surface systems pollutants have to diffuse into the benthic sediments before the plants have access to them. In these systems, most of the removal occurs due to the interaction of the pollutants with attached microbial communities called biofilms. In floating systems both the roots and the biofilm have more direct access to the compounds in the water column.

Although this may be true, Sasser et al. (1996) found that the nutrient compounds and within the vicinity of the floating mat was increased. However this was attributed to underlying benthic stratigraphy of the sludges and organic substances that collect beneath the floating mats. This is similar to the results Seitzinger (1991) observed in relation to the formation of algal blooms, where benthic sediments present in the water body were found to release nutrients into the water column.

Although these mechanisms could explain the decrease in EC over time, in this instance it is more likely that nutrients are being removed and bound in the FCW. This is because no sludge or benthic sediments were added to the treatments, nor were significant quantities formed during the experiment. However, it is important to note that EC can be used only as an indicator, similar to the total dissolved solid (TDS) measurement. This allows us to conclude that the presence of plants, in combination with the FCW system are involved in the

removal of ions from the water column. Furthermore, *Iris* and *Phragmites* apparently reduce the ions in the water column to the greatest degree.

As mentioned in Methods, the *Juncus* FCW's were constructed a number of months prior to the installation and start-up of the experiment. This results in a lessened removal effect when analysing EC. One explanation for this could be the increased root and shoot development in *Iris* and *Phragmites* in the early stages of the testing, resulting in an increased need for uptake of nutrient ions, leading to a more dramatic decrease in EC over time.

Although the statistical analysis showed limited significance in the hypertrophic treatment systems, over time a general decrease in EC was observed (Fig 3.6). The effect was made less pronounced due to the replenishment of nutrient ions on the 1st of August. The SPANOVA also did not detect a difference between treatments. As described in the results, if post-hock testing were valid, it may be possible that homogenous sub sets would be found. This is certainly supported graphically.

Similar results were found in the mesotrophic systems, however significant differences between the treatments were observed. Change in EC over time was similar in all treatments; however variability between treatments was found. This is likely to be induced by the greater drop in EC observed in the *Iris* and *Phragmites* mesotrophic systems over time with particular reference to the final stages.

3.4.2 Anti-algal Compound Formation – Phenolics, DOC and Characterisation of Properties

Phenolic release was hypothesised to act as a possible method for algal control due to inhibition of algal growth thereby acting as a natural algaecide (Pillinger et al. 1994). During the first 7 weeks of the experiment, water column phenolics concentration increased in all treatments, however the increases were observed to occur at different rates.

The control systems show a small increase in phenolics over time. These are likely to be phenolics generated by the algae rather than released from organic material. This occurs as a result of exudate released from the algal cells (Willoughby 1976). This is confirmed later with SUVA analysis which enables understanding of the molecular weight and humic to fulvic ratios of the phenolics.

In both hypertrophic and mesotrophic treatments, *Juncus* and control treatments indicated significantly lower phenolic content than the other treatments. The *Juncus* had been established for a period of time when most labile DOC forms were lost. Here DOC and phenolics leached from the rhizosphere during the stabilisation period. This indicates that

long-term phenol released from FCWs will reduce but remain effective during the lifespan of the system.

Production of phenolics was observed in control treatments, likely to be due algal cell exudate production. Vasconcelos & Leal (2008) discuss exudates of marine algae and cyanobacteria and the potential for such exudates to act as allelopathic substances contributing to the chemical environment around the producer. Exudates are a means of protecting the cells against external causes of stress, for example extreme pH. Such substances affect and influence the development, and in some instances growth, of other organisms. These exudates contain carbon and are likely to contain small amounts of phenolic compounds.

Furthermore, algal control and bloom prevention may be gained as a result of the macrophyte root exudate. Hypotheses stated phenol oxidase activity would correlate with ROL from arenchyma tissues, and resultant phenolics in the water column. However, if we forgo phenol oxidase activity; differences in water column phenolic content may be due to the composition of macrophyte root exudates.

Considerable evidence supports differences in chemical composition and concentration of plant root exudates. Larue et al. (2010) describe how *Iris, Typha* and *Phragmites* exhibit varying concentrations of intracellular root tissue phenolics. This is especially evident in *Iris* during the spring, where intracellular phenolic concentrations are found to be in excess of 10 times greater than in *Phragmites*. During this season *Iris* is producing large amounts of root and shoots biomass and resultant root exudate.

In hypertrophic planted systems greater water column phenolic concentrations were observed in *Phragmites* compared to other species. Under mesotrophy *Iris* was marginally more concentrated than *Phragmites.* All rhizosphere organic substrates used in the experiment were equal, and although the nutrient concentrations vary significantly between hypertrophic and mesotrophic treatments there was little difference in observed phenolics between the two trophic levels. FCW also exhibited high phenolics, particularly in the initial phase of the experiment.

Statistical analysis showed that there were significant differences in phenolic concentrations between species and also over time for both nutrient regimes. One of the limitations of SPANOVA is that differentiation between homogenous sub sets within the data cannot be made. Although this limitation applies, the results from the secondary control (unplanted FCW) were unexpected. During long periods of the testing the FCW treatment exhibits high levels of phenolics. Various reasons for this are proposed. Plant nutrient assimilation in FCW

does not occur due to the lack of plants. This leads to increased potential for algal bloom formation and subsequent increased algal phenolic production. Similarly, changes in biological or chemical oxygen demand within the rhizosphere potentially lead to anaerobic decomposition of organic matter. Carbon is a key constituent driving denitrification and is being used up more easily in systems where plants are present. These issues also link into other parameters measured.

Phenolic compounds of any description are based around an aromatic carbon ring and are classed as a component of the total DOC pool in the water columns and rhizospheres of the FCW mesocosms. DOC production is of importance due to system processes reliant on its presence such as microbial driven denitrification. Although the release of DOC is generally seen as a negative impact in terms of the risk of formation of trihalomethane by-products in drinking water (Gallard & Von Gunten 2002) or the problems associated with the release of recalcitrant carbon stores from peat (Freeman et al. 2001). The type of DOC released from the FCWs should have a lesser effect due to the labile nature of the carbon released. Much of the carbon released from the FCWs is in the form of phenolics which are also generally short lived (Welch et al. 1990) and readily broken down into compounds such as quinones.

In both trophic systems an approximate linear increase in DOC is observed. Similarly a pulse of DOC production is observed following nutrient replenishment on the 1st of August. Nutrient influx may have increased macrophyte biological processing increasing DOC or exudate production.

Smith & Kalin (2000)(in Headley et al. 2006) report that DOC could be released from the rhizosphere of FCWs in significant, and biologically useful, quantities. DOC and particularly particulate organic matter released from such systems could be enough to act as a source of carbon for biomineralisation of metal pollutants. In these tests, DOC production may be a product of available nutrients in the water column following nutrient replenishment, this is indicated by the doubled DOC values in the hypertrophic tests.

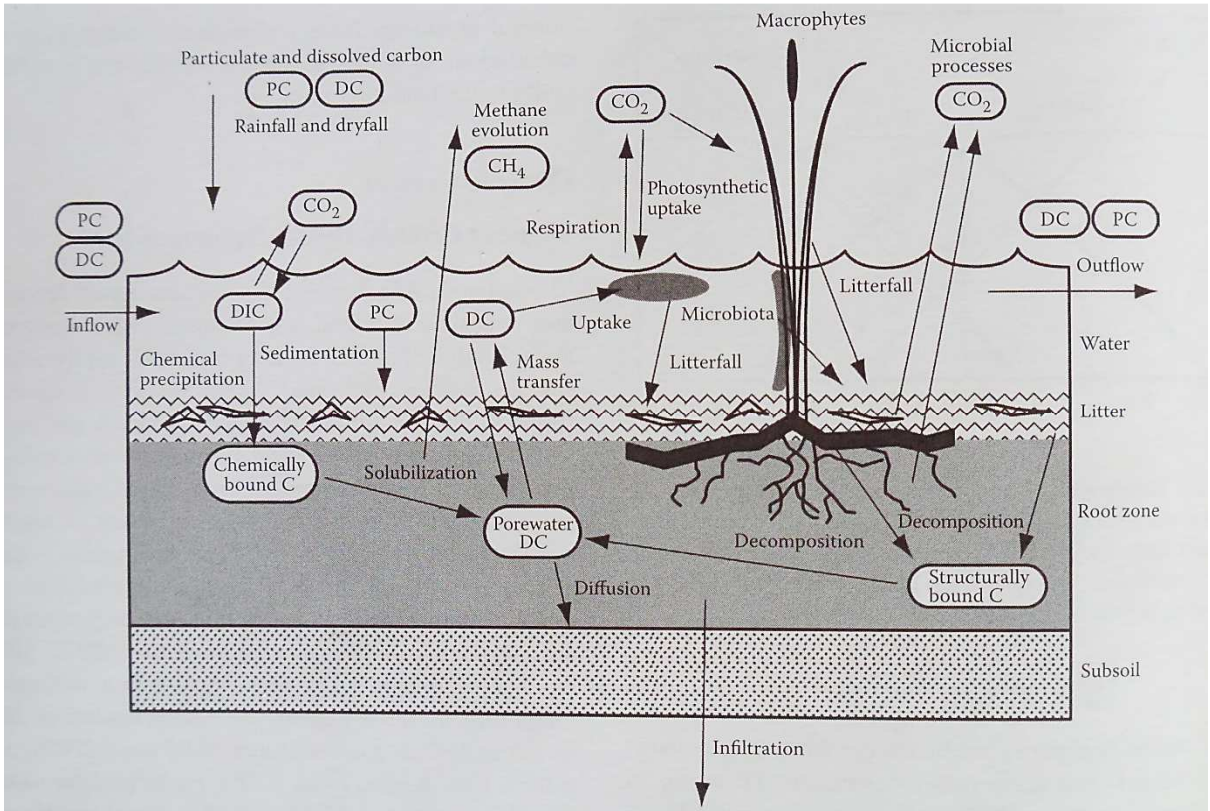


Figure 3.25 - Schematic diagram of Carbon storages and transformations in wetland systems (Kadlec & Wallace 2008)

Kadlec & Wallace (2008) provide a simplified schematic diagram (fig 3.25) of the main processes occurring in a treatment wetland that contribute to the rhizosphere and pore water DOC pool. The processes included are solubilisation of chemically bound carbon and the decomposition of structurally bound carbon. Here there is no reference to the component of the carbon pool produced or removed by the plants, however, Koretsky & Miller (2008) report that total organic carbon is typically higher in un-vegetated sites. This was found to be the case in the rhizosphere of an estuarine species of the *Juncus* genus. Similar results were observed in both the nutrient regimes of the FCW system (Fig 3.10 and 3.11), although this result was not significant.

Gaseous nitrogen species analysis may provide confirmation that the denitrification process was occurring at a greater rate within the planted systems. This process relies on microbial colonisation by denitrifiers and readily available carbon source (Kadlec & Wallace 2008). As plants are known to release carbon based compounds from their root tissues thereby modifying the environment in which they grow, increased denitrification may occur as a result of increased available DOC in the water column.

3.4.3 Nutrient Pollutant Biochemical Cycling – Phosphorus and Nitrogenous Compounds

Phosphate is predominantly a limiting factor governing primary production during eutrophication events in freshwater bodies. In both the nutrient regimes a release of phosphorus was detected in early stages in the unplanted FCW system. It is likely organic material is acting as a source of phosphorus, leaching into the water column. This leads to inferences about the total removal capability in the planted systems. Planted systems reduced the phosphate added to the systems at the start, as well as the phosphorus leached by the organic material as observed in the FCW treatments.

Nutrient concentrations in the containers were originally designed to achieve nutrient values characterised by hypertrophy and mesotrophy. Although the nutrient concentrations did not comply with concentrations reported in the literature (Wetzel 2001; Smith et al. 1999; Nürnberg 1996; Dodds et al. 1998) algal bloom formation did occur. Variation in trophic levels was achieved and resulted in differences in water quality parameters observed.

Whilst mesotrophic phosphate reduction reached extreme low levels by 22/06/2011 in the planted systems, the removal rate in the hypertrophic system was more rapid. This relationship can be explained by a method developed by Kadlec and Wallace (2009) for the design and scaling of CWs for water pollution control. The authors developed a method utilising the P-k-C* equation. This method relies on knowledge of hydraulic efficiency within the CW and evenness of mixing referred to as the Tanks in Series model known by the parameter P, temperature driven compound degradation rates, k, and importantly wetland background concentration given as C* which is given as a system cycling and rerelease parameter. This final parameter can cause challenges in CW scaling, due to the fact that as the designed effluent concentration approaches C* value, the more difficult it becomes for a pollutant to become removed or degraded. The rate at which phosphate is removed in the hypertrophic system during 14/06/11 - 22/06/2011 is similar to that achieved in the much lower phosphate concentrations observed in the mesotrophic system during initial stages.

Interestingly, a peak of phosphate was observed in week 10 of the control. Mesotrophic control indicated a slow reduction in phosphate concentration, which is most due to algal uptake and subsequent bloom formation, as indicated in the water column chlorophyll analysis. Phosphorus release coincides exactly with the senescence phase of the algal bloom. This supports research carried out into algal bloom senescence, post bloom formation. Here classic effects of eutrophication occur due to the degradation and breakdown of the algal bloom (Wetzel 2001; Schlesinger 1997; Vitousek et al. 1997; Smith et al. 1999). Zhu et al. (2013) analysed the breakdown of algal blooms from lake Taihu in the Yangtze River delta. Water samples were analysed for nutrient pollutant release and effect upon DO. During

degradation, phosphate rose rapidly from zero within 15 days of sample collection with a corresponding drop in DO.

Release of phosphate was not observed in any of the planted treatments. Two reasons are proposed; assuming no algal bloom was formed, no subsequent release of phosphate would occur in the senescence phase (Fig 3.14 and 3.15). Direct plant uptake is therefore highly likely to be the main driver for phosphate removal.

Headley and Tanner (2006) describe the multiple benefits of using FCWs rather than more conventional systems. Not only can FCWs be retrofitted into aquatic environments and situations where extreme fluctuations in water level are observed, but also in floating systems there is a significantly greater rate of plant uptake available due to the direct suspension of the roots of the system suspended into the water requiring treatment, much like hydroponic horticulture. In FWS systems the macrophytes are rooted into the bed media meaning phosphorus must first diffuse into the soils at the base of the system before being up taken into the biomass (Brix 1993). The rate of uptake is such that bloom formation is hindered significantly due to phosphorus limitation.

Although freshwater system productivity is limited by phosphorus inputs, nitrogen based compounds significantly affect water chemistry and as such the potential for algal bloom formation. As mentioned nitrogen containing compounds are crucial to vegetation biomass development. Wetlands of various types are able to process a range of nitrogen containing pollutants. Primary mechanisms include nitrification and denitrification by microbial communities in the rhizosphere of the system (Mitsch & Gosselink 2000). Nitrification takes place when oxygen demands for the process can be met by radial oxygen loss from arenchymous tissues in the plant root (Sorrell et al. 2000; Armstrong 1980) whereas denitrification occurs when labile carbon is plentiful and reducing conditions prevail (Vymazal 2007; Sprent 1987). Treatment wetlands are able to modify the rhizosphere environment allowing for reducing and oxidising conditions to prevail within the same “reactor” (Wiessner & Kuschk 2006). Furthermore, as well as the substrate mixture of *Calluna vulgaris*, peat and Coir acting as a source of anti-algal phenolics, some of the more labile DOC compounds probably added to the carbon pool available for driving denitrification as well as plant root exudates (this is quantified in Chapter 6).

Significant differences in nitrite removal were found between the control groups and planted treatments in the hypertrophic systems. As with phosphate, the unplanted FCW control leached nitrite. It is certainly possible that microbial breakdown of nitrate to nitrite has occurred (Sprent, 1987). Although it is expected that the systems would become quickly

colonised it is unlikely that the colonisation by denitrifying microbes had occurred at this stage. Only microbes contained within the peat substrate added to the FCW during system construction could be a responsible for an immediate resident population of denitrifying microbes.

Mesotrophic systems show remarkably similar responses to leaching or production of nitrite by the FCW control as hypertrophic. In the mesotrophic containers nitrite concentration was similar to the initial nitrite concentration in the hypertrophic system. However, in the mesotrophic system there is a lesser leaching or production effect shown in the control. All unplanted FCWs were identical, which raises questions regarding the increased presence of nitrite within the hypertrophic system. Heffernan & Fisher (2012)who studied the establishment phase of a wetland stream system describe how in some more developed or steady state environments plants and microbes will compete for nutrients until the biomass demand is met. Their results from early successional stages of the wetland show that plant uptake is inversely proportional to denitrification by the microbial community.

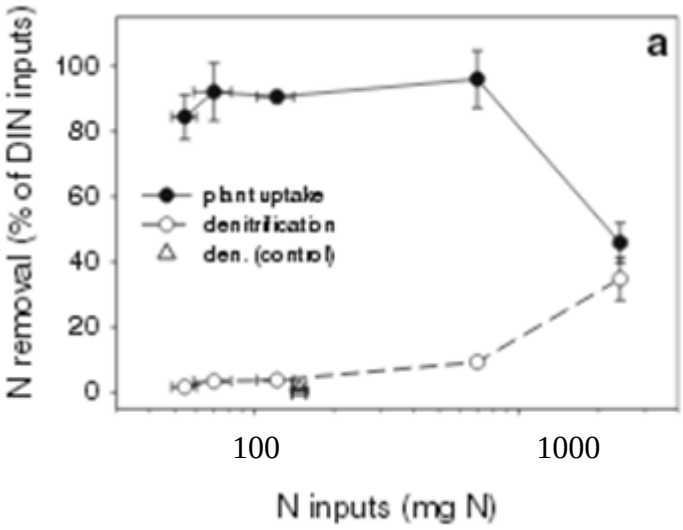


Figure 3.26 - microbial nitrogen removal against plant uptake.

Graph shows removal percentage of Dissolved Inorganic Nitrogen (DIN) (Adapted from Heffernan and Fisher (2012))

Figure 3.26 shows how microbial removal linked to denitrification does not increase until plant uptake begins to fall. However in the control mesocosm systems there are no plants to outcompete the microbial community for nitrogen. This is a possible explanation as to why the peak in nitrite is observed. This factor, i.e. free use of nitrogen without the pressure of competition from plants may explain a larger nitrite production peak in the hypertrophic system, proposed to be due to increased available nitrate in the water column as a substrate for microbial denitrification.

From figures 3.18 and 3.19, nitrate removal is clearly observed in the FCW control. As this cannot be due to plant uptake, microbial reduction to nitrite or organic matter binding must be occurring. Post-Hoc analysis shows the planted systems, FCW control and standard control to be in individual homogenous sub sets respectively. Although rhizosphere oxygenation levels will be discussed later, it was found that over the course of the experiment the FCW was significantly lower in DO throughout the 11 weeks. This factor supports microbial reduction of nitrate to nitrite, as this initial step in the denitrification process requires not only a sufficient and suitable carbon source but also anaerobic or reducing conditions within the rhizosphere (Vymazal 2007).

Again, algal bloom dynamics will be discussed in detail at a later stage, however the bloom events observed in the FCW control and the standard control seem to coincide with the production of nitrite as a result of microbial denitrification. In all the planted systems algal densities are kept to low levels in both nutrient regimes. Whereas in the unplanted FCW systems, as the nitrate became reduced and nitrite was produced the algal density began to increase.

These results suggest that nitrite is far more available to algae than nitrate. This is supported by early research undertaken by Grant & Turner (1969) who analysed nitrogen uptake by six species of green phytophagellate algae. Although the data supports a greater uptake of nitrite-N, nitrate-N is also up taken directly by the algae. However in the FCW control systems no significant increase of algae was found to develop in the early stages when nitrate is being reduced and nitrite is being produced. The 'preferential' uptake of nitrite as shown by Grant and Turner (1969) could explain why a greater density of algal bloom is observed in the FCW control as compared to the standard control where nitrate remains high for 4 weeks. Depletion only occurs when the algal bloom begins to increase in density.

It is notable that in all treatments and both nutrient regimes, nitrite and nitrate are removed rapidly following secondary dosing at 03/08/2011. During this phase of the experiment plant, algal and microbial biomass was visually observed to be greatly developed in all of the systems.

3.4.4 Effects of Radial Oxygen Loss on Dissolved Oxygen and Observed Effects upon DOC Production

Wiessner *et al* (2006) discuss how arenchyma tissues can account for up to 60% of the tissue volume in wetland macrophytes. Degree of vascularisation has been proven to enhance the capability of wetland plants to grow in anaerobic and waterlogged, (Jackson & Armstrong 1999). Wetland plants are able to withstand varying degrees of saturations and anoxia

dependant on the position along the aquatic to terrestrial continuum where they reside. And the degree to which root tissues lose oxygen radially through the root surface varies with species. Although every effort was made during the experiment to balance vegetation biomass, differences in growth were observed over the course of the testing. This has potential implications for root biomass, ROL, root exudate release and nutrient uptake. However, differences in the parameters observed mimics natural systems allowing valid conclusions to be drawn.

Hypertrophic water column DO showed statistically significant differences between treatments. From Post-Hoc analysis of homogenous sub sets the FCW control treatment was found to have significantly lower DO values for the duration of the experiment and the standard control significantly higher in a separate sub set. All the planted treatments showed relatively similar water column DO concentrations, whilst greatest DO was observed in the standard control. It is highly likely that this high DO value is generated due to photosynthetic processes occurring within the bloom and as a result of macrophyte gas transport. However, as discussed later, higher values of chlorophyll-a were observed in the FCW control that the standard control or planted treatment, This raises the question why are DO levels in the FCW control are lower than that of the standard control? Two hypotheses for this are suggested; shading of the water column due to the installation of the FCW system and secondly the effect of media degradation and carbon use in conjunction with the denitrification process and the lack of DO transport into the rhizosphere.

Wetland soils are known for the anaerobic conditions they possess (Mitsch and Gosselink, 2000; Wiesner et al, 2006) and the reducing conditions that are induced due to waterlogging of the rhizosphere (Vymazal, 2007). When analysing the pore water DO values, FCW control was observed to be significantly lower due to the lack of radial oxygen loss. The inclusion of macrophytes significantly increased the DO concentrations in the rhizosphere confirmed by low concentration in the control.

3.4.5 Effect of Water Chemistry on Algal Bloom Density

In both trophic regimes chlorophyll density in the planted treatments was consistently lower than that observed in either of the controls. Therefore planted FCW systems are successful at preventing the formation of ecologically harmful algal blooms. With regard to statistical analysis of bloom formation, under hypertrophic nutrient regimes bloom density characteristics showed significant changes over time. Similarly high levels of significance were observed between the treatment groups. Rates of algal density increase observed were

statistically classified into a homogenous sub set. From this it can be concluded that planting FCW systems aids the primary goal of algal bloom reduction.

In the mesotrophic systems no statistically significance was found over time when analysing bloom density characteristics. Although the p-value was 0.054 indicating significance was not found may the case statistically, however biological significance may still be drawn. This is likely to be caused by tight grouping of the other treatments and a modest increased algal density observed in the FCW control only. Low concentrations of Chlorophyll-a, could signify that the blooms observed under mesotrophy were potentially limited by nutrients. The nutrient concentrations observed in the hypertrophic mesocosms was great enough to create significant increases in algal bloom forming potential when planted mesocosm systems are not present.

3.6. Conclusions

It was hypothesised that significant differences in the nutrient sequestration rates of different plant species would be observed. While the analysis also focussed upon the FCW system as a whole; including the effect of anti-algal phenols (Wingfield et al. 1985; Welch et al.(1990; Pillinger et al. 1994), phenolic breakdown by phenol oxidase (Pind et al. 1994; Freeman et al. 2001; Freeman et al. 2004) and the effect dissolved oxygen in the rhizosphere as a result of macrophyte radial oxygen (Armstrong 1980; Brix 1993; Jackson & Armstrong 1999; Sorrell et al. 2000; Wiessner & Kuschk 2006).

Previous mentioned research highlighted that organic matter may act as a substrate for denitrifying bacteria (Wingfield et al. 1985) from which a labile carbon source could be extracted and used in the denitrification process. However, this experiment found that firstly oxygen levels are lowered as a result of introducing an organic substrate mixture and secondly that only partial denitrification seems to occur.

The phenomenon of significant reductions in water column DO is one of the negative effects that occur as a result of algal bloom senescence. If organic phenol releasing substrates are introduced into a eutrophic water body, results show that partial denitrification from NO_3^- to NO_2^- would occur. As shown, this could result in an exacerbation of the problems caused by algal blooms due to nitrite being more bioavailable to green algae, particularly the algae which colonised the mesocosm systems Heffernan & Fisher (2012).

Secondly Pillinger et al. (1994) suggested that polyphenols oxidised to monophenols may have a greater anti-algal effect than larger phenol containing organics. Although this phenomenon is well documented, in relation to this investigation it can only be considered as

plausible. Freeman *et al* (2004) describe how phenol oxidases are one of the few enzymes capable of breaking down phenolics in oxygenated conditions. Pind et al. (1994) studied the relationship between oxygen saturation and phenol oxidase activity and found positive correlations. Here there was a significant lack of phenol oxidase within the rhizosphere of the FCW systems as quantified by the L-DOPA method. This lack of extracellular phenol oxidase activity could have led to incomplete or non-existent breakdown of the polyphenolics clearly present in the water columns surrounding the rhizospheres of the FCWs. For this reason, although there was significant enhancement of the DO concentrations both within the rhizosphere and the exterior water column by the wetland macrophytes, it is likely this did not enhance the production of oxidised polyphenols with anti-algal properties.

The addition of wetland macrophytes is a crucial component of biochemical processing within the FCWs; including pollutant and nutrient uptake, intake of suspended solid material into root associated biofilms and oxygenation of the rhizosphere. Wetland macrophytes may significantly modify the rhizosphere environment to the extent that polyphenols may become oxidised. However, it is postulated that in short term monitoring, the colonisation of phenol oxidase producing microbes was insufficient in order for the macrophytes to modify the environment and promote successful breakdown by the enzyme. Long term analysis may provide insights into the significant colonisation of microbes and subsequent oxidation to anti-algal phenolics. Under the conditions prevalent in this experiment the primary method of bloom prevention appears to be the removal of nutrient pollutant rather than the effect of anti-algal phenolics.

Chapter 4 - The use of constructed treatment wetlands for addressing eutrophication of oligotrophic rich-fens: case studies for Cors Erddreiniog and Cors Bodeilio National Nature Reserves, Anglesey, North-west Wales

4.1 Introduction - The Use of Constructed Wetlands for Water Quality Amelioration at Three Locations

Rich-fen sites subject to restoration through the Anglesey & Llyn Fens LIFE project are oligotrophic systems sensitive to nutrient enrichment from catchment activities. Constructed wetlands have been found to provide an effective means of reducing nutrient concentrations in water entering the sites via defined surface pathways such as streams and localised seepages. A range of constructed wetlands have been designed to suit specific locations and conditions. System design and installation was informed by identifying nutrient species and concentrations in water entering the fens, knowledge of target nutrient levels, and the characteristics of the fen sites and their capacity to receive outflow from constructed wetlands. Three systems are discussed and conclusions regarding total pollutant removals are drawn.

4.2 Introduction to The Fen Sites and Natura 2000 Conservation Project

Natural Resources Wales (NRW) is the statutory body responsible for the management of two rich-fen Special Areas of Conservation (SAC) on Anglesey and Llyn peninsula. The scheme sets out to restore favourable management to 751 hectares of rare fen communities reliant upon oligotrophic and primarily ground water inputs. Mismanagement and neglect has led to the degradation of fen habitat within the conservations sites. The NRW LIFE acts under the umbrella of Natura 2000, which developed actions needed to effectively target restoration of the sites.

These targets aimed to minimise and prevent the effects of neglect and mismanagement, whilst also ameliorating wider problems such as nutrient enrichment directly affecting the fens. Combined problems have resulted in the invasion of Graminoid and competitive wetland species such as *Typha latifolia,* which prevent the successful maintenance and promotion of the rare indicator species of the conservation sites such as *Shoenus nigrigans*. The target communities on the sites are a key component of NRW's sites status as SSSI, SAC and RAMSAR sites. Intrinsic wetland management has not been the sole driver for the actions taken by the LIFE project, wider catchment issues such as enrichment caused by an array of diffuse and point source pollutants was a significant contributor of the effects observed. To this end, the set of actions granted by the LIFE project were as follows; mowing, controlled burning, grazing, hydrological works, peat removal, science and technical actions and constructed wetlands.

The following paper discusses the background, purpose, modelling, design, implementation and monitoring of bespoke constructed wetland systems, as part of hydrological works, in order to alleviate enrichment pressures on the LIFE sites of Anglesey.

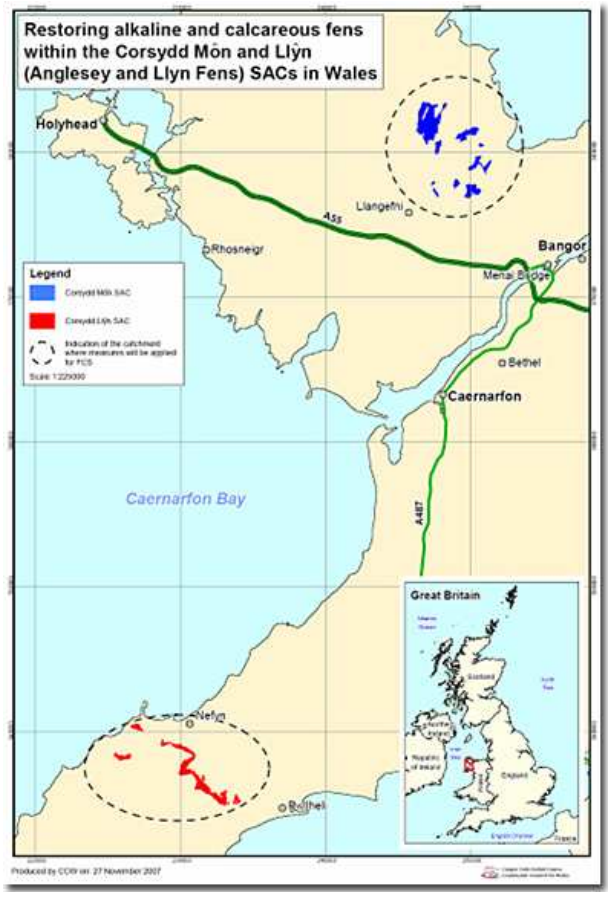


Figure 4.1 –Locations where fen restoration is occurring throughout North Wales.

Constructed Wetlands discussed in this thesis refer to 2 separate Anglesey fens, Cors Bodeilio and Cors Erddreiniog (Blue.) Source Anglesey and Llyn fens Life project

4.3 Background of Water Quality Issues and Effect on Conservation Status

The Anglesey & Llyn Fens SACs have been designated because they provide a western UK stronghold for important examples of two oligotrophic rich-fen habitats listed under Annex I of the Habitats Directive, namely 'alkaline fen' (H7230) and 'Calcareous fen with *Cladium mariscus* and species of the *Caricion davallianae*' (H7210). Both of these habitats are associated with groundwater-fed contexts rich in calcium but oligotrophic in terms of the availability of the key plant macronutrients N and P (Wheeler et al. 2009). Recent work undertaken to define threshold levels for N in groundwater feeding oligotrophic rich-fens suggests that concentration should not exceed 4.5 mg/l nitrate-N (UKTAG 2012, Farr et al. 2013), with a more specific target of 2 mg/l nitrate-N proposed for the Anglesey & Llyn SACs. Reducing N concentrations in groundwater discharge may require a range of management interventions and is needed both to deliver favourable condition for the rich-

fens, but also to ensure good groundwater status under the Water Framework Directive (WFD).

4.4 Effect of Nutrient Pollutants on the Conservation Site, the Need for Treatment and Constructed Wetlands as a Solution

Nutrient pressures occur on the sites due to a history of intensive agriculture across Anglesey. Many springs feeding the sites exhibit elevated concentrations of nitrate which are suspected to reflect continued agricultural pressure. Secondly, due to the nature of the aquifers below ground it is likely that the nitrogen legacy within this large underground body of water, which directly supplies the fens, will last for a significant period of time. The nutrient pressures on the fen result in enrichment of the site. Localised eutrophication is observed in areas where both Annex I features exist, to the extent that the conservation status of the sites is now unfavourable.

Constructed wetlands were identified as a potential solution for alleviating nutrient pressures early in the project, particularly for locations where water entry to the sites from runoff and/or groundwater is focussed. Nutrient pollutants (N and P) were targeted for removal, particularly in areas of the conservation sites where peat re-profiling works and hydrological re-connection works were being planned.

The constructed wetlands offer an immediate solution to the problem of N and P enrichment, although targeting the problem at source should still be paramount. These small bespoke systems are able to optimise and intensify mechanisms by which the pollutants can be removed from the water column. Planted treatment systems in particular offer a suitable microhabitat where biological processing of the pollutants can take place.

4.5 Design Tools Utilised for Constructed Wetland Scaling, System Selection and Layout

Although treatment wetlands are capable of significant pollutant removal, as the treatment system design becomes more passive, area requirement is increased. At all times during the design of the systems no mechanistic control of water flow would be available. Maintaining completely passive system design influenced the area of the CWs used increasing area required significantly. Development of knowledge throughout the project has led to the use of a series of design tools for treatment wetland modelling. Modelling systems became progressively more advanced, developing from nominal and actual hydraulic retention time (HRT) calculations though to advanced aerial calculations as outlined in Kadlec & Wallace (2008). Specifically, the P-k-C* model was rearranged in order to provide a necessary treatment wetland area.

Here the P-k-C* equation allowed for the modification within the model for system area, water volume treated and pollutant removal achieved. With this tool the conservation aspect, in terms of providing maximum areas for fen restoration could be achieved, balancing conservation with CW treatment potential. Specifically, water treatment capability and conservation area for restoration could be balanced effectively.

This rearrangement of the P-k-C* equation allowed for flexibility between treatment per unit area and manipulation of CW area until treatment requirements were met. The equations are detailed in equation 4.2 and rearranged form equation 4.3. The advanced modelling method calculation controls for intrinsic system temperature, hydraulic loading rate (HLR), pollutant specific rates of biological degradation (k) and hydraulic flow efficiency (P), desired outflow concentration in relation to inflow pollutant levels and system production of pollutants from degrading biological material in the system (C*).

4.6 Hydraulic Calculations and Area Quantification Modelling

Hydraulic Retention Time (HRT) calculations are based on simple models of treatment wetland volume and effective treatment space once porosity of the system is accounted for. This tool was used during the initial phases of treatment wetland design. Here a nominal (theoretical calculated time) retention time of the inflowing water was based on 48hours of water-system contact time.

nHRT is a theoretical time which pollutant laden water spends in the treatment wetland system. The calculation comprises system volume where the theoretical or nominal time within the system is deduced by factoring how long it will take for the water in the system to be replaced by the inflow water. This is based on the system being fully mixed, hence theoretical HRT where no short circuiting occurs. Factors that influence nHRT are primarily area and depth, the final parameter is porosity of the system (ε) (Kadlec and Wallace, 2009). This results in the following equation.

4.6.1 nHRT Calculation

Equation 4.1

$$nHRT = \frac{Vol\ \varepsilon}{Flow\ Rate\ (Q)} = \frac{L\ W\ D\ \varepsilon}{m^3/d}$$

Equation 4.1 is the simplest form of the model but a key concept that must be included even when more complex models are used to form the primary design parameters. Moreover, the porosity of the system depends firstly upon the system selection Horizontal Sub-Surface Flow

(HSSF) where bed media of the system will account for a percentage of the system volume of the system or Free Water Surface (FWS) where increased amounts of system volume is water and a small percentage taken up by biomass. Typically FWS systems have a porosity value of approximately 0.95 fractional volume when filled with water (Kadlec and Wallace, 2008).

