Nutrient removal with integrated constructed wetlands

Microbial ecology and treatment performance evaluation of full-scale integrated constructed wetlands

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Declaration

I hereby declare that the research documented in this Thesis is my own work, except where otherwise stated, and has not been submitted in any form for any other degree or professional qualification.

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Abstract

Wastewaters from intensive agricultural activities contain high concentrations of nitrogen and phosphorus that contributes to water management problems. During the past few years, there has been considerable interest in the use of constructed wetlands for treating surface water runoff from farmyards. If the contaminated runoff is not treated, this wastewater along with other non-point sources of pollution can seriously contaminate the surface water and groundwater. Integrated Constructed Wetlands (ICWs) are a type of free water surface wetlands. They are engineered systems that are designed, constructed and operated successfully for treating farmyard runoff in the British Isles. However, the long-term treatment performance of these systems, the processes involved in contaminant removal and the impact on associated water bodies are not well-known.

The aims of this project were to assess the performance of full-scale integrated constructed wetlands and understand nutrient removal in them. Performance evaluation of these systems through physical, chemical and microbiological parameters collected for more than 7 years showed good removal efficiencies compared to international literature. The monitored nutrient concentrations in groundwater and surface waters indicate that ICW systems did not pollute the receiving waters. The role of plants (*Typha latifolia*) and sediment in removing nutrients was also assessed. More nitrogen and phosphorus were stored in wetland soils and sediments than in plants. The results demonstrate that the soil component of a mature wetland system is an important and sustainable nutrient storage compartment.

A novel molecular toolbox was used to characterise and compare microbial diversity responsible for nitrogen removal in sediment and litter components of ICW systems. Diverse populations of nitrogen removing bacteria were detected. The litter component of the wetland systems supported more diverse nitrogen removing bacteria than the sediments. Nitrogen removing bacteria in the wetland systems appeared to be stochastically assembled from the same source community.

The self-organising map model was applied as a prediction tool for the performance of ICW and to investigate an alternative method of analysing water quality performance indicators. The model performed very well in predicting nutrients and biochemical oxygen demand with easy to measure and cost-effective water quality parameters. The results indicate that the model was an appropriate approach to monitor wastewater treatment processes and can be used to support management of ICW in real-time.

Publications

Journal papers

Scholz M., Harrington R., Carroll P. and Mustafa A. (2007), The Integrated Constructed Wetlands (ICW) Concept, Wetlands, 27 (2), 337-354.

Carty A., Scholz M., Heal K., Gouriveau F. and Mustafa A. (2008), The Universal Design, Operation and Maintenance Guidelines for Farm Constructed Wetlands (FCW) in Temperate Climates, Bioresource Technology, 99 (15), 6780-6792.

Zhang L., Scholz M., Mustafa A. and Harrington R. (2008), Assessment of the Nutrient Removal Performance in Integrated Constructed Wetlands with the Self-organizing Map. Water Research, 42 (13), 3519-3527.

Zhang L., Scholz M., Mustafa A. and Harrington R. (2008), Application of the Self-organizing Map as a Prediction Tool for an Integrated Constructed Wetland Agroecosystem Treating Agricultural Runoff. Bioresource Technology, 100 (2), 559-565.

Mustafa A., Scholz M., Harrington R. and Carroll P. (2009), Long-term Performance of a Representative Integrated Constructed Wetland Treating Farmyard Runoff. Ecological Engineering, 35 (5), 779-790.

Scholz M., Harrington R., Carroll P. and Mustafa A. (2010), Monitoring of nutrient removal within integrated constructed wetlands (ICW), Desalination, 250 (1), 356-360.

Kayranli B., Scholz M., Mustafa A. and Hedmark Å. (2010), Carbon Storage and Fluxes Within Freshwater Wetlands: a Critical Review. Wetlands, 30 (1), 111-124.

Kayranli Birol., Scholz M., Mustafa A., Hoffman O., Harrington R. Performance Evaluation of Integrated Constructed Wetlands treating Domestic Wastewater. Water Air & Soil Pollution. (accepted).

Conference papers

Scholz M., Harrington R., Carroll P. and Mustafa A. (2007), Development of the Integrated Constructed Wetlands (ICW) Concept. Kungolos A., Aravossis K., Karagiannidis A. and Samaras P. (Eds.). In Proceedings of the Society for Ecotoxicology Conference and the International Conference on Environmental Management, Engineering, Planning and Economics (24-28/06/2007), ISBN 978-960-89818-0-5, Vol. 2, pp. 1107-1112, Skiathos Island, Greece. Grafima Publishing, Thessaloniki, Greece.

Mustafa A., Scholz M., Harrington R. and Carroll P. (2008), Control of Nutrients with Integrated Constructed Wetlands. Crighton K. and Audsley R. (Eds.). In Proceedings of the Scottish Agriculture College and Scottish Environment Protection Agency Conference (26-27/03/2008). ISBN 1-85482-867-3, Scottish Agriculture College and Scottish Environment Protection Agency Edinburgh, UK, 6 pages

Mustafa A., Scholz M., Zhang L., Harrington R. and Carroll P. (2008), Assessment of the Long-term Performance of a Representative Integrated Constructed Wetland for Treating Farmyard Runoff, 10th British Hydrological Society Symposium 2008 (15-18 September 2008), Sustainable Hydrology for the 21st Century, Exeter, UK, pp. 445-451.

Mustafa A., Scholz M. and Harrington R. (2009), Characterization of Microbial Communities Removing Nitrogen within an Integrated Constructed Wetland Treating Rural Runoff. In J. M. Bayona and J. Garcia (Eds.), Proceedings on CD of 3rd Wetland Pollutant Dynamics and Control WETPOL 2009 (20-24/09/09), Barcelona, Spain. ISBN-10: 978-84-692-5587-2. pp. 111-112.

Kayranli B., Scholz M., Mustafa A and Hedmark Å. (2009), Review of Carbon Storage within Wetlands. In J. M. Bayona and J. Garcia (Eds.), Proceedings on CD of 3rd Wetland Pollutant Dynamics and Control WETPOL 2009 (20-24/09/09), Barcelona, Spain. ISBN-10: 978-84-692-5587-2. pp. 329-330.

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Chapter 1 Introduction

1.1. Background to the project

During the last three decades, there has been heavy investment worth billions of pounds in constructing wastewater treatment facilities worldwide to control water pollution. Whilst there have been considerable successes in managing pollution from point sources, but no significant investments have been made to control pollution from rural sources mainly arising from agricultural activities. This has resulted in pollution, lower water quality in sensitive catchments and polluted water supplies to urban areas (D'Arcy et al., 2000). Several alternative treatment options, such as ponds, constructed wetlands and buffer zones, are available to reduce the risk of water pollution from agriculture.

The use of wetland systems for the treatment of wastewater has increased dramatically in the last decade with an estimated more than 50,000 applications worldwide (Kadlec, 2004). Constructed wetlands are a type of treatment wetland divided into two major types – surface flow wetlands and subsurface flow wetlands. A number of factors, such as enhanced enforcement of environmental standards and sustainability issues, have provided the impetus to use constructed wetlands for wastewater treatment in both the developed and the developing world (Wallace and Knight, 2006). These systems have been used worldwide to treat different categories of wastewater including domestic, industrial, acid mine drainage, agricultural runoff and landfill leachate (Scholz, 2006; Carty et al., 2008a; Kadlec and Wallace, 2009). In contrast to conventional wastewater treatment systems they are accepted as low-tech, economical, green, efficient and sustainable systems that are capable of treating different types of wastewater, because of their inherent capability to act as sinks for different contaminants (Mitsh and Gosselink, 2007). These systems not only improve the water quality but also provide habitat for a wide diversity of plants and animals (Kadlec and Knight, 1996; Wallace and Knight, 2006; Mitsh and Gosselink, 2007).

The Integrated Constructed Wetland (ICW) concept has been developed in Ireland over the past 15 years. It is largely based upon mimicking natural shallow emergent vegetated type wetlands. Using a multi-celled configuration ensures that contaminants are sequentially removed, with the area and configuration of the wetland relative to influent volume determining removal efficiencies. More than sixty ICW systems have been constructed during the past 15 years in Ireland, predominantly treating runoff from agricultural activities.

Although the application of wetland systems to treat different categories of wastewater has been widespread with the numbers increasing over the course of time, there is a lack of knowledge of the detailed removal pathways for most of the contaminants. There is a great need to unravel the black box system of constructed wetlands to understand the overall removal processes.

Understanding of wetland treatment processes is still evolving, even though the wetland technology has been studied since 1952 (Siedel, 1973) and has been in full-scale operation since 1974 (Kickuth, 1977). The present state-of-the-art design approaches can be described as semi-empirical i.e. partly empirical and partly theoretical. There are different approaches for designing these systems in which intricate physical, chemical and biological processes take place. Models have been developed based on the analysis of input/output data or mass balance relationship converging to a general form of a first-order plug-flow model. They do not take account for the interactions and complex reactions that take place in the wetlands. The areal loading models developed by Kadlec and Knight (1996) and the volumetric models as developed by Reed et al. (1995) have certain limitations, for example, none of the accepted wetland treatment performance models are capable of consistent descriptions with invariant parameters (Kadlec, 2000). It is very important to understand the variables that affect the cycling of pollutants in order to design systems that can meet the environmental guidelines.

Although researchers have conducted experiments to determine the impact of different wetland components on pollutant removal, little work has been carried out on full-scale systems. Experiments have been conducted on microcosm-, or mesocosm-scales using sections of pipes, flasks, pots, tubs and troughs. Although comparative results from these systems are useful, but the treatment performance data from these systems, cannot be used, to understand and design full-scale systems. When systems are small, they are subject to significant edge effects (Kadlec and Wallace, 2009). Moreover, most of the past studies have been conducted on juvenile ecosystems which did not have enough time to mature. As wetland technology continues to develop, there is a need to understand the long-term variability of cycles within these systems. The treatment variability strongly influences the wetland design, hence knowledge of factors that influence long-term performance like biogeochemical cycles, become vital in understanding the functioning of treatment wetlands.

Constructed wetlands are mechanically simple treatment systems but the passive treatment processes that remove contaminants including nutrients are intricate. The plants, microorganisms and the soil matrix all play important roles in the retention and removal of pollutants. Worldwide researchers have carried out investigations of wetland nutrient removal efficiency. Nevertheless, they do not agree on a commonly accepted statement of the impact of wetland age on the nutrient removal efficiency. There is disagreement in the interpretation of variables, reactions and the impact of wetland age on nutrient removal.

Modelling and predicting treatment processes are significant for elucidating the complex nutrient removal mechanisms and assessing the corresponding water treatment potential of wetland systems, including ICWs. It is necessary to model and predict the nutrient removal processes to optimise the design, operation, management and water quality monitoring strategy of a wetland system. Moreover, for management purposes and to meet the regulatory requirements, it is essential to monitor the performance of these dynamic systems in real time. However, it is comparatively difficult to model and predict the performance of constructed wetlands due to the intricacies and complicated processes taking place within the system.

This thesis addresses these issues pertinent to the treatment of farmyard runoff rich in nutrients. The treatment performance of full scale integrated constructed wetlands will be assessed along with the impact of these systems on the associated water bodies.

1.2. Rationale, aims and objectives of this research

The rationale of the research project is to assess the long-term performance of wetland systems through studying treatment performance of full-scale mature wetland systems and determining their impact on the associated water bodies. This will provide information on the role of these structures on a catchment scale and address the arguments of practitioners and researchers. The overall aim of this project is to enhance understanding of the pollutant transformation and removal processes in full scale integrated constructed wetlands.

Detailed monitoring of wetland water quality and associated groundwater and surface waters was carried out for three integrated constructed wetland systems that were designed to collect and treat farmyard runoff. This study evaluated the long-term assessment of various full-scale ICW systems, and impact of these systems on the surrounding water bodies and groundwater. The application of novel molecular microbiological techniques provided an opportunity to gain insights into the microbial transformations responsible for nutrient removal while the use of a self-organising map (SOM) model was used for prediction purposes. The overall goal of the thesis is to assess the performance of ICW treatment systems in terms of pollution control and to identify the main factors which influence the performance in order to increase the benefits and overcome the limitations of ICWs.

The specific research objectives are:

1. to assess the performance of full-scale integrated constructed wetlands treating farmyard runoff and receiving high loads of biochemical oxygen demand (BOD), suspended solids (SS) and nutrients;
2. to investigate potential contamination of nearby surface waters and ground water;
3. to assess the role of wetland plant and sediment in removing nutrients;
4. to investigate the nitrifying and denitrifying bacterial communities in the sediment and litter compartments of integrated constructed wetlands; and

5. to apply a self-organising map model to predict water quality parameters to support management decisions.

1.3. Outline of thesis contents

This thesis consists of four main parts. The first part focuses on the treatment potential of integrated constructed wetlands and their impact on associated water bodies. The second part focuses on the role of plants and sediment in nutrient removal, while the third part covers investigation of bacteria responsible for nitrogen removal in integrated constructed wetland systems. The fourth part focuses on the application of a self-organising map model for the prediction of wetland performance. The thesis is organised into nine chapters.

Chapter 1 Introduction

In this chapter, a brief background and, the aims and objectives of this study are described.

Chapter 2 Constructed Wetlands

This chapter presents an overview of constructed wetlands, their types and pollutant removal mechanisms. The components of constructed wetlands and the removal processes are also described in this chapter.

Chapter 3 Integrated Constructed Wetlands

This chapter describes the novel concept of Integrated Constructed Wetlands. It also discusses the application of ICWs for remediation of agricultural runoff.

Chapter 4 Materials and Methods

This chapter describes the study sites along with the study design, data collection and subsequent analysis. Methods adopted to cover various facets of the study are described.

Chapter 5 Overall Treatment Performance

This chapter focuses on the performance treatment of three full-scale integrated constructed wetlands. The characteristics and description of the study sites along with the monitoring scheme are described. The factors that influence the removal of nutrients from these systems have been investigated. The impact of these systems on associated surface and groundwaters is also discussed.

Chapter 6 Role of Plants and Sediment

This chapter focuses on the role of plants and sediment in removing nutrients from wetland systems. The impact and importance of these components in the wetland setting is discussed. In addition, the contribution of nutrient uptake by emergent macrophytes and nutrient storage in the accumulated sediment and new accretions in a mature integrated constructed wetland is described.

Chapter 7 Microbial Ecology

This chapter outlines the application of molecular techniques to characterise bacteria that are responsible for the removal of nitrogen in integrated constructed wetlands. The chapter also investigates using a theoretical framework the species composition of nitrogen removing bacteria in different wetland systems.

Chapter 8 Application of Self-Organising Map Model

The chapter describes the application of an artificial intelligence technique to support integrated constructed wetland management decision. The application of the self-organising map (SOM) in predicting nutrients and other water quality parameters such as BOD that are expensive to monitor in real time is demonstrated.

Chapter 9 Conclusions and Recommendations

The final chapter presents the conclusions by summarising the key findings from this research along with recommendations for potential further reseach.

Chapter 2 Constructed Wetlands for Farmyard Runoff Management

2.1. Introduction

An overview of constructed wetlands is presented in this chapter. The definition of wetlands, types of constructed wetlands, the components of free water surface constructed wetlands and contaminant removal processes within these systems which are analogous to those of integrated constructed wetlands are described in this chapter.

The structure of this chapter is as follows. Sections 2.2, 2.3 and 2.4 summarise the definition, types, components and removal mechanisms of contaminants in constructed wetlands. Section 2.5 describes the ICW concept and its application to control nutrient-rich runoff from farmyards.

2.2. Definition of wetlands and constructed wetlands

In this section the definitions of wetlands and constructed wetlands are presented because they are very important for the scientific understanding of these systems.

2.2.1. Wetlands

The Convention on Wetlands (Ramsar, Iran, 1971), called the "Ramsar Convention", which directed international attention to the importance and protection of wetlands proposed the following definition: "*wetlands are areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water* *that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at high tide does not exceed six metres"*.

Generally, "wetlands are defined as lands where saturation with water is the dominant factor determining the nature of soil development and the types of plant and animal communities living on the surface" (Cowardin et al., 1979).

The U.S. Army Corps of Engineers (Corps) and the U.S. Environmental Protection Agency (EPA) jointly define wetlands as *"Areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs, and similar areas."*

2.2.2. Constructed wetlands

Constructed wetlands are man-made systems that are designed to emphasise specific characteristics of the wetland habitat for improved water treatment capacity (Wallace and Knight, 2006). They may be built as part of a treatment train that includes processes encompassing physical/chemical treatment methods.

2.3. Wetland systems versus conventional wastewater treatment systems

In contrast to conventional wastewater treatment systems the constructed wetland systems are accepted as low-tech, economical, green, efficient and sustainable systems that are capable of treating different types of wastewater, because of their inherent capability to act as sinks for different contaminants (Mitsch and Gosselink, 2007). These systems not only improve the water quality but also provide habitat for a wide diversity of plants and animals (Kadlec and Knight, 1996; Wallace and Knight, 2006; Mitsch and Gosselink, 2007). Compared to conventional wastewater treatment systems, wetlands have a higher rate of biological activity which enables conversion of many of the pollutants that are contained in the wastewater into non-toxic by products or essential nutrients that can be reused for additional biological activity (Kadlec and Wallace, 2009).

2.4. Types of treatment wetlands

The single main purpose of treatment wetlands is to improve the water quality. The use of wetlands to treat wastewater and polluted water is an intriguing concept involving the building of a partnership between humanity (our wastes) and an ecosystem (wetlands) (Mitsch and Gosselink, 2007). There are generally two types of treatment wetlands: natural wetlands and constructed wetlands.

2.4.1. Types of wetlands

Natural wetlands (Figure 2-1) can be broadly classified into the following types:

- Coastal or marine wetlands (Tidal salt marsh, tidal freshwater marsh, mangrove wetlands)

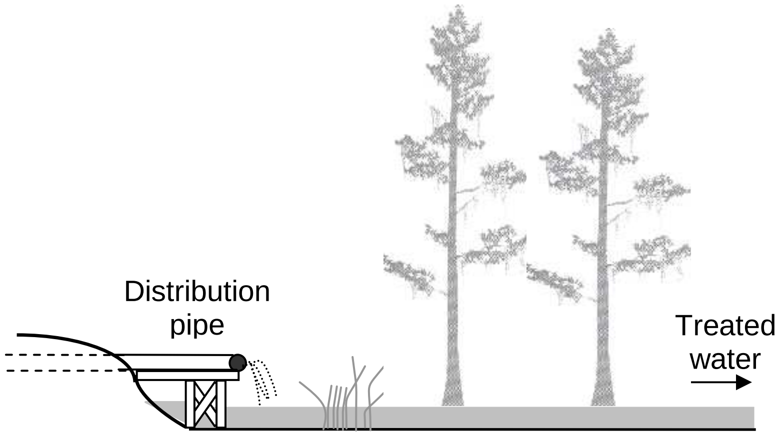


Figure 2-1. Natural wetland (after Mitsch and Gosselink, 2007).

- Inland wetlands (Palustrine; forested and scrub land, emergent, non-vegetated wetlands, area less than 8 ha.)
- Riverine wetlands
- Lacustrine wetlands (Lacking trees, shrubs, persistent emergent vegetation, with greater than 30 percent areal coverage, area more than 8 ha.)

2.4.2. Types of constructed wetlands

Constructed wetlands can be categorized into three basic types:

1) Free water surface (FWS)

These closely resemble natural wetlands and contain areas of open water, emergent plants and floating vegetation. FWS also provide ancillary benefits in the form of biodiversity enhancement and habitat for insects, molluscs, fish, amphibians, birds, reptiles and mammals. The wastewater destined for wetland treatment may be released directly into the wetland system or may travel through a treatment train. As the wastewater passes through these systems the contaminants are reduced by various physical, chemical and biological mechanisms in the water column and the soil matrix. Compared to other wetland types they require a larger footprint, and hence are capital cost-competitive but with low operating costs.

The first engineered constructed wetland treatment pilot systems were constructed in 1973 at Brookhaven National Laboratory near Brookhaven, New York, USA. In contrast to Europe, North America has more FWS, because of abundant space availability. For example, Florida in the USA has one of the largest constructed wetland treatment areas in the world. The most common applications of FWS are for polishing secondary treated effluent such as from activated sludge systems, trickling filters. Integrated Constructed Wetlands are classified as FWS systems (Kadlec and Wallace, 2009). The components of a FWS system are shown in Figure 2-2.

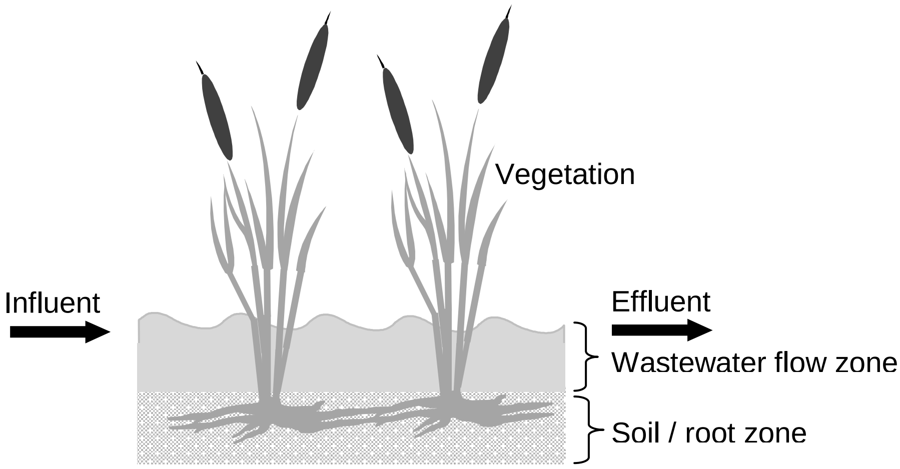


Figure 2-2. Schematic of free water surface horizontal flow constructed wetland with emergent macrophytes.

2) Sub-surface flow (SSF)

The system consists of gravel or soil beds planted with wetland vegetation that is limited to emergent vegetation. The wastewater in these systems stays beneath the surface of the media. The flow may be either horizontal or vertical through the system corresponding to horizontal sub-surface flow (HSSF) or vertical subsurface flow (VSSF), respectively. Compared to FWS they require less land area but are generally they are more expensive than FWS wetlands because of the gravel media and do not provide ancillary benefits of biodiversity and wildlife habitat. Most of the European countries prefer SSF wetlands because they require less area and space is much more at a premium in Europe than in North America. These systems were initiated in the Max-Planck Institute, Germany, in 1953 with the pioneering work of Käthe Seidel. Later Reinhold Kickuth developed a HSSF wetland process known as the root zone method (RZM) which involves a soil media composed of typically clay loam and planted with *Phragmites* (Kickuth and Könemann, 1987). The first full-scale RZM wetland became operational in 1974 at Liebenburgh-Othfresen, Germany.

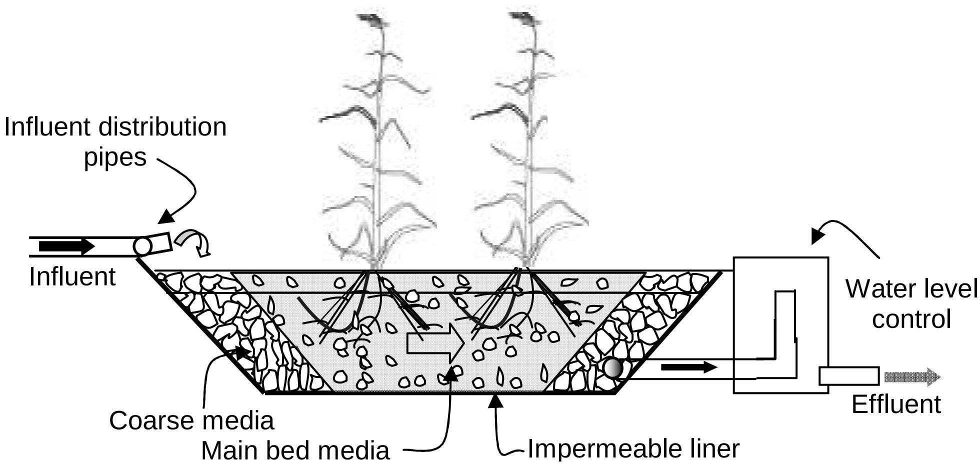


Figure 2-3. Longitudinal section of a horizontal subsurface flow constructed wetland (after Wallace and Knight, 2006).

In the UK SSF are commonly called reed bed treatment systems. Presently, there are more than 1000 systems in the database compiled by the Constructed Wetland Association of the United Kingdom. A schematic of SSF with horizontal flow is shown in Figure 2-3.

3) Hybrid system

A hybrid system (Figure 2-4) is a combination of two or more different systems. For example a VSSF wetland may be used in combination with a HSSF wetland or a free water surface wetland. VSSF provide conditions in which higher levels of oxygen can be transferred producing a nitrified effluent. HSSF have a limited capacity to produce nitrified effluents, thus VSSF can be used in conjunction with HSSF or FWS wetlands to create nitrification-denitrification treatment trains. An example of the hybrid constructed wetlands approach can be found at Kõo, Estonia, where the system consists of two VSSF beds followed by a HSSF bed and two FWS wetlands (Mander et al., 2003).

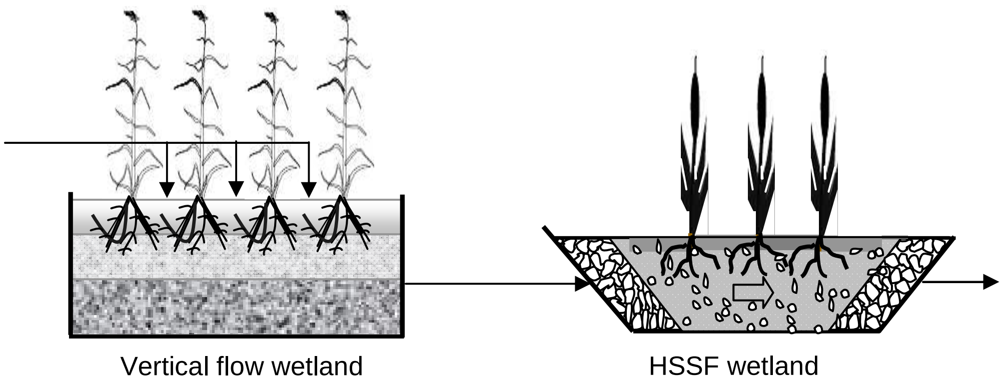


Figure 2-4. A hybrid (vertical flow + horizontal subsurface flow) wetland system.

2.5. Wetland hydrology

In wetlands, water is added from natural or anthropogenic sources and is also subtracted. This addition and subtraction of water controls the flow and depth. The movement of water in wetlands is important and influences the reduction of pollution in these systems.

Figure 2-5 represents the components of a wetland water budget. The flow exiting a wetland system depends on several factors including evapotranspiration, precipitation, infiltration and extraneous sources. The flow rate exiting the wetland can be calculated as follows:

$$Qout = Qin + Qc - Qgw - ET + P \qquad (2\text{-}1)$$

Where:

Q_{out} = flow exiting the wetland, m^3/d

Q_{in} = flow entering the wetland, m^3/d

ET = evapotranspiration, m^3/d

P = precipitation, m^3/d

Qgw = infiltration, m^3/d

Qc = catchment runoff rate, m^3/d

The hydrologic conditions not only determine the plant species composition but also have an effect on the soils and nutrients (Mitsch and Gooselink, 2007). Hydrology also controls the biogeochemical characteristics of wetlands (Reddy and Delaune, 2008). For example, when soils are flooded the oxidised species are converted to reduced forms through microbial processes and this produces anaerobic conditions in wetland soils which in turn support nitrogen removal processes like denitrification.

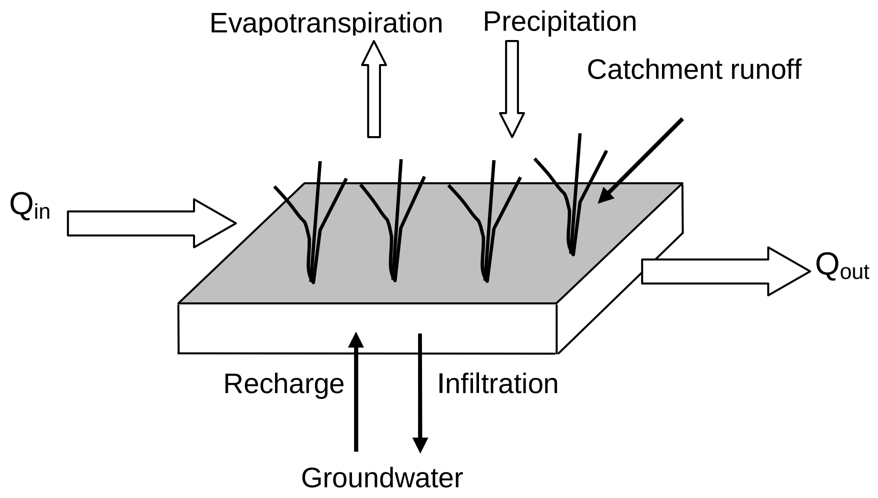


Figure 2-5. Constructed wetland water budget.

The hydrology of wetland systems is much more complex than that of conventional wastewater treatment systems. The mean water depth calculation involves a detailed survey of the water elevation plus a survey of wetland bottom topography. If h is the water depth, H is the local water elevation and G is the local ground elevation, then the local depth can be calculated as the difference between H and G;

$$h = H - G \qquad (2\text{-}2)$$

The water depth in the Houghton Lake wetland project, Michigan, USA, was determined by the difference between ground elevation and stage recordings (Kadlec, 2009). The nominal wetland water volume is defined as the volume enclosed by the upper surface of water and the bottom and sides of the containment structure. Detention time is defined as the ratio of wetland water volume to the volumetric flow rate. In wetlands, conservative tracer studies provide a direct measure of the actual detention time (Dierberg and Debusk, 2006; Sua et al., 2009).

The treatment effectiveness of surface flow wetland systems including integrated constructed wetlands (ICWs) is typically based on having appropriate hydraulic residence times, which depend very much on the specific site conditions (Harrington et al., 2005). In shallow, emergent and vegetated wetlands, such as ICWs, this hydraulic residence time depends on having sufficient functional wetland area with an appropriate length to width ratio and a high vegetation density.

2.6. Groundwater infiltration

Infiltration of polluted water can degrade the quality of groundwater and is therefore important and a matter of concern for regulators, practitioners and researchers. Most treatment wetlands are therefore lined either with synthetic liners or clay. The soil lining the wetland base must adequately impede infiltration to protect groundwater (Dunne et al., 2005a,b; Keohane et al., 2005; Scholz et al., 2007). Where low permeability soils suitable for the construction of a wetland (such as clays) are not found on site, alternative measures should be taken. Additional soils can sometimes be found locally, but this may incur additional costs for transportation, particularly if located away from the site. The use of an artificial plastic liner, similar to that required for a landfill, can also be considered. However, it will incur a much greater cost and may require replacement in the future.

Infiltration in wetlands occurs by vertical flow and depends on soil conditions. If the soil is fully saturated then a water mound may form above the shallow aquifer. Conversely, if the soil is unsaturated then the water may percolate downwards beneath the wetland. During its downward movement, the wetland water may receive some additional treatment by microbial degradation.

2.7. Contaminant removal mechanisms

Wetland systems are exposed to different pollutants, such as phosphorus, nitrogen, pathogens, pesticides, heavy metals, oestrogenic compounds, in varying quantities. There are wide arrays of physical, chemical and biological mechanisms that transform and distribute pollutants in the multiple abiotic and biotic components of wetland systems. As wastewater enters the wetland, the velocity is drastically reduced resulting in settling of suspended solids. Nutrients increase plant biomass production whilst the growth, dieback and decomposition of plant biomass create internal storage compartments. Removal of pollutants occurs through different processes such as sedimentation, filtration, microbial degradation, plant uptake, and adsorption. (Kadlec and Knight, 1996; IWA, 2000). ICWs which are the main focus of this thesis are shallow emergent-vegetated free water surface wetlands which are detailed in the following sections.

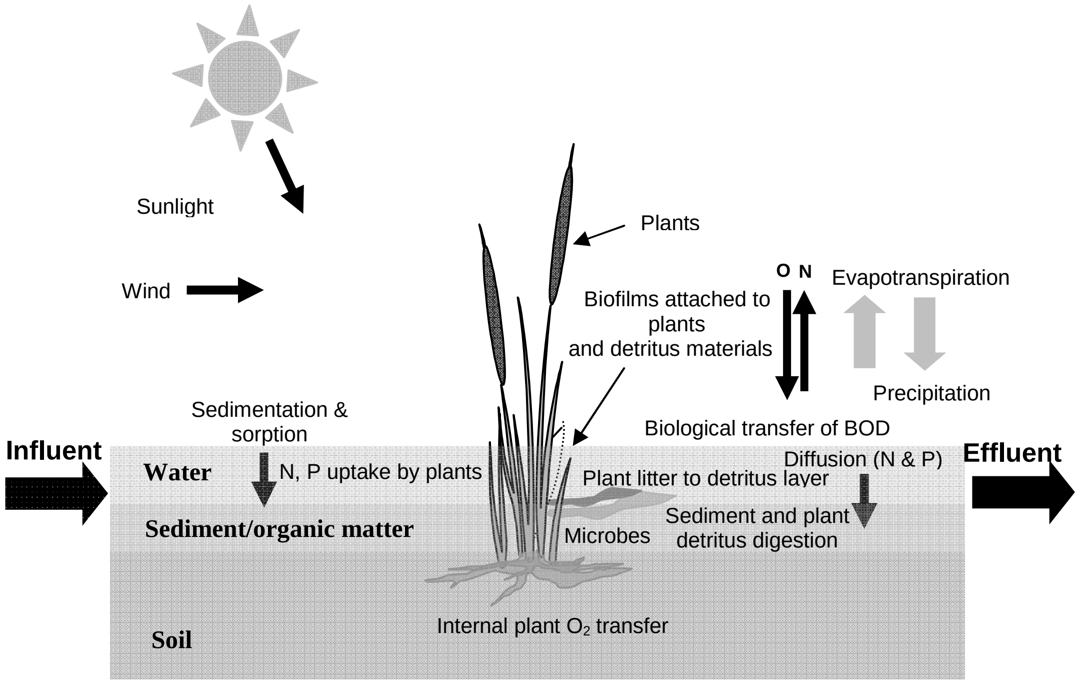


Figure 2-6. Constructed wetland components and ecological processes (after Wallace and Knight, 2006).

2.8. Components of FWS

To understand the treatment processes it is important to know about the components of a wetland system because all the processes occur within each or multiple components. Different components along with the ecological processes of constructed wetlands are shown in Figure 2-6. FWS have three components: a fixed component, a water component and an atmospheric component (Breen, 1990).

The fixed component includes the wetland substrate, wetland vegetation, accumulated litter and detritus, and microbial biofilms, while the water component comprises the influent, the water column, the effluent and the associated pollutants. The atmospheric component regulates the movement of gases into and out of the water column (Wallace and Knight, 2006).

2.9. Physical removal processes

Physical processes play a vital role in the reduction of both dissolved and particulate pollutants. The main physical processes involved in pollutant removal from wetland systems are gravitational settling, diffusion and volatilisation. Gravitational settling is an important process that is responsible for suspended solids removal. The diffusion process facilitates oxygen transfer from the atmosphere into the water column resulting in a thin layer of nearly-saturated DO at the top of the water column. Volatilsation occurs when compounds with significant vapour pressures change to the gaseous state and leave the water column.

2.10. Chemical removal processes

Chemical processes play an important role in the removal of phosphorus and dissolved metals. The major chemical removal mechanisms are adsorption, ultraviolet (UV) radiation and chemical precipitation. The accumulated plant detritus is the wetland substrate and during adsorption the pollutant is adsorbed on the adsorption media (substrate). If the adsorbed material can be microbially degraded, as for organic compounds, then the sorption sites can be renewed. If the material cannot be microbially degraded, such as phosphorus then the sorption sites will finally become saturated and removal through this mechanism will cease.

The UV radiation that enters the water column from sunlight chemically affects the molecules. For example, the viability of pathogens is affected. The process of chemical precipitation takes place when reactions within the wetlands result in the formation of insoluble compounds. Metals such as iron, copper and nickel are removed by hydroxide or sulphide precipitation within the wetland system (Wallace and Knight, 2006).

2.11. Biological removal processes

Wetlands house a wide variety of microorganisms such as bacteria, fungi and algae. These organisms are responsible for the breakdown and consumption of different pollutants in particular organic matter and nutrients.

2.12. Removal mechanisms

2.12.1. Suspended solids removal

Suspended solids are removed by physical processes known as discrete and flocculent settling as well as filtration/interception mechanisms (Hammer et al., 1993, Tanner et al., 1995, Kadlec and Knight, 1996; Wallace and Knight, 2006). Sedimentation is promoted by plant detritus through reduction in water column mixing. Plant detritus also drastically reduces the shear forces, thus enhancing flocculation and settling performance. Settling of suspended particulate matter by gravity is optimised by low flow velocity and long residence time. FWS systems are very efficient at removing suspended solids, with removal rates >90%, but these systems are complicated and sensitive because of the resuspension of previously settled solids by bioturbation (Gearheart et al., 1992; Hey et al., 1994; Wallace and Knight, 2006).

2.12.2. Organic matter degradation

Organic matter degradation may occur either through aerobic or anaerobic processes. In the upper reaches of the water column, aerobic conditions tend to prevail while anaerobic conditions will occur in the plant/detritus layer at the water column base. The oxygen needed to support aerobic processes is supplied through atmospheric diffusion, photosynthesis, internal oxygen transport within emergent wetland plants and rainfall reaeration (Moshiri et al., 1993). Wetland systems also have their internal organic matter loads from growth, dieback and decomposition of biomass produced by wetland plants. The following figure shows carbon cycling in wetland systems.

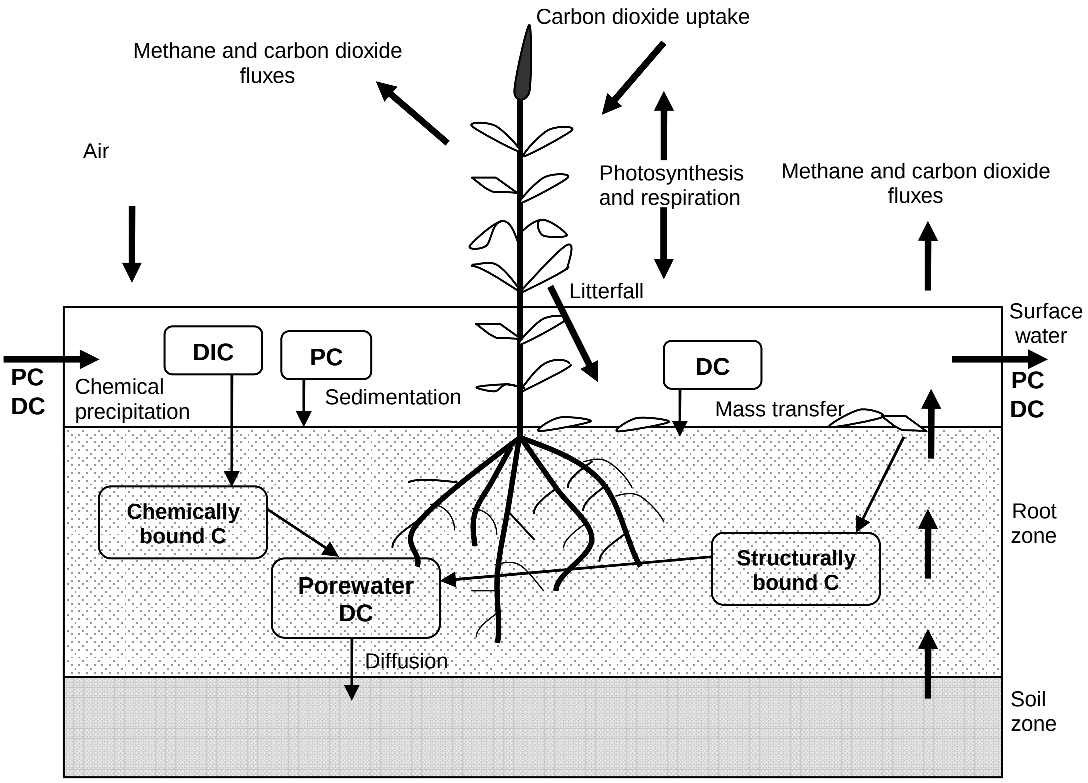


Figure 2-7. Schematic diagram showing the major components of the carbon cycle in wetland systems. DIC, dissolved inorganic carbon; DC, dissolved carbon; PC, particulate carbon

2.12.3. Nitrogen removal

2.12.3.1. Nitrogen occurrence in constructed wetlands

Different compounds of nitrogen are among the most important constituents of wastewater and of concern to engineers and scientists because of their polluting and toxic effects on receiving waters and species present. In wetlands, nitrogen exists in two forms: inorganic nitrogen compounds and organic nitrogen compounds. Ammonia, nitrite, nitrate and nitrogen gas or dinitrogen are included in inorganic nitrogen compounds, while amino acids, urea and uric acids, and purines and pyrimidines are a variety of compounds that make up the organic nitrogen compounds. Some of the important inorganic nitrogen compounds are discussed below.

2.12.3.2. Ammonia nitrogen

NH_4^+ (ionized ammonia), also known as ammonia nitrogen, is predominant in wetland systems (Kadlec and Wallace, 2009). Controlling and reducing the ammonia concentrations is a key consideration in the design of wetland treatment systems because ammonia is the principal form of nitrogen in wastewater and has the potential to degrade the environment.

2.12.3.3. Nitrite

NO_2^- is chemically unstable and is found in low concentrations. Detectable levels of nitrite are an indication of the presence of an anthropogenic source and incomplete nitrogen assimilation.

2.12.3.4. Nitrate

NO_3^- is chemically stable and remains untransformed in the numerous energy-consuming biological nitrogen transformations that occur (Kadlec and Knight, 1996). It is an essential nutrient for plant growth.

2.12.3.5. Microbial transformations of nitrogen

Nitrogen is interchanged between the atmosphere, organic matter and inorganic compartments through dynamic processes involving microorganism-mediated chemical (biochemical) reactions (Figure 2-8). The understanding of nitrogen transformations and the environmental factors that control these transformations is essential for the design of wetland systems to treat nitrogen (Kadlec and Wallace, 2009).

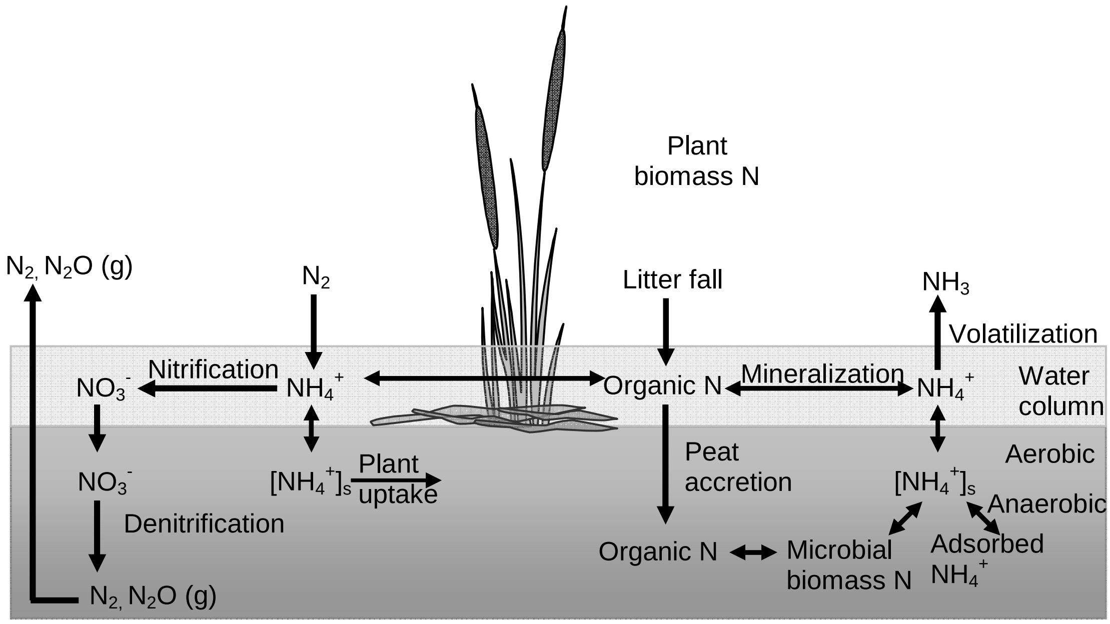


Figure 2-8. Schematic diagram showing the major nitrogen transformations (adapted from Reddy and DeLaune, 2008)

2.12.3.6. Nitrogen transformations in wetlands

Nitrogen chemistry in constructed wetlands is not only governed by classical nitrogen pathways but also by the alternative path of anaerobic ammonium oxidation plays a vital role in nitrogen transformations within the constructed wetland systems. The important processes responsible for nitrogen transformations and removal are as follows:

1) Ammonification 4) Anammox

2) Nitrification 5) Nitrogen assimilation

3) Denitrification 6) Nitrogen fixation

1) Ammonification

This is a complex biochemical process in which organic nitrogen is converted to inorganic nitrogen, ammonia. Ammonification is also the first step in mineralisation of organic nitrogen (Reddy and Patrick, 1984). This process occurs through microbial breakdown of organic tissues and hydrolysis of urea and uric acid. The process proceeds more rapidly in aerobic conditions than in anaerobic conditions. A typical ammonification reaction is as follows:

$$NH_2CONH_2 + H_2O \longrightarrow 2NH_3 + CO_2$$

2) Nitrification

Nitrification is a microbially mediated process which occurs in two steps. It is one of the most important mechanisms that reduce the concentration of ammonia nitrogen. The first step is ammonia oxidation mediated by bacteria of the genus *Nitrosomonas* (Wallace and Nicholas, 1969) while the second step is aerobic nitrite oxidation and is mediated by the bacteria of the genus *Nitrobacter* (Wallace and Nicholas, 1969). Energy is released in these steps which is utilised by cells for their growth.

$NH_4^+ + 1.5\ O_2 \xrightarrow{\textit{Nitrosomonas}} NO_2^- + 2\ H^+ + H_2O \quad [\Delta G^{o'} = -275\ kJ/mol]$

$NO_2^- + 0.5\ O_2 \xrightarrow{\textit{Nitrobacter}} NO_3^- \quad [\Delta G^{o'} = -74\ kJ/mol]$

Both *Nitrosomonas* and *Nitrobacter* are obligate aerobes and chemolithotrophic i.e. they can utilise inorganic materials as electron donors in oxidation reactions to yield energy that is required for metabolic processes.

3) Denitrification

Denitrification is a biochemical process in which oxidized nitrogen compounds (nitrate or nitrite nitrogen) are reduced to nitrogen gases (N_2 and N_2O). In this process the enzyme nitrate reductase allows facultative bacteria like genus *Pseudomonas* to utilize oxygen atoms bound in nitrate and nitrite molecules as final electron acceptors. Denitrifying bacteria can function under anaerobic conditions. A carbon source is needed that can be provided by the decaying plant detritus and carbonaceous biochemical oxygen demand CBOD present in the incoming wastewater. The nitrate reduction pathway is as follows:

$$4\ (CH_2O) + 4\ NO_3^- \longrightarrow 4\ HCO_3^- + 2\ N_2O\ (g) + 2H_2O$$

$$5\ (CH_2O) + 4\ NO_3^- \longrightarrow H_2CO_3 + 4\ HCO_3^- + 2\ N_2\ (g) + 2H_2O$$

where (CH_2O) represents the biodegradable organic matter.

4) Anaerobic Ammonium Oxidation (Anammox)

Anammox is a process which involves the oxidation of ammonium and nitrite to nitrogen gas. Anammox is mediated by a group of *Planctomycete* bacteria.

$NH_4^+ + 1.32\ NO_2^- + 1.066\ HCO_3^- + 0.13\ H^+ \longrightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$ [$\Delta G^{o'} = -357$ kJ/mol]

Anammox bacteria utilise the nitrite for ammonia oxidation (Jetten et al., 2001, Tal et al., 2006, Kuenen, 2008).

5) Nitrogen Fixation

Nitrogen fixation is the process by which atmospheric nitrogen gas diffuses into solution and is reduced to ammonia nitrogen. This reduction takes place by autotrophic and heterotrophic bacteria, cyanobacteria and higher plants. Some important N_2-fixing organisms in wetland systems include free-living bacteria, cyanobacteria and N_2-fixing bacteria associated with plant roots.

Previous studies by Dierberg and Brezonik (1984) concluded that nitrogen fixation was an insignificant component of TN loading to the treatment wetland studied. Moreover, Kadlec and Wallace (2009) concluded that previous estimates of nitrogen fixation in treatment wetlands by various researchers are insufficient for quantifying them.

6) Nitrogen Assimilation

In nitrogen assimilation inorganic nitrogen forms are converted via a variety of biological processes into organic compounds that serve as building blocks for cells and tissues. Ammonia and nitrate-nitrogen are the two forms of inorganic nitrogen that are generally assimilated. The various biological assimilation processes occur in: macrophyte growth, microorganisms and algae.

Compared to natural wetlands, constructed wetlands are nutrient-enriched because of the high phosphorus and nitrogen loadings present in the wastewater. Greenway (2005) reported high nutrient content in plant tissues in constructed wetlands. Nutrients are also assimilated from the sediment by macrophytes, and from the water by free-floating macrophytes (Wetzel, 2001).

2.12.3.7. Nitrogen removal/retention mechanisms

Nitrogen transformations have been described above. Some of the processes described only transform nitrogen while others ultimately remove nitrogen from the wastewater. For example, nitrification is the principal transformation mechanism that reduces the concentration of ammonia nitrogen by converting it to oxidized nitrogen. On the other hand, denitrification is considered a major removal mechanism for nitrogen in most types of constructed wetlands. Ammonia volatilization, denitrification, plant uptake (with biomass harvesting), ammonia adsorption, anammox and organic nitrogen burial are the mechanisms that ultimately remove nitrogen from wastewaters (Vymazal, 2007).

Biological nutrient removal systems in natural environments are quite different and more complex compared to those in conventional wastewater treatment plants. There are more genera possibly involved in natural systems. For example, ammonia-oxidising bacteria in natural systems include *Nitrosospira* and *Nitrococcus* in addition to *Nitrosomonas* (Bothe et al, 2000). Austin et al. (2003) reported *Nitrosospira* along with *Nitrosomonas* and *Nitrococcus* in tidal flow wetlands. They

Table 2-1. Key processes and microbes in the Nitrogen Cycle (adapted from Madigan and Martinko, 2006)

Processes	Organisms
Nitrification ($NH_4^+ \longrightarrow NO_3^-$)	
$NH_4^+ \longrightarrow NO_2^-$	*Nitrosomonas*
$NO_2^- \longrightarrow NO_3^-$	*Nitrobacter*
Denitrification ($NO_3^- \longrightarrow N_2$)	*Bacillus, Paracoccus, Pseudomonas*
N_2 Fixation ($N_2 + 8H \longrightarrow NH_3 + N_2$)	
Aerobic	*Azotobacter*
Free-living $\longrightarrow$	Cyanobacteria
Anaerobic	*Clostridium*, purple and green bacteria
Symbiotic	*Rhizobium*
	Badyrhizobium
	Frankia
Anammox ($NO_2^- + NH_3 \longrightarrow 2N_2$)	*Brocadia*

Nitrosospira much more prevalent than *Nitrosomonas* in a treatment wetland. Heterotrophic bacteria such as *Paracoccus denitrificans and Pseudomonas putida* are also capable of nitrification (Bothe et al, 2000). Table 2-1 shows the processes involved in nitrogen cycling and the associated microbes.

2.12.4. Phosphorus removal in constructed wetlands

2.12.4.1. Phosphorus occurrence in constructed wetlands

Different compounds of phosphorus are among the most important constituents of wastewater and of concern to engineers and scientists because of their polluting and toxic effects on the receiving waters and species present. In wetlands phosphorus exists in two forms, inorganic phosphorus compounds and organic phosphorus compounds. *Orthophosphate* (PO_4-P) is the general term used for inorganic phosphate ions. If phosphorus readily combines with dissolved organic materials, then it is termed dissolved organic phosphorus (DOP). Some of the organics in DOP are readily hydrolyzed by soil enzymes, and together with PO_4-P are termed soluble reactive phosphorus (SRP). Phosphorus associated with suspended particles is known as particulate phosphorus (PP).

2.12.4.2. Phosphorus removal

The removal of phosphorus occurs through physical, chemical and biological processes including sedimentation of incoming particulate P, sorption and precipitation reactions along with biological uptake (Mitsch et al.,1995; Kadlec and

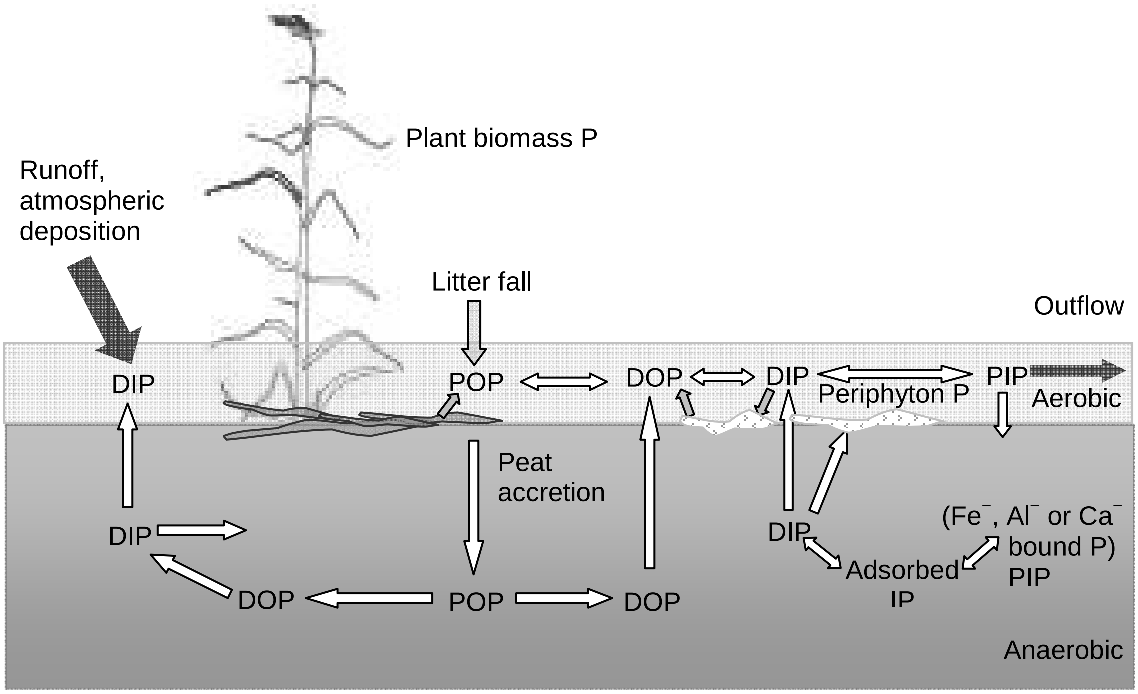


Figure 2-9. Schematic diagram showing the major phosphorus transformations (adapted from Reddy and DeLaune, 2008)

DIP, dissolved inorganic phosphorus; DOP, dissolved organic phosphorus; POP particulate organic phosphorus; PIP particulate inorganic phosphorus; IP inorganic phosphorus

Knight, 1996; Axt and Walbridge, 1999; Reddy et al., 1999; Bridgham et al., 2001; Braskerud, 2002). The long term sustainable removal of phosphorus in a wetland system is through accumulation on and burial in the bottom sediments (Craft and Richardson, 1993).

Plants grow, die and decay, returning the cycled phosphorus back to the water column. However, phosphorus is retained in those plant components that resist decay. It is this retention that plays an important role in the long-term storage of phosphorus (Kadlec, 1994b). Figure 2-10 shows phosphorus cycling in a constructed wetland system.

Wetlands provide a setting for the interconversion of all phosphorus forms. Plants take up soluble reactive phosphorus (SRP) and convert it to tissue phosphorus. SRP may also be sorbed to wetland soils and sediments. If the organic matrix is oxidized then the organic structural phosphorus may be released as soluble phosphorus. Some of the important phosphorus removal processes in wetland are as follows:

Sorption. This mechanism of phosphorus removal has a saturation threshold. Kadlec and Rathbun (1984) have described sorption as a two-step process.

1) Phosphorus swiftly exchanges between the soil pore water and soil particles or mineral surfaces (adsorption).
2) Phosphorus gradually penetrates into solid phases (absorption).

Sorption occurs until the entire wetland soil is loaded to the concentration corresponding to that of the concentration in water in the wetland. The time period to

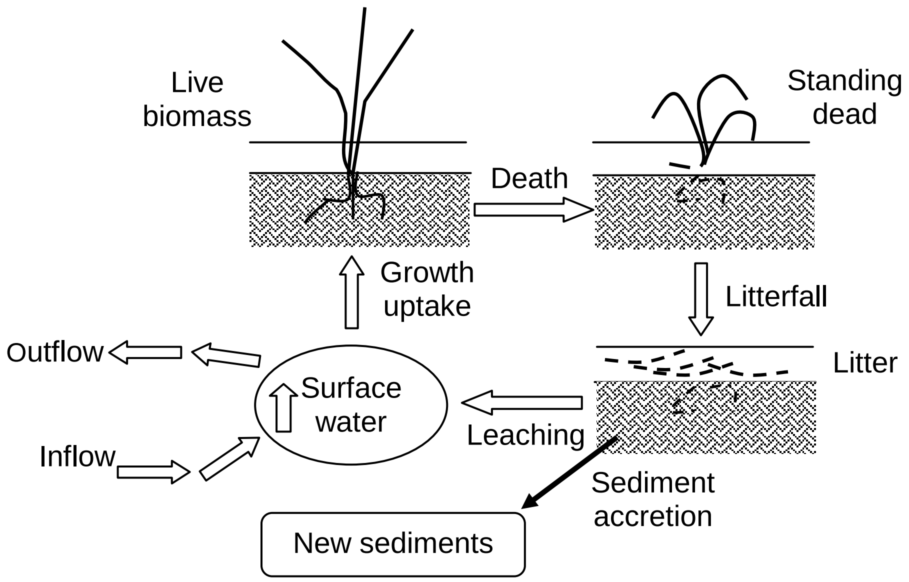


Figure 2-10. Phosphorus cycling in wetlands (after Wallace and Knight, 2006).

saturate can be long for soils with high sorption capacity and vice versa. The maximum sorbed concentration of soils for holding phosphorus has been found to rely strongly on the amount of iron and aluminium in the soil (Reddy et al., 1998).

Precipitation. Precipitation is an important mechanism for phosphorus removal. Aluminium and iron are the major constituents of mineral wetland soils and under oxidised conditions a coating of hydrated ferric oxides forms on clay or silt particles. Several forms of phosphate, such as ferric phosphate, aluminium phosphate and calcium phosphate, are attached to this coating. In calcareous wetland soils, high concentrations of calcium result in formation of complex calcium phosphate compounds such as calcium phosphate, dicalcium phosphate and beta-calcium phosphate. Precipitation reactions occur on the surfaces of soil particles and the amount of phosphorus that will precipitate depends on the amount of exposed surface (Reddy and DeLaune, 2008).

Biomass storage and accretion. Plants use phosphorus for growth. Plant decomposition processes release phosphorus back into the water column. Hence, the plant growth cycle seasonally stores and releases phosphorus. Plants are composed of components that decay and components that resist decay. The latter create new stable residuals, which accrete in the wetland. The biomass storage mechanism has a finite phosphorus retention capacity whereas accretion is a sustainable process. The phosphorus associated with the new stable residuals is retained in the system and contributes to the long-term storage of phosphorus.

Microbial phosphorus. Wetland soils contain microbial communities which decompose the organic material, and play an important role in remobilization and cycling of nutrients. The life cycle of microorganisms is short, and decomposition is swift. Therefore it is likely that most of the microbial uptake of P is returned as DOP and PP and hence only a small fraction is permanently buried.

2.12.4.3. Phosphorus removal/retention mechanisms

Phosphorus removal mechanisms described above play an important role in removing phosphorus from the water column. In addition to the described processes above there are also secondary processes like particulate settling and movement among storage components. In addition, the hydrologic regime also influences phosphorus transfer and storage within wetlands.

2.13. Summary

This chapter has presented the basic components and working of constructed wetlands that are mechanically simple but ecologically engineered systems. Nutrient removal which is the main focus of this research has been described in detail.

Chapter 3 Integrated constructed wetlands

3.1. Introduction

This chapter presents an overview of integrated constructed wetlands (ICWs). It introduces the novel concept of integrated constructed wetlands that are efficient for removing contaminants including nutrients. The later part of this chapter investigates how this innovative and novel wetland design methodology can be applied for farmyard runoff management.

The structure of this chapter is as follows. Sections 3.2 to 3.8 summarise the concept, advantages and limitations, hydrology and hydraulics, vegetation, processes design considerations, groundwater infiltration and maintenance of ICWs. The final section 3.9 describes the application of ICWs to control runoff from farmyards that are rich in nutrients.

3.2. ICW concept

The Integrated Constructed Wetland (ICW) Initiative was developed in Ireland with an approach that endeavoured to achieve 'water treatment', 'landscape fit' and 'biodiversity enhancement' targets by an innovative wetland design methodology. The conventional practice in Ireland for managing farmyard dirty water is land spreading. This method has resulted in increased levels of nitrogen and phosphorus in surface and ground waters (Healy et al., 2007). The ICW concept (Figure 3-1) is founded on the holistic use of land to control water quality. These systems are areas of land-water interface that form an integral part of the environmental and ecological structure of the landscape (Scholz et al., 2007). They

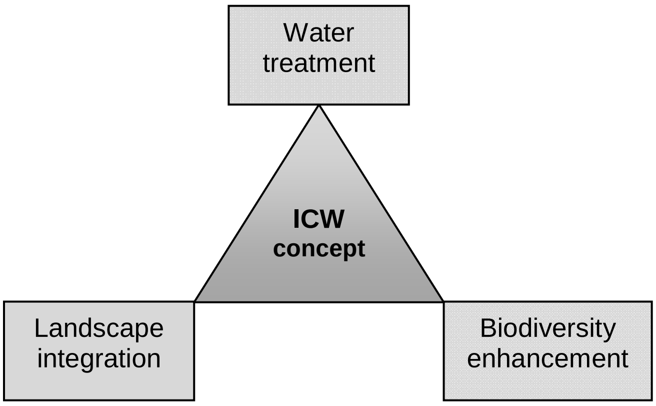


Figure 3-1. Integrated constructed wetland conceptual framework.

act as buffer lands that control the transfer and storage of farmyard dirty water rich in nutrients.

Integrated Constructed Wetlands mimic to a large extent the structures and processes found in natural wetlands. They are shallow and densely vegetated free-water surface flow wetlands with horizontal flow. This concept explicitly combines the objectives of cleansing and managing water flows with that of integrating the wetland infrastructure into the landscape and enhancing its biological diversity. The technology envelope for ICW is summarised in Figure 3-2. ICWs are used to treat a wide array of wastewaters. Some applications are:

- Animal wastewater
- Diffuse pollution
- Farmyard wastewater
- Food industry
- Landfill leachate
- Quarry and mining wastewater
- Sewage (also septic tank effluent)
- Sludge dewatering
- Trade effluents
- Urban/road runoff

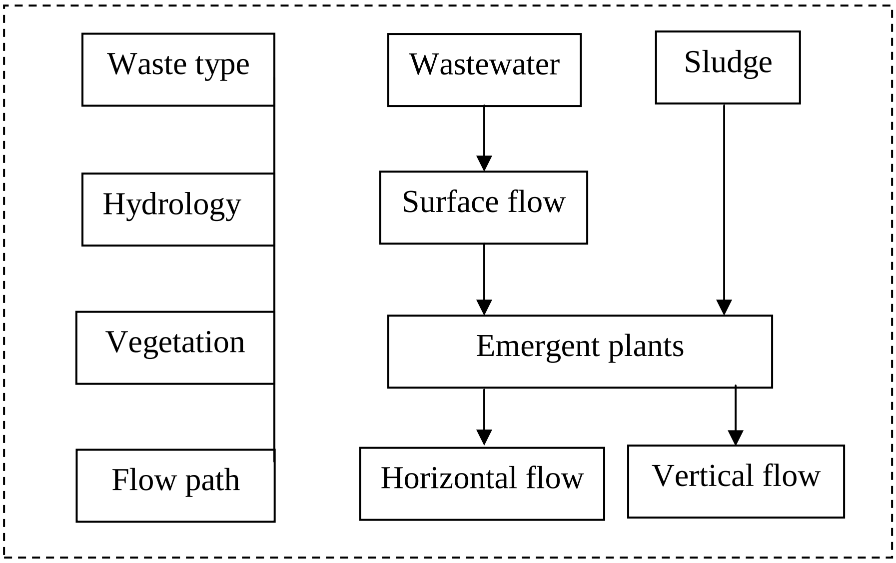


Figure 3-2. Integrated constructed wetland technology envelope.

3.3. Advantages and disadvantages

3.3.1. Advantages

Integrated constructed wetlands have several advantages: They are an effective multi-cellular system for treatment of various kinds of wastewaters. The robust and segmented system design increases life and makes management easy. They are constructed using local materials with minimum ‘external costs’ and are sustainable over a long lifetime (>50 years). They have low maintenance requirements, low energy demands and fit to the landscape and enhance scenery. They reduce odours produced, due to factors such as shallow surface flow and dense plant cover. The captured nutrients can be recycled for land management and the treated water can be reused.

3.3.2. Disadvantages

Integrated constructed wetlands also have some disadvantages. They require dedicated and relatively large land areas (typically 1-2% of farm area), competent skills for design, site analysis and characterisation, and construction, planning permission and discharge licences. Their performance is not consistent through out the year. The construction and establishment of vegetation may be weather dependent. If deep areas of water are included, there is a potential water hazard. If inadequately designed, constructed, or managed, they may pose a threat to surface and ground waters.

3.4. Hydrology and hydraulics

The wastewater flows entering ICW systems are exceptionally variable and the variations are stochastic. In places with high rainfall events as in Ireland, fluctuations in flow are an important factor that dictates the overall size of the wetland. The cleansing effectiveness of surface flow wetland systems is typically based on having appropriate hydraulic residence times. In shallow, emergent, and/or vegetated wetlands, such as ICW, this depends on having sufficient functional wetland area with an appropriate length to width ratio and an emergent vegetation density. Surface hydraulic effectiveness of the ICW depends on 1) segmentation of the wetland into a number of wetland cells of appropriate configuration, 2) avoidance of preferential flow (i.e., a direct and linear flow path between inlet and outlet), 3) vegetation stand density, and 4) managing the water depth to ensure optimal functioning.

Infiltration of wetland waters from wetland bed and bank surfaces, along with evapotranspiration, creates increased storage capacity (freeboard), thereby increasing hydraulic residence times. Such water loss buffers the impact of precipitation-generated fluxes through the ICW system. The flow types which occur in ICWs include plug, batch, mixed, sheet and laminar. The combined effect of flows, openness of these systems to precipitation and variable fluxes makes it difficult to determine accurate hydraulic residence times. A general hydraulic discharge equation from an ICW can be determined (Harrington and McInnes, 2009):

$$Q = (A + B) - (C + D) \quad (3\text{-}1)$$

where Q = discharge (m^3/annum); A = intercepted yard area (m^2) × annual rainfall (m) + livestock wastewater volume (m^3); B = ICW area (m^2) × annual rainfall (m); C = ICW area (m^2) × annual evaporation (m); D = ICW area (m^2) × annual infiltration rate (m).

3.5. Vegetation

The primary vegetation types used in ICW systems are emergent plant species (helophytes). They have special tissues that facilitate oxygen storage and its transportation from the leaves through the stem to the roots. Water and soil characteristics influence the variety and performance of plant species in each segment of an ICW. Plants may have year-round growth or be seasonally deciduous. While more than a hundred native species can be used, in general about 9 species are most commonly used. Their genera are: *Carex, Typha, Sparganium, Glyceria, Juncus, Eleocharis, Iris, Veronica, Ranunculus*.

ICWs that are shallow water wetlands (typically <200 mm) with emergent-vegetation such as the dominant plant genera *Typha, Glyceria* and *Carex* assist in water capture. Additionally, the plants provide shade and lower turbulence which decreases potential evapotranspiration. These characteristics facilitate the retention of water within ICW systems (van der Valk, 2006). Figure 3-3 shows the

Figure 3-3. Establishment of C*arex riparia* in an integrated constructed wetland cell (photo from Dr Rory Harrington).

establishment of plants in an ICW cell.

Phragmites australis is the most common species used in reed-beds. This species is not generally recommended for ICW systems as it is invasive and may eventually dominate the whole system thus decreasing biodiversity (Lee and Scholz, 2007). Moreover, their deep roots tend to open up pathways through the soil. Initial plant establishment exerts the dominant influence on the vegetation structure of an ICW.

3.6. Processes within ICWs

Within an ICW, the contaminated effluent is treated through various physical, chemical and biological processes involving aquatic plants, algae, microorganisms, water, soil and sun. The main processes are as follows:

- Physical filtration of suspended solids by wetland vegetative biomass acting as a hydrological baffle to incoming flows (optimised by high vegetation density and low flow velocity).

- Settling of suspended particulate matter by gravity (optimised by low flow velocity, low wind speed, low disturbance and long residence time).
- Uptake, transformation and breakdown of nutrients, hydrocarbons and pesticides by biomass, plants and microbes (increased by a relatively high temperature, long residence time, contact with micro-organisms and plants, high micro-organism and plant density, and a relatively high organic matter content).
- Accumulation and decomposition of organic matter, which is important for nutrient cycling (optimised by low velocity and availability of adsorption sites on suitable aggregates).
- Microbial mediated processes such as nitrification (aerobic) and denitrification (anaerobic), which are important for the cycling of nitrogen.
- Chemical precipitation and sorption of nutrients such as phosphorus by soil (influenced by the availability of sorption sites, pH and redox potential).
- Predation and natural die-off of pathogens; e.g. *Escherichia coli* and Cryptosporidium (optimised by high diversity and density of natural predators (e.g. protozoa), and increased exposure to ultraviolet light).

3.6. Design considerations

ICW systems are designed not only to improve the water quality but also to integrate the structure naturally into the landscape and enhance biodiversity (Figure 3-4). The ICW design philosophy is in agreement with the guidelines proposed by Carty et al. (2008b). Information on topography, hydrogeology, surface water, groundwater and subsoil are obtained for assessing the suitability of sites for an ICW prior to the design and construction phase.

A detailed site-specific assessment combining site investigation and desk study is conducted to determine the site characteristics, and assess any potential impacts on groundwater and surface waters. The site assessement is based on characteristics such as topography, geology, soils and subsoils, hydrogeology, hydrology, flora and fauna, archaeological and architectural features, and natural interest. A topographical survey of the farmyard and the proposed ICW site is conducted which includes contours (0.5 m contours), location and use of buildings, boundaries, and hydrological features. The nearby surface water features such as rivers, streams and drains are also noted during the survey. The suitability of the nearby stream as a potential discharge point is also assessed. During the site assessment, information on wells, springs, water table elevation, aquifers, nearby

Figure 3-4. An example of an ICW in Carlow, Ireland (photo from Dr Rory Harrington).

surface and groundwater supplies and connectivity with surface water features is also gathered. The soil and geological conditions are also studied.

In ICW design, sizing is an important element. The sizing formula for the ICW treatment area has been developed from the need to address robustness and sustainability with special regard to phosphorus. The size is based mainly on farmyard intercepted precipitation events that may be > 50 times the average washwater volume. The functioning area of an ICW for effective treatment of farmyard runoff requires an area twice the associated farmyard interception area with a further land area allowance of about 25 percent. This additional allowance is for auxiliary embankment areas. The ICW area is typically 1-2% of any individual farm area (Dr Rory Harrington, pers. comm.).

3.7. Groundwater infiltration

ICW are not lined with artificial liners, but are constructed using in situ soils. Site subsoils are used and reworked to line the wetland bed and bank surfaces, and topsoil is redistributed for plant establishment. This helps to impede infiltration from the bottoms and sides of cells. ICW are underlain by at least 1.5 m of subsoil, with the upper 0.5 m enhanced where necessary to a hydraulic conductivity of 10^{-8} m/s (Dunne et al. 2005a, b). Inflows of farmyard dirty water contain high nutrient, biochemical oxygen demand and suspended solids concentrations. As this water passes through the ICW system, suspended organic material is typically deposited onto wetland soil surfaces, which also helps to impede infiltration from wetland cells (Kadlec and Knight 1996, Scholz 2006). Rowsell et al. (1985) and Bouwer et al.

(2001) have shown that the permeability of lagoons receiving animal waste may decrease over time due to sealing by organic matter and sediment accumulation.

3.8. Maintenance

The success of an ICW depends on the maintenance and operation of the system. While an ICW is designed to be as self-maintaining as possible, it is crucial that a maintenance program is adopted to ensure continued effective water treatment and 'rejuvenation' of the system.

Pipe maintenance. All inlet and outlet pipes within the ICW system are visually inspected weekly for blockages, sediment accumulation and debris. Blockages affect the hydraulics of the system, while sediment accumulation may indicate inadequate solids separation further up in the system. Any blockages and sediment or debris accumulations around the inlet or outlet pipes should be cleared (Scholz, 2007).

Flow control. During prolonged dry periods, water depths within the ponds decrease, especially in down-gradient regions of the wetland. It is essential to ensure that soils are flooded (to at least 50 mm). Integrated constructed wetlands are usually not allowed to dry out as cracks may form in the base, which may cause higher infiltration rates in the short term when effluent re-enters the cell. Any adjustment of pipes must be carried out gradually as these movements may cause huge surges of effluent to subsequent wetland cells or receiving surface or groundwaters.

Vegetation maintenance. Water levels are maintained at less than 300 mm to ensure good plant growth. However, most macrophytes tolerate short periods of increased water depth (up to 500 mm), such as associated with high rainfall, as well as low water depths or even no water during dry weather. Major changes in vegetation cover and composition are noted as a possible indicator of change (i.e. degradation or improvement) in wetland performance.

Removal of sediment. The removal of accumulated sediment is usually confined to the first cell (Scholz et al., 2007). For a heavily loaded system, the inclusion of an open water pond at the initial stage of the ICW as a sediment trap may extend the operational life of subsequent cells before the removal of material is required. Any pond located within the initial wetland cell would require relatively frequent material removal. The most appropriate way of managing the material removed is likely to be land spreading on the farm in accordance with good farm management practice. Information on the solid content and nutrient composition, particularly phosphorus, are required to ensure that the usage complies with farm nutrient management requirements (Bowmer and Laut, 1992; Carty et al., 2008a,b).

Inspection of pond embankments. The wetland operator undertakes regular visual inspection of the internal and external faces of the wetland embankments to check for any water leakage, slippage or distortion. The internal embankment face is checked by walking along the embankment crests and external embankment faces by walking along the external boundary of each cell. Any defects such as leakages, slippages or distorted areas should be addressed immediately.

Access and security checking and maintenance. Access around the wetland is maintained by managing vegetation growth on the embankments. Under normal operating conditions, growth on some pond crests needs to be cut biannually using a mower or topper. Security and safety considerations for both humans and livestock are incorporated into the design of the ICW.

Monitoring of the final effluent and receiving watercourses. The monitoring of the ICW and the final effluent allows the operator to assess the performance of the wetland system and to detect any malfunction. The general appearance of the final effluent is noted, paying particular attention to water colour, smell and any evidence of plant material in the discharge. If the final discharge water appears to be heavily discoloured, polluted or contains plant material, then the outlet pipe is isolated immediately by closing the gate valve. However, water that is visibly clear may also have a high nutrient load, which can only be determined by laboratory analysis.

The condition and appearance of the receiving waters at the point of discharge is checked on a monthly basis and following extreme events such as high rainfall. The operator assesses the condition and appearance of water, both upstream and downstream of the discharge location. Heavily discoloured water or the appearance of sludge type material may indicate an upstream pollution source. Foaming immediately downstream of the discharge point may indicate pollution in the final effluent. The outlet pipe from the ICW should be isolated immediately by closing the gate valve in the event of any suspected pollution incident and advice from a suitable agricultural advisor and the regulator should be sought (Carty et al., 2008b).

3.9. Integrated constructed wetlands for farmyard runoff treatment

3.9.1. Farmyard runoff treatment

During the past few years there has been considerable interest in the use of constructed wetlands for treating surface water runoff from farmyards because agricultural pollutants cause problems in downstream water bodies, due to eutrophication (Braskerud, 2001; Poe et al., 2003).

Farmyard runoff emanates from a wide array of farming activities. It is composed of runoff from silos, yards and other areas of hard-standing and dairy cow access tracks. It may contain slurry (occasional), raw milk and washings from pesticide sprayers and dipping equipment. If the contaminated runoff is not treated, this runoff along with other non-point sources of pollution can seriously contaminate the associated surface water and groundwater.

The conventional practice for management of farmyard dirty water in Ireland is land spreading of (Tunney et al., 1997). Storage and spreading are governed by rules to prevent water pollution. However, this practice requires considerable labour and machinery resources, as well as storage infrastructure. Improper storage and spreading has been linked to water pollution, particularly to increased levels of nitrogen and phosphorus in surface and ground waters (Healy et al., 2007). In contrast, the ICW concept is founded on the holistic use of land to protect and improve water quality. Figure 3-5 shows a farm and an associated ICW in Waterford, Ireland.

Figure 3-5. Integrated constructed wetland treating farmyard runoff in the Anne valley near Waterford, Ireland (photo from Dr Rory Harrington).

3.9.2. Nutrients in farmyard runoff

Nitrogen and phosphorus within farmyard runoff are associated with the animal wastes which forms the major soluble part. Table 3-1 shows the high levels of nutrients associated with various categories of farm animals. Morse et al. (1992) conducted a nutrition study and found that cows excrete 88.2% of phosphorus consumed daily of which 68.6% is in faeces, 1.0% in urine and 30.3% in milk.

When nutrient-rich wastewater enters and travels through the shallow vegetated cells of a constructed wetland, various physical, chemical and biological processes gradually remove nutrients from the wastewater. The vegetation provides surface area for biofilm growth and assists in the cycling of nutrients. The shallow water depth in conjunction with emergent vegetation provides stable aerobic and anaerobic zones supporting nitrification and denitrification, respectively; processes that play an important role in nitrogen removal. Phosphorus is removed through

Table 3-1. Comparison of approximate waste quantities from animals and human. (adapted from data in CH2M Hill, Payne Engineering,1997)

Type	Classification	Nitrogen (g/d.100 kg)	Phosphorus (g/d.100 kg)	BOD (g/d.100 kg)
Dairy cattle	Lactating cow	44	7	161
	Dry cow	35	5	119
	Heifer	31	4	130
Beef cattle	Forage feeder	31	11	137
	Energy diet feeder	29	9	137
	Yearling	31	10	130
	Cow	33	12	119
Human	70 kg	19	2.3	26

sorption onto exchange sites in the sediment. Nevertheless, sustainable removal of phosphorus is through accumulation and burial in sediments. The dead plant material undergoes decomposition but some portions resist decay and form stable new accretions that store phosphorus.

3.9.3. Application of integrated constructed wetlands

Farmyard runoff, which is rich in nutrients and organic matter, is a source of diffuse pollution, and potentially a serious risk to receiving watercourses by contributing to eutrophication (Cleneghan, 2003). The loss of nutrients and contaminants from agricultural land, farmyards, dairy parlours, tracks and roofs to rivers, lakes and groundwater, can have a detrimental impact on water quality. Both point and diffuse sources of pollution from agriculture contribute to the degradation of water quality and aquatic ecosystems (e.g. fish kills and loss of habitats) through eutrophication, contamination of groundwater, siltation and direct toxicity to organisms which consequently affect biodiversity, fisheries, recreation and public health. Polluted farm runoff also affects farmers, exposing them to fines and prosecutions, and the wider community by the subsequent degradation and loss of water supply within affected watersheds (Harrington and Ryder, 2002; Mantovi et al., 2003; Scholz et al., 2007). A variety of methods, including ponds, lagoons, storage structures, filters and sediment basins, and constructed wetlands, are used to manage water quality from agricultural activities.

In Ireland, 25% of the land area is used for dairy farming and it is estimated that the agricultural sector is the source of approximately 32% of pollution in rivers and streams (Jennings et al., 2003). All types of farming operations generate high levels of nutrients and there is a need to intercept and reduce these contaminant loads prior to discharge to a water body. One of the earliest wetland systems to treat runoff from animal yards was built in 1930 in Dallas County, Iowa, USA. A two year study of two-cell FWS system, which served a 26-ha feedlot comprising of 7000 cattle, indicated good removal efficiencies of 87% for BOD, 60% for SS and 58% for TKN.

Pond systems have been used to manage water from farmyards, with a significant portion of nutrients returned to pasture land via periodic desludging. The application of constructed wetlands to manage water from farm activities has been a topic of considerable research over the past decade (Newman et al, 2000; Hunt and Poach, 2001; Shamir et al, 2001; Ibekwe et al, 2003; Forbes et al, 2004; Dunne et al, 2005, Healy et al., 2007; Edwards et al., 2008). From 2000 to date, more than 15 FWS wetlands (integrated constructed wetlands) have been commissioned in the

Table 3-2. Characteristics of 12 ICWs in Anne Valley, Ireland treating farmyard runoff and concentration reduction efficiency for selected nutrients (Scholz et al., 2007).

ICW	Farmyard area (m^2)	ICW area (m^2)	ICW to farmyard area ratio	Number of cows	NH_4-N reduction %	MRP reduction %
1	4500	3906	0.9	60	99	99
2	14750	22966	1.6	60	99	98
3	5400	10288	1.9	50	98	81
4	9200	10237	1.1	100	98	93
5	4000	3940	1.0	35	99	98
6	9800	12691	1.3	80	99	99
8	2300	3940	2.0	n/a	99	97
9	4800	7964	1.7	55	98	96
10	2100	4375	2.1	50	99	99
11	5000	7676	1.5	77	99	92
12	13600	10748	0.8	85	99	99
13	5000	5610	1.1	n/a	99	93

NH_4-N, ammonium-nitrogen; MRP, molybdate reactive phosphorus.

Anne Valley of County Waterford located in the south-east of Ireland.

A summary of the concentration reduction efficiency of 12 ICW sites is presented in Table 3-2. These systems have provided good water quality improvements most likely because of their large sizes and hence increased retention time.

3.10. Summary

This chapter has presented the intriguing and novel integrated constructed wetland concept. Infiltration of wetland water into the ground which is a major concern for practitioners has also been discussed. Moreover, the maintenance aspects and application of these systems for treatment of nutrient enriched farmyard runoff have also been described.

Chapter 4 Materials and Methods

4.1. Introduction

This chapter explains the study sites and various methods used in the research. It contains information on the methodology adopted to meet the study objectives. The first section describes the study sites while the subsequent sections describe the details of methodology. The second section describes the water quality monitoring scheme and the sediment and macrophyte sampling and analysis. The third section describes the sampling and analysis protocol for the molecular work to characterise nitrogen removing bacteria in sediment and litter components of the selected study sites. Finally the last section describes the Self-Organising Map (SOM) model and its application for the prediction of water quality parameters.

4.2. Experimental set-up

4.2.1. Study sites

Thirteen ICW systems were constructed to treat agricultural wastewater and improve the water quality of Annestown stream located in Waterford in southeast Ireland. The ICW treatment systems are located in a temperate zone with a mean annual temperature and precipitation of 11.4ºC and 1094 mm respectively (Met Éireann, 2007). Mean seasonal temperatures for the region in 2008 were as follows: winter, 7.8ºC; spring, 10.3ºC; summer, 14.9ºC; and autumn, 12.2ºC. The study was carried out at three representative sites, ICW 3, 9 and 11 (Figure 4-1). The site suitability was assessed by identifying different indicator variables such as good

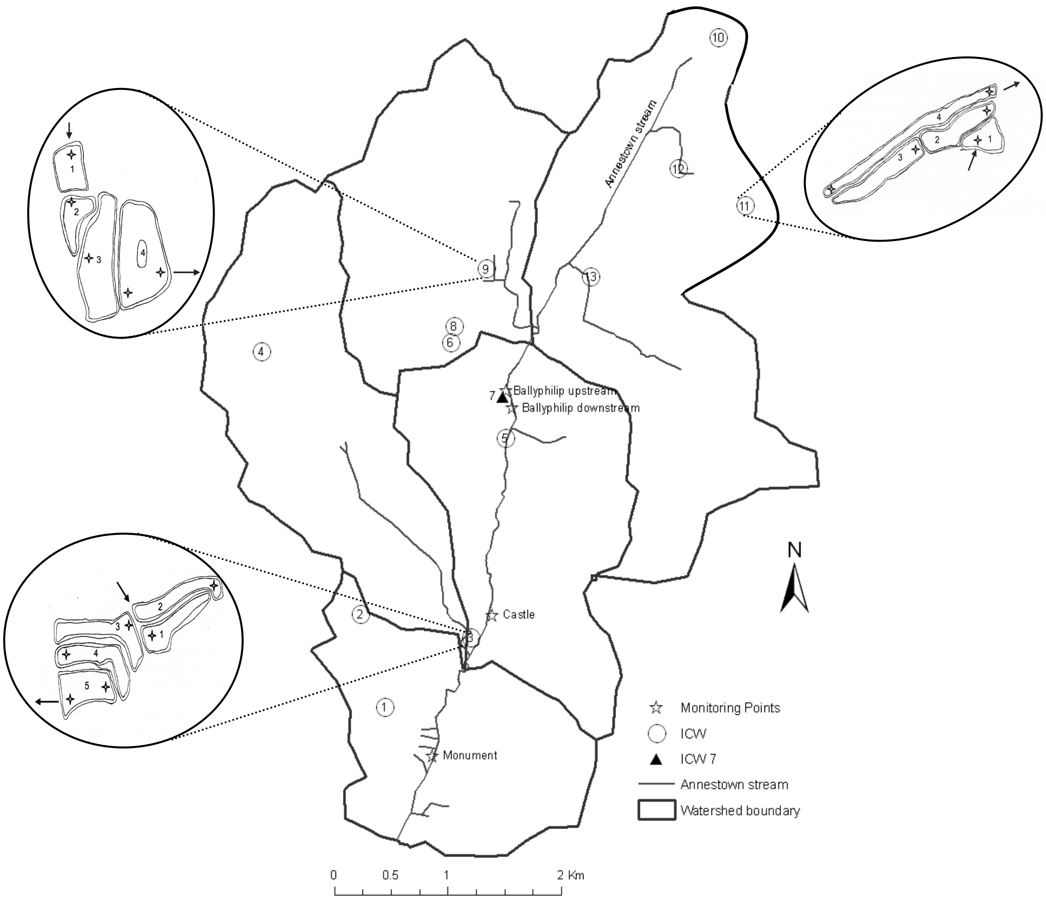


Figure 4-1. Study sites in Annestown catchment.

agricultural practice, site access and historical data availability. Based on this assessment, ICW 3, 9 and 11 were selected as priority sites. All three systems were constructed in 2000 and commissioned in 2001 to treat farmyard runoff comprising of yard and dairy washings, and rainfall on open yard and farmyard roofed areas along with occasional silage and manure effluents.

Prior to construction of these wetland systems, there was no treatment and the runoff was spread onto the adjoining fields. The wetland systems have a multi-cellular configuration with a minimum number of four cells. The systems operate as a set of sequential containment structures that intercept and control the contaminant gradient. The wastewater contained farmyard and roof runoff occasionally contaminated by manure, and was conveyed to the ICW system by gravity through pipes. The key features of wetlands were horizontal surface flow and intermittent hydraulic loading.

The ICW systems were based on four cells (ICW 9 and 11) and five cells (ICW 3) operated in series. A single influent entry point was located at the first cell. Each cell had one inflow and one outflow structure. The water flow between each cell was by gravity through a PVC pipe. The cells preceding the last cell had depths of approximately 1 m, while the last cell was approximately 1.5 m deep.

The ICW cells were not lined with an artificial liner. However, the subsoil was reworked and used as a natural liner. The cells were only partly planted with vegetation naturally available on the site. Further plant growth occurred by natural colonisation. The established emergent vegetation for the three sites comprises *Typha latifolia*, *Carex riparia*, *Glyceria maxima*, *Phalaris arundinacea*, *Carex riparia* and *Juncus effusus.*

Integrated constructed wetland 3

The first system ICW 3 is located in the southern part of the catchment at a grid reference of E250526, N103743. The wetland system has a total area of 1.02 ha and the primary vegetation types are emergent plant species (helophytes). The associated dairy farm had an area of 0.54 ha and was operated for 50 cows. According to the soil classification by Irish Forest Services (IFS), ICW 3 site has soils derived from mainly acidic parent materials. The subsoil of ICW 3 has a texture of alluvium undifferentiated soils. Figure 4-2 presents an aerial view of ICW 3.

Figure 4-2. Aerial view of integrated constructed wetland 3, with Annestown stream flowing parallel, Waterford, Ireland (April, 2008).

Integrated constructed wetland 9

The second system ICW 9 (Figure 4-3) is in the north-western part of the catchment at a grid reference of E250405, N100663. It has a total area of 0.79 ha while the associated farm has an area of 0.48 ha and was operated for 55 cows. According to the soil classification by Irish Forest Services (IFS), the ICW 9 site has soils derived from mainly mineral alluvium composition. The subsoils of ICW 9 are classified as a combination of undifferentiated alluvium and acid volcanic till.

Figure 4-3. Last pond of integrated constructed wetland 9 (December, 2008).

Integrated constructed wetland 11

The third system ICW 11 (Figure 4-4) is located in the north-eastern part of the catchment at a grid reference of E253088, N104696. It has a total area of 0.76 ha while the associated farm has an area of 0.5 ha and was operated for 77 cows. The details of established vegetation for this site are summarized in Table 4-1. According to the soil classification by Irish Forest Services (IFS), the ICW 11 site has soils derived from a mainly mineral alluvium. The subsoil has a texture of alluvium undifferentiated soils.

Table 4-1. Cell and vegetation cover details for integrated constructed wetland 11.

Cell number	Area (m^2)	Vegetation cover (%)	Vegetation type no.	Vegetation cover by species
Cell 1	1208	100	2	*Typha latifolia* (80%) and *Carex riparia* (20%)
Cell 2	1906	100	3	*Glyceria maxima* (50%), *Carex riparia* (35%) and *Typha latifolia* (15%)
Cell 3	2126	100	5	*Glyceria maxima* (40%), *Phalarius arundinacea* (25%), *Carex riparia* (20%), *Juncus effusus* (10%) and *Typha Latifolia* (5%)
Cell 4	2435	1	1	Open water (99%), *Juncus effusus* (0.8%) and others (0.2%)

Figure 4-4. Main inlet of integrated constructed wetland 11 (cell 1), Waterford, Ireland (February, 2007).

4.3. Water quality monitoring

4.3.1. Wetland water quality

In order to evaluate the water treatment potential and contaminant reduction in ICW, the systems were regularly monitored for various physical, chemical and microbiological parameters. Grab samples at each wetland cell inlet and outlet were taken on an approximately fortnightly basis by Waterford County Council personnel.

4.3.2. Groundwater quality

To assess the impact of ICWs on groundwater, the water quality in groundwater-monitoring wells was regularly monitored. For ICW 3, three piezometric groundwater-monitoring wells were placed up-gradient of (one well, 5 m depth) and within (two wells, 5m and 3m depths) the ICW site in autumn 2004.

These wells were sampled on a quarterly basis from 2004 to 2008. The day before sample extraction, all wells were purged and subsequent analysis was carried out according to APHA (1998).

For ICW 11, four piezometric groundwater-monitoring wells were placed up-gradient of (one well, 5 m depth), within (two wells, 3 m and 5 m depths) and down-gradient (one well, 5 m depth) of the ICW site (Figure 4-5) in autumn 2004. These wells were also sampled on a quarterly basis from 2004 to 2008. The day before sample extraction, all wells were purged prior to sampling and subsequent analysis was carried out according to APHA (1998).

4.3.3. Stream water quality

The water quality of the receiving stream for ICW11, which is a tributary of the Annestown Stream, was monitored (Figure 4-1). Grab water samples were collected from twenty one points along the stream located adjacent to the ICW. Three points were sampled up-stream, up to sixteen parallel to cell 4 and two points down-stream to assess the effect of the ICW system on the associated receiving watercourse (Figure 4-5). In 2007, grab samples were taken predominantly during an intense period for monitoring the water quality of the receiving watercourse. The sampling scheme was designed to monitor the water quality during periods of relatively low and normal flows. Weekly samples were collected in late spring and during the following summer. Later on, monthly samples were collected during autumn and winter. Nutrient analysis was conducted at the County Council water laboratory using standard methods (APHA, 1998).

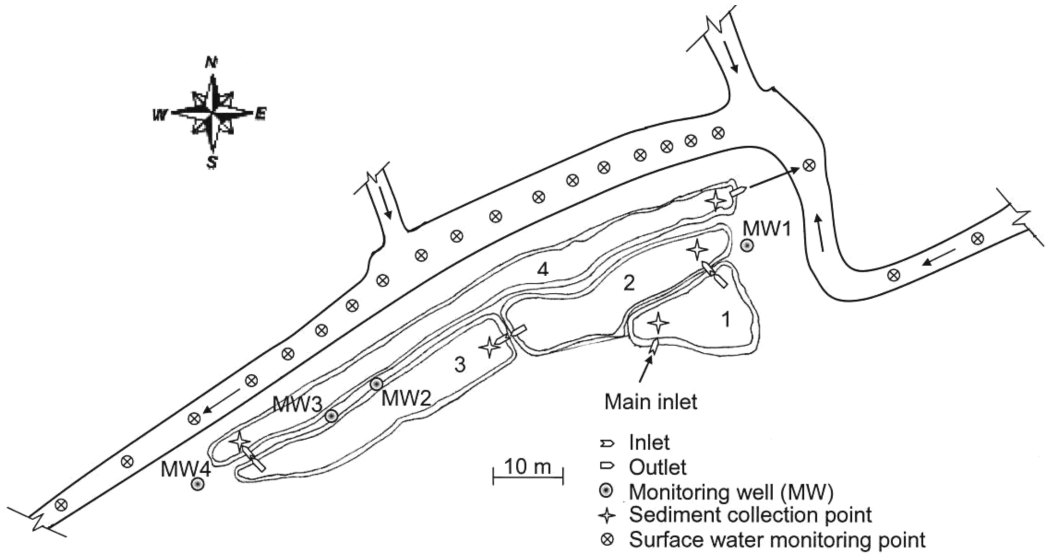


Figure 4-5. Surface water and groundwater monitoring, sediment collection, inlet and outlet points for the selected integrated constructed wetland (ICW 11).

4.4. Wetland water quantity

For ICW 11, continuous flow measurements were undertaken between April 2003 and June 2004. From June 2004, spot measurements were made of flows into and out of each ICW cell. Furthermore, flows entering and leaving ICW cells were monitored and recorded using standard Greyline flow meters and associated data loggers. The standard Greyline Area-Velocity Flow Meter uses a submerged ultrasonic sensor to continuously measure both level and velocity to calculate flow in the influent/effluent pipe. Data are stored in the data logger. The stored flow logs are transferred to laptop and saved in the database.

4.5. Sediment and plant sampling for nutrient analyses

Sediment and plant samples were collected (Figure 4-6) from the first cell of ICW 11, as the major contaminant removal takes place in the first cell of the ICW. Over the years plant biomass has been accumulating in the ICW and is important for nutrient cycling. A total of 18 samples of sediment and plants from the first ICW cell were collected and analysed for nutrients. The above- and -below biomass of *Typha latifolia* in the wetland cell were sampled and analysed for nutrients in summer and winter 2008**.** Three locations, one near the inflow, one at the middle of cell and one near the outflow were selected in the ICW cell (Figure 4-7) and three quadrats were positioned (0.25 m^2 plots) at each location. Hence, plants were harvested in 9 quadrats of the ICW cell during the collection period for a total of 18 quadrats (9 quadrats x 2 seasons). For above ground biomass evaluation, the plots were harvested and biomass was separated from the upper zone (top-leaves and stem) while for the below ground biomass evaluation, it was separated from the lower zone (bottom-roots and rhizomes). The samples were sorted and washed in the

Figure 4-6. Sediment and macrophyte sampling at an integrated constructed wetland site.

laboratory and later on dried in an oven at 80^{o}C for a minimum of 48 hours. After drying the samples were weighed and the plant material was ground to less than 1 mm (Planetary Ball Mill PM 100, Retsch, Germany) for nutrient analysis, using standard methods. Plant cover was assessed by measuring the area occupied by each species in the wetland.

Sediment samples (from the same locations as macrophyte sampling points) were also collected to assess the sediment depth and quality. The sampling was conducted along transects across each wetland cell; three points along the primary axis from the main inlet to outlet of each pond, and also perpendicular to this. The water depth was measured and sediment cores were collected in a plastic liner (diameter 48 mm) within a stainless steel aquatic sediment sampler (Wildco hand corer, length 0.51 m), equipped with a Lexan nosepiece and a rubber flutter valve to provide suction. The corer was attached to a steel extension rod and driven into the sediment by hand for sample collection. The sampling points were marked with

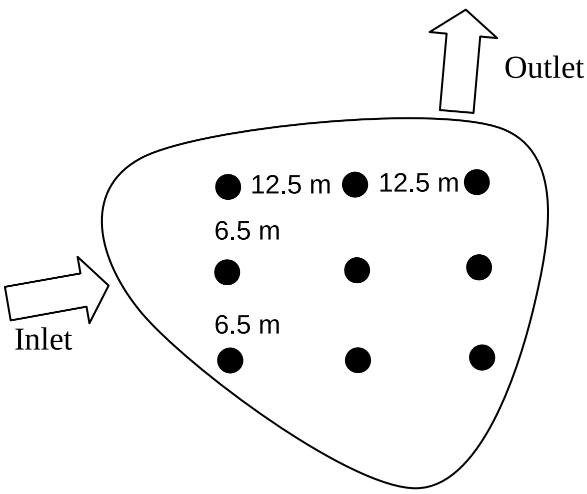


Figure 4-7. Plan view of the integrated constructed wetland cell showing sediment and plant sampling locations.

permanent markers and ropes, to facilitate and ensure that the sediment samples were collected from the same locations for the two seasons, to enable comparison of results between seasons. Samples were divided into five subsections; sediment top, sediment middle, sediment bottom, clay top and clay bottom. The samples were transferred to sample bags and retained for laboratory analysis.

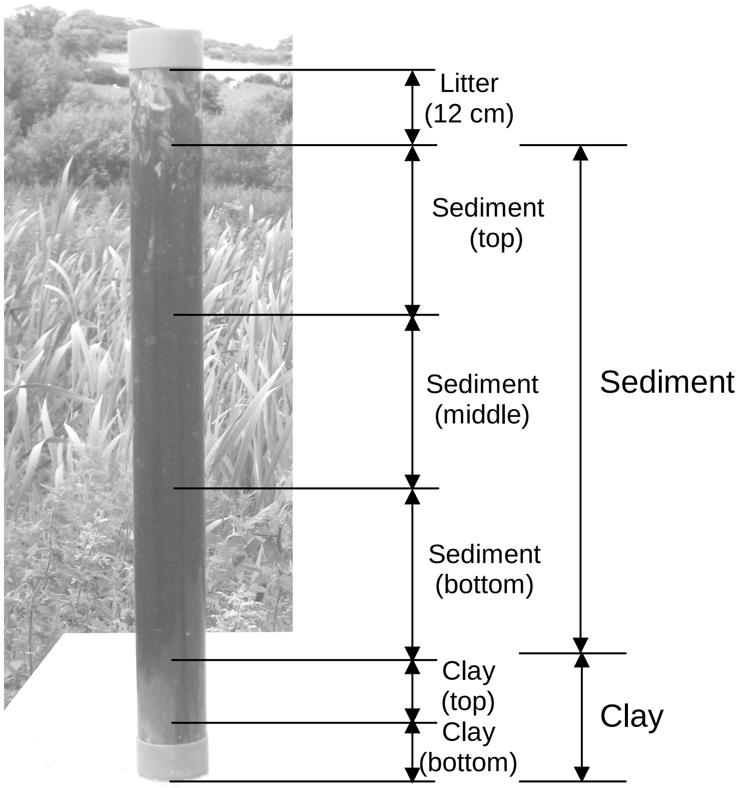


Figure 4-8. Sediment core samples collected from cell 1 of ICW 11 (not to scale).

4.6. Sediment and litter sampling for characterising bacteria

The analysis was carried out at two universities; Linköping University, Sweden, and Newcastle University, United Kingdom.

I –Sampling for analysis at Linköping University, Sweden

To better understand nutrient removal processes in different parts and components of selected ICW examples, sediment and litter samples were collected in April and May 2007 from three different wetlands (ICW 3, 9 and 11), frozen and sent to Linköping University, Sweden, for subsequent molecular microbiological analysis. Samples from ICW 3 and 9 were taken on 24 April 2007, while samples for ICW 11 were taken on 8 May 2007. Mostly triplicate field sediment and litter samples were collected 1 m to the left, 1 m in front and 1 m to the right of each sampling point located near the inlet of each ICW cell and the outlet of each ICW system. Sediment samples were collected with a sediment sampler (Ø 4 cm). Green plant material was removed from litter samples before collection. Samples were stored at −20 °C before analysis.

II – Sampling for analysis at Newcastle University, United Kingdom

In April 2008, litter and sediment samples were collected from two ICW systems, ICW 3 and 11. Duplicate field litter and sediment samples were collected from each wetland cell. For each sampling location all buried litter in an area of 0.2 m^2 was collected, while sediment samples were collected from the same area with a sediment sampler (Ø 4cm) below the sediment-water interface with the upper 3 cm being used for analysis.

The samples were collected near the influent point of each cell with an additional sample at the outlet of the last cell. For ICW 3, a total of twenty four (twelve each of litter and sediment) samples were collected from six sampling points while for ICW 11 a total of twenty (ten each of sediment and litter samples) were collected from five sampling points. All samples were frozen immediately after collection and transported to University of Newcastle for molecular microbiological analysis.

4.7. Analysis

4.7.1. Water quality parameters

Liquid samples were analysed for variables including the five-days at 20ºC N-allythiourea biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), suspended solids (SS), ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), molybdate reactive phosphate (MRP; equivalent to soluble reactive phosphorus), total phosphorus (TP), total coliforms (TC) and *Escherichia coli* (*E. coli*) at the Waterford County Council water laboratory using American Public Health Association standard methods (APHA, 1998) unless stated otherwise. The Waterford County Council Water Laboratory is accredited by the Environmental Protection Agency (EPA).

MRP and TP were analysed in Waterford Council laboratory. MRP was measured by automated colorimetry (Method 4500 E) and TP was measured using the ascorbic acid method following digestion using nitric acid and hydrogen peroxide (Method 4500-P E). NH_4-N (Method 4500 H), nitrate nitrogen (NO_3-N) (Method 4500-NO_{3-}H) and total nitrogen (TN) (Method 5210 B) were analysed in Waterford

Council laboratory, using automated colorimetry.

Suspended solids were assessed by filtering a known volume of wastewater through a glass fibre filter (Method 2540 D). The filter is initially weighed and then wastewater is passed through this filter paper. The wet filter is dried in the oven at 105^{o}C and weighed. The difference in weight between the dry filter paper before and after filtration, divided by the volume of water filtered gives the SS concentration.

Biochemical oxygen demand (BOD_5) was analysed using a manometric measurement device, supplied by Lennox, Ireland (Method 5210 B). In this respirometric method oxygen consumed by the microorganisms from an air or oxygen enriched environment in a closed vessel under conditions of constant temperature and agitation is measured. During metabolism, bacteria produce carbon dioxide that is chemically bound by the potassium hydroxide solution contained in the seal cup in the bottle. This results in a pressure drop in the system, that is directly proportional to the BOD value and is measured by the Lovibond® BOD sensor. The BOD concentration is then displayed directly in mg/l.

Chemical oxygen demand was analysed using the colorimetric reflux method (Method 5220 D). The colorimetric closed reflux method is a more economical option than the open reflux method as the quantity of reagents required, the amount of hazardous waste generated and the labour required are all lower. The test measures the oxygen equivalent of the amount of organic matter oxidized by potassium dichromate in a sulphuric acid solution. Samples are analysed using Hach dichromate COD vials. The wastewater samples are added to the vials and then placed in the preheated reactor block (150°C) for two hours. After the oxidation step is completed, the COD results are determined colorimetrically.

pH was measured by a pH meter , electrical conductivity and dissolved oxygen are measured by a multimeter, while temperature was measured using a digital thermometer (unless stated otherwise). Conductivity data were obtained from a PTI-18 digital conductivity meter, and referenced against a standard solution of 1 mol dm^{-3} KCL (Method 2510B). Total coliforms and *E. coli* were analysed using the membrane filter method (Method 9222 B).

4.7.2. Nutrients in sediment and macrophytes

For determination of total nitrogen and phosphorus, the samples were milled and oven dried. Kjeldahl digests were prepared using the procedure devised by Taylor (2000). For laboratory quality control, all analyses were performed with certified reference materials. Total nitrogen and phosphorus concentrations were subsequently determined by automated colorimetry (Auto analyser, Bran + Luebbe, Model AA3).

Carbon was determined in the soil and litter samples using a Carlo Erba NA2500 Elemental Analyser. The method is based on the complete and instantaneous oxidation of the sample by flash combustion which converts all organic and inorganic substances into combustion products. The resulting gases pass through a reduction furnace and are swept into the chromatographic column by the carrier helium gas. The gases are separated in the column and detected by the thermal conductivity detector which gives an output signal proportional to the concentration of the individual components of the mixture.

4.7.3. Characterisation of bacteria in sediment and litter

A summary of molecular approaches adopted to analyse bacteria removing nitrogen in ICWs is shown in Figure 4-9. DNA extraction and PCR was run for sediment and litter samples at Linköping University, Sweden. Samples for this part of the study were collected from the three representative ICW systems in 2007. In 2008, DNA was extracted from sediment and litter samples collected from two ICW systems; PCR was run, DGGE was performed and finally sequencing was done to characterise nitrogen removing bacteria at Newcastle University.

4.7.3.1. Deoxyribonucleic acid extraction and purification from litter and sediment

I – Linköping University, Sweden

DNA was extracted from sediment and litter using a FastDNA® Spin Kit for Soil (Bio 101 Inc., La Jolla, CA, USA). Samples (0.25 g) were suspended in a sodium phosphate buffer supplied with the FastDNA® Spin Kit as stipulated by the manufacturer, and homogenised for 180 s with a hand-held blender (DIAX 900 Homogeniser Tool G6, Heidolph, Kelheim, Germany).

II –Newcastle University, United Kingdom

DNA was extracted from duplicate sediment and litter samples using a FastDNA® SPIN kit for Soil (MP Biomedical Inc., USA) according to the manufacturer's protocol with slight modifications. The extracted DNA was stored at -20ºC.

4.7.3.2. Polymerase chain reaction (PCR) amplification

I – Linköping University, Sweden

The ammonia-oxidising bacterial community was investigated using group-specific polymerase chain reaction (PCR) primers while the denitrifying bacterial community was assessed using the functional gene nitrous oxide reductase (nosZ), which is the gene for the terminal enzyme in denitrification (Hallin et al., 1999; Sundberg et al., 2007a).

The extracted DNA from all samples was diluted 10-fold to avoid inhibition of the PCR by humic substances. This dilution ratio was determined by testing different dilution ratios. Polymerase chain reaction amplification was undertaken using forward and reverse primers for ammonia-oxidising bacteria. The PCR was performed in a 50 μl mixture (Sundberg et al., 2007a) on a PTC-100TM thermal cycler (MJ Research Inc., San Francisco, CA, USA).

For nitrous oxide reductase, the forward and reverse primers targeting the nosZ gene were used in the subsequent PCR. The PCR was performed on a PTC-100TM thermal cycler in a 50 μl mixture including 1.33 U of Taq polymerase and 5 μl of the supplied buffer (including 1.5 mM $MgCl_2$; Roche Diagnostic GmbH, Manheim, Germany), each nucleotide at a concentration of 200 μM, the primers at 0.125 μM each, 600 ng μl^{-1} bovine serum albumin and 2 μl of the DNA template (Sundberg et al., 2007b).

The PCR products of DNA extraction and PCR reactions were examined by agarose gel electrophoresis. A mixture of agarose and buffer was heated and poured into the agarose gel casting tray. The solidified gel was covered with an electrophoresis buffer before running electrophoresis. The electrophoresis buffer was the same as the one used to prepare the agarose. The PCR products and dye supplied with the DNA extraction kit (2 μl of dye and 4 μl of PCR products) were placed into the loading wells formed by the gel comb. The first well of each row was loaded with 2 μl of Gene Ruler (1 kb DNA ladder; 1000 base pairs for ammonia-oxidising bacteria and nitrous oxide reductase nosZ) and 4 μl of distilled water. The electrophoresis was run for 40 min at 120 V (Owl Scientific, Inc., Woburn, MA, USA). In a fume cupboard the gel was then immersed for 15 min in ethidium bromide solution and washed subsequently with tap water. The ethidium bromide stained gel was then visualised by UV illumination.

II – Newcastle University, United Kingdom

The ammonia-oxidising bacterial community was investigated using the forward primer (CTO189fC-GC) together with the reverse primer (CTO654r), as described by Kowalchuk et al. 1997. The primers are designed to amplify a 465 base pair (bp) sequence covering the V2-V4 region of the 16SrRNA gene of AOB belonging to the β-protebacteria. PCR was performed on a Px2 Thermal Cycler (Thermo Hybaid Inc., Massachusetts, USA) in 47 μl of PCR Mega Mix manufactured by Microzone Ltd. (UK), 2 μl of primer mix (CTO 189/654) and 1 μl of DNA extract. The PCR amplifications included an initial denaturing step for 3 min at 95^{o}C, followed by a total of 30 cycles: 1 min at 95^{o}C, 1 min at 57^{o}C and 1 min at 72^{o}C, with a final primer extension step of 10 min at 72^{o}C.

The denitrifying bacterial community was assessed using the functional gene primers. Polymerase chain reaction amplification was undertaken using the forward primer (FlaCu) and reverse primer (R3Cu) targeting the nirK gene while forward primer (cd3af) and reverse primer (R3cd) targeting the nirS gene were used in the subsequent PCR. The PCR was performed on a Px2 Thermal Cycler (Thermo Hybaid Inc., Massachusetts, USA) in 47 µl of PCR Mega Mix manufactured by Microzone Ltd. (UK), 2 µl of primer mix {(FlaCu/R3Cu) *nirK*, (cd3af/R3cd) *nir*S} and 1 µl of DNA extract. The PCR amplifications included an initial denaturing step for 2 min

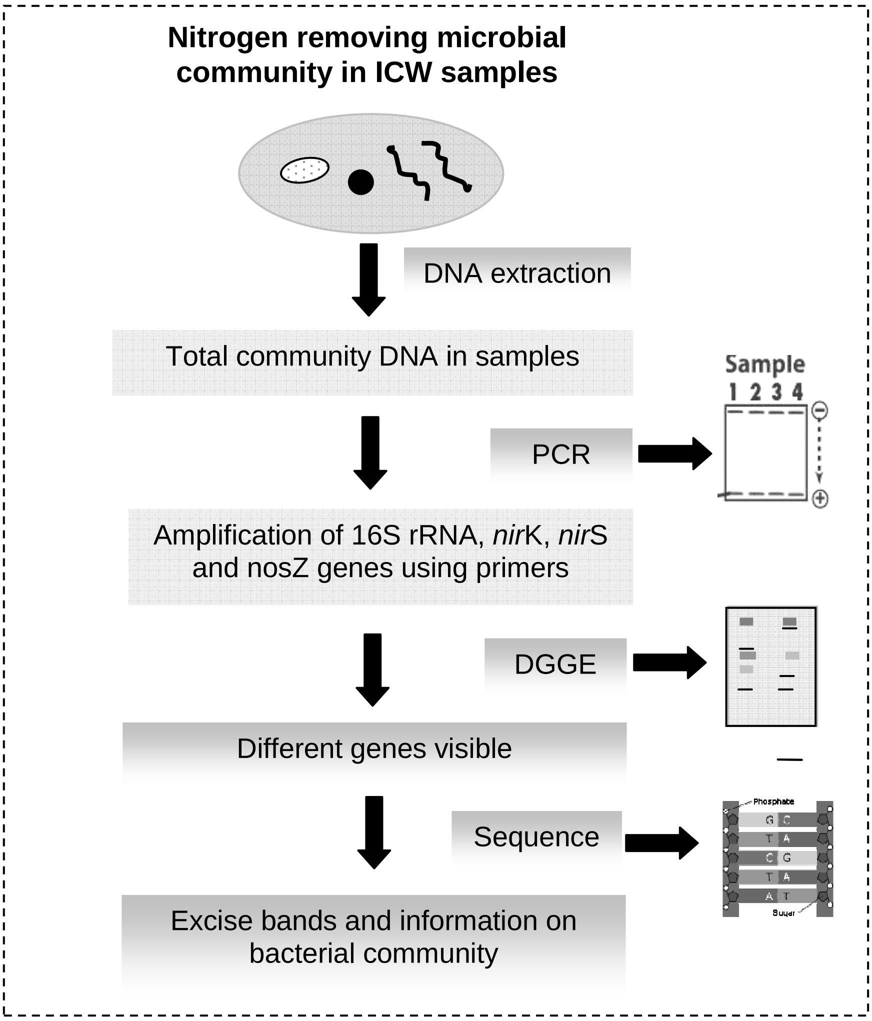


Figure 4-9. Molecular microbiological approaches adopted to analyse nitrogen removing bacterial communities in ICWs.

at 94°C, followed by a total of 35 cycles: 1 min at 94°C, 1 min at 51°C and 1 min at 72°C, with a final primer extension step of 10 min at 72°C. The presence of PCR products was confirmed by analyzing 5 µl of product on 1% agarose gels stained with ethidium bromide using a Bio-Rad Fluor-S ® MultiImager (Bio-Rad, UK).