Developments in treatment wetland knowledge led to the use of the P-k-C* model outlined below (Kadlec & Wallace 2008)

4.6.2 P-k-C* Calculations

Equation 4.2 - P-k-C*

$$\frac{C_o - C^*}{C_i - C^*} = \frac{1}{(1 + k / (Pq)^P)}$$

Parameter	Use in P-k-C*equation
P (pTIS)	denotes hydraulic flow efficiency and mixing (dimensionless)
K	pollutant specific weathering rate standardised for 20°C
C*	system pollutant cycling and release (standardised concentration)
Q	Assumed average Flow volume per day (m^3/day)
Temp	system temperature factor (°C)
Theta factor	modifier of temperature effect away from K_{20} rate (dimensionless)
K_{temp}	Temperature adjusted removal rate
area	Area (m^2)
C_i	Influent pollutant concentration (standardised concentration)
C_o	Effluent concentration achieved under the above parameters (standardised concentration)

Figure 4.2 Explanation of parameters utilised in the P-k-C*

The format used for analysis of treatment on an aerial rather than volumatric basis with fixed volume and inflow concentration allowed for manipulation of area until a suitable outflow concentration was achieved therefore maximising smace for habitat creation and minimising constructed wetland footprint. For this the P-k-C* model was manipulated as follows-

4.6.3 Rearrangement of P-k-C* for Outlet Concentration

Equation 4.3

$$C_o = C^* + (C_i - C^*)(1 + k/ (Pq)^{-P}$$

Once a suitable CW area had been set as determined by the UKTAG guidelines (Section 4.3) the design process could be undertaken.

4.7 Constructed Wetland and Conservation Site Specific Pollutant Removal Processes

Although constructed wetlands have been used as a biological tool for removing a variety of pollutants from the water column including calcium (Mayes et al. 2009a)and other heavy metals (Ye et al. 2001), animal waste water (Hunt & Poach 2001) and road runoff, including petrochemicals (Shutes et al. 1999), the primary aim of the NRW treatment wetlands is the removal of nitrogen, phosphorus and DOC (due to the sites acting as a catchment for local drinking water reservoirs, preventing chlorination issues at the treatment plant.), whilst maintaining calcium. Phosphorus has found its way into the water courses supplying the conservation site, but this tends to be as a direct result of adjacent agriculture. These point sources of phosphorus, in the form of phosphates, may eventually be targeted by CWs. However, currently addressing the problem at source is of primary concern. Nitrogen enrichment is more prevalent in the water supplied to the fen. This, as mentioned, is due to the connectivity of the underground aquifers of Anglesey. Beamish & Farr (2013)describe the connectivity of the sites due to a carboniferous limestone aquifer. The water falling on the adjacent land supplies the aquifer with water which surfaces as springs supplying the fen. This is the source of the calcium rich water. However, when intensive agriculture and fertiliser application are undertaken on the land much of the nutrient content seeps down into the aquifer (Farr et al. 2013). This manifests itself as enrichment in the site.

CWs are a means by which optimisation and intensification of biological transformations of nitrogen and phosphorus containing compounds can be achieved, thereby removing them from the water column. Each biological breakdown or removal process is pollutant specific. Therefore the system must be designed in order to optimise conditions for biological transformations. Primary pollutants targeted in the systems described in this work are nitrate, ammonium, nitrite and phosphate.

4.7.1 Nitrate Removal in Constructed Wetlands

Nitrate is the dominant form of nitrogen based pollutant in the spring waters supplying the conservation site(Gilman & Newson 1982; Farr et al. 2013). In order to assess the way in which to maximise nitrate removal the nitrogen cycle must be considered. Shapleigh (2013) discusses the nitrogen cycle and suggests that removal of nitrate can be undertaken by microbial dentitrification. System design must reflect the conditions required for efficient pollutant removal, the system specifics which affect the processes involved are described in Chapter 1.

4.7.2 Potential for Ammonium Nitrification

A number of water input locations around the conservation sites are thought to contain ammonium and are affecting the localised area it is supplying. It is discussed in Chapter 1 that ammonium nitrification is primarily an aerobic process biologically controlled and undertaken by microbes. In order to achieve high oxygenation rates, FWS wetlands or vertical flow (VF) pulse flow wetlands where the system is allowed to become saturated with air between pulses tend to be utilised. These systems may be employed in order to deal with ammonium in the water supplying the fens. However, complexity and lack of passive design in the latter system may add increased need for maintenance and monitoring, and also increased system set up costs.

4.7.3 Phosphorus Removal

Phosphorus removal processes are discussed in Chapter 1. However, inclusion of additional parameters in the P-k-C* equation can account for the loss of phosphorus from the system. The component which accounts for loss is the C* detailed in equation 2 and 3. The C* is used as a modifier to the inflow and outflow concentrations to model area required for successful pollutant removal.

4.8 Water Flow Volume Calculation and Estimation

In CW systems installed in more natural situation the primary driver for flow rate consideration is linked directly to precipitation and the associated catchment lag time before arriving at the influent of the CW system. Groundwater fed springs are typically more stable than surface waters, however conventionally, treatment wetlands are designed to a reasonably stable or predictable volume flowing into the systems. The Anglesey Fens treatment wetlands influent points have had flow monitoring in place on many of the springs feeding the fens. This was the basis for the hydraulic element of system design, where most inflows indicated a flow rate which only fluctuated heavily in storm flow or summer drought events. The systems were designed around average flow rate which meant reduction in treatment potential would be observed during the higher flow periods. This was seen to be the case when water chemistry data ware analysed.

4.9 System specific Plant Selection and Unitisation of Locally Abundant Species

Vascular wetland macrophytes were selected on the basis of submergence capability in relation to system water level design. This is primarily determined by the presence of arenchyma tissues in the plant. However, the primary cause for plant selection was based

around site availability. Harvesting from within the site was undertaken due to the potential risk of introduction other species not currently native to the sites.

Plant harvesting from within the site was used to prevent the possible introduction of non-native species. This may have occurred if seedlings had been introduced to the site. Plant harvesting varied in success due to the localised plant availability. Access for harvesting machinery, harvesting and wetland location distances occasionally led to limited planting of the system. All methods of harvesting and planting resulted in successful development of biomass stand within the treatment system. Species used included *Iris pseudacorus, Phragmites australis* and *Typha latifolia.*

4.10 System Specific Design, Operation and Monitoring (2012-13)

4.10.1 Cors Bodeilio NNR, Site Parameters and Design Considerations

Previous water quality analysis identified a number of locations of nutrient enrichment on Cors Bodeilio. Also influx of competitive wetland macrophytes was observed at many points where ground and surface water irrigate the fen.

Two primary locations for CWs and a third location which would be granted based on the success of the two primaries were required. In both the locations nutrient pressures were significant yet not acute. Moreover, each of the inflows identified were targeted to their location in relation to agricultural runoff. Specifically, both inflows could potentially see concentrated spikes of nutrient pollutant enrichment during phases of agricultural fertilizer application. Thus installed systems would help buffer against peaks whilst reducing lesser nutrient pressure throughout the year. The streams emerge as diffuse field runoff is collected into a ditch and piped onto the site. CWs were situated within the site boundary where flows enter the site.

4.10.1.1 Bodeilio FWS Constructed Wetland Flows and Pollutant Loading

The FWS system was identified to ameliorate an agricultural drain with an average peak flow of 0.5L/s resulting in a total Q of 43.2 m^3/day. Sampling of the systems inflow continued throughout the lifetime of the project to monitor enrichment effects. Water quality sampling was undertaken on a monthly basis.

Parameters were measured in the field and in laboratory situations. Field measurements undertaken included Dissolved oxygen (DO), pH, Electrical Conductivity (EC) and temperature. Laboratory analysis comprised primarily of Ion chromatography from which nutrient pollutants could be quantified, UV-Vis analysis using spectrophotometry, Dissolved organic carbon (DOC) and phenolic constituent.

4.10.1.2 System Modelling - Bodeilio FWS HRT

A FWS system utilised attached microbial biofilms colonising the roots, rhizomes and stems of the plants in the system for degradation of nutrient pollutants (see figure 1.2 Chapter 1). These types of constructed wetland typically exhibit water depths of 0.15-0.50m and are planted with species that can tolerate waterlogged conditions due to the presence of arenchyma tissues enabling oxygen transfer into the roots. Submerged aquatic vegetation (SAV) may also be utilised for biofilm colonisation. These systems differ from Horizontal Sub Surface Flow (HSSF) systems (explained later) where a porous media is relied upon as an attachment surface for the microbial communities.

The FWS system at Bodeilio was the first system to be implemented. Nominal HRT was calculated for the system. Based on a Q of 43.2 a nHRT was set at 24hours. This flow rate would encompass the majority of peak flow events arising from agricultural runoff.

The system was scaled to a volume of 50 m^3 (10m x 10m), therefore a 6.8m^3 buffering capacity capable of dealing with higher flow rates was achieved. Secondly unplanted inlet and outlet zones were used to minimise short circuiting.

Due to timescale and system installation, system area calculations were retrospectively undertaken to confirm system pollutant removal (Fig. 4.20)

4.10.2 Bodeilio FWS System

Figure 4.3 - The Bodeilio FWS system.

The image shows the system after one growing season, *Phragmites australis* was harvested from site and used in the treatment wetland system

4.10.2.1 Bodeilio CP Nitrabar/ HSSF Hybrid System

The second inflow point to the site at Cors Bodeilio was identified and a HSSF system was selected. This design utilised a gravel media for the colonisation of microbial biofilms in order to achieve successful pollutant degradation. Liaison with EA Wales led to the inclusion and hybridisation of the NitraBAR system (NITRABAR FIELD REPORT 2009) into the design of the CW used, the prototype system developed by the EA aimed to aid nitrate reduction.

This modification to conventional HSSF design resulted in the addition of an organic carbon media of wood chips which acted as a carbon source in order to promote microbial dentitrification. *Iris pseudacorus* sourced from the site was used to plant the system due to its specific water logging tolerance. Limestone gravel 20-40mm in diameter was used to cap the wood chip media. This was undertaken to allow flow through the wood chips as indicated in figure 1.1 chapter 1.

4.10.2.2 HSSF System Scaling and Design

nHRT modelling used a 24 hour retention time using average flow volumes (Q) of 0.5L/s or 46.2 m^3/day although seasonal variation occurs. The area available comprised 170m^2 (as measures using GPS) of space which comprised of a depression formed in the site naturally holding water prior to intervention. The system was 0.5m in depth and comprised a gravel and woodchip matrix for biofilm colonisation. Total system volume was calculated to be 85m^3 and a treatment volume of 42.5m^3, due to 0.5 estimated porosity value. nHRT calculations indicated a retention time of just under 24hours. This was deemed suitable due to the low concentrations of pollutants. Hybridisation would produce an anaerobic and carbon rich environment for optimal denitrification. Identical water quality parameters were measured in both systems to assess implications system design selection.

4.10.2.3 Bodeilio HSSF

Figure 4.4 - Bodeilio HSSF system NitraBAR hybrid planted with Iris pseudacorus.

Two inflows from agricultural runoff (Red, arrow denotes location of secondary inflow, out of shot) were piped into a distribution channel (Yellow). Following system installation, water level in the receiving fen was increased by means of ditch blocking. This resulted in a proportion of the CW becoming flooded.

4.10.3 Cae Gwyn FWS system Located at Cors Erddreiniog

The Flagship project of the Anglesey LIFE Fens project was a peat land restoration project at Cae Gwyn, a section of land at Cors Erddreiniog SSSI. This project comprised hydrological reconnection works, peat reprofiling, installation of water distribution system, drawdown prevention mechanisms and constructed wetlands (figure 4.5 and 4.6) to alleviate enrichment pressure on the reprofiled bare peat.

The calcareous water issuing from the springs was vital to the restoration of calcareous fen on Cae Gwyn. Whilst water issuing from the springs is calcium rich, it also contains significant concentrations of nitrogen. The nitrogen here may inhibit the re-colonisation of *Shoenus nigricans* dominated fen community M13 vegetation community. Maximum recorded nitrate in the spring water reached 22.34mg/L whilst 129.21mg/L of essential ionic calcium was recorded. The task was to reduce nitrate levels in the spring water significantly using a constructed wetland; however the area constraints on the system were significant due to the localised presence of marl substrate, prime conditions for M13 colonisation.

The Cae Gwyn system was designed around a FWS area of 680 m^2, the system has an average depth of 0.3m resulting in a 194 m^3 system once vegetation colonisation is taken into account (0.95 porosity). The system is also designed around a 2L/s flow rate resulting in a 54 hour theoretical retention time.

Enriched spring water was treated by the CW in order sufficiently oligotrophic. This was to prevent the colonisation of competitive graminoid species which do not represent the vegetation of the SSSI. Here, modifications of the treatment wetland system, as scaled using P-k-C* equation, allowed for partial treatment of the enriched water whilst maintaining a sufficient area for colonisation of M13 habitat.

Parameters used in the P-k-C* model were as follows;

Parameter	Value
pTIS	5
K	35
C*	3
Q	172.8
Temp	10
Theta factor	1.05
K_{temp}	21.48
area	680
C_i	24
C_o	5.80

Figure 4.5 - parameters for P-k-C* model at Cae Gwyn.

Figure 4.6 – View from the inlet zone during construction phase with civil engineering contractors at Cae Gwyn FWS CW.

Following excavation additional soil was added to the base of the system to act as rooting media. This was undertaken due to the presence on compacted lay in the base of the system. Although this is an excellent material for retaining water and preventing hydraulic drawdown, it is not suitable for macrophyte colonisation.

Figure 4.7 – Completed system at Cae Gwyn.

Limited planting was undertaken; much of the growth achieved was due to natural colonisation.

4.11 Constructed Wetland Water Quality Mitigation Results April 2012 to April 2013

4.11.1Cors Bodeilio FWS Nitrate Observations

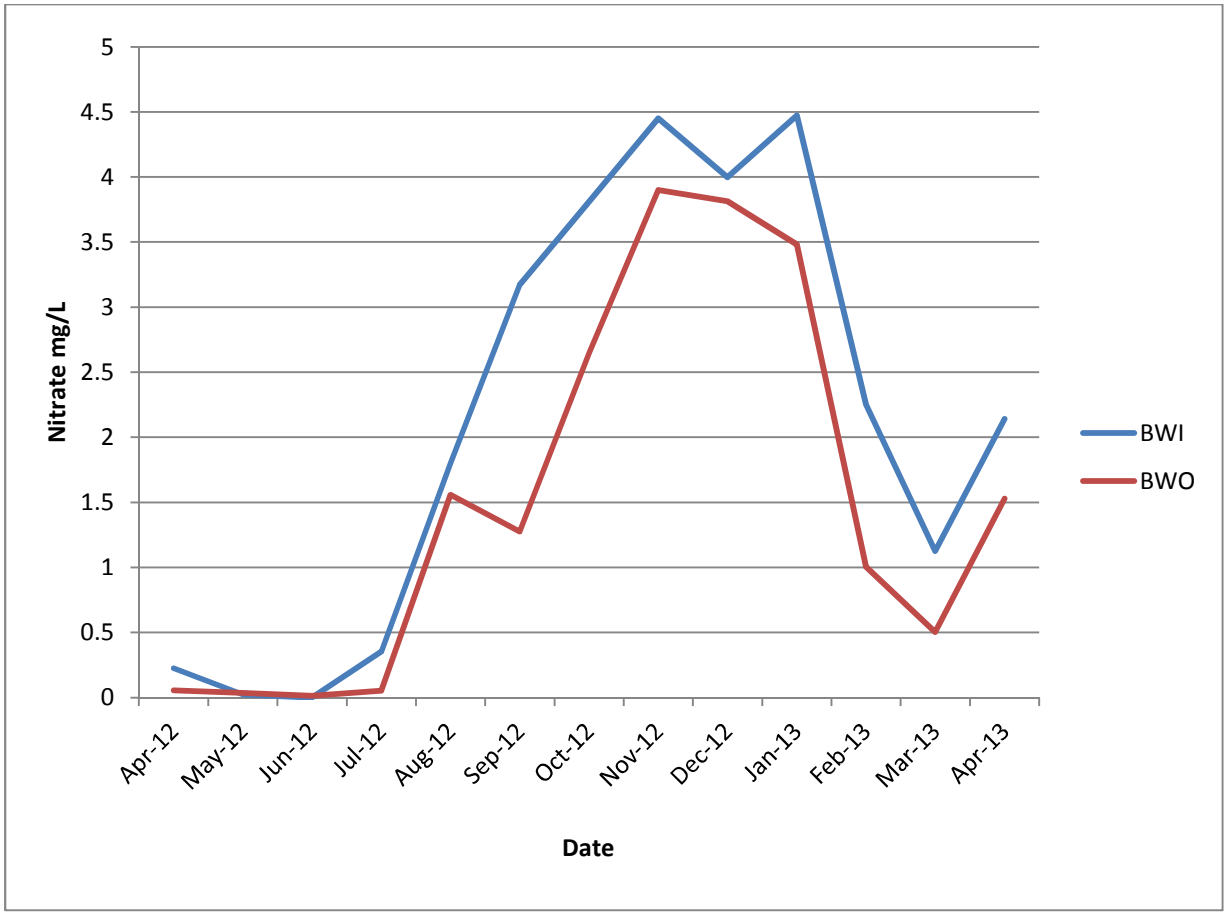


Figure 4.8 – Bodeilio FWS nitrate concentration over time.

BWI and BWO denote Inflow and outflow respectively. Although the surface water inflow at Bodeilio FWS does not indicate excessive nitrate concentrations compared to Cae Gwyn, the CW was successful at reducing nitrate concentrations for the duration of monitoring. Maximum percentage removal was observed in July where 85% nitrate reduction was achieved. No error bars were added to the graph due to single monthly samples from the CW.

4.11.2 Bodeilio FWS Nitrite Observations

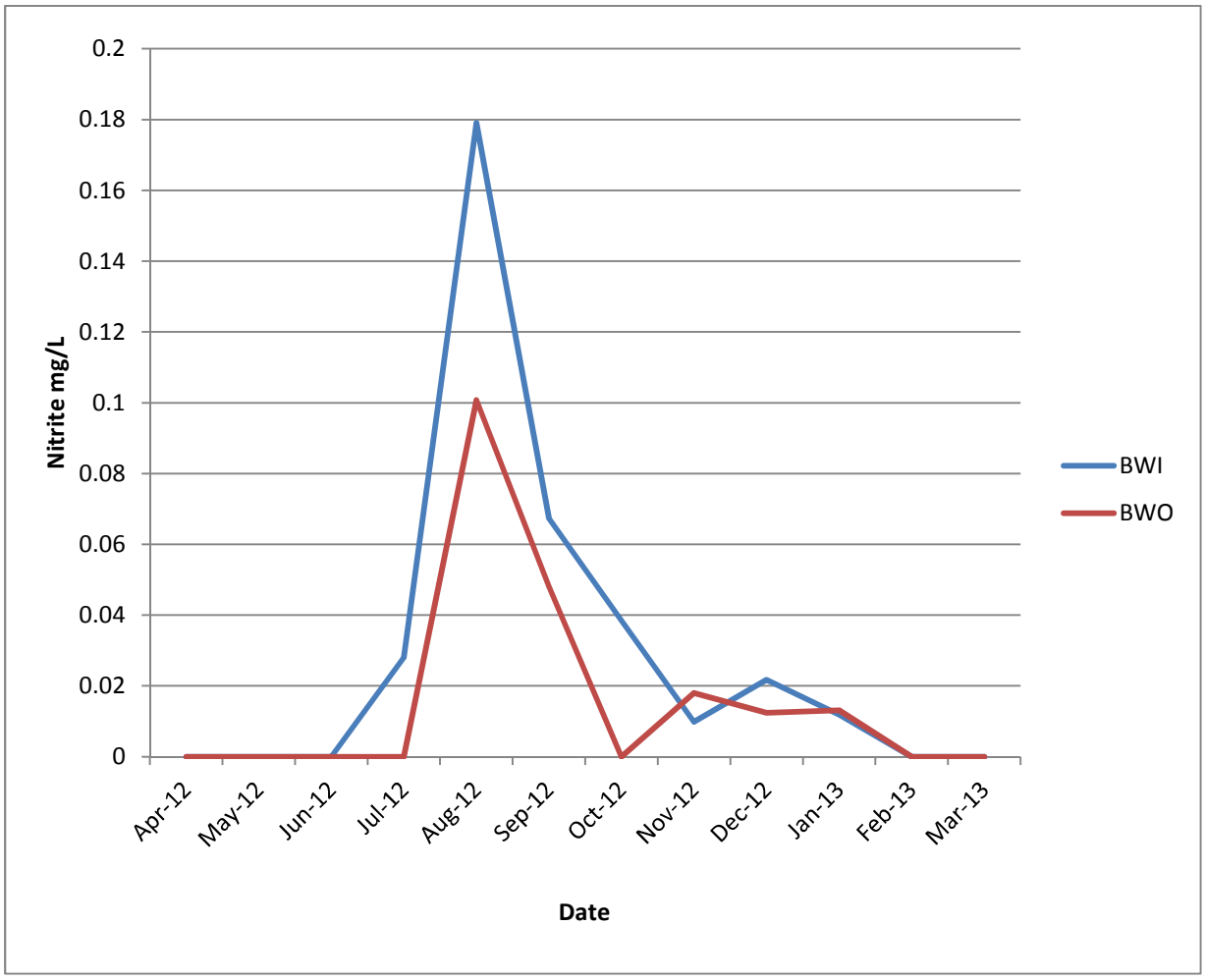


Figure 4.9 - Bodeilio FWS nitrite over time.

The Graph shows the nitrite concentration of the inflow and outflow of the constructed wetland. The CW was observed to be a source of nitrite on a single occasion in November.

4.11.3Phosphorus Removal Bodeilio FWS

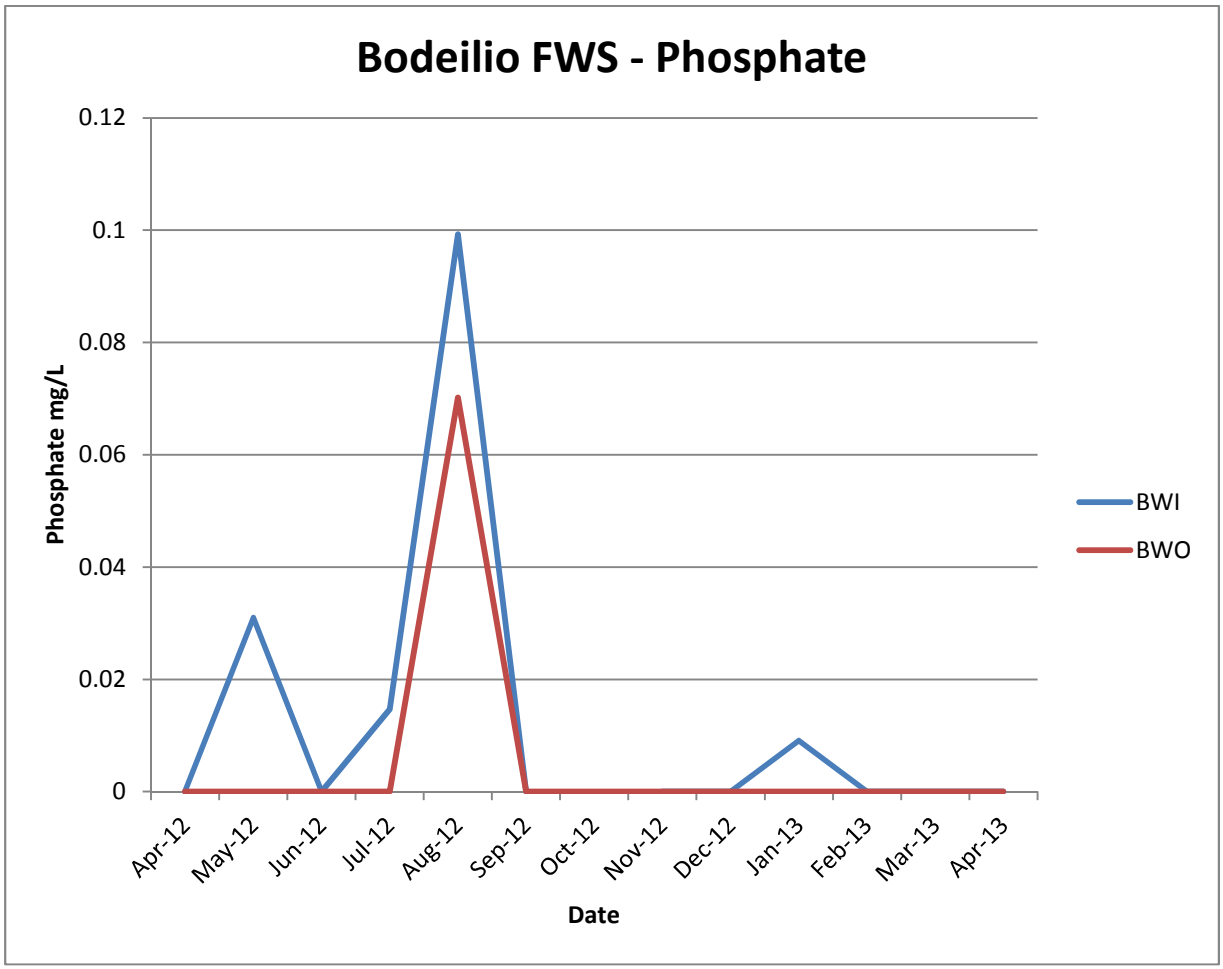


Figure 4.10 - Bodeilio FWS Phosphate over time.

The graph shows the phosphate concentration in the inflow and outflow of the CW. Four separate phosphate events are observed on the sampling days. Phosphate is noticeably low concentration at all times, however the treatment system was capable of removing 100% of the phosphate from the outflow, with the exception of August when only 30% removal was achieved.

4.11.4 System Maintenance of Alkalinity and Calcium

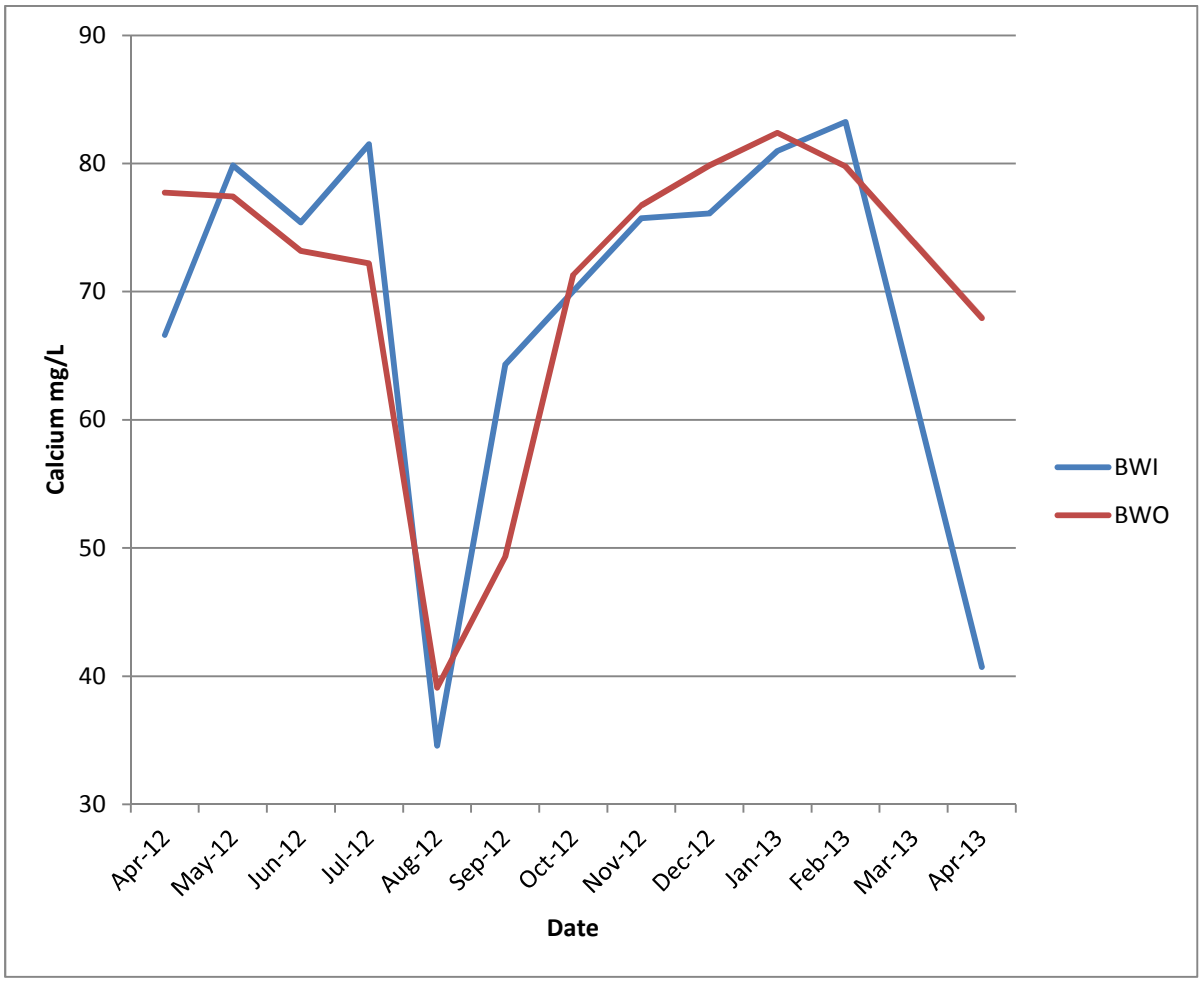


Figure 4.11 - Bodeilio FWS calcium over time.

Calcium concentration is of primary concern on the life conservation sites as it is a driver for the presence of M13 and alkaline fen vegetation communities. Influent and effluent calcium concentrations are markedly similar. Continual fluctuation is observed in calcium concentration. However, in the final month of sampling calcium release from the system is significantly higher than the influent concentration observed.

4.12 Pollutant removal HSSF NitraBar Hybrid

4.12.1 Nitrate

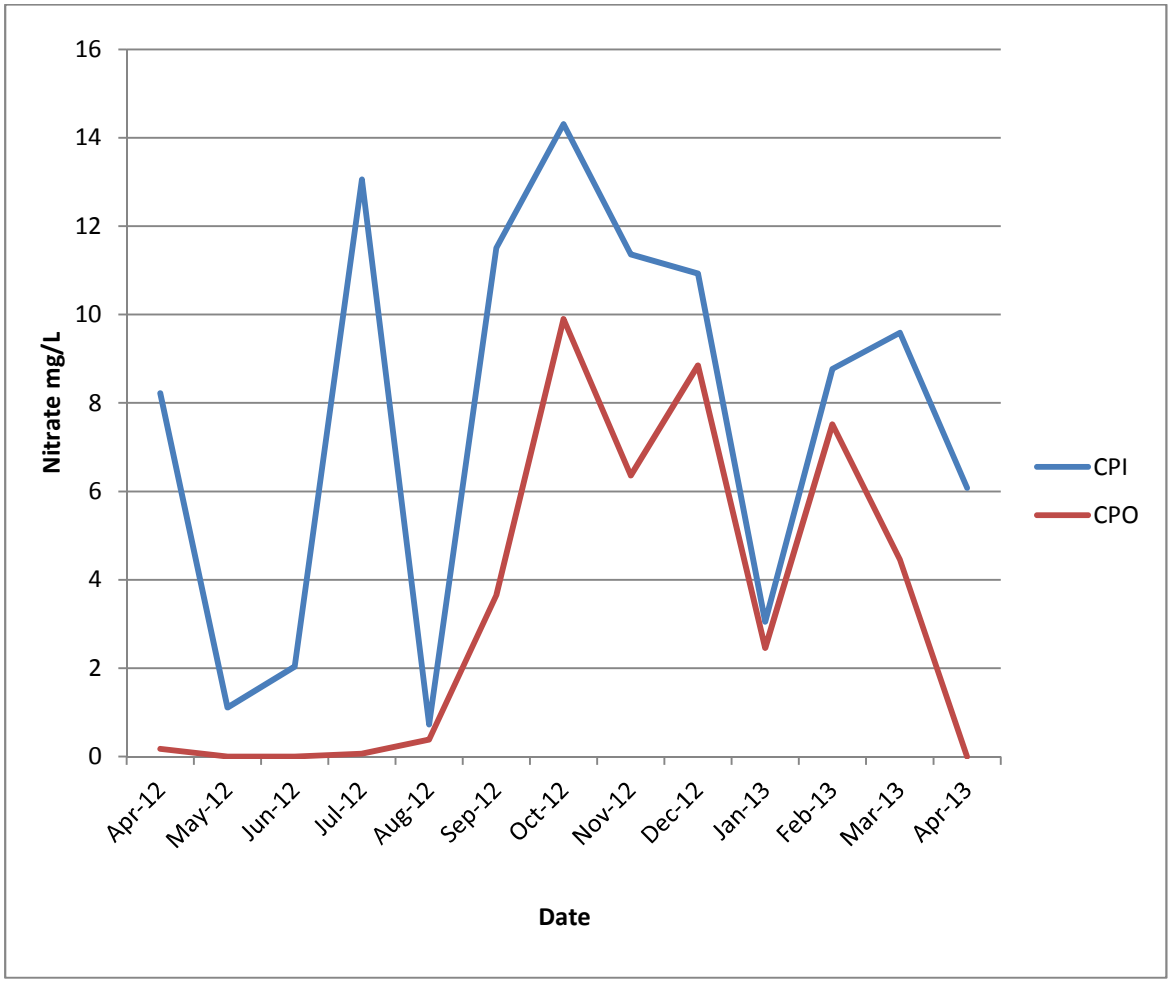


Figure 4.12 – Bodeilio HSSF nitrate over time.

CPI and CPO indicate the mean inflow nitrate concentration from the two inflows and outflow nitrate concentration respectively. The results indicate a sporadic influx and range of nitrate concentrations from the highest observed concentration of14.31mg/L nitrate to the lowest observed concentration of 0.721 mg/L. Again the system design achieved maximum removal of 99% in July of the first year of system operation.

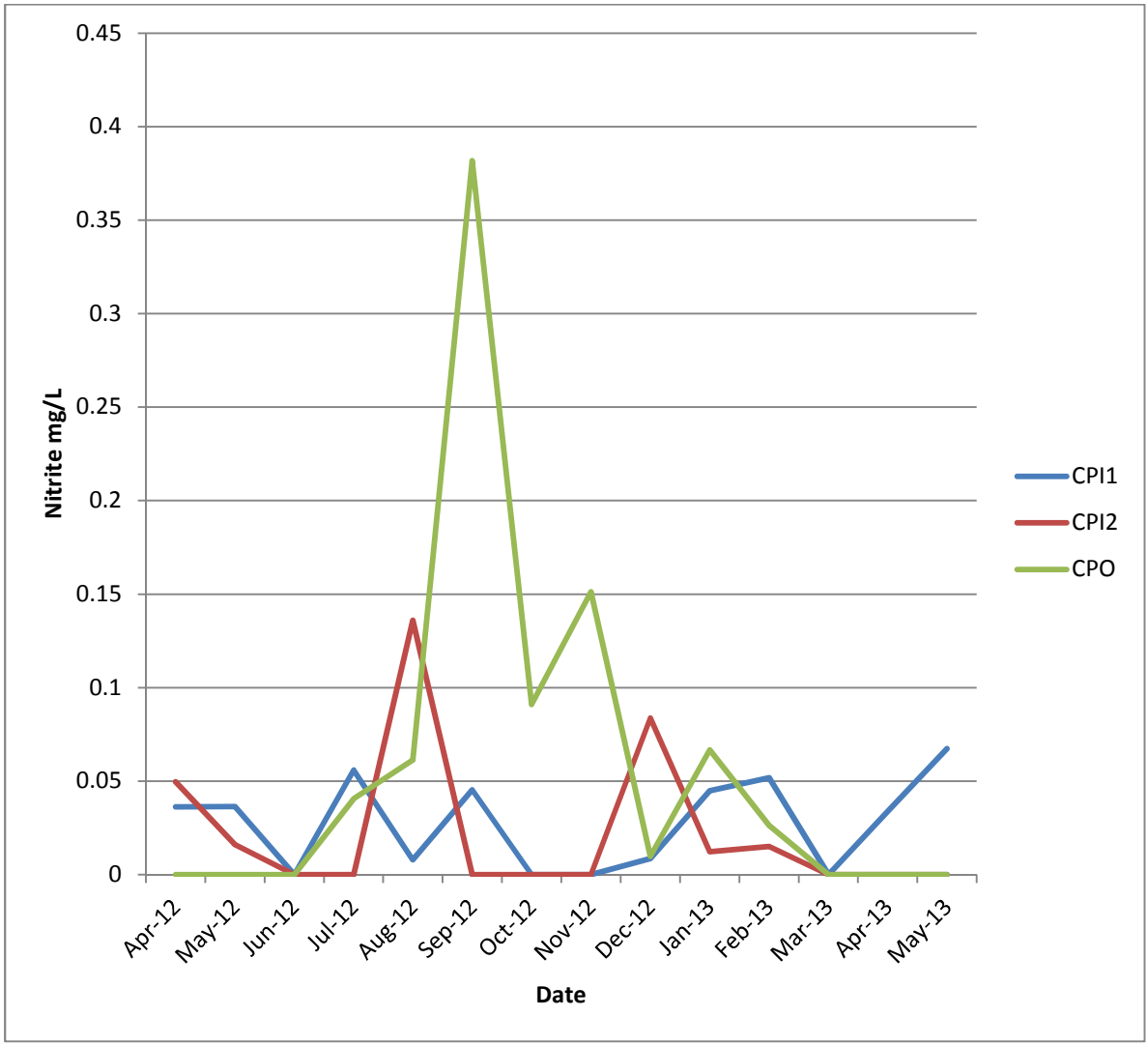


Figure 4.13 - Bodeilio HSSF nitrite over time.

The Graph shows nitrite concentration in the inflow and outflow of the system. From August to November the HSSF system at Bodeilio is acting as a source of nitrite. Although nitrate reduction is continually occurring as shown in figure 4.12, nitrite is not being completely lost from the system. Very low levels of nitrite are present in the inflow throughout the year.

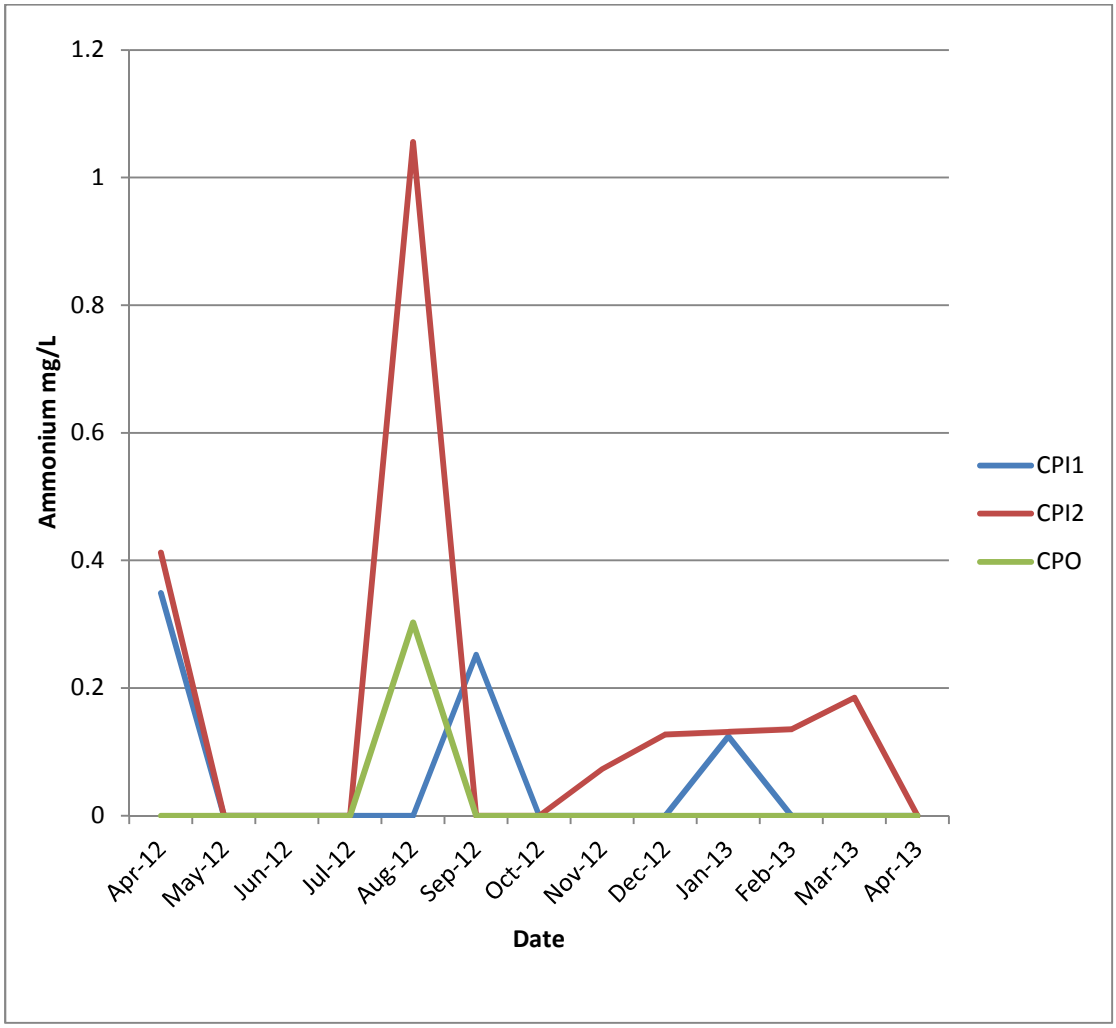


Figure 4.14 - Bodeilio HSSF Ammonium over time.

The graph shows the presence of dissolved ammonium in the inflow and outflow of the HSSF system. On multiple occasions the primary inflow (CPI1) of the HSSF system indicated ammonium presence. Sustained detection of ammonium is observed in the secondary inflow to the system (CPI2). Ammonium was removed from the water column throughout the monitoring of the system; however the largest peak of 1.056 mg/L observed in the secondary inflow in August resulted in detection in the outflow.

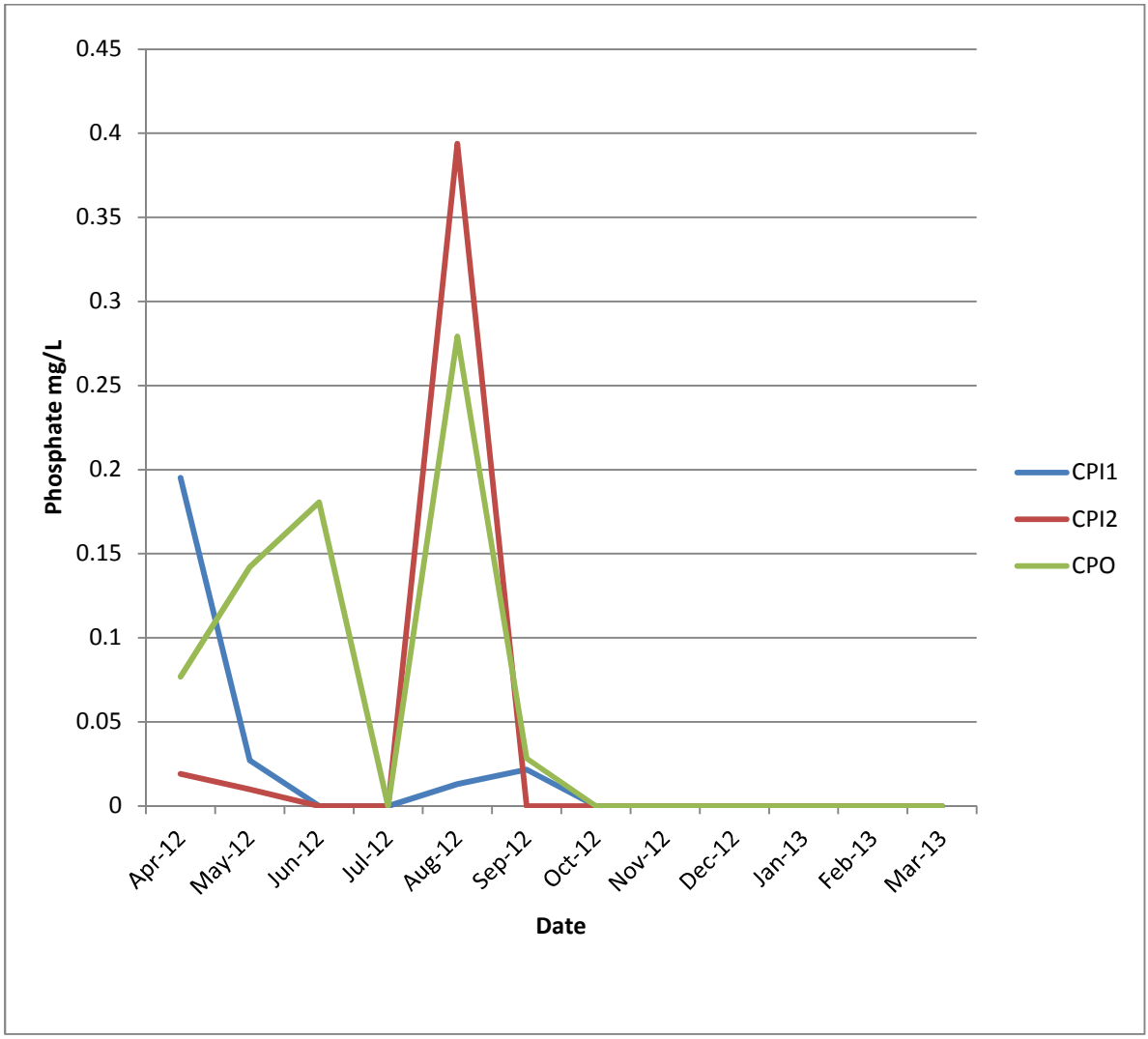


Figure 4.15 - Bodeilio HSSF Phosphate over time.

The graph shows that during the initial phases the system acted as a source of phosphate. The system was a source of P from installation until July. Very low concentrations of phosphate are observed in both of the inflows to the system during this period. Following initial stabilisation, the system offers some buffering to phosphate peak phosphate inputs of 0.4 mg/L Phosphate during August and lower input concentrations in September. No phosphate was detected at all during the following month.

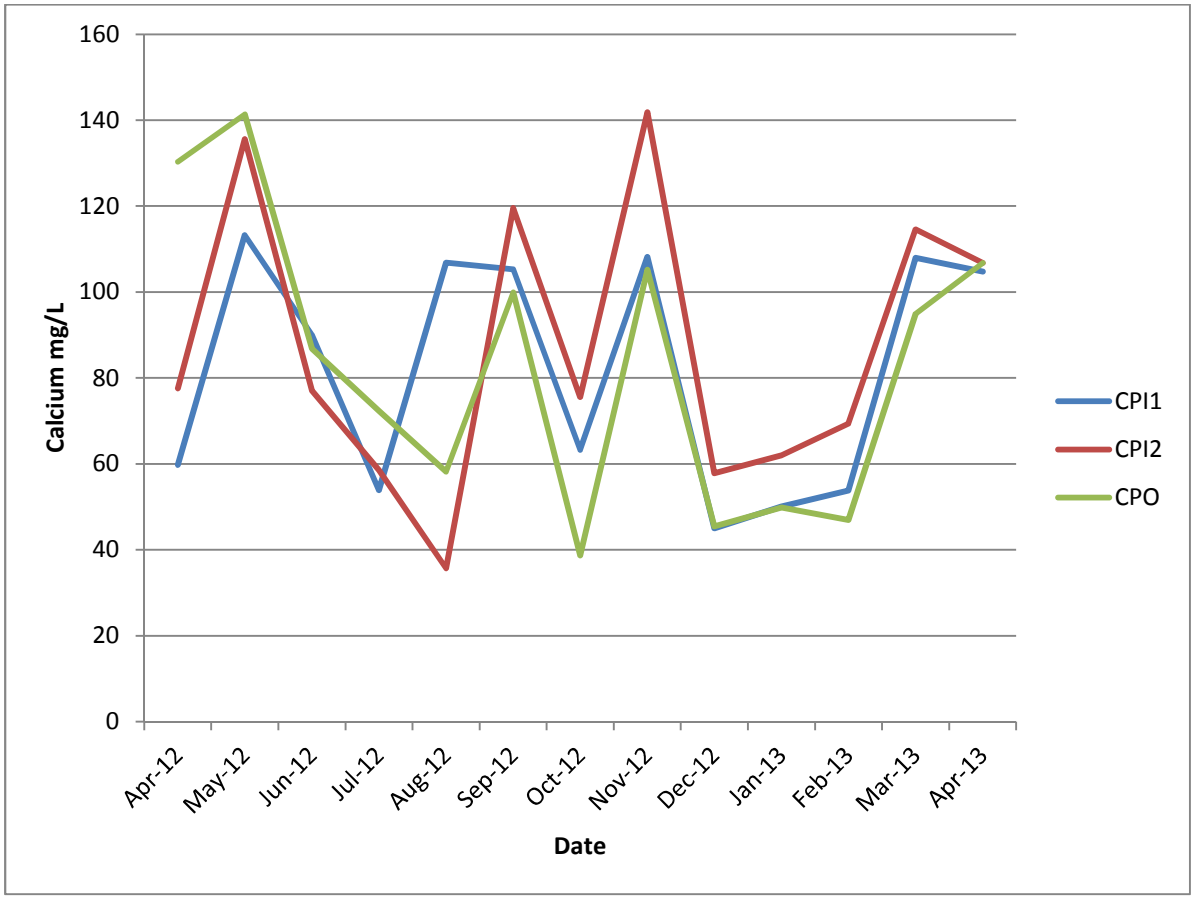


Figure 4.16 - Bodeilio HSSF Calcium over time.

The Graph shows that the installation of a constructed wetland does not significantly affect the concentration of calcium in the outflow from the system. From installation to August, calcium is being released from the system; whilst during September to completion of monitoring calcium is being contained within the system indicated by lower concentration.

4.13 Cae Gwyn

4.13.1 Pollutant Removal – Cae Gwyn FWS

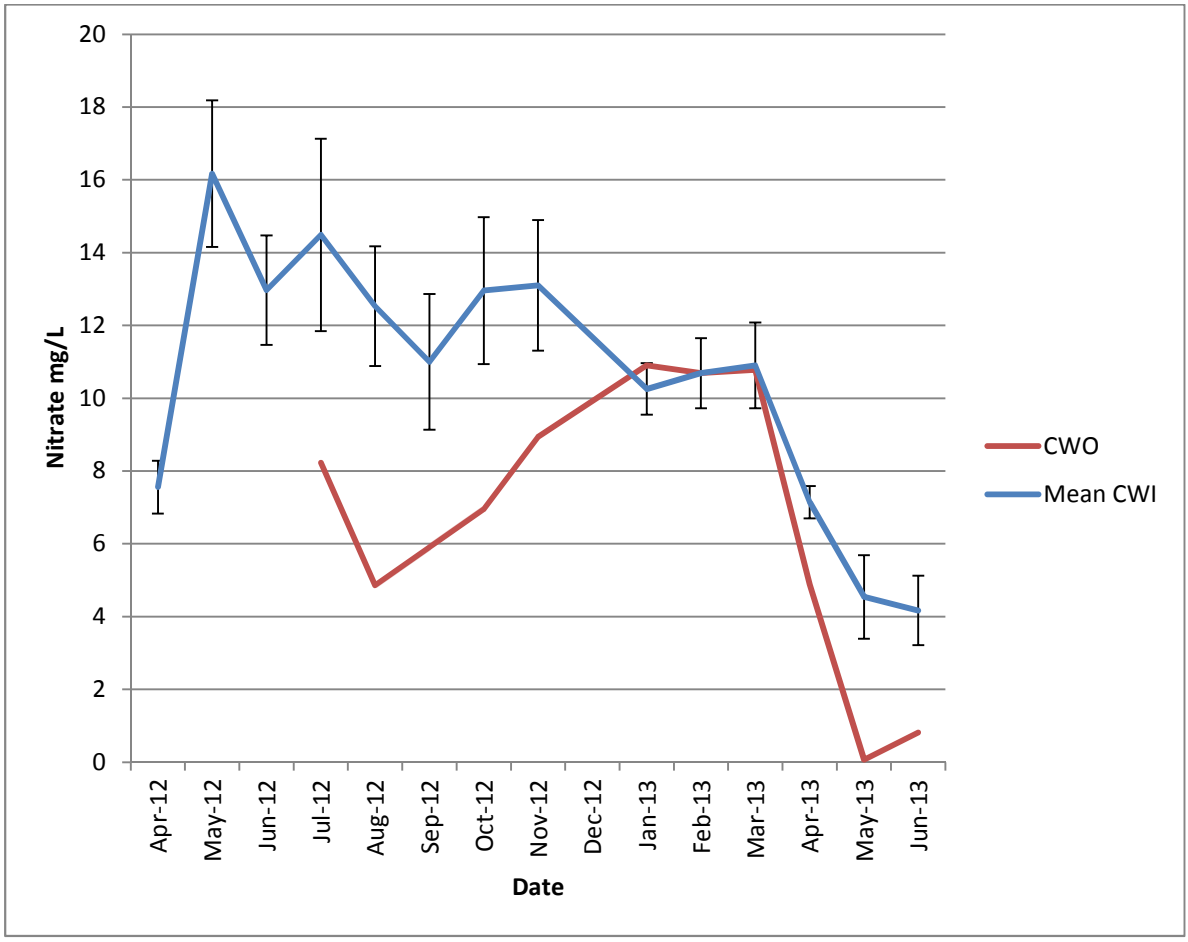


Figure 4.17 - Cae Gwyn Nitrate over time.

The graph indicates the nitrate reduction the system achieved in the initial stabilisation year. Reduced nitrate reduction is observed January to March whilst increased removal of nitrogen is observed for the rest of the year. Outflow was established on system completion in July. Inflow concentrations are averaged from 3 contributing flows where error bars represent standard error of the mean.

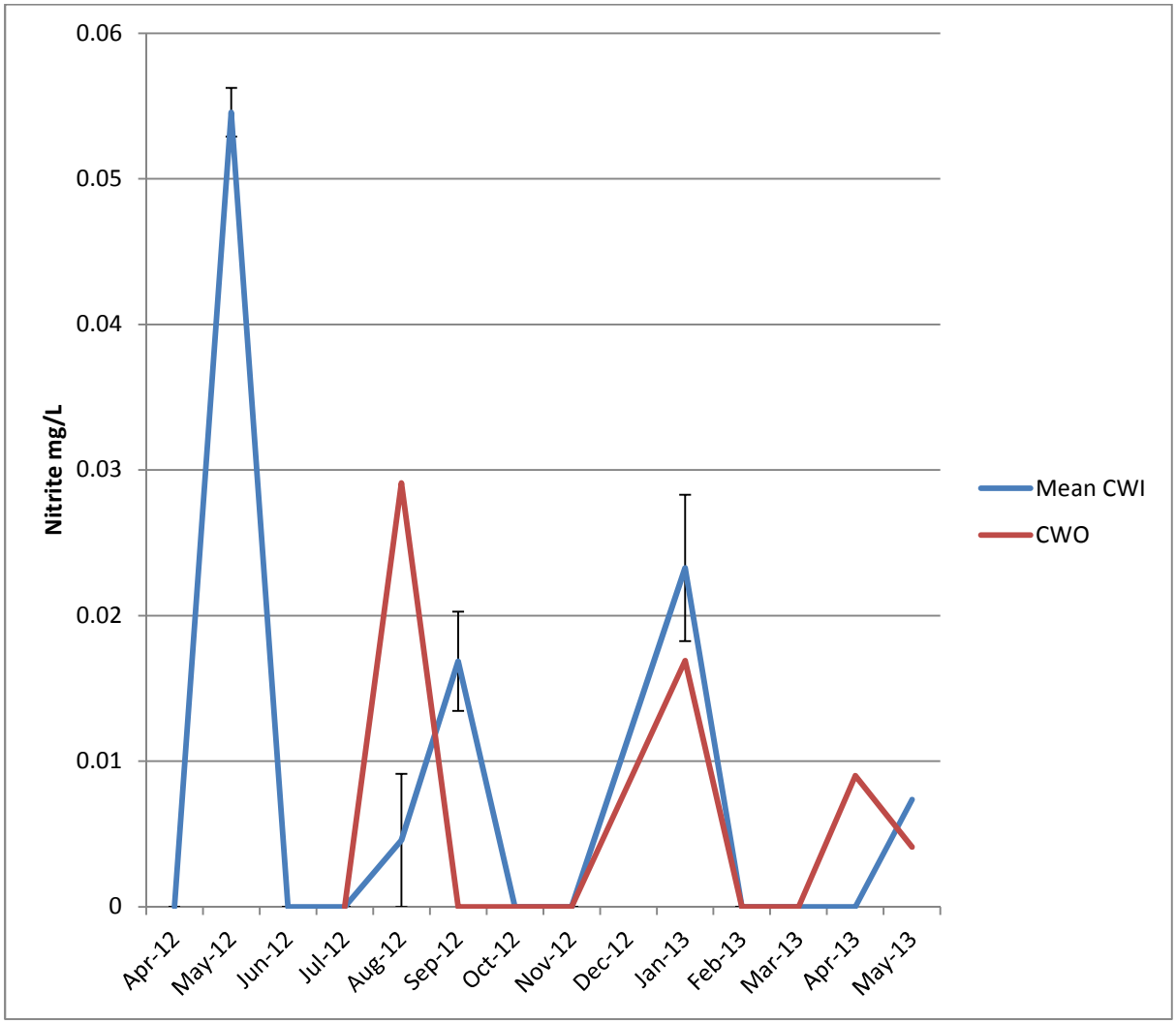


Figure 4.18 - Cae Gwyn Nitrite over time.

Analysis of dissolved nitrite indicates events in raised nitrite in the inflow. As in the Bodeilio systems, nitrite peaks occur in August and in January. Over the course of the analysis 4 peaks of nitrite were detected whilst 2 peaks of nitrite were detected in the outflow. Error bars represent standard error.

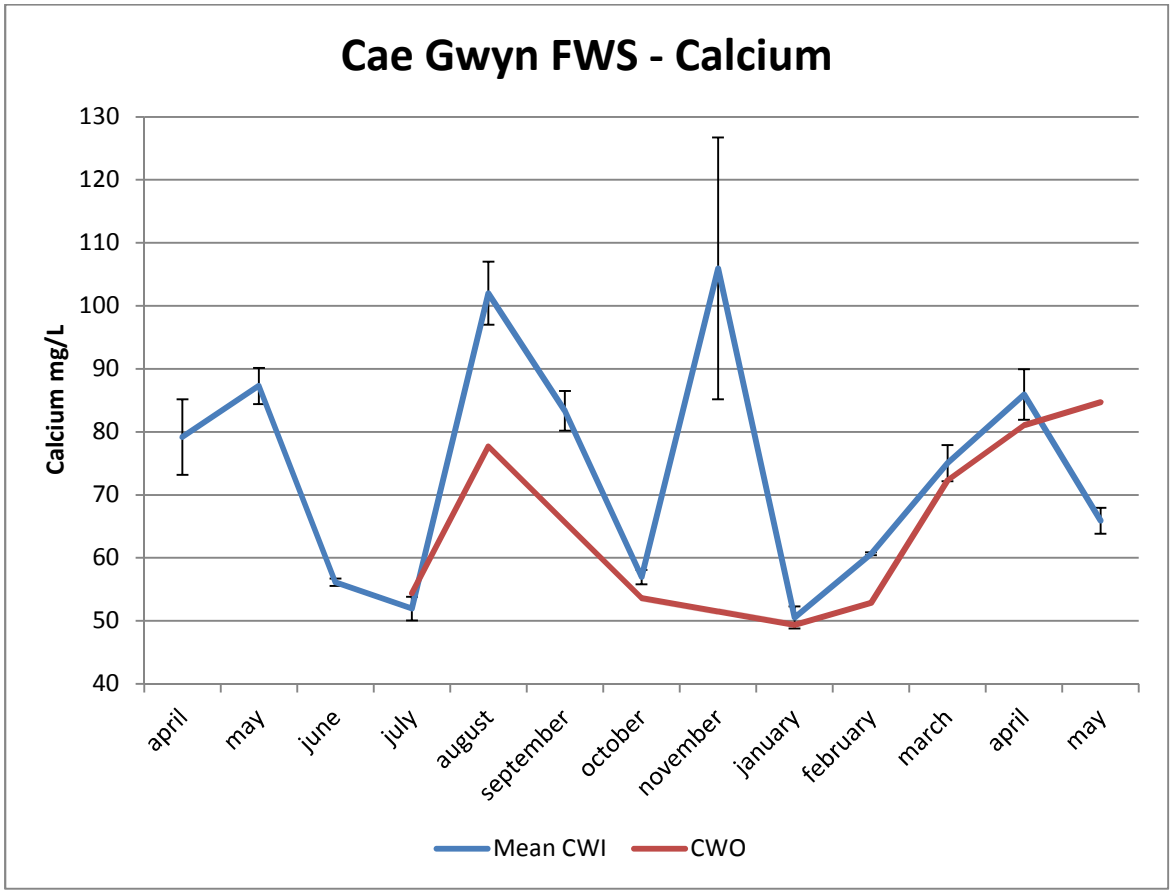


Figure 4.19 - Cae Gwyn Calcium over time.

The graph shows dissolved calcium ions within the inflow and outflow of the FWS system; here reductions in calcium are more notable than previous systems. Marked reductions in calcium are observed in August and November.

4.14 Bodeilio FWS Discussion

Despite the use of basic retention time scaling, on average 34% of nitrate was removed. The mean removal percentage value is low; however CW area was significantly restrained by the presence of good quality NVC wet fen meadow adjacent to the system, limiting CW size. A nHRT of 24 hours was utilised in the design minimised the effect on localised vegetation stands. This system has proved that although full nitrate removal was not achieved, water quality improvements are pronounced and significant.

Over 12 months of observed system operation, only a small number of *Phragmites* reached good level of growth (Figure 4.3). In FWS system, development of plant shoots, stems and rhizomes are fundamental to biofilm colonisable area. It is suggested that in following years, when density is increased, wetted plant surface area will rapidly increase resulting in increased biofilm colonisation and potential denitrification within the anaerobic zones of the biofilms.

Nitrite was also observed to enter the CW (figure 4.9). It is proposed that, due to a considerable distance that the water supplying the fen at this location travels from the enriched agricultural land to the CW, some denitrification has already been achieved and/or it formed a constituent of the fertilisers applied to the agricultural land. Nitrite, as described earlier, is a natural breakdown product in the nitrogen cycle. The CW can be seen to be reducing nitrite concentrations on passing through the system, further reducing the enrichment effect.

Phosphate, although reasonably low in concentration (0.1 mg/L maximum concentration observed), was largely removed. As highlighted earlier, the primary mechanisms involved in removing dissolved phosphorus from the water column are achieved by precipitation of phosphorus (Kadlec & Wallace 2008; Vymazal 2007)mechanisms that cause sedimentation. Primary mechanisms include binding of phosphorus with sediments in order to cause flocculation within the system. Precipitation as $CaPO_4$ is also a possibility considering small observed reduction in dissolved ionic calcium (Fig. 4.11)

Crucially, calcium was not significantly affected by passage through the CW system. This is of great benefit to the receiving conservation site where calcium is an important macronutrient to the plants that designate the conservation site as an SAC.

Parameter	Value
pTIS	3
K	35
C*	0.5
Q	43.2
Temp	10
theta	1.05
K_{temp}	21.48
area	80
C_i	4.5
C_o	1.16

Figure 4.20 - Parameters used for retrospective calculation of the P-k-C* model for the Bodeilio FWS system.

The values calculated for outflow concentration of nitrate are similar to the concentrations observed in the treatment wetland system.

4.15 Bodeilio HSSF Discussion

Figure 4.12 Bodeilio HSSF system indicates significantly higher nitrate concentrations than the previous system and greater removal is also achieved. However, fluctuating inflow provides concentrations provide an insight into nitrogen transformations in the catchment.

Reduced nitrate removal was observed from December to February where temperature dependant rate of biological degradation (k) is compromised. Similarly, increased winter rainfall may have reduced retention times due to increased flow. The inflow Q was estimated to infease by ~150% during storm events, significantly reducing retention time and negatively effecting pollutant removal. However temperature may have also had an effect on any denitrification occurring in the system.

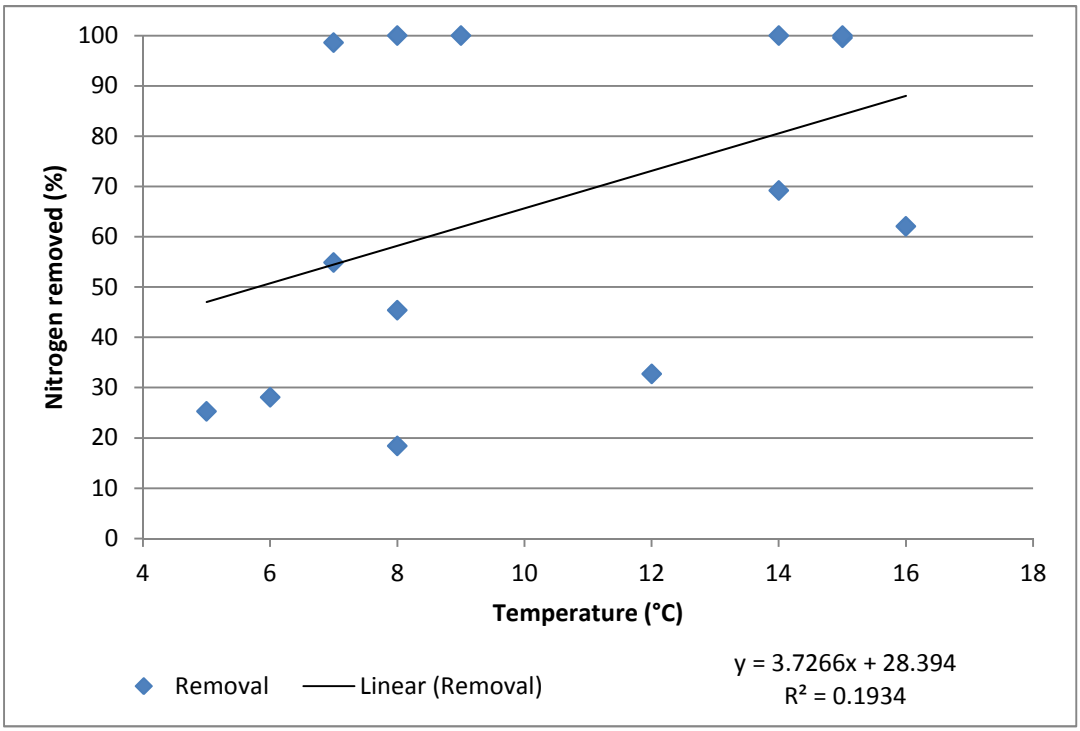


Figure 4.21 - Correlation between percentage removal of nitrate and temperature.

A degree of correlation between temperature and Nitrogen removed exists (R=0.1934). Temperatures accessed Accuweather (02/05/2014)

This positive relationship of rate of nitrate removal with increasing temperature related to the Theta factor of 1.05. This value indicated to what degree the rate of break down (k) is linked to temperature. A Theta value of 1.0 suggests no effect of temperature whilst the value of 1.05 indicated positive temperature dependence (Kadlec & Wallace 2008). Nitrite production is observed to occur during periods of increased nitrate in the influent, specifically August to November. This suggests during periods of excessive nitrate influx, complete breakdown of nitrate to gaseous forms of nitrogen is limited. Carbon availability, anaerobic conditions do

not seem to be limiting the rate of breakdown. Instead it is proposed that flow through the system is no longer entirely subsurface (Figure 4.4). It can be seen that the system shows a significant area of FWS at the terminal (Left) end of the CW. This is likely to have occurred due to the compression of the wood chip based carbon source media of the NitraBar hybrid system. Once FWS establishes in the HSSF system, hydraulic resistance is substantially reduced. This manifests itself in water flowing through the area of least resistance in the system, the surface water, rather than through the CW matrix, reducing contact with microbial denitrifiers.

Ammonium is suitably buffered and while the system was primarily designed with denitrification of nitrite in mind nitrification of ammonium is probable. This precursor to nitrate in the nitrogen cycle may provide an explanation to nitrite production and the reduced removal of N as gaseous substances. The increase in nitrite production coincides with periods of ammonium influx. It is proposed that when reconsidering the cycle as described by Shapleigh (2013) the system provides insufficient retention time for adequate pollutant breakdown of the ammonium constituent of the total nitrogen before denitrification can take place. When additional ammonium enters the system nitrification steps must first occur. This means incomplete breakdown for a percentage of the nitrate produced by system nitrification.

Phosphorus was only observed in significant concentrations during a single sampling visit in the inflows of the HSSF system. Early sampling indicated that the system was acting as a source of phosphate for the initial 3 months of operation. This is likely to be due to the addition of the wood chip media and breakdown of its more labile compounds resulting in a pulse of phosphorus being lost. However, positive results can still be observed in relation to phosphorus. No P was detected in the outflow of the system for the remaining months. This, in combination with no inflow phosphate confirms the system ceased to act as a source for phosphorus, secondly buffering capacity is demonstrated in August.

Calcium concentrations indicated that system acting as a source of dissolved calcium for the primary part of system stabilisation whilst in the latter part small reductions in calcium were observed. This may be due to the addition of limestone media releasing calcium in the early stages and potential formation of carbonates as a result of anaerobic conditions in the treatment bed in the latter stages. It can also be observed that during the initial growth phase of the plants during the summer months calcium is also lower in concentration, possibly due to the demand as a macronutrient in the tissues of the CW macrophytes.

4.16 Cae Gwyn Discussion

At the FWS system installed at Cae Gwyn, the biomass stand took longer to naturally develop due to low density planting of the system. Similar to the two previous systems, reduced nitrate removal was observed during January to April. The system utilises three large streams that issue from 3 separate groundwater fed springs, supplied by a reasonably large catchment. This effect results in high inflow observations during winter storm events, this factor combined with low temperatures affect the rate of pollutant degradation significantly, as well as decreasing nominal retention time.

No ammonium or phosphate was detected in any of the inflow or outflow samples taken from the system. This is supported by the ground water dependant nature of the springs supplying the fen and CW.

It is to be noted that the nitrate concentration within the spring water being treated by the CW is far more stable than that observed in the agricultural runoff systems seen on Bodeilio SAC. These results are also lower than expected when comparing them to initial monitoring data where concentrations of nitrate exceeded 24 mg/L (itnitial monitoring period – data not shown). It is proposed that some of the nitrate is being transformed or taken up as it passes over an area of fen above the CW in the catchment. The 3 contributing springs flow across a length of fen ranging from approximately 40-80m where transformations of nitrate may be occurring. This manifests itself as reduced concentrations of nitrate observed at the inflow confluence to the CW. This process could be confirmed by taking samples along a transect along the flow path of the spring water, from the confluence zone to the individual spring heads.