4.7.3.3. Denaturing gradient gel electrophoresis

The PCR products generated using different primers were analysed by denaturing gradient gel electrophoresis. The gels were run to analyse PCR products amplified using AOB specific primers (Kowalchuk et al., 1997), and the functional gene primers (Throback et al., 2004). Polyacrylamide gels (120 x 120 x 1 mm) composed of 37:5:1 acrylamide: bisacrylamide (7% w/v polyacrylamide) prepared with a denaturing gradient spanning 30-60% were cast with a gradient marker (GM-40, C.B.S. Scientific Company Inc., Del Mar, CA, USA). The composition of 100% denaturant was defined as 7M urea and 40% (vol/vol) formamide (Muyzer er at., 1993). The gels were polymerised with 15 µl of TEMED and 150 µl of ammonium persulphate. Samples were prepared by mixing 11 µl of PCR products with 11 µl of loading buffer and loaded onto the DGGE gel. The gels were run in 1 x TAE buffer at 65°C for 4.5 h at 200 V. Subsequently, the gels were stained in a solution containing 200 ml of 1 x TAE buffer and 20 µl of SyBr green I (Sigma, Poole, UK; diluted 1/10000 in 1 x TAE) for 30 min according to the manufacturer’s protocol and photographed under ultraviolet light using a Bio-Rad Fluor-S ® MultiImager (Bio-Rad, UK).

4.7.3.4. Sequencing

The DGGE bands were excised using a sterile tip, transferred to 30 µl of TE buffer and stored at -20°C. The samples (excised DGGE bands plus TE buffer) were melted in a heating block at 95°C for 10 minutes. PCR was performed as described above using the respective primers (CTO, FlaCu and cd3af) without a GC clamp. 5 µl of post-PCR reaction product was mixed with 2 µl of Exonuclease I/Shrimp Alkaline Phosphate (ExoSAP-IT), initially incubated at 37°C for 15 min to degrade remaining primers and nucleotides and later incubated at 80°C for 15 min to inactivate ExoSAP-IT. The cleaned PCR products were then sequenced. The sequences were then BLAST analysed using a database containing over 33000 sequences (Cole et al., 2003). The DNA sequences of the samples were trimmed at both the 5' and 3' ends using the Chromas software (Technelysium Pty. Ltd, Helensvale, Australia). The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nih.gov) was used to find closely related gene sequences in public databases.

4.8. Application of Self-Organising Map (SOM)

The SOM toolbox (version 2) for Matlab 7.0 developed by the Laboratory of Computer and Information Science at Helsinki University of Technology was used for prediction of water quality parameters. The toolbox is available online at http://www.cis.hut.fi/projects/somtoolbox (Vesanto et al., 1999). The SOM model was applied for ammonia-nitrogen, MRP and BOD removal data to better understand the corresponding removal mechanisms. It was also applied to fill missing values and replace outliers in the ICW data set.

The component planes for each variable of the SOM model are shown in an output map. The unified distance matrix (U-matrix) representation of the SOM visualises the distances between the map neurons (Vesanto et al., 1999; Lee and Scholz, 2006). The distances between the neighbouring map neurons are calculated, and subsequently visualised. This helps to identify and subsequently illustratively show the clusters in the input data. The component plane shows the value of the variable in each map unit (Lee and Scholz, 2006).

The data obtained from the ICW system sites 3, 9 and 11 were combined and subsequently used for prediction of nutrients. For prediction of BOD_5, data from ICW 11 system was used. The inexpensive and easy to measure SOM input water quality variables of the outflow were DO (mg/l), temperature (ºC), pH (-), chloride (mg/l), conductivity (μS/cm). The corresponding expensive and time-consuming to measure model output parameters were outflow ammonia-nitrogen (mg/l) or MRP (mg/l). All statistical analyses were performed using the standard software packages Origin 7.0 and MATLAB 7.0. Significant differences (P<0.05, if not stated otherwise) between data sets are indicated where appropriate.

4.9. Summary

This chapter documents the description of three full-scale integrated constructed wetland sites. The details of various experimental methods undertaken have been described. Table 4-2 summarises the methods related to this study and shows the contribution of various people. This chapter summarises the methods used to analyse various water quality parameters and also methods to determine nutrients in plants and sediments. The molecular toolbox applied to investigate nitrogen removing bacteria in wetland components has also been described. Finally the application of SOM model for water quality prediction purposes has been described.

Table 4-2. Methods related to study and contribution of different people.

Project facet	Contribution
1) ICW performance evaluation	
Water quality sampling	W + A
Water quality analysis	W
Data treatment	AM
Data analysis	AM
2) Role of plant and sediments	
Plant and sediment sampling	W + AM
Laboratory analysis	AM
Vegetation cover	W + AM
Sediment depth	W + AM
Data analysis	AM
3) Microbial ecology	
Litter and sediment sampling	W + AM
DNA extraction, PCR	AM
DGGE	AM + NC
Sequencing	NC
Data analysis	AM
4) Application of SOM model	
Data collection	W
Data treatment	AM + VC
SOM model application	AM + VC

A occasional assistance by the author; AM author himself; NC Newcastle University staff; VC visiting Chinese PhD student; W Waterford County Council staff

Chapter 5 Treatment Performance

5.1. Introduction

This chapter investigates treatment potentials and mechanisms for BOD_5, COD, SS and nutrient removal in three full-scale integrated constructed wetlands. Annual variations of influent and effluent water quality are presented and performances of the three wetland systems are assessed by evaluating the constituent concentrations.

A neural network model (self-organising map) was used to explain the effect of water quality variables on the BOD_5, SS and nutrient removal. The performance of a selected wetland system was also statistically compared to examine the removal performance of full-scale integrated constructed wetlands. Parts of this chapter have been published as articles in Bioresource Technology, Ecological Engineering, Water Research and Wetlands.

The major objectives of this chapter are to assess the performance of full-scale integrated constructed wetlands treating nutrient enriched wastewater emanating from farmyards and to investigate potential contamination of nearby surface waters and ground water.

5.2. Inflow water quality

Table 5-1 presents the mean inlet concentrations of the monitored water quality variables. For ICW 3, the mean inflow BOD_5, COD and suspended solids values were 469.20 ± 978.07 mg/l, 1663 ± 2793.22 mg/l, 623.18 ± 1669.18 mg/l, respectively. For ICW 9 values were 314.79 ± 725.12 mg/l, 753.11 ± 1061.40 mg/l, 583.27 ± 1337.71mg/l, respectively. The mean inflow values for ICW 11 were as follows; BOD_5, 615.30 ± 692.886 mg/l; COD, 1646.23 ± 1978.52 mg/l; suspended solids, 460.86 ± 2091.99 mg/l. ICW 11 had high mean BOD_5 values compared to

Table 5-1. Summary data of water quality variables of Integrated Constructed Wetlands (ICWs) influents between 2001 and 2008.

Variable	**Statistics**	**ICW**		
		3	**9**	**11**
Dissolved oxygen (mg/l)	Mean	2.4	3.8	9.3
	SD	2.3	2.5	2.0
	N	59	47	41
Temperature (ºC)	Mean	12.6	13.7	13.2
	SD	3.0	3.6	2.9
	N	56	46	37
pH (-)	Mean	6.8	7.1	7.7
	SD	0.7	0.4	1.0
	N	72	69	70
Electrical conductivity (μS/cm)	Mean	1118	1014	1403
	SD	876	468	745
	N	50	45	38
Suspended solids (mg/l)	Mean	623	583	461
	SD	1669	1338	2092
	N	69	75	79
Biochemical oxygen demand (mg/l)	Mean	469	315	615
	SD	978	725	693
	N	58	65	66
Chemical oxygen demand (mg/l)	Mean	1663	753	1646
	SD	2793	1016	1979
	N	81	79	88
Ammonia-nitrogen (mg/l)	Mean	52.0	29.4	38.6
	SD	38.4	31.1	36.4
	N	96	120	137
Molybdate reactive phosphorus (mg/l)	Mean	19.2	8.7	10.5
	SD	20.3	12.7	7.9
	N	98	121	140
Chloride (mg/l)	Mean	91.1	123	113
	SD	52.6	102	54.6
	N	75	87	104

SD standard deviation; N number of samples

ICW 3 and ICW 9, while the latter two had high mean inflow suspended solids concentrations compared to the former. There were more animals (70 cows) on the farm associated with ICW 11 compared to 50 and 55 cows in farms associated with ICW3 and ICW 9, respectively. Concerning nutrients, ICW 3 had higher influent concentrations (NH_4-N, 52 mg/l; MRP, 19.2 mg/l) compared to ICW 9 (NH_4-N, 29.4 mg/l; MRP, 8.7 mg/l) and ICW 11 (NH_4-N, 38.6 mg/l; MRP, 10.5 mg/l). ICW 9 had a higher mean chloride concentration, 123 mg/l compared to ICW 3 and ICW 11 which had 91 mg/l and 113 mg/l, respectively. Overall in terms of physical and chemical parameters, ICW 3 receives more contaminated influent compared to ICW 9 and ICW 11. In general, Table 5-1 illustrates the very high variability in the quality of farmyard runoff entering the ICW systems.

5.3. Outflow water quality

Table 5-2 presents the mean outlet concentrations of the monitored water quality variables. For ICW 3, the mean outflow BOD_5, COD and suspended solids values were 18.5 ± 20.9 mg/l, 91.1 ± 45.9 mg/l, 15.5 ± 16.10 mg/l, respectively, and for ICW 9 values were 10.0 ± 12.0 mg/l, 57.6 ± 47.9 mg/l, 21.9 ± 76.7 mg/l, respectively. The mean outflow values for ICW 11 were as follows: BOD_5, 11.4 ± 10.1 mg/l; COD, 63.39 ± 75.2 mg/l; suspended solids, 14.27 ± 16.86 mg/l. Concerning nutrients, for ICW 3, the mean outflow NH_4-N and MRP values were 1.7 ± 3.8 mg/l, 3.5 ± 2.3 mg/l, respectively, and for ICW 9 values were 0.8 ± 1.0 mg/l, 0.6 ± 0.5 mg/l, respectively. The mean outflow values for ICW 11 were as follows: NH_4-N, 0.4 ± 0.6 mg/l; MRP 0.9 ± 0.6 mg/l.

With regards to pH, the ICW effluent was circumneutral for all three wetland systems (ICW 3, 7.2; ICW 9, 7.6 and ICW 11, 7.5). Kadlec and Wallace (2009) reported that vegetated free-water surface wetlands produce effluent with pH just above neutrality. The effluent dissolved oxygen concentrations increased for ICW 3

Table 5-2. Summary data of water quality variables of Integrated Constructed Wetlands (ICWs) effluents between 2001 and 2008.

Variable	**Statistics**	**ICW**		
		3	**9**	**11**
Dissolved oxygen (mg/l)	Mean	3.9	8.6	5.4
	SD	3.0	2.9	3.4
	N	40	33	52
Temperature (ºC)	Mean	12.0	10.8	13.9
	SD	4.4	4.2	4.8
	N	39	34	47
pH (-)	Mean	7.2	7.6	7.5
	SD	0.9	0.6	0.6
	N	58	55	80
Electrical conductivity (µS/cm)	Mean	404	368	378
	SD	146	57.3	45.0
	N	34	32	47
Suspended solids (mg/l)	Mean	15.5	21.9	14.3
	SD	16.1	76.7	16.8
	N	58	53	89
Biochemical oxygen demand (mg/l)	Mean	18.5	10.0	11.3
	SD	20.9	12.0	10.1
	N	54	52	69
Chemical oxygen demand (mg/l)	Mean	91.0	57.6	63.4
	SD	45.9	47.9	75.2
	N	69	61	98
Ammonia-nitrogen (mg/l)	Mean	1.7	0.8	0.4
	SD	3.8	1.0	0.6
	N	91	85	148
Molybdate reactive phosphorus (mg/l)	Mean	3.5	0.6	0.9
	SD	2.3	0.5	0.6
	N	92	84	152
Chloride (mg/l)	Mean	49.9	45.5	37.4
	SD	14.3	8.5	7.3
	N	63	58	113

SD standard deviation; N number of samples and ICW 9 but decreased for ICW 11 compared to the influent. The wetland environment is complex and sediments and litter components consume oxygen during decomposition. Moreover wetlands have their own oxygen demand, for example DO plays a vital role in driving the nitrification and aerobic decomposition.

5.4. BOD removal

5.4.1. Removal performance

Integrated constructed wetland 3

Figure 5-1 shows the time series of BOD_5 concentrations in the inlet and outlet of ICW 3. The BOD_5 inlet and outlet values in the initial year of operation were relatively unstable. Wetland systems have biomass storage components which take time to mature. Hence, it is most likely that the system was maturing in the initial year of operation. All the outlet BOD_5 concentrations in later years were below the threshold of 25 mg/l set for discharge from Irish wastewater treatment

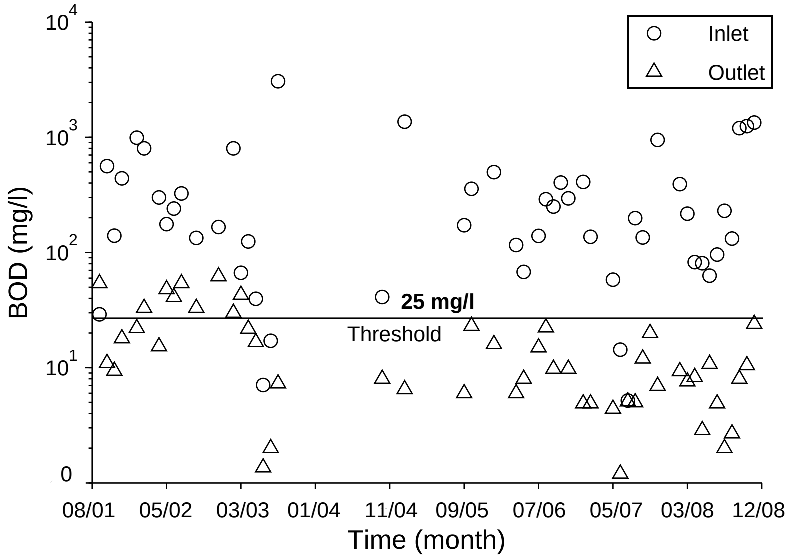


Figure 5-1. BOD_5 concentrations entering and leaving ICW 3 (2001-2008).

plants. The maximum BOD_5 inlet value was 3068 mg/l while the maximum effluent BOD_5 value was 62 mg/l. The average effluent BOD_5 concentration was 18.54 mg/l (Table 5-2) which is well below the 25 mg/l Irish discharge standard from wastewater treatment plants.

Integrated constructed wetland 9

Figure 5-2 shows the time series of BOD_5 concentrations in the inlet and outlet of ICW 9. The effluent BOD_5 concentrations in the initial year of operation were higher than the threshold of 25 mg/l set for discharge from Irish wastewater treatment plants. It is most likely that the system was maturing. The majority of outlet BOD_5 concentrations in later years were below the threshold of 25 mg/l. The maximum BOD_5 inlet value was 4500 mg/l while the maximum effluent BOD_5 value was 38 mg/l. The average effluent BOD_5 concentration was 10.8 mg/l (Table 5-2) which is well below the 25 mg/l Irish discharge standard from wastewater treatment plants.

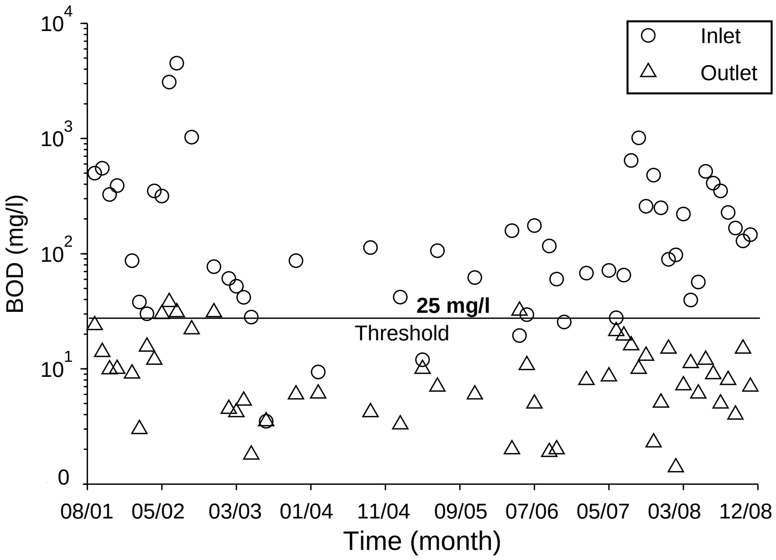


Figure 5-2. BOD_5 concentrations entering and leaving ICW 9 (2001-2008).

Integrated constructed wetland 11

Figure 5-3 shows the inlet and outlet BOD_5 concentrations for ICW 11. The BOD_5 outlet concentration in the initial months of operation was occasionally higher than the 25 mg/l threshold set for discharge from Irish wastewater treatment plants. It is most likely that the system was maturing and the aerobic and anaerobic degradation processes were becoming established. Most of the outlet BOD_5 concentrations in later years were below the threshold of 25 mg/l. The maximum BOD_5 inlet value was 2481 mg/l while the maximum effluent BOD_5 value was 34 mg/l. Average influent and effluent BOD_5 concentrations for the monitoring period (August 2001-December 2008) were 572.6 mg/l and 11.36 mg/l, giving an average concentration reduction efficiency of 98%.

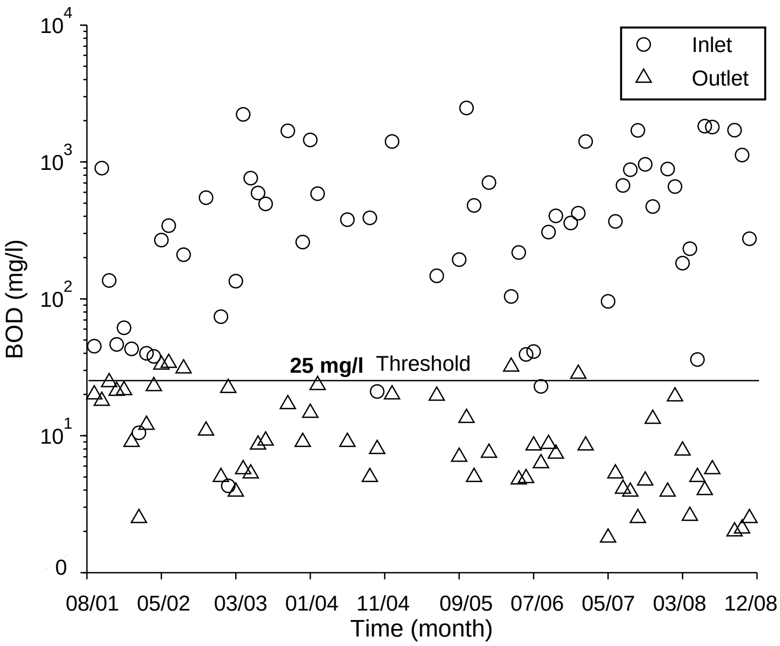


Figure 5-3. BOD_5 concentrations entering and leaving ICW 11 (2001-2008).

5.4.2. Comparison of annual BOD removal performances

The mean annual biochemical oxygen demand removal efficiencies for the three wetland systems are shown in Figure 5-4. The BOD_5 removal efficiency varied with time. BOD_5 was consistently removed from the three ICW systems with mean removal efficiencies between 96% and 65%, 90% and 98%, and 85% and 97% for ICW 3, 9 and 11, respectively. The removal efficiency of ICWs is higher in comparison to other wetlands treating similar type of influents: 65 and 76%, Connecticut (Knight et al., 2000, Newman et al., 2000, respectively); 40-50% New Zealand (Tanner et al., 2005); 80% Oregon (Skarda et al., 1994). Overall, ICW 11 was more efficient in BOD_5 removal compared to ICW 3 and 9. The mean effluent BOD_5 concentrations for the three wetland systems studied; ICW 3 (16.87 mg/l); ICW 9 (9.48 mg/l) and ICW 11 (15.43 mg/l) were well below the threshold of 25 mg/l for discharges from Irish wastewater treatment plants.

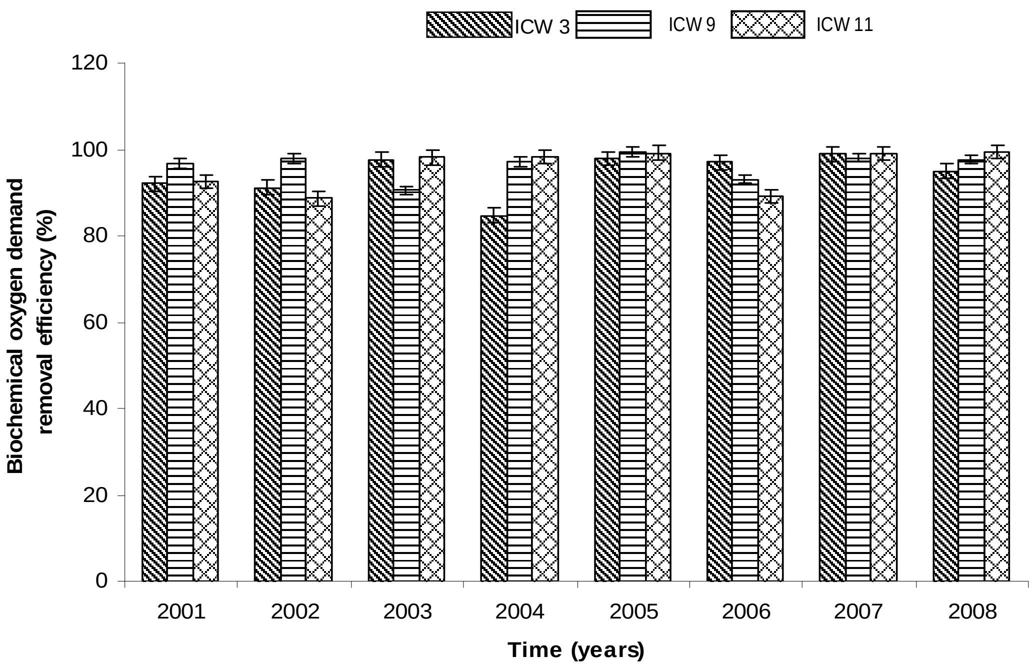


Figure 5-4. Mean and annual BOD_5 removal efficiencies for ICW 3, 9 and 11 (2001-2008).

The ratios of BOD_5 to COD for the ICW effluents were close to 0.2 (ICW 3 was 0.2; ICW 9 was 0.17 and ICW 11 was 0.17). Crites and Tchobanoglous (1998) reported a ratio of 0.21-0.23 for free-water surface wetland effluents located in Columbia, Missouri. Moreover the BOD_5/COD ratio in the inlets and outlets decreased over time for all three systems indicating that they were efficiently removing the organic matter even after operating for more than 7 years.

In ICW, the BOD_5 removal mechanisms include sedimentation and microbial degradation. Sedimentation is a physical process which not only removes suspended solids but also provides sufficient contact time that is essential for subsequent chemical and biological processes to take place for the removal and degradation of contaminants. Nevertheless, the wetland environment is very complicated; for instance respiration occurs in aerobic zones, fermentation and nitrate reduction occur in anoxic or anaerobic zones while methanogenesis occurs in the anaerobic zones. Some of the carbon is processed above water, as the standing dead material oxidizes, while some of the submerged litter and sediments are processed into soluble organic compounds that add carbon to water. These complex processes result in the nonzero background BOD concentration in wetlands.

5.4.3. BOD removal kinetics

In treatment wetlands, concentration profiles decline in an approximately exponential pattern over distance from the inlet. Kadlec and Knight (1996) discussed the removal of BOD in wetlands using the first-order kinetic model (the k-C* model). First-order kinetics dictates that the removal rate of a particular pollutant is directly proportional to the remaining concentration at any point within the wetland cell. If J is the constituent reduction rate (g/m^2/year), k is the first-order rate constant (m/year), C is the constituent concentration (mg/l) and C^* is the background constituent concentration (mg/l), then the simplest model will be as follows:

$$J = k\,(C - C^*) \qquad (5\text{-}1)$$

The mixing theory asserts that when the concentration of the reactant decreases along the length of the flow path through a reactor then it is classified as plug flow. Plug flow provides a more suitable description of the flow pattern in constructed wetlands. The methodologies adopted for the design of wetland systems have been derived from conventional chemical reactor principles and hence assume first-order degradation kinetics and a plug flow regime. Incorporating the plug flow regime concept into equation 5-1, the final equation becomes as follows:

$$\ln\left(\frac{C_{out} - C^*}{C_{in} - C^*}\right) = -\frac{k}{q} \qquad (5\text{-}2)$$

where

C_{out} is the outlet concentration (mg/l);

C_{in} is the inlet concentration (mg/l);

C^* is the background concentration (mg/l);

k is the first-order rate constant (m/d); and

q is hydraulic loading rate (m/d).

However, these simple and widely used models do not encompass the complex wetland hydraulics and processes. Kadlec (2000) has discussed the issue in his paper entitled “The inadequacy of first-order treatment wetland models”. Researchers have proposed more sophisticated models like the Tank in Series (TIS) model or Plug flow with dispersion (PFD) model. These models simulate non-ideal hydraulics (Kadlec 2003). Nevertheless, Rousseau et al. (2004) reviewed various wetland design approaches and pointed out that despite its deficiencies, the plug flow model remains the best available method.

Wetland hydraulics is complicated. Specifically, ICW systems are semi-natural and open; flow rates are therefore partly unknown. However, based on the available data, the first-order rate constant for ICW 11 was determined (Table 5-3). Data collected in the database were and used to calculate the k value. A background concentration (C*) of 4 mg/l was used for ICW 11 which has an area of 7676 m^2. A summary of the rate constant is presented in Table 5-3. Also provided in the table are mean k values computed by Knight et al. (2000) using the Livestock Wastewater Treatment Database (LWDB) and Jamieson et al. (2007) for wetlands treating dairy wastewater.

The hydraulic loading rate q (Q/A) is one of the key wetland design parameter. This factor for ICW systems is very complicated because ICW are not fully engineered treatment wetlands where the inflow and outflow rates are known, and where losses to groundwater are zero. Moreover, ICW are purely driven by rainfall events and not by rather constant inflow rates, which are common for

Table 5-3. Mean inlet and outlet BOD_5 concentrations and first order reaction rate constants.

BOD_5in (mg/l)	BOD_5out (mg/l)	k (m/yr)	Reference
668.5	4.95	15.2	ICW 11
263	93	22	Knight et al. (2000)
1747	34	9.7	Jamieson et al. (2007)

constructed wetlands treating domestic wastewater. The value of k computed in this study is lower than the rate constant computed by Knight et al. (2000) using data from LWDB and higher than that computed by Jamieson et al. (2007). The values reported by Knight et al. (2000) have been corrected for temperature using the modified Arrhenius equation and are presented as k_{20} rate constants. For the current study, a temperature correction was not made as the latest literature suggests that BOD removal is not improved at higher wetland water temperatures (Kadlec and Wallace, 2009). The different climate may also be one of the reasons for the different k value in this study and moreover at times the ICW receive very high organic loads which might have caused anoxic conditions. Furthermore, generation of farmyard runoff is intermittent, as it relies on precipitation events. The intermittent application of wastewater results in an unsteady system and hence influences the k value.

5.4.4. Factors affecting BOD removal

The SOM model was applied to the ICW 11 data to identify the relationship between the influent water quality variables and BOD removal (Figure 5-5). The SOM model is highly suitable for visualising relationships in complex biochemical data sets (Garcia and González, 2004). The relationships between most variables in biochemical processes are complex and non-linear, and SOM's visualisation capabilities can be utilised for understanding these intricate relationships.

The most important clusters present in the process data are identified in the U-matrix. While, the component planes show the behaviour of a given input variable

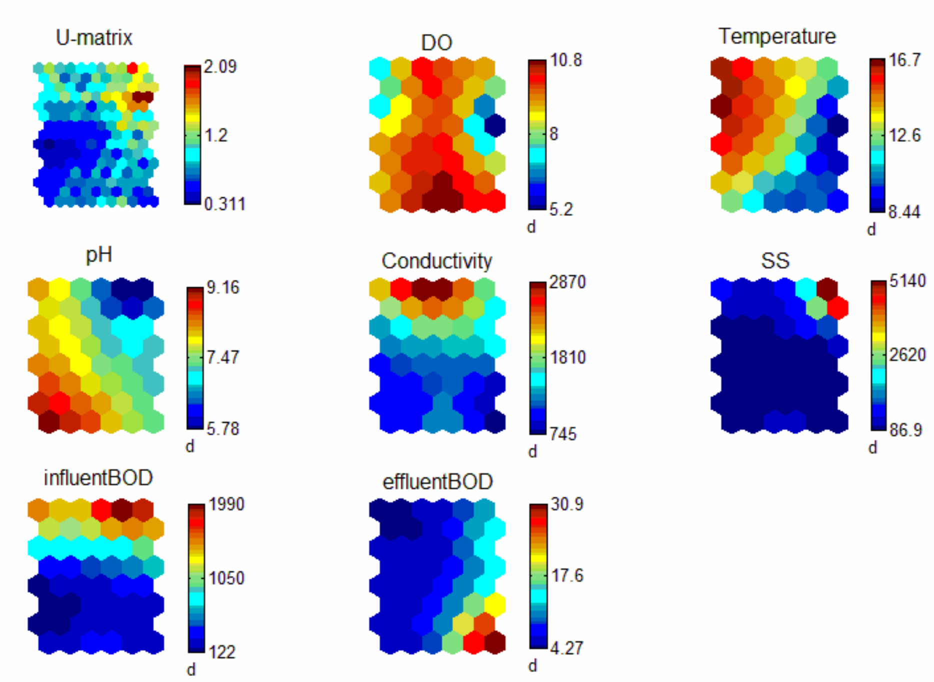


Figure 5-5. Visualisation of relationships between influent water quality indicators and effluent BOD_5 concentrations in ICW 11. The U-matrix is at the top left, followed by the component planes. The eight figures are linked by position: in each figure, the hexagon in a certain position corresponds to the same map unit.

throughout the whole data set. Figure 5-5 shows that high effluent BOD_5 concentrations (>20 mg/l), are associated with relatively low pH (<7) and high influent BOD_5 concentrations (>100 mg/l). The SOM map does not show any clear relationship between the effluent BOD_5 concentrations and conductivity, and temperature. From the U-matrix, it can be seen that these data units are small. Klomjek and Nitisoravut (2005) reported that high salt concentration is a major factor causing poor BOD treatment by leading to plant stress and affecting the metabolism function of the organism. However, the SOM map shows that high conductivity did not have an impact on BOD_5 removal in the present study. Salt concentration refers to conductivity and chloride concentrations present in the farmyard runoff.

Compared to traditional wastewater treatment systems (activated sludge, trickling filters, etc.), treatment wetlands have very complex processes and are composed of biotic components that exhibit spatial heterogeneity. In most wastewater treatment systems, the first-order model has been reliable for predicting removal rates of organic matter. The modified Arrhenius relationship is used to adjust the removal coefficient.

$$k_{v1} = k_{v1,20}\,\theta^{(T-20)} \tag{5-3}$$

where

k_{v1} = rate constant at temperature T, d^{-1};

$k_{v1,20}$ = rate constant at 20°C, d^{-1};

θ = modified Arrhenius temperature factor, dimensionless; and

T = water temperature,

Overland flow and stabilisation ponds are the two most closely related companion technologies to wetlands for BOD reduction. Kadlec and Reddy (2001) reported temperature coefficients close to 1 for both systems. Abis (2002) also reported the lack of temperature effect in ponds. Kadlec and Wallace (2009) reanalysed the temperature effect on performance of several wetland systems and reported a theta value of 0.985 ± 0.021. This value indicates a slightly worse performance at higher temperatures. They concluded that in wetland systems BOD removal is not improved at higher wetland temperatures. The carbon processing in wetlands is very complex and many other processes in wetland soils, sediments and biomass can affect BOD removal. BOD_5 influent and effluent data (C_i and C_e , respectively) from ICW 3, ICW 9 and ICW 11 were used to determine the relationship between influent and effluent BOD_5 concentrations. The linear relationship of inlet and outlet concentration is

ICW BOD_5 correlation $\quad C_e = 0.0017\ C_i + 11.831$ (5-4)

Many wetland variables such as vegetation type, water depth, climate, size and shape, are not taken account of in the above equation.

5.5. Suspended solids removal

5.5.1. Removal performance

Integrated constructed wetland 3

Figure 5-6 shows the time series of SS concentrations in the inlet and outlet of ICW 3. The majority of outlet SS concentrations except for the initial months and some in later were below the threshold of 35 mg/l as set for discharge from Irish wastewater treatment plants. The maximum SS inlet value was 6065 mg/l while the maximum effluent SS value was 64 mg/l. The average effluent SS concentration was 15.5 mg/l (Table 5-2) which is well below the 35 mg/l Irish discharge standard from wastewater treatment plants.

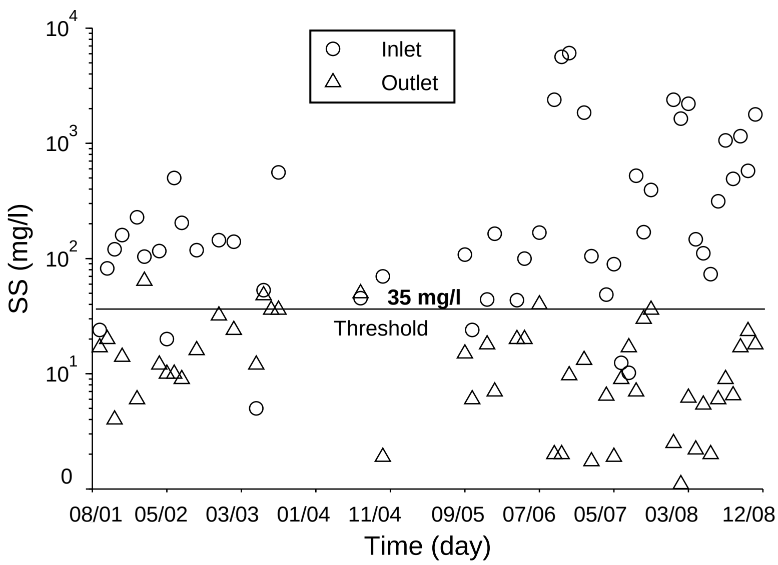


Figure 5-6. SS concentrations entering and leaving ICW 3 (2001-2008).

Integrated constructed wetland 9

Figure 5-7 shows the inlet and outlet SS concentrations for ICW 9. All the outlet SS concentrations except for the initial months and one in 2006 were below the threshold of 35 mg/l as set for discharge from Irish wastewater treatment plants. The maximum SS inlet value was 7840 mg/l while the maximum effluent SS value

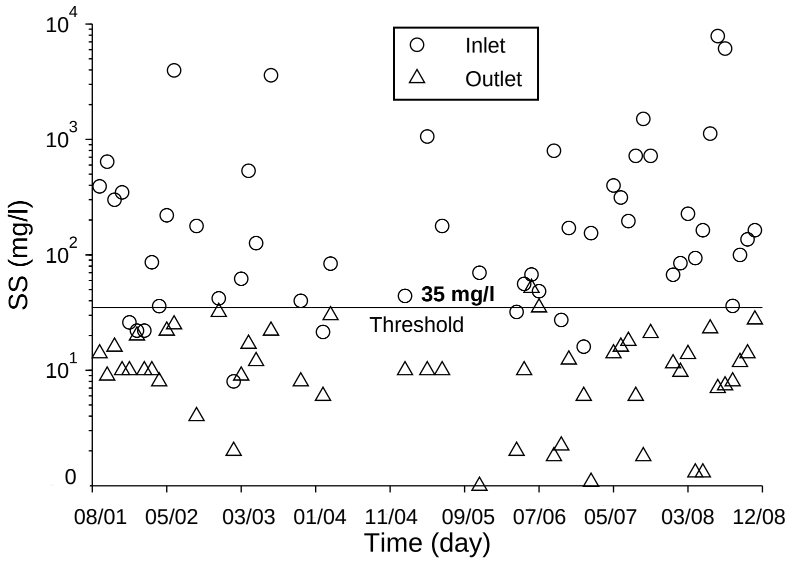


Figure 5-7. SS concentrations entering and leaving ICW 9 (2001-2008).

was 51.6 mg/l. The average effluent SS concentration was 12.71 mg/l (Table 5-2) which is well below the 35 mg/l Irish discharge standard from wastewater treatment plants.

Integrated constructed wetland 11

Figure 5-8 shows the inlet and outlet SS concentrations for ICW 11. Most of the outlet SS concentrations were below the threshold of 35 mg/l set for discharge from Irish wastewater treatment plants. The maximum SS inlet value was 8640 mg/l while the maximum effluent SS value was 100 mg/l. The average effluent SS

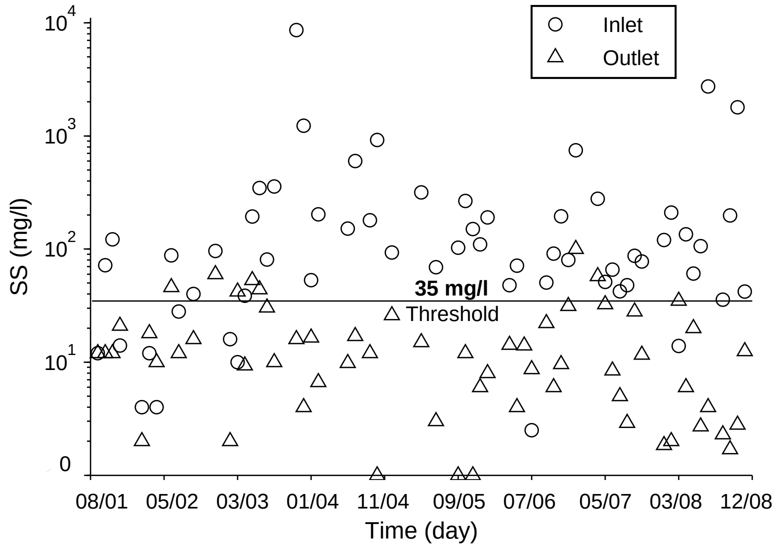


Figure 5-8. SS concentrations entering and leaving the ICW 11 (2001-2008).

concentration was 16.44 mg/l (Table 5-2) which is well below the 35 mg/l Irish discharge standard from wastewater treatment plants.

5.5.2. Factors affecting suspended solids removal

The removal of suspended solids is one of the major functions performed by wetland ecosystems. Suspended solids are removed by physical processes such as sedimentation, aggregation and interception. Wetland plants play a major role in the removal of solids. They reduce water column mixing and cause flocculation of smaller colloidal particles into larger particles which can settle easily.

The sizes of wetland cells also influence the removal of SS. The larger the cell size, the higher the retention time and the greater the SS removal. All ICW systems have high SS removal efficiencies: ICW 3, 97.6%; ICW 9, 96.2%, and ICW 11, 90.8%. The last cell of ICW 9 in comparison to those of ICW 3 and ICW 11 has denser vegetation and hence lower SS concentrations in the effluents: 12.71 mg/l compared to 15.64 mg/l and 16.44 mg/l. Moreover, a pond forms the final element in

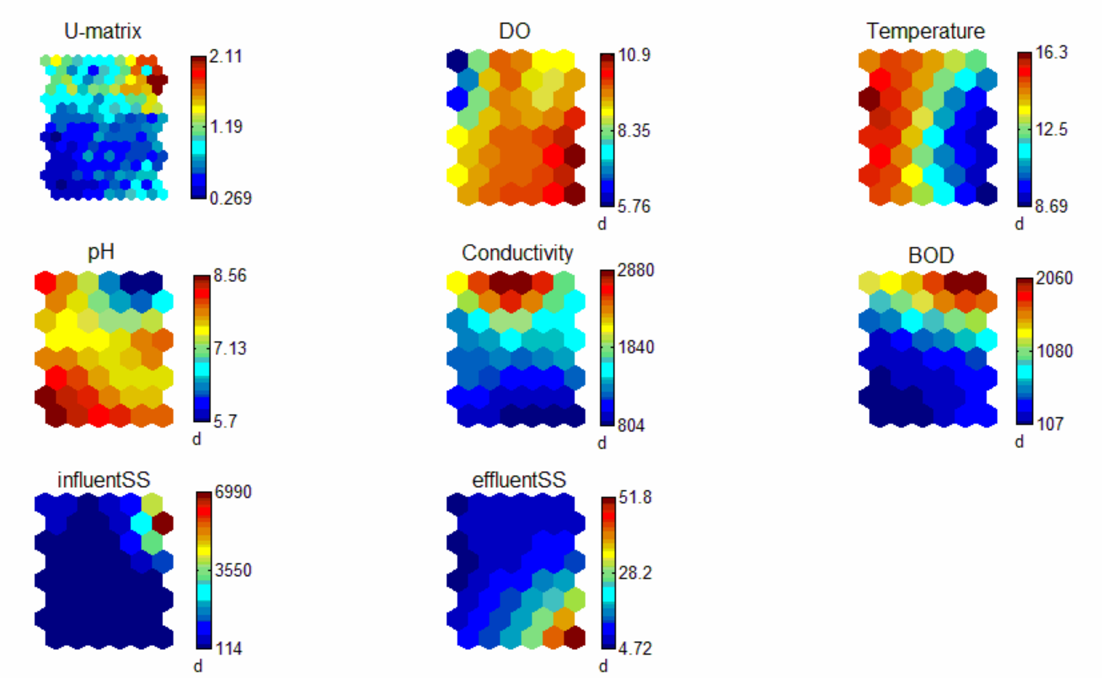


Figure 5-9. Visualisation of relationships between influent water quality indicators and effluent SS concentrations in ICW 11. The U-matrix is at top left, followed by the component planes. The eight figures are linked by position: in each figure, the hexagon in a certain position corresponds to the same map unit.

ICW 11 and this has resulted in comparatively higher values of SS from this wetland.

Kadlec and Wallace (2009) discouraged the use of a pond as the final element in constructed wetland systems. However, ICW are designed not only for water treatment but other objectives including integration into the landscape and biodiversity enhancement.

Birds, fish and mammals can resuspend solids leading to high concentrations of SS in wetland effluents due to bioturbation. Wetlands also produce sediments internally. For example, SS originate from stem and leaf litter. A portion of this material undergoes microbial decomposition and hence contributes to the SS. The final effluent SS is linked to the internal processes taking place within the system which may be physical or biological and are random in nature. Thus, SS from wetlands are impacted by stochastic variability (Kadlec and Wallace, 2009).

5.6. Nutrient removal

5.6.1. Removal performance

Integrated constructed wetland 3

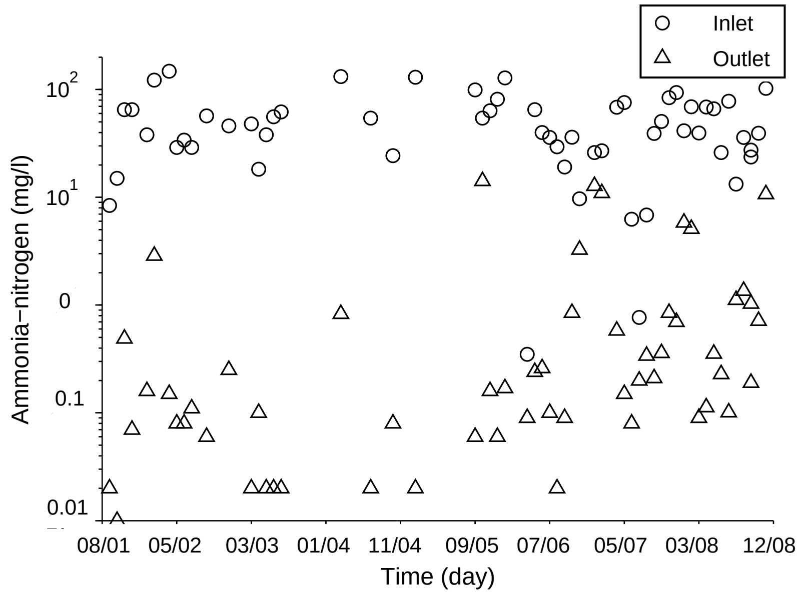


Figure 5-10. NH_4-N concentrations entering and leaving ICW 3 (2001-2008).

Figure 5-10 shows the time series of ammonia-nitrogen concentrations in the inlet and outlet of ICW 3the performance of ICW 3 for reduction. The maximum ammonia-nitrogen inlet value was 160 mg/l while the maximum effluent value was 14.20 mg/l. The average effluent ammonia-nitrogen concentration was 1.37 mg/l which is low. Significant discharges of ammonia can be toxic to aquatic life and the low effluent value shows that the ICW effluent is not impacting on the receiving water courses and associated life.

Integrated constructed wetland 9

Figure 5-11 shows the inlet and outlet ammonia-nitrogen concentrations for ICW 9. The maximum ammonia-nitrogen inlet value was 245 mg/l while the maximum effluent value was 4.55 mg/l. The average effluent ammonia-nitrogen

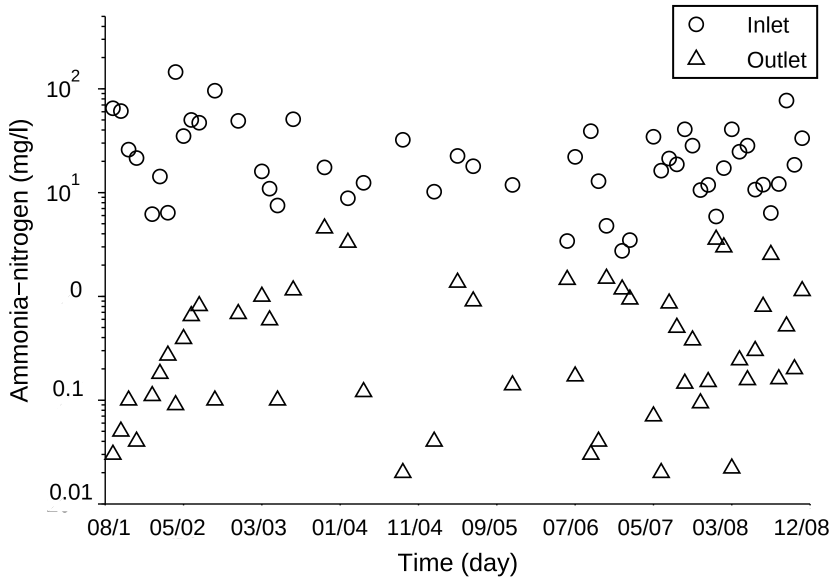


Figure 5-11. NH_4-N concentrations entering and leaving ICW 9 (2001-2008).

concentration was 0.71 mg/l which is low. Significant discharges of ammonia can be toxic to aquatic life and the low effluent value shows that the ICW effluent is not impacting on the receiving water courses and associated aquatic life.

Integrated constructed wetland 11

Figure 5-12 shows the inlet and outlet ammonia-nitrogen concentrations for ICW 11.

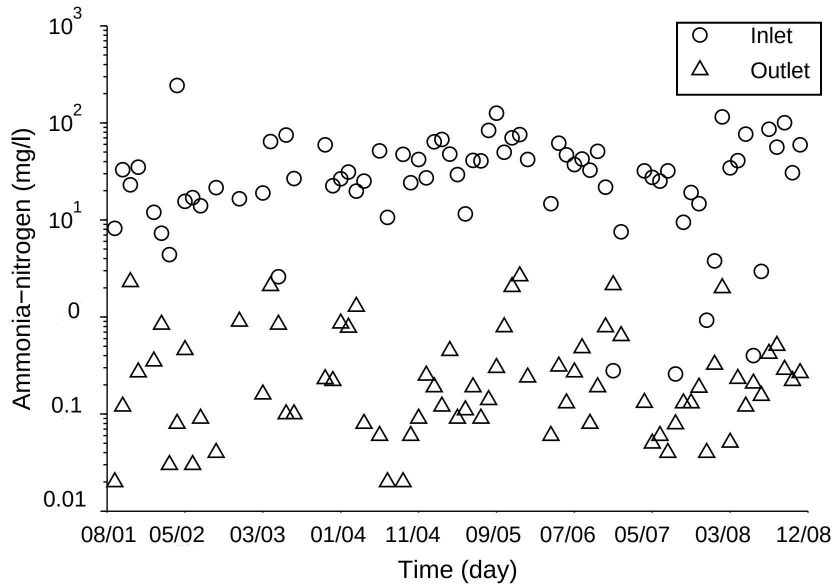


Figure 5-12. NH_4-N concentrations entering and leaving ICW 11 (2001-2008).

The maximum ammonia-nitrogen inlet value was 243 mg/l while the maximum effluent value was 2.65 mg/l. The average effluent ammonia-nitrogen concentration was 0.42 mg/l which is low. Significant discharges of ammonia can be toxic to aquatic life and the low effluent value shows that the ICW effluent is not impacting on the receiving water courses and associated aquatic life.

Integrated constructed wetland 3

Figure 5-13 shows the inlet and outlet molybdate reactive phosphorus concentrations for ICW 3.

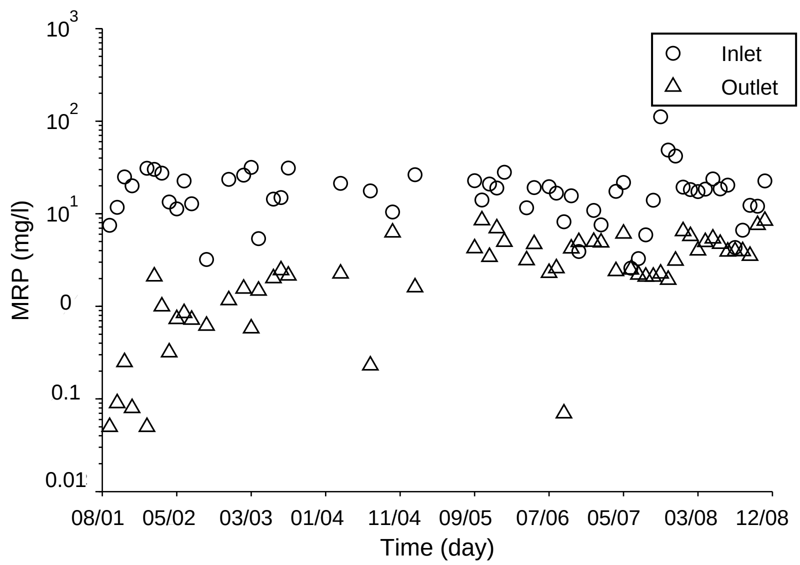


Figure 5-13. MRP concentrations entering and leaving ICW 3 (2001-2008).

The maximum MRP inlet value was 111.40 mg/l while the maximum effluent value was 8.53 mg/l. The average effluent MRP concentration was 3.04 mg/l and the average percentage reduction in MRP concentration over the monitoring period was 84.2%.

Integrated constructed wetland 9

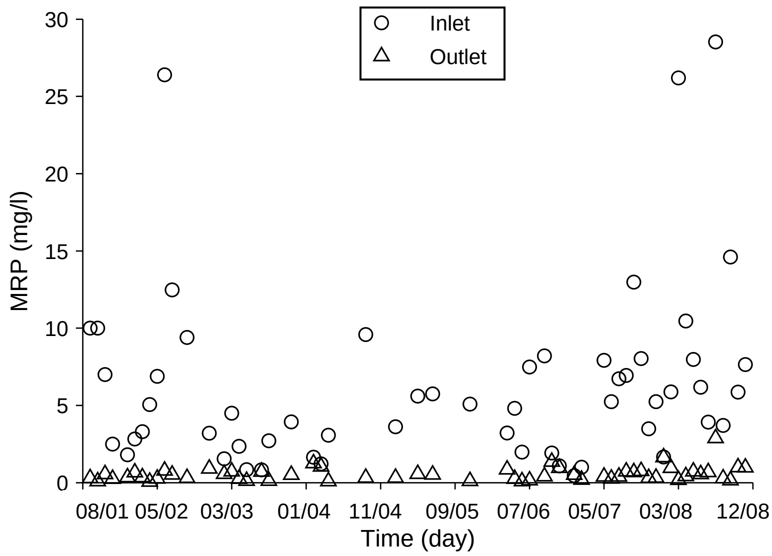


Figure 5-14. MRP concentrations entering and leaving ICW 9 (2001-2008).

Figure 5-14 shows the inlet and outlet molybdate reactive phosphorus concentrations for ICW 9. The maximum MRP inlet value was 124.70 mg/l while the maximum effluent value was 2.88 mg/l. The average effluent MRP concentration was 0.58 mg/l and the percentage reduction in MRP concentration over the 8 years monitoring period was 93.0%.

Integrated constructed wetland 11

Figure 5-15 shows the inlet and outlet molybdate reactive phosphorus concentrations for ICW 11. The maximum MRP inlet value was 37.5 mg/l while the maximum effluent value was 2.60 mg/l. The average effluent MRP concentration was 0.93 mg/l and the average percentage reduction in MRP concentration over the monitoring period was 91.2%.

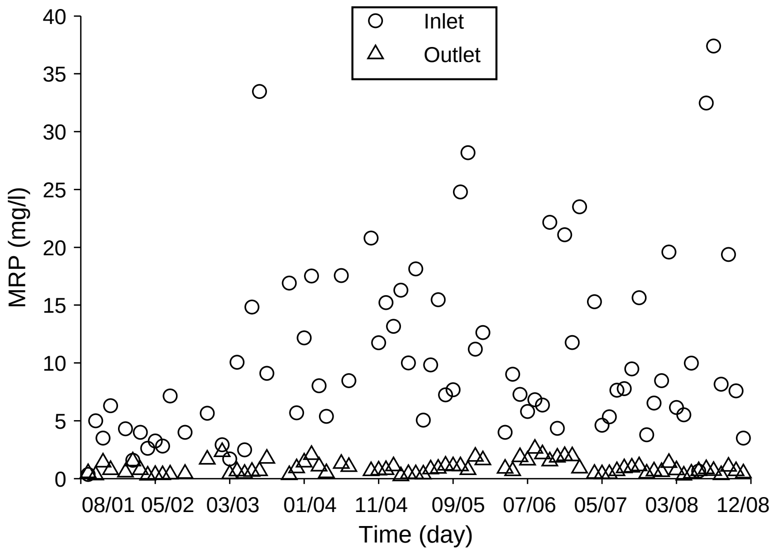


Figure 5-15. MRP concentrations entering and leaving ICW 11 (2001-2008).

5.6.2. Comparison of annual nutrient removal performances

A common measure of wetland contaminant removal effectiveness is the percentage reduction in contaminant concentration, or the ‘removal efficiency’. The removal efficiency for various parameters was calculated using Equation 5-5.

$$RE\ (\%) = \left(\frac{Cin - Cout}{Cin}\right) \times 100 \qquad (5\text{-}5)$$

where; RE = removal efficiency, and Cin and Cout are the mean influent and effluent concentrations, respectively.

The mean ammonia-nitrogen and molybdate reactive phosphorus removal efficiencies for the three integrated constructed wetlands are shown in Figure 5-16. It shows the annual treatment performances of three different ICW systems. The nutrient removal efficiency varied with time. Ammonia-nitrogen was consistently removed from all of the ICW systems with mean annual efficiencies between 80% and 99%, 92% and 99%, and 97% and 99% for ICW 3, 9 and 11, respectively.

Mean ammonia-nitrogen reduction in ICW 11 was higher than reductions in ICW 3 and 9. Throughout the monitoring period the mean annual removal efficiency for ICW3 was lower compared to ICW 9 and 11. For ICW 3 the ammonia nitrogen removal during the first year of operation increased by approximately 10% and was greater than 97% for the four succeeding years; however it then decreased to 72% and 74% during the sixth and seventh year of operation. The low removal efficiencies during this period (2006-07) were due to the entry of poultry wastes originating from a farm situated nearby. For ICW 11, there were no significant annual trends in mean ammonia-nitrogen reduction as discussed below. During the eighth year of operation, ICW 11 had a high ammonia-nitrogen removal efficiency, 98% compared to 94% and 92% for ICW3 and 9, respectively. Molybdate reactive phosphorus was also consistently removed with mean annual efficiencies between 67% and 99%, 77% and 98% and, 81% and 94% for ICW 3, 9 and 11, respectively. However, there was a reduction in the molybdate reactive phosphorus removal efficiencies in the eighth year for all three ICW.

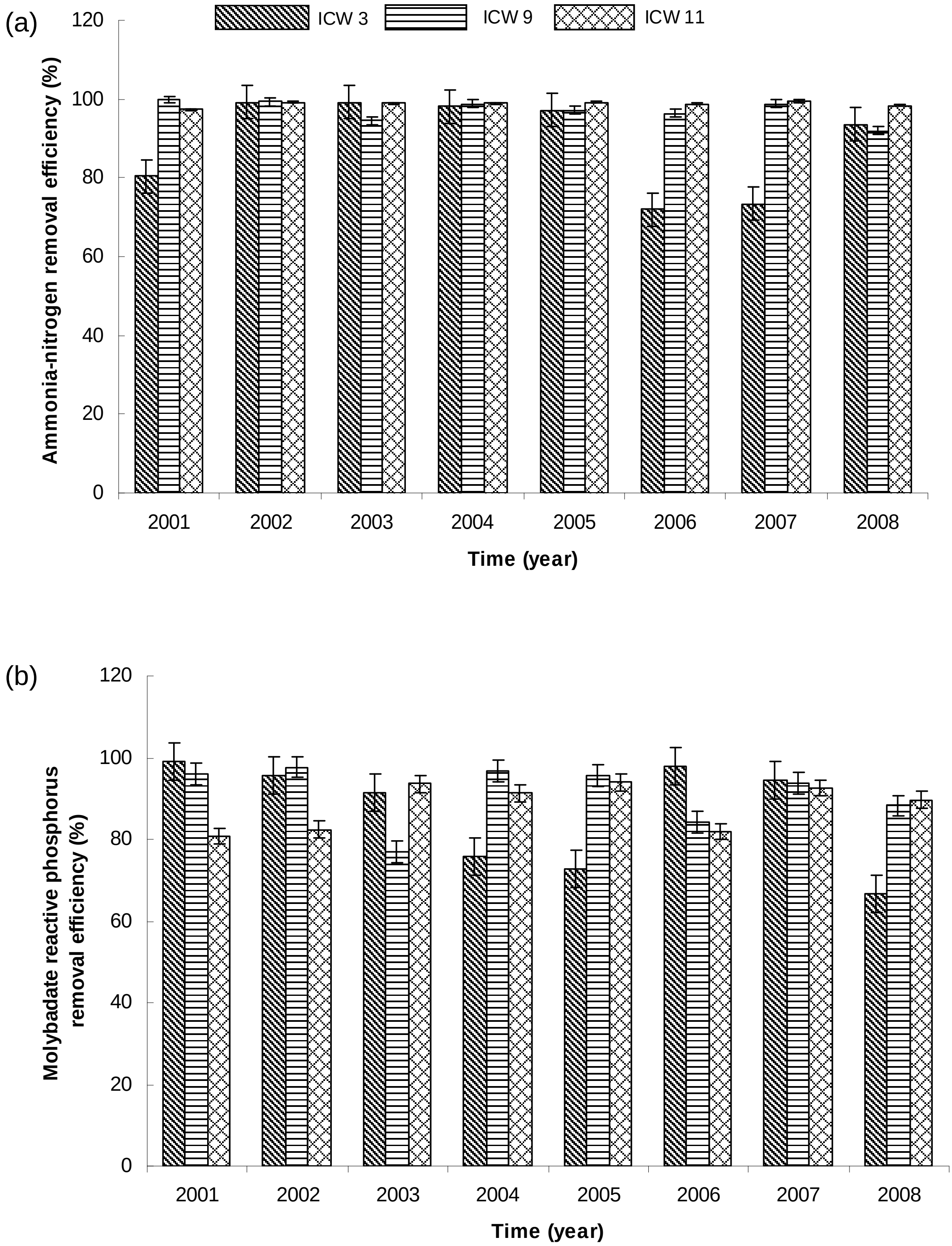


Figure 5-16. Annual nutrient removal efficiencies in ICW 3, ICW 9 and ICW 11 for (a) ammonia-nitrogen (b) molybdate reactive phosphorus. Error bars indicate standard errors.

5.6.3. Long-term nutrient removal performance

Annual treatment performance. In order to assess the annual and seasonal variations in nutrient removal, ICW 11 was selected, based on the historical data. Table 5-4 shows the annual treatment performances of the ICW system in terms of nutrient removal from 2001 to 2008. There were no significant annual trends in mean ammonia-nitrogen and MRP reduction. However, for nitrate-nitrogen the mean reduction decreased by 6% in 2007, which was, although this was not statistically significant. In 2008, there was an abrupt decrease in the nitrate-nitrogen removal. Figure 5-17 shows cell-wise reduction of nitrate-nitrogen concentrations. The figure illustrates that more than 91% and 66% of nitrate-nitrogen is removed in the first and third cells of the ICW system respectively. In contrast, nitrate-nitrogen concentrations increase in cells 2 and 4, which is most likely due to the presence of field drains. Water from the drain adjacent to the cell 4 has high nitrate concentrations, and it is likely that it may have percolated into cell 4 and cell 2 which is parallel to cell 4 (Figure 5-24). The MRP reductions were similar to the SS reductions, which might indicate that phosphorus is often bound to particulate matter within the inflow water (Scholz, 2006).

As the ICW system continued to mature, the microbial communities and aquatic vegetation became more established, resulting in a stable system with high pollutant removal capacity. The overall nutrient removal efficiency of the system was high: 99% for ammonia-nitrogen, 87% for nitrate-nitrogen and 93% for MRP, even in the seventh year of its operation. This finding contrasts with the common notion that the nutrient removal efficiency of constructed treatment wetlands decreases with age, especially for phosphorus removal as the mineral sediment becomes saturated; i.e. no free adsorption sites remain (Kadlec, 1999; Kent, 2000). This may imply that ICW cannot be compared with traditional treatment wetlands in terms of their capacity to retain nutrients.

The soil characteristics have an important influence on the magnitude of phosphorus losses. Different factors such as particle size distribution, organic content, and iron and aluminium concentrations influence the ability of wetland soil to hold phosphorus (Sharpley, 1995; Leinweber et al., 1999; Daly, 2000). In general, soils with high clay content have a higher capacity to bind phosphorus than those with sandy and organic soils (Sharpley, 1995; Maguire et al., 1997; Leinweber et al., 1999; Daly, 2000). Also soils with higher iron and aluminium content were found to have a greater capacity to bind phosphorus (Daly, 2000). Dunne et al. (2005b) demonstrated with the help of an intact soil/water column study that the phosphorus sorption parameters were significantly related to the amorphous forms of iron and aluminum oxides in soils. Previous research conducted by Dunne et al. (2005a,b) showed that the soil in the current study site area contains high proportions of silt and clay (54 ± 2.0% silt and 33 ± 1.7% clay), and most likely also relatively high amounts of iron and aluminium, resulting in a greater phosphorus binding capacity of the soil.

Furthermore, as the wetland ages, the continuous accumulation of senescing plant biomass increases the phosphorus storage capacity of the ICW system (Wallace and Knight, 2006). Moreover, the wetland plants were not harvested, resulting in the accumulation of organic matter. The wetland subsequently changes from an initially mineral based system to an organic based system with higher phosphorus removal capacity.

Table 5-4. Annual nutrient concentrations and removal efficiencies (RE) for ICW 11 2001 to 2008.

Variables	2001	2002	2003	2004	2005	2006	2007	2008
Ammonia-nitrogen (mean±standard deviation)								
Influent (mg/l)	24.8 ± 12.2	39.0 ± 76.6	40.1 ± 39.3	33.2 ± 20.2	51.7 ± 51.0	33.9 ± 22.5	21.0 ± 27.2	48.8 ± 38.4
Effluent (mg/l)	0.68 ± 1.0	0.30 ± 0.35	0.40 ± 0.58	0.34 ± 0.46	0.45 ± 0.66	0.39 ± 0.59	0.11 ± 0.13	0.77 ± 1.15
N	4	9	12	27	46	16	23	13
RE (%)	97.2	99.1	99	98.9	99.1	98.8	99.5	98.4
Nitrate-nitrogen (mean±standard deviation)								
Influent (mg/l)	-	-	-	-	2.91 ± 3.82	3.02 ± 3.47	2.58 ± 2.95	2.71 ± 3.06
Effluent (mg/l)	0.01 ± 0.02	1.3 ± 1.5	5.4 ± 2.9	1.04 ± 1.3	0.19 ± 0.31	0.21 ± 0.33	0.63 ± 0.26	2.15 ± 1.81
N	4	8	12	27	46	16	23	13
RE (%)	-	-	-	-	93.4	93	86.8	21.2
Molybdate reactive phosphate (mean±standard deviation)								
Influent (mg/l)	3.8 ± 2.5	3.9 ± 1.6	12.6 ± 13.4	13.1 ± 10.3	14.3 ± 10.9	9.34 ± 7.23	8.04 ± 5.83	12.81 ± 11.3
Effluent (mg/l)	0.73 ± 0.48	0.7 ± 0.5	0.80 ± 0.59	1.14 ± 0.80	0.87 ± 0.42	1.69 ± 0.60	0.52 ± 0.18	1.25 ± 1.26
N	4	9	12	30	46	16	23	13
RE (%)	80.7	82.4	93.6	91.2	93.9	81.9	92.6	90.2

N sample number

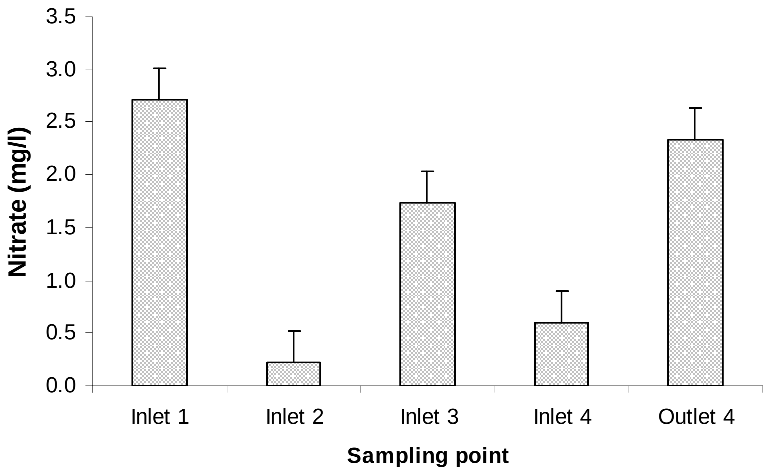


Figure 5-17. Nitrate concentration variations in ICW 11 during 2008.

Seasonal performance. Seasonal variations in nutrient removal performance were observed (Table 5-5). The ammonia-nitrogen concentrations within the inflow were relatively high in summer and autumn compared to winter and spring. In comparison, the ammonia-nitrogen outlet concentrations were higher during fall compared to other seasons. Therefore the mean removal efficiencies were higher during spring (99.4%) and summer (99.7%) and slightly lower during fall (98.7%) and winter (99.3%). Although the mean removal efficiencies in fall and winter were relatively high, the mean concentrations of the inflow drastically increased during these two seasons and the mean concentrations in the outflow were 0.92 ± 0.769 and 0.60 ± 0.201 mg/l, respectively. The ammonia-nitrogen concentrations in the outflow were significantly greater ($p < 0.05$) during fall than summer. This can be explained by the observation that there was reduced outflow and a longer retention time in summer compared to fall (see below).

In comparison to previous constructed treatment wetland studies (Gottschall et al., 2007; Newman et al., 2000), the ICW system has very high nutrient removal efficiencies. Molybdate reactive phosphorus was also efficiently removed in spring (95.7%), summer (90.7%), fall (94.9%) and winter (93.2%). The MRP outlet concentrations were significantly greater during fall than during summer, because there was reduced outflow and a longer retention time in summer.

Table 5-5. Seasonal comparison of nutrient concentrations for the integrated constructed wetland (ICW 11) treating farmyard runoff.

Nutrient	Spring	Summer	Autumn	Winter
Ammonia-nitrogen (mean ± standard deviation)				
Influent (mg/l)	28.09 ± 21.389*	50.99 ± 56.640*	72.09 ± 62.597*	38.15 ± 22.960*
Effluent (mg/l)	0.17 ± 0.286*	0.16 ± 0.201**	0.92 ± 0.769***	0.24 ± 0.197*
RE (%)	99.4	99.7	98.7	99.3
Molybdate reactive phosphorus (mean ± standard deviation)				
Influent (mg/l)	12.23 ± 11.654*	10.54 ± 6.430*	21.62 ± 13.113*	15.08 ± 6.555*
Effluent (mg/l)	0.52 ± 0.235*	0.98 ± 0.147‡	1.09 ± 0.511ˆ	1.02 ± 1.503*
RE (%)	95.7	90.7	94.9	93.2

RE, removal efficiency; means followed by the same symbol are not statistically significantly different ($p<0.05$)

5.6.4. Nutrient removal kinetics

Based on the available data first-order rate constants for nutrients (NH_4-N and TP) were computed (Table 5-6). Background concentrations (C*) of 0.05 and 0.02 mg/l were used for NH_4-N and TP, respectively. A summary of the computed rate constants is presented in Table 5-6. Also provided in the table are mean k values computed by Knight et al. (2000) using the Livestock Wastewater Treatment Database (LWDB) and Jamieson et al. (2007) for wetlands treating dairy wastewater.

Table 5-6. Mean inlet and outlet nutrient concentrations and summary of first order reaction rate constants computed for ICW 11.

Parameter	Mean C_{in} (mg/l)	Mean C_{out} (mg/l)	k (m/yr)	Jamieson et al. (2007)	Knight et al. (2000)
NH_4-N	20.94	0.16	12.2	7.0	14
TP	9.14	0.78	5.8	4.3	8

The values of rate constant k computed in this study are lower than the rate constant computed by Knight et al. (2000) using data from LWDB and higher than those computed by Jamieson et al. (2007). The values reported by Knight et al. (2000) have been corrected for temperature using the modified Arrhenius equation and are presented as k_{20} rate constants. For the current study, temperature correction was not made. There are many reasons for the fluctuating k values. At times the ICW receive very high organic loads which might have caused anoxic conditions. Furthermore, generation of farmyard runoff is intermittent, as it relies on precipitation events. The intermittent application of wastewater results in an unsteady system and hence may have influenced the k values.

5.6.5. Factors affecting nutrient removal

The SOM model was applied to identify the relationships between the outflow ammonia-nitrogen concentrations and other water quality variables. The component planes for each variable of the SOM model are shown in Figure 5-18. The unified distance matrix (U-matrix) representation of the SOM visualizes the distances between the map neurons (Vesanto et al., 1999; Lee and Scholz, 2006). The distances between the neighbouring map neurons were calculated, and subsequently visualized. The component plane shows the value of the variable in each map unit (Lee and Scholz, 2006).

The component plane helps to visualise the relationships between ammonia-nitrogen and other variables. High outflow ammonia-nitrogen concentrations (>1.16 mg/L) are linked to high chloride concentrations (>39.4 mg/L), high conductivity values (>386 μS/cm) and low temperatures (<12.9 °C). Ammonia nitrogen concentrations do not reveal an obvious association with DO concentrations and pH. Low reduction rates are apparently associated with high outflow ammonia-nitrogen concentrations as shown in Table 5-7. High levels of conductivity and chloride represent high salt concentrations in the runoff. The linear relationship between effluent conductivity and chloride concentration is shown in Eq. (5-6). Furthermore, Eqs. (5-7)–(5-9) show regression equations for ammonia-nitrogen. It can be seen that ammonia-nitrogen removal was influenced by high salt concentrations.

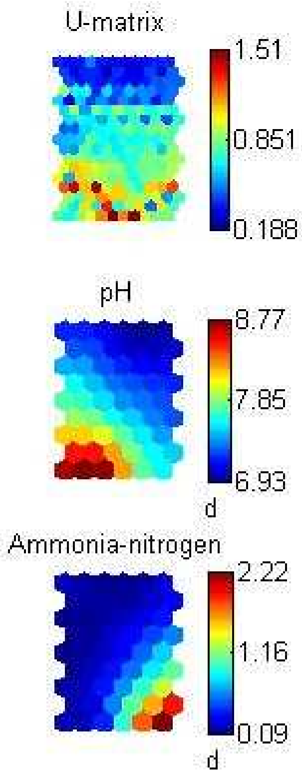


Figure 5-18. Abstract visualisation of the relationships between outflow ammonia-nitrogen (NH_4-N, mg/l), and outflow dissolved (DO, mg/l), temperature (ºC), pH (dimensionless), chloride (mg/l) and conductivity (µS/cm) using a self-organising map model.

Conductivity = 3.94 x chloride + 229.5 (5-6)

R^2 = 0.30 and $p < 0.01$

Ammonia-nitrogen = 0.05 x chloride – 1.2, (5-7)

R^2 = 0.20 and $p < 0.01$

Ammonia-nitrogen = 0.003 x conductivity – 0.13, (5-8)

R^2 = 0.15 and $p < 0.01$

Ammonia-nitrogen = 0.03 x chloride + 0.0005 x conductivity (5-9)

R^2 = 0.20 and $p < 0.01$ (chloride and conductivity)

Chapanova et al. (2007) demonstrated that ammonia conversion is sensitive to the salinity of the wastewater to be treated; after adding salinity to the input wastewater, ammonia degradation was markedly reduced. However, Dincer and Kargi (1999) showed that salt concentrations of 42% resulted in significant reductions in both nitrification and denitrification. In contrast, the outflow temperature is negatively correlated (R = -0.689) with the ammonia-nitrogen concentration, suggesting that temperature had a positive effect on the ammonia-nitrogen removal. The elevated water temperature can enhance volatilisation of nitrate. Relatively high temperatures (>12.9 °C) are better for both nitrification and denitrification, compared to temperatures <12.9 °C (US EPA, 2000). Chapanova et al. (2007) reported that at 5 ºC the ammonia-nitrogen removal rate was on average three to five times lower than at temperatures between 15 and 25 ºC. Nitrification is greatly affected by temperature; nitrification rates are slow in cold compared to warm climates (Chapanova et al., 2007; Vymazal, 2007).

No obvious correlation (R = - 0.07) between pH and ammonia- nitrogen could be identified (Figure 5-18). Most outflow pH values were between 7.0 and 8.0 at temperatures <17.6 °C. Ammonia-nitrogen concentrations did not reduce at this pH range. Ammonia-nitrogen may be found in the unionized form (NH_3) or ionized form ($NH4^+$) depending on water temperature and pH. The ionized form is predominant in wetlands; e.g. at pH 7.0 and 25 ºC, the percentage of unionized ammonia is approximately 0.6% (US EPA, 2000). It was also reported that at high pH between 8.0 and 8.5, the proportion of ammonia might increase to between 20 and 25% at 20 °C, if surface turbulence is high due to wind action.

Table 5-7. Correlation coefficients and corresponding p values (in brackets) related to correlation analysis comprising input (column headings) and target (row headings).

Variables	Dissolved oxygen (mg/l)	Temperature (°C)	pH (dimension less)	Chloride (mg/l)	Conductivity (μS/cm)
Ammonia-nitrogen	0.118	-0.689	-0.07	0.348	0.424
(mg/l)	(0.256)	(<0.01)	(0.343)	(<0.01)	(<0.01)
Molybdate reactive	-0.275	-0.422	0.093	0.513	0.435
phosphorus (mg/l)	(<0.01)	(<0.01)	(0.231)	(<0.01)	(<0.01)

Significant losses of nitrogen may occur in open water areas via ammonia gas (NH_3) volatilization (US EPA, 2000; Camargo Valero and Mara, 2007).

Many papers (Schaafsma et al., 1999; US EPA, 2000; Noorvee et al., 2007; Iamchaturapatr et al., 2007) indicate that DO significantly influences the removal rate of ammonia-nitrogen in constructed wetland systems. However, the DO concentrations had no obvious impact on ammonia-nitrogen removal in ICW based on the visualization of the relationship between outflow ammonia-nitrogen and DO ($R = 0.11$). Therefore, it can be seen that ammonia-nitrogen removal was largely influenced by salt concentrations and temperature. The variables pH and DO seemed to be of less importance.

Phosphorus

The visualisation of relationships between the outflow MRP concentrations and other water quality parameters of the SOM model is shown in Figure 5-18. High outflow MRP concentrations (>1.39 mg/L) are linked to high chloride concentrations (>40.3 mg/L) and high conductivity values (>338 μS/cm). MRP removal was largely influenced by the DO and salt concentrations, and correlated comparatively weakly with temperature and pH.

Chloride (R = 0.51) and conductivity (R = 0.435) were correlated positively with MRP, indicating that elevated salt concentrations had a negative impact on MRP removal. With increasing salt concentrations, the phosphorus removal rates of the tested ICW systems decreased. This was probably because phosphate-accumulating microorganisms were sensitive to salinity (Scholz, 2006). The salt accumulation in phosphate-accumulating microorganism cells might have caused a significant increase in osmotic pressure in cells resulting in decreased phosphorus removal. Diminished phosphate accumulation capabilities subsequently result in reduced removal efficiencies as discussed previously by Carucci et al. (1997); Panswad and Anan, (1999); Wang et al., (2007).

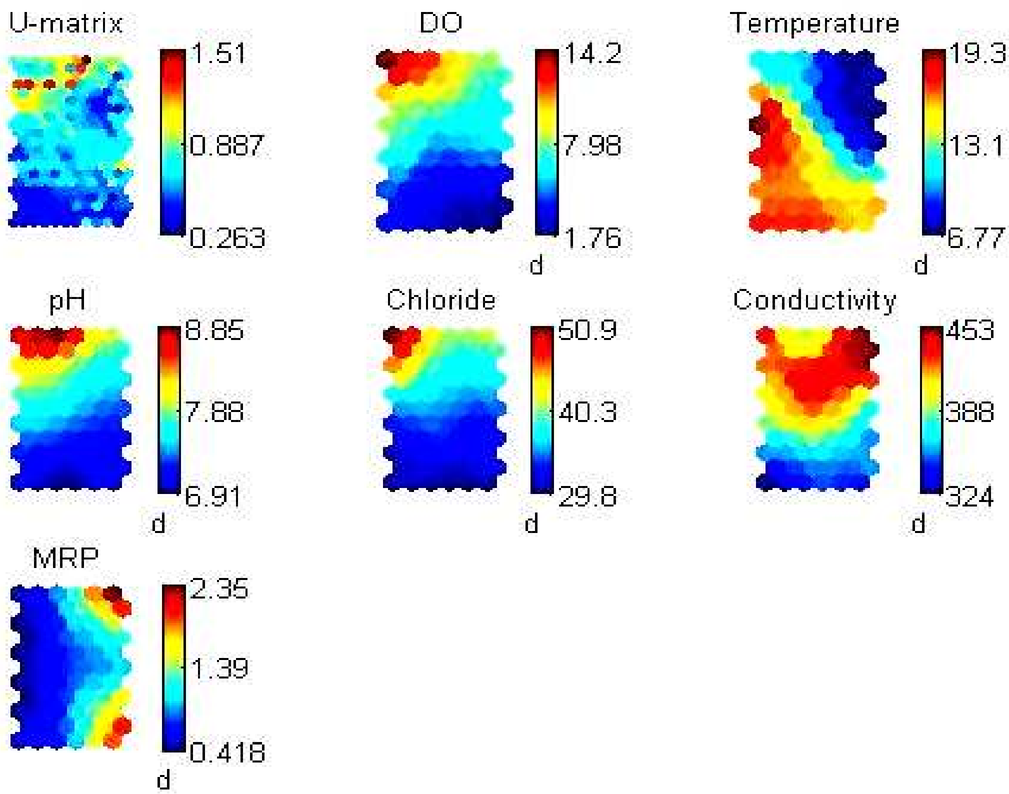


Figure 5-19. Abstract visualisation of the relationships between outflow molybdate reactive phosphorus (MRP, mg/l), and outflow dissolved (DO, mg/l), temperature (ºC), pH (dimensionless), chloride (mg/l) and conductivity (μS/cm) using a self-organising map model.

In contrast, DO is negatively correlated (R = -0.28) with MRP indicating that high DO concentrations had positive effects on MRP removal. Dissolved oxygen is an important variable influencing phosphorus removal in ICW. Studies by Girija et al. (2007) revealed that the phosphorus concentrations decreased from 6.0 to 0.1 mg/l as DO concentrations increased from 0.1 to 8.6 mg/l. Low DO concentrations can cause the release of phosphorus from sediment, causing an increase in MRP (Golterman, 1995; Maine et al., 2007). Furthermore, Wang et al. (2007) reported that phosphorus concentrations between 0.22 and 1.79 mg/l within a biological reactor effluent could be obtained when the corresponding influent phosphorus concentration ranged between 15 and 20 mg/l. The DO was controlled at 3.0 ± 0.2 mg/l during the aerobic phase and pH was maintained at 7.0 ± 0.1. Phosphorus removal of 90% was achieved in the reactor.

Phosphorus might precipitate as calcium phosphate or co precipitate with iron colloids or with calcium carbonate (Golterman, 1995). For example, the US EPA (2000) reported that phosphorus might precipitate as calcium phosphate within sediment pore water or in the water column near active phytoplankton growth at pH values >7.0. Furthermore, as pH decreases, MRP sorption to carbonates decreases while adsorption to iron increases (Golterman, 1995). Concerning the ICW 3, 9 and 11 study, the correlation (R = 0.10) between pH and MRP was weak, indicating that a high pH had a small positive influence on MRP removal. Since overall pH values were comparatively low (Figure 5-19), the influence of pH on MRP removal was weak. However, the chemical composition of the three ICW systems and their effluents is complex.

Pietro et al. (2006) observed that phosphorus removal was weakly correlated with water temperature in a freshwater marsh located in the Southern Florida, USA. In comparison, high MRP concentrations in ICW are associated with low temperatures (R = -0.42). However, the influence of temperature was lower for MRP removal than for ammonia-nitrogen removal. In addition to the factors described above, nutrients especially nitrogen, are also removed biologically through microorganisms present in wetland systems. The role of microbes in removing nitrogen is discussed in Chapter 7.

5.6.4. Correlation analysis

The Shapiro-Wilk Normality Test was used to determine whether or not a data set was normally distributed (Shapiro and Wilk, 1965). If the raw data set did not follow a normal distribution data were transformed using a simple log transformation method (Eq. (5-10)).

$$x(new) = \log(x + a)\,;\ a \in R \tag{5-10}$$

where

$x(new)$, pre-processed data; and x, raw data.

The data sets for ammonia-nitrogen (a=0), MRP (a=0.5), SS (a=0) and BOD_5 (a=0) were transformed using this method (for a, see Equation 5-10). Raw COD data, which had a normal distribution, and transformed ammonia-nitrogen, MRP, SS and BOD_5 data were used in the subsequent analyses. The SOM model was applied to identify the relationships between the transformed input data set.

High log (ammonia-nitrogen) values (>-0.84) were linked to high log (MRP + 0.5) (see Equation 5-10; *a*=0.5) values (>0.07), indicating that ICW outflow ammonia-nitrogen concentrations were positively correlated with MRP concentrations. The relationships between log (ammonia-nitrogen) and the other three variables were weak. High log (MRP + 0.5) values (>0.07) were associated with high log (ammonia-nitrogen) values (>-0.84), high COD values (>56.6) and low log (SS) values (<0.88), indicating that ICW outflow MRP concentrations were positively correlated with ammonia-nitrogen and COD concentrations, but negatively correlated with SS concentrations. In contrast, the MRP concentrations did not show obvious relationships with BOD_5 concentrations. High log (SS) values (>0.88) were positively correalted to high log (BOD) values (>0.93), indicating that ICW outflow SS concentrations were positively related to BOD concentrations. However there was no significant correlation between log (SS) values and COD values. Chemical oxygen demand values were positively correlated with log (BOD) values, indicating that ICW outflow COD concentrations were linked to BOD concentrations.

Table 5-8 summarises the results from a correlation analysis for the input variables. The mathematical relationships between all variables were relatively weak. However, the relationships between SS and BOD_5, and between MRP and COD were reasonably strong after data transformation. Unlike the SOM model, the missing values of each pair were disregarded in this analysis. Findings were in agreement with the key relationships revealed by the SOM. The correlations between log (ammonia-nitrogen) values and log (MRP + 0.5) values, log (MRP + 0.5) values and log (SS) values, log (MRP + 0.5) values and COD values, log (SS) and log (BOD)

Table 5-8. Correlation analysis showing the correlation coefficient *R* for the input ICW11 dataset.

First variable	Second variable	Pairs	*R*	*P*
Log (ammonia-nitrogen)	Log (MRP+0.5)	140	0.35	<0.0001
Log (ammonia-nitrogen)	Log (SS)	67	-0.02	0.86
Log (ammonia-nitrogen)	COD	86	0.14	0.21
Log (ammonia-nitrogen)	Log (BOD)	57	0.19	0.16
Log (MRP+0.5)	Log (SS)	68	-0.29	0.02
Log (MRP+0.5)	COD	85	0.42	<0.0001
Log (MRP+0.5)	Log (BOD)	57	0.08	0.54
Log (SS)	COD	59	0.02	0.89
Log (SS)	Log (BOD)	41	0.41	0.01
COD	Log (BOD)	54	0.31	0.02

MRP, soluble reactive phosphate; SS, suspended solids; COD, chemical oxygen demand; BOD, biochemical oxygen demand.

values, and COD values and log (BOD) values, were statistically significant (P<0.05).

5.6.5. Statistical models

Design methodologies for wetlands treating wastewaters have assumed that plug flow conditions and first order degradation prevails in these systems. However, Kadlec (2000) reported that the first-order models are simple to use, but fail to characterise the complex processes that occur in wetland systems. Statistical models present an alternative to the first-order model. The statistical models presented below are a simple way to explain wetland parameters. Cheap and easily measurable variables such as DO, T, pH and EC were used to determine and expensive and time consuming water quality variables such as nutrients and BOD_5 with the aid of multivariate linear regression models. Statistical models for ammonia-N reduction can be an alternative to first-order models. These models are capable of predicting reduction rate, as well as explaining factors important for reduction. Braskerud (2002), also presented statistical models. Multiple regression analysis was carried out, including influent water quality variables such as ammonia-N concentrations, temperature, conductivity, pH and DO. Subsequently, influent ammonia-N concentrations, conductivity and temperature were selected as the optimal regressors.

Statistical models for ICWs 3, 9 and 11 are presented in Table 5-9. As shown in this table, ammonia-N concentrations decreased with increasing influent conductivity. This may be attributed to an undesirable effect of salt on the microorganisms. Such a salt inhibition effect on nutrient removal has been reported in previous studies as discussed above. The models also show that effluent ammonia-N concentrations are likely to decrease under high temperature conditions.

Table 5-9. Statistical models for ammonia-nitrogen reductions

ICW	Regression equation[a]	R^2
3	Ce = 11.79 -0.014Ci - 0.002T - 0.61EC	0.15
9	Ce = 1.7 + 9.22×10^{-4}Ci - 0.10T - 0.02 EC	0.16
11	Ce = 2.60 + 1.77×10^{-4}Ci -0.16T - 3.12×10^{-5}EC	0.29

[a] Ce = effluent ammonia-N concentration, Ci = influent ammonia-N concentration, T= influent wastewater temperature. EC = influent electrical conductivity

Uygur and Kargi (2004) found that ammonia-N removal efficiency decreased from 96 % to 39 % when salt content increased in a sequencing batch reactor. Tseng and Wu (2004) also reported that in a submerged biofilter experiment, the ammonia removal rates were positively affected by temperature and influent ammonia concentration. Although the models presented above show some relationships between various parameters, the R^2 values are low, indicating that the models are weak. Also, nitrogen processing in treatment wetlands is very intricate and encompasses various physical, chemical and biological processes in different compartments.

5.7. Pathogen removal

Animals are a source of pathogenic organisms to integrated constructed wetlands. The faeces of farm animals contain high number of bacteria. For example, cows produce 10^5 – 10^7 faecal coliforms per gram. Wetland birds also contribute coliforms to the wetland environment. Treatment wetlands have a potential to reduce high numbers of incoming total coliforms (Kadlec and Wallace, 2009). The net removal of pathogenic organisms is by various processes including solar disinfection, predation, settling and filtration. For monitoring purposes, indicator organisms like total coliforms and *Escherichia coli* are frequently used.

The concentrations of total coliforms and *E.coli* in the three integrated constructed wetlands are presented in Table 5-10. Average concentrations of total coliforms in the influent for ICW 3, ICW 9 and ICW 11 were 1.3×10^5, 1.5×10^5 and 9×10^5 cfu/100ml respectively. Total coliforms were reduced by an average of 99.6%, 99.8% and 99.4% for the three ICW systems respectively. The average removal of the indicators analysed (total coliforms and *E. coli*) were in the range 99.93–99.99%, showing a very high efficiency of the ICW systems for removing pathogens.

Log_{10} removals of indicator bacteria (total coliforms) by integrated constructed wetlands in the current study are better than those reported previously for treatment of dairy water by constructed wetland technology at Tucson, Arizona, USA (Karpiscak et al., 1999). The removal of *E. coli.* in ICW was better than a reed bed system treating wastewater produced from a resort hotel in Florence, Italy, as reported by Masi et al., 2007. In the current study, comparison between the three integrated constructed wetland systems revealed differences in the overall levels of removal.

Table 5-10. Reduction of pathogenic organisms (mean ± standard deviation) in various free water surface (FWS) wetlands.

System	Inlet (CFU/100ml)	Outlet (CFU/100ml)	Reduction (log_{10})	Reference
Total Coliforms				
ICW 3	$1.3 \times 10^5 \pm 9.6 \times 10^4$	$4.4 \times 10^3 \pm 5.6 \times 10^3$	1.98	This study
ICW 9	$1.5 \times 10^5 \pm 7.7 \times 10^4$	174 ± 86	1.99	This study
ICW 11	$9.0 \times 10^5 \pm 9.6 \times 10^5$	$5.6 \times 10^3 \pm 8.5 \times 10^3$	1.99	This study
Tucson, Arizona	$0.66 \times 10^7 \pm 1.2 \times 10^7$	$1.4 \times 10^6 \pm 2.9 \times 10^6$	1.89	Karpiscak et al. (1999)
Escherichia coli				
ICW 3	$1.7 \times 10^5 \pm 1.7 \times 10^5$	93 ± 66	1.99	This study
ICW 9	$7.6 \times 10^4 \pm 1.1 \times 10^5$	52 ± 50	1.99	This study
ICW 11	$7.3 \times 10^5 \pm 9.1 \times 10^5$	27 ± 29	1.99	This study
Florence, Italy	3.4×10^6	1.8×10^2	1.99	Masi et al. (2007)

5.8. Reuse

The arrangement of multiple treatment environments within constructed wetlands can provide water quality appropriate for reuse. The treated water from ICW may discharge into surface water or groundwater. It may also be used for various applications such as agricultural, flushing barns and, environmental and recreational uses. Greenway (2005) reported that constructed-wetland technology is a viable option not only reduces nutrients but it performs the function of disinfection, rendering the treated wastewater a resource to irrigate crops, playing fields, parks and gardens, or golf courses.

House et al. (1999) successfully combined constructed wetlands and aquatic and soil filters for reclamation and reuse of water for toilet flushing, landscape irrigation and aesthetic water features. If the treated water is used for agricultural irrigation of non-food crops then treatment and water quality requirements are less stringent because of the reduced opportunity of human exposure to the water as outlined by US EPA (2004). The reclaimed water quality and treatment requirements for irrigation of non-food crops are shown in the Table 5-11. The treated water can also be used for environmental and recreational uses. The various types of application include development and restoration of existing wetlands, groundwater recharge, stream flow augmentation, lakes and ponds. When water is reused for environmental purposes in addition to protecting public health, care is required for to protect ecosystems (Asano, 2007). According to the microbiological criteria outlined in the Irish Bathing Water Quality Regulations 2008, inland waters are designated excellent if there are less than 500 *E. coli.* per 100 ml and 200 intestinal enterococci

Table 5-11. Reclaimed water quality and treatment requirements for irrigation of non-food crops. (adapted from USEPA, 2004).

Parameter/State	Arizona	California	Nevada	Washington
Treatment	Secondary treatment and disinfection	Secondary, oxidized[b] and disinfected	Secondary treatment and disinfection	Oxidized and disinfected
BOD	NS[a]	NS	30 mg/l	30 mg/l
TSS	NS	NS	NS	30 mg/l
Turbidity	NS	NS	NS	2 NTU (avg) 5 NTU (max)
Coliform	Fecal	Total	Fecal	Total
	200/100 ml (avg)	23/100 ml (avg)	200/100 ml (avg)	23/100 ml (avg)
	800/100 ml (max)	23/100 ml (max)	400/100 ml (max)	240/100 ml (max)

[a]NS – Not specified by state regulations
[b]Oxidised means the wastewater is treated with a biological process

per 100 ml based on a 95th percentile evaluation (http://www.environ.ie/en/Legislation/ Environment). Currently in Ireland there are no guidelines for water reuse. The treated wastewater from ICW fulfils the Italian regulations for reuse for the indicator pathogen *E. coli* (80 percentile equal to 50 cfu/100 ml and maximum admitted value equal to 200 cfu/100 ml). The effluent water from the ICW is also of a quality that can be reused for irrigation of non-food crops after disinfection and for restricted recreational reuse.

5.9. ICW Water balance

Observations for ICW site 11

Mean monthly inflows and outflows from the ICW system during the monitoring period (2003–2007) were 180 ± 217.5 m^3/month and 45 ± 132.3 m^3/month, respectively (Figure 5-20). Although inflow to the ICW system occurred between August and December, there was no corresponding outflow during most

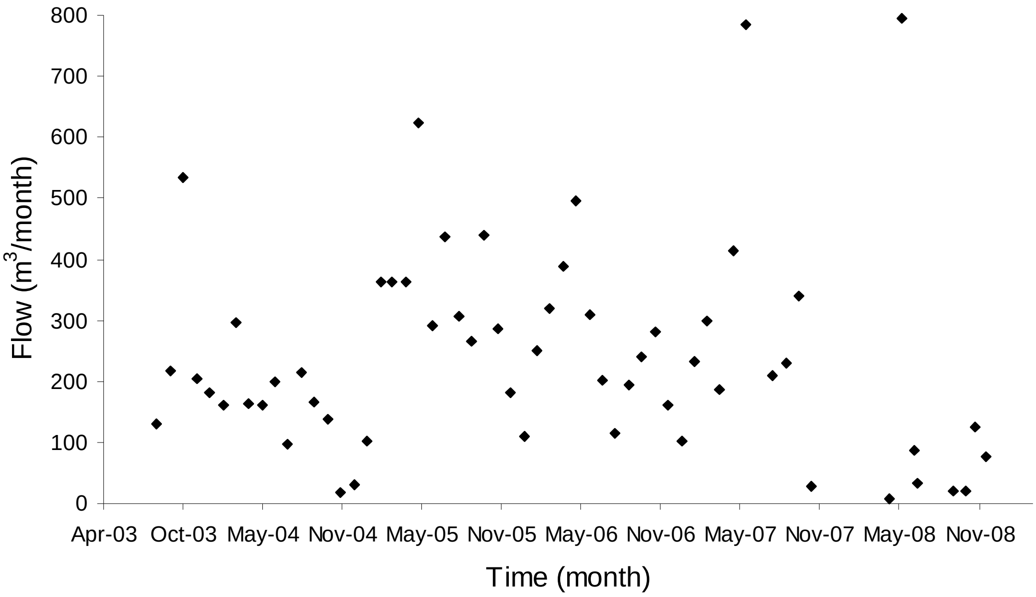


Figure 5-20. Mean monthly inflow rates into the integrated constructed wetland (ICW 11).

of these months indicating that there was limited discharge to the receiving watercourse.

The flows through the wetlands decreased during summer, and there was no discharge from the ICW system to the receiving watercourse. Flow monitoring indicates that approximately 25% of the influent into the ICW system is subsequently discharged at the outlet as effluent. Based on flow and weather monitoring data, the sources of inflow and outflow are summarised in Figure 5-21. During dry periods, increased storage capacity was created within the ICW cells due to losses via evapotranspiration and infiltration. These processes provided additional storage capacity for runoff within the ICW system during storm events.

The intermittent nature of the outflow and the great loss of partially treated runoff to the ground have a great impact on calculating and interpreting treatment performances. Consequently it is very difficult to accurately determine constituent masses. Therefore, calculations such as removal performances are based on constituent concentrations. Moreover, the lack of outflow data during drier compared to wetter periods is likely to result in less accurate interpretations for summer than winter data.

The microbial transformations within the wetland systems are a function of the available area for biofilm growth. In ICW systems, the dense vegetation stands and the associated litter provide a large surface area for biofilm biomass and hence enhanced treatment capability. The biofilm entraps both organic and inorganic solids. The formation of biomass is greatest at the inlet of the wetland cell where the organic loading is highest (Ragusa et al., 2004; Scholz, 2006), and decreases progressively through the system. The biofilm not only reduces the pollutant concentrations but also decreases the hydraulic conductivity by reducing the pore volume.

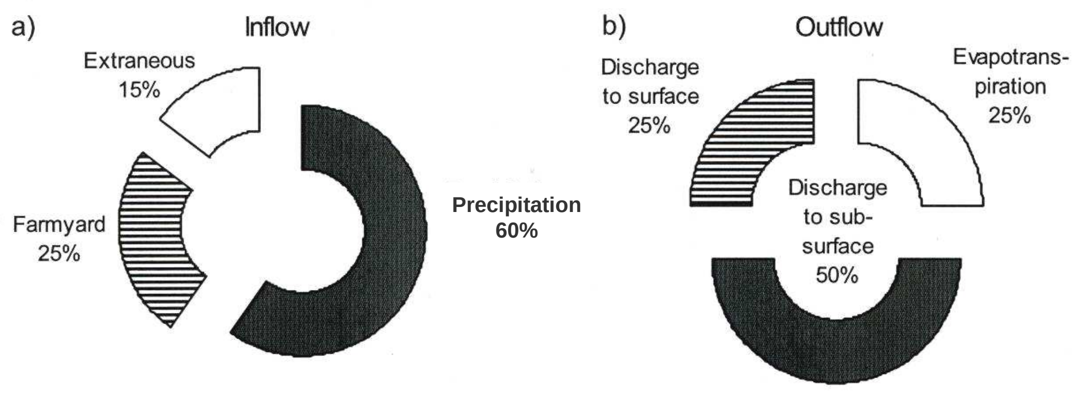


Figure 5-21.Composition of integrated constructed wetland inflow and outflow.

In addition to biofilm formation, organic matter and humic substances, which develop rapidly within wetland soils, increase the availability of carbon supporting denitrification and the biological feedback mechanisms that secure water retention. These processes result in flow impedance, and subsequently self-sealing of the ICW cell. This leads to a progressive contaminant reduction within the discharge to the groundwater.

5.10.Surface water quality

5.10.1. Receiving stream water quality

In order to evaluate the effects of ICW on the associated receiving waters, grab samples were taken during an intensive campaign to monitor the surface water quality of the receiving water courses associated with ICW 3 and ICW 11.

Integrated constructed wetland 3

The construction and commissioning of the ICW systems led to the transformation of a non-point-source to an identifiable point source of potentially polluted water (i.e. if treatment is insufficient). Presently, there are no discharge standards for the final effluent in Ireland.

The final effluent of the ICW 3 is discharged into a small tributary of the associated stream (Figure 5-22) through the outlet from cell 5. The small tributary meets the Annestown stream. The ICW is flanked by the Annestown stream and its tributary as shown in the figure below. At the discharge point, mean outlet ammonia-nitrogen concentrations were 0.172 ± 0.077 mg/l, while nitrate-nitrogen and MRP concentrations were 0.096 ± 0.272 mg/l and 4.409 ± 1.039 mg/l, respectively.

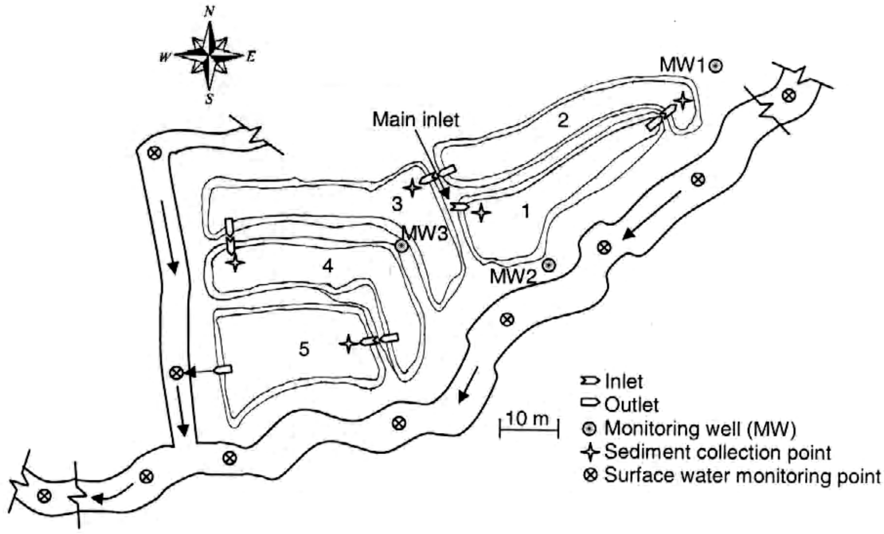


Figure 5-22. Surface water and groundwater monitoring, sediment collection, inlet and outlet points for ICW 3.

The nutrient concentrations of the Annestown stream monitoring points designated as U/S in Figure 5-23 were very stable. The ammonia-nitrogen and molybdate reactive phosphorus concentrations at the points preceding the point of confluence between the outlet of the final cell 5 and the receiving tributary were less than ICW effluent concentrations. However, nitrate-nitrogen concentrations of the upstream points were higher than the ICW outlet concentrations. Further downstream where the ICW outlet tributary meets the Annestown stream, the measured nutrient concentrations decrease indicating that the stream has enough assimilative capacity to buffer the ICW outflow concentrations. Overall, the points monitored on the Annestown stream had a median ammonia-nitrogen concentration of 0.047 mg/l, nitrate-nitrogen concentration of 4.234 mg/l and MRP concentration of 0.028 mg/l. Phosphorus, which is a limiting nutrient, is in compliance with the Irish Phosphorus Regulations (1998). The key nutrient indicator for Irish rivers is MRP, which should have an annual median concentration <0.03 mg/l.

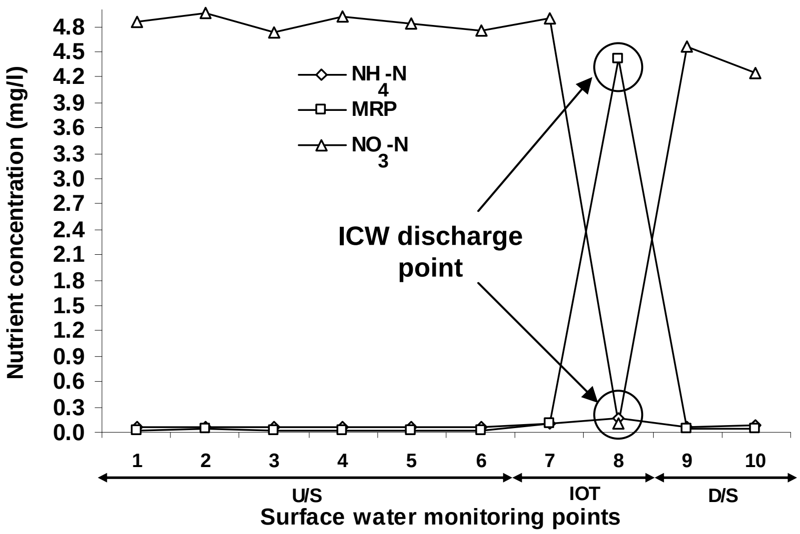


Figure 5-23. Fluctuations of nutrient concentrations within the stream and river adjacent to ICW 3. NO_3-N, nitrate-nitrogen; MRP, molybdate reactive phosphorus; NH_4-N, ammonia-nitrogen; U/S upstream; IOT integrated constructed wetland outflow tributary; D/S downstream.

Integrated constructed wetland 11

The final effluent of the ICW is discharged into a small stream (Figure 5-23) through the outlet from cell 4. At the discharge point, mean ammonia-nitrogen concentrations were 0.37 ± 0.562 mg/l, while mean nitrate-nitrogen and MRP concentrations were 0.99 ± 1.812 mg/l and 0.94 ± 0.628 mg/l, respectively.

Most nitrate-nitrogen concentrations at the point of confluence between the outlet of the final ICW cell 4 and the receiving stream and downstream of this point are lower than at the upstream sampling points (Figure 5-23 and 5-24). Further down-stream, some measured nutrient concentrations decrease indicating that the stream has sufficient assimilative capacity to buffer the ICW outflow concentrations(Figure 5-25).

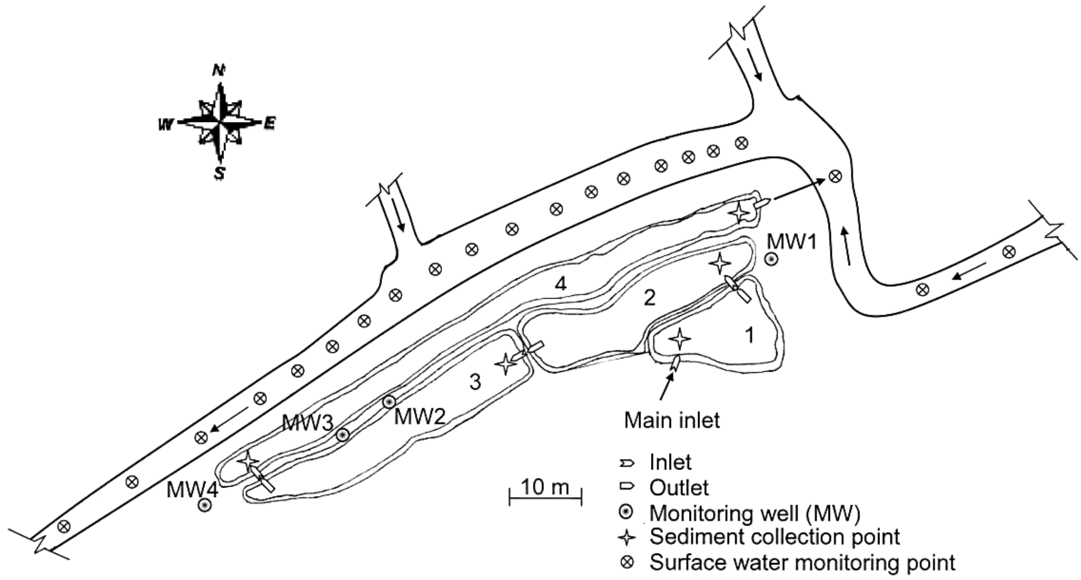


Figure 5-24. Surface water and groundwater monitoring, sediment collection, inlet and outlet points for ICW 11.

The exception is sampling point 14 due to the presence of a field drain discharging water rich in nutrients and the narrowing of the stream at that point However, the trend reversed further down-stream because field drains were absent.

Overall, the monitored stream had a median ammonia-nitrogen concentration of 0.05 mg/l, nitrate-nitrogen concentration of 0.134 mg/l and MRP concentration of 0.029 mg. Phosphorus, which is a limiting nutrient, complies with the Irish Phosphorus Regulations (1998). The key nutrient indicator for Irish rivers is MRP, which should have an annual median concentration <0.03 mg/l.

The ICW effluent is variable and predominantly depends on the patterns of precipitation. Outflows from the system are highly variable (1.5 ± 4.41 m^3/d), and the inflow quality and quantity of the ICW system is predominantly a function of precipitation intensities and patterns. Furthermore, there is no outflow from the system during periods of very low inflows, indicating that there is a short

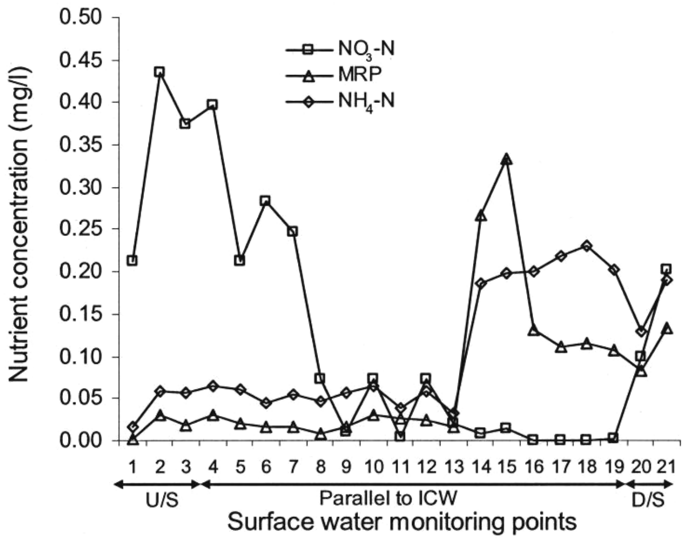


Figure 5-25. Fluctuations of nutrient concentrations within the stream adjacent to ICW 11. NO3-N, nitrate-nitrogen; MRP, molybdate reactive phosphorus; NH4-N, ammonia-nitrogen; U/S upstream; D/S downstream.

discharge period to the receiving stream. Discharge from the ICW system during periods of high precipitation generally occurs during periods when the buffering capacity of the receiving water is enhanced by an increased dilution ratio.

Annestown stream

Improvement in the Annestown stream water quality coincided with the operation of ICW systems (Table 5-12), which treat approximately 75% of all farmyard dirty water generated within the watershed. Seven ICW systems representing more than 80% of farmyard discharge were located upstream of Ballyphilip bridge and Dunhill village (i.e., approximately half way along the stream and midway in the catchment). At the Ballyphilip upstream monitoring point the mean ammonia-nitrogen concentrations were 0.0645 ± 0.0487 mg/l, while nitrate-nitrogen and MRP concentrations were 4.854 ± 1.429 and 0.0326 ± 0.0393 mg/l, respectively.

The concentrations of ammonia-nitrogen at Ballyphilip upstream and Castle were almost identical, 0.0645 ± 0.0487 and 0.0645 ± 0.0481 mg/l, respectively. However, there was a significant increase in ammonia-nitrogen concentrations to 0.115 ± 0.159 mg/l at Ballyphilip. The increase is most likely due to the discharges from ICW treating sewage at Dunhill. This ICW system is overloaded because of the increase in the number of houses over the past few years resulting in increased load to the system. Further downstream, there was a significant increase in ammonia-nitrogen concentrations to 0.0982 ± 0.1140 mg/l at Monument which is the last monitoring station. The increase may be attributed to intensive animal farming which in the lower part of catchment.