Calcium flowing into the Cae Gwyn CW fluctuates significantly, whilst the outflow concentrations of calcium are observed to be more stable. Similarly to phosphorus binding with sediment, calcium is also able to bind with sediments to form calcitic muds or marl. Potentially higher periods of flow and suspended sediment could contribute to the reductions in calcium observed in the system. Secondly, the flows produced by the spring above the CW in the catchment were too large to pass totally through the CW. Lowest concentrations observed in the outflow of the CW were 50 mg/L, and although this is a significant reduction, the water bypassed the system may still hold sufficient calcium for the development of M13 communities and alkaline fen. Although this has implications for enrichment associated with the “un-treated” water, significant reductions in nitrate were seen in a large percentage of the spring water flowing through the system. Similarly the vegetation in the CW will represent a significant calcium demand during plant growth phases of the CW, potentially quantifiable by tissue calcium measurements

One of the additional suggestions for the reductions in calcium observed is linked to the colonisation of the CW by a calcareous fen indicator species *Chara variegata*. This species of stonewort relies heavily on calcium rich waters and becomes covered with and contains large amounts of calcium carbonate (CEH 2004). *C. variegata* was found in dense mats all through the constructed wetland. This species may be responsible for the large reductions in calcium observed. However, this may be of benefit to the pollutant reduction capabilities of the system by producing large amounts of wetted plant surface area for the colonisation of microbial biofilms, which play an important role FWS wetlands. Indeed, Kadlec and Wallace (2009) mention that many large wetland systems in the USA rely entirely on submerged aquatic vegetation. The presence of a continual depth of water which is unshaded may help maintain this species within the treatment system and associated biofilm colonisation (CEH 2004)

Figure 4.22 and 4.23 - *Chara variegata*, sourced directly from the inflow of the FWS system.

4.17 Conclusions and Total Removal

Using mean inflow volume of 2 L/s for each CW, the calculated mean nitrogen compound removal for each CW based on inflow outflow data, averaged for the duration of the monitoring period, totalled for all 3 of the CWs installed it can be concluded that more than 243 kg of elemental nitrogen has been removed from the water column in 14 months of system operation. When putting this into the context of the agricultural enrichment pressures in the catchment, this is equivalent to 1200 kg of bulk fertiliser (although nitrogen contents of fertilisers vary widely by manufacturer).

If the treatment wetland systems had not been implemented by the Natural Resources Wales EU LIFE Fens project this nitrogen would have found its way directly into the conservation site, severely affecting the biology of the fens. Treatment wetlands have in the past proven to be a suitable tool for pollutant degradation. Here, even where available space is at a premium on the conservation sites, it has been shown that improvements in water quality can be achieved.

Although highly technical and engineered systems can be used for industrial pollutant removal applications where processes are significantly intensified, passive natural looking systems are highly desirable in a conservation setting. Moreover, the systems discussed here have proved to be capable of performing the LIFE project targets for water quality amelioration. In scenarios such as these, total pollutant removal, are not likely to be achieved due the pressure for space. Intensifying biological treatment may have been a method by which increased treatment could be observed, however systems such as this are more costly in terms of set up and maintenance costs. The passive and inexpensive systems on the Anglesey conservation sites have fulfilled their purpose despite not achieving complete pollutant removal.

Chapter 5 - Investigation into Constructed Wetlands for Water Quality Remediation into Calcium and pH Sensitive Fen Sites – Preliminary Feasibility Study

5.1 Introduction - Calcium Mobility and Dependant Conservation Communities

Constructed wetlands (CWs) have been used as a cost effective, passive and semi natural method by which water quality can be improved or manipulated. As discussed in Chapter 4, the Anglesey and Llyn Fens LIFE project run by the Natural resources wales (NRW) is one such example where this technology has been shown to be advantageous. The LIFE project set out to restore 751 hectares of wetlands to a favourable or recovering state after a period of mismanagement and neglect. There is a great potential for these sites to fully recover, many of which have already been classed as SAC's or SSSI.

NRW's conservation strategy is linked to the category of wetland habitat in question and environmental pressures upon it. One of the rarest mire sub-communities found on the LIFE sites is M13 Mire. The LIFE sites are unique because both alkaline fen and calcareous fen habitats (listed under Annex I of the EU Habitats and Species Directive) occur together. This location is the sub community's western UK stronghold. This community is described by the Elkington et al. (2001) in the National vegetation classification; "*M13 dominated by Shoenus nigricans, the black bog rush. This community occurs only on peat or mineral rich, specifically calcium, soils. For this community to occur, irrigation by calcareous base rich water with an oligotrophic nutrient content is essential. Water flow-type also seems to be highly important as communities are most regularly found where shallow, sheet flow or flushing is present. This may occur at a spring or similar issue, seepage faces or flush lines. Water rich in calcium also flows from many of the springs on the sites; this is due to the presence of limestone within the aquifers supplying the spring heads. Calcium becomes dissolved within the water due to chemical weathering of limestone within the underground aquifers. Other topogenous areas may be suitable, providing close contact with water high in lime based substrates.*"

These communities thrive in oligotrophic nutrient conditions; therefore problems occur when nutrient pollutants increase. Many of the aquifers, springs and streams supplying the fens are highly concentrated with nutrient pollutants such as nitrate, which cause eutrophication within the fens. Currently, one of the main pressures being exerted upon the LIFE sites is the addition of nutrient pollutants such as nitrogen and phosphorus, arriving into the wetlands via a large number of issue points, including spring heads, streams and field drains. Pollutants arrive in the watershed from many different sources primarily caused by heavily fertilised agricultural land. This is of great concern when trying to improve and conserve the habitats and vegetation exhibited in the conservation sites which rely on oligotrophic irrigation. The observed addition of nutrient pollutants to the conservation sites has driven the invasion of competitive species such as *Phragmites australis* and *Cladium jamaicense* and *Typha*

latifolia, which are species that possess high biomass accumulation resulting in soil building in the system.

CWs have the potential to alleviate some of the pressure placed on these communities. It is essential that as the nutrient enriched water passes through the CW and nutrient pollutants are removed from the water column, this is a primary function in conventional CWs. Excessively high levels of nitrogen are removed via denitrification, following nitrification where necessary. This can also be achieved by soil building caused by the slow decomposition of organic matter or sedimentation.

However, part of the requirement for calcium by the mire communities which will receive the treated water necessitates that calcium must remain in solution (Elkington et al. 2001). It is desirable that the build-up of calcium within the CW is minimised due to its demand by the M13 communities. As yet it is unknown if M13 will develop when conditions are oligotrophic and calcareous, or whether the community is most reliant upon calcareous water alone. It is reasonable to deduce that if nutrients are not minimised M13 will be unable to colonise due to the rapid invasion of more competitive graminoid species.

This is the crucial difference between this piece of research and that which has been undertaken previously. Much similar research has been undertaken involving water quality improvement in relation to calcium content in a range of different research settings, but more often calcium is addressed as the pollutant. For example the work of Mayes et al (2009), where extreme concentrations of calcium were observed leaching from lime rich industrial spoil heaps. This was undertaken in order to assess the ability of CWs to neutralise high alkalinity, generated by the weathering of lime. The rationale for reducing strong alkalinity is due to the direct toxicity caused by heavily alkaline waters. Alkalinity values in excess of pH 9 have been shown to be toxic to higher aquatic fauna (Mayes et al. 2009a). Wetlands soils were selected as a suitable method to remove calcium; this is due to the high concentration of CO_2 observed in the wetland environment, enabling the precipitation of calcium as calcium carbonate. High respiration rates of heterotrophic microbial communities are responsible for the production of anaerobic CO_2 rich wetland environment (Mitsch & Gosselink 2000). Precipitation of the calcium with CO_2 successfully leads to the lowering of pH in the outflows and receiving waters.

Using knowledge gained in the Ca removal study system, modifications to CW design may be introduced in order to preserve calcium in solution. Precipitation of calcium highlighted in Mayes et al (2009), relies upon its combination with CO_2 arising from microbial respiration. If CWs are designed to minimise microbial respiration and therefore CO_2 production, this

may result in the reduction of calcium precipitation within the treatment wetland, allowing it to remain available to calcium dependant plant species.

Biogeochemical cycling of calcium tends to become dominant when a lack of iron is observed, resulting in calcium carbonate formation (Kadlec & Wallace 2008). This process has occurred over time at a number of the LIFE sites, where calcium carbonate deposits have become combined with sediments to form calcitic muds or marl. Observations of oxidised Iron have been recorded on parts of the site but not in locations where Marl is present.

In most instances, calcium is considered to be a micro nutrient to most plant species, whilst exhibiting marked seasonal fluctiations in availability and spatial dynamics (Wetzel 2001). It is generally recognised that around 0.77% of dry weight of wetland plants is made up of calcium (Mitsch & Gosselink 2000) where the main repositories for such accumulated ions is the central vacuole and cell membranes (Wetzel 2001). Generally in treatment wetlands little change is observed in calcium content from inflow to outflow. However marked changes in biogeochemical cycling of calcium can occur when iron is observed to be lacking (Kadlec & Wallace 2008).

There are a number of studies that have been undertaken in order to understand the preservation of calcium in solution; however these have not been undertaken in the field of biogeochemistry. One such example arises in the analysis of mineral uptake in the field of nutrition, where inositol phosphate breakdown compounds were found to increase the solubility of calcium via chelation (Shen et al. 1998). Here the study concluded that once the phosphorylation of phytate occurred, its solubility increased. This allowed for the increase in binding strength to Ca^{2+}, in turn allowing greater absorption from the mammalian digestive tract. Inositol is a low molecular weight carbon based molecule dominated by a cyclic carbon structure. This compound was found to have an greater ability to promote calcium uptake into the blood by chelating calcium.

Secondly, the research concluded that, of a number of forms of inositol phosphate, molecules containing fewer phosphate functional groups were able to increase calcium uptake more readily. Less phosphorylation was required to optimise solubilisation of the calcium. This could mean that LMW cyclic hydrocarbons, especially those that are unsaturated, may be able to chelate calcium and prevent precipitation.

CWs are known for their ability to act as sinks for heavy metal pollutants (Mitsch & Gosselink 2000). And indeed, many wetland plants are able to take up heavy metal pollutants into their tissues (Weis & Weis 2004). Numerous avenues have been explored for the use of CWs for iron removal from acid mine drainage where the main contaminants are sulphur and

Iron II (Johnson & Hallberg 2005). However one particular piece of research may provide a method by which calcium could be maintained in solution, as opposed to the research carried out by Mayes et al (2009), where calcium was considered the pollutant and precipitation with CO_2 was required. White et al (2011) based their research on the ability of wetland plants to aid the oxidation of iron II to iron III. Plant processing and biomass metal accumulation was questioned due to the small percentage of metal in dry biomass. White et al (2011) found other locations where iron in solution actually increased on passing through the wetland.

White et al (2011) discuss how DOC availability leads to the increase in solubility and availability of metals. The study concluded that ferrous iron could remain in its reduced form in the presence of DOC. In particular a fraction of DOC known as phenolics, which are of low molecular weight, inhibited the oxidation of ferrous iron. Iron was shown to form complexes or chelate with this fraction of the DOC maintaining its solubility.

Ferrous iron arising from the drainage of disused mine shafts has an ionic value of 2+, identical to that of calcium. It is proposed that DOC or a molecular weight fraction thereof, may also allow for calcium chelation or complex formation in order to maintain solubility a prevent precipitation with CO_2 within the treatment wetland.

From the above evidence it can be hypothesised that DOC may allow for chelation or complex formation with ionic calcium. This hypothesis may be expanded upon; in that there will be an optimum molecular weight range that allows calcium chelation. Inhibition of microbial respiration may reduce the possibility of calcium carbonate formation within the CW allowing the calcium to successfully pass through the CW in solution. This allows for uptake or precipitation within the calcareous and alkaline fen conservation sites.

This research will not only provide an insight into calcium mobility, behaviour and fate, but also elucidate the appropriate DOC releasing organic material to be used in CWs, where maintenance of calcium is essential.

5.2 Preliminary Investigation into Calcium Association with DOC of Varying Molecular Weights

In order to begin to understand best practice for the installation of CWs on the LIFE sites, used for the maintenance of calcium, the possibility of affecting the calcium concentrations by manipulating DOC must first be examined. This was the purpose of the preliminary investigation detailed here.

5.2.1 Rationale for Methods Utilised in Multiple Stage Analysis

There were a number of stages required to understanding calcium chelation with DOC. The first of which was to investigate if there is indeed, any chelation or association with DOC released from some form of natural substrate that chelates with calcium. Preliminary findings may influence the system set up of the mesocosm experiment. The final stage of the research involved mesocosm CW design in order to ensure calcium solubility through the course of the systems detailed in chapter 6. Results may be able to inform design for field scale best management practice on the LIFE sites.

According to the literature, calcium chelation should occur in calcium laden water samples that have DOC added to them, whilst no chelation is expected to be observed where de-ionised water was added as a control.

From previous research it could be predicted that DOC leached from *Calluna vulgaris* would provide DOC at a number of different molecular weight groups. Initially the organic source was *Calluna* due to the relevant successful release of DOC from this material in the previous experiment relevant to Floating Constructed Wetlands (FCW) in Chapters 2 and 3. The FCW chapter analysis concluded the release of phenolics, among others, were dominant fractions of the DOC released.

The organic material was collected and placed into a container of water to allow DOC to leach out; this liquor was then filtered through 0.45µm filters. Calcium chelation was assumed by increased total Calcium concentration associated with the specific MW DOC. This association was achieved by using 3500 Dalton pore size dialysis tubing, into which the DOC solution was added. Only DOC with molecular weight greater than 3500Da was retained in the tubing. The experiment was designed to allow calcium to freely diffuse across the membrane reaching equilibrium inside and outside the dialysis tube. Where chelation occurs, it is expected that the concentration gradient will be altered allowing more ionic calcium to equilibrate across the membrane into the dialysis tube, resulting in increased total calcium, whilst ionic calcium will be balanced.

Total calcium was measured by flame photometry, an electrical or high temperature method whereby light emission at 422.4nm from the burned chemical to be analysed determines the concentration (Barnes et al. 1945).

Once DOC-calcium chelation had been proven, the DOC was analysed using Size Exclusion Chromatography. This was done in order to assess if, as in White *et al* (2011), there is stronger chelation with a specific molecular weight range. However, this is discussed in the following Chapter (Chapter 6).

5.3 Methods

5.3.1Calcium Solution

A stock solution of 40 g/L Calcium Chloride was prepared and added to ultrapure water in order to achieve a working solution of 100mg/L of Ca^{2+}. This solution was added to the test container at a later stage.

5.3.2 DOC Stock Solution for Stage 1 *Calluna* Only

A stock solution of DOC was created by leaching soluble carbon from approx. 250g of *Calluna vulgaris* material into 700ml of ultrapure grade water. This produced a solution of 358mg/L^{-1} using a 0.45 µm membrane filter and DOC of 351mg/L^{-1} after filtration through 0.2 micrometres. No sparging of DIC was undertaken as organic carbon was assumed to be sufficiently dominant. The pH of this stock was 5.5.

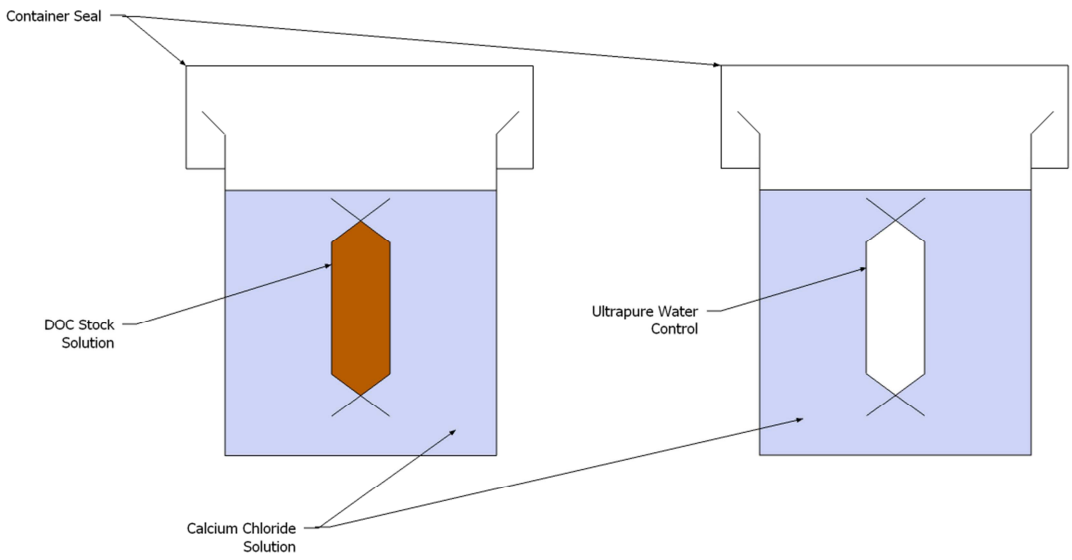
The DOC solution was diluted to 40mg/L^{-1} for testing to mimic field concentrations of DOC measured at a NitraBar system on Cors Bodeilio, Anglesey.

5.3.3 Stage 1 Experimental Setup

60ml of 100mg/L^{-1} calcium chloride was added to a sealable container. Into which a section of 3500 Molecular Weight Cut-Off (MWCO) or Dalton (Da.) dialysis tubing filled with 20 ml of DOC stock solution was added. Calcium should pass freely across the dialysis tubing membrane whereas the DOC solution was retained within the tubing. Therefore any net gain in total calcium should be due to DOC-calcium chelation, rather than diffusion.

Samples were taken from within the dialysis tubing and from the test container. Comparisons in total calcium allowed analysis of chelation level of the DOC solution

Five replicates for DOC chelation and 5 control replicates using ultrapure deionised water were used. The containers were left in a temperature control room at 10 degrees to mimic field temperature.



Twenty five millilitre samples were taken immediately after addition, 24 and 48hours after addition. 48 hours was the maximum time period, this was to replicate parameters observed at current NitraBar systems at Bodeilio SAC (Chapter 4).

5.3.4 Stage 2 Calcium Release from Associated DOC

The MWCO of the dialysis membrane is 3500 Da, therefore it is possible that DOC of this molecular weight or smaller may reach equilibrium between the solution inside the tubing and the external solution. The second stage of the test involved taking the bag from experiment 1 containing DOC >3500Da and placing it into ultra-pure water container. This process was used in order to assess the association of calcium with any <3500 Da DOC that remained in the tubing at stage 1.

If association with the lower MW rage is consistent, calcium will be observed to pass through the membrane with the associated lower MW fraction of the DOC retained in the tubing from experiment 1.

5.3.5 Stage 3 Calcium Association with DOC Mixture

In order to further confirm any association between DOC and calcium, an increased range of MW DOC fractions was created by the combination of DOC from various organic substrates. The initial experiment was repeated utilising a 24 hour experiment period. This was achieved by leaching DOC into ultra-pure water using organic barley straw, peat based compost, heather (as used in stages 1 and 2) and Coir, a material derived from coconut fibre, in the same method as described in section 5.3.1.3.

5.4 Analytical Methods

5.4.1Total Calcium Analysis

Total calcium was measured using an atomic emission flame photometer. Standards were created using Calcium Chloride consisting of 0, 20, 40, 60 80 and 100mg/L of Ca.

5.4.2 Analysis of DOC Molecular Weight

Molecular weight range was analysed using a Polymer Laboratories high performance liquid chromatograph set up for size exclusion chromatography (HPLC SEC). Samples are carried by a mobile phase of a phosphate mobile phase. On coming into contact with the solid phase of the column low molecular weight (LMW) molecules become more strongly bound to the solid phase of the column than higher molecular weight (HMW) fractions. Therefore HMW fractions are observed earlier than LMW fractions. A wide range of standards are used to act as retention time standards, 150K, 77K, 32K, 13K, 4.3K and vitamin B12 as 1.356K Daltons

This process will provide evidence to support calcium chelation with specific molecular weight ranges. Response is represented as millivolts and is proportional to amount of molecules of a particular weight, whilst time in minutes is proportional to weight.

5.4.3 Statistical Analysis

ANOVA analysis was used to determine any statistically significant difference in the data observed. Following the method stated in Chapter 2.

5.5 Results

5.5.1 Stage 1 Results

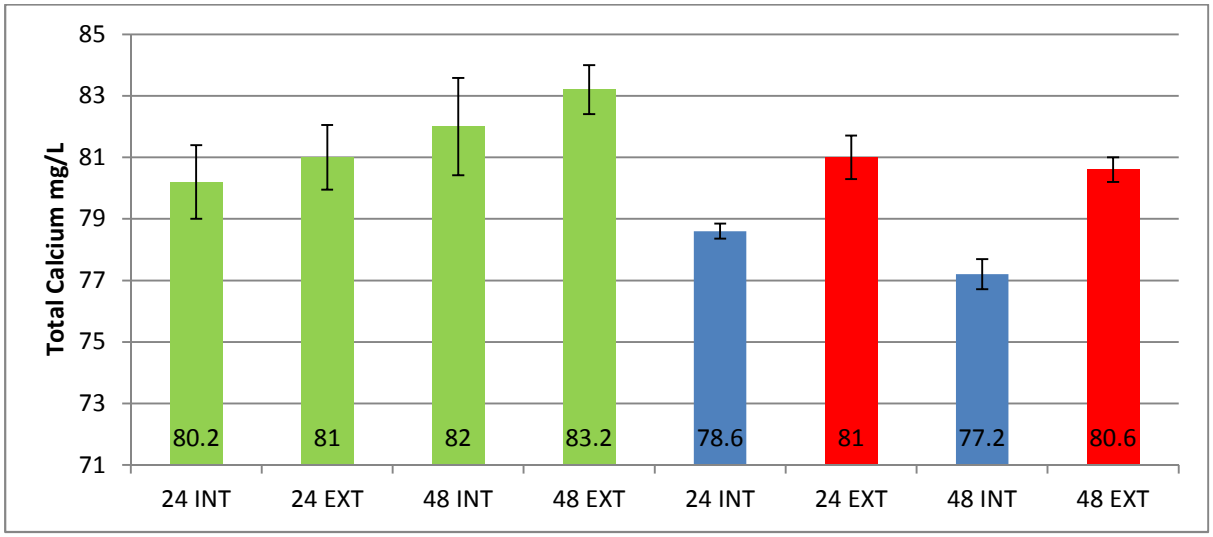


Figure 5.1- Total calcium for both DOC treatment (Blue internal and Red external) and the control (Green) dialysing experiments. INT refers to total calcium within the dialysis tubing whilst EXT refers to the solution within the test container. 24 and 48 refer to the time period in hours between treatment or control solution addition.

Both of the controls for both of the time periods showed no violation of having equal variances. No significant difference lies between the INT or EXT total calcium concentrations at the $p<0.05$ level of significance. However, significant differences were detected at both time periods for the DOC test solutions. Lower Calcium concentrations were observed inside the dialysis tubing, indicating increased association with the lower MW constituent located externally of the tubing. The calcium has 'actively' moved across the membrane in association with < 3500Da DOC.

5.5.2 Stage 2

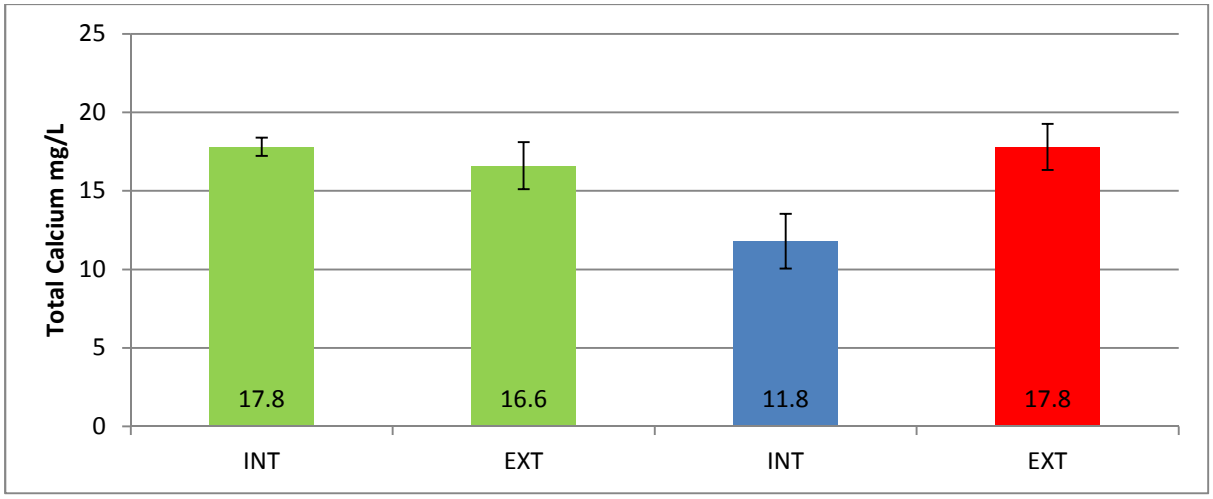


Figure 5.2 - Total calcium re-release test.

Dialysis tubing removed from the stage 1 and replaced into ultrapure water. Calcium concentrations observed in the control indicate similar values whilst greater calcium is detected outside of the dialysis tubing (EXT Red). A significant difference in calcium concentration was observed between internal and external calcium concentration in the DOC treatment, whilst no significance was found in the control .

5.5.3 Stage 3 - Combination of Mixed DOC Sources Results

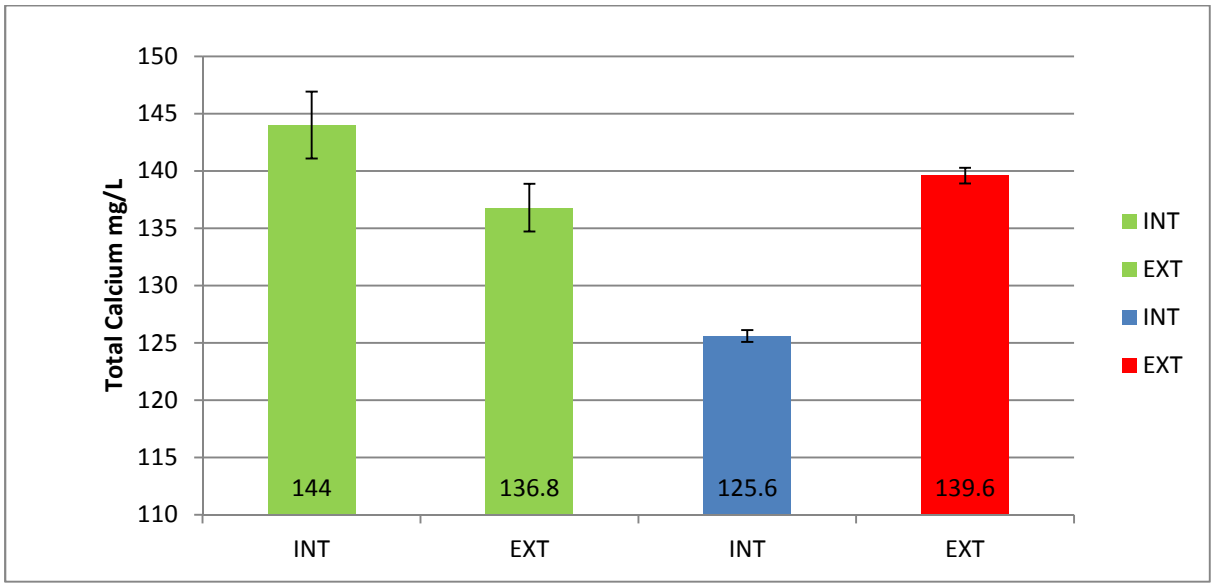


Figure 5.3 – Calcium association with DOC of differing MW's utilising DOC from multiple organic sources over 48 hours.

The graph indicates greater calcium concentration associated with Lower MW DOC (EXT Red) than high MW DOC retained in the dialysis tubing. Control samples indicate a greater concentration of calcium inside the dialysis tube; however this was not shown to be significant. Calcium concentrations show a similar results compared to the results from Stage 1 and 2. Increased calcium is associated with DOC <3500Da in the DOC treatment and was shown to be significant.

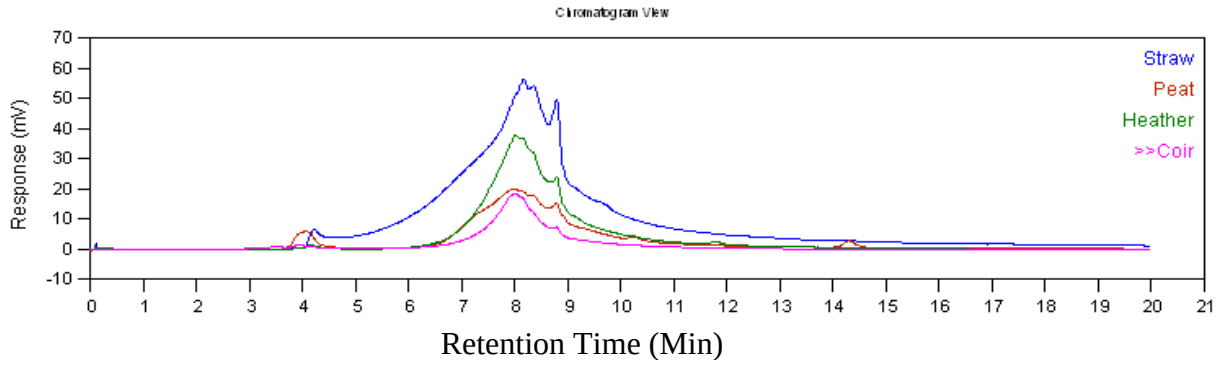


Figure 5.4 - HPLC analysis of each separate source DOC released from respective organic material.

The DOC analysed The Chromatograph demonstrates the diversity of molecular weights present in each type of DOC produced. Also an insight is given into relative concentration of each molecular weight fraction of the DOC as indicated by the strength of the response in millivolts.

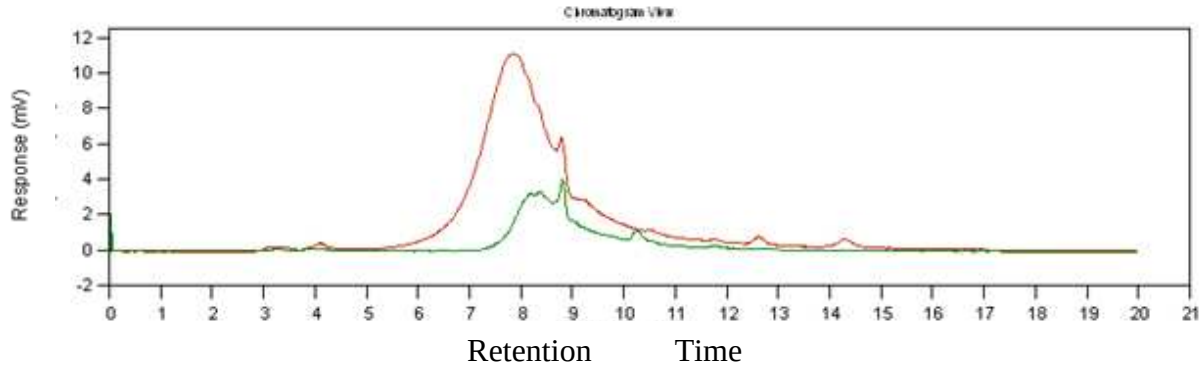


Figure 5.5 - Internal and external dialysis membrane DOC molecular weight.

The graph represents the DOC molecular weight spectra following dialysis. The Red line indicates the MW spectra of the DOC within the dialysis tubing <3500Da at 7.5 minutes whereas the GREEN line represents the DOC outside of the dialysis tubing. In this instance no DOC is observed before approximately 7mins 30sec. These data confirm the separation of the HMW DOC, confining it to the dialysis tubing.

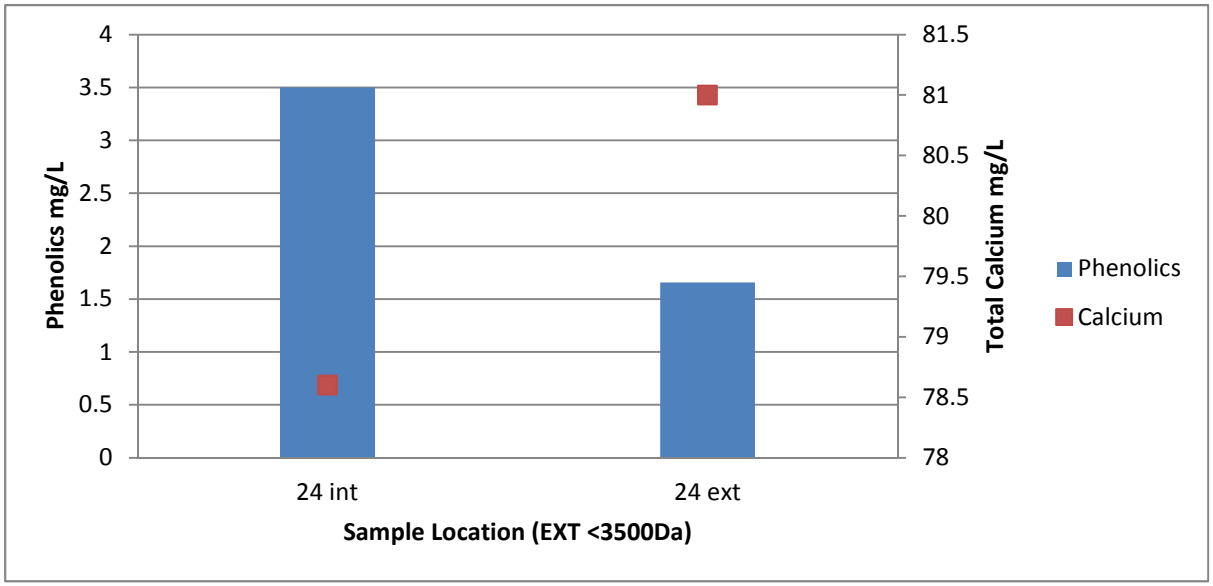


Figure 5.6 – Phenolic-calcium relationship.

Calcium is observed in greater concentrations where <3500Da DOC exists. The effect is especially pronounced when plotted against phenolic concentration. Concentration of phenolics was statistically significantly lower in EXT 24 samples.

5.6 Discussion

In all 3 levels of experiments calcium was found to associate, chelate or bind with DOC of LMW (<3500MWCO). It is proposed that the lower molecular weight DOC possesses more potential sites for chelation or binding (Weishaar et al. 2003).

Secondly, it was expected that the presence of phenolics would act as a means of chelation. This idea was explored by White *et al* (2011) where Fe^{2+} was found to chelate with phenolics in an acid mine drainage wetland. It is reasonable to suggest that Ca^{2+} may bind in a similar way. However this does not immediately seem to be the case if we consider figure 5.6 where lower phenolics were associated with greater calcium concentrations. However, taking SEC peak height as a proxy for total DOC observed internally and externally with respect to the dialysis tubing (Fig 5.5), it can be seen that the total DOC within the dialysis tube is of greater concentration than that of the external DOC samples. Given that phenolics are a constituent of DOC is the potential for phenolic binding cannot be excluded due to the presence of these compounds on both sides of the membrane. Similarly in Peacock et al. (2013) the phenolic fraction of DOC is discussed, the authors infer a positive linear relationship between DOC and phenolics. If this is applied to the experiment in this chapter, reduced phenolics and increased calcium could be an artefact of reduced DOC concentration.

Clearly there is an increased potential for chelation of calcium to LMW DOC due to associated concentrations observed. But from the above, it can be proposed that phenolic content may provide an explanation for calcium chelation potential, but molecular weight may be more influential.

5.7 Conclusion

In short this experiment found the following

1. LMW DOC indicated increased calcium association or chelation potential.
2. Phenolic binding as discussed in White, Freeman, & Kang, (2011) cannot be excluded at this stage and will require further mesocosm studies to confirm

This chapter has acted as justification to further detailed investigation into the effect of phenolic content and molecular weight spectra on calcium mobility. This chapter has provided validation to theories and hypotheses developed from the literature. It can be concluded that experimentation using mesocosms, where DOC MW and phenolic content express different concentrations as a result of system design, will be used to further understand calcium mobility within the CW environment. The investigation at mesocosm scale following the results presented in this chapter will form the basis for including primary

CW functions of water quality improvement with further investigation into mobility and fate of calcium, especially when applied to management of the Anglesey and Llyn LIFE Fens project

Chapter 6 - Investigation onto the Effects of Various Types of Constructed Treatment Wetlands on Mobility and Fate of Calcium in the System

6.0 Introduction - Scope for Calcium Solubility Maintenance

Following experiments at bench scale (Chapter 5), it was concluded that calcium became associated with or chelated to low molecular weight DOC, typically of <3500mwco, and low phenolic content. Therefore manipulation of DOC and phenolics may affect Calcium in the in the Constructed Wetlands (CW) environment and is potentially a viable method for preventing calcium precipitation. But this is needed to be combined with the CW principle target of enrichment alleviation with calcium concentration maintenance as set out by the Llyn and Anglesey fens LIFE project.