The mean MRP concentration at Ballyphilip upstream were 0.0326 ± 0.0393 mg/l. The concentration increased to 0.052 ± 0.153 mg/l at Ballyphilip downstream which may be due to discharges from an ICW system treating sewage. The discharges from this ICW system enter the stream upstream of the monitoring point. MRP concentrations at Ballyphilip downstream and Monument are almost the same: however the concentrations at Castle are higher. This increase is not statistically significant. The water quality of the Annestown stream complied with the target phosphorus concentration of a median annual concentration <0.03 mg MRP/l as required by the Irish Phosphorus Regulations (1998). However, the stream's monitoring sites located downstream of Ballyphilip bridge and Dunhill village did not comply with this regulation. This lower section of the stream is affected by nutrients originating from intensive animal farming and discharges from the Dunhill village sewage works. Furthermore, less than 70% of farmyard runoff in this lower section of the stream is intercepted by ICW. The biological water quality status of the stream has improved from an overall water quality rating of Q2 (seriously polluted) in 1999 to a water quality rating of Q3/4 (slightly polluted) in 2001 (EPA 2002). Further evidence suggests that the water quality rating has since improved to Q4 (unpolluted). Sea trout (Salmon trutta) have returned to the stream after many decades of absence. The common newt (Triturus vulgaris) has become abundant in all ICW of the catchment. Although invertebrate monitoring within the catchment is ongoing, preliminary results indicate great ecological habitat enhancement.

Table 5-12. Mean concentrations ± standard deviations (2001-2008) for nutrients in Annestown stream .

Nutrients	Bally Phillip Upstream	Bally Phillip Downstream	Castle	Monument
NH_4-N	0.064 ± 0.049*	0.115 ± 0.159**	0.064 ± 0.048*	0.098 ± 0.114**
NO_3-N	4.85 ± 1.43*	4.75 ± 1.28*	5.19 ± 1.30**	4.55 ± 1.76*
MRP	0.033 ± 0.039*	0.052 ± 0.153*	0.097 ± 0.430*	0.050 ± 0.113*

Means followed by the same symbols are not statistically significantly different ($p < 0.05$)

5.11. Groundwater quality

The contamination of groundwater by infiltration through ICW substrate is a key concern. The ICW sites were constructed using *in situ* soils. The subsoil was reworked and used to line the bed and banks of all cells to reduce the risk of infiltration. As the contaminated farmyard runoff passes through the ICW system, the suspended matter settles on the soil surface and subsequently hinders infiltration of contaminants through the wetland cells (Kadlec and Knight, 1996; Scholz, 2006; Wallace and Knight, 2006).

At ICW 3, the mean ammonia-nitrogen concentration of the groundwater monitoring well located up-gradient was 35.95 mg/l, which indicates a high value. Chloride can serve as a tracer of water movement (Kadlec and Wallace, 2009). Chloride concentrations in this well were stable and did not increase over the monitoring period. This confirms that pollution in the well does not originate from the ICW system. The mean nitrate-nitrogen and MRP concentrations in the well were 0.53 mg/l and 0.14 mg/l, respectively (Table 5-13). The mean ammonia-nitrogen concentration for the well located between the stream and the ICW cell was 15.83 mg/l, which is less than in the well located up-gradient. The ammonia-nitrogen concentration for the well located between ICW cells was 6.50 mg/l, which is lower than the other wells located up-gradient and between the ICW cell and stream. Hence ammonia-nitrogen from the ICW is not impacting negatively on groundwater. MRP concentrations determined down-gradient were below the detection limit of 0.02 mg/l. Mean nitrate-nitrogen concentrations were 0.53 mg/l for the well up gradient and the one located between the stream and ICW cell, while the well located between cells had a slightly higher mean concentration of 0.57 mg/l. The nitrate concentrations were all below internationally recommended thresholds of approximately 25 mg/l nitrate.

At ICW 11, the mean ammonia-nitrogen concentration of the groundwater monitoring well located up-gradient was 0.79 mg/l, which indicates pollution but not originating from the ICW system. The mean nitrate-nitrogen and MRP concentrations were 0.02 mg/l and 0.12 mg/l, respectively (Table 5-13). The mean ammonia-nitrogen concentration for the down-gradient well was 0.50 mg/l, which is less in comparison to the other wells located up-gradient and between ICW cells. MRP concentrations in the down-gradient well were below the detection limit of 0.02 mg/l. Thus ammonia-nitrogen from the ICW is not impacting negatively on groundwater.

This finding is confirmed by an assessment of the nutrient data for the two wells between cells 3 and 4. The wells located between the ICW cells have a relatively high ammonia-nitrogen concentration because their sampling points are most influenced by the proximity of the cell sediments, which are rich in ammonia-nitrogen. The nitrate concentrations are also well below internationally recommended thresholds of approximately 25 mg/l nitrate.

Generally, the presence of high phosphate concentrations in groundwater is an indication of a shallow subsoil depth and the presence of preferential flow paths through the subsoil. However, in this case, where approximately 50% of the ICW water discharges to groundwater, the mean nitrate-nitrogen and MRP concentrations in all the down-gradient monitoring wells were <0.03 mg/l indicating that the groundwater in the vicinity of the ICW system is not polluted by infiltration from the ICW cells. The soil investigation results at the time of installation of the ground water monitoring wells indicated the presence of clay in the substratum of the ICW: clay over rock at 4.2 m for monitoring well 1; clay over gravel at 1.7 m, and clay over rock at 4.3 m for monitoring well 2; clay over gravel at 4.3 m, and clay over rock at 4.7 m for monitoring well 3; clay over rock at 2.5 m for monitoring well 4. Furthermore, the observation of hydrogel formed on the detritus confirms the low.

Table 5-13. Mean nutrient concentrations in groundwater monitoring well samples between 2004 and 2008 (number of samples 30)

Monitoring well number	Position relative to the stream/ICW	Depth (m)	NH_4-N (mg/l)	NO_3-N (mg/l)	MRP (mg/l)
		ICW site 3			
1	Up-gradient	5	35.95	0.53	0.14
2	Within ICW	5	15.83	0.53	<0.02
3	Within ICW	3	6.50	0.57	<0.02
		ICW site 11			
1	Up-gradient	5	0.86	0.14	0.12
2	Within ICW	3	2.74	0.17	0.03
3	Within ICW	5	2.53	0.19	<0.02
4	Downgradient	5	0.48	0.09	<0.02

NH_4-N, Ammonia- nitrogen; NO_3-N, Nitrate-nitrogen; MRP, Molybdate reactive phosphorus

mobility of nutrients in subsoil, and shows the important role that the subsoil plays in attenuating pollutants.

Moreover, there are many biogeochemical processes that play essential roles in impeding the infiltration of pollutants to groundwater. For example, the ICW vegetation provides a large surface area to support microbial biofilms (Wallace and Knight, 2006). Detritus provides a carbon source to microbes for denitrification and assists in the long-term sequestration of phosphorus (Wallace and Knight, 2006). The study of characterisation of nitrogen removing bacteria in components of ICW systems (Chapter 7) clearly indicates the presence of various ammonia-oxidising and denitrifying bacteria that play an important role in removing nutrients. The production of methane during anaerobic metabolism inhibits the loss of water through capillary-pore structures (Kellner et al. 2004; Tokida et al. 2005).

Nutrient removal occurred in virtually all cells in ICW 11 (Table 5-14). Nutrient enrichment of groundwater beneath the ICW system is of great concern because high concentrations may have a direct effect on sensitive receptors (EPA, 2002). The transportation and attenuation of pollutants in ICW cells predominantly depend on wetland soil, physical impedance and underlying geological formations; e.g. attenuation of ammonia during migration in the subsurface is known to occur (Erskine, 2000). The key processes are sorption (cation exchange) and biological degradation (Buss et al. 2004). In wetland peat, methane bubbles originating from microbial anaerobic processes lower the hydraulic conductivity (Kellner et al., 2004). The biofilm matrix formed on detritus acts like a hydrogel, which withstands changes in fluid shear stresses (Harrison et al., 2005).

Table 5-14. Comparison of cell-by-cell mean nutrient concentration reductions (%) for ICW 11.

Variable	Unit	Cell 1	Cell 2	Cell 3	Cell 4
Ammonia-nitrogen	(mg/l)	73.30	15.20	3.37	6.16
Molybdate reactive phosphorus	(mg/l)	73.60	2.64	-2.13	12.80

5.12. Limitations of the analysis

ICW are always different from each other and hence there was no true replication in this study. The seasonal effects of water quality improvement were tested by analysis of variance (ANOVA) for ICW 11 only. The data were tested for normality, and transformed to meet normality if necessary and the analysis did not violate the assumptions of ANOVA. However, experimental error has been reduced due to an assessment of potential outliers and sub-sampling where required. The limitations associated with the statistical analysis suggest that the outcomes of a particular case study presented may only be valid for the specific ICW system assessed and very similar systems.

The ICW systems studied are semi-natural and open. Considering that flow rates are therefore partly unknown and that flow estimations would result in very inaccurate constituent mass estimations, the display of constituent concentrations was chosen instead. This is common practice for less engineered and rather natural systems.

It has to be emphasised that ICW are not fully engineered treatment wetlands where the inflow and outflow rates are known, and where losses to groundwater are zero. Moreover, ICW are purely driven by hydrology (i.e. storm events) and not by rather constant inflow rates, which are common for constructed wetlands treating, for example, domestic wastewater.

5.13. Summary

This chapter has demonstrated that integrated constructed wetlands outperformed other types of wetland systems in terms of nutrient removal. In the integrated constructed wetland (ICW) systems studied all contaminant concentrations present in farmyard runoff decreased as follows: ICW 3, NH_4-N (89.1%), MRP (86.7%); ICW 9, NH_4-N (97.1%), MRP (91.2%), and ICW 11, NH_4-N (98.8 %), MRP (88.3%).The ICWs act as a sink for nutrients. Furthermore, the biochemical oxygen demand, chemical oxygen demand, suspended solids, total coliforms and *E. coli* values were also reduced within the ICW system.

The long-term assessment demonstrates that the example ICW systems can be considered an effective and sustainable wastewater treatment option for farmyard runoff rich in nutrients. The ICW systems had neither polluted the groundwater nor decreased the water quality of the receiving watercourse.

Chapter 6 Role of plants and sediment

6.1. Introduction

This chapter investigates the role of plants and sediment in removing nutrients from water being treated in ICW. The structure of this chapter is as follows: Sections 6.2, 6.3 and 6.4 review the importance of plants and sediment and their role in removing nutrients from constructed wetlands. Section 6.5 onwards presents the findings of a study conducted in ICW 11 to assess the role of *Typha latifolia* and sediment in nutrient accumulation.

6.2. Plants and sediment in wetlands

The plants and sediment in wetlands have many important functions. In addition to water quality improvement, plants also have various physical and ecological functions. The physical functions comprise transpiration, particulate trapping and flow impedance, while the ecological functions include human use values and wildlife habitat (Kadlec and Wallace, 2009). Some of the main effects of vegetation on water treatment in wetlands are as follows:

- Plants follow their life cycle and hence store nutrients during growth periods and release nutrients during senescence and periods of die-back.
- Plants also influence the oxygen supply to the water. For instance, emergent vegetation blocks the wind and hence lowers reaeration of water through movement.

Figure 6-1. Dense stands of emergent vegetation in integrated constructed wetlands (*Glyceria maxima* and *Carex riparia*).

- The litter from decaying macrophytes provides considerable surface area for the attachment of biofilms.
- Plant litter has a high carbon content and fulfils the energy requirements of denitrifiers.

Vegetation is an essential component of a CW (Figure 6-1) and increases the efficiency of contaminant removal. In free water surface constructed wetlands, vegetation influences the treatment mechanisms; it reduces water column mixing, hence increasing sedimentation, and also provides surface area for biofilm attachment (US EPA, 2000; Wallace and Knight, 2006). Karathanasis et al. (2003) reported higher BOD and suspended solids removal in planted systems compared to unplanted systems. Moreover, the vegetation in these systems requires nutrients for growth and wetland plants utilise nitrogen and phosphorus for their growth and reproduction. In this way a portion of nutrients in the water column is transferred to plants, contributing to water quality improvement by reducing nutrient concentrations of the wastewater flowing through the wetland system.

Important plant terminology (defined by Mueleman et al., 2002) is as follows: phytomass refers to all living plus dead vegetative material, biomass refers to all living vegetative material while necromass refers to all dead vegetative material. Tanner (2001) summarised the role of plants as ecosystem engineers in his paper entitled "Plants as ecosystem engineers in subsurface-flow treatment wetlands".

Plants mainly influence treatment performance by enhancing the main nutrient transformation processes (e.g. nitrification and denitrification) by two pathways: root-zone oxygen release and supply of organic matter. Moreover, cycling and build up of plant-derived organic matter provides a sustained supply of organic carbon for microbes, and also sequester organically bound nutrients, and acts as a barrier to nutrient release.

Studies have indicated that improved nutrient removal occurs in planted wetlands compared to unplanted systems (DeBusk et al., 1989; Soto et al., 1999; Tanner et al., 1995; Tanner 2001). However, these studies involved comparatively immature systems where plant uptake and sediment adsorption pools still had filling capacity. Borin and Tocchetto (2007) investigated the performance of a constructed surface flow wetland in reducing diffuse N pollution. They estimated that over a 5-year period the wetland had a 90% N-removal efficiency and found that most of the removal was due to plant uptake. Moreover, Gottschall et al. (2007) found that plant uptake was significant for overall nutrient removal in a well-established constructed wetland treating agricultural wastewater since 1996.

Case studies of animal wastewater treatment wetlands have shown varying nutrient removal rates, 45-98% N and 35-96% P, with generally higher nutrient removal in recently established wetlands and those with lower loading rates (Hammer et al. 1993; Hunt and Poach, 2000; Newman et al. 2000; Schaafsma et al., 2000). However, most of the studies were conducted when vegetation was still establishing, so there is a lack of information concerning long-term nutrient storage within the vegetation pool in constructed wetlands. There are also concerns about long-term phosphorus removal as the sorptive capacity of wetland soil becomes saturated over time. Consequently practitioners, regulators, ecological and environmental engineers, and wetland scientists are sceptical and have concerns about the long-term performance of these systems, especially in terms of nutrient removal.

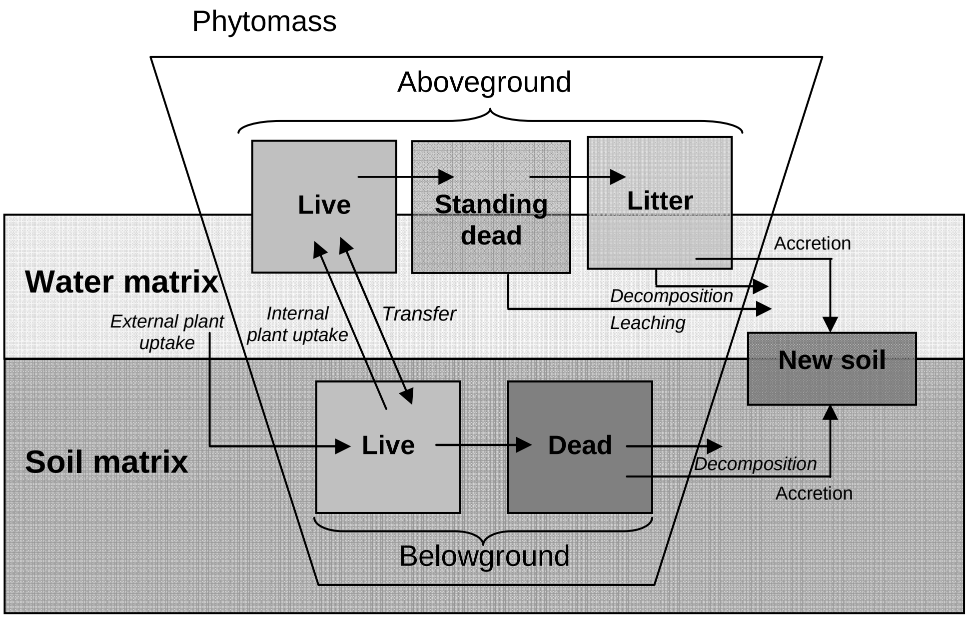


Figure 6-2. Transfer of materials and formation of new soils in the biosphere of integrated constructed wetlands (after Kadlec, 2005).

However as the wetland ages, creation of new soils and sediments occurs (Figure 6-2). The incoming solids settle and simultaneously plant litter decomposes. Both plant shoots and roots and rhizomes senesce and decay. Some portions of necromass (above-ground and below-ground) resist decay and form new accretions that are stable. New sediments comprise the remnants from plant stems, leaf debris, dead roots and rhizomes, and also from undecomposable fractions of dead algae, fungi, invertebrates and bacteria. These new stores of nitrogen and phosphorus are assumed to be decomposition resistant. The least studied aspect of nitrogen and phosphorus transfer in wetlands is these new stores (Kadlec and Wallace, 2009). The amount of accretion in wetlands has been quantified using atmospheric deposition markers (radioactive cesium or lead) or introduced horizon markers (feldspar or plaster). The accretion rates and nutrient burial for various wetlands in North America are shown in Table 6-1.

Kadlec (2009c) described the development of changes in sediment and soils in a study encompassing three decades of full-scale operation of a lake wetland treating wastewater. He found that long-term storages of nutrients in the studied wetland were dominated by the formation and accretion of the new soils. He concluded that after a period of approximately 5 years, virtually all of the added phosphorus was stored in new soils and sediments. The aim of this study was to assess the role of plants (*T. latifolia*) and sediment in full-scale integrated constructed wetlands treating farmyard runoff. The objective was to investigate the contribution of nutrient uptake by emergent macrophyte and nutrient storage in the accumulated sediments and new accretions in a mature integrated constructed wetland treating runoff from an animal farm and also to identify the presence or absence of ammonia-oxidising and denitrifying groups of organisms in wetland litter and sediments. The study builds on previous investigation of the treatment performance of full-scale integrated constructeds wetland treating agricultural wastewater (Mustafa et al., 2009).

Table 6-1. Accretion rates and nutrient burial in various free-water surface wetlands (adapted from Kadlec and Wallace, 2009).

Location	Method	Water nutrients (mg/l)		Accretion (cm/yr)	Burial ($g/m^2.yr$)		Reference
		Nitrogen (NH4-N)	Phosphorus (TP)		Nitrogen	Phosphorus	
Louisiana	Feldspar	0.05	<0.1	0.14	-	0.36	Rybczyk et al. 2002
Michigan	Lead 210	0.1	<0.1	0.20	-	0.24	Kadlec and Robbins, 1984
Everglades WCA2A	Cesium 137	0.3	0.1-1.0	0.16	11.6	0.06	Craft and Richardson, 1993
Sacramento, California	Visual	16	>1.0	1.50	44	0.51	Notle and Associates, 1998
Michigan	Resurvey	10	>1.0	1.80	56	13.7	Kadlec, 1997

6.3. Vegetation effects on nutrient cycling

Nitrogen. With regards to nitrogen processing, plants have two important effects. Plants follow a growth cycle that stores and release nitrogen seasonally and they also assist in the creation of new and stable residuals accreting in wetland systems. Nitrogen is contained in these residuals as part of their structure, and thus accretion is a burial process for nitrogen.

Ammonia and nitrate-nitrogen are the two most important forms of nitrogen that are generally used for assimilation. Ammonia uptake is favoured by wetland plants over nitrate uptake, except for cases in which incoming waters have high levels of nitrate. *T. latifolia* are very able to utilise either nitrate or ammonia (Brix et al., 2002) but there is a seasonal variation. For example, Brisson and Chazarenc (2008) reported that in summer *Carex rostrata* removed more ammonium than *T.* l*atifolia*, but *Typha* remover more total nitrogen removal in winter. Different plant species respond differently, for example Zhu and Sikora (1994) conducted a short-term study on several SSF gravel wetland microcosms and found that 70-80% of the entire nitrate loss was by plant uptake; species wise, 85% of the nitrate was taken by bulrush (*Scripus atrovirens georgianus*), 75% by cattail (Typha *latifolia*) and 70% by common reed (*P. australis*). Moreover, in newly constructed wetlands the development of new vegetation generates a demand for nitrogen that continues only during the growth period. For example, Sartoris et al. (2000) conducted a two-year study of a 9.9 ha FWS constructed wetland at Hemet, California and reported that as the plant coverage went from near zero (planted clumps at 1.2 m spacing) to about 80%, and vegetation density increased by 67%, there was a decrease in ammonia load from the wetland from 98 to 15 gN/m^2.yr. Subsequently, they found that plant uptake was a primary sink for nitrogen. Nevertheless, the biomass in wetlands has a finite capacity to retain nutrients. Kadlec (2009b) reported that at the Houghton lake wetland project, removal of nitrogen was controlled by processes involving vegetation and associated biota. Approximately, 20% of the added nitrogen was sequestered in the new, larger standing crops.

Phosphorus. With regards to phosphorus processing plants have two important effects: storage and release, and sediment accretion. Plants follow a growth cycle in which phosphorus is stored and released seasonally and they also assist in the creation of new and stable residuals accreting in wetland systems. Phosphorus is contained in these residuals as part of their structure, and thus accretion is a burial process for phosphorus. The biomass compartment in wetlands has a finite capacity to retain nutrients but accretion is a sustainable process. Kadlec (2009b) reported that at the Houghton lake wetland project, removal of phosphorus was controlled by processes involving vegetation and associated biota. Approximately, 14% of the added phosphorus was sequestered in the new, larger standing crops.

6.4. Role of soil and sediment in nutrient cycling

Soil/sediment is an important matrix in wetland systems. The soils in wetlands are termed as hydric, i.e. they are formed under conditions of saturation or flooding and are saturated long enough to develop anaerobic conditions in the upper part of soil (Reddy and DeLaune, 2008). Saturation or flooding conditions promote anaerobic biogeochemical processes. In wetlands, carbon is the primary driver for all the biogeochemical processes. Plants are an important source of organic matter and are important in regulating the biogeochemical wetland cycles. Wetland plants have unique characteristics like physiological, anatomic and morphological adaptations which allow them to adapt to wastewater stresses and to the oxygen-deficient conditions of saturated soils. Oxidised forms of chemical species dominate the drained systems while in flooded systems reduced forms dominate (Figure 6-3). The presence of reduced forms indicates anaerobic soil conditions and hence can be used as an indicator of hydric soils. The saturated soil conditions support microbial populations. Aerobic microbial populations are restricted to zones where oxygen is available while anaerobic microbial populations adapt to anaerobic environments.

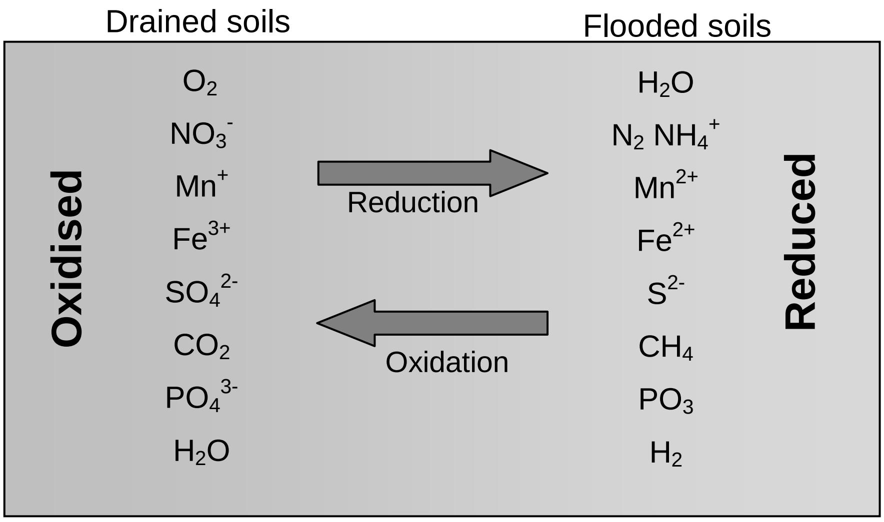


Figure 6-3. Redox forms of chemical compounds dominating in drained and flooded soils (after Reddy and DeLaune, 2008).

Later part in this chapter the presence of both aerobic and anaerobic nitrogen removing microbial communities is confirmed in the sediments of the studied wetland systems.

Nitrogen. Nitrogen processing by wetlands is complex. Various nitrogen reactions effectively process inorganic nitrogen through processes lsuch as nitrification, denitrification, ammonia volatilisation and plant uptake. In wetlands nitrification (ammonium oxidation) is restricted to the water column, surface aerobic layer and the aerobic root zone.The nitrification process in these zones is regulated by two factors: the fraction of soil volume with oxygen and availability of ammonium (Reddy and Patrick, 1994). The supply of oxygen to the aerobic soil-water interface depends on diffusion of oxygen through the water column or photosynthetic production by wetland vegetation (Figure 6-4). Denitrification occurs in anaerobic regions of the soil profile. During this process, the oxidation state of nitrate-nitrogen is reduced from +5 to 0, when nitrate-nitrogen is converted to molecular nitrogen

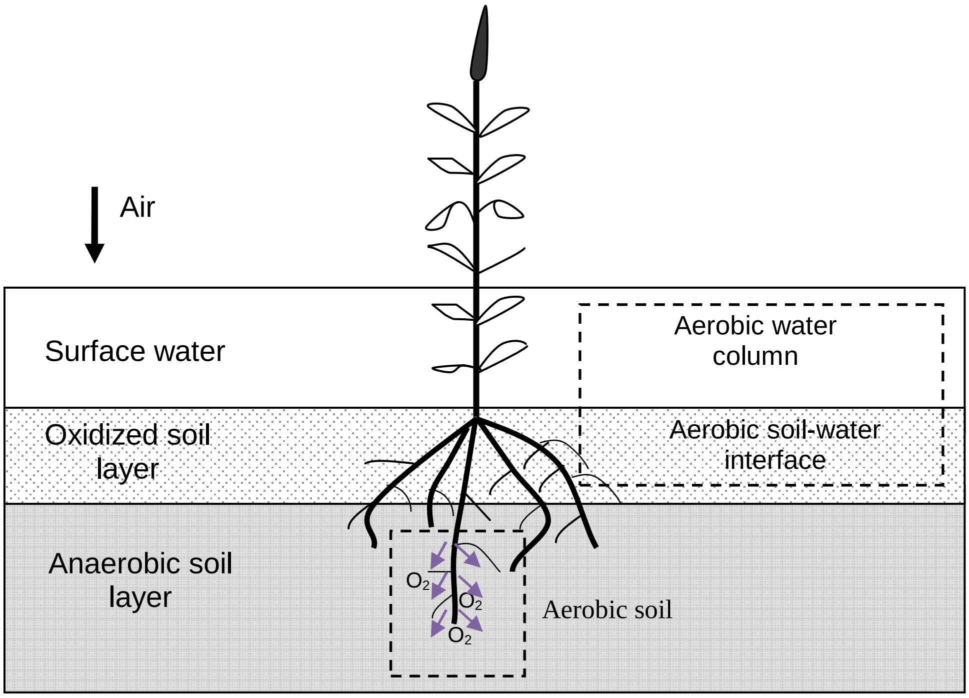


Figure 6-4. Dotted rectangles show examples of potential nitrification sites in surface flow wetlands.

with the transfer of five electrons.

In wetland systems there is exchange of nitrogen species (dissolved) between the soil and water matrices. For instance, nitrification in the aerobic soil layer is maintained by ammonium flux from the anaerobic layer and denitrification in the anaerobic soil layer is maintained by nitrate flux from the aerobic soil layer and water column. The ammonium flux occurs by advection, diffusion, mixing and bioturbation at and near the soil-water interface. The anaerobic soil layer contains high concentrations of dissolved ammonium and soluble organic nitrogen and this assists in establishing steep gradients between the soil and water matrices. The ammonium flux from the anaerobic zone where NH_4^+ concentration is high to the aerobic zone where concentration is low is controlled by factors such as the ammonia concentration gradient, ammonium production rate, soil reduction intensity,

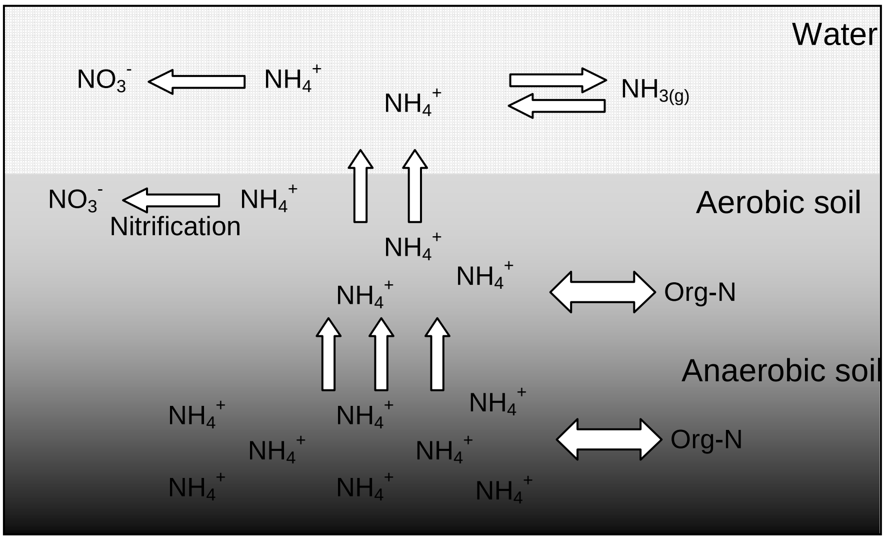


Figure 6-5. Schematic showing flux of ammonium from the anaerobic soil layer to the aerobic soil layer and overlying water column (after Reddy and DeLaune, 2008).

temperature, bioturbation and mixing, adsorption-desorption. The ammonium flux from the anaerobic soil layer to the aerobic soil layer and overlying water column is show in the Figure 6-5.

The nitrate flux form the aerobic soil portion is governed by the thickness of the aerobic soil layer, water column depth, mixing and aeration in the water column, nitrate concentration and temperature. The aerobic soil layer contains high concentrations of nitrate and this assists in establishing sharp gradients between the water and soil matrices. The nitrate flux from the aerobic zone where NO_3^- concentration is high to the anaerobic zone where concentration is low and the demand for electron acceptors is high is controlled by factors such as the nitrate concentration gradient, nitrification rate, reduction or denitrification rate, and bioturbation and mixing. The nitrate flux from the aerobic soil layer to the anaerobic soil layer and overlying water column and denitrification in the anaerobic soil layer is shown in Figure 6-6.

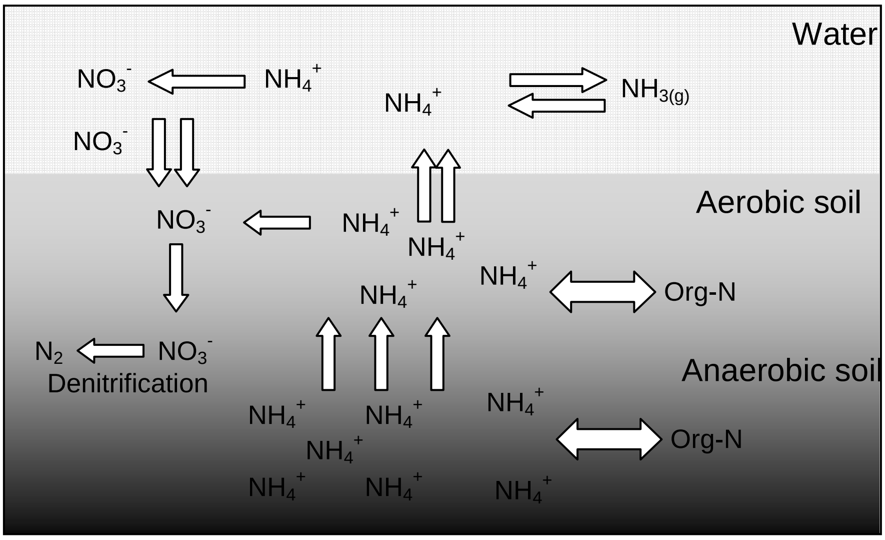


Figure 6-6. Schematic showing flux of nitrate nitrogen from the aerobic soil layer to the anaerobic soil layer and overlying water column (after Reddy and DeLaune, 2008).

Phosphorus. In wetland soils, phosphorus exists in organic and inorganic forms. Inorganic phosphorus is found in combination with aluminium, iron, calcium and magnesium. In alkaline soils, the availability of phosphorus is determined by the solubility of calcium compounds in which phosphorus is found. In acid soils, the aluminium and iron minerals control the solubility of inorganic phosphorus. With the passage of time, stable phosphate minerals are formed as a result of continuous interactions of soluble phosphorus with acid soils containing Fe and Al minerals in acid soils and Ca compounds in alkaline soils.

The main retention mechanisms for inorganic phosphorus are adsorption and precipitation. The retention of phosphorus in wetland soils is regulated by a variety of physicochemical properties including pH, redox potential, phosphorus loading, iron, aluminium, and calcium contents of soils, organic matter content and background soil phosphorus concentrations.

Organic phosphorus is derived from a variety of sources including microbes, algae, vegetation, detritus and organic matter in soil. In wetlands where soils predominantly organic, approximately 50-90% of the total phosphorus are in the inorganic form while in wetlands where mineral soils dominate approximately 10-50% of the total phosphorus is in organic form.

The availability of phosphorus for retention in soils and assimilation by plants is affected by the transport processes between soil and the overlying water column. Phosphorus is mobilised between sediment or soil and the overlying water by various transportation processes including advection, dispersion, diffusion, seepage, resuspension, sedimentation and bioturbation.

The concentrations of dissolved inorganic and organic phosphorus in soils are usually much higher than in the overlying water column. This results in a flux of these components (dissolved) from soil to the overlying water column. In wetlands there are sharp gradients between the soil and the overlying water column, thus suggesting a large flux of soluble phosphorus from soil to the overlying water column.

Particulate phosphorus (PP) generated in the water column settles on the soil surface, thus the flux of particulate phosphorus is from the water column to soil. The settling of PP provides long-term retention by wetlands and the flux of dissolved components into the water column provides phosphorus to biotic communities. Overall in wetlands, net P flux is always from the water column to the soils or sediments. The phosphorus retention in wetland is complex and involves intercoupled physical, chemical and biological processes that eventually retain phosphorus.

A review of wetland science literature identifies the role of accretion as the main long term storage for phosphorus but much of the literature does not address the method of soil-building as a way of nutrient immobilisation. For example, Craft and Richardson, (1993); Reddy et al., (1993); Rybczyk et al., (2002), Kadlec, (2009c) have identified accretion as a principal long-term storage for phosphorus. Others, for example USEPA, (1999) states: “New constructed and natural wetlands are capable of adsorbing phosphorus (P) loadings until the capacity of the soils and new plant growth is saturated”. Crites et al. (2006) state: “Adsorption and precipitation reactions are the major pathways for phosphorus removal…” (Kadlec, 2009c). The long-term study by Kadlec (2009c) clearly demonstrates that some authors have ignored the process of soil accretion for P storage. Nevertheless, interestingly USEPA (2000) correctly identifies accretion and burial as a sustainable mechanism. The burial of phosphorus in new accretions of sediments and soils provides a means for sustainable removal (Kadlec, 2009c).

6.5. Nutrient uptake by vegetation in an ICW

This section and the subsequent sections present the results of a study of nutrient accumulation in *Typha latifolia* and sediments in the first cell of ICW 11 where maximum contaminant reduction takes place.

The nutrient content of the above-and below-ground *Typha latifolia* tissues varied noticeably. In summer, at most locations the above-ground tissues had a higher nutrient content than the below-ground tissues (Figure 6-7). Conversely, in winter the below-ground tissues had a higher content than the above-ground tissues. Two-way ANOVAs (Table 6-2) were conducted to test the significance of differences over the two seasons and between various sampling locations. With regards to location of sampling points there were no significant differences observed for both TN (p=0.413) and TP (p=0.530).

Differences by sampling dates were found to be significant for TN only (p = 0.001). With the change in seasons, there is translocation of nutrients within the plant. Prior to senescence in autumn, the important ions are translocated from the shoots to the roots and rhizomes. The stored nutrients are then used in early spring growth (Garver et al., 1988).

Table 6-2. Two-way ANOVA results for nutrient content of *Typha latifolia* at the integrated constructed wetland (2008). The effects of location and date were tested.

Factor	d.f.	F-static	p-value
Total Nitrogen (Main effects)			
Location	8	0.687	0.413
Date	1	85.99	0.000
Interaction			
Location × Date	8	0.687	0.413
Total Phosphorus (Main effects)			
Location	8	0.401	0.530
Date	1	2.172	0.150
Interaction			
Location × Date	8	0.401	0.530

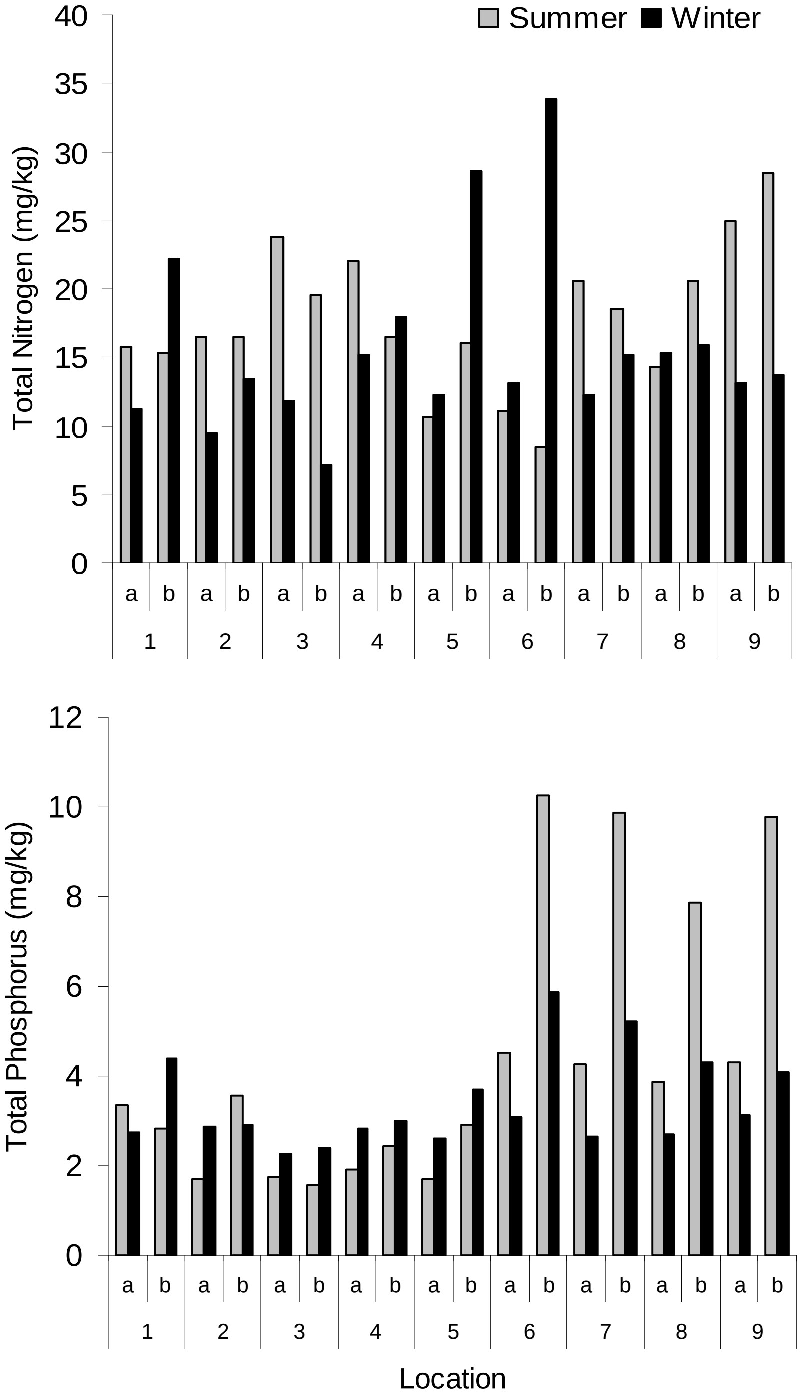


Figure 6-7 Average concentration of nutrients (N and P) in the above- and below-ground plant tissues of the studied integrated constructed wetland system during summer and winter (2008); a represents the stem and leaves, b represents the roots and rhizomes while numbers represent the sampling quadrats.

To find the relationship between surface water quality and plant nutrients (TN and TP) uptake, regression analyses were conducted. The regression analyses revealed no significant relationships between surface water nutrients and the concentrations of nutrients in plants (Table 6-3). Positive and significant relationships between NH_4^+ concentrations in the wastewater and TN concentration in plant tissues were observed, with R^2 values ranging from 0.89 to 0.38.

Table 6-3. Regression analyses for plant and water quality data at the studied integrated constructed wetland (2008).

Season	Dependent variable	Predictor	Significant difference	R^2	p-value
Summer	TN in A and B	NH_4^+	Above	0.89	0.008
			Below	0.5	0.01
	TN in A and B	$NO3^-$	Above	-0.09	0.52
			Below	0.48	0.35
	TP in A and B	MRP	Above	-0.47	0.65
			Below	-0.25	0.58
Winter	TN in A and B	NH_4^+	Above	0.88	0.006
			Below	0.38	0.03
	TN in A and B	$NO3^-$	Above	-0.44	0.64
			Below	-0.57	0.69
	TP in A and B	MRP	Above	-0.47	0.65
			Below	-0.89	0.82

A previous study of the seasonal variations in ICW performance for nutrient removal indicated high ammonia-nitrogen efficiencies during spring and summer (Mustafa et al., 2009). The sampling for this study was carried out in mid summer, a period when the plants had fully grown and utilised the nutrients in rebuilding their tissues. Gottschall et al. (2007) found significant relationships between NH_4^+ wastewater concentrations and TN in plants.

The ICW system studied here was dominated by ammonia-nitrogen, and previous studies by various researchers have shown that NH_4^+ is the preferred form of nitrogen for cattails (*Typha latifolia).* The first cell of this wetland system had the highest ammonia-nitrogen concentrations and it is likely that during the growing season, i.e. in spring when the plants rejuvenate, plant uptake was driven by wastewater NH_4^+ concentrations. A study by Gottschall et al. (2007) also confirms this. Moreover, Brix et al. (2002) found higher nutrient uptake and growth rates by *T. latifolia*, when supplied with NH_4^+ rather than NO_3^-, as the exclusive nitrogen source.

Figure 6-8 represents nutrient content changes in tissues of *T. latifolia*. There was a marked difference among above- and -below ground tissues during the two sampling times. More nutrients (N and P) were stored in the below-ground tissues in summer as compared to winter for the obvious reason that there is translocation of nutrients. Gotschall et al. (2007) also found nutrient content changes in plant tissues with respect to seasons. Differences by sampling date were found to be significant for TN only and not for TP (Table 6-2). The results corroborate with those of Gotschall et al. (2007).

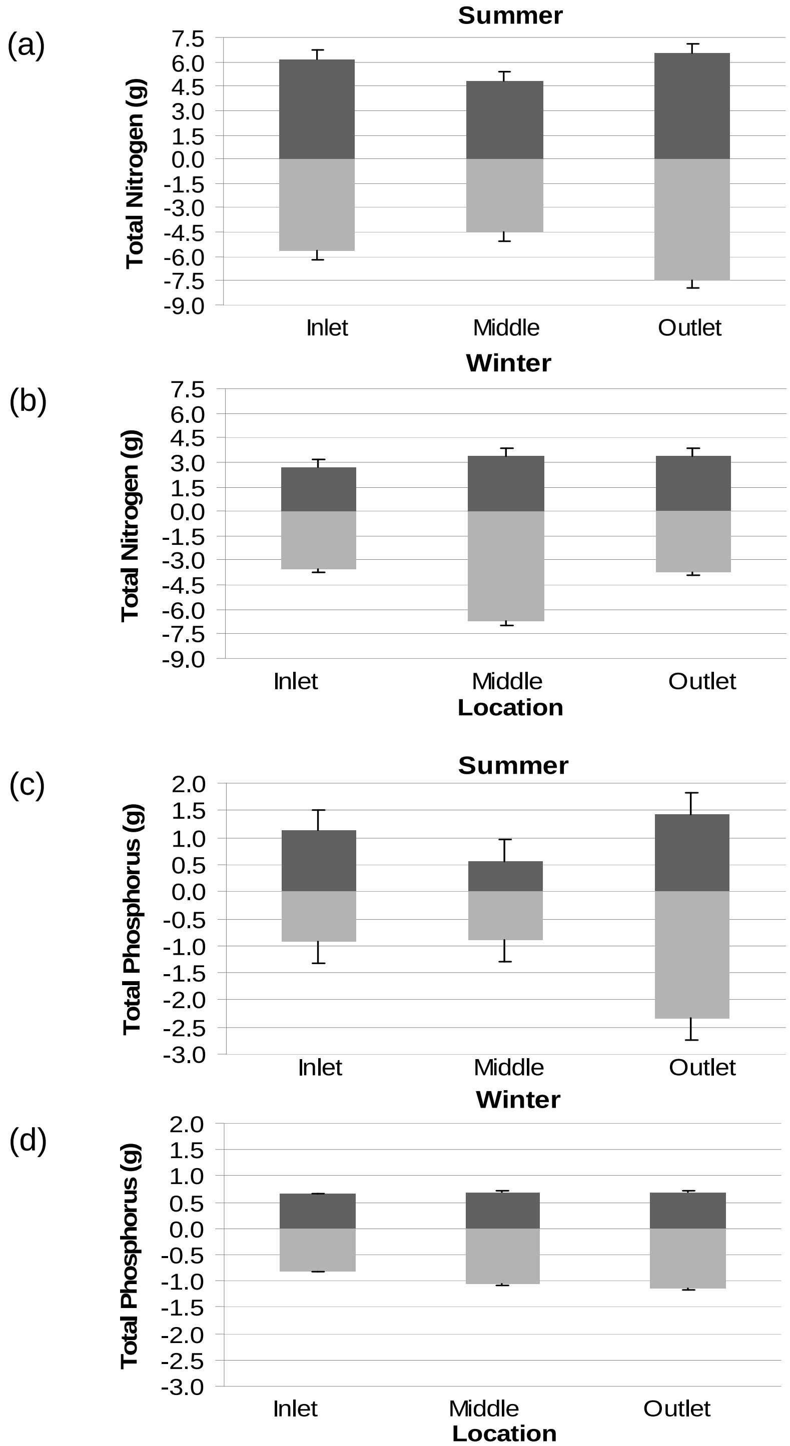


Figure 6-8. Changes in total nitrogen and total phosphorus contents of *Typha latifolia* plant tissues in 2008 in cell 1 ICW 11. Above-ground tissues are shown as positive values while below-ground tissues are shown as negative values. Nutrient content is expressed in g/0.25m^2, representing the average of sample quadrats (n=3); standard error estimates are included. (a) Total nitrogen in summer (b) Total nitrogen in winter (c) Total phosphorus in summer (d) Total phosphorus in winter.

6.6. Nutrients in litter

Mean total N and P concentrations in litter were 17.3 g/kg and 1550 mg/kg, respectively. Debusk and Reddy (2005) conducted a study of litter decomposition and nutrient dynamics in the Everglades marsh and found mean nutrient concentrations in cattail and saw grass litter were 22 g/kg and 1153 mg/kg, respectively. Some nitrogen is utilised by plants and remaining nitrogen uptake is either leached from biomass or necromass into water in a soluble form or remains in dead plants (Kadlec, 2005). The poorer autumn performance of integrated constructed wetlands in ammonia removal (Mustafa et al., 2009), is most likely due to return of soluble N to the water column. However, partially decomposed litter of cattail is characterised by low N and P content, primarily due to leaching. When this partially decomposed litter is added to the wetland soil surface it acts as a strong nutrient sink because of its high C as compared to C in soil layers below. Debusk and Reddy (2005) reported that the high sink strength of litter in the Everglades marsh was due to the combination of an extremely low nutrient content and relatively high C quality.

6.7. Ammonia-oxidising and denitrifying bacteria in litter and sediments

Nitrogen transformation processes

In comparison to ammonia-oxidizers, denitrifiers were more abundant in most of the litter and sediment samples (Table 6-4). Since the nitrate concentrations in water within the ICW systems were low, it is likely that oxygen and nitrate served as electron acceptors in the lower layer of the wetland cells, and this might have promoted the growth of denitrifying bacteria. Each ICW system contained denitrifying bacteria, but they were present in varying proportions. For example, ICW 11 had a lower proportion of denitrifying bacteria than both ICW 3 and ICW 9. Samples analysed from ICW 3 and ICW 9 did not indicate the presence of ammonia-oxidising bacteria. Most denitrifiers are heterotrophs. The supply of organic carbon by macrophytes raised the overall heterotrophic activity, leading to the consumption of oxygen. Thus, oxygen availability in the sediment was reduced, and subsequently denitrification was supported (Bastviken et al., 2005). Ammonia-oxidising bacteria were present at ammonia-nitrogen concentrations between approximately 5 and 20 mg/L. Denitrifying bacteria were observed at nitrate-nitrogen concentrations between 0.1 and 4.5 mg/L. In comparison to ammonia-oxidising bacteria, more denitrifiers were present in most ICW systems, while ammonia-oxidising bacteria were found in samples collected from ICW 11 only.

Table 6-4. Bacterial community abundance in integrated constructed wetland (ICW) litter and sediment samples compared to the corresponding mean ammonia-nitrogen and nitrate-nitrogen concentrations in surface water.

ICW no.	Ammonia-oxidising bacteria[a]	Denitrifying bacteria[a]	Ammonia-nitrogen (mg/L)	Nitrate-nitrogen (mg/L)
3	0	73	13.95	0.27
9	0	80	5.20	1.69
11	27	53	9.73	1.73

[a]Relative presence (%) of bacterial community (0 = absent; 100 = present).

Comparison of ammonia-oxidising and denitrifying communities

Interesting differences between three selected ICW example sites are discussed in this section (see Table 6-4). Samples analysed from ICW 3 and ICW 9 did not indicate the presence of ammonia-oxidising bacteria. ICW3 had lower denitrifying bacteria numbers than ICW 9. Since, ICW 9 had a higher aquatic plant cover density than ICW 3, it is suggested that the decaying macrophytes within ICW 9 increased organic matter which is a source of carbon and therefore energy for denitrifying bacteria. Samples taken from ICW 11 indicated the presence of ammonia-oxidising bacteria. There was a reduced availability of organic matter at the bottom of ICW 11, which led to decreased numbers of heterotrophic bacteria and consequently created conditions in which ammonia-oxidising bacteria proliferated.

ICW 9 contained a higher proportion of denitrifying bacteria than ICW 11 and had a higher plant density than ICW 11. The high number of denitrifying bacteria was linked to high concentrations of nitrate. The decaying plants contributed to organic matter that became a source of energy for denitrifying bacteria.

6.8. Nutrient accumulation in soils and sediment

The amount of sediment accumulation, rate of build-up, removal frequency, sediment composition, and management of removed sediment are the most important items of information for the design engineer (Scholz et al., 2007). Surveys to determine sediment accumulation was conducted on two occassions. The first survey was conducted in 2006, five years after commissioning of the integrated constructed wetland (ICW 11). Sediment accumulations were measured for ICW 11 to obtain information on the nature and rate of sediment build-up in the ICW systems. The second survey was conducted in 2008, at the time of core sampling. The mean depth of sediment for the ICW 11 was 13.6 ± 10.12 cm.

The first wetland cell of ICW 11 which is approximately 100 cm deep: allowing a minimum water depth of 20-cm and a further 20-cm freeboard, this leaves 60 cm depth for sediment accumulation before removal is required. Therefore, at a sedimentation rate of 3 cm per annum, removal would be required at intervals of approximately 20 years if the sediment was equally dispersed throughout the entire wetland. This was not the case, however, as the rate of sedimentation varied considerably between individual wetland cells, and the removal frequency required for each cell will therefore vary accordingly. Due to variations in rates of sediment accumulation, the desludging frequency will also vary from cell to cell in any ICW system. The first cell has the highest rate of sediment accumulation. The sediment depth in the first cell after 5 and 7 years of operation was 35 cm and 45 cm respectively. This shows that desludging of the first pond appears to be necessary approximately every ten years.

The surveys conducted to determine sediment accumulation rate showed increases in depth of the wetland soil. The soil investigation results at the time of construction indicated the presence of clay in the substratum of the ICW. Since the start of its operation there was an average increase in depth of 0.45 m of new material in the first cell of the studied wetland system. Over this 7-year operation period the accretion rate was approximately 6.4 cm/yr.

Soil cores from ICW 11 collected in summer and winter of 2008 were divided into sections by depth and analysed for nutrients. N and P were stored in the wetland soil. After 7 years of operation, the levels of nitrogen and phosphorus in wetland soils were found to be 21.9 ± 5.01 g/kg and 3.41 ± 0.85 g/kg respectively

(Table 6-5). The average NH_4-N and TP concentration in the influent were 38.6 mg/l and 14.4 mg/l, respectively.

The concentrations of both nitrogen and phosphorus varied with depth. The top sediments had higher concentration of nutrients while the levels decreased progressively with depth for most of the samples. There were differences observed with respect to seasons. Higher nutrient concentrations were observed in summer than in winter (Table 6-5). This may be because of the changes in hydrological regimes during the two seasons. There was much reduced or no outflow during summer and much higher flows in winter compared to summer. Song et al. (2007) conducted a wetland microcosms study and found that rewetting of soil followed by drying released phosphorus by desorption of previously adsorbed phosphorus.

The first cell from which core samples were collected received the highest concentration of nutrients (N and P) which has resulted in higher biomass. Compared to other cells, the first cell had an obviously higher biomass resulting in a higher necromass and more accretion. N and P concentrations were higher near the inflow and varied with distance (Table 6-5). An average of 70,000 m^3 of wastewater entered the integrated constructed wetland during 7 years of operation. The influent contained 15 mg/l of TP, 10.5 mg/l MRP, 140 mg/l of TN, 41.9 mg/l of dissolved inorganic nitrogen (DIN); 38.6 mg/l of NH_4-N, 2.5 mg/l of NO_3-N and 0.76 mg/l of NO_2-N. N and P were successfully stored in the wetland soils and sediments. Over the 7 year period, approximately 1046 kg of phosphorus entered the wetland system. A total of 63 kg were exported, thus creating a mass removal of 94%. 780 kg of phosphorus was stored in the first cell, thus the soils and sediments in the first cell stored 74% of the incoming phosphorus load.

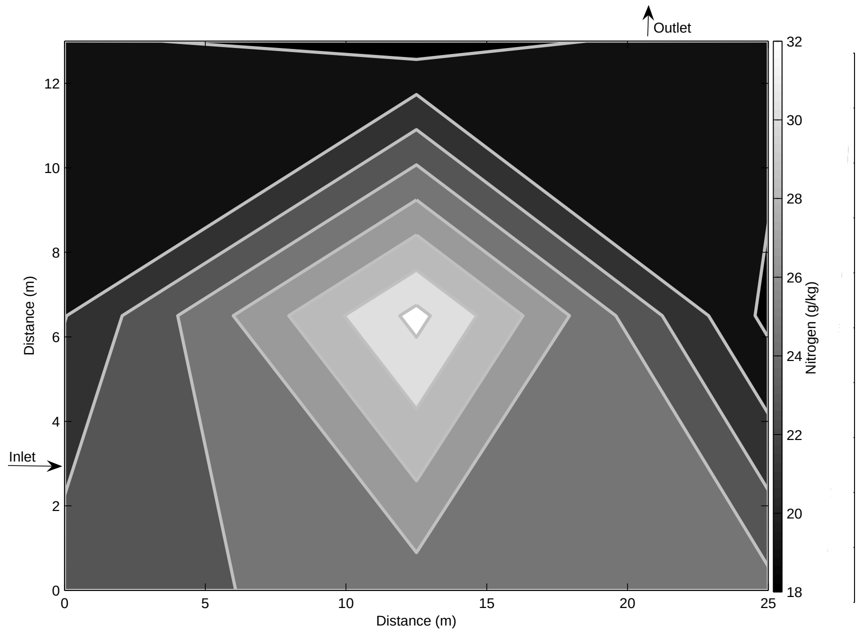
Table 6-5. Concentration of nutrients (N and P) at various depth ranges in the soils and sediment of the studied integrated constructed wetland system ICW 11.

Season/sampling point	Distance from inflow (m)	N g/kg				P g/kg			
		0-15 cm	15-30 cm	30-45 cm	0-45 cm	0-15 cm	15-30 cm	30-45 cm	0-45 cm
Summer									
1	8.5	15.8	5.74	3.90	25.4	2.99	1.15	0.88	5.02
2	6.0	14.1	11.9	4.31	30.3	2.14	1.89	0.78	4.81
3	8.5	16.0	11.8	1.83	29.6	2.19	1.64	0.25	4.08
4	20	8.17	9.83	1.67	19.6	0.82	1.05	0.68	2.55
5	18	18.5	16.1	4.26	38.8	2.17	1.86	0.84	4.87
6	20	9.23	4.32	2.14	15.6	2.31	1.56	0.99	4.86
7	35	8.50	6.12	4.96	19.6	0.98	0.91	0.74	2.63
8	33	6.27	5.12	4.04	15.4	0.90	0.88	0.65	2.43
9	35	9.74	7.22	3.92	20.8	2.00	1.75	1.11	4.86
Average ± standard deviation, N (g/kg) 23.9 ± 7.79 and P (g/kg) 4.01 ± 1.14									
Winter									
1	8.5	9.09	7.16	4.50	20.7	1.82	1.48	0.71	4.01
2	6.0	9.75	6.27	3.57	19.6	1.80	1.14	0.55	3.49
3	8.5	8.24	7.41	3.96	19.6	1.80	1.22	0.56	3.58
4	20	10.6	5.32	4.25	20.2	1.39	0.85	0.68	2.92
5	18	15.7	8.32	2.32	26.3	2.02	1.09	0.18	3.29
6	20	14.07	4.11	0.99	19.2	1.31	0.53	0.16	2.00
7	35	8.24	5.16	3.81	17.2	1.12	0.77	0.47	2.36
8	33	9.23	7.25	1.99	18.4	0.94	0.44	0.15	1.53
9	35	8.63	5.31	3.42	17.4	1.02	0.62	0.41	2.05
Average ± standard deviation, N (g/kg) 19.8 ± 2.71 and P (g/kg) 2.80 ± 0.85									
Average of Summer and Winter, N (g/kg) 21.9 ± 5.01 and P (g/kg) 3.41 ± 0.85									

Accumulation of nutrients in the soil of the first cell of ICW 11 is depicted in Figure 6-9. The maximum nitrogen accumulation is in the centre while overall the figure shows an almost symmetrical distribution pattern. For phosphorus, the distribution is asymmetrical with maximum concentrations dispersed in a triangular shape from the inlet. The different spatial patterns of nutrient accumulation in sediments show that variations may have been influenced by the wetland hydraulics. For nitrogen, low values (18-22 g/kg) are found at points close to the inlet and outlet, while for phosphorus medium values (3.5 g/kg) are found near the inlet with comparatively low values near the outlet (2.5 g/kg). Generally, nutrient accumulation depends on factors like hydrology, hydraulics and vegetation.

Dolan et al. (1981) found in a pilot-scale treatment wetland that soil was the most significant compartment for P storage, followed by plant roots and rhizomes, and plant litter. Most of the P stored in wetland surface soils is in organic matter (Reddy et al., 1998; Axt and Walbridge, 1999; Graham et al., 2005). Dunne et al. (2007) found that storage of phosphorus in surface soils (0–10 cm) was greatest (> 87%) relative to the sum of all other ecosystem compartments. Debusk and Reddy (2005) studied nutrient dynamics in the Everglades marsh and reported mean total N concentrations in soil of 28 and 29 g/kg for the 0-10 and 10-30 cm depth intervals while P concentrations were 1.15 and 0.64 g/kg, respectively. Kadlec (2009c) reported a long-running study (30 years), discussing the role of wetland soils and sediments in removing nutrients at Houghton lake wetland project. He reported that over that period of time, 30 cm of new soil developed which stored nutrients.

a)



b)

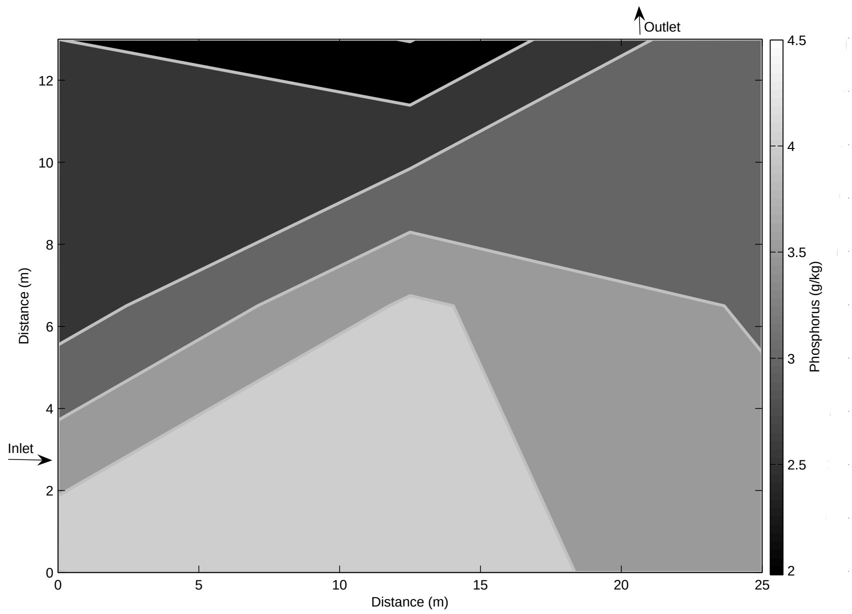


Figure 6-9. Contour map showing accumulation of nutrients in sediments of cell1, ICW 11. (a) nitrogen (b) phosphorus

Phosphorus concentrations were approximately 2000 mgP/kg and the nitrogen concentration was 2-3% DW. The results reported here also show that soil is the most important wetland component for long-term storage of phosphorus.

Tanner et al. (1998) reported that under suitable conditions in surface-flow constructed wetlands, high rates of plant-derived organic matter accretion may provide substantial long-term immobilisation and storage of nutrients, and a sustained carbon supply for microbial denitrification. In general, the upper soil of the wetland (0-15 cm) had higher phosphorus concentrations than the bottom layers (Table 6-5). In these systems the wetland plants were not harvested, resulting in the accumulation of organic matter. Some of the detritus decomposes but recalcitrant portions have most likely resulted in accretion of new sediments. The accretion of new sediments assists in the long-term sequestration of phosphorus (Wallace and Knight, 2006). Nitrogen in this study was also stored in the soils and sediment, approximately 50% (in the first cell). Borin and Tocchetto (2007) evaluated the performance of a constructed surface flow wetland treating diffuse N pollution from croplands and estimated the five year water and nitrogen balances. They found that the wetland soil accumulated more than quarter of the incoming nitrogen load.

Moreover, the integrated constructed wetland subsequently changes from an initially mineral based system to an organic based system with higher phosphorus removal capacity. In acid soils, inorganic P can be adsorbed onto iron (Fe) and aluminium (Al) oxides (Faulkner and Richardson, 1989; Rhue and Harris, 1999). In organic soils, such as those present in wetland ecosystems, inorganic P can react with Al and Fe associated with organic matter (Rhue and Harris, 1999). The organic matter accumulation and subsequent accretion are important for long-term P retention (Kadlec, 1989; Craft and Richardson, 1993; Mitsch and Gosselink, 1993; Pant and Reddy, 2001). Five upper soil layer samples from ICW 11 were randomly selected and the iron content was determined. The iron content in the samples showed a strong relation with P (R^2=0.74). Dunne et al. (2005a), also reported that phosphorus sorption was significantly related to the iron (Fe) content of soils.

Carbon in litter and sediments. Carbon accumulation in mg/kg was measured for six samples in the direction of flow. C concentrations decreased from the inlet towards the outlet zone. Average C accumulation in the upper 6 cm of the soils and sediments was 181.3 ± 27.5 g/kg. This is high when compared to values reported in literature. Vohla et al. (2007) reported C concentrations in soil samples collected from a horizontal subsurface flow constructed wetland of 2.2 to 5.7 g/kg. The reason for lower levels of carbon accumulation in the HSSF CW may be due to factors such as the characteristics of the soil, which was coarse sand and the type of wastewater, septic tank effluent. Conversely ICW 11 has soil that is rich in organic matter and wastewater from farmyard which is rich in nutrients. The litter on top of the sediments had a high C content of 362.2 ± 75.5 g/kg. Obviously the litter from decaying macrophytes provides considerable surface area for the attachment of biofilms, and plays an important role in supporting microbial processes in wetlands (Brix et al., 1994). Also wetland sediments have been shown to be important habitats for microorganisms supporting denitrification (Bastviken et al., 2003). Hence, sediment and associated litter are components that support microbial-mediated processes. Chapter 7 on microbial ecology reports that the litter component supports a more diverse microbial community than sediments. During cell synthesis, microbes assimilate carbon and nitrogen. For aerobic decomposition of plant detritus a C:N ratio of 25 is required and the litter in this study had a C:N ratio of >40 for most of the collected samples.

According to Reddy and Laune (2008) if the C:N ratio of litter >25 then net immobilisation of inorganic nitrogen will occur as a result of assimilation of nitrogen by microbes during decomposition. In this case immobilisation will be greater than ammonification. Immobilisation is a process in which inorganic nitrogen is converted into organic forms while ammonification is a process in which organic nitrogen is broken down to ammonium (Figure 6-10).

Ammonification/N-mineralisation

Organic N *Ammonium*

$$^{-}HOOC - \underset{\substack{|\\ H}}{\overset{\substack{R\\ |}}{C}} - NH_2 \longrightarrow NH_4^+$$

N-immobilisation

Inorganic *Organic N*

$$\begin{matrix} NH_4^+ \\ NO_3^- \end{matrix} \longrightarrow {}^{-}HOOC - \underset{\substack{|\\ H}}{\overset{\substack{R\\ |}}{C}} - NH_2$$

Figure 6-10. Transformation and conversion processes of ammonia in soils (after Reddy and DeLaune, 2008). R is a radical attached to carbon while NH_2 is the amine functional group.

6.9. Nutrient storage in vegetation and soils

The total nutrient storage in ICW 11 was calculated (Table 6-6). There was a decrease in plant nutrient storage from summer (July) to winter (December). For TN, the reduction was about 31% from 50.9 kg in summer to 35.2 kg in winter. For TP, the reduction was around 44.5 % from 13.7 kg in summer to 7.6 kg in winter. Overall TN and TP storage in plants decreased from summer to winter. Nutrient uptake studies have been conducted at various geographical locations. Comin et al. (1997) studied naturally restored wetlands treating agricultural runoff in Spain, Europe and found that the plant uptake (T. *latifolia*) accounted for over 66% of nitrogen removal. Greenway and Woolley (2000) conducted a study on a constructed wetland treating municipal wastewater in Australia and found 24-47% of TN and 47-56% of DP removal was due to plant uptake. Newman et al. (2000) studied the seasonal performance of a dairy wastewater system in Connecticut, USA and found that

Table 6-6. Nutrient storage estimates in vegetation and soil/sediments in ICW 11.

	Summer kg	Winter kg	Difference (Summer-Winter) kg
Total nitrogen			
Plant	50.9	35.2	15.7
Soil and sediment	6057	4201	1856
Total	6107.9	4236.2	1871.7
Total phosphorus			
Plant	13.7	7.6	6.1
Soil and sediment	899	662	237
Total	912.7	669.6	243.1

plant uptake (T. *latifolia* and *Phragmites australis Cav.*) accounted for approximately 3% of nitrogen removal. All the three case studies discussed above were conducted on wetlands which were all under five years old, in which vegetation is still being established.

One of the similar evaluations of plant nutrient uptake comes from Gottschall et al. (2007). They conducted a study to elucidate the role of plants in the removal of nutrients at a well-established constructed wetland treating agricultural wastewater since 1996. Overall, plant uptake accounted for 0.7% of TKN removal. When considered separately, 9% of TKN and 5% of TP removal were due to an increase in plant storage in cell 2 of the wetland system. In this study, plant uptake in the first cell accounted for 0.4% of TKN removal and 0.8% of TP removal. The lower nutrient storage by plants in cell 1 could be as the result of Brix's (1997) argument that plant uptake is only significant under low nutrient loading conditions. It is clear that the first cell receives the most contaminated influent and highest load as the untreated wastewater enters the system and the plants in this part of the wetland system are most likely to store small amounts of nutrient as compared to other cells.

Analysis revealed that total nitrogen in the soil samples of ICW 11 was higher that the total phosphorus. The storage of nitrogen in new soils and sediments was approximately 6 times more than phosphorus. Kadlec (2009a) found that nitrogen accretion in new soils and sediment at Houghton lake wetland was 10 times more than phosphorus. The new soil layers were formed in the studied integrated constructed wetland system through the accumulation of incoming solids entering along with the nutrient enriched wastewater, macroscopic accretions (macrophyte detritus) and microscopic accretions (algal, bacterial and microbial detritus). Consequently, new residuals from various sources were deposited on the wetland soil surface (Kadlec, 2005).

Since 2001, the wastewater discharge added approximately 9760 kg of nitrogen to ICW 11. A total of 223 kg were exported, so mass removal was 97.7%. 5175 kg of nitrogen was stored in the first cell, thus the soils and sediments in the first cell, i.e. 52% of the incoming nitrogen load.

6.10. Summary

This chapter described the role of plants and sediment in removing nutrients from an integrated constructed wetland treating agricultural wastewater for more than 7 years. More N and P were stored in wetland soils and sediments than in plants. The first cell had the highest depth of sediment accumulation (45 cm). Over the 7-year operation period the accretion rate was approximately 6.4 cm/yr. With regards to management, desludging of the first wetland cell of ICW 11 appears to be necessary in 2011. An average of 10,000 m^3 per year of wastewater entered the integrated constructed wetland. Approximately, 74% (780 kg) of the phosphorus and 52% (5175 kg) of the nitrogen that entered the wetland system was stored in the wetland soils and sediments. Plants stored a small percentage of nutrients as compared to soils (<1% both N and P). This study demonstrates that the soil component of a mature wetland system is an important and sustainable nutrient storage component.

Chapter 7 Microbial ecology

7.1. Introduction

This chapter reports on the use of novel molecular methods to gain an insight into nitrogen removing microbial communities present in two full-scale integrated constructed wetlands. The rationale of the research was to explain spatial variation of the nitrogen-removing bacterial community structure within the wetland litter and sediment using denaturing gradient gel electrophoreses (DGGE). This was the first field-scale investigation of nitrogen removing microbial communities for full-scale ICW. The contents of this chapter have been submitted as a manuscript to Water Research.

The research investigated two hypotheses. The first was that the community composition is likely to change throughout the different stages of wastewater treatment within an ICW. The second hypothesis was that any nitrogen removing community composition similarities measured can be explained by the chance occurrence of the same organisms in two wetland systems. In other words, the community composition similarities would be no greater or smaller than if they had been randomly drawn from the same source of taxa. The aims of this chapter are as follows:

- to characterise and compare microbial diversity responsible for nitrogen removal within different wetland cells of selected ICW;
- to characterise and compare microbial diversity responsible for nitrogen removal in different components (sediment and litter) of wetland systems; and

- to compare the species composition of nitrogen removing bacteria in different wetland systems within a theoretical framework.

7.2. Molecular microbial tool box

Traditionally, microorganisms and their physiologies have been studied in isolation by pure culture techniques. But with the passage of time researchers realized that these techniques are biased since only a small fraction (1%) of the total cell population is cultivable (Amann et al., 1995). Therefore, methods which did not involve cultivable techniques were needed to study microbial diversity.

Woese and colleagues researched and produced seminal publications which revolutionized the world of microbial ecology (Woese and Fox, 1977; Woese, 1987, Woese, 1994). Their pioneering findings motivated the development of new molecular techniques which made it possible to study the structure, function and dynamics of microbial communities. Presently there are a number of nucleic acid-based techniques to determine the genetic diversity of microbial communities in the environment. The advent of these techniques has made it possible to evaluate spatial and temporal changes in microbial communities. In order to evaluate these changes, samples from different locations have to be analysed or microbial ecosystems need to be investigated over days, months and years. A summary of commonly applied molecular techniques to determine abundance and diversity in environmental samples is shown in Figure 7-1.

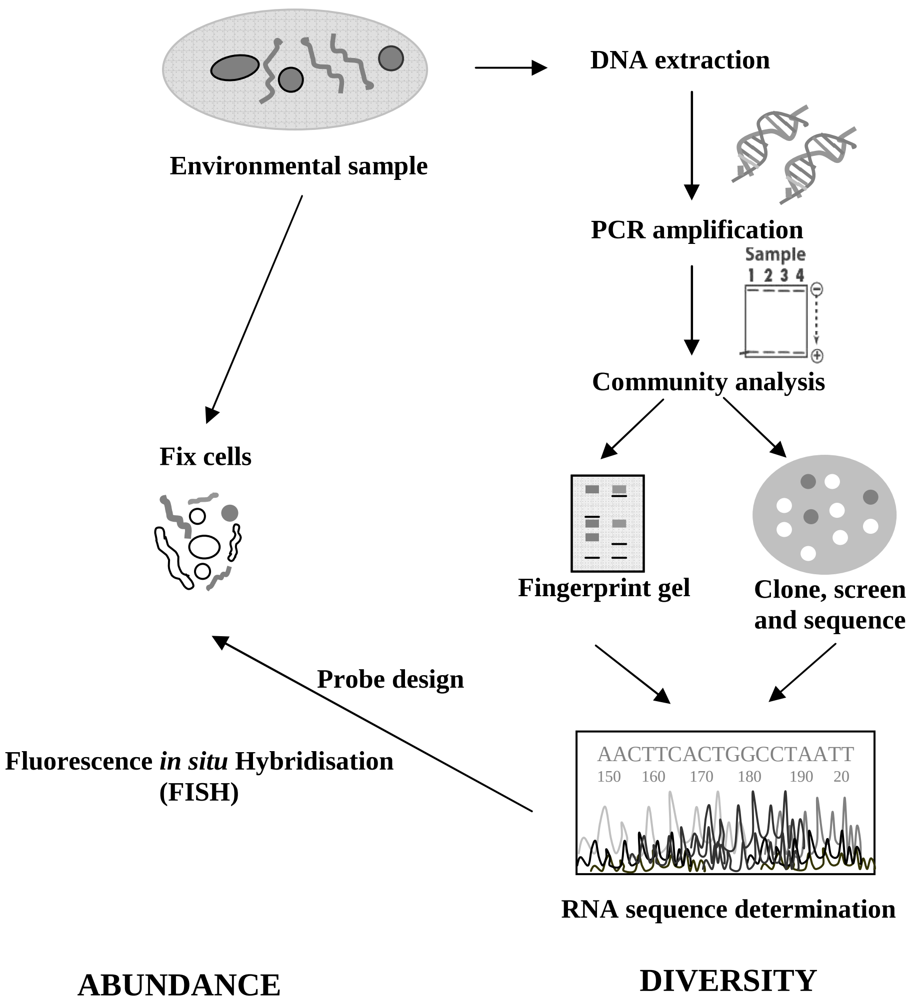


Figure 7-1 Summary of most commonly applied techniques for the study of microbial diversity, community structure, identity and abundance (adapted from Head et al., 1999).

The analysis of bacterial communities using nucleic-acid based techniques has made it possible to overcome the biases of cultivation-dependent methods commonly known as the "great plate count anomaly". For this purpose, specific genes are used to assess biodiversity in the microbial community. Specific genes are often linked to specific organisms and the presence of a particular organism can be confirmed by the detection of a specific gene. The most commonly applied techniques for microbial studies are shown in Figure 7-1. The application of cloning and sequencing has been successfully applied to explore microbial communities, but it is laborious and time consuming to analyse a large number of samples. However, genetic fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE) are remarkably suitable for comparison of large number of samples, especially for studies focussing on spatial and temporal variation of bacterial communities in relation to environmental factors. In microbial ecology, Muyzer et al. (1993) first introduced DGGE as a convenient tool for the assessment of microbial diversity in natural samples. There are many environmental studies in which nitrogen removing bacteria have been investigated by various fingerprinting methods including DGGE, restriction fragment length polymorphism (RFLP) and terminal restriction fragment length polymorphism (T-RFLP).

It is important to understand how microorganisms and mixed microbial consortia function in various engineered and natural environments since this can provide an opportunity for engineers to manipulate these environments to induce the necessary metabolic responses to accomplish removal of various pollutants. With this enhanced understanding, models can be developed for the design of these treatment systems, leading to improvements in design and operations procedures.

Molecular tools are helpful to explain chemical movement, fate, and impact in all engineered and natural systems. Additional research opportunities with molecular biology tools include assessing the impact of environmental conditions and anthropogenic inputs on microbial community structure, assessing the assimilative capacity of systems in the context of bioremediation, and developing approaches for predicting rates and extent of biodegradation. Recently, there have been enormous advances in methods for detection of microorganisms in the environmental matrices. Molecular methods are now being successfully applied to study the microbial ecology of wastewater treatment systems. For example, studies of different bacterial groups important in activated sludge wastewater treatment systems (autotrophic ammonia-oxidizing bacteria - AOB - and mycolata involved in foaming) have revealed how differences in operational parameters and plant configuration affect the composition and abundance of bacterial communities and may result in damaging effects and process failure (Head et al., 1999).

Extraction of nucleic acids

Nucleic acids are found in the cell in two forms: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). They are composed of monomers called nucleotides. A nucleotide is composed of three components: a molecule of phosphate, a molecule of sugars either ribose in RNA or deoxyribose in DNA, and a nitrogen base (adenine, guanine, cytosine, thymine or uracil). DNA and RNA are informational macromolecules as they contain genetic information in their sequences. Extraction of DNA or RNA from environmental samples is the first step in PCR based methods in the molecular toolbox. The total community DNA is extracted using commercially available kits that yield highly purified DNA from soil and other complex habitats (Madigan and Martinko, 2006).

Polymerase chain reaction (PCR)

PCR was developed by Kary Mullis (1983). PCR is an *in vitro* technique that multiplies DNA molecules by up to a billion fold (Madigan and Martkin, 2006). It depends on the natural DNA replication mechanism to amplify a specific sequence of DNA. It is a rapid and simple method for amplifying selected molecular markers by using specially designed primer combinations. A primer is a nucleic acid strand that serves as a starting point for DNA replication, and is required because most DNA polymerases (i.e. enzymes that catalyze the replication of DNA) cannot synthesize *de novo* DNA.

Each PCR round has three stages: denaturation, annealing and extension. In the first stage, known as denaturation, the hydrogen bonds that hold together the two strands of DNA are broken through heating (between 93ºC and 95 ºC). In the second stage, annealing, the temperature is lowered (within 40ºC and 72ºC) to an ideal level so that the primers can anneal to their target sequences (Sambrook et al., 1989). The last step is extension, during which the temperature is increased to the optimal (usually 72 ºC), which extends the DNA fragments to which the primers are annealed.

Denaturing gradient gel electrophoresis (DGGE)

In microbial ecology, DGGE was first introduced by Muyzer et al. (1993) as a convenient tool for the assessment of microbial diversity in natural samples. DGGE is a powerful tool for studying the community structure and sequence diversity of microbial systems over space and time. It is a method based on electrophoresis of PCR amplified 16S rDNA fragments in polyacrylamide gels. The gels contain a linearly increasing gradient of denaturant which cause the dissociation of the double stranded DNA based on differences in nucleotide composition. The result is a gel with several bands located at different points vertically in the gel. To determine the identity of organisms, individual bands can be excised and sequenced. The sequence obtained can then be compared with sequences from known organisms in public databases. Moreover, the banding patterns can act as community fingerprints. The number of bands gives information on the diversity while the intensity and position of bands gives insight into the community structure.

Fluorescence in situ Hybridisation (FISH)

It is a technique used to detect specific organisms in biological samples. Specific organisms can be detected using fluorescently labelled oligonucletide probes. Using a suite of probes, each designed to react with a specific organism and each containing its own fluorescent dye, this technique can be used for characterisation of microorganisms in environmental samples. If FISH is combined with confocal microscopy it becomes a valuable tool for studying the abundance of specific microbial populations in space and time.

Fluorescence in situ Hybridisation is carried out in four main steps. The first step is permeabilisation and fixation of cells, the second is hybridisation of the probe with target cells, the third is washing off excess and unbound probe and the fourth step is using a fluorescence microscope for analysis.

7.3. Nitrogen removing bacteria

Ammonia-oxidising bacteria (AOB)

Nitrification is a two step process in which ammonia oxidises to nitrite, and then nitrite oxidises to nitrate (Figure 7-2). It results from the activity of nitrifying bacteria which are aerobic, autotrophic and chemolithotrophic. Carbon dioxide (CO_2) is the main source of carbon for nitrifying bacteria, which is fixed via the Calvin cycle (Prosser, 1989). The nitrifiers are slow growing compared to heterotrophs

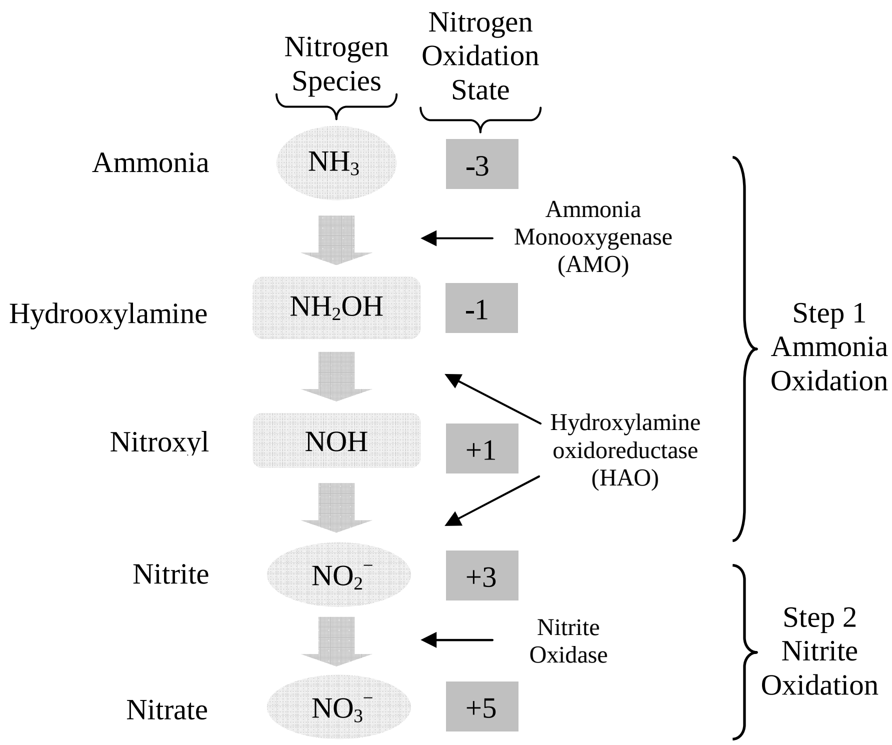


Figure 7-2 Steps in nitrification (after Vaccari, 2006).

making it difficult to isolate and grow them in the laboratory. Therefore, molecular methods are appropriate for studying AOB communities. PCR based methods followed by DGGE are most commonly used for studying the composition of ammonia-oxidising bacteria. The complete oxidation of ammonia is not carried out by a single chemolithotroph but two separate groups of bacteria, the ammonia-oxidising bacteria and the nitrite-oxidising bacteria, are responsible for the oxidation of ammonia to nitrate. Ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are the two major guilds in nitrification.

The use of molecular techniques based on 16S rRNA gene as a molecular marker classifies ammonia-oxidisers into three genera *Nitrosomonas, Nitrosospira* and *Nitrosococcus*. *Nitrosomonas* and *Nitrosospira* belong to the β subgroup of

Proteobacteria while *Nitrosococcus* belongs to the γ subgroup of *Proteobacteria*. The nitrite-oxidisers include *Nitrobacter, Nitrococcus, Nitrospina* and *Nitrospira. Nitrobacter, Nitrococcus, Nitrospina* belong to the α, β and γ subgroup of Proteobacteria, respectively. *Nitrospira* is a member of Xenobacteria.

Population studies of ammonia-oxidising bacteria (AOB) show that *Nitrosomonas* sp. dominate in engineered systems. For example, Schramm et al. (1996) detected a predominance of *Nitrosomonas europea*-like organisms on biofilms in ammonium-rich trickling filters, while Juretschko et al. (1998) reported the predominance of *Nitrosomonas mobilis* within activated sludge originating from industrial wastewater. However, Ibekwe et al. (2003) detected a predominance of *Nitrosospira*-like organisms in samples from sub-surface horizontal flow wetlands treating dairy wastewater.

Denitrifying bacteria

Denitrification is a stepwise process in which nitrate is reduced to nitrogen gas (Figure 7-3). In this process gaseous N_2 is formed biologically. A wide variety of taxonomic groups have been found to have the capacity for denitrification. Approximately 130 species of bacteria and archaea can denitrify (Zumft, W. G. 1992). Most of the denitrifying bacteria are facultative, anaerobic and chemoheterotrophs. They use organic compounds as electron donors and nitrogen oxides as terminal electron acceptors.

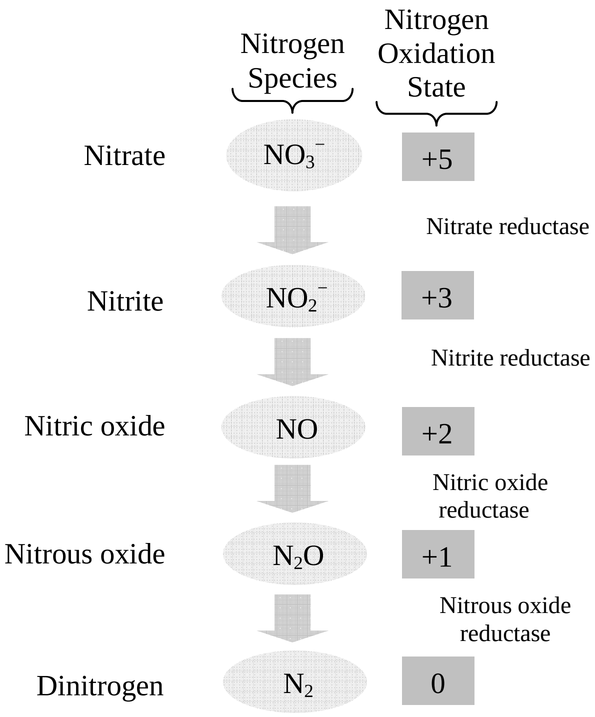


Figure 7-3 Steps in denitrification (after Vaccari, 2006).

In denitrification, nitrite is reduced to nitric oxide and this step distinguishes denitrifiers from other nitrate-respiring bacteria. This reaction is catalysed by different types of nitrite reductases: Cu-containing enzyme encoded by *nirK* gene or a heme-containing cd_1 enzyme encoded by *nirS* gene. The reduction of nitrous oxide to nitrogen is the last step in the denitrification pathway (Figure 7-3). This step is catalysed by nitrous oxide reductase encoded by the *nosZ* gene (Throback et al., 2004). Molecular techniques based on 16S rDNA gene sequences avoid the limitations of culturability and have been used to detect and analyze a larger portion of the bacterial community in environmental samples. However, the high diversity among denitrifiers excludes a 16S rDNA-based approach and instead identification requires the use of functional markers of the genes involved in the denitrification process, e.g. *nirK* gene, *nirS* gene or *nosZ* gene (Braker et al., 2001).

Denitrifying bacteria assist in removing excess nitrogen from wastewater and also in the degradation of organic pollutants. Most bacteria characterised by this functional trait belong to the wide array of diverse subclasses of Proteobacteria (Throback et al., 2004). Hallin et al. (2006) found *Paracoccus* sp., *Thauera spp.*, *Azoarcus* sp., *Alcaligenes faecalis*, *Bradyrhizobium japonicum* and *Blastobacter denitrificans* in activated sludge samples collected from full-scale and pilot-scale plants treating municipal wastewater. Moreover, Ruiz-Rueda et al. (2007) detected *Azoarcus tolulyticus spp.*, *Ralstonia eutropha* and *Azospirillum brasilense* in sediment samples collected from a free-water surface flow constructed wetlands treating secondary treated wastewater from a treatment plant. Sundberg et al. (2007b) detected a diverse denitrifying bacterial community comprising *Thiobacillus denitrificans*, *Rhodopseudomonas palustris*, *Burkholderia mallei*, *Azosprillium* sp. and *L. lactis* sp. in soil samples from a compact constructed wetland treating landfill leachate. Previous studies indicate the presence of diverse denitrifying bacterial communities in both engineered and natural wastewater treatment systems.

7.4. Biological processes science

Traditionally, the science behind biological processes in wastewater treatment systems has been empirical rather than theoretical (Curtis et al., 2003). The community composition in biological wastewater treatment systems can be evaluated using classical ecological work as discussed by Simberloff (1978). Curtis et al. (2003) have promoted the viewpoint that both theories and models developed for classical ecology could be related to microbial communities living within biological wastewater treatment systems such as activated sludge (Curtis et al., 2007; Graham and Smith, 2004). Van der Gast et al. (2006) employed the species-area model, which is based on one of the oldest biological laws, to study the relationship between bacterial taxa richness and reactor size. They also employed the theory of island biogeography to study the assembly and development of bacterial diversity in membrane bioreactors. Moreover, Baptista et al. (2008) conducted a study on laboratory-scale wetlands and compared the size and the species composition of Eubacteria, sulphate-reducing bacteria and the Archea within a theoretical framework using the classical Theory of Island Biogeography. They found that the three functional groups appeared to be stochastically assembled from the same source community.

7.5. Nitrogen removal in integrated constructed wetlands

In ICW systems, the litter from decaying macrophytes provides considerable surface area for the attachment of biofilms, and is therefore important for microbial processes such as the transformation of nutrients (particularly nitrogen) in wetlands (Brix et al., 1994). In treatment wetlands, sediments have been shown to be important habitats for microorganisms supporting denitrification (Bastviken et al., 2003). Hence, sediment and associated litter are components that play a vital role in supporting microbial-mediated processes. It has long been recognised that microorganisms are responsible for the decomposition of organic matter and nutrients in wastewater treatment facilities. However, very little work has been done to characterise nitrogen removal by microbial communities in free surface water constructed wetlands treating agricultural wastewater.

Case Studies

7.5.1. Site description

The study was carried out at two representative sites: ICW 3 and ICW 11. Both systems were built in 2001 to treat farmyard runoff comprising yard runoff (main component), roof runoff and dairy washings. The yard runoff was occasionally contaminated by silage and manure. Prior to construction of these wetland systems, there was no treatment, and the runoff was spread onto the adjoining fields. The wetland systems had a multi-cellular configuration with a minimum number of four cells. The systems operated as a set of sequential containment structures that intercept and control the contaminant gradient (Scholz et al., 2007).

ICW 3 has a total area of 1.00 ha and receives runoff from a farm comprising 70 dairy and beef cows, while ICW 11 has a total area of 0.76 ha and receives runoff from a farm comprising 77 dairy cows. Emergent plant species (helophytes) were the primary vegetation type in the ICW. Farmyard runoff was conveyed into the constructed wetlands by gravity through pipes. The key features of the constructed wetlands were horizontal flow and intermittent hydraulic loading. ICW 3 consisted of five cells, while ICW 11 comprised four cells. The cells have a linear sequential arrangement with a single influent entry point located in the first cell. The water between cells is conveyed through PVC pipe (diameter of 18 cm).

7.5.2. Water quality

The water quality in both case study systems has been monitored since operation began in 2001 (Scholz et al., 2007). Data collected between 2007 and 2008 were used for this study. Inflows and outflows to each cell were sampled approximately every two weeks. Water analysis for parameters including standard five-day biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), ammonia-nitrogen, nitrate-nitrogen and molybdate reactive phosphorus (MRP), was conducted as discussed in chapter 4; 4.7.1.

7.5.3. Application of molecular tools

In April 2008, litter and sediment samples were collected from both ICWs. Duplicate litter and sediment samples were collected from each wetland cell, details of which have been described in chapter 4, section 4.6.

Deoxyribonucleic acid extraction and purification from litter and sediment. The duplicate sediment and litter samples were subjected to deoxyribonucleic acid (DNA) extraction as discussed in chapter 4, section 4.7.3.1.

Polymerase chain reaction (PCR) amplification. The ammonia-oxidising bacterial community was investigated using forward and reverse primers as described by Kowalchuk et al. (1997) while the denitrifying bacterial community was assessed using functional gene primers. Polymerase chain reaction amplification was undertaken using the respective forward and reverse primers as discussed in 4.7.3.2.

Denaturing gradient gel electrophoresis (16S rRNA, nirK and nirS genes). The PCR products generated using different primers were analysed by denaturing gradient gel electrophoresis as discussed in 4.7.3.3.

Sequencing. The DGGE bands were excised using a sterile tip and sequencing was carried as outlined in section 4.7.3.5.

7.6. Analysis of DGGE data

The scanned images of DGGE gels were analysed using the following procedure: Stained gels were viewed using an ultraviolet transilluminator (UVP, San Gabriel, California, USA) and photographed with a Polaroid camera (CU-5, GRI, Great Dunmoor, Essex, UK). The presence and intensity of the bands in the DGGE gels were analysed using Bionumerics 4.0 (Applied Maths BVBA, Keistraat, Belgium). The software produced normalized composite gels with reference to markers included on the DGGE gels (van Verseveld and Röling, 2004). Subsequent analysis was carried out by exporting the band-matching data to a Microsoft Excel spreadsheet. Bray-Curtis similarity matrices, for non-metric multi-dimensional scaling (MDS) were constructed using Primer 6 for Windows (Version 6.1.5, Primer-E Ltd, Plymouth, UK), and later followed by analysis of similarities (ANOSIM) using the band designation as variables of presence and absence to ascertain statistical significance (Clarke and Warwick, 2001).

The Bray-Curtis (BC) index is often used to determine similarities between samples. MDS produces plots by clustering of similar samples. To verify if the observed clusters are statistically significant, a pair wise ANOSIM is conducted on the similarity matrix. The ANOSIM calculates an R-statistic by comparing the mean distances within user-defined groups and between groups. R-statistic takes values between 0 and 1. An *R* value of 1 indicates that the communities are completely different among defined groups, and an *R* of 0 indicates no difference among groups.

The number of DGGE bands for the ammonia-oxidising and denitrifying bacteria were compared with each other. The nature of the assembly of the bacterial community within the two ICW was evaluated using a methodology proposed by Simberloff (1978), using the Raup and Crick index (Raup and Crick, 1979). Band intensity data, obtained using gel analysis software (Bionumerics 4.0), was used for the calculation of the band relative abundance (pi). The band frequency was obtained by dividing the number of samples for each band by the total number of samples. Each band in the DGGE gel was assumed to be an operational taxonomic unit (OTU). The Shannon-Weaver diversity index (*H*; equation 7-1), was also computed to determine and compare the diversity of microbial communities.

$$H = \sum (p_i)(\log p_i) \qquad (7\text{-}1)$$

where *H* is the Shannon-Weaver diversity index, and p_i is calculated as n_i /N, where n_i is the band intensity for an individual band and N is the sum of band intensities.

7.7. Theoretical framework

Until the past decade, the science behind biological wastewater treatment systems in the field of environmental engineering was mostly empirical without any theoretical underpinning. In contrast, in the domain of civil engineering, after the failure of several engineered structures, the structural engineers have applied the laws of physics to their designs, marking a new era of safe and sound structures, in which empirical design is coupled with theory (Curtis et al., 2003). However, in the realm of biological wastewater treatment systems, there is no comprehensive theoretical foundation to steer design. Prosser et al. (2007) suggested that with the increasing dependence on specific microbial processes in wastewater treatment, it is incumbently essential and crucial to understand the factors that control these processes. This can be attained by generating theory that is based on existing observations and subsequently validated by experiments. According to Curtis et al. (2003), the last major theoretical advances in wastewater treatment engineering were made more than four decades ago in the 1960s by Downing et al. (1964) and then Lawrence and McCarty (1970).

7.7.1. Existing theories

Various theories exist in ecology, such as the theory of island biogeography which describes the processes of community assemblage and development, or nonlinear growth dynamics which explains the impacts of the nonlinear nature of biological growth on process stability. These theories can assist in improving the better understanding of biological wastewater treatment systems. In real-life conditions, there are millions of individual cells in each micrometer that form a consortium of microorganisms on biofilms in engineered (conventional wastewater treatment plants) and semi-engineered (wetland) systems. The fluctuating diversity of this microbial community leads to fluctuating performances in full-scale treatment systems which often comprise reactors of hundreds of cubic meters volume.

However, there is a poor understanding of the assembly of microbial communities within wastewater treatment systems.

7.7.2. Engineered biological systems

There is a need to test models of microbial community assembly to achieve the objective of rationally engineered biological systems. There are two models of microbial community assembly: (a) The microbial communities may arise through the selection of specific organisms that are best adapted to the surrounding conditions in a biological wastewater treatment system (deterministic selection); and (b) The microbial communities may arise through immigration by organisms present in the peripheral environment, and the class of organisms that colonise a biological wastewater treatment system is dictated by their arrival and propagation within the system (stochastic selection). Both Raup and Crick (1979) and Simberloff (1978) suggested that to test whether similarities in communities observed for two systems are a result of deterministic selection, the observed data must be statistically tested against a null hypothesis. The null hypothesis of this study is that community composition similarity would be no greater or smaller than if they had been randomly drawn from the same source of taxa.

7.7.3. Raup and Crick indices

The Raup and Crick index of similarity evaluates the observed number of species common to two sites with the number of species common to two sites that would be expected if they were selected randomly from the source population. The differences between the two data (observed and randomised) correlate with the level of similarity or dissimilarity between the two sites. The similarity index can be defined by the probability that the expected similarity would be greater than or equal to the observed similarity. Thus, a similarity value between 0.05 and 0.95 indicates a random occurrence of the same organisms in two samples, thereby validating the null hypothesis. In contrast, values below 0.05 and above 0.95 indicate deterministic selection thereby falsifying the null hypothesis (Raup and Crick, 1979).

The Raup and Crick index is generated from presence or absence data supplied by DGGE gels. The calculation is done using the PAST (PAlaeontological STatistics) programme (Hammer et al., 2001). For this study, the Raup and Crick indices were calculated and used to compare the DGGE data from within and amongst the ICW. Simberloff (1978) suggested that to determine whether island colonisation was stochastic, two different types of data could be used. Type 1 data consist of a species list, sampled for the group of islands on one visit, while Type 2 data is a species list for the same island, sampled at successive times. For this study, Type 1 data were collected; i.e. samples were collected from a group of islands (wetland cells) forming the archipelago (wetland) on one visit.

7.8. Treatment performance

The inflow and outflow water quality for the two wetlands is summarised in Table 7-1. The values show the very high variability of the farmyard water entering the two ICW systems. For ICW 3, the mean change between the influent and effluent was 96.60% for ammonia-nitrogen and 74.31% for nitrate-nitrogen, while for ICW 11, removal efficiencies of 98.65% and 67.53% for ammonia-nitrogen and nitrate-nitrogen, respectively, were recorded.

Nitrogen concentrations in different cells of the two ICW systems is shown in Figure 7-4. Most of the nutrients are removed in the first two cells of both systems. For example, in ICW 3, approximately 88% of nutrient removal takes place in the first two wetland cells. In the last wetland cell (i.e. cell 4) of ICW 11, the nitrate concentration was higher compared to the ammonia-nitrogen concentration implying that microbial diversity and vegetation cover may have an impact on the removal of nitrogen compounds in the ICW. The ammonia-nitrogen concentrations within the two wetland systems decreased progressively from the inflow to the outflow as wastewater passed through the various wetland cells indicating stable nitrification in both systems.

There were fluctuations between the inflow and outflow nitrate-nitrogen concentrations for both systems. For ICW 3, the nitrate-nitrogen concentration decreased from 2.38 mg/l to 0.079 mg/l between the first and second wetland cell,

Table 7-1. Mean influent and effluent water characteristics and corresponding reduction rates (RR %) for the studied integrated constructed wetlands 2007-2008.

Parameter	Units	ICW 3			ICW 11		
		Inlet	Outlet	RR	Inlet	Outlet	RR
Temperature	ºC	12.9 ± 2.58	13.4 ± 3.95	-	13.8 ± 3.06	14.9 ± 4.28	-
pH	-	6.76 ± 0.87	7.27 ± 0.95	-	8.12 ± 0.73	7.37 ± 0.54	-
EC	µS/cm	1190 ± 1092	359 ± 100	-	1469 ± 760	373 ± 44.1	-
SS	mg/l	411 ± 705	8.46 ± 9.39	97.9	78.4 ± 71.1	15.2 ± 29.7	80.5
BOD_5	mg/l	641 ± 1767	6.77 ± 5.14	98.9	593 ± 546	5.78 ± 5.10	99.0
COD	mg/l	2079 ± 3817	88.5 ± 28.3	95.7	1341 ± 1207	50.3 ± 27.4	96.2
NH_4–N	mg/l	44.6 ± 34.6	1.51 ± 1.87	96.6	28.6 ± 33.1	0.39 ± 0.898	98.6
NO_3–N	mg/l	2.39 ± 3.89	0.61 ± 1.45	74.5	2.60 ± 3.05	0.83 ± 1.44	68.0

EC, electrical conductivity; SS, suspended solids; BOD, five-day biochemical oxygen demand; COD, chemical oxygen demand; N, nitrogen but subsequently increased to 0.56 mg/l between the second and third cell, and then decreased progressively between the following pairs of cells. However, there was a marked increase in the final effluent concentration, which was 0.61 mg/l. Also for ICW 11, there were also fluctuations in the nitrate-nitrogen concentrations following almost the same pattern as for ICW 3. The mean inflow nitrate-nitrogen concentration was 2.57 mg/l, and the corresponding outflow concentration was 0.83 mg/l. There was a high reduction in nitrate-nitrogen concentration (>96.6 % for ICW 3; >93.7% for ICW11) in the first wetland cell of both ICW systems. Denitrifying bacteria are mostly regulated by the availability of organic matter (Bastviken et al., 2003). In the first cells, the high organic loading coupled with dense vegetation stands and associated litter provided large surface areas for the growth of biomass associated with attached biofilms. These factors would have created conditions, which are favourable for denitrification, and resulting in an effective reduction of the nitrate-nitrogen concentrations in the first cell. There was an abrupt increase in nitrate-nitrogen concentration between the second and third cells, but the pattern reversed for the following cells except for the last cell of both systems. This observation indicates an increase in nitrate-nitrogen concentrations at the outflow.

The increase of nitrate-nitrogen concentration between the second and third cells may be because of lower organic matter availability as most of the BOD (>85%) has been reduced through biodegradation in the first cell of the ICW system (Mustafa et al., 2009). Subsequently, as the system becomes stable, there is a progressive decline in the concentration of nitrate-nitrogen. The high concentration of nitrate-nitrogen within the outflow may be because of the impact of vegetation. Ibekwe et al. (2007) reported that in a free surface wetland, a treatment pond with

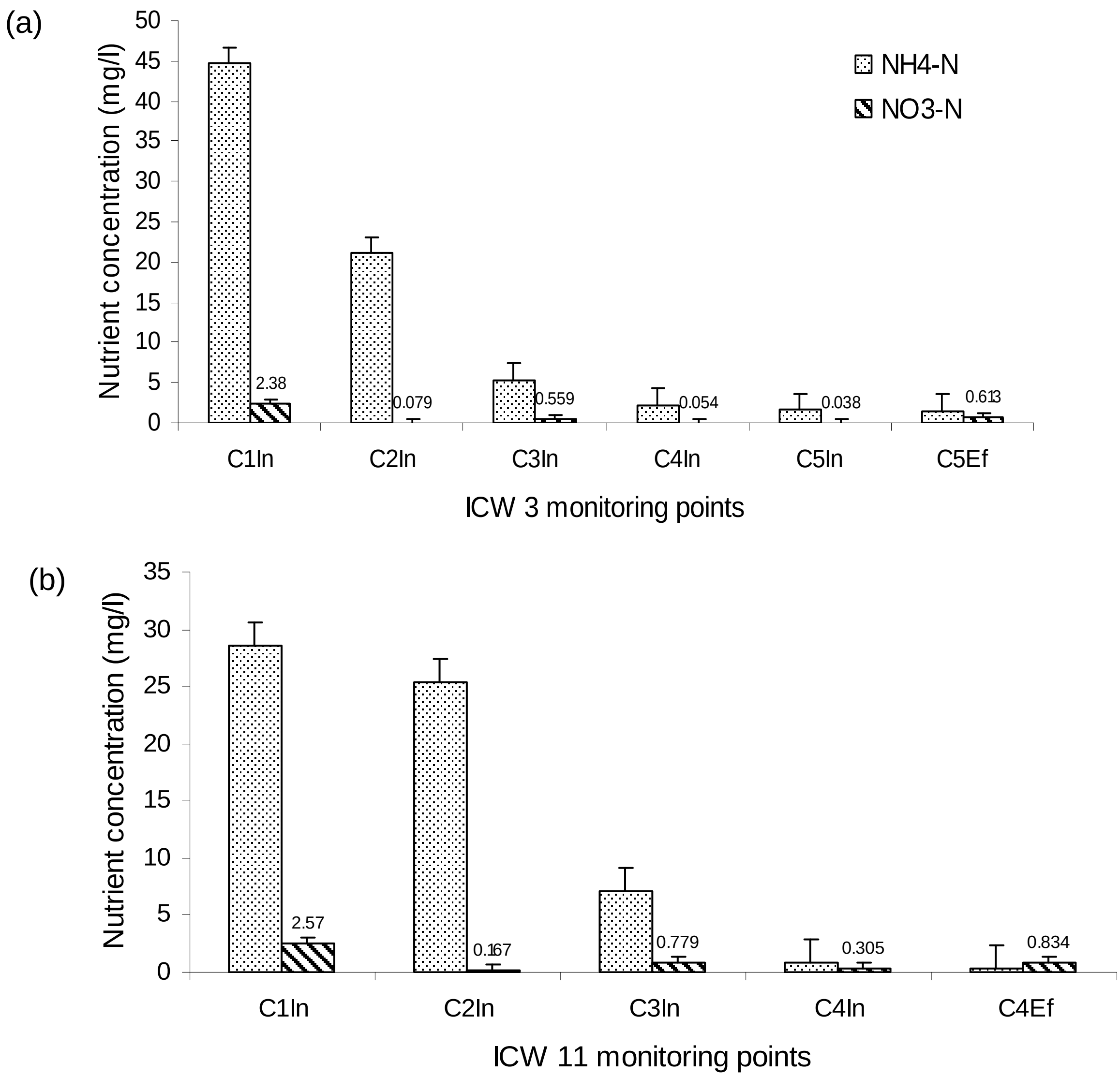


Figure 7-4. Mean nitrogen concentrations in various cells of integrated constructed wetlands (a) ICW 3 (b) ICW 11. Error bars indicate standard error.

50% plant cover had a nitrate removal efficiency of 96.3%. The last wetland cells of both systems had very low vegetation densities (<2%). This is most likely the reason for the high nitrate-nitrogen concentrations in the outflow.

Even though ammonia removal efficiency was good overall (96.6% for ICW 3 and 98.6% for ICW 11), the specific nitrate production rate was much lower than the ammonia consumption rate in the ICW. This indicates that full nitrification did not consume all of the ammonia (Table 7-2). Moreover, nitrite was not detected in any of the samples measured along the transect of the ICW systems. Loss of ammonia through volatilisation is also unlikely, since the pH was < 8.5 in all cells of the ICW. It is therefore probable that the ammonia was biologically removed, through a combination of diverse mechanisms.

Table 7-2 Nitrogen removal and conversion rates and possible mechanisms for nitrogen removal in each cell of the integrated constructed wetland ICW 11

	Cell 1	Cell 2	Cell 3	Cell 4
% of total NH_4-N removed	90	-54	42	21
NH4-N removal rate (mol g/BOD.h)	0.014	-0.004	0.025	0.008
NO_3-N production rate (mol g/ BOD.h)	0.0013	-0.0008	0.00003	0.0001
% Accounted for by full nitrification	9	17	0.13	2
% Accounted for by other means	91	83	99.87	98

7.9. Bacterial community compositions

The use of CTO (primer used to characterise AOB) and functional gene primers in PCR-amplification of DNA extracts from litter and sediment samples from both ICW gave products of the expected size. Banding patterns from litter and sediment samples were analysed using Bionumerics 4.0 software (see above). The litter and sediment samples had their own unique profiles, indicating variation in microbial community composition between the two components and also amongst various cells of the two wetland systems.

7.9.1 Ammonia-oxidising bacteria

DGGE analysis of the PCR products yielded patterns consisting of one to five bands. The ICW cells exhibited variation in the band patterns. A more diverse population was noted for litter compared to sediment samples. Interestingly, both *Nitrosospira* and *Nitrosomonas* populations were detected in the ammonia-rich environment of the two wetland systems. The litter samples from ICW 3 had one dominant band (C2) migrating in the range of *Nitrosomonas* sp. Nm59, while samples from ICW 11 had two dominant bands (C5 and C6) migrating in the ranges of *Nitrosospira* sp. Nsp12 and *Nitrosospira* sp. En271. The dominating band C2 exhibited 97 % similarity to *Nitrosomonas* sp. Nm59. Bands C5 and C6 showed signs of 99% and 100% similarity to *Nitrosospira* sp. Nsp12 and *Nitrosospira* sp. En271, respectively (Table 7-3a). Overall, two *Nitrosospira*-like sequences and only one *Nitrosomonas*-like sequence were detected in the litter and sediment samples. Sediment samples from the two systems had one dominant band migrating in the range of *Nitrosospira* sp. Nsp12. The dominating band C1 exhibited 100 % similarity to *Nitrosospira* sp. Nsp12. Sundberg et al. (2007) also reported the presence of only *Nitrosospira*-like populations in sediments of a compact constructed wetland treating landfill leachate. Ibekwe et al. (2003) reported that subsurface horizontal flow wetlands treating dairy wastewater had greater *Nitrosospira* sp. diversity. They showed that the majority of the wetland samples were phylogenetically related to *Nitrosospira* and clustered into two main groups, including that of *Nitrosospira* sp.

strain Nsp12, which was also detected in the sediment and litter samples of the present study.

The first, third and last cell of ICW 3 contained both *Nitrosospira* sp. and *Nitrosomonas* sp. populations, while the fourth cell contained only Nitrosomonas sp. Nm59. *Nitrosospira* sp. was detected in the first, third and fourth cells while *Nitrosomonas* sp. was detected in the second cell of ICW 11. Several previous studies have suggested that environmental factors such as salinity and ammonia concentrations determine the presence of certain ammonia-oxidising bacteria.

7.9.2. Denitrifying bacteria

DGGE analysis of the PCR products yielded patterns consisting of one to ten bands for *nir*K (PCR product bands not shown). There was a variation between the ICW cells in the band pattern, and more diverse populations were observed within litter as compared to sediment samples. One to six bands for *nir*S were noted. The litter samples from both ICW systems 3 and 11 had one dominant band (C21-nirK) migrating in the range of *Rhizobium* sp. R-24663. The dominating band C21 exhibited 82% similarity to the *Rhizobium* sp. R-24663 There was one dominating band visible (C25-nirS) migrating in the range of *Dechloromonas* sp. R-28451. The dominating band exhibited 90 % similarity to *Dechloromonas* sp. R-28451 (Table 7-3b).

With regards to *nirK*- and *nirS*-containing denitrifier bacterial communities, *Paracoccus*, *Pseudomonas*, *Rhizobium* and *Dechloromonas* were identified in the samples. *Paracoccus* and *Dechloromonas* were present in all samples collected from the five cells of ICW 3, while in ICW 11 samples *Pseudomonas* was detected in addition to *Paracoccus* and *Dechloromonas*. *Pseudomonas* and *Dechloromonas* were detected in the first two cells of ICW 11, while for the third and fourth cells only *Dechloromonas* was identified, whereas *Paracoccus* was detected in the first, second and last cell of ICW 11. *Dechloromonas* was identified in the samples collected from all wetland cells of ICW 3.

Paracoccus-like bacteria were detected as the dominant group in sediment samples collected from the various cells of ICW 3 and ICW 11. Neef et al. (1996) reported that the genus *Paracoccus* was responsible for the high denitrification activity in a denitrifying sand filter reactor. *Paracoccus*-like bacteria use reduced sulphur compounds for electron donors during the denitrification process. It is most likely that reduced sulphur is present within the soil and sediments of ICW, which are similar in this respect to other wetland systems (Knight and Wallace, 2009).

The litter samples collected from cells 1 to 5 of ICW 3 contained *Dechloromonas* and various uncultured bacteria. *Pseudomonas*, *Dechloromonas* and uncultured bacterial strains were detected in the first two cells of ICW 11, while the third and fourth cells contained *Dechloromonas*. For ICW 11, only one strain of *Dechloromonas* was identified in the last cell 4, which had only 5% vegetation cover and a higher concentration of nitrate compared to ammonia-nitrogen implying that diversity and vegetation has an impact on the removal of nitrogen compounds.