6.1 Molecular Weight and Phenolic Content

Based on prior bench scale DOC MW observations as well as increased calcium solubility associated with in LMW DOC compounds in the field of nutrition by Shen et al., (1998), it was hypothesised that CWs exhibiting lower MW ranges of DOC may show increased calcium chelation and therefore mobility. Binding to LMW DOC was proposed to prevent complexation with carbonate and hence prevent precipitation.

Further testing of calcium precipitation inhibition by the presence of phenolics is required to determine whether the findings of White, Freeman, & Kang, (2011) where Fe^{2+} precipitation was inhibited hold true with Ca^{2+}. Although findings from Chapter 5 indicated an association of calcium with low phenolics, this was attributed to the phenolic group's constituent of the DOC pool on either side of the dialysis membrane. Greater concentrations of phenolics were linked to DOC of greater molecular weight; however this was attributed to total DOC concentration observed.

6.1.1 Achieving Differing DOC Molecular Weights in Constructed Wetland Mesocosms

Methods for achieving DOC MW manipulation within the CW environment was proposed by CW design, associated mesocosm nitrogen processing, resultant DOC demand and breakdown. CWs where denitrification is required generally exhibit anaerobic conditions and a labile carbon source (Mitsch & Gosselink 2000; Kadlec & Wallace 2008). By utilising both Free Water Surface (FWS) and Horizontal Subsurface Flow (HSSF) design varying rates of denitrification and carbon breakdown were expected due to oxygen conditions in the systems. Increased denitrification, utilization and subsequent breakdown of carbon during microbial respiration in HSSF were therefore expected to result in lower MW DOC. Whilst lower carbon utilisation and breakdown was expected in the FWS systems and therefore reduced LMW DOC availability.

6.1.2 Achieving Phenolic Concentrations Range in Constructed Wetland Mesocosms

Research into phenolic production in wetland systems may elucidate a mechanism for the mobility of calcium, developing on the observations of Fe^{2+} mobility with phenolics (White et al. 2011). Evidence suggests that phenolic content of root exudates and root tissues varies between species (figure 6.1) and has been suggested to occur as a result of adaptive processes. For example macrophyte root phenolic production, emerging as root exudate as a defence mechanism against compounds harmful to macrophyte growth such as xenobiotics and other compounds which occur in the rhizosphere (Larue et al. 2010).

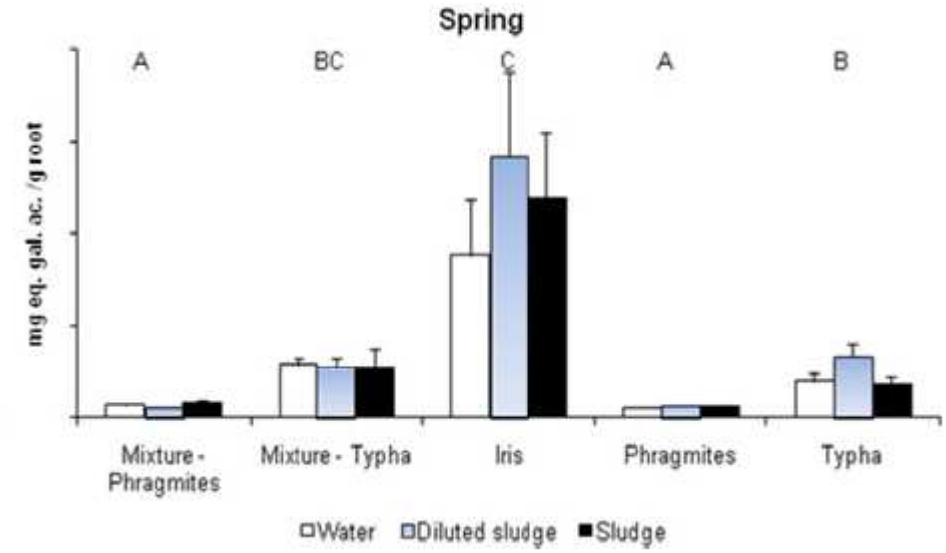


Figure 6.1 – Variation in phenolics produced by wetland species.

The graph above shows significantly higher phenolics in Iris as compared with other species. The figure above focusses on the significant increase in phenolic material observed in the roots and root exudates of the species *Iris psuedacorus.*

The findings of Larue et al., (2010) indicate variation in phenolic concentrations, as observed in chapter 3, figures 3.8 and 3.9. Despite that the experiments of Larue et al., (2010) and the results of chapter 3 indicate variation in absolute values for total phenol production, as well as species identified as possessing most prolific phenol production, phenol varied significantly between tested species in both experiments. Therefore further species specific analysis was undertaken to assess potential phenol production in a mesocosm scenario. Two species were selected in order to produce different phenolic concentrations within the rhizosphere, expected to influence calcium mobility.

6.1.3 Total CW DOC, Phenolics and the Effect on Conservation Sites

Application of the calcium mobility findings in this chapter may affect the design and plant species selected in future systems installed on the NRW conservation sites. The CWs must reduce nitrogen, phosphorus and DOC, whilst maintaining calcium. Due to these stipulations it was important to find a suitable type of DOC and/or phenolics that were highly efficient at maintaining calcium mobility whilst minimising DOC inputs.

6.2 Hypotheses for the Promotion of Calcium Mobility by Varying Phenolics and DOC MW

1. LMW DOC provides greater calcium chelation capability, preventing precipitation and maintaining mobility
2. Wetland macrophytes produce different concentrations of phenolics as root exudates, which have the potential to chelate calcium, preventing precipitation and maintaining mobility
3. It may be that wetland macrophytes may have different demands for calcium for incorporation into tissues and biomass and therefore calcium uptake and removal.

Final application of CW design utilised on the NRW conservation sites will be the choice of the NRW team and be based on calcium delivery to the site versus total DOC inputs.

6.3 Methods

6.3.1 Plant Species and System Design Selection

As in the study by Larue et al., (2010) *Iris psuedacorus* was used as a known producer of phenolic substances within the roots and rhizomes. *Phragmites australis* was selected due to the popularity of its use in various types of treatment wetland across the globe. Both plants species are readily available for use and harvesting from the field sites on Anglesey.

In order to produce variable rates of denitrification and subsequent carbon source breakdown in relation to the degree of oxygen depletion, free water surface (FWS) and subsurface flow (SSF) treatment systems were installed. Saturated SSF systems are used in the final stages of constructed wetland systems in order to complete the final step of denitrification (Kadlec & Wallace 2008), as increased denitrification is achieved in anaerobic conditions.

6.3.2 Water Inflow Chemistry and Control

Small linear wetlands were created using 7 litre containers into which a daily volume of 1 litre acted as pulse fed inflow. Clean, non-limestone, naturally rounded gravel was used as SSF media, the presence of which reduces the amount of direct atmospheric diffusion of

oxygen into the water column. However, when calculating hydraulic retention time (HRT) for each of the systems the retention time will be dramatically decreased due to the reduction in available water volume in the subsurface flow system. This void space was calculated to be 63% of the total 7 litres in the linear test containers and was to be accounted for when comparing the treatments calcium budget and mobility, as well as nitrogen removal rates.

In order to replicate exact field water chemistry, water was extracted directly from a ground water fed spring on Anglesey and transported to the laboratory. The site in question was Waun Eurad SSSI, here water is known to be of high Calcium concentration and highly charged with Nitrate, and occasionally phosphate. Calcium becomes dissolved in the ground water arising from a spring from a large aquifer running though carboniferous limestone before emerging at the surface.

Treatments applied were as follows:

Plant species	*Phragmites australis*	*Phragmites australis*	*Iris psuedacorus*
System type	SSF	FWS	FWS
Hypothesis No. explored	1	1 and 2	2

Figure 6.2 – table of treatment types applied and plant species used. Five replicates of each treatment type were set up resulting in a total of 15 linear mesocosm treatment wetlands.

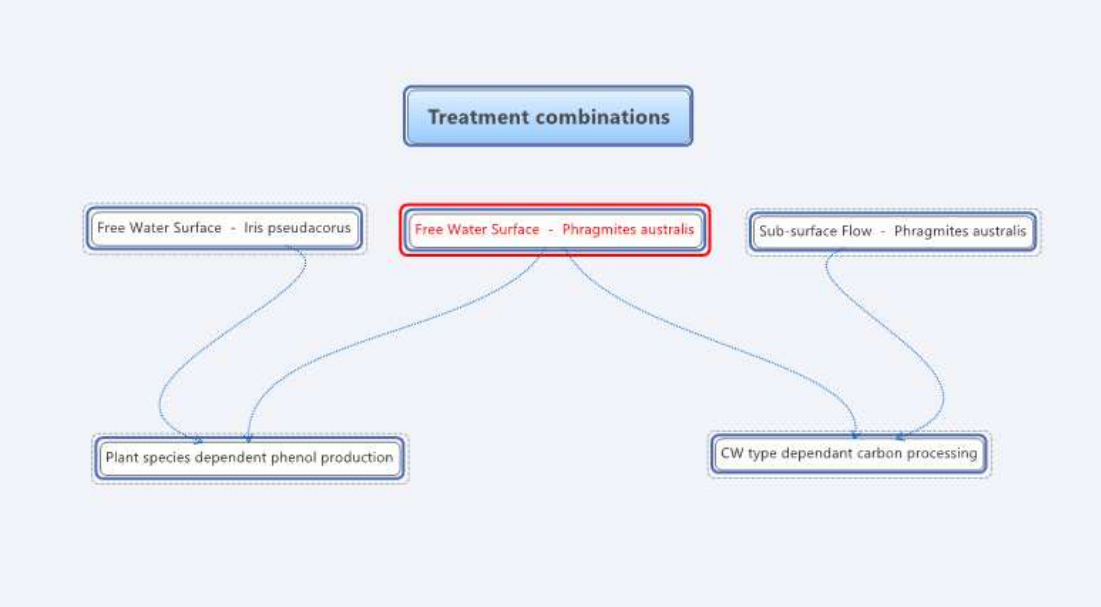


Figure 6.3 –method by which the FWS *Phragmites australis* treatment is utilised for both system type carbon processing analysis and species phenolic production analysis.

6.3.3 Water Distribution System and Randomised Design for Lighting and Temperature

The laboratory experiment utilised site water fed from a tanked reservoir via an electric water pump into a temperature controlled growth chamber under a 12:12 hour photoperiod. Water was evenly distributed onto each wetland mesocosm by means of taps along the length of an irrigation pipe. This way control was gained in water flow supply between each of the mesocosm systems.

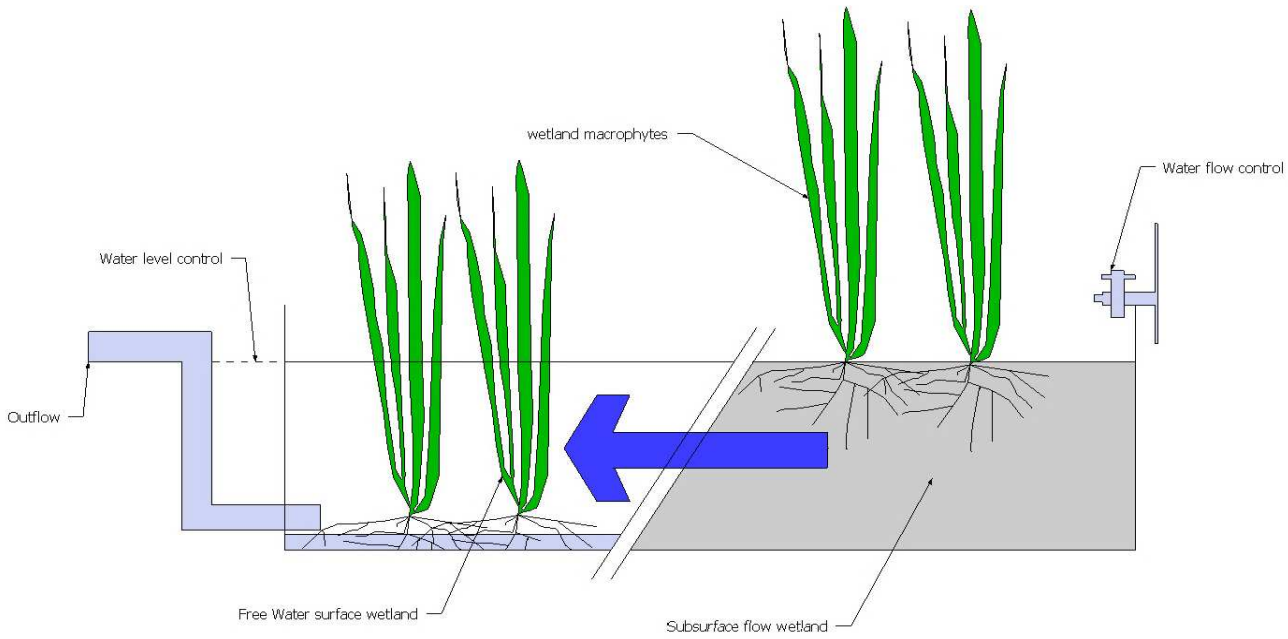


Figure 6.4 –Wetland Mesocosm set up.

The diagram indicates water level control. The mesocosms were designed with inflows allowing water onto the surface at the inlet zone and outflows that release water from the deepest part of the treatment system. This set up ensures that water does not simply flow across the surface of the wetland and is forced to interact with the rhizosphere or standing biomass, meaning water pollutants flow through the microbial biofilms allowing remediation.

Figure 6.5 – Images of Mesocosms.

Clockwise from top left; water level control and sample collection system, *Phragmites* SSF, *Iris* FWS and *Phragmites* FWS

6.3.4 Water Sampling

Samples were collected weekly from the outflow of all of the mesocosms during the timed pulse of water inflow. Samples were collected weekly for a period of 6 weeks. Electrical conductivity (EC) and pH were measured for all 15 systems before filtration. Inflow water chemistry analysis was undertaken on the reservoir tank containing site water within the growth chamber.

6.4 Water Chemistry

6.4.1pH and Electrical Conductivity (EC) Measurements

pH was analysed using a laboratory standard probe calibrated to pH 7 and 4. The pH probe used corrected for any temperature fluctuations.

The EC was measured immediately after pH analysis using a laboratory standard EC probe calibrated to 1314 m Sieverts.

6.4.2 Sample Preparation

All samples taken including inflow water samples were filtered through Whatman 0.45µm syringe filters and stored at 3-4 $^{\circ}$C until required for analysis.

6.4.3 DOC Quantification and Analysis

DOC was measured using an Analytical Sciences Thermalox TOC/TN analyser. In order to measure DOC the samples were acidified to between pH 2 and 3 and sparged with oxygen for 2 minutes in order to remove inorganic carbon compounds. The instrument was calibrated using standards of known concentration (0, 5, 10, 15, 20, 30 and 40 mg L-1) potassium hydrogen phthalate was used as the standard compound.

Machine replicates using the Thermalox are unnecessary due to multiple sample injections during analysis. For this analysis the multiple injection number was 5 injections.

6.4.4 Phenolic Compound Quantification

Phenolic concentrations were determined using a method adapted from Box (1983). 250µl of sample was added to a clear micro plate well. 12.5 µl of Folin-Ciocalteau reagent was added followed by 37.5 µl of Na_2CO_3 (200 g/L). Samples were spun in a centrifuge at 1000 rpm for 1 minute. After 1.5 hours the absorbance was measured at 750nm on a Molecular Devices M2e Spectramax plate-reader. Phenolic concentrations were then derived from the preparation of a standard curve using tannic acid phenol standards of known concentration (0, 1, 2, 4, 6, 8, 10, 15, 20 mg/L).

6.4.5 Dissolved Ion Chromatography

A suite of ions were analysed using ion chromatography by means of a 850 Professional IC and 858 auto-sampler, Thermo Fisher AS14A anion column and Metrohm C4 cation column. A range of standards were used separately for anions and cations using Fluka Multi-ion standard including Sodium, Ammonium, Potassium, Calcium, Magnesium, Fluoride, Chloride, Nitrite, Bromide, Nitrate, Phosphate and Sulphate .

6.4.6 Total Calcium Analysis, Flame Photometry and Sample Specific Calibration Curves

Total calcium analysis was undertaken using flame photometry atomic emission spectroscopy at 422.4 nm. Calibration curves were generated from a range of standards and blanks (0, 20, 40, 60, 80, 100, 150 and 200 mg/L). Standards were prepared using calcium chloride dissolved into ultrapure water. From the calibration curve total calcium concentration in the samples were back calculated.

A number of elements can interfere during the burning of the sample in the atomic emission spectrophotometer (AES) causing shifts in the absorbance values due to changes in the colour of the flame emitted (Willard et al. 1988). Following trials for correlation with a number of suspected elements that could cause interference, a method by which total calcium could be

correctly calculated was applied. The method used was adapted from Harvey (2012) where addition of known concentrations of calcium were added sequentially to volumes of the sample in order to generate sample specific calibration curves.

Matrix correction by standard addition allowed for the correction of pre-calibrated samples. Each sample was combined and spiked to increase in total calcium by 50, 100 and 150 mg/L. From the expected versus observed concentrations, values generated by standard additions created a sample specific calibration, which allowed formulae to be derived in order to correct the original sample concentration. The formulaic alteration to the original concentrations were as though an x=y increase was to be observed as expected with a linear set of standards added.

6.4.7 UV-Vis Analysis Indicating, DOC Composition Inferences

Three analyses of DOC composition were used: SUVA as discussed in chapter 2. Plus additional aromaticity and humic content analysis undertaken by absorbance ratios at set wavelengths. Aromaticity is estimated by Absorbance at 250nm divided by absorbance at 365nm (E2:E3), whilst humic content is estimated by absorbance at 465nm divided by absorbance at 665nm (E4:E6).

6.4.8 Organic Compound Molecular Weight (HPLC SEC)

MW range was analysed using high performance liquid chromatograph size exclusion chromatography. Samples are carried by a mobile phase of a phosphate based solution. On coming into contact with the solid phase of the column low molecular weight (LMW) molecules become more strongly bound to the solid phase of the column than higher molecular weight (HMW) fractions, therefore compounds detected at later retention times equate to LMW compounds.

A wide range of standards are used to act as retention time standards, 150K, 77K, 32K, 13K, 4.3K and vitamin B12 as 1.356K Daltons

The results generated following peak detection produce three values;

1. Mp-Peak molecular weight
2. Mn-Molecular weight value at which equal number of molecules are present above and below the Mn value
3. Mw-Similar to Mn however, the value at which equal mass is observed above and below the value

Here the main important biologically active carbon compounds are generally of MW<1000Da. Calibration allowed identification of the retention time where DOC with MW <1000Da was released from the solid phase of the column and detected in the chromatograph. The area under the curve following the retention time associated with LMW DOC was used to calculate percentage LMW DOC of the samples.

6.5 Results

6.5.1 pH

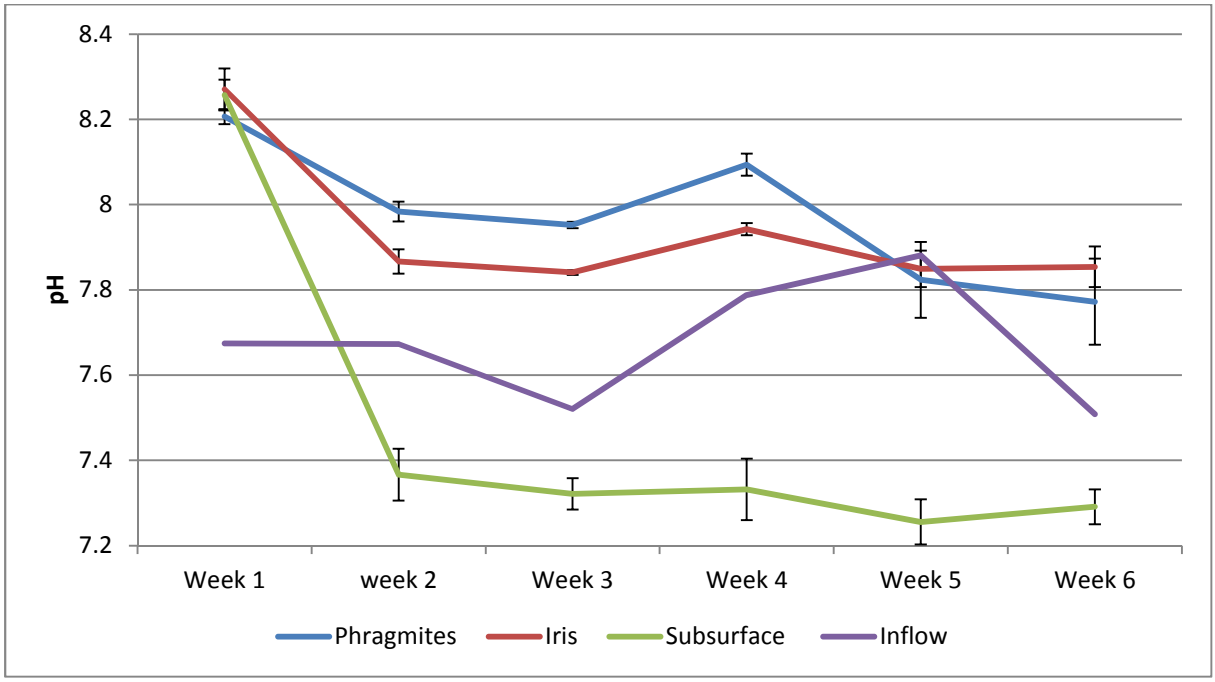


Figure 6.6 – Calcium Mesocosm pH.

The graph indicates pH over the course of the 6 week experiment. In both of the planted FWS treatments pH become more alkaline than inflowing water for the duration of the experiment although in week 5 no increase in alkalinity was observed. The SSF system showed more acidic outflow conditions following passage through the system. SPANOVA on the above data indicates a significant difference in the pH of the treatments over time ($p<0.005$). Analysis of Between-Subjects effects indicates that the response between treatments are different ($p<0.001$)

6.5.2 EC

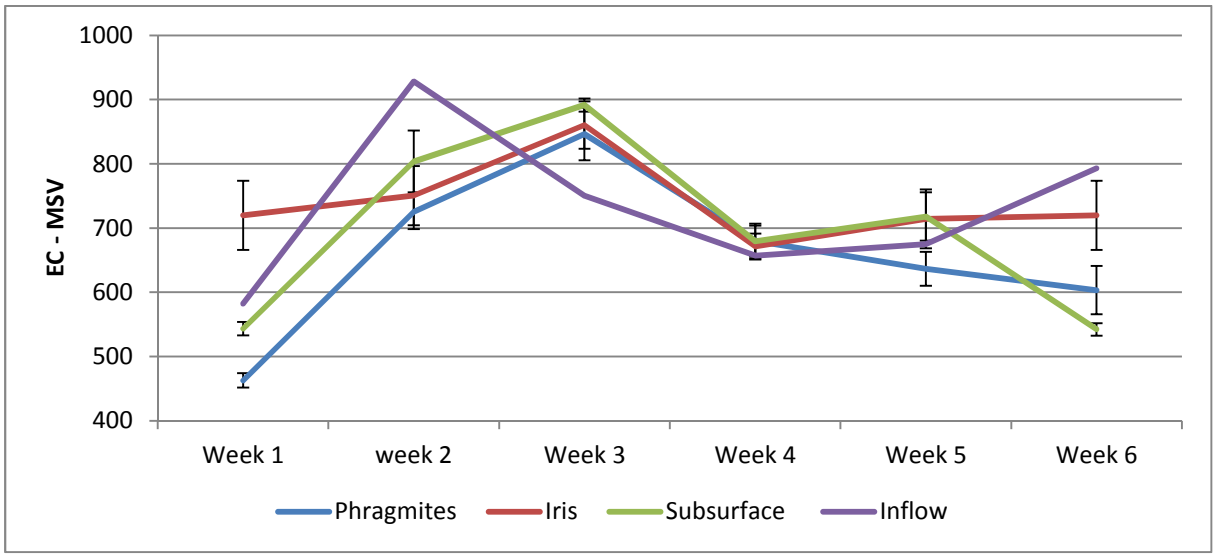


Figure 6.7 – Calcium mesocosm EC.

The graph indicates EC over the course of the 6 week experiment. Although all treatments initially show lower EC values than the inflow, no obvious difference between treatment and inflow EC was observed for the remainder of the experiment. SPANOVA analysis of the EC data showed a significant difference over time ($p<0.001$). Between subjects analysis showed there to be no significance in the relationships between the treatments over time ($p<0.055$).

6.5.3 Dissolved Oxygen

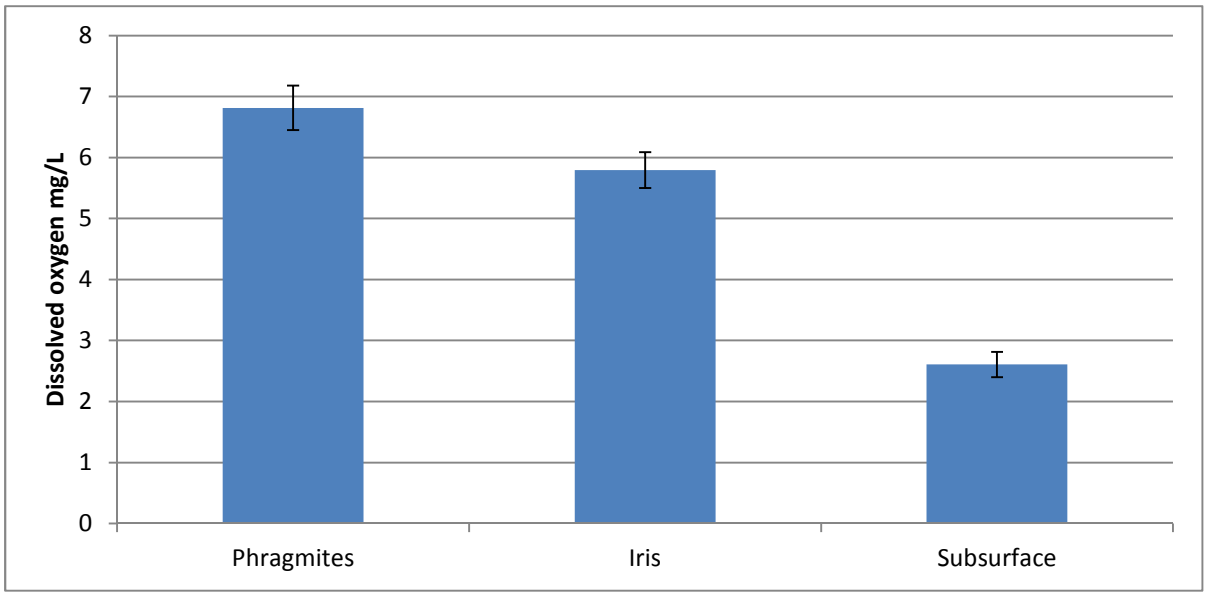


Figure 6.8 – Calcium mesocosm DO.

The graph shows mean DO for the experiment. The SSF system shows 75% lower dissolved oxygen as compared with the two FWS planted systems. (15%) increased DO is observed in the *Phragmites* system as compared to the *Iris* system.

6.5.4 Nutrient Pollutant Compounds Ion Chromatography

6.5.4.1 Nitrate

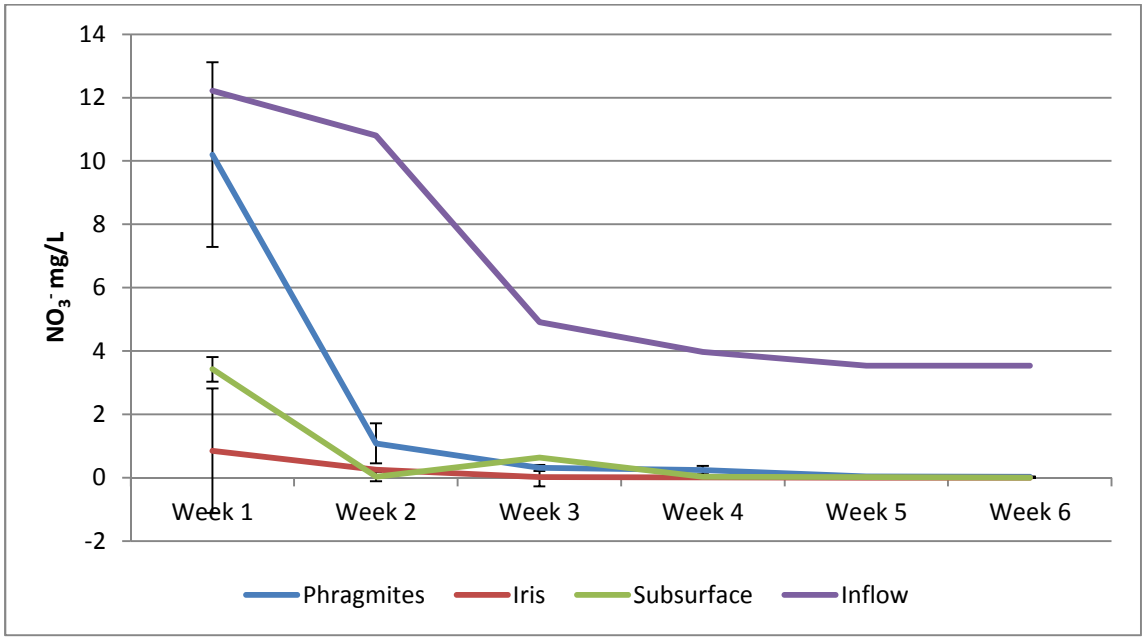


Figure 6.9 – Calcium mesocosm nitrate removal.

The graph indicates a near total removal of nitrate from the outflowing water samples confirming primary wetland function for pollutant removal. The treatments showed a significant difference in nitrate over time ($p<0.001$). All 3 treatments indicated values for nitrate that approach zero at week 2 onwards. The Between-groups SPANOVA indicates significance between the treatment types is detected ($p<0.005$). The standard error for all of the planted treatments was shown to be small.

6.5.4.2 Nitrite

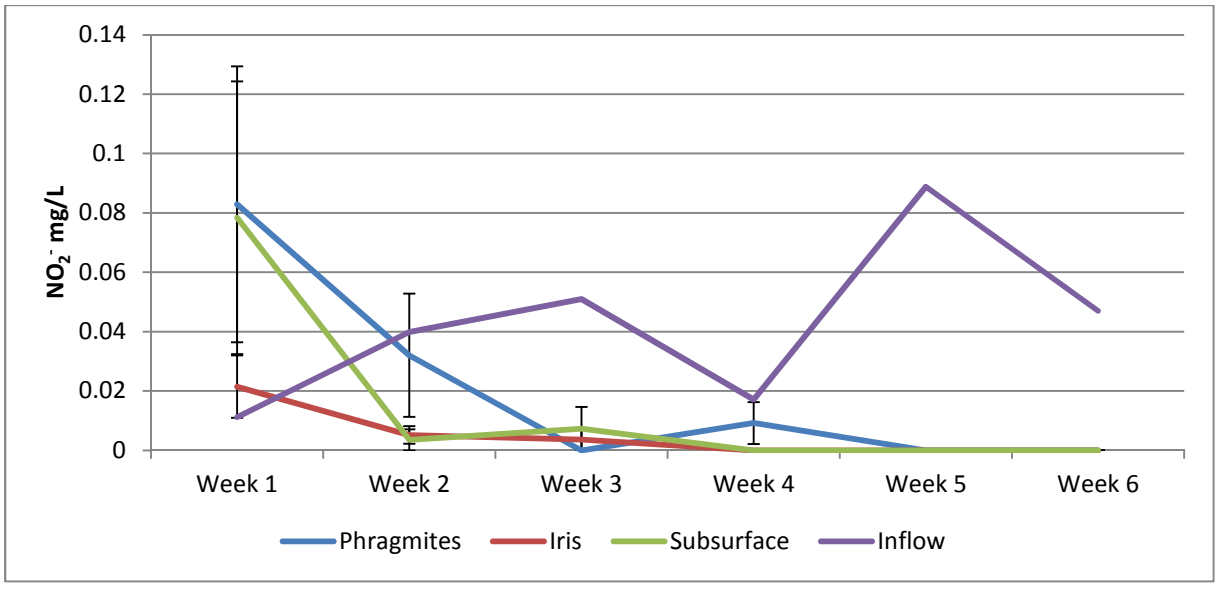


Figure 6.10 – Calcium mesocosm nitrite removal.

Nitrite data indicates a less tightly grouped pattern than nitrate in the previous figure. SPANOVA analysis, suggests significance over time ($p<0.002$). No significant difference exists between the treatments. However, Nitrite concentrations exhibited in the treatment mesocosms are generally very low. This low concentration can result in an increased potential for error.

6.5.5 DOC

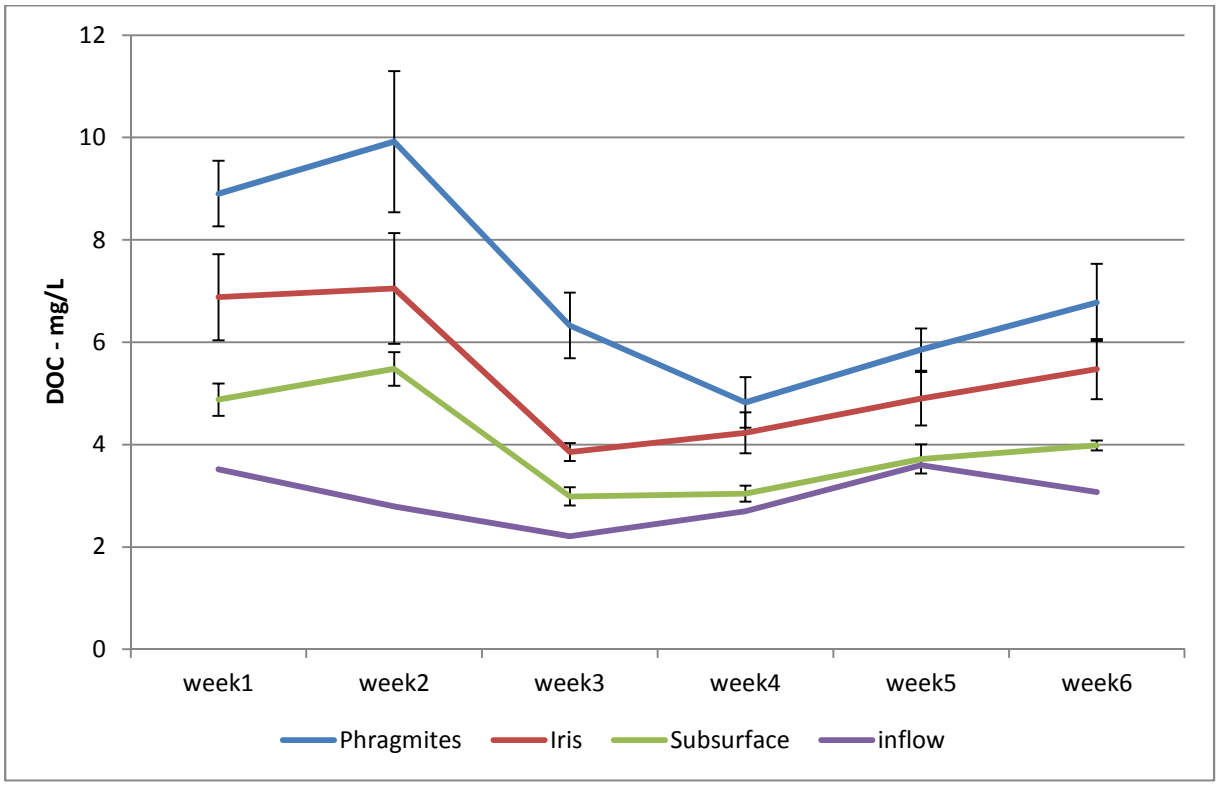


Figure 6.11 – Calcium mesocosm DOC.