7.10. Community diversity changes within and amongst wetland systems

7.10.1. Diversity index

The DGGE data were assessed using two approaches: (1) Richness, Evenness and Shannon-Weaver diversity indices, and (2) non-metric multidimensional scaling (MDS) ordination plots. The Richness, Evenness and Shannon's diversity indices revealed significant differences between the two contrasting ICW ecosystems. The overall mean diversity of litter and sediment samples for the three genetic populations was 1.38 for ICW 3 compared to 1.50 for ICW11 (Table 7-4; $p<0.05$). In general, ICW 11 had higher diversity indices compared to ICW 3. The Shannon-Weaver diversity indices in the litter samples were higher than those for sediment samples. For ICW 3, the diversity of ammonia-oxidising bacteria and diversity of nitrite reductase (*nirK* and *nirS*) gene fragments were higher in samples collected near the outlet than those collected near the inlet of the wetland system. In contrast for ICW 11, the diversity was higher near the inlet than the outlet. For both systems, the bacterial diversity was higher in the litter than the sediment samples. Based on the Shannon-Weaver diversity index, the data indicated that litter had the most diverse group of denitrifying bacteria.

Table 7-3. Sequence analysis of bands excised from DGGE gel derived from bacterial 16S rRNA extracted from wetland sediment and litter samples (a) Ammonia-oxidising bacteria

Sequence	**ICW 3**												**ICW 11**										Accession number	% Similarity	Strain
	C1In		C2 In		C3 In		C4 In		C5 In		C5Ef		C1 In		C2 In		C3 In		C4 In		C4Ef				
	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S			
C1	+				+						+												AY123801	99	*Nitrosospira* sp. Nsp12
C2	+				+		+		+		+												AY123811	98	*Nitrosomonas* sp. Nm59
C3					+		+				+												DQ676290	100	*Methylophilus* sp.
C4															+		+						AY123811	97	*Nitrosomonas* sp. Nm59
C5		+		+									+	+			+			+			AY123801	99	*Nitrosospira* sp. Nsp12
C6													+				+						AY727031	100	*Nitrosospira* sp. En271
C7													+				+						AY792265	98	*Methylophilus* sp.

(b) Denitrifying bacteria

Sequence	**ICW 3**												**ICW 11**										Accession number	% Similarity	Strain
	C1In		C2 In		C3 In		C4 In		C5 In		C5Ef		C1 In		C2 In		C3 In		C4 In		C4Ef				
	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S			
nirK																									
C8			+		+		+		+		+		+		+								EU448024	81	Uncultured denitrifying bacterium clone T23_D5 nitrite reductase (nirK) gene, partial cds
C9											+		+		+								EU448024	81	Uncultured denitrifying bacterium clone T23_D5 nitrite reductase (nirK) gene, partial cds
C10											+		+		+								FM209186	86	*Pseudomonas aeruginosa* LESB58 complete genome sequence
C11															+								AY345247	78	*Pseudomonas aeruginosa* strain DN24 copper-dependent nitrite reductase
C12																						+	EF623501	100	Uncultured bacterium clone LK22mK-28 nitrite reductase (nirK) gene, partial cds
C13		+				+		+		+		+		+						+			AM230857	77	*Paracoccus* sp. R-26824 nirK gene for nitrite reductase
C14																				+			DQ783326	96	Uncultured bacterium clone T1R2_0-7cm_038 NirK (nirK) gene, partial cds
C16								+		+				+				+					AM419485	89	Uncultured organism partial nirK gene for putative copper containing dissimilatory nitrite reductase, clone Fin28
C18			+		+								+										EF615316	86	Uncultured bacterium clone P1m_nirK-33 nitrite reductase (nirK) gene
C19			+		+		+						+										DQ337794	87	Uncultured bacterium clone S12m_nirK-33 NirK (nirK) gene
C21	+		+		+		+		+		+		+		+		+				+		AM230832	82	*Rhizobium* sp. R-24663 nirK gene for nitrite reductase
C22															+								DQ337762	89	Uncultured bacterium clone P7m_nirK-25 NirK-lke (nirK) gene, partial sequence
C23	+		+		+				+		+		+		+		+						DQ304404	88	Uncultured bacterium clone Ag100-6 putative nitrite reductase (nirK) gene, partial cds
nirS																									
C24			+																				AY078267	85	*Thauera terpenica* strain 21Mol putative dissimilatory nitrite reductase (nirS) gene, partial cds
C25	+		+		+		+		+		+		+		+		+		+				AM230919	90	*Dechloromonas* sp. R-28451 nirS gene for nitrite reductase
C26													+		+								AM230913	84	*Dechloromonas* sp. R-28400 nirS gene for nitrite reductase

Table 7-4 shows the Richness, Evenness and Shannon's diversity indices of CTO, *nir*K and *nir*S in ICW. The mean Shannon's diversity indices of CTO in litter samples were 0.58 and 0.68 for ICW 3 and ICW 11, respectively. The Shannon's diversity indices of *nir*K and *nir*S in litter samples were 1.78 and 2.05 for ICW 3 and 2.04 and 2.31 for ICW 11, respectively. For sediments, the Shannon's diversity indices of *nir*K and *nir*S were 0.49 and 2.01 for ICW 3 and 0.89 and 1.60 for ICW 11, respectively. Statistical analysis showed that the diversity in ICW 11 was higher than in ICW 3 for two genes; CTO and *nirK*. The *nir*S diversity index of ICW 3 sediment was higher than that of ICW 11. This may be due to the higher nitrate concentration at the inlet of ICW 11, 2.60 mg/l compared to 2.39 mg/l for ICW 3.

Table 7-4. Diversity indices for the ammonia-oxidising and denitrifying bacterial communities in sediment and litter of the ICW systems (mean ± SD)

Primer/ Genes	ICW no./ Component	Richness	Evenness	Shannon's (H)
CTO	ICW 3 / litter	2.17 ± 1.17	0.91 ± 0.05	0.58 ± 0.50
	ICW 11 / litter	3.50 ± 2.45	0.85 ± 0.03	0.68 ± 0.80
	ICW 3 / sediment	1.00 ± 0.00	-	-
	ICW 11 / sediment	1.00 ± 0.00	-	-
*nir*K	ICW 3 / litter	8.42 ± 3.22	0.85 ± 0.09	1.70 ± 0.45
	ICW 11 / litter	10.5 ± 2.86	0.87 ± 0.05	2.02 ± 0.20
	ICW 3 / sediment	1.83 ± 0.75	0.92 ± 0.05	0.74 ± 0.21
	ICW 11 / sediment	4.00 ± 3.24	0.79 ± 0.09	1.12 ± 0.73
*nir*S	ICW 3 / litter	9.86 ± 2.37	0.90 ± 0.03	2.00 ± 0.27
	ICW 11 / litter	11.71 ± 2.41	0.90 ± 0.03	2.19 ± 0.18
	ICW 3 / sediment	10.0 ± 4.58	0.90 ± 0.04	2.01 ± 0.47
	ICW 11 / sediment	7.40 ± 4.51	0.86 ± 0.09	1.60 ± 0.68

Ruiz-Rueda et al. (2007) reported that sediment samples from high nitrate conditions at the inlet of a constructed wetland had lower *nir*S diversity. Furthermore, the *nir*S diversity was higher than that of *nir*K in both ICW systems. Compared to other studies, the diversities of *nir*K and *nir*S in ICW systems were lower than those in natural environments. Priemé et al. (2002) studied the diversity of nitrite reductase (*nirK* and *nirS*) gene fragments in forested upland and wetland soils and reported high diversity indices of *nir*K and *nir*S of 3.55 and 5.27, respectively. This may be due to differences in carbon source in the semi-engineered ICW environment and the natural soil environment. However, the *nir*S diversity for both ICW 3 and ICW 11 was > 2 and was similar to the value reported (2.015 maximum) by Ruiz-Rueda et al. (2007).

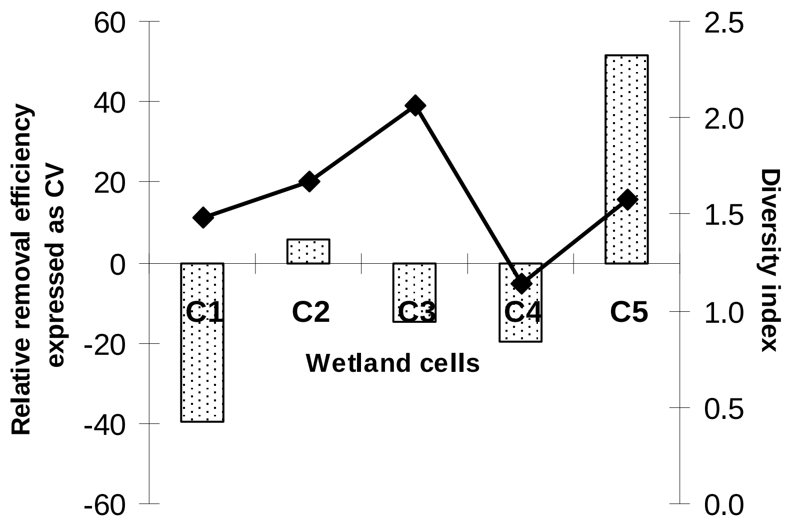


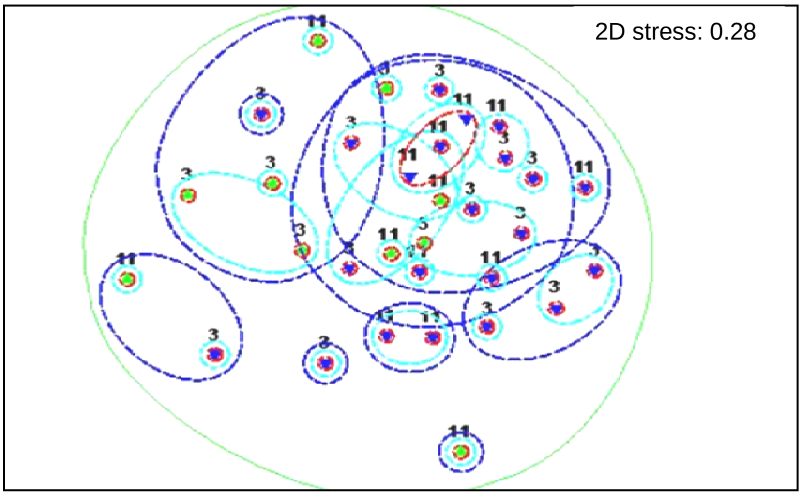
Figure 7-5. Mean relative removal efficiency of nitrate and the corresponding diversity indices (*nir*K and *nir*S) in various cells of ICW 3. RE, removal efficiency; CV, coefficient of variation.

The difference in nitrite reductase gene diversity between ICW 3 and ICW 11 may be because of the different soil and plant characteristics of the two systems. The dissolved oxygen concentrations may also have influenced the diversity as it plays an important role in controlling the denitrifying community structure. Comparing the nitrogen removal efficiencies of ICW 3 and ICW 11, the removal efficiency of ICW 11 was found to be more stable over the period of monitoring. The influence of diversity on the stability of wastewater treatment processes is debatable; Rowan et al. (2003) have suggested that the level of bacterial diversity within a wastewater treatment facility has a major influence on process stability. In this study, the relative removal efficiencies (expressed as coefficient of variation) indicated high variability (Figure 7-5).

7.10.2. Multidimensional scaling analysis of banding patterns

Differences between bacterial community structures within the two ICW ecosystems were monitored and analysed using the ordination technique Non-metric Multidimensional Scaling (MDS) to understand the relationship between the bacterial community and the ICW nitrogen removal performance. MDS is one of the several methods that tackle the problem of non-linear relationships, and was selected because it does not make any of the distributional postulations, of other ordination techniques. Hence, MDS is more appropriate for environmental data (McCune and Grace, 2002). In the MDS plots, the distance between the points

(a)



(b)

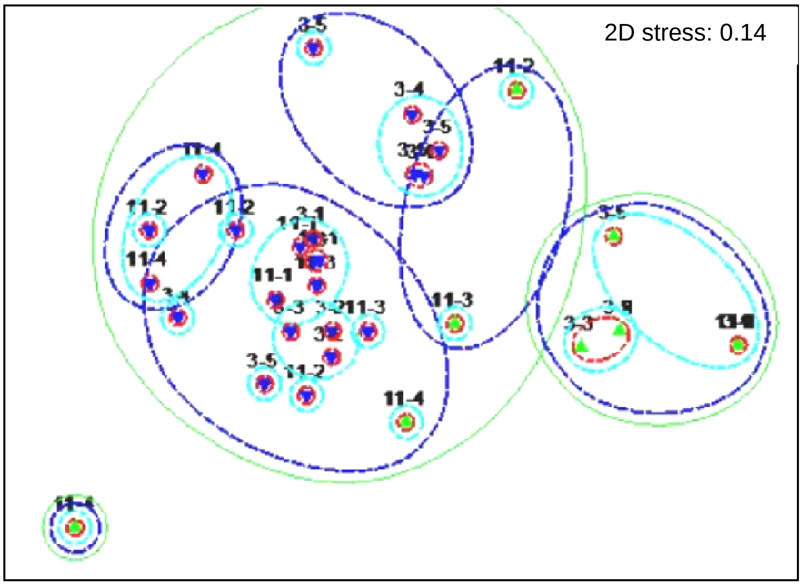


Figure 7-6. Multidimensional scaling analysis of denaturing gradient gel electrophoresis profiles of (a) nirS and (b) nirK fragments derived from ICW 3 and ICW 11. Coloured symbols indicate sediment (green triangles) and litter samples (blue inverted triangles). The position of symbols show the differences between DGGE profiles based on their distance in a two-dimensional plot. Distance was derived from Bray-Curtis similarity coefficients calculated from the DGGE profiles. The enclosed elliptical shapes represent percent similarity amongst the samples; light green 20%, blue 40%, cyan 60% and red 80%. 11-2, ICW 11 cell 2; ICW, integrated constructed wetlands.

reflects the similarity of the DGGE profiles at a given sampling time. Similar community structures are closer together. The MDS plots for the nitrogen-cycling bacteria are shown in Figure 7-6.

Figure 7-6 a and b show that the samples from the two sites formed tight clusters that were separate from each other in two dimensional space. The MDS map indicates that there were distinct microbial communities with respect to site and source (sediment and litter). Nevertheless, the communities in the two wetland sites are on the whole no more similar or different than if they had been assembled by chance. Many of the highly abundant taxa were found in most of the wetland cells.

Figure 7-7 looks at the similarities in microbial communities along the transect in the direction of flow from cell 1 to cell 5 of ICW3. For example, litter communities in cell 1 and 2 are distinct, i.e. there is a shift in litter community. In

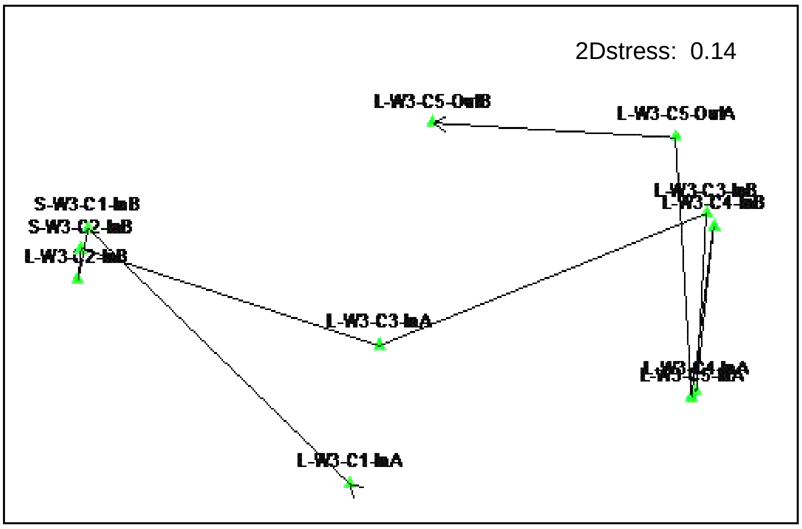


Figure 7-7. MDS analysis of DGGE profiles of 16S rRNA gene fragments derived from ICW 3.

contrast the sediment community looks more similar, for example the sediment communities in cell 1 and cell 2 are the same. Overall, the MDS plot shows significant differences in litter communities between the wetland cells.

A comparison of the two groups of nitrogen removing bacteria by ANOSIM confirmed that the communities among the two ICW vary as the R statistic was >0 (Table 7-5). There were significant differences in the nitrogen removing bacterial communities between replicates, the communities in ICW 3 and ICW 11, and the communities present in litter and sediment. However, the biggest statistical difference was between sample source i.e. sediment or litter.

Table 7-5. Results of analysis of similarities for different factors.

Factors	p	R-statistic	Stress
Ammonia-oxidising bacteria			
Between replicates	<0.040*	0.405	0.01
Between ICW3 and ICW11	<0.007*	0.311	0.01
Between sediment and litter	<0.070*	0.248	0.01
*Denitrifying bacteria (nir*K)			
Between replicates	<0.001*	0.535	0.14
Between ICW3 and ICW11	<0.032*	0.109	0.14
Between sediment and litter	<0.007*	0.140	0.14
*Denitrifying bacteria (nir*S)			
Between replicates	<0.007*	0.407	0.26
Between ICW3 and ICW11	<0.267	0.025	0.26
Between sediment and litter	<0.001*	0.279	0.26

ICW, integrated constructed wetland; Significant analysis of similarities (ANOSIM) results are denoted by *

7.11. Microbial community assembly

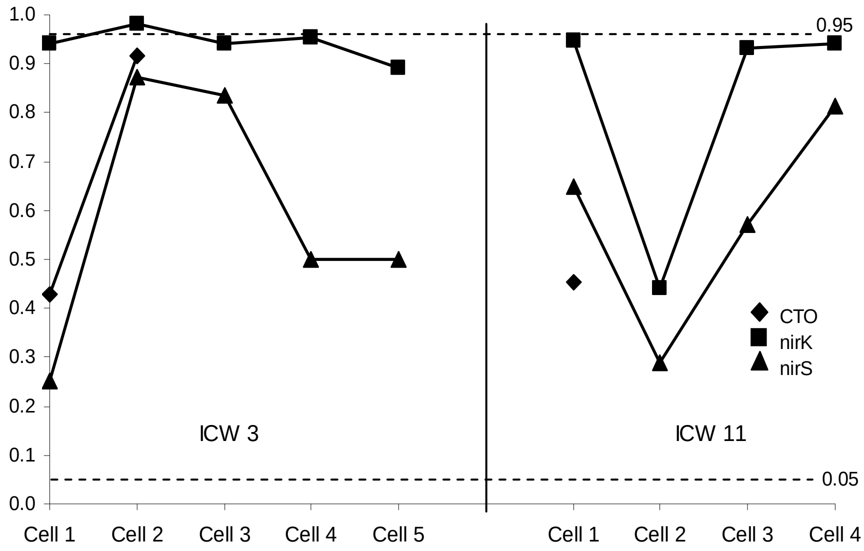
7.11.1. Raup and Crick indices

The average values of the Raup and Crick indices obtained from comparison of both ICW 3 and ICW 11 litter samples were 0.58 ± 0.25 for the ammonia-oxidising bacterial communities and 0.54 ± 0.26 and 0.64 ± 0.26 for the denitrifying bacterial communities, *nir*K and *nir*S, respectively. The average indices values for sediment samples were 0.60 ± 0.27 for the ammonia-oxidising bacterial communities and 0.86 ± 0.17 and 0.61 ± 0.22 for the denitrifying bacterial communities, *nir*K and *nir*S genes, respectively. The finding supports the null hypothesis of this study which proposes that the microbial communities in the two wetland systems were no more similar or different than if they had been stochastically assembled from the same taxa source. Baptista et al. (2008) also found that the microbial diversity of laboratory-scale wetlands appeared to be randomly assembled.

7.11.2. Comparison of microbial communities between the ICW cells

The Raup and Crick indices for both ICW 3 and ICW 11 litter samples were between 0.05 and 0.95 for the three genetic populations, CTO, *nir*S and *nir*K (Figure 7-8b). There was no significant similarity (>0.95) except for one point (*nir*S gene), cell 4 of ICW 3, and also no significant dissimilarity (<0.05) between cell 1 and subsequent sampling points. Overall, the data suggest that the similarities in ammonia-oxidising and denitrifying bacterial communities in different cells of the two ICW systems are no greater than can be accounted for by chance.

(a)



(b)

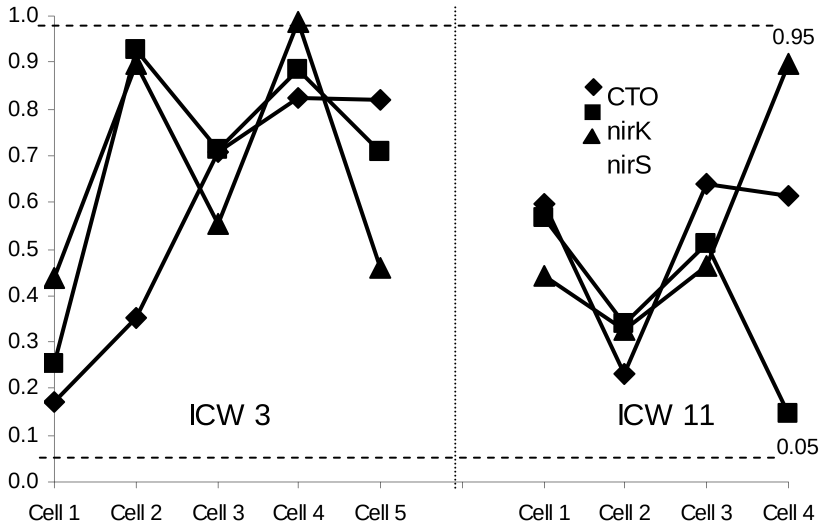


Figure 7-8. A comparison of Raup and Crick indices for the three bacterial populations (a) sediment (b) litter

For sediment samples, the Raup and Crick indices for both ICW 3 and ICW 11 were between 0.05 and 0.95 for the three genetic populations, CTO, *nir*S and *nir*K (Figure 7-8a). There was no significant similarity (>0.95) except for one point (*nir*K gene), cell 2 of ICW 3, and also no significant dissimilarity (<0.05) between adjacent sampling points. Overall, the results suggest that the ammonia-oxidising and denitrifying bacterial communities in different cells of the two ICW systems were stochastically assembled.

7.11.3. Comparison of microbial communities down the ICW profile

The Raup and Crick indices for both ICW 3 and ICW 11 were between 0.05 and 0.95 for all sampling points for the three genetic populations except for *nir*K gene (cell 1 and adjacent sampling point) of ICW 11 (Figure 7-9). The data suggest that selection for ammonia-oxidising and denitrifying bacteria in ICW was random. There appears a gradient in AOB and DNB diversity down the profile of ICW as clearly shown in Figure 7-9. Rowan et al. (2003) also reported a gradient in AOB

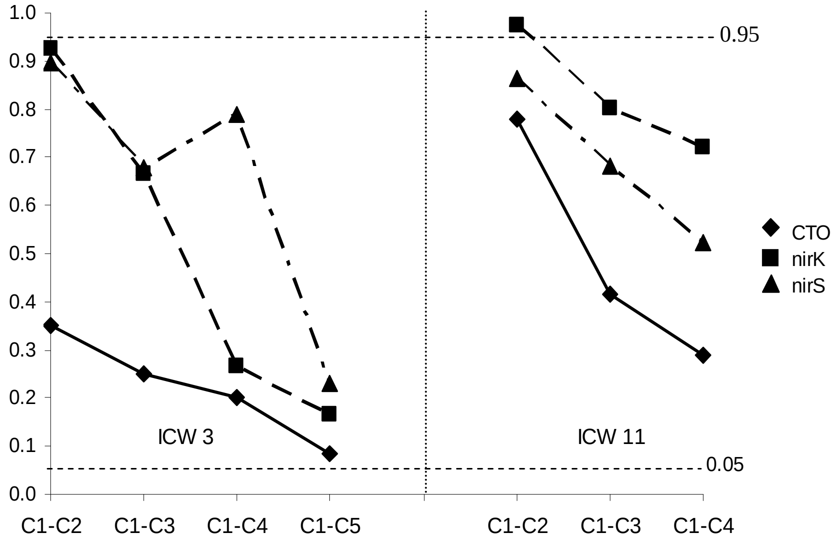


Figure 7-9. A comparison of Raup and Crick indices for the ammonia-oxidising and denitrifying bacterial DGGE data (litter samples) between cell 1 and the adjacent sampling points.

diversity down a reactor. However, they reported similarities in AOB communities as statistically greater than could be expected from chance, i.e. non-random, contrary to the results of this study. The difference may be due to the controlled conditions in fully engineered wastewater treatment reactors compared to uncontrolled conditions in semi-engineered treatment wetland systems fully exposed to the natural environment.

7.12. Summary

For ammonia-oxidising bacteria, both *Nitrosospira* and *Nitrosomonas* were detected in the wetland systems studied. The denitrifying bacteria, *Paracoccus, Pseudomonas, Rhizobium* and *Dechloromonas* were identified. There were considerably diverse populations of nitrogen removing bacteria in the studied wetland systems compared to conventional wastewater treatment systems. The litter component of the two wetland systems supported more diverse nitrogen removing bacteria (ammonia-oxidising and denitrifying) than the sediments. Nitrogen removing bacteria in the two full-scale wetland systems appeared to be stochastically assembled from the same source community.

Chapter 8 Application of self-organising map model

8.1. Introduction

This chapter presents the application of the self-organising map (SOM) model for the prediction of full-scale integrated constructed wetlands performance. The model was used to predict outflow nutrient and biochemical oxygen demand concentrations in order to assess protection of receiving watercourses. The SOM model was also utilised to fill in the missing values and replace outliers from the ICW data set. The contents of this chapter have been published as research articles in Water Research and Bioresource Technology.

More specifically, section 8.2 describes the application of neural networks to wastewater treatment systems. Section 8.3 describes the aims and objective of this chapter. Section 8.4 describes the SOM model. Sections 8.5, 8.6 and 8.7 show the model application for prediction of BOD and nutrients, and filling missing values and replacing outliers in the ICW dataset.

8.2. Application of artificial neural networks

In the realm of engineering, conventional approaches to modelling depend on mathematical tools such as differential equations and transfer functions. These tools emphasise exact description of each quantity involved and are suitable when the system is simple or well-defined. When the system under consideration is complicated like treatment wetlands, mathematical tools become less effective. To overcome problems faced by conventional modelling, neural network modelling has been proposed as a feasible option and successfully applied in different areas including wastewater treatment engineering where conventional approaches fail to provide satisfactory solutions.

In ICW systems various biochemical reactions take place that remove organic matter and nutrients through processes such as biochemical oxygen demand removal, nitrification, anammox and denitrification. With increasingly stringent regulations of effluent quality, process monitoring and control in order to meet the requirements of the Water Framework Directive (WFD), the control of effluent has become more important.

The wastewater treatment occurring in the ICW has unique characteristics. Variable flows and concentrations of contaminants enter into systems making them dynamic. Moreover, the constructed wetland systems designed and commissioned in different parts of the world have variable performances and because of this unpredictability, the knowledge and experience attained from one system cannot be applied to another. The performance of ICW depends on many factors, for example, the variability between design and type, influent and hydrologic conditions, aquatic plant type and local climate.

Various parameters like BOD, COD, SS, NH_4-N, NO_3-N and MRP are monitored for maintenance purposes, efficiency evaluation and compliance with standards (Lee et al., 2005; Scholz, 2006). Artificial neural networks (ANN) have proven useful for describing extensive scientific datasets, and are especially capable of solving nonlinear relationships which cannot simply be explained by explicit mathematical models. Therefore, the Self-Organising Map (SOM), which is an artificial neural network has been used as a prediction tool.

Kohonen (2001) classified neural network architecture into three categories:

1) Feedforward networks
2) Feedback networks
3) Unsupervised networks

In the feedforward networks, sets of input signals are transformed into sets of outputs. The transformation is determined by external, supervised adjustment of parameters. In feedback networks, the input information identifies the initial activity of the system. After state alterations, the asymptotic final state is defined as the result of the computation. In the last category of unsupervised (self-organising) networks, neighbouring neurons in the network compete and develop recursively particular detectors for different input signal patterns. SOM is an unsupervised neural network technique.

Modelling and predicting treatment processes is significant for elucidating the complex nutrient removal mechanisms, and assessing the corresponding water treatment potential of ICW. It is necessary to model and predict the nutrient removal processes in order to optimise the design, operation, management and water quality monitoring strategy of an ICW. An important model aim is frequently to predict expensive and time consuming to measure parameters using other parameters, which are more cost-effective, quicker and easier to measure (Lee and Scholz, 2006). For example, biochemical oxygen demand (BOD) is an important parameter to characterize the biodegradable components of organic matter in wastewater, but it is time-consuming to measure (at least five days). For water quality real time control purposes, an accurate inferential model for BOD prediction is therefore required.

Data quality is also a crucial factor for modelling, because it affects the accuracy of the corresponding modelling results. In general, it is difficult to assemble a large, robust and multivariate dataset. No matter how well an experiment is planned, there will always be times when something goes wrong unexpectedly, resulting in gaps and outliers in the data (Burke, 1999). Since most conventional modelling approaches require complete input datasets, missing values and outliers will therefore require a reduction in data available for subsequent modelling. Therefore an appropriate method for filling missing values and replacing outliers is required. For example, the self-organising map (SOM) model is not affected by missing values, and can process with incomplete input datasets. Based on this characteristic, a SOM model can be developed for an incomplete data set to predict missing values in the input dataset.

The self-organising map is based on an unsupervised neural network algorithm and has been used to analyse, cluster and model various types of large databases (Kohonen et al., 1996; Lee and Scholz, 2006; Kalteh et al., 2008). Astel et al. (2007) and Scholz (2008) applied SOM models successfully for classification of large water and environmental datasets. The SOM model, which has not been as often implemented in water treatment process control strategies in comparison to traditional neural networks, was successfully used for the first time as a prediction tool for heavy metal removal in constructed wetland systems by Lee and Scholz (2006). The SOM model has also been used successfully to predict water quality parameters in activated sludge wastewater treatment plants (Rustum and Adeloye, 2007; Rustum *et al*., 2008).

However, the SOM model has never been applied to model and predict the nitrogen and phosphorus removal efficiencies within constructed wetland systems such as ICW. The model can be used by wetland engineers and managers to monitor wastewater treatment processes in ICWs.

8.2.1. Self-organising map

The self-organising map (SOM) is a neural network model and algorithm that implements a characteristic non-linear projection from the high-dimensional space of sensory or other input signals onto a low-dimensional array of neurons (Figure 8-1), and has been widely applied for visualisation of dimensional systems and data mining (Kohonen et al., 1996). The SOM is a competitive learning neural network and based on unsupervised learning, which means that no human intervention is

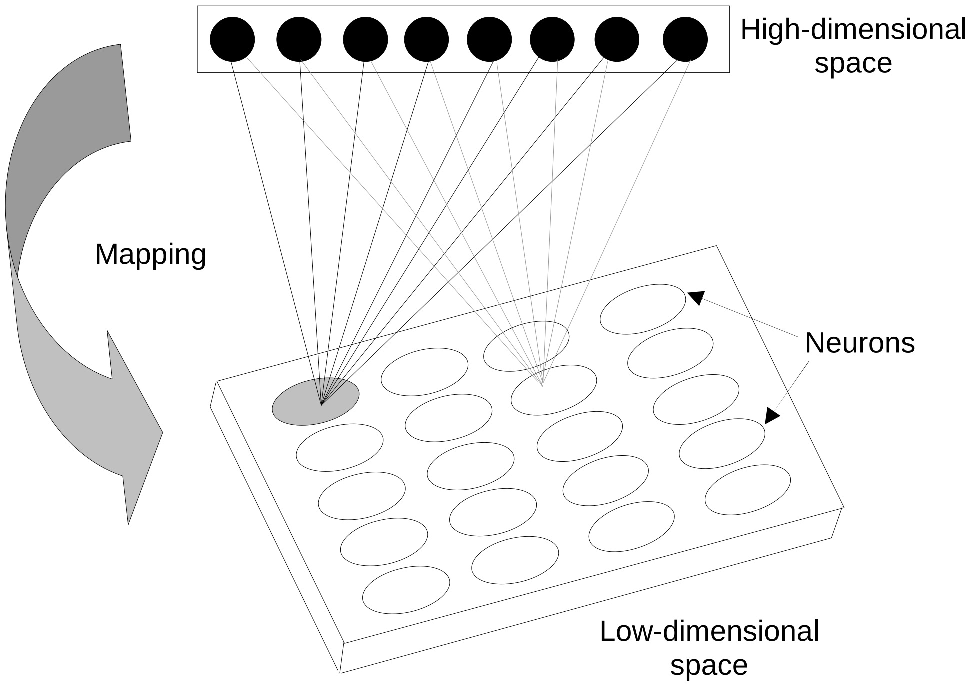


Figure 8-1. Mapping of a high-dimensional vector into a low-dimensional vector space in the SOM. A eight dimensional input vector (high-dimensional) is projected into two-dimensional (low-dimensional) space.

required during the learning process and that little needs to be known about the characteristics of the input data (Alhomiemi et al., 1999). In the SOM algorithm, the topological relations and the number of the neurons or nodes are fixed from the beginning. Each neuron *i* is represented by an n-dimensional weight, or model vector $m_i = [m_{i1},\ldots,m_{in}]$ (*n*, dimension of the input vectors). Each neuron contains a weight vector. At the start of the model, the weight vectors are initialized to random values. During training, the weight vectors are calculated using a distance measure such as the Euclidian distance, which is defined in Equation 8-1.

$$D_i = \sqrt{\sum_{j=1}^{n}(x_{ij} - m_{ij})^2} \text{ ; i = 1, 2,…, M} \qquad (8\text{-}1)$$

where

D_i = Euclidian distance between the input vector and the weight vector i;

x_{ij} = j^{th} element of the current input vector;

m_{ij} = j^{th} element of the weight vector i;

M = number of the neurons in the self-organising map; and

n = dimension of the input vectors.

Node *c* (Equation (8-2)), whose weight vector is closest to the input vector, is chosen as the best matching unit (BMU). When the BMU is found, the weight vectors m_i are updated. The BMU and its topological neighbours are moved closer to the input vector. The update rule of the weight vector is shown in Equation (8-3).

$$\|x - m_c\| = \min\{\|x - m_i\|\}, \qquad (8\text{-}2)$$

where

x = input vector;

m = weight vector; and

$\|\bullet\|$ = a distance measure.

$$m_i(t+1) = m_i(t) + \alpha(t)h_{ci}(t)[x(t) - m_t(t)]\,, \tag{8-3}$$

where

$m(t)$ = weight vector indicating the output unit's location in the data space at time t;

$\alpha(t)$ = learning rate at time t;

$h_{ci}(t)$ = neighbourhood function centre in the winner unit c at time t; and

$x(t)$ = input vector drawn from the input data set at time t.

After this competitive learning exercise, the clusters corresponding to characteristic features can be shown on the map. The quality of the mapping is usually measured with the quantization error and the topographical error. Since the codebook vectors of the SOM represent the local mean of the input vector, the SOM can be used for the prediction of missing components of an input vector. A prediction can be made by seeking the BMU for a vector with unknown components. The predicted values can be obtained from the BMU. The application of the SOM for prediction purposes is illustrated in Figure 8-2. The model is trained using the training dataset, which is removed from the vector to predict a set of variables as part of an input vector. The depleted vector is subsequently presented to the SOM to identify its BMU. The values for the missing variables are then obtained by their corresponding values in the BMU (Rustum et al., 2008).

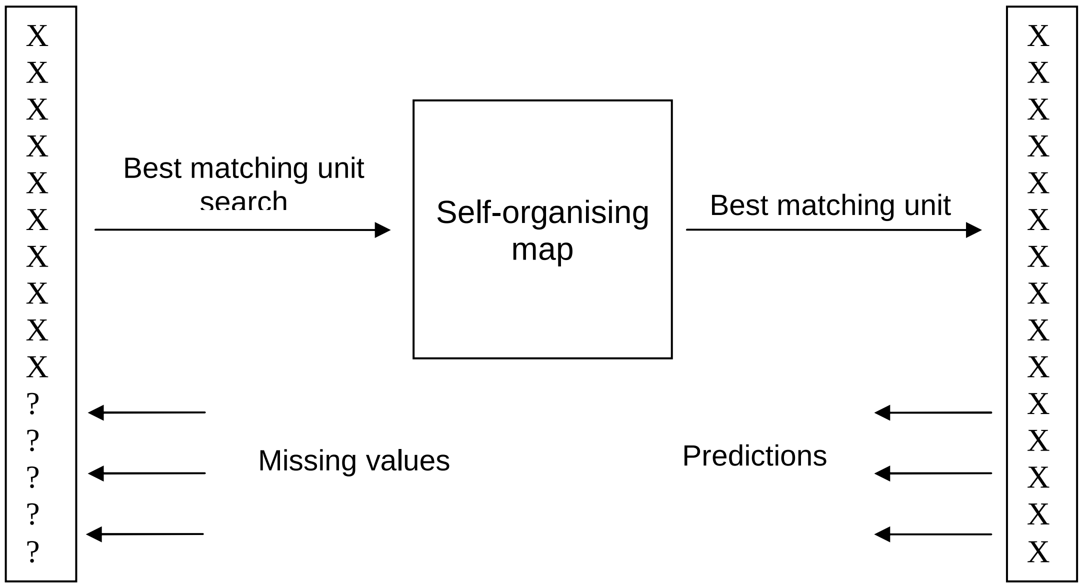


Figure 8-2. Predicting missing components of the input vector using the self-organising map.

8.3. Aim and objectives

The aim of this multi-disciplinary research was to propose simple and robust models suitable for providing real time control of the treatment performances of integrated constructed wetlands. The corresponding objectives are as follows:

1. To predict the nutrient concentration removal performances with the SOM model using water quality parameters, which are more cost-effective, quicker and easier to measure;
2. To predict the time-consuming and expensive variable BOD with incomplete input datasets using a SOM model based on faster and easier to measure inexpensive water quality parameters;

3. To fill missing values and replace outliers in the ICW data set with a SOM model capable of making accurate predictions; and
4. To compare the overall modelling approach with conventional techniques.

8.4. Data and variables

8.4.1. Nutrient and BOD prediction

The data used for prediction of nutrients and BOD were discussed in Chapter 4; 4.8.

8.4.2. Missing values and outliers

Missing values are very common in large environmental datasets provided by regulators. There are many reasons for missing values. Even if experiments have been planned well, there will always be missing values when something goes wrong, such as equipment malfunctioning and human error. The best solution for estimating missing values is to repeat the experiment and to generate a new complete dataset (Burke, 1999). However, some experiments can not be repeated because of limited time or costs, or due to the high variability of a complex environmental system such as an ICW. Prediction of missing values by modelling is an alternative method when it is not feasible to repeat the work.

Outliers are data that appear to be not consistent with the entire dataset. The reasons causing outliers may include measurement errors, recording errors and extreme measurement conditions (Iglewicz and Hoaglin, 1993). There are many methods for identifying outliers, such as visual inspection methods, calculation of z-scores and modified z-scores, boxplots, Rosner's test and Grubbs' test (Iglewicz and Hoaglin, 1993; Burke, 1999). Real outliers are usually removed from the data

Table 8-1. Summary statistics of the main outflow variables of ICW 11

Variable	Measurements						Number of Missing Values	Number of outliers
	Unit	Mean	Standard deviation	Median	Minimum	Maximum		
Ammonia-nitrogen	mg/l	0.37	0.565	0.12	0.00	3.15	6	0
MRP[a]	mg/l	0.89	0.550	0.81	0.00	2.60	3	1
SS[b]	mg/l	15.1	15.94	10.0	0.7	60.0	72	9
COD[c]	mg/l	55.2	18.25	55.0	10.0	100.0	57	5
BOD_5[d]	mg/l	11.2	9.20	8.0	0.6	34.0	86	6

[a]molybdate reactive phosphorus, [b]suspended solids, [c]chemical oxygen demand, [d] Five days biochemical oxygen demand

set, and treated as missing values in statistics. Table 8-1 summarises the outflow variables of ICW 11.

8.4.3. Training and testing of datasets

For prediction of nutrients. For modelling purposes, the ICW 3, 9 and 11data sets were randomised and then subdivided into two sets. The first subset was used as a training dataset, and the second subset was used as a testing dataset. Training and test datasets are summarised in Table 8-2. The model was verified with the test dataset. For example, when predicting the ICW treatment performance for ammonia-nitrogen removal, the corresponding ammonia-nitrogen data entries were omitted from the test dataset, implying that ammonia-nitrogen concentrations were in fact missing values. After running the simulation, the predicted ammonia-nitrogen concentrations were subsequently compared with the actual values.

Table 8-2. Summary statistics of the datasets used for prediction when applying the self-organising map model

Statistics	Ammonia-nitrogen prediction	Molybdate reactive phosphorus prediction
Number of training datasets	240	250
Number of test datasets	74	84
Correlation coefficient	0.934	0.951
Mean absolute scaled error[a]	0.015	0.048

[a] Mean absolute scaled error (MASE) = $((\frac{1}{n}\sum_{i=1}^{n}(a_i - p_i))/(\frac{1}{n-1}\sum_{i=1}^{n}|a_i - a_{i-1}|))$; a_i = actual values; p_i = predicted values; and n = number of test data sets.

For prediction of BOD. The SOM model was tested to predict the ICW 11 outflow BOD concentrations to monitor and control the outflow water quality in real time. In general, the input variables used for prediction should be time-efficient and easy to measure. Based on this consideration, the other four variables, which can be measured within several hours, were used for predicting five-day BOD. Only 58 data records for BOD with no missing values were used for modelling. However, these 58 data records were incomplete as there were missing values for the other four parameters. The data set was mixed in a random order and the odd row subset was used as a training dataset, and the even row subset as a testing dataset. After training with the training data set, the model was verified with the test dataset. The BOD data entries were omitted from the test data set, implying that BOD data were in fact missing values. After running the simulation, the predicted BOD data were subsequently compared with the actual values.

8.5. Nutrient prediction

The SOM model was applied to predict the ammonia-nitrogen and MRP removal

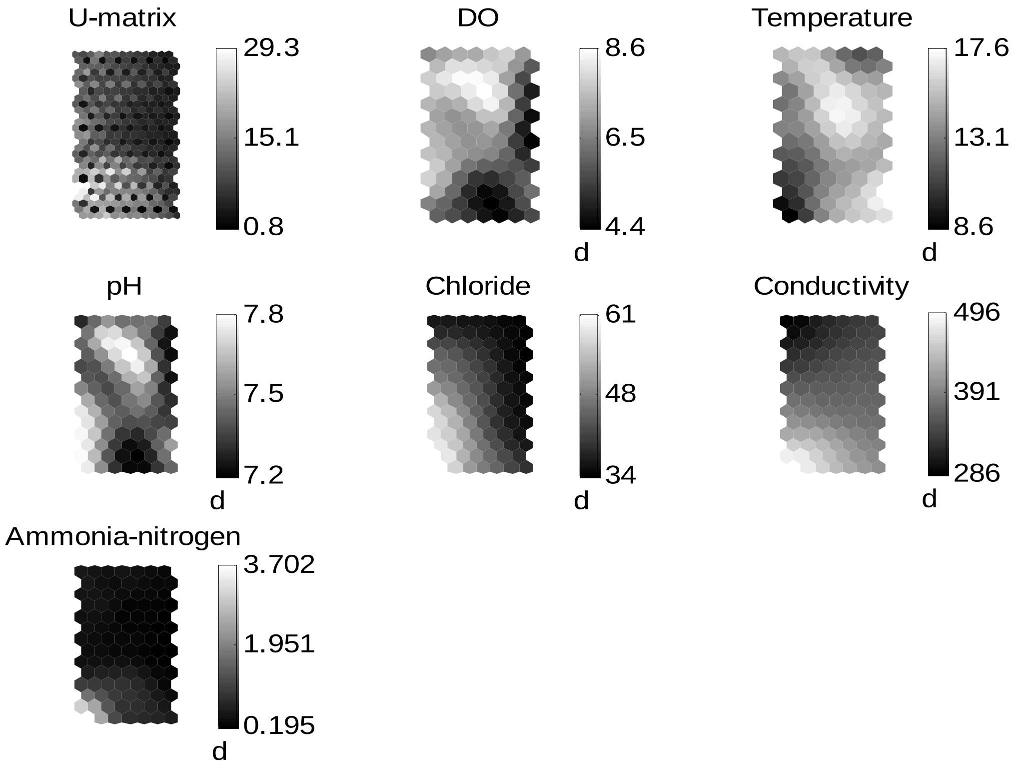


Figure 8-3. Abstract visualisation of the relationships between outflow ammonia-nitrogen (mg/L), and outflow dissolved oxygen (DO, mg/L), temperature (ºC), pH (dimensionless), chloride (mg/L) and conductivity (µS/cm) using a self-organising map model.

performances of ICWs. Table 8-3 summarises the results from a correlation analysis comprising the input variables DO, temperature, pH, chloride and conductivity, and the target variables ammonia-nitrogen and MRP. Findings are in agreement with Figures 8-3 and 8-4 which highlight the key relationships revealed by the SOM. For example, it can be seen that ammonia-nitrogen concentrations were highly correlated with temperature, chloride and conductivity. In comparison, MRP concentrations were highly correlated with DO, chloride and conductivity.

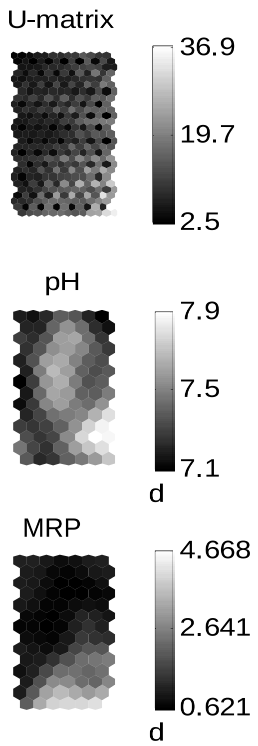


Figure 8-4. Abstract visualization of the relationships between outflow molybdate reactive phosphorus (MRP, mg/L), and outflow dissolved oxygen (DO, mg/L), temperature (ºC), pH (dimensionless), chloride (mg/L) and conductivity (µS/cm) using a self-organising map model.

In general, the measurements of input variables used for prediction should be more cost-effective and time-efficient, and easier in comparison to those of the target variables. Based on this consideration, temperature (R=-0.376) and conductivity (R=0.428) were selected as input variables to predict ammonia-nitrogen in the outflow of the ICW. DO (R=-0.463) and conductivity (R=0.562) were selected as input variables to predict MRP. Considering the relatively high costs and long time associated with most chloride measurement techniques, chloride was not selected for predicting either ammonia-nitrogen or MRP concentrations, even though it had comparatively strong correlations with ammonia-nitrogen (R=0.384) and MRP (R=0.477).

Figure 8-5 shows the actual and predicted outflow ammonia-nitrogen and MRP concentrations. The SOM modelling performances for predicting outflow ammonia-nitrogen and MRP concentrations are shown in Table 8-2. It shows the summary statistics of the SOM model for the test. The SOM model has a comparatively lower mean absolute scaled error indicating its relatively high accuracy in prediction if compared to previous results (Lee and Scholz, 2006). In general, the SOM model performed very well in predicting the nutrient concentrations in ICW systems.

Table 8-3. Correlation coefficients and corresponding *p* values (in brackets) related to a correlation analysis comprising input (column headings) and target (row headings) variables (n = 314 for Ammonium-nitrogen and n = 334 for MRP).

Variables	Dissolved oxygen (mg/L)	Temperature (ºC)	pH (-)	Chloride (mg/L)	Conductivity (µS/cm)
NH_4-N[a](mg/L)	-0.016 (0.779)	-0.376 (<0.01)	0.096 (0.088)	0.384 (<0.01)	0.428 (<0.01)
MRP[b](mg/L)	-0.463 (<0.01)	-0.206 (<0.01)	-0.163 (<0.01)	0.477 (<0.01)	0.562 (<0.01)

[a]Ammonium-nitrogen, [b]Molybdate reactive phosphorus.

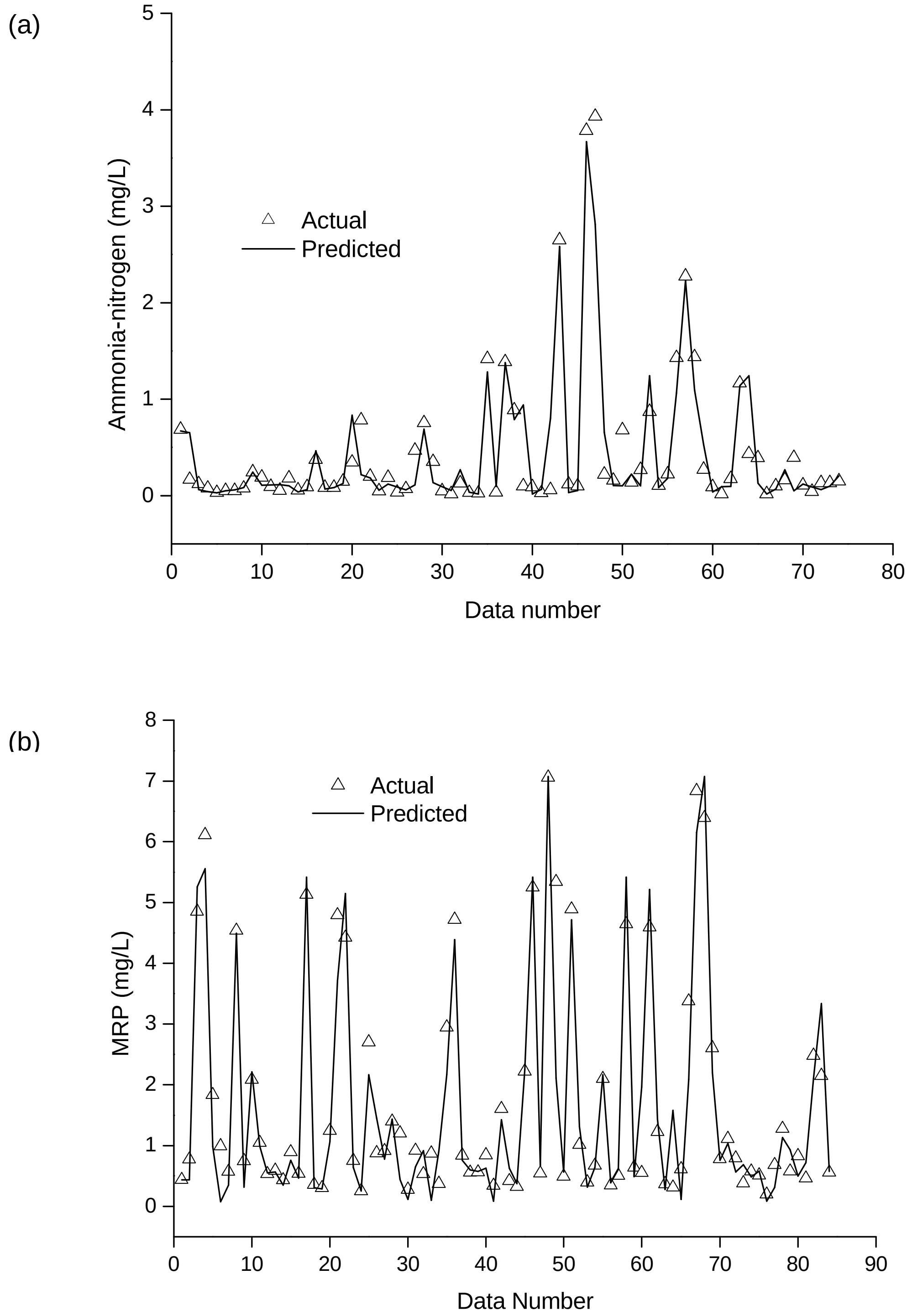


Figure 8-5. The actual and predicted outflow (a) ammonia-nitrogen and (b) molybdate reactive phosphorus (MRP) concentrations.

8.6. BOD prediction

BOD is an important water quality parameter for characterising the biodegradable components of organic matter in wastewater, but it is not suitable for real time water quality monitoring, because of the long time associated with BOD measurement techniques (Scholz, 2006). The SOM modelling performance in terms of predicting outflow BOD concentrations is shown in Figure 8-6.

For water quality control purposes, it is important to assess whether or not outflow concentrations meet water quality standards for discharge. In this study BOD concentrations were divided into three bins by common international threshold values: A threshold for BOD of 25 mg/L is used for discharges from Irish wastewater treatment plants (Smith *et al*., 2004), and a BOD concentration of 10 mg/L is used for conservation of the living environment (MOE, 1989). A predicted BOD value was regarded as accurate if it was in the same bin as the actual value.

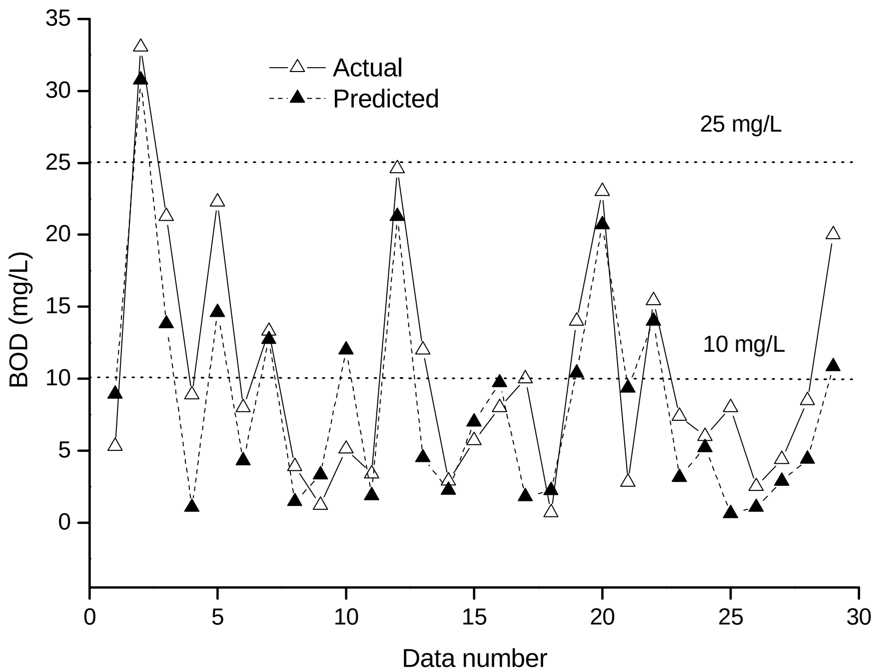


Figure 8-6. The actual and predicted biochemical oxygen demand (BOD) in ICW 11 outflow.

Table 8-4 shows the summary statistics of the SOM model performance for prediction of BOD. Correct predictions for bins 1 to 3 were relatively high, 95, 89 and 100%, respectively. The corresponding mean was approximately 93%. This study is just an example of how BOD concentrations can be predicted with SOM to monitor and control outflow water quality in real time. When applying the SOM model to other scenarios, new thresholds would need to be selected. In general, the SOM model performed very well in predicting BOD concentrations. Real time BOD control is therefore possible for ICW systems.

Table 8-4. Performance of the self-organising map model for the prediction of biochemical oxygen demand (BOD).

	Mean	Standard deviation	Total number	Accurately predicted number/total number of each BOD bin		
				Bin 1[a]	Bin 2[b]	Bin 3[c]
Actual	10.4	8.2	29	18/19	8/9	1/1
Predicted	8.1	7.3	29	(94.7%)	(88.9%)	(100%)

[a] Bin 1 (BOD<10 mg/L), [b] Bin 2 (BOD=10-25 mg/L), [c] Bin 3 (BOD>25 mg/L).