DOC was observed to be greater in the outflow samples in all treatments. *Phragmites* FWS showed the highest level of DOC at all sample times, on average 164.4% greater than inflow across the treatments. These data indicate DOC varies with treatment type. A significant difference in the treatments over time was observed ($p<0.005$); therefore DOC changed from week 1 to week 6. Between groups analysis also indicated a significant result ($p<0.003$).

6.5.6 DOC Character and Composition

6.5.6.1 MW Spectra

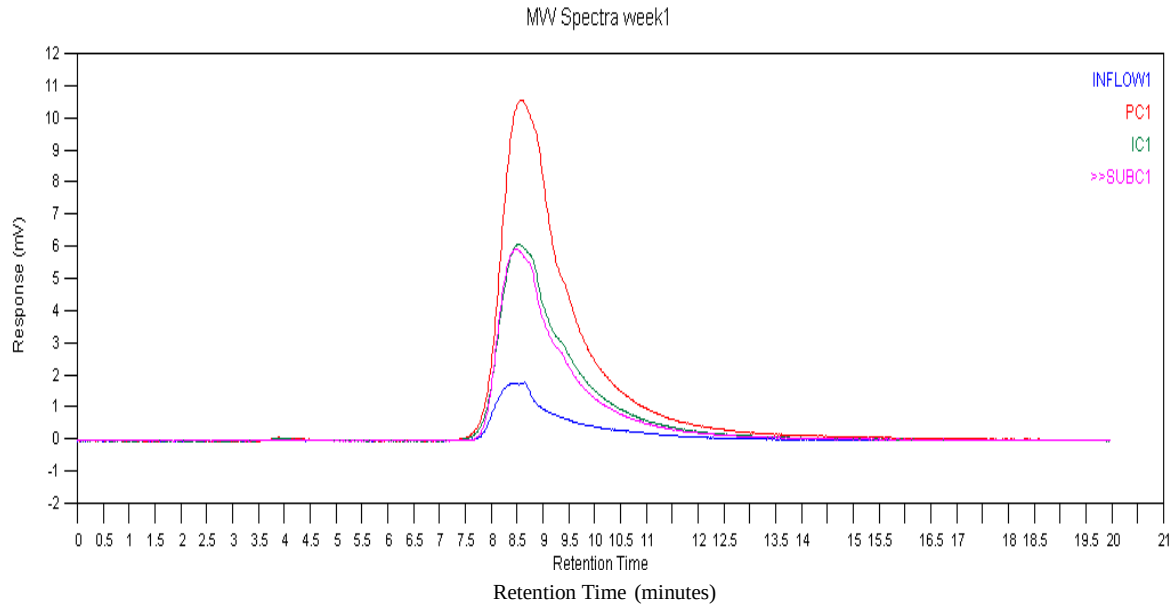


Figure 6.12 – Calcium mesocosm SEC for DOC.

Analysis at week 1 indicates the different MW properties of the DOC compounds being produced within the treatment mesocosms. Response (mV – y-axis) is an indication of the concentration of each molecular weight compound passing through the column. Whilst retention time (minutes – x-axis) indicates decreasing molecular weight with time. These peaks are indicative of the concentrations observed in the samples analysed for DOC.

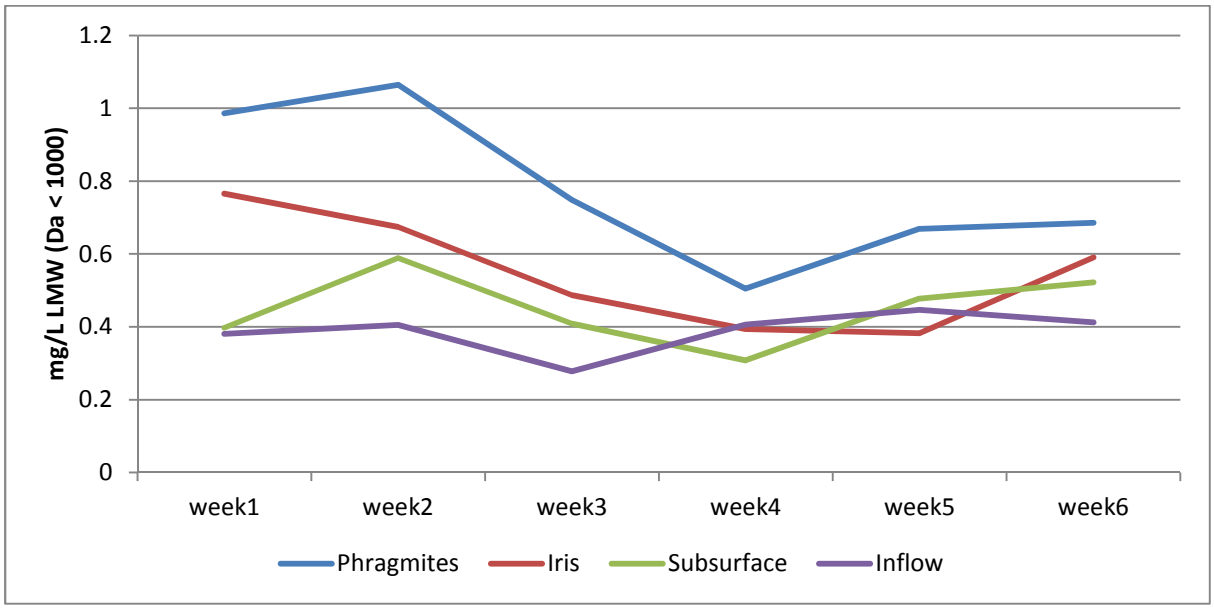


Figure 6.13 – Calcium mesocosm, total produced LMW DOC.

The graph describes the LMW concentration of the DOC as milligrams per litre of sample error bars are not included due to the values being calculated from existing figures. There appears to be little difference between the concentration of LWM DOC between the

inflowing water and that of the SSF system. Similarly, *Iris* appears to be grouped with the two aforementioned treatments up until week 4, remaining marginally above for the preceding weeks. *Phragmites* remains as a separate treatment for the duration of the experiment, exhibiting an increased concentration throughout, typically around 0.1-0.2 mg/L above the other treatments.

6.5.6.2 Phenolics

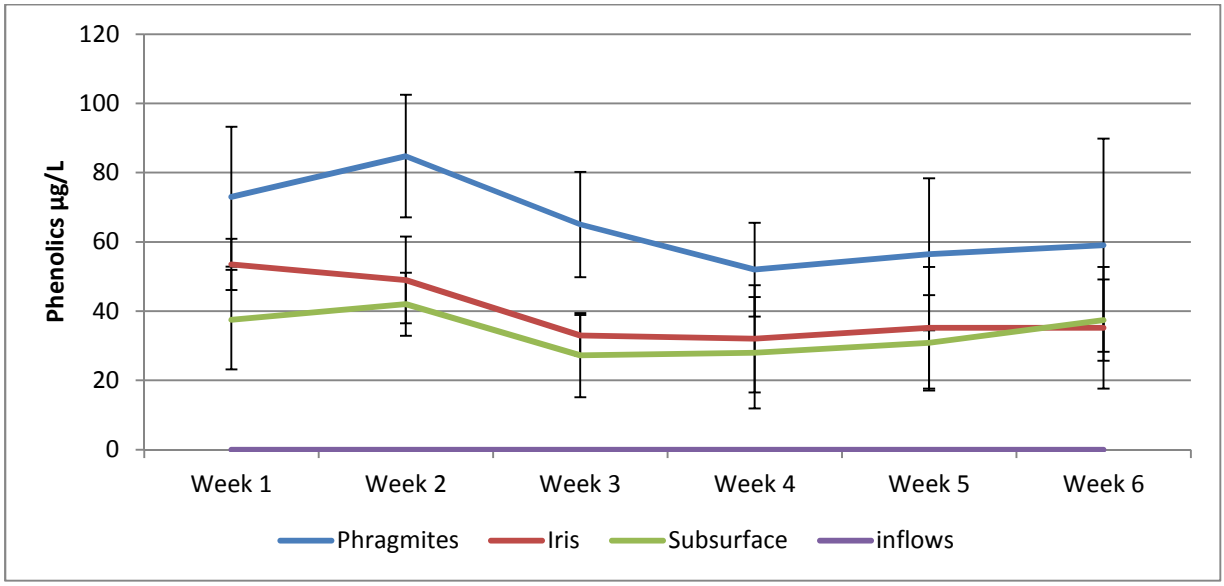


Figure 6.14 – Calcium mesocosm phenolic production.

Phenolics are continually highest in the *Phragmites* samples. Inflow is characterised by the absence of any phenolic containing compounds. All of the phenolic material must therefore be produced by the system. The data replicates the relationship observed in figure 6.11. No significant difference is observed in the treatments over time ($p<0.15$). The between groups analysis indicates significance ($p<0.001$).

6.5.6.3 UV-Vis Analysis

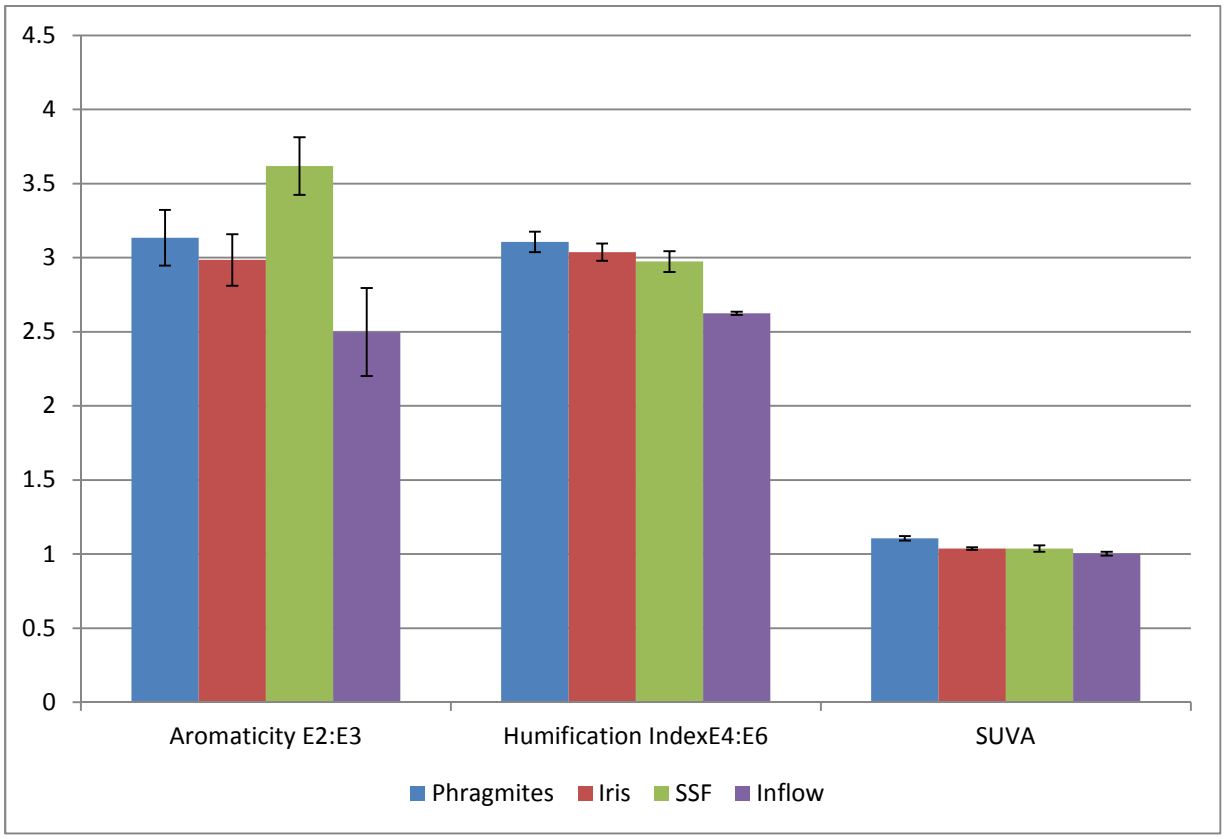


Figure 6.15 – Characterisation of the aromatic, humic and specific ultraviolet absorbance properties of the DOC

Aromatic content was found to be greatest in the subsurface flow systems. Aromaticity was increased in all the planted treatments as compared to the inflowing water chemistry. Initial inspection groups the FWS planted systems together, achieving similar levels or aromaticity. The SSF system indicated an average increase in aromaticity of 16% over the two FWS systems.

Humic content of the treatments are all increased as a result of passage through the treatment wetland mesocosms. Here little difference appears between the treatments but the control inflow is markedly less humic in character.

Little difference in SUVA is detected between all the treatments and the inflowing water chemistry of the control.

6.5.7 Calcium Mobility

6.5.7.1Ionic calcium

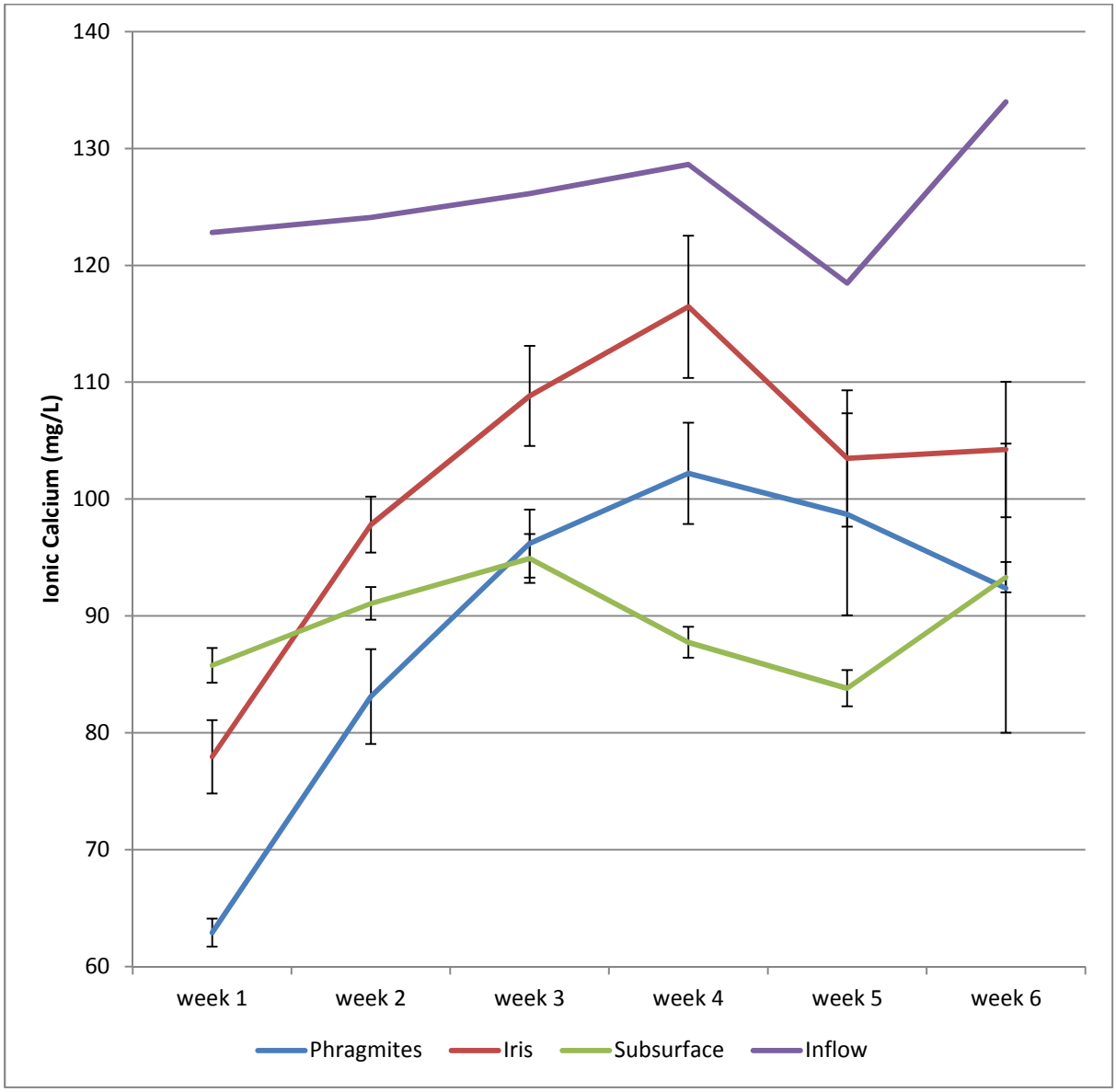


Figure 6.16 – Calcium mesocosm ionic calcium.

All samples taken from the inflow possess ionic calcium concentrations in excess of 118mg/L. For the duration of the experiment the *Iris* treatment has the least effect upon ionic calcium dissolved in the water column as compared with both the *Phragmites* planted systems. Calcium is consistently reduced in the SSF system remaining between 84 and 95 mg/L. Initial reductions in ionic calcium are observed in both FWS systems during the initial 2 weeks of the experiment. Differences are detected by SPANOVA analysis in the treatments over time ($p<0.009$). Although overlap exists between the planted mesocosm system ionic calcium values, significant differences are still observed between groups ($p<0.002$).

6.5.7.2 Total Calcium

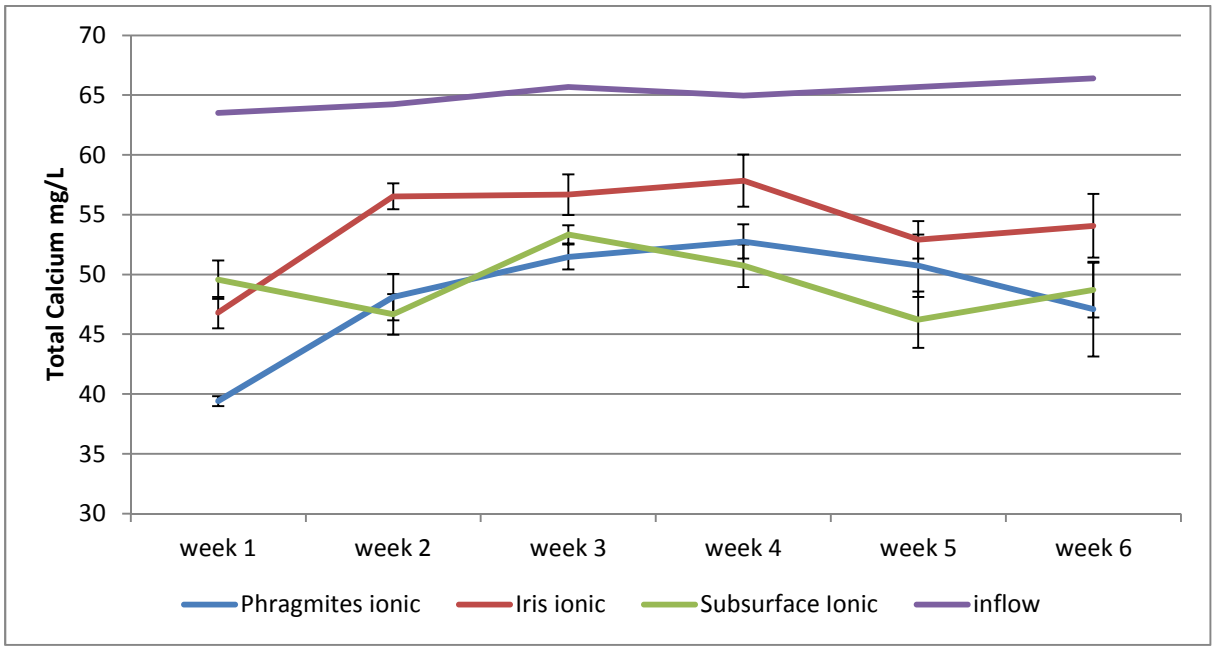


Figure 6.17- Atomic Emission Spectrophotometry Total calcium.

It was observed that total calcium was analysed to be less concentrated than ionic calcium under all treatments. Due to interference at the atomisation phase of AES, low total calcium values were observed; matrix correction was applied as explained in the methods section (6.4.6) shown in figure 6.18.

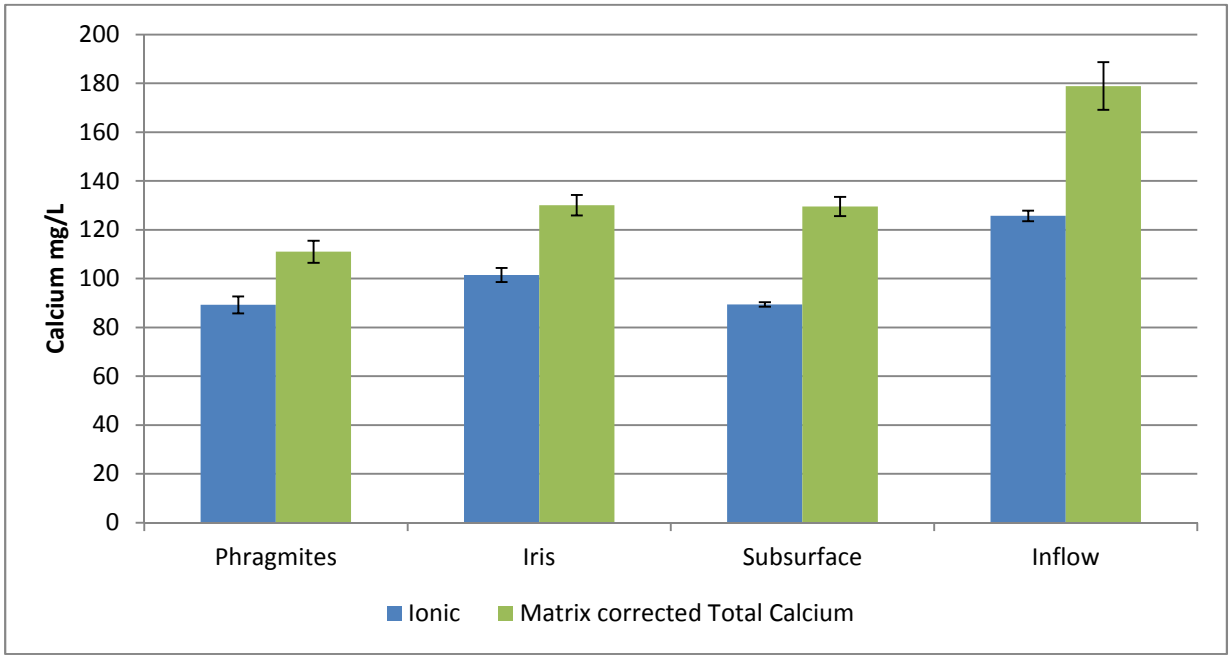


Figure 6.18 – Mean Total Calcium, Post Matrix Correction.

Calcium mesocosm ionic and total calcium values averaged for each treatment for the duration of the experiment following matrix additions of known standards.

6.5.7.3 Total Calcium – Matrix Adjusted

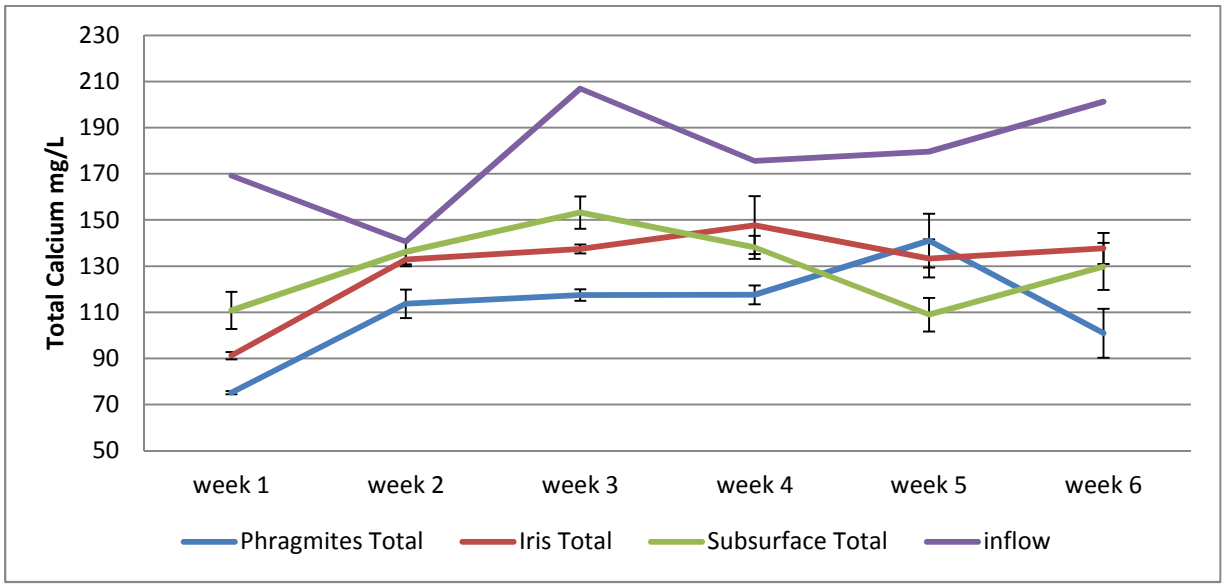


Figure 6.19 - Post matrix correction total calcium.

The Graph indicates corrected values for total calcium as observed in the treatment mesocosms. All of the treatments reduce the concentration of total calcium dissolved within the outflow of the mesocosms in comparison to the inflow values. No clear relationships between treatment type and total calcium value were apparent. However, SPANOVA analysis indicated differences are detected over time and between treatments ($p<0.001$ and $p<0.001$ given respectively). Selection of a suitable time period under which to undertake an ANOVA could result in a skewing of the homogenous Sub-sets. For example in week 2 a homogenous sub-set analysis may group the data with *Phragmites* as an outlier during that time period, whilst during weeks 3 through to 6, the control would act as a singular homogenous sub-set accounting for the significant difference observed in the between treatments analysis of the SPANOVA.

6.5.8 Total Chelation

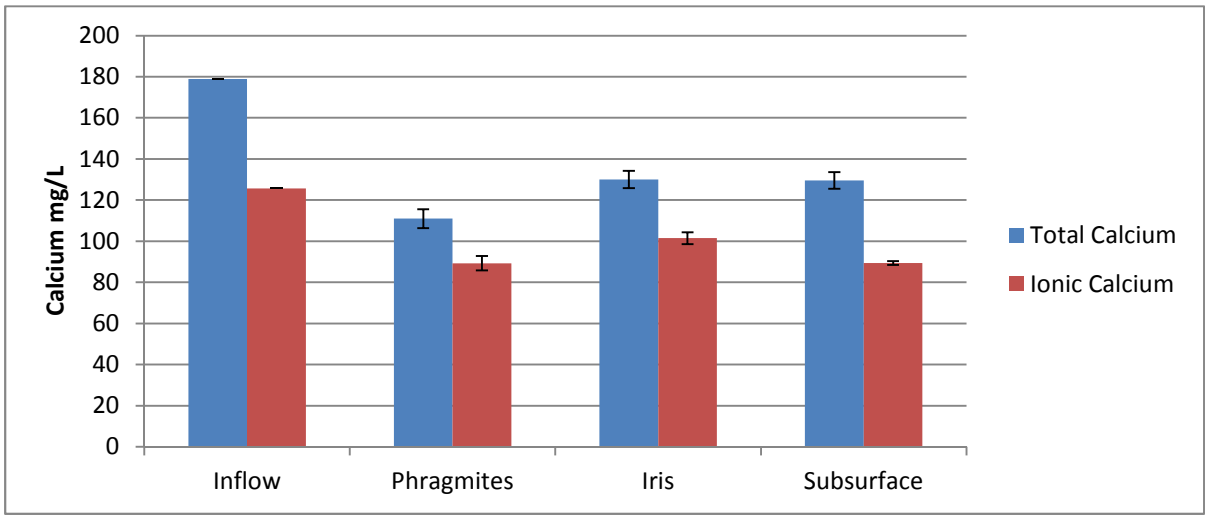


Figure 6.20 - Mesocosm calcium modification.

The graph indicates total and ionic calcium for each of the treatments and indicates the inflow values before passage through the mesocosm systems. The data shows that more calcium was removed from the systems containing *Phragmites* than *Iris*; this is the case for both Total and ionic in the *Phragmites* FWS and ionic calcium in the SSF system. Total calcium for *Iris* and the subsurface flow systems are relatively similar however the ionic values in the SSF system are significantly lower. Statistical analysis was not performed on this data set because the between treatments analysis of the SPANOVA already indicated significant differences (Figure 6.19) Considering total calcium minus the ionic calcium gives an insight into the bound calcium in the outflow water, knowledge can be gained into the fate and mobility of calcium in the system in relation to treatment types.

6.5.9 Increased Ca present

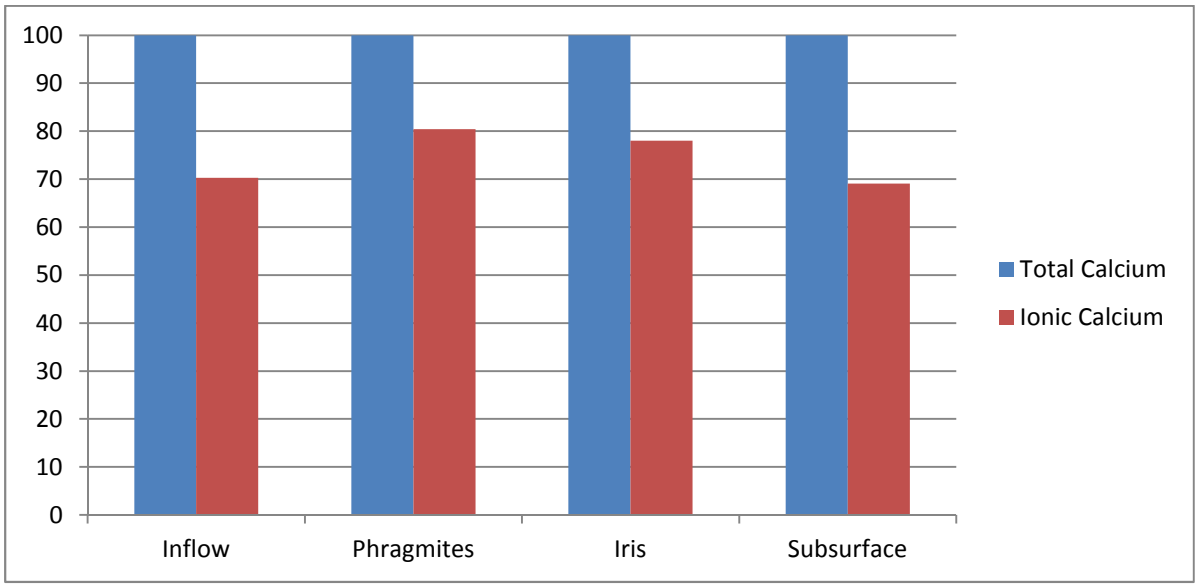


Figure 6.21 – Ionic calcium as a percentage of the total calcium in the system.

In the *Phragmites* FWS system ionic calcium constitutes the largest percentage of the three planted treatments, whereas the SSF system planted with *Phragmites* shows the lowest concentration of post mesocosm ionic calcium, although differences between treatments are all very small.

6.6 Discussion

6.6.1 Water Chemistry Parameters

The pH of the water is acidified to a small degree resulting from passage through the SSF system. Significance was also detected between each of the treatments and is likely to lie with

the SSF system as the Phragmites and Iris do not show large differences in pH compare to the inflow water.

Primary function of treatment systems, as represented by the mesocosms, is for nitrogen and phosphorus removal. As such, specific pH ranges exist for nitrification and denitrification facilitated by microbial communities. Denitrifiers are able to perform maximum denitrification potential between pH 6.5-7.5 (Kadlec & Wallace 2008). This range of values most fitting with the SSF system and is indeed the type of treatment wetland most readily associated with denitrification in conventional CW systems and also provides suitable redox conditions for the process to be undertaken.

Higher pH values are observed in both of the FWS systems. Although removal of nitrogen was detected (See below), this was found to be outside the optimum range (Kadlec & Wallace 2008). Suggestions for the increased alkalinity are discussed in the previous chapter, one of which is algal colonisation of the water column. This process can only occur in the FWS systems rather than the SSF due to the ability for light to pass into the water column. The process put forward was; CO_2 uptake into algal biomass (Schippers et al. 2004) decreasing the acidic balance of the water chemistry (Willoughby 1976).

Assessing the SSF data it is difficult to assign a singular cause for the reduction in pH. Although the result is not considered to be typically acidic, it could be proposed that the slight acidification was due to increased DOC production by microbial biofilms, anaerobic conditions or reductions in ionic calcium. Ionic was found certainly to be lowest in the SSF for the duration of the experiment.

FWS system treatments both have far lower ionic calcium values for the initial two weeks before stabilising in week 3, approximately 100 mg/L (Figure 6.16). It is suggested that this is simply a result of exposing the plants to water chemistry containing significant nutrient content. Calcium is an essential micronutrient for growth of plants regularly amounting to up to, and occasionally in excess of 0.7% of dry biomass of higher plants (Jones and Lunt, 1967; Mitsch & Gosselink, 2000). It is reasonable to deduce that nutrient rich conditions initiate rapid initial growth, resulting in nutrient uptake, including that of calcium.

Calcium loss and pH reduction may be contributed towards by dissolved oxygen (analysed in detail later). DO is linked to redox potential and allows for conditions resulting in calcium carbonate precipitation and reducing alkalinity.

EC is a method of water chemistry analysis that is able to quickly provide a measure of electrical carrying capacity of the solution elucidating the quantity of dissolved ions within

the sample. This is most beneficial in that early inferences can be made into changes in the dissolved ionic makeup of the solutions following passage through the wetland mesocosms systems.

Primary ions of concern include nitrate, nitrite and phosphate as well as calcium in its ionic form. Following the analysis undertaken on the EC data (Figure 6.7) it was concluded that the treatments did not significantly affect the electrical conductivity of the water chemistry. Although fluctuating EC was recorded over time, similar values were observed in the treatments as observed in the inflow which may relate to limited change in total ions.

The primary driver for the investigation into calcium maintenance was the investigation carried out by Mayes et al (2009) where CWs were used for calcium removal. Calcium acted as a pollutant and was ameliorated by passing the water through the wetland in order to precipitate calcium in large quantities. Carbonate formation is essentially abiotic and is influenced by oxygen availability (Kadlec & Wallace 2008).

DO concentrations and the associated redox state are linked to a number of processes that occur within the CW environment. There are two processes that are influenced by the oxygen availability in CWs; these are the formation of carbonate precipitates with calcium and microbial denitrification. Oh & Silverstein (1999) discuss denitrification and DO availability. Although the situation in which the aforementioned authors discuss denitrification potential in relation to DO is very different to the mesocoms used, namely simulated activated sludge, the relationship between DO and denitrification remains. Although overall denitrification was not significantly affected, the rate at which nitrate was denitrified was significantly different. The data discussed in Oh & Silverstein (1999) can be applied to the treatments used in this experimental set up.

The FWS mesocosms sit just outside of the DO values suggested by Oh and Silverstein (1999)(>5.6mg/L), whilst SSF expressed DO values of approximately 2.5mg/L, meaning denitrification could be potentially occurring at a significant rate. Although denitrification is an important process to maintain within the treatment wetland the primary interest in this piece of research is calcium maintenance.

6.6.2 Ion transformations in Mesocosm Calcium Mobility Analysis

Nitrogen and phosphorus containing compound removal is the primary target for the installation of CWs on the conservation sites of Anglesey and the Llyn peninsula conservation sites. Therefore, it is of the upmost importance that when examining the

implications of calcium mobility within treatment wetland systems that these processes are unaffected by the additional functions required by the system.

No phosphorus containing compounds were found in the Waun Eurad spring water used as the inflow of the mesocosms supply water, this was therefore not discussed on this occasion.

As seen in figure 6.9 all treatments significantly reduced nitrate on passing through the CW mesocosms. This in itself elucidates successful pollutant removal from the systems and in all treatment types, nitrogen has either been assimilated into plant biomass or has been successfully transformed into other compounds, for example nitrite or full denitrification to gaseous nitrogen. The two main processes in nitrogen removal, nitrification and denitrification, each require significantly different conditions for these processes to take place. Such conditions are governed by oxygen availability or redox potential which has similar effects on the precipitation of calcium in wetlands.

Nitrite in the inflowing water exhibited sporadic fluctuation in concentration. However the systems were able to buffer against these fluctuations, all be it at a small magnitude. Nitrite was not always completely removed or taken up into the system.

When looking at the details of possible nitrate and nitrite transformations there are limited transformations that can physically take place (Sprent 1987). Nitrite known to be an intermediate step in both nitrification and denitrification. The processes involved in nitrification are transformation from ammonium into an intermediate compound hydroxylamine and subsequently production of nitrite. Nitrification continues from here to form nitrate (Shapleigh 2013). Denitrification interestingly reforms nitrate through nitrate reductase expressing microbes. This microbial pathway relates to removal of nitrogen on strictest terms from the treatment wetland system and is the main method by which nitrogen leaves the system. Otherwise nitrogen is merely stored in the biomass of the plants and microbes inhabiting the system, which due to decay cycles can potentially be re-released into the water column. It is worth noting that these processes need to be accounted for when designing CW systems as indicated by the C* value in the P-k-C* equation (Kadlec & Wallace 2008).

Other possible transformation of nitrate and nitrite include assimilatory reductions of the above compounds back to the intermediate hydroxylamine and reformation of ammonium (Shapleigh 2013). It is to be noted that no ammonium was detected in any of the treatments nor in the inflow of the experiment. This means that the only processes involved in nitrogen compound removal are denitrification or plant uptake.

Hypothetically, even if all the nitrogen removal over the course of the experiment was due to plant uptake and no microbial denitrification had taken place the potential for microbial colonisation is present. This means that the system was still succeeding in nitrogen removal from the water column during the calcium mobility analysis despite not being fully mature.

6.6.3 DOC and Phenolic Dynamics in Relation to Calcium

Analysis of DOC is important as the presence of these compounds, especially in labile forms, is relied upon for microbial denitrification and in relation to this experiment, potential calcium mobility by chelation.

DOC was increased by all the treatments to varying magnitudes (Figure 6.11). Inflow DOC influenced the concentrations observed flowing through the mesocosms, whereby treatment concentrations seemed to track the changes observed in inflow DOC. The additional DOC found in the outflow was due to system production. At all times the *Phragmites* system produced the most DOC whilst the subsurface system produced lowest concentrations.

Although planting density was controlled, DOC in the SSF systems was significantly less concentrated compared to FWS systems (46%). This significant reduction may be attributed to the reduction in void space within the system. This resulted in a reduced nHRT within the mesocosm container. Porosity analysis undertaken on the SSF gravel media concluded a porosity value of 0.40, reducing void space by 60%. Therefore reduced nHRT is observed, decreasing the potential for DOC concentration due to flushing effect.

However, biofilm colonisation in the SSF system may be greater due to larger surface area. Discussed previously, anaerobic conditions where microbial denitrification conditions prevail are associated with subsurface flow systems rather than FWS, although the process is still able to occur. It is also known that organic carbon removal is achieved as a bi-product of denitrification due to its requirement by microorganisms in the process (Kurzbaum et al. 2010). The primary difference is that in SSF systems the majority of the microbial community is sessile and bound in a polysaccharide matrix where they rely on metabolic processes of other microbes in the community (Costerton et al. 1995). It is proposed that the large increase in potential colonisable surface area within the SSF mesocosm may be acting as an additional means of DOC removal from the system, either in terms of microbial biomass accumulation or to meet demand during denitrification. Therefore wetland design will inevitably influence DOC processing and potentially calcium mobility. Similarly, variations in DOC concentration ultimately affect the possibility for calcium chelation or association with calcium. The potential for the hypothesised chelation in relation to chemical

make-up of the DOC is discussed later. However, the sheer difference in DOC concentrations may be enough to overcome any effect of chelation potential.

Figure 6.12 provides an insight into the molecular weight properties of the DOC being released from the mesocosms. It was originally hypothesized that the MW of the DOC being produced or transformed within a wetland system may provide a mechanism for the mobilisation of calcium. And from the preliminary calcium mobility experiment (Chapter 5), calcium associated with DOC of <3500 Dalton Molecular Weight Cut off (DaMWCO).

In the mesocosm experiments it was found that *Phragmites* produced the greatest quantity of <1000Da MWCO DOC for the duration of the experiment. Strobel, Christian, Hansen, Borggaard, & Andersen (2001), discuss reactivity of LMW carbon based compounds and their reactivity within the soil water interface. They found that in the case of soil DOC in forests, LMW DOC made up less than 10% of the total DOC pool (Van Hees et al. 1996; Westergaard Strobel et al. 1999; Strobel et al. 2001) similar to that found in the wetland mesocosms. Strobel *et al* (2001) found that DOC released various bound and chelated metal ions and that different magnitudes of each metal type were released at different rates. However, DOC produced as root exudate by plants in a CW scenario may result in the fresh uptake or chelation of metal onto the DOC molecule as opposed to the release observed in research undertaken by Strobel *et al* (2001). The release of metals from the DOC was chemically induced; therefore binding, chelation or sorption of metal onto the DOC molecule must have been achieved prior. DOC produced as a result of photosynthetic processes or root exudates for modification of the rhizosphere (Larue et al. 2010) may provide DOC molecules that have not as yet been bound to metal ions, allowing calcium-DOC molecules to form where Ca^{2+} dominates.

Acid mine drainage remediation wetlands are becoming increasingly popular as a means of removing reduced forms of iron from the mine drainage water and increasing the pH to more ecologically safe levels (Hancock 1973; Hedin, Nairn and Kleinmann 1994; White et al. 2011). It was proposed by White et al (2011) that the presence of phenolics prevents the oxidation of Iron due to its chelation onto the phenolic group of the DOC. This chelation prevents the iron from becoming oxidised and forming a precipitate resulting in flocculation.

Tu *et al* (2004) undertook analysis into the mobilisation of Arsenic, among other metals, by various forms of DOC from 2 fern species. One of the species produced 3-5 times more oxalic acid than the second. Oxalic acid is a typical form of LMW DOC, and if present in the root exudate of the test species used in this mesocosm experiment could reasonably be attributed as a form of the mobilisation of calcium.

DOC characterisation was achieved in this experiment by SEC analysis, indicating molecular weight. Although the *Phragmites* treatment showed significantly more LMW DOC being produced detailed organic compound analysis may provide a better insight into chelation properties.

Phenolics are a constituent of the DOC pool; however no phenolic containing material was detected at any time in the inflow water samples. This finding concurs with system production of phenolic containing DOC molecules as found by White et al (2011). Phenolic production is a function of mesocosm processing. This was also in agreement with, and the basis for, multiple species used in the experiment to analyse production of phenolics between species and its effect on calcium mobility, as found in Fletcher & Hegde (1995).

Lower phenolics concentrations were observed in *Phragmites* SSF treatment compared to *Phragmites* FWS system. Shorter retention time and flushing within the SSF system is considered to be the driver for lower concentrations of phenolics. Additional *in vitro* analysis of phenolics per unit biomass per unit DOC is examined in the following chapter. However, differences in phenolic content were observed here that contradicted Larue et al (2010) who found *Iris pseudacorus* produced significantly more phenolic material per gram of biomass. Differences in biomass in the current study may have occurred resulting in reduced phenolic content observed in the *Iris* mesocosms. Although plant biomass was balanced at system start up by controlling planting density, dry biomass may have changed significantly over time, potentially affecting observed phenol concentrations in the rhizosphere.

6.6.3.2 Characterisation and Composition of DOC by UV-Vis

It was expected that different species would produce difference MW ranges and also exhibit different chemical compositions of the DOC. UV-Vis absorbance showed that the most apparent differences in composition lie with aromatic components. Whilst both planted systems increased the aromatic content of the DOC, most noticeable effect was indicated in the SSF system, despite shorter system retention time.

Costerton et al (1995) found that increased levels of aromatic DOC were linked to increases in microbial density of biofilms. The SSF mesocosms used in this experiment provided a greater surface area for attached microbial communities due to the magnitude of surface area on the gravel bed media compared to the surface area in the FWS systems. Although, microbial densities may be different in fully functioning field scare systems.

6.6.3.3Linking aromatic content to original hypotheses for greater calcium association with LMW DOC

UV-Vis techniques for aromiticity, defined by the E2:E3, ratio show that that as molecular weight increases the compounds absorb more UV at longer wavelength therefore decreasing the ratio value (Helms et al. 2008; De Haan & De Boer 1987). Therefore, high aromaticity shown in the SSF system (figure 6.15) whilst retaining low total DOC (figure 6.11) may be a suitable method to maintain calcium mobility whist reducing DOC output from the system. SSF systems may not be viable in all field situations due to potential $CaCO_3$ formation and suspended solid material in the inflow water. *Phragmites* FWS produced significantly more DOC in the mesocosm than the *Iris* treatment however the aromatic values are similar. This may provide a more viable option in field conditions Preliminary studies (Chapter 5) concluded that calcium became associated with LMW DOC, furthermore evidence in the literature and characterisation of the mesocosm aromatics support the potential for chelation (Helms et al. 2008; De Haan & De Boer 1987; Weishaar et al. 2003).

Humic DOC molecules are generally of high molecular weight, are in abundance in terrestrial and aquatic systems, and often have the greatest effect upon water colour (Willoughby 1976). Many types of DOC have been associated with the mineral dissolution and precipitation reactions (Weishaar et al. 2003). Petrovic, Kastelan-Macan, & Horvat (1999) discuss the possibility that humic compounds facilitate heavy metals (namely Pb^{2+}, Zn^{2+} and Cu^{2+}) binding to soils and minerals where the number of binding cites on the humic acid regulates the potential sorption. This research indicates the possibility for calcium binding or complex formation with humic compounds produced in the wetland mesocosm. However binding site characteristics of the humic compounds may induce binding calcium to soils which may be a negative effect in the context of the research in this chapter.
The results for humic content in figure 6.11, show all treatments produced a similar humic content. However if this is related to the DOC produced in the mesocosm systems, the DOC released from the *Phragmites* FWS treatment is more concentrated followed by *Iris* thes SSF system. This may have direct implication for the humic content per unit DOC produced in the systems.

In the context of CWs installed on the Anglesey and Llyn fens, one of the targets was to reduce DOC and maintain Calcium whilst removing nitrogen. *Phragmites* FWS stands produce low humic content per unit DOC compared to SSF and *Iris*, therefore potential chelation properties are reduced. DOC with high chelative properties whilst exhibiting low total concentration should provide most calcium mobility maintenance with minimum DOC output, resulting in efficient mobility maintenance with regard to DOC released.

Little difference is observed in the SUVA values between treatments. This may be due to the correction for DOC concentration. Unlike aromatic and humic content, SUVA at 254 nm is

normalised against DOC concentration. SUVA values correlates strongly with aromatic values per unit DOC, confirmed by research correlating SUVA 254 against percentage aromaticity as determined by C^{13} labelling (r^2>0.97) (Weishaar et al. 2003). Weishaar *et* al (2003) found correlations between SUVA and aromatics whilst Helms *et al* (2008) found that the reason for decreasing E2:E3 ratio was due to increased HMW in the sample. If we apply this to the data in figure 6.15 we see that the SUVA for the SSF system is similar to the other treatments; however the treatment shows a low DOC value (figure 6.11) suggesting a low 254 nm absorbance in order to provide a similar SUVA value. In order to observe an a significant increase in aromatics, the E3 value and linked higher MW for the SSF system must be equally low in order to generate a large aromatic value. This shows that the composition of the DOC arising from the SSF system has a low total DOC content however the aromatic, and inferred LMW DOC, percentage is greater than the other 2 treatments meaning there is a potential for high chelation with low carbon output.

Following this analysis of DOC characteristics suggestions can be made into CW design and species selection. SSF systems seem to facilitate processes in the rhizosphere, linked to greater LMW DOC production per unit DOC. This type of DOC is by its nature more labile and readily used in biological processes such as denitrification and may possess increased chelation properties for calcium. Secondly, *Iris* produces similar quantities of LMW DOC per unit DOC for calcium chelation and mobilisation. Using systems with *Iris* may allow for maximum calcium chelation and mobility maintenance with the minimum DOC production. The production of LWM labile DOC also means that the DOC will have less impact upon downstream water bodies due to increased utilisation in the conservation sites.

6.6.4 Calcium Analysis

Various calcium components were analysed in order to understand potential for chelation and fate of calcium in the system. Maximum possible calcium concentration flowing from the systems is desirable, whether this is as a chelate with DOC compounds or otherwise. It was expected that differences in calcium concentration may occur due to plant species nutrient uptake requirement, flocculation, carbonate formation or chelation to DOC resulting in mobility maintenance.

All treatments in the mesocosm study reduced the concentration of ionic calcium. As calcium is an essential nutrient for plant growth (Mitsch & Gosselink 2000) a portion of the ionic calcium removal can be attributed to plant growth. The significance of this can be seen especially well when the first 3 weeks of calcium data are observed in the FWS planted systems (figure 6.16). Over the course of the initial 3 weeks of the experiment, calcium

utilisation by the plants or otherwise, decreases rapidly. Ionic calcium values stabilised from week 4 to the end of the experiment. Uptake of calcium is one transformation that can occur in wetland systems, however binding to DOC may be occurring by week 4 leading to reduced detection of calcium in its ionic form.

Kadlec and Wallace (2008) discuss the formation of carbonates and the conditions in which this reaction takes place. Formation of carbonates occurs due to the precipitation of HCO_3^- with Ca^{2+}. However the reaction is mediated by high pH. As the reaction occurs H^+ is released from HCO_3^-. It is suggested that $CaCO_3$ may have been formed within the SSF mesocosm, this means that the produced H^+ may still be in solution driving down the pH in the outflow (figure 6.6), although this is thought to be minimal due to the pH of the SSF balancing around 7.3. Low DO concentrations are known to induce carbonate formation resulting in pH reductions. $CaCO_3$ is less bioavailable to plants and could explain stunted growth observed in the SSF *Phragmites* plants. Reduced hydraulic retention time in the system could account for reduced contact time between bioavailable calcium in its ionic form and the plant roots. Approximately 30% reduction in ionic calcium is observed in the SSF system for the entirety of the experiment: limited uptake and carbonate formation seems likely.

Although $CaCO_3$ formation within the treatment wetland could be problematic and reduce calcium output from the systems, potential sorption properties of the calcium carbonate for phosphorus binding (Prochaska & Zouboulis 2006)can also be seen to be beneficial to the project.

All of the treatments significantly reduced the total concentration of calcium in the water column (Figure 6.19) however; *Phragmites* is continually less concentrated with the exception of week 5. This seems to correlate with lower ionic values as seen in figure 6.20. Total calcium was more concentrated in *Iris* and SSF treatments and may be attributed to uptake or precipitation. This data is important to the development of calcareous fen on the Llyn and Anglesey Fens project conservation sites. If substantial calcium is being removed from the water column the development of calcareous fen habitat will be ultimately limited. Moreover, the selection of plants may be more crucial than first thought. It may be plant calcium requirement that drives the availability of calcium in the water column of the CW.

6.6.4.1Total Chelation and Increased Calcium presence

It has already been concluded that the presence of *Phragmites* in the system reduces total calcium concentration significantly, however further support for the potential chelation with DOC as produced in the *Iris* mesocosms is illustrated by figure 6.21. *Iris* has a greater value

for total calcium and furthermore exhibits in greater (2.4%) concentration of bound calcium. Moreover, Weis & Weis (2004) refer to the regularity of macrophyte metal utilisation in treatment wetlands, where “nutrient pumps” (Odum 1988) are the pathway by which heavy metal pollutants are taken up into plant biomass. It is proposed that *Phragmites* exhibits a greater percentage biomass of calcium and therefore has the potential to exhibit greater numbers of calcium channels in the roots of the plant than *Iris.*

6.6.6 Conclusions

All of the treatment wetland types significantly removed nutrient pollutants from the water column as set out as the primary function of the treatment wetlands systems installed by the Llyn and Anglesey LIFE Fens project. Biological mobilisation and immobilisation was found to vary between constructed treatment wetland system designs, in this case FWS and SSF treatment systems, and also between the plant species used in this experiment. System design and plant species can also affect the amount of calcium passing through the systems. However, it is uncertain if this is primarily due to plant species and the production of DOC with chelative properties or DOC modification due to microbial activity within denitrification optimised subsurface flow systems. Calcium is a known plant, however this experiment was short term, mesocosm scale and under controlled conditions. Field measurements of tissue calcium content may elucidate a species present on the Llyn and Anglesey fens suitable for CW use whilst exhibiting a low biomass demand for calcium itself.

Further knowledge needs to be gained on the mechanisms involved in calcium mobility which is explored in chapter 7. *In vitro* experimentation with plant species specific root exudate DOC was undertaken in chapter 7 to confirm any chelation properties linked with the root exudate DOC. Secondly, whether the chelation or solubility maintenance is an artefact of root exudate DOC production or microbial DOC processing as a result of system type.

However, this research can influence decision making and advise best practice for the design and installation of CWs to treat diffuse agricultural pollution in the form of nitrates and phosphates where Calcium maintenance and DOC reduction are required. *Iris* FWS systems are able to maintain high pH, perform good nitrogen removal, prevent large DOC losses from the system, produce low phenolic concentrations, allow for ionic and total calcium maintenance, and release a high proportion of the calcium in a bound form potentially chelated to the DOC produced in the system. By selecting this set up for nutrient enrichment and DOC reduction, whilst maintaining calcium, the NRW LIFE Fens Project targets may be met. However this is in conjunction with proper scaling of the treatment systems in order to achieve the primary target.

Chapter 7 - Investigation into the Effect of Plant Species on Rhizosphere DOC Production

7.1 Introduction - Root Exudate Contribution to Calcium Mobility

Total and ionic calcium concentrations in wetlands are potentially affected by 3 main pathways; plant uptake, precipitation in or binding to the system components and thirdly by chelation. There is a potential for plant selection within treatment wetlands based on species specific calcium requirement or the facilitation of calcium mobility maintenance by chelation to LMW DOC and phenolic compounds produced by root exudates, as described in Chapter 5 and 6. Thus exudate compounds were collected from *Phragmites australis* and *Iris pseudacorus* in order to determine the potential for calcium chelation and resultant mobility maintenance.

Hypothesis development

Chapter 5 suggested that Ca^{2+} became associated with or chelated to DOC of MW <3500 Daltons. Chapter 6 indicated that *Iris* produces more LMW DOC per gram of total DOC, and that *Iris* treatments resulted in greater ionic and total calcium compared to *Phragmites* treatments. Therefore the following hypotheses were developed to quantify the mechanisms involved in calcium mobility highlighted in the previous chapters.

1. *Iris pseudacorus* produces DOC based root exudates that contain more phenolics and LMW compounds per unit DOC than *Phragmites australis*
2. Due to the above, DOC produced by *Iris pseudacorus* has more potential for chelation; therefore will maintain mobility and preventing calcium precipitation within the constructed wetland environment.

7.2 Methods

7.2.1 Plant Selection and Preparation

Maturing *Phragmites* and *Iris* plants were extracted from the FWS treatments used in the mesocosm study(chapter 6), FWS systems plants were selected in order to minimise root damage. Plants were selected based upon similar root and shoot sizes and degree of development. Each of the plants was washed in order to remove the majority of the remnant soils and to remove biofilms wherever possible (figure 7.1 and 7.2). The plants were weighed individually in order to produce 5 replicates of a similar weight for each species.

7.2.2 Root Exudate Collection

Plants were set up hydroponically in order to produce solutions containing the root exudate DOC and phenolic material. In order to prevent against biological processes resulting from the manipulation and disturbance of the roots, the plants were placed into ultrapure water for

two days prior to being moved and suspended hydroponically into 1 litre of ultrapure grade water for exudate collection. This period also helped remove any remaining soils from the roots (figure 7.3 and 7.4.

Above ground parts of the plant were allowed access to direct sunlight and the atmosphere. The plants were secured by the addition of soft packing around the base of the stems in order to support the plant in the collection bottles. A foil covering was placed around the bottles in order to prevent sunlight penetration into the root zone.

Following a period of 10 days the plants were removed from the bottles, the water in the collection bottles was filtered through 0.45µm Whatman filters and 1L stored at 3°C until analysis. The plants were measured for wet biomass, and also dried at 45°C for 48 hours before dry biomass was recorded. The dried plants were weighed in terms of their below water level (i.e. roots and rhizomes) and shoot biomass individually and also as below water level and shoot dried biomass. This was undertaken in order to assess the production of DOC and phenolics standardised against the biomass of each replicate.

Figures 7.1 and 7.2 Five replicates of *Iris pseudacorus* and *Phragmites australis* (Left and right respectively)

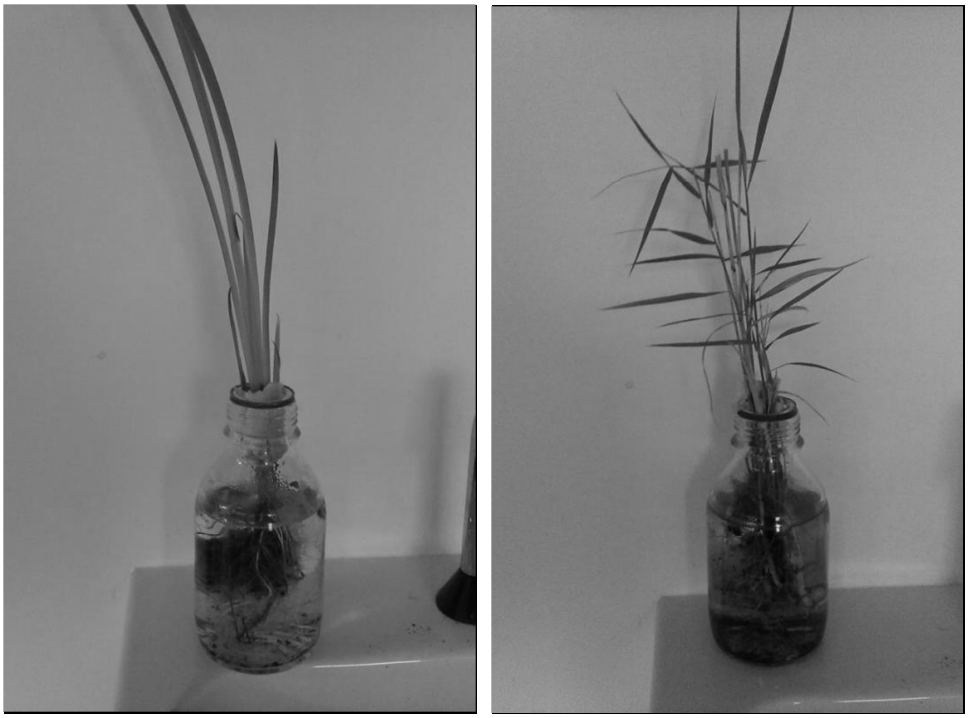


Figure 7.3 and 7.4 *Iris pseudacorus* and *Phragmites australis* (Left and right respectively).

Note- the pictures were taken during the 2 day stabilisation phase where immersion in water aided final soils removal from the roots and rhizomes, which was discarded before exudate collection.

Figures 7.5 and 7.6 - Root and shoot biomass for both plant species pre and post drying (Left and right respectively).

7.2.3 Data Collection

From the root exudate solution the following information was collected as specified in Chapter 6

1. DOC concentration
2. Phenolic content
3. Molecular weight Spectra
4. UV VIS absorbance scan 200-800nm

7.2.4 Calcium Addition

Following filtration and analysis of root exudate samples, $CaCl_2$ solution was prepared to 300mg/L Ca^{2+}. $CaCl_2$ solution was added in equal measures to 150ml of root exudate DOC from each replicate. Thorough mixing of the containers using an oscillating table was undertaken for 24 hours to allow mixing of the exudate with the $CaCl_2$ solution. Assumptions were made at this stage that no additional calcium had leached from the plant roots during exudate collection and that calcium concentration would be due to the artificial addition of calcium chloride only.

Following calcium addition and mixing, the ionic and total calcium values were assessed using the aforementioned methods of analysis. Ion chromatography was undertaken for the same suite of ions as used in earlier chapters, however only ionic calcium data was used here.

7.3 Results

7.3.1 DOC

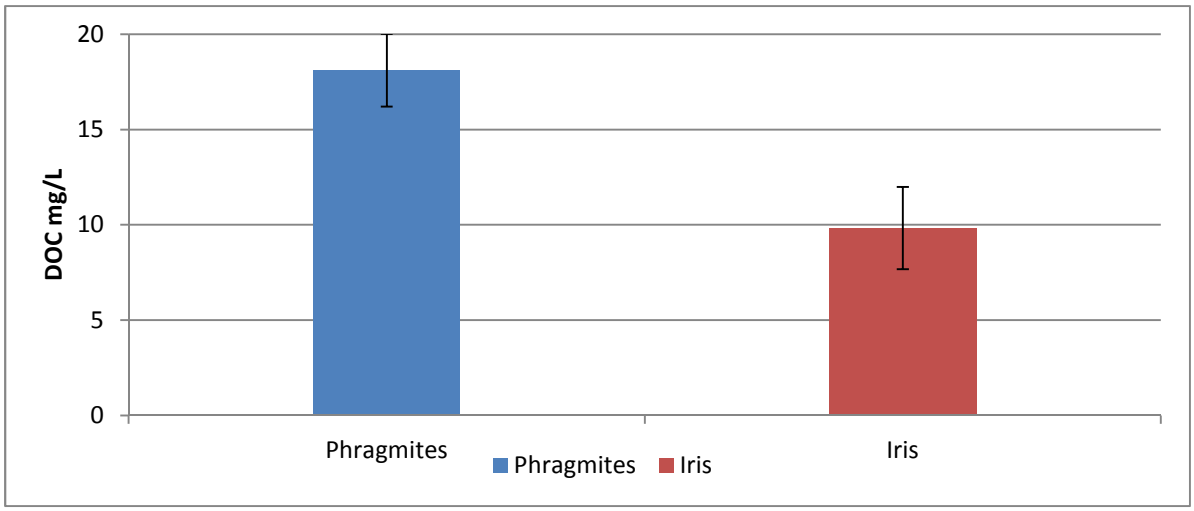


Figure 7.7 – Total DOC produced by the test species during hydroponic root exudate collection. Error bars indicate standard error of the data.

Although plant biomass was estimated to be relatively similar, *Phragmites* was shown to produce approximately 7mg/L more than the *Iris* substrate collection. T-test analysis for DOC produced in the root exudate showed significant differences in the concentration of

DOC within the collection bottles. However, additional data including tissue biomass was also required in order to draw to express DOC per unit biomass.

7.3.2 DOC Standardised per Unit Plant Biomass

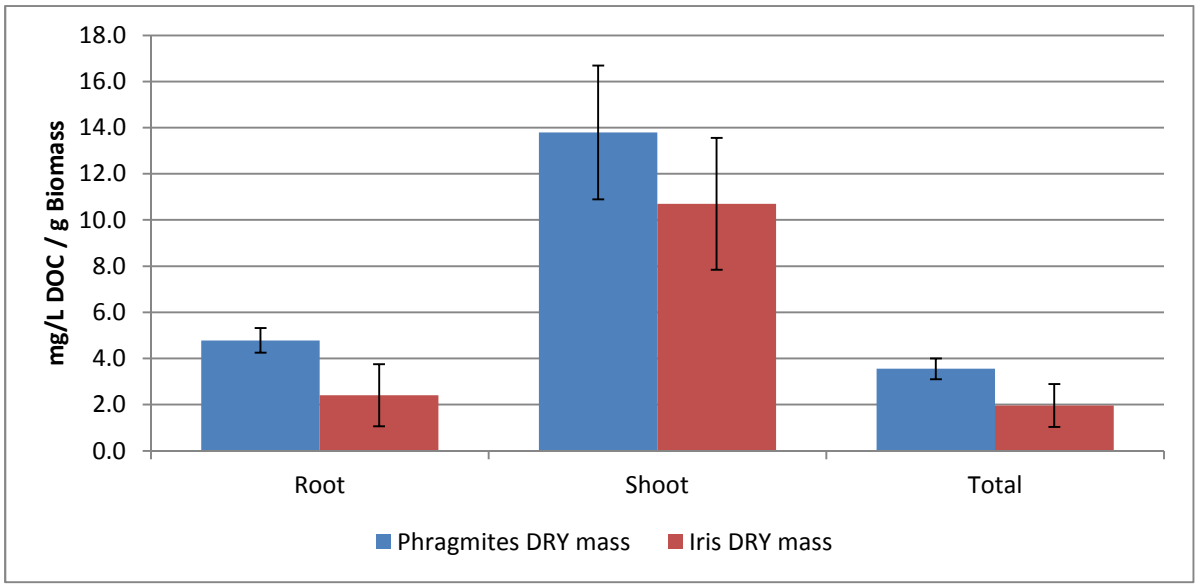


Figure 7.8 DOC produced per unit dry biomass of *Phragmites* and *Iris*.

Total DOC produced as root exudate was standardised against biomass to assess the input to the DOC pool. In all instances *Phragmites* produced greater concentrations of DOC per unit biomass. DOC standardised for total biomass indicated a statistically significant difference in root exudate DOC production ($p<0.020$).

7.3.3 Phenolic Constituent of the DOC Pool

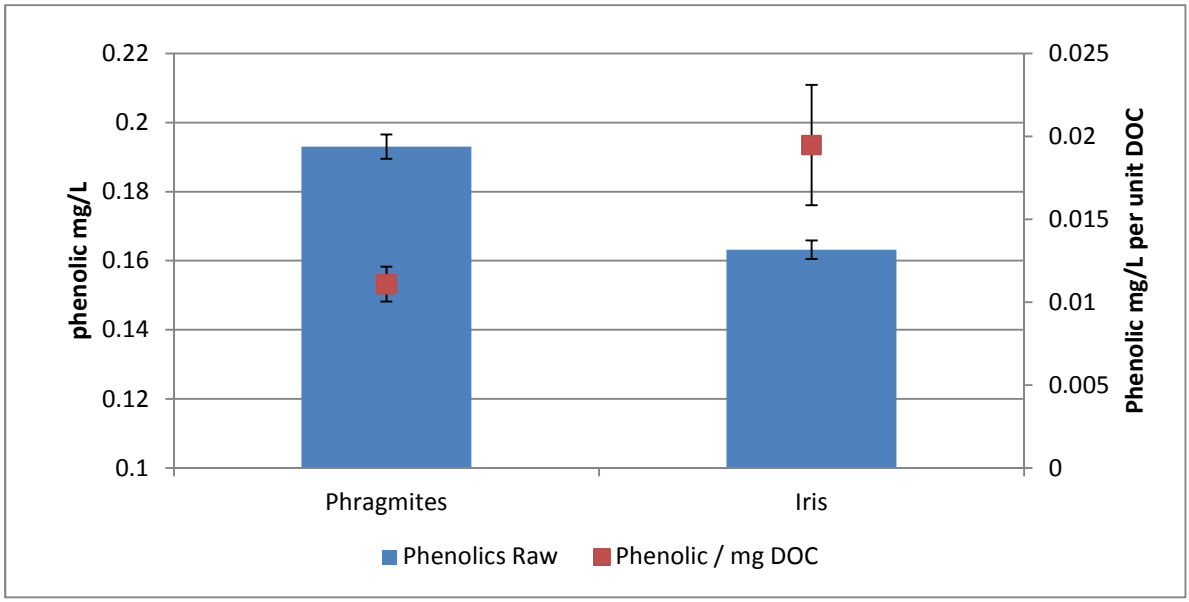


Figure 7.9 - Phenolics produced by *Phragmites* and *Iris* as root exudate.

Phragmites produces more raw phenolics consistent with DOC (figure 7.7). However when phenolics are standardised per unit DOC *Iris* produces a greater concentration. ANOVA analysis indicated a statistically significant difference between the phenolic content of the DOC produced in the root exudate collection ($p<0.001$). *Phragmites* produces statistically more phenolic, however, *Iris* is observed to produce more phenolic compounds per unit of DOC although this was not significant ($p<0.057$).

7.3.4 Low Molecular Weight Fraction of DOC

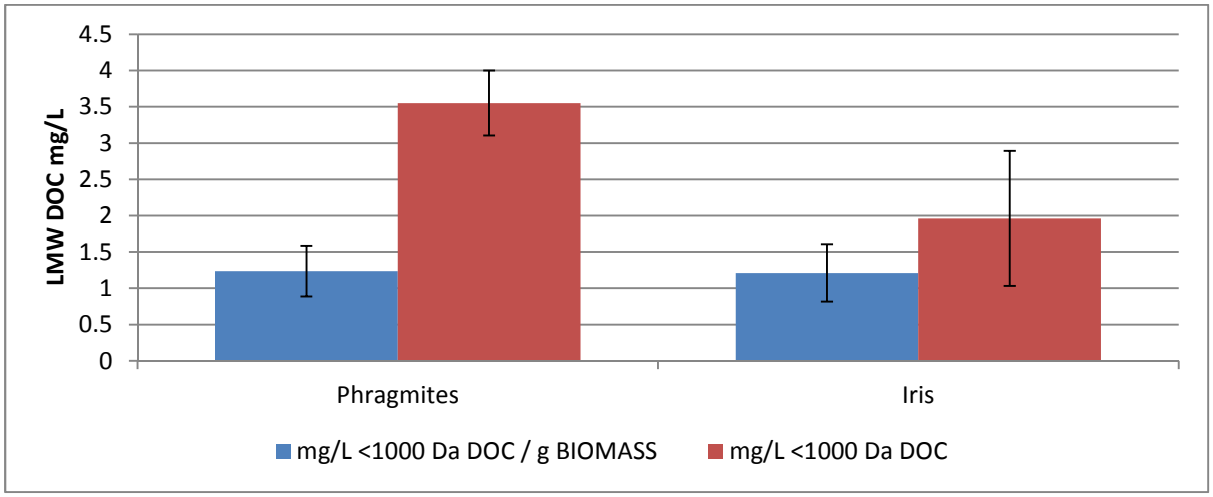


Figure 7.10 - Low molecular weight constituents of root exudate DOC.

The data shows that Phragmites produces greater concentration of total LMW DOC concurrent with figure 7.7, however when standardised per gram of biomass Phragmites and Iris produce similar concentrations.

The data from the LMW DOC per unit biomass was found not to be normally distributed and the variances between the treatments were not similar enough in order ANOVA. A T-Test was performed in order to compare means for significant differences. The T-test indicated significant difference was found between the *Iris* and *Phragmites* when analysing the LMW DOC before the biomass element was included. A T-statistic of $p<0.930$ was found indicating strong significance between the treatments.

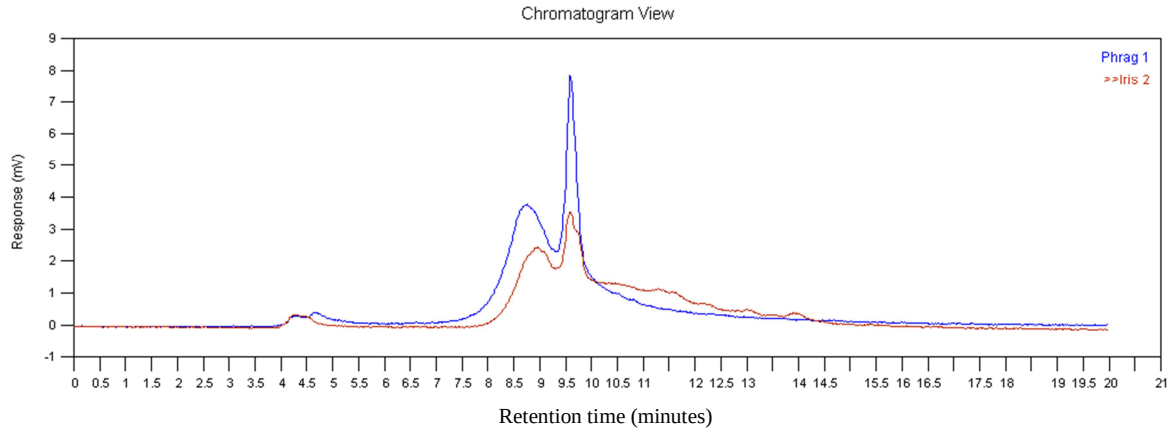


Figure 7.11 - *Phragmites* and *Iris* size exclusion chromatograph.

Phragmites shows a greater response during the early stages of compound detection, when higher molecular weight groups are desorbing from the solid phase of the SEC column. At 10 minutes *Iris* 2 (Red) shows a greater concentration of molecules within the lower molecular weight range. The data presented here supports figure 7.7 for total DOC, and confirms evidence from the LMW data (figure 7.10) supporting that *Phragmites* produces more total DOC whilst *Iris* produces more LMW DOC per unit/g biomass/mg DOC.

7.3.5 UV-Vis Spectroscopy

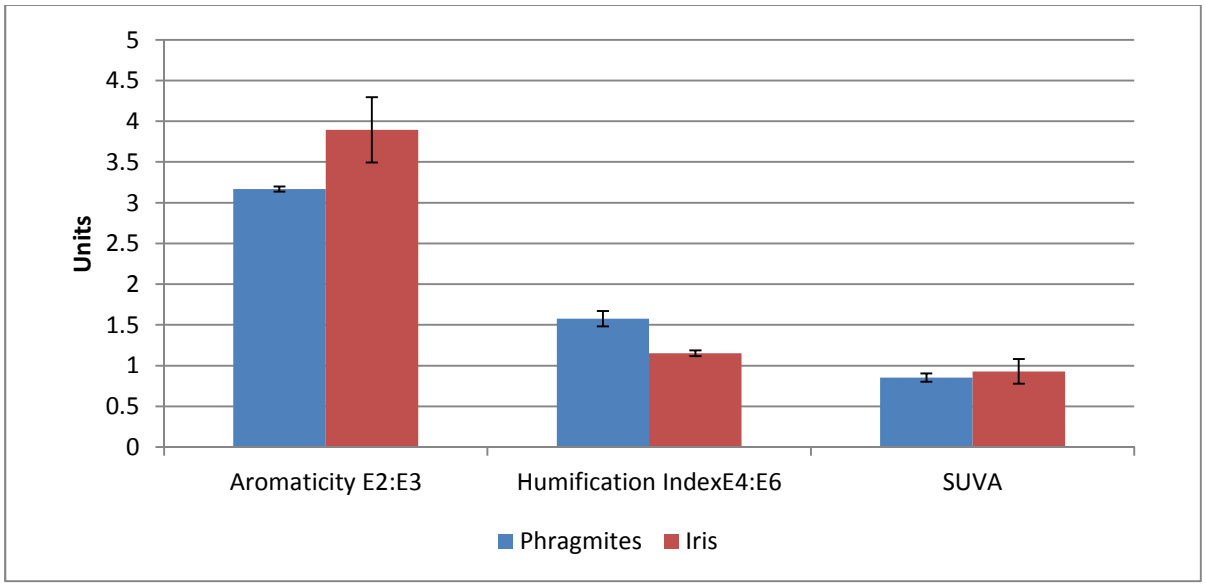


Figure 7.12 – UV-Vis component analysis of root exudate DOC.

The graph above contradicts the data seen in figure 6.15 of Chapter 6, where the FWS *Phragmites* system produced DOC with 15% lower aromatic values in the CW mesocosms. When analysing root exudate DOC contribution alone, *Iris* produces distinctively greater concentrations of aromatic carbon than *Phragmites*, although it is more variable. Humic level follows the trend observed in Chapter 6, indicating an approximately 50% greater

concentration in *Phragmites* root exudate values. Similarly SUVA per unit of DOC does not suggest differences between the two species under analysis.

7.3.6 Calcium Concentration

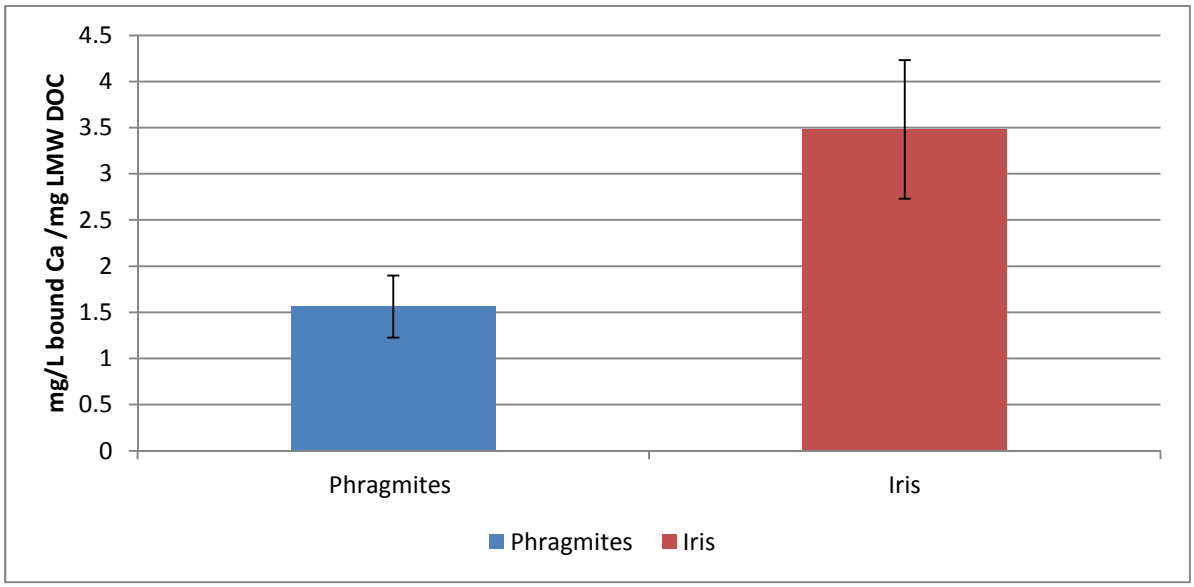


Figure 7.13 – Bound (non-ionic) calcium per mg of root exudate.

The *Iris* data suggests a significant greater concentration of bound Calcium than *Phragmites* ($p<0.048$).

7.4 Discussion

7.4.1 DOC Compounds

On analysis of the DOC produced as root exudate (figure 7.7), as reported in chapter 6 *Phragmite*s produced significantly more DOC (80%) than Iris ($p<0.03$). Detection of concentrations up to 20 mg/L DOC in the root exudate collection experiment confirms that DOC is contributed to significantly by root exudate. Achieving the lowest possible DOC concentration in exported waters is a target for the LIFE Fens project. Even when DOC production was standardised against biomass *Phragmites* produces approximately 50% more DOC per unit biomass. Based on DOC production, both total and per unit biomass (figure 7.8), *Iris* is more suitable for use in the CWs on the Anglesey and Llyn conservation sites due to the requirement for lowering DOC output.

Morphological variation, photosynthetic by-products used as exudate, root surface area and degree of suberisation vary between the species analysed. It is proposed that increased production of DOC is a factor that is potentially controlled by root surface area. When the roots of each species are considered, morphological variation is clear. Attention is drawn to

Figure 7.5 and 7.6 where plants used in the root exudate experiment are pictured. *Phragmites australis* is seen to have a larger amount of roots generally, however the biomass weight is similar to that of the *Iris pseudacorus*. *Iris* posessed a heavily suberised main root which is highly dense, rhizomes project from this main root and have limited surface area in comparison to the Phragmites roots. This variation in implied surface area may contribute towards reduced DOC per unit biomass observed in *Iris.*

7.4.2 Phenolics

Understanding of potential chelation, highlighted by the mesocosm experiment may include chelation of Ca^{2+} to the phenol molecules on the DOC compouds. White, Freeman, & Kang (2011) found strong correlation between phenolics and iron complex formation preventing oxidation. Similarly, if calcium is capable of chelation with the phenolic group, increased phenolic content per unit DOC may be advantageous. Greater phenolic production suitable for calcium binding and mobility maintenance in the wetland system also supports the Anglesey and Llyn LIFE Fens focus for reducing total DOC output. Reduced decomposition in the fen may be observed due to inhibitory characteristics of phenols and low phenol oxidase activity (Freeman et al. 2004). The build-up of phenolics in combination with reduced bimolecular oxygen caused by rewetting of the fens should reduce overall decomposition and associated DOC release.

The results of the phenolic analysis (figure 7.9) indicated that *Phragmites* produced the greatest concentration of phenolics. However, standardised per unit DOC, *Iris* is observed to produce more phenolic compounds. If the observations in metal binding properties by White et al (2011) apply to chelation of calcium, then the DOC produced as a root exudate by *Iris* has a greater calcium carrying capacity whilst reducing DOC output to the conservation site.

7.4.3 Molecular Weight Variation

LMW DOC constituent was analysed due to its potential for chelation, as found in the calcium dialysis experiment (Chapter 5). The *in vitro* experiment showed that *Phragmites* and *Iris* produce statistically similar quantities of LMW DOC per unit biomass (Figure 7.10). This investigation elucidates the importance of per unit biomass correction. If we reconsider evidence for LMW DOC production in figure 7.10, *Phragmites* was observed to produce significantly greater concentrations of LWM DOC. Figure 7.11 also shows that the DOC MW spectral distribution of each of the species studied.

7.4.4 UV-Vis Spectroscopy

Analysis of aromatic character, humification index and SUVA showed that although humic content and SUVA showed similar results to the mesocosms, Iris aromatic content was significantly greater in the *in vitro* experiment.

Costerton et al. (1995) found relationships between aromatic root exudate production and colonisation of biofilms. It is proposed that during the setup of the *in vitro* experiment, biofilms previously colonising the roots of the plants was washed off. This induced the production of labile aromatic DOC by the roots which promote the colonisation of biofilms allowing the plants to benefit from the microbial symbioses that arise (Walker et al. 2003).

7.5 Calcium Mobility and Dynamics with Regard to Root Exudate DOC

Revisiting hypothesis 1, it was confirmed that *Iris* produced greater phenolics and LMW DOC per unit biomass per unit DOC than *Phragmites*. However, this alone did not provide the mechanisms by which DOC interactions with dissolved calcium. Analysis of DOC-Ca^{2+} interacts was quantified by calcium addition to the root exudate samples detailed below. It was hypothesised that wetland macrophytes produce different concentrations of DOC, LMW carbon and phenolic compounds dependent on rhizosphere chemistry, plant morphology, microbial communities colonising the roots and resultant symbioses. Understanding the mechanisms involved in potential DOC chelation of calcium was paramount in the *in vitro* testing. However, the experimental set up also allowed for the collection of root exudates, this allowed for separation of within the system and root exudate specific factors. Mechanisms of chelation driven by root exudates were analysed independently of contributions made by system design specific DOC modifications, which was observed in chapter 6.

Figure 7.13 shows that DOC produced by *Iris* is capable of binding significantly more calcium per unit that DOC produced by *Phragmites*. This evidence indicated that root exudates root exudates produced by *Iris* have a greater calcium carrying capacity. In terms of effect on the water chemistry within the CWs on the conservation sites, this means that maximum calcium mobility is achieved for minimum DOC output.

Following confirmation of hypothesis 1, hypothesis 2 was revisited. *Iris* not only produces greater concentrations of phenolics and LMW DOC compounds per unit biomass per unit DOC (hypothesis 1), the calcium detected in the *Iris* treatments was also found in greater concentrations in a bound form than *Phragmites*. This therefore has the potential to prevent complex formation of calcium with carbonate, resulting in calcium mobility maintenance.

7.6 Conclusion

In vitro analysis of DOC supports the conclusions drawn from the mesocosm experiment of chapter 6. Calcium increasingly forms association with DOC originating from the root exudates produced by *Iris pseudacorus*. This species produces significantly lower amounts of DOC per unit biomass. When installing CWs to treat enriched spring water supplying the alkaline and calcareous fens of the Anglesey and Llyn LIFE fens project it is of benefit to install systems that contain primarily *Iris*. Selecting *Iris* as the primary macrophyte will aid in preventing elevated DOC within the fen, and similarly in the catchment the fen supplies. This is particularly the case at Cae Gwyn, Anglesey, where the fen directly supplies a drinking water reservoir. Chlorination used in the disinfection process at water treatment works can lead to the release of disinfection by-products when specific types of DOC are present (Cotruvo 1981). Reducing DOC production with the CWs supplying the fen by selecting for *Iris* rather than *Phragmites* may therefore lead to reduced health risk (Brennan & Schiestl 1998).

Increased phenolic content of the DOC produced by *Iris* may also prevent the decomposition of material within the treatment systems by inhibition of hydrolases linked to reduced phenol oxidase activity (Freeman et al. 2004). Greater hydrological control and rewetting operations undertaken in future within the conservation site may suppress the activity of phenol oxidase resulting in a build-up of phenolics which inhibit decomposition by hydrolases, as mentioned in previous chapters. This could lead to further aquatic carbon capture reducing health risk further.

Although the exact mechanism for calcium chelation has not been exhaustively characterised evidence suggests that the increased phenolic content will indeed chelate more calcium as seen in White et al (2011). Greater LMW constituent exhibited in the *Iris* DOC may have implications into calcium mobility and microbial use within the fen conservation sites, as labile carbon may be targeted as a carbon source for microbial processes, including dentitrification.

Potential chelation, binding or association of calcium with DOC produced by *Iris pseudacorus* will allow for the maintenance and or colonisation of M13 communities within the conservation site (Elkington et al. 2001). Calcium maintenance drove research into intrinsic system qualities that could be engineered into CW design and best management practice. This target driven research has provided a suitable option for calcium maintenance to the fens.

Calcium mobility analysis and experiments were based upon *in vitro* water chemistry observations. In order to successfully quantify and characterise the potential for maintaining Ca^{2+} mobility in the CW environment, characterisation of the DOC and phenolic compounds needs to be undertaken in order to understand the mechanism of chelation. Also, if chelation of Ca^{2+} occurred on the DOC molecules, the chelation binding site should be identified.

Carboxylic acids are a key component of naturally produced DOC compounds (Oliver et al. 1983). And are regularly a constituent of LMW DOC compounds (Strobel et al. 2001). This supports the Ca associations with LMW observed in Chapters 5 and 6.

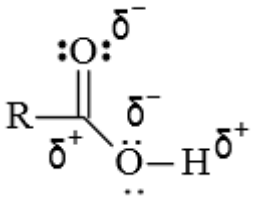


Figure 7.14 – Schematic diagram of carboxylic acid (De Ruiter 2005).

It is proposed that the chelation site could be linked to the polar region of the carboxylic acid as described below (figure 7.15). Ionic Calcium is prevalent in a Ca_2^+ form when dissolved. Therefore association or chelation may occur as follows.

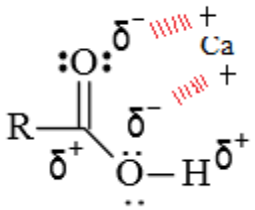


Figure 7.15 – Schematic diagram of proposed calcium chelation site on the carboxylic acid group.

Calcium chelation may allow for increased transport of calcium through the constructed wetland. Secondly, the exploration of calcium chelation mechanisms which exploit intrinsic system DOC and phenolic production, due to system specific and plant derived sources both showed potential for increasing calcium mobility in CWs.

These data have shown that FWS systems planted with *Iris pseudacorus* offer a suitable option for not only calcium maintenance or reduced calcium uptake, but also systems that will successfully minimise enrichment pressures caused by the influx of eutrophic water chemistry supplied to the conservation sites from the spring issue points. Targeting the nutrient enriched, calcium concentrated springs with treatment wetlands as set out in this

research may offer the opportunity for significant conservation success on the Anglesey and Llyn Fens LIFE project and other systems characterised by high nutrient removal and calcium maintenance requirement.

Chapter 8 - Synthesis and Conclusions

8.1 Introduction

The use of Constructed Wetlands (CWs) for water quality improvement has been explored in this thesis, with emphasis on two major subject areas. First, the combination of anti-algal phenol release from degradation of organic matter (Wingfield et al. 1985; Welch et al. 1990; Pillinger et al. 1994) in association with Floating Constructed Wetland (FCW) technology building on the work of Headley, Tanner, & Council (2006). Secondly, maintenance of calcium mobility within CW systems and the supply of optimal water chemistry by means of CWs.

Joint Nature Conservation Committee (2008) discusses the issue of eutrophication in freshwater water bodies in the UK. Estimates of the percentage of standing water bodies with eutrophic status was 80% for England, 40% in Wales and 15% in Scotland, totalling an area of 845 km^2. These values highlight the effects of nutrient enrichment across the UK. Biogeochemical cycling of Nitrogen (N) and Phosphorus (P) is being significantly disrupted by a range of processes undertaken largely by man (Herath 1997), and significant problems are being caused by agriculture (McDowell & Wilcock 2008).

Not only are 845 km^2 of standing water bodies being negatively affected in terms of their ecology, water chemistry and ecosystem function, but wider implications of nutrient enrichment are being observed in the watershed. This has certainly been observed in the water chemistry of the Anglesey and Llyn sites fed by such waters, where the majority of the catchment consists of intensive agriculture. Rather than the production of algal blooms in a water body, invasion of competitive graminoid species reliant on high nutrients has been documented.

A number of methods exist which may be capable of minimising enrichment and pollutant loading, both in standing water bodies and the wider catchment. One of the primary methods being increasingly applied is Catchment Sensitive Farming (Natural England 2011). However, in the case of the Anglesey and Llyn sites, even immediate halting of intensive farming may still lead to enriched water affecting the conservation sites. This is due to the N, and in some cases P (Holman et al. 2008)legacy within the aquifers and catchments supplying the fens (Beamish & Farr 2013). This has the potential to continue the supply of enriched spring water for a considerable amount of time ranging from years to decades (Farr et al. 2013). As presented in the preceding chapters, CWs offer an immediate environmental engineering based tool to reduce nutrient enrichment pressures in freshwater ecosystems, particularly in catchment-scale conservation projects, but also by means of inclusion and retrofit into standing water bodies suffering eutrophic effects.

8.2 Success

8.2.1 Floating Constructed Wetlands

Research undertaken in this thesis into floating CWs (FCWs) provided evidence of the benefits for system use for the prevention of algal bloom formation. The hybrid system merged FCW technologies with anti-algal phenolics and successfully reduced nutrient concentrations and algal bloom densities. At the most concentrated phase of the algal bloom in the control treatment, planted floating systems exhibited 90% or 630mg/L less chlorophyll-a. The reduction in algal bloom density reduced the degradation of aquatic ecosystems. Eutrophication is said to become a significant problem when the algal bloom formed due to nutrient overloading has a primary production value of 150g of C/m^2 (Herath 1997; Jost et al. 1991), severely deteriorating water quality affects the health of an aquatic ecosystem and is one of the major problems caused by excess nutrient loading.

Socio-economic factors such as loss of primary biological function (Yang et al. 2008b), filter blockages in water treatment plants (Welch et al. 1990) and potential health hazards including increased concentrations of methemoglobinemia (Fewtrell 2004) are significant motives for nutrient load reduction in freshwater bodies and prevention of algal bloom formation. The use of anti-algal releasing compounds (Wingfield et al. 1985; Welch et al. 1990; Pillinger et al. 1994), incorporated into a floating wetland system together with the inclusion of wetland macrophytes offered a successful mechanism for water quality improvement in Chapter 3.

Systems as implemented in a manner to mimic Wingfield et al (1985), where macrophytes were not present, showed marked decreases in Dissolved Oxygen (DO). This was attributed to the breakdown of organics and DOC, including phenol release (Chapter 3). Planting resulted in greater concentrations of oxygen detected in the rhizosphere as a result of ROL (Armstrong 1980; Sorrell et al. 2000), compensatory to any oxygen demand for breakdown of organics added to the system as substrate. Here, it is postulated that greater degradation of phenolics and associated release into the water column increases in relation to greater phenol oxidase activity (Freeman et al. 2001). However, limited phenol oxidase activity was detected throughout. Despite low phenol oxidase concentrations, phenolics were still observed in the water column, water quality was significantly improved in the planted systems and biological control over algal density and nutrient loading was obtained.

Phenolics and DOC release was examined further in Chapters 6 and 7, where plant root exudates were found to be a considerable contributing factor to biological processing in CWs. The input from macrophytes into CW biogeochemical cycling has also been examined by Zhai et al. (2013) where root exudates were found to contribute to denitrification processes.

The input from the plants may be contributing to algal control as observed in Chapter 2 and 3 and also in terms of calcium mobility in Chapters 5, 6 and 7.

Additionally, the installation of an un-planted FCW system, utilizing phenolic releasing organic matter alone, was found to worsen the effects of eutrophication in the mesocosms. When considering denitrification in treatment wetlands, a substrate for microbial community attachment and labile carbon sources are needed (Kadlec & Wallace 2008; Shapleigh 2013; Vymazal 2007). Here, only partial denitrification was observed, which manifested itself as increased nitrite in the water column. This compound was found to be more bioavailable to green pelagic algae as described in Heffernan & Fisher, (2012) and resulted in the observation of greater densities of algae.

8.2.2 Nutrient Removal Case Studies

Following investigations into water body enrichment, this PhD sought to contribute to the NRW Anglesey and Llyn Fens Project. Here, enrichment and eutrophic water chemistry was affecting large areas of peat and fenland, contributing considerably to site degradation. The installation of CWs would provide an insight and direct benefit to catchment scale conservation projects where enrichment is a significant problem.

Chapter 4 details the three primary systems installed on the Anglesey conservation sites. All systems designed, installed and monitored in the project significantly reduced N inputs to the fen. The systems installed were monitored for a period of 14 months, allowing for estimates of total elemental N removed from the water to be quantified. Averaging values from the 3 systems, based on mean flow, inflow and outflow N compounds and differences observed, suggests that 243kg of N were removed from the water flowing onto the conservation sites. This quantity is approximately equivalent to a 1.2 tonne bulk bag of “Straight” inorganic fertilizer.

Although P is generally considered the limiting factor for plant productivity, and healthy systems tend to possess a C:N:P ratio of 106:16:6 (Mitsch & Gosselink 2000) P was not readily detected at the locations where enrichment, vegetation community shifts and eutrophication were being observed. N compound removal was therefore the target for the CWs installed.

Hydraulic retention based calculations (nHRT), hybridisation with NitraBar Systems (NitraBar Field Report, 2009) and first order areal degradation rate coefficient modelling and integration of the P-k-C* model (Kadlec & Wallace 2008) were systematically included and developed through the project. Although nHRT calculations are a basic method of CW

scaling, once knowledge had been developed in P-k-C*scaling, recalculation of systems designed using nHRT and inclusion of the systems parameters into the P-k-C* equation correlated with the observed improvement in water chemistry.

Ca supply to the receiving fen communities (Joint Nature Conservation Committee 2008) was an important factor at all 3 of the CW systems. The main focus was the Cae Gwyn Free Water Surface (FWS) system, due to the installation of the CW being part of hydrological reconnection works carried out by NRW. The CW system here was implemented in order to connect water from Ca rich springs to a major peat re-profiling scrape for fen habitat restoration. Ca mobility, in particular preventing the formation of calcium carbonate in the CW, was of concern for the Llyn and Anglesey fens project as well as maintaining treatment performance with regard to conventional nutrient removal.

Using information from Mayes et al. (2009), where CWs were used for the removal of calcium at pollutant levels, hypotheses for reversal of processes were explored. Knowledge developed in Chapters 5, 6 and 7 can be used in new CW systems where calcium mobility is required and for the modification of systems the NRW Life Fens CWs where needed.

8.2.3 Calcium Mobility Maintenance

In order to quantify the potential for maintaining Ca mobility, mesocosm systems were used to manipulate system design and plant selection. The use of mesocosms allowed for the use of multiple replicates in a controlled environment. This process meant mechanistic analysis could be undertaken to distinguish the processes involved.

White et al. (2011) proposed binding of Fe II to phenolics, whilst Shen et al (1998) proposed methods of calcium solubility by binding to low molecular weight carbon based molecules, especially inositol phosphate. DOC and phenolic compounds are regularly observed in the influent and effluent of the Anglesey CWs, additional compounds of this nature are also discussed by Zhai et al (2013) in terms of plant production. Here, a method by which calcium could be prevented from forming a precipitate with CO_2 would be the primary mechanism for solubility maintenance optimised by utilising LMW DOC containing phenolic groups produced as plant root exudate.

The preliminary study was used to observe the effect of molecular weight (MW) of DOC molecules on potential binding or association with Ca. The results showed Ca was associated with low MW DOC of high phenolic content. This evidence informed hypotheses in relation to constructed wetland design, i.e., Horizontal Sub-surface Flow (HSSF) or Free Water Surface (FWS) and plant selection. Larue et al. (2010) analysed the phenolic content of a

number of wetland macrophyte species and found variation in the tissues and root exudates. This evidence was the driver for the investigation into species selection for future systems installed on the Anglesey and Llyn Fens.

Iris pseudacorus FWS was found to maintain Calcium concentrations to the greatest degree on passage through the wetland mesocosms. The contribution of the plants in terms of root exudate was further studied by *in-vitro* analysis of collected root exudates detailed in Chapter 7. The collection of root exudates and consequent testing of ionic and total calcium concentrations allowed for assessment of a potential chelation method. The driver for calcium maintenance and DOC reduction was highlighted in chapter 4. These targets were set due to the Anglesey SSSI sites acting as the catchment for a local drinking water reservoir it supplies. Currently, there are various means of treating water; this involves the use of chemicals and physical processing. Usually, water is treated by the addition of a disinfectant chemical. These include chlorine, ozone, chlorine dioxide and chloramines (Krasner et al. 2006). However, the use of these halogen based chemicals can result in the formation of trihalomethanes (THMs), which have been linked to negative impacts on human health (Chow et al. 2003). Therefore, although DOC and phenolics could provide a chelation and mobility mechanism, increasing this could result in a trade off in terms of negative impacts on drinking water quality. The cost and benefits of habitat protection and drinking water supply therefore need to be analysed depending on the site in question. Further studies in Chapter 7 however, indicated that per unit biomass *Iris pseudacorus* produces less total DOC, similar concentration of low MW (<1k Dalton) DOC and increased phenolics than *Phragmites australis*. These characteristics make *Iris pseudacorus* a suitable plant species for selection in CW systems where calcium maintenance in important.

This finding may tie into research undertaken in Chapters 2 and 3 where phenolic production potentially affected the proliferation of algae in a eutrophic mesocosm. The results from Chapter 3 showed that phenolic concentrations exhibited in *Iris pseudacorus* mesocosms were similar to concentrations exhibited by *Phragmites australis.* Whereas in the *in-vitro* experiment (chapter 7), differences in phenolic production were observed. The FCW experiment suggested rhizospheric and biochemical interactions that led to similar effects observed in treatment effectivity, however mechanistic analysis at *in vitro* scale proves otherwise. This evidence highlights the importance of understanding processes from *in vitro* to field scale.

8.3 Approach taken

The research undertaken in this PhD was scaled at *in vitro* microcosm mesocosm and full scale for both key aspects of the research. Detailed mechanistic analysis was undertaken in mesocosms whilst the installations of full catchment scale systems were also employed.

Mesocosms have been questioned by many researchers (Carpenter 1996; Schindler 1998; Ahn & Mitsch 2002) regarding their limitations. Edge effects and limited environmental interaction are seen as intrinsic problems. However, when analysing mechanisms involved in the biochemical processing in the CW system, replication and control must be gained. Mesocosms were certainly deemed appropriate for the FCW and Ca chelation experiments, however differences in mesocosm location were considered carefully.

The FCW experiments, although controlled were undertaken in such a way as to be exposed to environmental inputs. All water chemistry nutrient additions, installation times, FCW set up and monitoring could be undertaken in a highly controlled manner, however environmental effects allowed for increased potential for algal colonisation than in a laboratory environment.

A full scale prototype FCW was produced and installed into a eutrophic lake on Anglesey as part of an ongoing project. Preliminary data suggested increased concentrations of phenolics localised to the system

Figure 8.1 - Dense Algal Bloom observed in Anglesey eutrophic lake

Figure 8.2 - Mesh netting filled with organic barley straw, although not used in FCW experiment, more available at large scale

Figure 8.3 - *Phragmites australis* seedlings added to the FCW system

Figure 8.4 - Addition of the system to the eutrophic lake

Figure 8.5 - Final settled position of the FCW. New *Phragmites* shoots beginning to grow from the FCW

Installation of CWs as part of the NRW project was experimental to some degree; however these systems also needed be functional. Nutrient reductions were observed in all of the CWs and all 3 of the systems, irrespective of scaling methodology, were deemed successful. Further improvements in pollutant removal capacity could have been achieved with modification to the designs of the CWs used on the fens, however, as the Llyn and Anglesey sites are conservation sites first and foremost, balances between quality fen habitat and CW area protecting the fen's plant communities must be considered. The Cae Gwyn system reached an average removal of approximately 75% nitrate. If increased available space were accessible for CW use this figure could be greatly increased near to the background concentration value (C*). Using the same parameters as outlined in Chapter 4, a 320m^2 increase in wetland area could increase nitrogen removal to the C* value.

Full scale treatment wetlands installed on the Anglesey site proved the concept was viable on a large scale with the inclusion of a CW system in the flow path from enriched springs to conservation site. However, mechanistic analysis of Ca mobility could not be suitably tested on a field-scale project due to the control and replication needed when studying these

processes using mesocosms and controlling environmental factors allowed for a more systematic approach. Further examination of mechanisms was achieved by *in-vitro* root exudate testing. The approach taken in Chapters 5, 6 and 7 have now prompted the installation of two replicate cells of a treatment wetland system on Cors Bodeilio NNR. The proposed systems will comprise two cells created identically in terms of influent chemistry and loading. A single enriched spring will be divided into the two cells. Retention time, depth, width and other scaling parameters will be identical also. The findings from Chapter 7 in particular will be integrated by means of plant species selection. As a continuation of the research *Phragmites australis* and *Iris pseudacorus* will be used in the respective cells. The findings from water quality monitoring will then continue to influence the installation of future CWs in the project.

8.4 Improvements to Analytical Methods and Potential Additional Research

8.4.1 Floating Constructed Wetlands

When considering the investigations as undertaken in Chapters 2 and 3, it became clear that a hydroponic style treatment maybe have been beneficial. These systems are detailed in Headley et al (2006) and this method may have elucidated the effect of macrophyte installation alone. Two methods of control tests were used in Chapter 3, however, additional hydroponic systems may have produced a more complete picture of the mechanisms involved in algal bloom mitigation thus determining the relative inputs of anti algal DOC compounds versus nutrient removal mechanisms facilitated by plants.

The experiments undertaken in Chapter 3 were subjected to two nutrient regimes, namely eutrophic and mesotrophic. These levels were suitable for observing differences in treatment potential, at high resolution, over limited nutrient concentration ranges. The values generated by varying trophic state were useful, however, it may become important to test total treatment potential to understand if there is a maximum pollutant loading rate at which the FCW can continue to influence the presence of algal blooms. A method by which this could be undertaken may utilise a regular nutrient dosing system in order to maintain high levels of nutrient rather than pulse feed. This method may also allow for the development of pollutant rate constants that could be used as a parameter for FCW specific P-k-C* computations. It is highly likely that the presence of roots and the effect on flow distribution would also need to be considered in order to produce suitable P values to compensate for hydraulic short circuiting within systems. This could be undertaken by using tracer tests on FCWs. Distribution of FCWs and system surface area to volume ratios would also need to be included in the research.

Similar to the testing of anti-algal effect of organic substrate types examined in Chapter 2, root exudate anti-algal effect would be useful in determining total effects on algal bloom formation. Here *in vitro* experiments may provide more information specific to root exudates produced and confirm any differences in species specific exudate production as observed in chapter 7 across a greater range of plant species. Two potential methods may be appropriate here. The first method proposed is the collection of exudate and subsequent balancing of DOC and phenolic concentrations by dilution. Once the exudates had been manipulated, the solutions could be added to eutrophic mesocosms and algal bloom formation monitored. Secondly the addition of root exudate directly to a uniform algal bloom culture would determine algaecidal effect.

8.4.2 Natural Resources Wales Llyn and Anglesey Fens Project

The systems installed on the Anglesey sites have been monitored for 14 months where 243kg of elemental N were removed. This monitoring must continue in order to determine medium and long term performance and aid in system servicing schedules. Further assessment of the CWs should really be undertaken in order to asses hydraulic flow uniformity. This was not undertaken within this thesis due to the sensitivity of the fen receiving the CW water. NRW did not want to introduce tracers into a site where colonisation by the target species was occurring. However, tracer testing may be utilised in future and achieved by means of chemical addition to the inflow of the CW in a single pulse (Kadlec & Wallace 2008). Regular monitoring of the outflow concentrations of the observed tracer would confirm nominal hydraulic retention time and volumetric efficiency values can be derived. This was not done due to constraints on releasing chemicals into the SSSI sites that are supplied by the CWs.

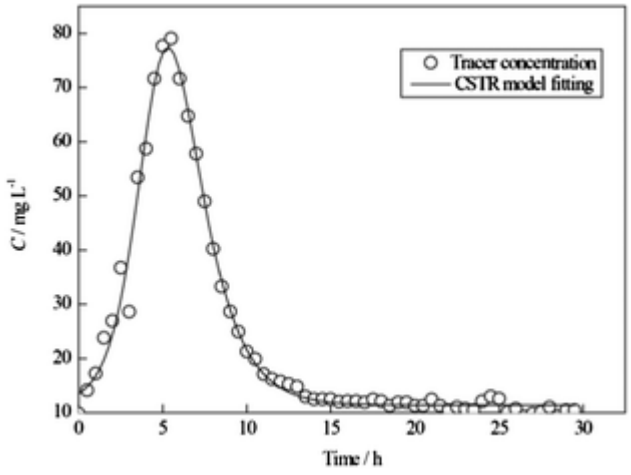


Figure 8.6 –Tracer response in HRT monitoring (Cui et al. 2012)

The figure above indicates the concentration of tracer compounds as they reach the outflow of the CW in question. Good hydraulic efficiency is shown by the highly resolved peak due to the short time from the first detection of the tracer to final.

Hydraulic efficiency in the Cae Gwyn system should be a particular focus due to the design of the inflow structures. Here, it is speculated that the distribution channel along the leading edge of the treatment system bay might be insufficiently deep in order to allow for efficient use of the constructed wetland area. Tracer testing on the system may provide information regarding designed HRT versus actual HRT, quantifying the dead space within the system.

The CWs used on Anglesey have been proven to be considerably beneficial. NRW will be continue to monitor the systems but also will undertake detailed vegetation monitoring. The inclusion of additional CWs around the margins of the sites is highly likely based on nutrient sequestration alone; however this may be further supported by evidence of desirable vegetation community re-establishment in the longer term.

The evidence presented in this thesis shows that passive wetlands systems, restricted by area, improve water quality supplied to conservation sites, both for the prevention of ecologically harmful algal blooms and the maintenance of calcium dependant, oligotrophic supply waters. The hybridised FCW systems offer a method by which the release of algaecidal phenolics combined with hydroponic macrophytes significantly reduced the potential for algal bloom formation.

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