8.7. Filling in missing values and replacing outliers

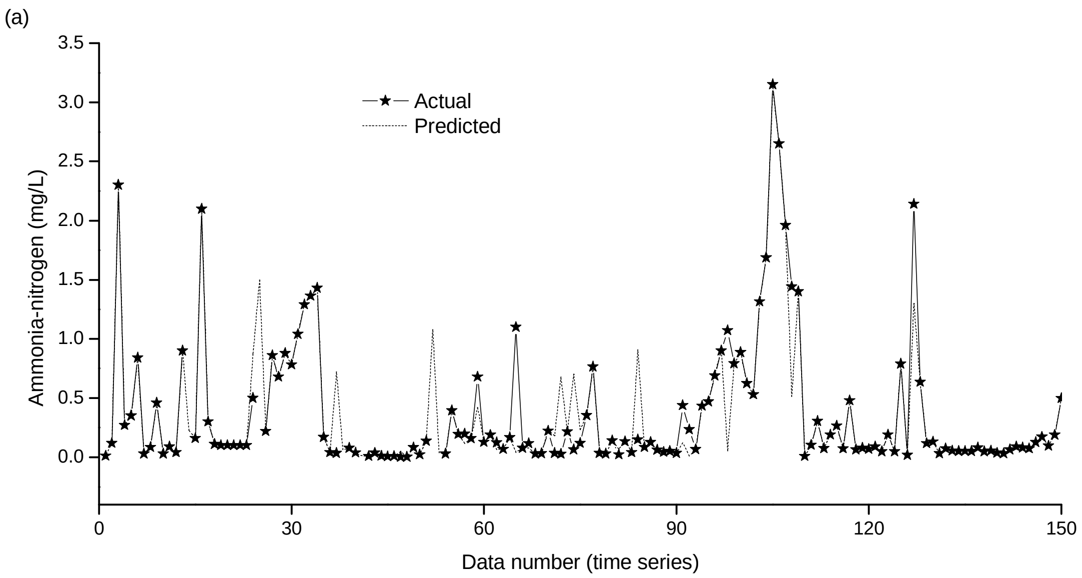
The SOM model was applied to the data (normalised) to predict missing values and to replace outliers in the ICW 11 inflow and outflow data set. Table 8-5 shows the performance of the SOM model. The correlation coefficients, mean square errors and mean absolute errors between actual and predicted values for all variables are shown in Table 8-5. The SOM model has relatively high correlation coefficients, lower mean square errors and lower mean absolute errors indicating its relatively high prediction accuracy if compared to previous studies (Chandramouli *et al*., 2007; Rustum and Adeloye, 2007).

Table 8-5. Performance of the self-organising map model during training.

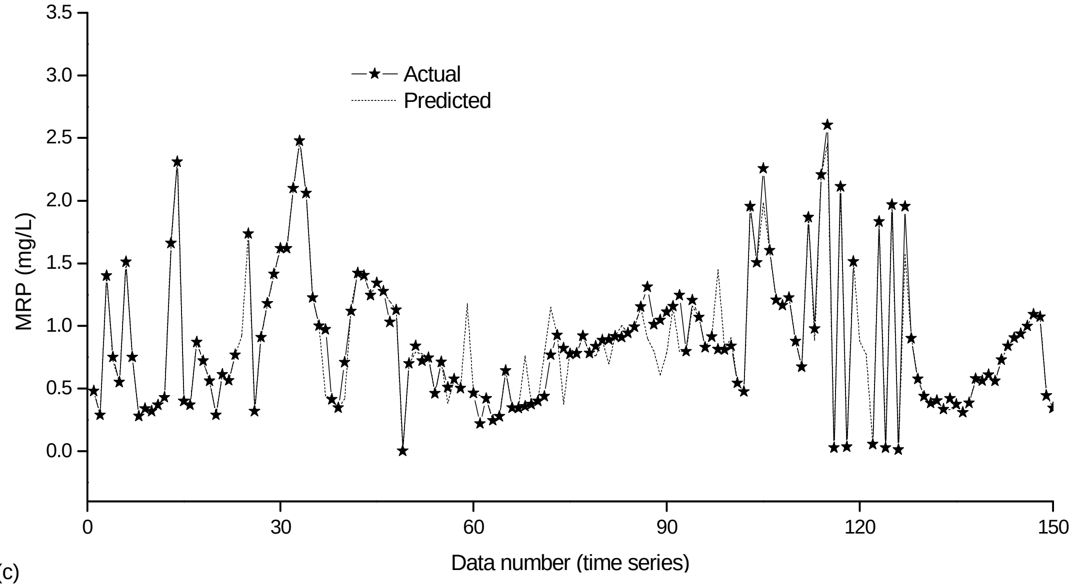
Variable (mg/L)	Correlation coefficient R	Mean relative absolute error	Mean square error
Ammonia-nitrogen	0.93	0.639	0.042
MRP[a]	0.97	0.074	0.017
Suspended-solids	0.95	0.238	23.325
COD[b]	0.95	0.071	30.088
BOD[c]	0.96	0.309	6.351

[a]molybdate reactive phosphate, [b]chemical oxygen demand, [c]biochemical oxygen demand.

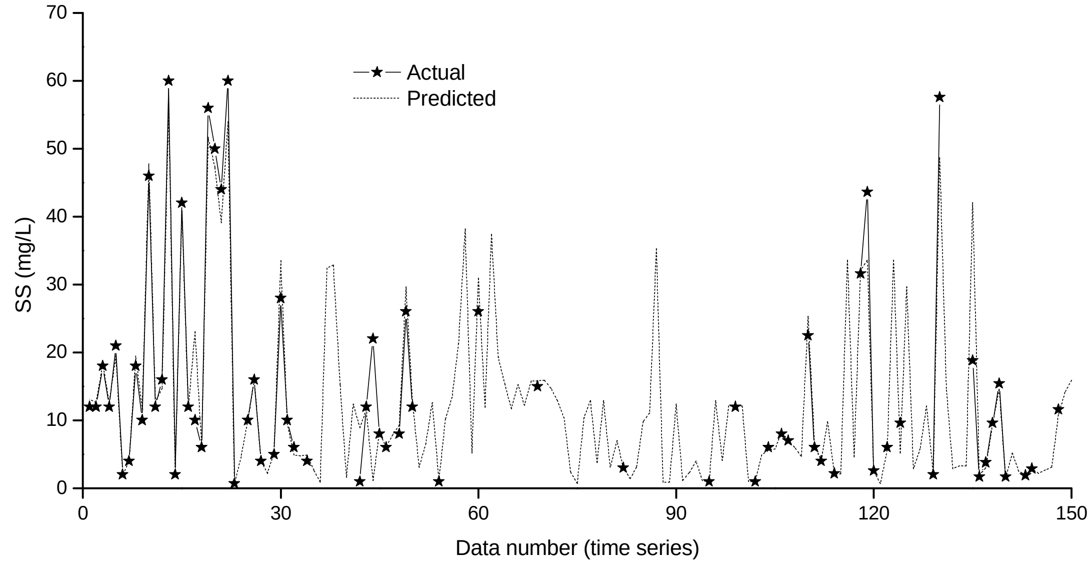
Figures 8-7a to 8-7e compare the actual and predicted values for ammonia-nitrogen, MRP, SS, COD and BOD concentrations, respectively. The trends for the predicted concentrations are in very similar to the actual concentrations. Hence missing values and outliers can be predicted and replaced by the predicted values.



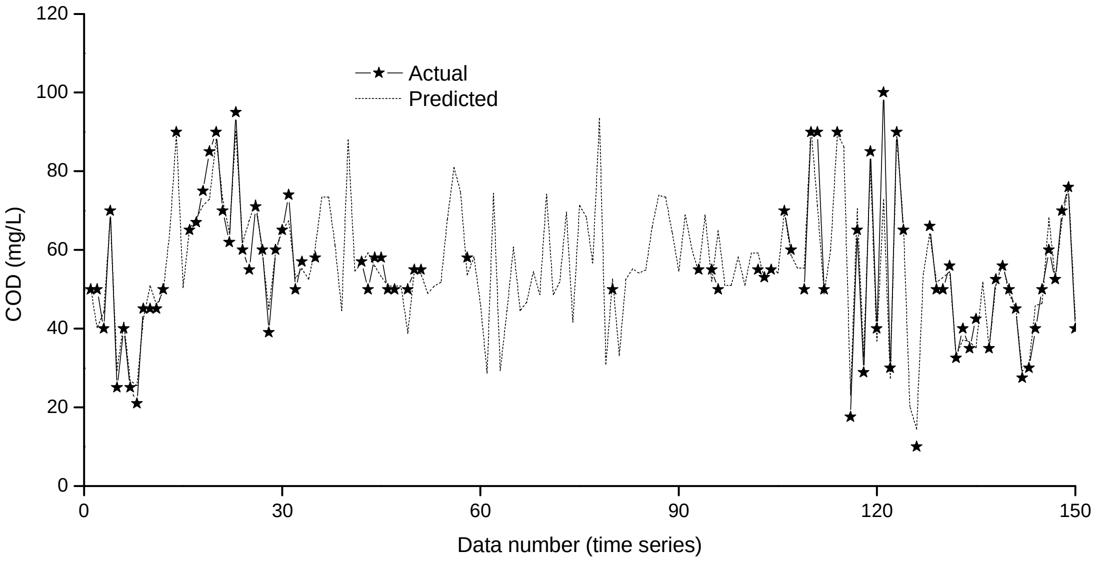
(b)


Actual
Predicted
MRP (mg/L)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
30
60
90
120
150
Data number (time series)

(c)


Actual
Predicted
SS (mg/L)
70
60
50
40
30
20
10
0
0
30
60
90
120
150
Data number (time series)

(d)


Actual
Predicted
COD (mg/L)
120
100
80
60
40
20
0
0
30
60
90
120
150
Data number (time series)

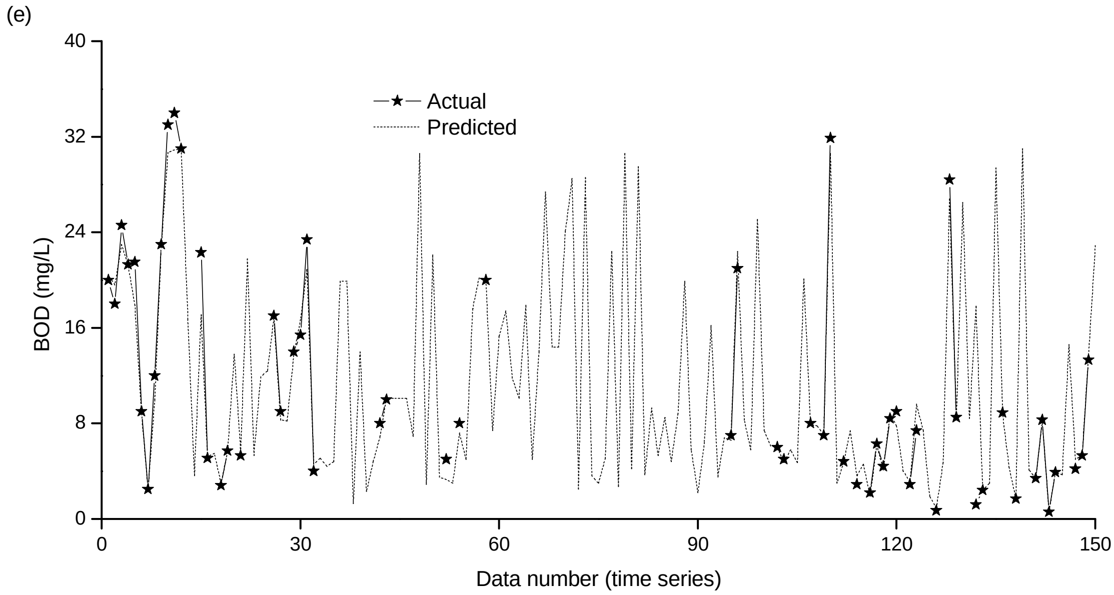


Figure 8-7. The actual and predicted values for (a) ammonia-nitrogen; (b) molybdate reactive phosphate (MRP); (c) suspended solids (SS); (d) chemical oxygen demand (COD); and (e) biochemical oxygen demand (BOD).

8.8. Summary

This chapter shows the application of SOM as a management tool for integrated constructed wetlands. The SOM model was successfully applied to predict water quality variables of integrated constructed treatment wetlands. It performed very well in predicting nutrient and biochemical oxygen demand (BOD) concentrations.

The self-organising map (SOM) showed that the ammonia-nitrogen outflow concentrations correlated with water temperature and salt concentrations (indicated by conductivity and chloride in farmyard runoff). High ammonia-nitrogen removal efficiency can be achieved if salt concentrations are low and temperatures are high. The SOM model also revealed that molybdate reactive phosphorus removal was predominantly affected by salt and dissolved oxygen. Molybdate reactive phosphorus can easily be removed within ICW if salt concentrations are low and dissolved oxygen, temperature and pH values are high.

The SOM performed very well in modelling and predicting nutrient removal in ICW. Nutrients such as ammonia-nitrogen and molybdate reactive phosphorus can be accurately predicted by other more cost-effective, rapid and easier to measure water quality variables such as temperature, conductivity and dissolved oxygen. Moreover, the SOM model performed very well in predicting biochemical oxygen demand (BOD) concentrations. Real time control of the water quality in ICW systems is therefore possible. Outflow five-day BOD concentrations can be predicted by other outflow parameters, which can be measured within several hours. Outflow BOD concentrations in 3 bins were accurate between 89 and 100%.

The missing values and outliers for the long-term ICW data set were replaced by the SOM model, which showed an excellent performance in predicting BOD. In comparison to traditional prediction models, the SOM was not affected by missing values and processed incomplete datasets relatively well, leading to good predictions. In conclusion, the results indicated that the SOM model is an excellent practical tool for predicting key outflow water quality parameters of integrated constructed wetlands.

Chapter 9 Conclusions and Recommendations

9.1. Conclusions

The most important conclusions resulting from this study are summarised as follows:

- The ICW systems studied reduced concentrations of contaminants present in farmyard runoff. Nutrients including ammonia-nitrogen and molybdate reactive phosphorus were effectively reduced even after >7 years of operation. Hence, the structures act as a sink (not source) for nutrients.

- The groundwater and surface water monitoring results indicated that the ICW systems had not polluted groundwater nor degraded the water quality of the receiving watercourse. Concentrations of nutrients including nitrate-nitrogen in receiving water bodies were lower down-stream than up-stream of the ICWs. The presence of denitrifying and ammonia-oxidising bacteria in wetland litter and sediments shows the potential of wetlands to remediate infiltrating wastewater.

- There were more diverse populations of nitrogen removing bacteria in the wetland systems studied compared to conventional wastewater treatment systems. The ammonia-oxidising bacteria, *Nitrosospira* and *Nitrosomonas* were detected in the studied wetland systems. The denitrifying bacteria, *Paracoccus, Pseudomonas, Rhizobium* and *Dechloromonas* were identified. The litter component of the two wetland systems supported more diverse nitrogen removing bacteria (ammonia-oxidising and denitrifying) than the sediments. Nitrogen removing bacteria in the representative full-scale wetland systems appeared to be stochastically assembled from the same source community.

- The study of role of plants and sediment and soil in removing nutrients from an integrated constructed wetland revealed that the soil component of a mature wetland system is an important and sustainable storage component for long-term nutrient storage. More N and P were stored in wetland soils and sediments than in plants. The first cell of a representative ICW had the highest rate of sediment accumulation (45 cm). Over the 7-year operation period the accretion rate was approximately 6.4 cm/yr. With regards to management guidelines, desludging of the first wetland cell of ICW 11 appears to be necessary in 2011. Approximately 74% (780 kg) of the phosphorus and 52% (5175 kg) of the nitrogen that entered the wetland system was stored in the wetland soils and sediments. Plants stored a small percentage of nutrients compared to soils (<1% both N and P).

- A self-organising map model was successfully used to predict water quality variables in integrated constructed wetlands. Nutrients such as ammonia-nitrogen and molybdate reactive phosphorus can be accurately predicted by other more cost-effective, rapid and easier to measure water quality variables such as temperature, conductivity and dissolved oxygen. BOD which is expensive to measure can be monitored cost-effectively, by applying the SOM model. The model also successfully filled in the missing values and replaced the outliers for the long-term ICW data set. The performance of the SOM model is encouraging and the model can be used to support management decisions in real-time.

9.2. Recommendations for further work

The findings have significant implications for the future operation, monitoring and management of integrated constructed wetlands for farmyard runoff treatment. While this study has demonstrated the positive role of integrated constructed wetlands in nutrient removal, nevertheless there is an obvious need to carry out further studies. Some important future research frontiers are as follows:

- The monitoring of ICW should continue to reinforce the multi-year water quality dataset. Pathogens, nutrients and carbon in ICW influent/effluent should be measured more regularly and frequently.
- More research is needed to better understand the processes responsible for the transformation and removal of nutrients. The ammonia-oxidation and denitrification potentials and their seasonal variations should be investigated. Important relationships could be inferred by combining these results with those of the previous study of characterisation of nitrogen removing bacteria.
- It would be interesting to quantify ammonia-oxidising and denitrifying bacterial communities using real-time PCR. This may contribute to deeper and broader understanding of underlying mechanisms in nitrogen removal processes.
- ICW maintenance guidelines suggest that the first cell of ICW 11 will require desluding in 2011. A schedule for dredging methodology and sediment disposal in a sustainable manner needs to be prepared and implemented. Moreover, the performance of the system after sediment

removal needs to be monitored. Performance pre and post-sediment removal will provide important information for ICW design, operation and maintenance guidelines.

- Greenhouse gas emissions, such as methane, carbon dioxide and nitrous oxide from ICWs need to be investigated.
- There is a need to evaluate the impacts of hydrological regimes on nutrient treatment, especially P. For example, when the outflow exceeds 100mm, P reductions are variable.
- Although the absence of an artificial liner makes ICW systems affordable detailed studies are needed to address the concerns of hydrogeologists. The present study has provided some evidence of these systems not polluting groundwater, but there is a need to conduct laboratory studies coupled with field investigations to address the concerns of practitioners.
- Contaminant removal in a constructed wetland occurs through a various range of exchanges between the three wetland components of soil, water and air matrices. The effectiveness and level of these interactions depends on the dynamics of water movement. Contact time between wastewater, soil and microorganisms is an important factor that dictates contaminant removal in wetland systems. The velocity of wastewater is also an important determining factor for the contaminant removal processes. Hence tracer studies should be conducted to gain insights into the internal hydraulics of integrated constructed wetlands.

References

Abis, K.L., 2002. The performance of facultative waste stabilisation ponds in the United Kingdom. PhD dissertation, University of Leeds, UK.

Alhoniemi, E., Hollmen, J., Simula, O., Vesanto, J., 1999. Process monitoring and modelling using the self-organizing map. Integrated Computer-Aided Engineering 6: 3−14.

Amann, R.I., Ludwig, W. and Schleifer, K., 1995. Phylogenetic identification and insitu detection of individual microbial cells without cultivation. Microbiology Reviews 59: 143−169

APHA,1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association/American Water Works Association/ Water Environment Federation, Washington DC, USA.

Asano, T., Burton, F.L., Leverenz, H.L., Tsuchihashi, R., Tchobanoglous, G., 2007. Water Reuse, Issues, Technologies and Applications. McGraw-Hill: New York, USA.

Astel, A., Tsakovski, S., Barbieri, P., Simeonov, V., 2007. Comparison of Self-organizing maps classification approach with cluster and principal components analysis for large environmental data sets. Water Research 41: 4566−4578.

Austin, D.C., Lohan, E., Verson, E., 2003. Nitrification and denitrification in tidal vertical flow wetland pilot project. Proceedings, WEFTEC 2003 National Conference, 76th Annual Conference and Exhibition; Water and Environment Federation; Alexandria, Virginia, USA.

Axt, J.R., Walbridge, M.R., 1999. Phosphate removal of palustrine forested wetlands and adjacent uplands in Virginia. Soil Science Society of America Journal 63: 1019–1031.

Baptista, J.C., Davenport, R.J., Donnelly, T., Curtis, T.P., 2008. The microbial diversity of laboratory-scale wetlands appears to be randomly assembled. Water Research 42: 3182−3190.

Bastviken, S. K., Eriksson, P. G., Martins, I., Neto, J. M., Leonardson, L., Tonderski, K.S., 2003. Potential nitrification and denitrification on different surfaces in a constructed treatment wetland. Journal of Environmental Quality 32: 2414−2420.

Bastviken, S.K., Eriksson, P.G., Premrov, A. and Tonderski, K.S., 2005. Potential denitrification in wetland sediments with different plant species detritus. Ecological Engineering 25: 183−190.

Borin, M.,Tocchetto, D., 2007. Five year water and nitrogen balance for a constructed surface flow wetland treating agricultural drainage waters. Science of the Total Environment 380: 38–47.

Bothe, H., Jost, G., Schloter, M., Ward, B.B., Witzel, K.-P., 2000. Molecular analysis of ammonia oxidation and denitrification in natural environments. FEMS Microbiology Reviews 24: 673−690.

Bouwer, H., Ludke, J., Rice, R.C., 2001. Sealing pond bottoms with muddy water. Short Communication. Ecological Engineering 18: 233−238.

Bowmer, K.H., Laut, P., 1992.Wastewater management and resource recovery in intensive rural industries in Australia. Water Research 26: 201–208

Braker, G., Ayala del Rio, H.L., Devol, A.H., Fesefeldt, A., Tiedje, J., 2001. Community structure of denitrifiers, Bacteria, and Archae along redox gradients in Pacific Northwest marine sediments by terminal restriction fragment length polymorphism analysis of amplified nitrite reductase (nirS) and 16S rRNA genes. Applied and Environmental Microbiology. 67, 1893-1901.

Braskerud, B.C., 2001. The influence of vegetation on sedimentation and resuspension of soil particles in small constructed wetlands. Journal of Environmental Quality 30: 1447−1457.

Braskerud, B.C., 2002. Factors affecting N retention in small constructed wetlands treating agricultural non-point source pollution. Ecological Engineering 18: 351−370.

Breen, P.F., 1990. A mass balance method for assessing the potential of artificial wetlands for wastewater treatment. Water Research 24: 689−697.

Bridgham, S. D., Johnston, C. A., Schubauer−Berigan, J.P., Weishampel, P., 2001. Phosphorus Sorption Dynamics in Soils and Coupling with Surface and Pore Water in Riverine Wetlands. Soil Science Society of America Journal 65: 577−588.

Brisson, J., Chazarenc, 2008. Maximizing pollutant removal in constructed wetlands: Should we pay more attention to macrophyte species selection? Science of the Total Environment 407: 3923−3930.

Brix, H., Dyhr−jensen, k., Lorenzen, B., 2002. Root-zone acidity and nitrogen source affects *Typha latifolia* L. growth and uptake kinetics of ammonium and nitrate. Journal of Experimental Botany 53: 2441−2450.

Brix, H., 1994. Functions of macrophytes in constructed wetlands. Water Science and Technology 29: 71−78.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands? Water Science and Technology 35: 11−17.

Burke, S., 1999. Missing values, outliers, robust statistics and non-parametric methods. LC*GC Europe Online Supp.lement, 19–24. Available online at http://www.lcgceurope.com/lcgceurope/data/articlestandard//lcgceurope/502001/4509/article.pdf.

Camargo, V. M. A., Mara, D. D., 2007. Nitrogen removal via ammonia volatilization in maturation ponds. Water Science and Technology 55: 87−92.

Carty, A., Scholz, M., Heal, K.V., Gouriveau, F. and Mustafa, A., 2008a. The universal design, operation and maintenance guidelines for farm constructed wetlands (FCW) in temperate climates. Bioresource Technology 99: 6780−6792.

Carty, A., Scholz, M., Heal, K.V., Keohane, J., Dunne, E.J., Gouriveau, F. and Mustafa, A., 2008b. Farm constructed wetland design manual for Northern Ireland and Scotland. Department of Environment (Northern Ireland) and Scottish Environment Protection Agency, UK, 61p.

Carucci, A., Majone, M., Ramadori, R., Rossetti, S., 1997. Biological phosphorus removal with different organic substrates in an anaerobic/aerobic sequencing batch reactor. Water Science and Technology 35: 161–168.

Chandramouli, V., Brion, G., Neelakantan, T.R., Lingireddy, S., 2007. Backfilling missing microbial concentrations in a riverine database using artificial neural networks. Water Research 41: 217−227.

Chapanova, G., Jank, M., Schlegel, S., Koeser, H., 2007. Effect of temperature and salinity on the wastewater treatment performance of aerobic submerged fixed bed biofilm reactors. Water Science and Technology 55: 159−164.

CH2M HILL and Payne Engineering, 1997. Constructed wetlands for livestock wastewater management. Literature review, database and research synthesis. Gulf of Mexico Program, Stennis Space Center, MI, USA.

Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd ed. PRIMER-E: Plymouth, UK.

Clenaghan, C., 2003. Phosphorus regulations; national implementation report. Environmental Protection Agency, Wexford, Ireland.

Cole, J.R., Chai, B., Marsh,T.L., Farris, R.J., Wang,Q., Kulam, S.A., Chandra, S., McGarrell, D.M., Schmidt, T.M., Garrity, G.M., 2003. The Ribosomal Database Project (RDP−II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Research 31: 442−443.

Cowardin, L., Carter, V., Golet, F. and LaRoe, E., 1979. Classification of wetlands and deepwater habitats of the United States. Office of Biological Services, US Fish and Wildlife Service.

Craft, C.B., Richardson, C.J., 1993. Peat accretion and N, P, and organic C accumulation in nutrient−enriched and unenriched Everglades peatlands. Ecological Applications 3: 446–458.

Crites, R., Tchobanoglous, G., 1998. Small and Decentralized Wastewater Management Systems. Mcgraw-Hill, New York, USA.

Crites, R.W., Middlebrooks, E.J., Reed, S.C., 2006. Natural Wastewater Treatment Systems. CRC Press, Boca Raton, FL., USA.

Curtis, T.P., Head, I.M., Graham, D.W., 2003. Theoretical ecology for engineering biology. Environmental Science and Technology 37: 64–70.

Curtis, T.P., 2007. Crystal ball – 2007, Theory and the microbial world. Environmental Microbiology 9: 1−11.

Daly, K., 2000. Phosphorus desorbtion from Irish soils. In: Tunney, H. (Ed) Quantification of Phosphorus Loss from Soil to Water. Environmental Protection Agency, Wexford, Ireland, pp. 59−101.

D'Arcy, B.J., Ellis, J.B., Ferrier, R.C., Jenkins, A. and Dils, R. (eds.), 2000. Diffuse Pollution Impacts. The Environmental and Economic Impacts of Diffuse Pollution in the UK.CIWEM/EA/SEPA joint publication. Terence Dalton, Lavenham, UK, 181p

Debusk, W.F., Reddy, K.R., 2005. Litter decomposition and nutrient dynamics in a phosphorus enriched everglades marsh. Biogeochemistry 75: 217–240.

DeBusk, T.A., Burgoon, P.S., Reddy, K.R., 1989. Secondary treatment of wastewater using floating and emergent macrophytes. In: Constructed Wetlands for Wastewater Treatment: municipal, Agricultural, and industrial, Hammer, D.A. (ed.). Lewis Publishers, Chelsea, Michigan, USA.

DeBusk, T.A., Langston, M.A., Burgoon, P.S. and Reddy, K.R., 1990. A performance comparison of vegetated submerged beds and floating macrophytes for domestic wastewater treatment. In: Constructed Wetlands in Water Pollution Control, P.F. Cooper and B.C. Findlater (eds.). Pergamon Press, Oxford, UK, pp. 301–308.

Dieberg, F.E., Brezonik, P.L., 1984. Nitrogen and phosphorus mass balances in a cypress dome receiving wastewater. In: Cypress Swamps, Ewel, K.C., Odum, H.T. (eds.) University of Florida Press, Gainesville, Florida, USA, pp. 112−118.

Dierberg, F.E., DeBusk, T.A., 2005. An evaluation of two tracers in surface-flow wetlands: rhodamine-wt and lithium. Wetlands 25: 8–25.

Dincer, A.R., Kargi, F.,1999. Salt inhibition of nitrification and denitrification in saline wastewater. Environmental Technology 20: 1147–1153.

Dolan, T.J., Bayley, S.E., Zoltek Jr., J., Hermann, A.J., 1981. Phosphorus dynamics of a Florida freshwater marsh receiving treated wastewater. Journal of Applied Ecology. 18: 205–219.

Dunne, E.J., Culleton, N., O'Donovan, G., Harrington, R., Daly, K., 2005a. Phosphorus retention and sorption by constructed wetland soils in Southeast Ireland. Water Research 39: 4355−4362.

Dunne, E.J., Culleton, N., O'Donovan, G., Harrington, R., Olsen, A.E., 2005b. An Integrated constructed wetland to treat contaminants and nutrients from dairy farmyard dirty water. Ecological Engineering 24: 221−234.

Dunne, E.J., Smith, J., Perkins, D.B., Clark, M.W., Jawitz, J.W., Reddy, K.R., 2007. Phosphorus storages in historically isolated wetland ecosystems and surrounding pasture uplands. Ecological Engineering 31: 16–28.

Downing, A.L., Painter, H.A., Knowles, G.J., 1964. Nitrification in the activated sludge process. Journal, Institute of Sewage Purification 64: 130-158

Edwards, A.C., Kay, D., McDonald, A.T., Francis, C., Watkins, J., Wilkinson, J.R. and Wyer, M.D., 2008. Farmyards, an overlooked source for highly contaminated runoff. Journal of Environmental Management 87: 551−559.

EPA, 2002. Interim Report: The Biological Survey of River Quality 2001. Environmental Protection Agency, Johnston Castle, Wexford, Ireland, pp. 145.

Erskine, A.D., 2000. Transport of ammonium in aquifers: retardation and degradation. Quarterly Journal of Engineering Geology and Hydrogeology 33: 161−170.

Harrington, R., Carroll, P., Carty, A., Keohane, J., Ryder, C., 2007. Integrated Constructed Wetlands: concept, design, site evaluation and performance, International Journal of Water 3: 243− 256.

Harrington, R., McInnes, R., 2009. Integrated Constructed Wetlands (ICW) for livestock wastewater management. Bioresource Technology 100: 5498–5505.

Harrison, J.J., Turner, R.J., Marques, L.L.R., Ceri, H., 2005 Biofilms: A new understanding. American Scientist, pp. 508−515.

Faulkner, S.P., Richardson, C.J., 1989. Physical and chemical characteristics of freshwater soils. In: Constructed Wetlands for Wastewater Treatment: municipal, Agricultural, and industrial, Hammer, D.A. (ed.). Lewis Publishers, Boca Raton, Florida, USA, pp. 41−72.

Fogler, H.S., 1992. Elements of Chemical Reaction Engineering. Second Edition, Prentice Hall, Englewood Cliffs, New Jersey, USA, pp.838.

Forbes, M.G., Dickson, K.R., Golden, T.D., Hudak, P., Doyle, R.D., 2004. Dissolved phosphorus retention of light−weight expanded shale and masonry sand used in subsurface flow treatment wetlands. Environmental Science and Technology 38: 892−898.

Garcia, H. L., Gonzalez, I. M., 2004. Self−organizing map and clustering for wastewater treatment monitoring. Engineering Applied Artificial Intelligence 17: 215–225.

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

Gearheart, R.A., Klopp,F., Allen,G., 1992. Use of constructed wetlands to treat domestic wastewater, City of Arcata,CA. Water Science and technology 26: 1625−1637.

Girija, T.R., Mahanta, C., Chandramouli, V., 2007. Water quality assessment of an untreated effluent impacted urban stream: the Bharalu Tributary of the Brahmaputra River, India. Environmental Monitoring and Assessment 130: 221−236.

Golterman, H.L., 1995. The labyrinth of nutrient cycles and buffers in wetlands: results based on research in the Camargue (Southern France). Hydrobiologia 315: 39−58.

Gottschall, N., Boutin, C., Crolla, A., Kinsley, C., Champagne, P., 2007. The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. Ecological Engineering 29: 54−163.

Graham, D.W., Smith, V.H., 2004. Designed ecosystem services: application of ecological principles in wastewater treatment engineering. Frontiers in Ecology and in the Environment 2: 199–206.

Graham, S.A., Craft, C.B., McCormick, P.V., Aldous, A., 2005. Forms and accumulation of soil P in natural and recently restored peatlands−Upper Klamath Lake, Oregon, USA. Wetlands 25: 594−606.

Graver, E.G., Dubbe, D.R., and Pratt, D.C., 1988. Seasonal patterns in accumulation and partioning of biomass and macronutrients in Typha spp. Aquatic Botany 32: 115−130

Greenway, M., Woolley, A., 2000. Changes in plant biomass and nutrient removal over 3 years in a constructed wetland, Cairns, Australia, Water Science and Technology 44: 303–310.

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

Hallin, S., Throbäck, I.N., Dicksved, J., Pell, M., 2006. Metabolic profiles and genetic diversity of denitrifying communities in activated sludge after addition of methanol or ethanol. Applied and Environmental Microbiology 72: 5445−5452.

Hallin, S., Lindgren, P.E., 1999. PCR detection of genes encoding nitrite reductase in denitrifying bacteria. Applied and Environmental Microbiology 65: 1652−1657.

Hammer, D.A., Pullin, B.P., McCaskey, T.A., Eason, J. and Payne, V.W.E., 1993. Treating livestock wastewater with constructed wetlands. In: Moshiri, G.A. (ed.), Constructed Wetlands for Water Quality Improvement. CRC Press, Boca Raton, Florida, USA, pp. 343−347.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: palentological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1−9.

Harrington R., Ryder C., 2002. The use of integrated constructed wetlands in the management of farmyard runoff and waste water. In: Proceeding of the National Hydrology Seminar on Water Resources Management Sustainable Supply and Demand. The Irish National Committees of the IHP (International Hydrological Programme) and ICID (International Commission on Irrigation and Drainage), Tullamore, Ireland.

Harrington, R., Dunne, E.J., Carroll, P., Keohane, J., Ryder, C., 2005. The concept, design and performance of integrated constructed wetlands for the treatment of farmyard dirty water. In: Dunne, E.J., Reddy, K.R., Carton, O.T. (Eds), Nutrient Management in Agricultural Watersheds: A Wetlands Solution, Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 179-188

Harrington, R., McInnes, R., 2009. Integrated Constructed Wetlands (ICW) for livestock wastewater management. Bioresource Technology 100: 5498–5505.

Harrison, J.J., Turner, R.J., Marques, L.L.R., Ceri, H., 2005 Biofilms: A new understanding. American Scientist, pp. 508−515.

Head, I., M., Saunders, J.R. and Pickup, R.W. 1998. Microbial evolution, diversity and ecology; a decade of ribosomal RNA analysis of uncultured microorganisms. Microbial Ecology, 35: 1−21.

Healy, M.G., Rodgers M., Mulqueen J., 2007. Treatment of dairy wastewater using constructed wetlands and intermittent sand filters. Bioresource Technology 98: 2268−2281.

Hey, D.L., Kenimer, A.L., Barrett, K.R., 1994. Water quality improvement by four experimental wetlands. Ecological Engineering 3: 381−398.

House, C.H., Bergmann, B.A., Stomp, A.M., Fredericket, D.J., 1999. Combining constructed wetlands and aquatic and soil filters for reclamation and reuse of water. Ecological Engineering 12: 27−38.

Hunt, P.G., Poach, M.E., 2000. State of the art for animal wastewater treatment in constructed wetlands. In: Proceedings of the Seventh International Conference on Wetland Systems for Water Pollution Control, International Water Association, Lake Buena Vista, Florida, 11–16 November, pp. 707–718.

Hunt, P.G. and Poach, M.E., 2001. State of the art for animal wastewater treatment in constructed wetlands. Water Science and Technology 44: 19−25.

Iamchaturapatr, J., Yi, S.W., Rhee, J.S., 2007. Nutrient removals by 21 aquatic plants for vertical free surface-flow (VFS) constructed wetland. Ecological Engineering 29: 287−293.

Ibekwe, A.M., Grieve, C.M., Lyon, S.R., 2003. Characterization of microbial communities and composition in constructed dairy wetland wastewater effluent. Applied and Environmental Microbiology 69: 5060−5069.

Ibekwe, A.M., Lyon, S.R., Leddy, M., Jacobson-Meyers M., 2007. Impact of plant density and microbial composition on water quality from a free water surface constructed wetland. Journal of Applied Microbiology 102: 921−936.

Iglewicz, B., Hoaglin, D.C., 1993. How to Detect and Handle Outliers. American Society for Quality Control (ASQC) basic references in quality control, Vol. 16. Milwaukee, Wisconsin, ASQC Quality Press.

Irish Phosphorous Regulations, 1998, Water Quality Standards for Phosphorous. Local Government (Water Pollution) Act 1977. S.I. No. 258 1998. Irish Government, Dublin, Ireland

IWA, 2000. Constructed wetlands for pollution control. Processes, performance, design and operation. Scientific and Technical Report No. 8. TJ International (Ltd), Padstow, Cornwall, UK, 156p.

Jamieson, R., Gordon, R., Wheeler, N., Smith, E., Stratton, G., Madani, A., 2007. Determination of first order rate constants for wetlands treating livestock wastewater in cold climates, Journal of Environmental Engineering and Science 6: 65–72.

Jennings, E., Mills, P., Jordan, P., Jensen, J-P.,Søndergaard, M., Barr, A., Glasgow, G., Irvine, K., 2003. Eutrophication from Agricultural Sources-Seasonal Patterns & Effects of Phosphorus.Environmental Protection Agency, Johnstown Castle, Co. Wexford, Ireland

Jetten, M.S.M., Wagner, M., Fuerst. J., van Loosdrecht, M., Kuenen,G., Strous, M., 2001.Microbiology and application of the anaerobic ammonium oxidation (‘anammox’) process. Current Opinion in Biotechnoogy 12: 283–288.

Juretschko, S., Timmermann, G., Schmid, M., Schleifer, K.-H. , Pommerening-Röser, A., Koops H.−P., Wagner, M., 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrosospira*-like bacteria as dominant populations, Applied and Environmental Microbiology 64: 3042–3051.

Kadlec, R.H., 2003. Pond and wetland treatment. Water Science and Technology 48: 1−8.

Kadlec R.H., 2005. Vegetation Effects on Ammonia Reduction in Treatment Wetlands. In: Natural and Constructed Wetlands: Nutrients, metals and management, Vymazal, J. (ed.) Backhuys Publishers: Leiden, The Netherlands, pp. 233−260.

Kadlec, R.H., 2009a.Wastewater treatment at Houghton Lake, Michigan: Hydrology and water quality. Ecological Engineering. 35: 1287–1311.

Kadlec, R.H., Bevis, F.B., 2009b. Wastewater treatment at the Houghton Lake wetland: vegetation response. Ecological Engineering 35: 1312–1332.

Kadlec, R.H., 2009c. Wastewater treatment at the Houghton Lake wetland: soils and sediments. Ecological Engineering 35: 1333–1348.

Kadlec, R.L., Knight, R.H., 1996. Treatment Wetlands. Lewis Publishers, Boca Raton, Florida, USA.

Kadlec, R.H., 1997. An autobiotic wetland phosphorus model. Ecological Engineering 8: 145−172.

Kadlec, R.H., 1999. The limits of phosphorus removal in wetlands. Wetlands Ecology and Management 7: 165−175.

Kadlec, R.H., 2000. The inadequacy of first−order treatment wetland models. Ecological Engineering 15: 105−120.

Kadlec, R.H., Reddy, K.R., 2001. Temperature effects in treatment wetlands. Water Environment Research 73: 543–557.

Kadlec, R.H., 2004. Wastewater treatment: Wetlands and reedbeds. In: Encyclopedia of Environmental Microbiology, Bitton, G. (ed.) Wiley.

Kadlec, R.H., 2005. Vegetation effects on ammonia reduction in treatment wetlands. In: natural and Constructed Wetlands: Nutrients, Metals, and Management, Vymazal, J., (ed.) Backhuys publishers, Leiden, the Netherlands, pp. 233−260.

Kadlec, R.H., Wallace, S.D., 2009. Treatment Wetlands, CRC press, Boca Raton, Florida, USA.

Kalteh, A.M., Hjorth, P., Berndtsson, R., 2008. Review of the self−organizing map (SOM) approach in water resources: Analysis, modelling and application. Environmental Modelling & Software 23: 835−845.

Karathanasis, A.D., Potter, C.L. and Coyne, M.S., 2003. Vegetation effects on fecal bacteria, BOD, and suspended solid removal in constructed wetlands treating domestic wastewater. Ecological Engineering. 20: 157−169.

Karpiscak, M.M., Freitas, R.J., Gerba, C.P., Sanchez, L.R. and Shamir, E., 1999. Management of dairy waste in the Sonoran Desert using constructed wetland technology. Water Science and Technology 40: 57−65.

Kellner, E., Price, S., Waddington, J.M., 2004, Pressure variations in peat as a result of gas bubble dynamics, Hydrological Processes 18: 2599−2605.

Kent, D.M., 2000. App.lied wetlands science and technology, second ed. CRC Press, Boca Raton, USA.

Keohane, J., Carroll, P., Harrington, R., Ryder, C., 2005. Integrated constructed wetlands for farmyard dirty water treatment a site suitability assessment. In: Dunne, E.J., Reddy, K.R., Carton, O.T. (eds.), Nutrient Management in Agricultural Watersheds: A Wetlands Solution. Wageningen Academic Publishers, Wageningen, pp. 196−206.

Kickuth, R., Könemann, N., 1987. Patent: Method for the purification of sewage waters. United States, Germany 4,793,929.

Kickuth, R., 1977. Degradation and incorporation of nutrients from rural wastewaters by plant hydrosphere under limnic conditions. In: Utilization of Manure by Land Spreading, London, UK, pp. 335−343.

Klomjek, P. and Nitisoravut, S., 2005. Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere 58: 585−593.

Knight, R.L., Payne, Jr. W.E., Borer, R.E., Clarke, Jr., RA, Pries, J.H., 2000. Constructed wetlands for livestock wastewater management. Ecological Engineering 15: 41−55.

Kohonen, T., Oja, E., Simula, O., Visa, A., Kangas, J., 1996. Engineering applications of the self organizing map. Proceedings of the IEEE 84: 1358−1384.

Kohonen, T., 2001. Self-organizing Maps, 3rd edition. Springer: Berlin, Germany.

Kowalchuk, G.A., Stephen, J.R., De Boer, W., Prosser, J.I., Embley, T.M.,Woldendorp, J.W., 1997. Analysis of ammonia−oxidizing bacteria of the β subdivision of the class *Proteobacteria* in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing PCR−amplified 16S ribosomal DNA fragments. Applied and Environmental Microbiology 63: 1489−1497.

Kuenen, J.G., 2008. Anammox bacteria: from discovery to application. Nature Reviews Microbiology 6: 320-326.

Langergraber, G. Simunek, J., 2005. Modelling variably−saturated water flow and multi−component reactive transport in constructed wetlands. Vadoze Zone Journal 4: 924−961.

Lee, B.−H., Scholz, M., 2006. Application of the self-organizing map (SOM) to assess the heavy metal removal performance in experimental constructed wetlands. Water Research 40: 3367−3374.

Lee, B.-H., Scholz, M. and Horn, A., 2005. Constructed Wetlands for the treatment of concentrated stormwater runoff (Part A). Environmental Engineering Science 23: 191-202.

Leinweber, P., Meissner, R., Eckhardt, K.−U., Seeger, J., 1999. Management effects on forms of phosphorus in soil and leaching losses. European Journal of Soil Science 50: 413–424.

Levenspiel, O.,1995. Chemical Reaction Engineering. 2nd edition, John Wiley & Sons, New York, USA.

Madigan, M.T., Martinko, J.M., 2006. Brock, Biology of microorganisms. 11th edition, Prentice Hall, New Jersey, USA.

Maguire, R.O., Edwards, A.C., Wilson, M.J., 1997. Comparison of chemical forms and distribution of phosphorus within cultivated and uncultivated soils; some implications for losses. In: Tunney, H., Carton, O.T., Brooks, P.C., Johnson, A.E. (eds.), Phosphorus Losses from Soil toWater. CAB Wallingford, UK, pp. 427–430.

Maine, M.A., Suñe, N., Hadad, H., Sánchez, G., Bonetto, C. 2006. Nutrient and metal removal in a constructed wetland for wastewater treatment from a metallurgic industry. Ecological Engineering 26: 341–347.

Mander, Ü., Kuusemets, V., Lõhmus, K., Mauring, T., Teiter, S., Augustin, J., 2003. Nitrous oxide, dinitrogen, and methane emission in a subsurface flow constructed wetland, Water Science and Technology 48: 135−142.

Manfield, M., Griffiths, R.I., Leigh, M.B., Fisher, R. and Whiteley, A.S., 2005. Functional and compositional comparison of two activated sludge communities remediating coking effluent. Environmental Microbiology 7: 715−722.

Mantovi, P., Marmiroli, M., Maestri, E., Tagliavini, S., Piccinni, S., Marmiroli, N., 2003. Application of a horizontal subsurface flow constructed wetland on treatment of dairy parlour wastewater. Bioresource Technology 88: 85–94.

Masi, F., Martinuzzi, N., Bresciani, R., Giovannelli, L., Conte, G., 2007. Tolerance to hydraulic and organic load fluctuations in constructed wetlands. Water Science & Technology 56: 39–48.

McCune, B., Grace, J. B., 2002. Analysis of Ecological Communities. Gleneden Beach , Oregon: MjM Software Design.

Met Éireann, 2007, http://www.met.ie/climate/climate−data−information.asp.

Mitsch, W.J., Gosselink, J.G., 2007, Wetlands, 4th edition, Wiley, New Jersey, USA.

Mitsch, W.J., Cronk, J.K., Wu, X., Narin, R.W., Hey, D.L., 1995. Phosphorus retention in constructed freshwater riparian marshes. Ecological Applications 5:830−845.

MOE, 1989. Quality of the Environmental in Japan. Ministry of the Environment (MOE), Government of Japan, Tokyo, Japan. Available online at http://www.env.go.jp/en/wpaper/1989/index.html.

Morse, D., Head, H.H., Wilcox, C.J., van Horn, H.H., 1992. Effects of concentration of dietary phosphorus on amount and route of excretion. Journal of Dietary Science 75: 3039–3049.

Moshiri, G. A., 1993. Constructed Wetlands for Water Quality Improvement. Lewis Publishers, CRC Press, Boca Raton, Florida, USA.

Mueleman, A.F.M., Beekman, J.P.H. and Verhoeven, J.T.A., 2002. Nutrient retention and nutrient−use efficiency in *Phragmites australis* stands after wastewater application. Wetlands 22: 712–721

Mustafa A., Scholz M., Harrington R., Carroll P., 2009. Long-term performance of a representative integrated constructed wetland treating farmyard runoff. Ecological Engineering 35: 779−790.

Muyzer, G., De Waal, E.D., and Uitterlinden, A.G.,1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction−amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59: 695−700.

Newman J.M., Clausen J.C., Neafsey J.A., 2000. Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut. Ecological Engineering 14: 181−198.

Noorvee, A., Poldvere, E., Mander, U., 2007. The effect of pre-aeration on the purification processes in the long−term performance of a horizontal subsurface flow constructed wetland. Science of the Total Environment 380: 229–236.

Notle and Associates, 1998. Sacramento regional wastewater treatment plant Demonstration WETLANDS Project: Five year summary report 1994−1998, Report to Sacramento Regional County Sanitation District, http://www.srcsd.com/cw.html, Notle and Associates.

Panswad, T., Anan, C., 1999. Impact of high chloride wastewater on an anaerobic/anoxic/aerobic process with and without inoculation of chloride acclimated seeds. Water Research 33: 1165–1172.

Pant, H.K., Reddy, K.R., 2001. Phosphorus sorption characteristics of estuarine sediments under different redox conditions. Journal of Environmental Quality 30: 1474–1480.

Pietro, K.C., Chimney, M.J., Steinman, A.D., 2006. Phosphorus removal by the Ceratophyllum/periphyton complex in a south Florida (USA) freshwater marsh. Ecological Engineering 27: 290–300.

Poe, A.C., Pichler, M.F., Thompson, S.P. and Paerl, H.W., 2003. Denitrification in a constructed wetland receiving agricultural runoff. Wetlands 23: 817−826.

Priemé, A., Braker, G., Tiedje, J.M., 2002. Diversity of nitrite reductase (*nir*K and *nir*S) gene fragments in forested upland and wetland soils. Applied and Environmental Microbiology 68: 1893−1900.

Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone M.K., Freckleton, R.B., Green, J.L., Green, L.E., Killham, K., Lennon, J.L., Osborn, A.M., Solan, M., van der Gast, C.J., Young, J.P.W., 2007. The role of ecological theory in microbial ecology. Nature Reviews Microbiology 5: 384−392.

Prosser, J. I., 1989. Autotrophic nitrification in bacteria. Advances in Microbial Physiology 30:125−181.

Ragusa, S.R., McNevin, D., Qasem, S., Mitchell, C., 2004. Indicators of biofilm development and activity in constructed wetlands microcosms. Water Research 38: 2865−2873.

Raup, D.M., Crick, R.E., 1979. Measurement of faunal similarity in palaeontology. Journal of Paleontology 53: 1213−1227.

Reddy, R. K., DeLaune, R. D., 2008. Biogeochemistry of wetlands: science and applications. CRC press, Boca Raton, Florida, USA.

Reddy, K.R., DeLaune, R.D., DeBusk,W.F., Koch,M.S., 1993. Long-term nutrient accumulation rates in the Everglades. Soil Science Society of American Journal 57: 1147–1155.

Reddy, K.R., Kadlec, R.H., Flaig, E., Gale, P.M., 1999. Phosphorus retention in stream and wetlands. Critical. Reviews in Environmental Science and Technology 29: 83−146.

Reddy, K.R., Wang, Y., DeBusk, W.F., Fisher, M.M., Newman, S., 1998. Forms of soil phosphorus in selected hydrologic units of the Florida Everglades. Soil Science Society of American Journal 62: 1134–1147.

Reddy, R. K., Patrick, W.H., 1984. Nitrogen transformations and loss in flooded soil and sediments. Critical Reviews in Environmental Science and Technology 13: 273−309.

Reed, S.C., Crites, R.W. and Middlebrooks, E.J., 1995. Natural systems for waste management and treatment. Second Edition. McGraw−Hill, New York, USA, 433p.

Rhue, R.D., Harris, R.G., 1999. Phosphorus sorption/desorption reactions in soils and sediments. In: K.R. Reddy, G.A. O'Connor and C.L. Schleske, Editors, Phosphorus biogeochemistry in subtropical ecosystems, Lewis Publishers, Boca Raton, pp. 187−206.

Rousseau, D.P.L., Vanrolleghem, P.A., Pauw, N. De., 2004. Model-based design of horizontal subsurface flow constructed treatment wetlands: a review. Water Research 38: 1484 −1493.

Rowan, A.K., Snape, J.R., Fearnside, D., Barer, M.R., Curtis, T.P., Head, I.M., 2003. Composition and diversity of ammonia−oxidising bacterial communities in wastewater treatment reactors of different design treating identical wastewater. FEMS Microbiology Ecology 43: 195–206.

Rowsell, J.G., Miller, M.H. and Groenevelt, P.H., 1985. Self−sealing of earthen liquid manure storage ponds: II. Rate and mechanism of sealing. Journal of Environmental Quality 14: 539−543.

Ruiz−Rueda, O., Trias, R., Garcia−Gil, L.J., Baneras, L., 2007. Diversity of the nitrite reductase gene *nir*S in the sediment of a free−water surface constructed wetland. International Microbiology 10: 253−260.

Rustum, R., Adeloye, J.A., 2007. Replacing outliers and missing values from activated sludge data using Kohonen self organizing map. Journal of Environmental Engineering 133: 909−916.

Rustum, R., Adeloye, A.J., Scholz, M., 2008. Applying Kohonen self-organizing map as a software sensor to predict biochemical oxygen demand. Water Environment Research 80: 32−40.

Rybczyk, J.M., Day Jr., J.W., Conner, W.H., 2002. The impact of wastewater effluent on accretion and decomposition in a subsiding forested wetland. Wetlands 22: 18–32.

Sambrook, J.; Fritsch, E.F. And Maniatis, T., 1989. In: Molecular Cloning: a laboratory manual. Cold Spring Harbor Laboratory press, Cold Spring Harbor Laboratory, New York, USA. 1659 p.

Sartoris, J., Thullen, J.S., Barber, L.B., Salas, D.E., 2000. Investigation of nitrogen transformations in a southern California constructed wastewater treatment wetland. Ecological Engineering 14: 49–65.

Schaafsma J.A., Baldwin A.H. Streb C.A., 2000. An evaluation of a constructed wetland to treat wastewater from a dairy farm in Maryland, USA. Ecological Engineering 14: 199−206.

Scholz, M., 2006. Wetland Systems to Control Urban Runoff. Elsevier, Amsterdam, The Netherlands.

Scholz, M., Harrington, R., Carroll, P., Mustafa, A., 2007. The integrated constructed wetlands (ICW) concept. Wetlands 27: 337−354.

Scholz, M., 2008. Classification of flood retention basins: the Kaiserstuhl case study. Environmental and Engineering Geoscience 14: 61−80.

Schramm, A., Larsen, L.H., Revsbech, P.N., Ramsing, B.N., Amann, R. Schleifer, H.K. (1996) Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes, App.lied and Environmental Microbiology 62: 4641–4647.

Shamir, E., Thompson, T.L., Karpisack, M.M., Freitas, R.J., Zauderer, J., 2001. Nitrogen accumulation in a constructed wetland for dairy wastewater treatment. Journal of American Water Resources Association 37: 315–325.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591−611.

Sharpley, A.N., 1995. Identifying sites vulnerable to phosphorus losses in agricultural runoff. Journal of Environmental Quality 24: 947–951.

Siedel, K., 1973. Patent: System for purification of polluted water. Washington D.C., United States 3,770,623.

Simberloff, D., 1978. Using island biogeography distributions to determine if colonization is stochastic. American Naturalist 112(986), 713−726.

Skarda, S.M., Moore, J.A., Niswander, S.F., Gamroth, M.J., 1994. Preliminary results of wetland for treatment of dairy farm wastewater. In: DuBowy, P.J., Reaves, R.P. (Eds.), Constructed wetlands for animal wastewater management. Proceedings of workshop, 4−6 April 1994, Purdue University, West Lafayette, Indiana, USA, pp.. 34−42.

Smith, D., Neill, O.N., Doris, Y., Moriarty, J., 2004. Urban waste water discharges in Ireland: a report for the years 2002 and 2003. Environmental Protection Agency, Office of Environmental Enforcement, Johnstown Castle Estate, County Wexford, Ireland. Available online at http://www.epa.ie/downloads/pubs/water/wastewater/epa_urban_waste_water_discharge_2002_2003.pdf.

Soto, F., Garcia, M., de Luis, E. and Becares, E., 1999. Role of Scirpus lacustris in bacterial and nutrient removal from wastewater. Water Science and Technology 40: 241–247.

Stern, D.A., Khanbilvardi, R, Alair, J.C., Richardson, W., 2001. Description of flow through a natural wetland using dye tracer tests. Ecological Engineering 18: 173–184.

Strous, M., Van Geerven, E., Zheng, P., Kuenen, J.G., Jetten, M.S.M., 1997. Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations, Water Research 31: 1955–1962.

Strous, M., 2001. Microbiology and application of the anaerobic ammonium oxidation (anammox) process. Current Opinion in Biotechnology 12: 283−288.

Sua, T-M., Yanga, S-C., Shihb, S-S, Lee, H-Y., 2009. Optimal design for hydraulic efficiency performance of free-water-surface constructed wetlands. Ecological Engineering 35: 1200–1207.

Sundberg, C., Stendahl, J.S.K, Tonderski, K., Lindgren, P.−E., 2007a. Overland flow systems for treatment of landfill leachates — potential nitrification and structure of the ammonia−oxidising bacterial community during a growing season, Soil Biology & Biochemistry 39:127−138.

Sundberg, C., Tonderski, K., Lindgren, P.−E., 2007b. Potential nitrification and denitrification and the corresponding composition of the bacterial communities in a compact constructed wetland treating landfill leachates. Water Science and Technology 56(3), 159−166.

Tal, Y., Watts, J.E.M., Schreierl, H.J., 2006. Anaerobic ammonium-oxidizing (anammox) bacteria and associated activity in fixed-film biofilters of a marine recirculating aquaculture system. Applied and Environmental Microbiology, 72: 2896–2904.

Tanner, C.C., 2001. Growth and nutrient dynamics of soft-stem bulrush in constructed wetlands treating nutrient-rich wastewaters. Wetland Ecology Management 9: 49–73.

Tanner, C.C., 1996. Plants for constructed wetland treatment systems – A comparison of the growth and nutrient uptake characteristics of eight emergent species. Ecological Engineering 7: 59−83.

Tanner, C.C., Nguyen, M.L., Sukias, J.P.S., 2005. Nutrient removal by a constructed wetland treating subsurface drainage from grazed dairy pasture, Agriculture, Ecosystems and Environment 105: 145–161.

Tanner, C.C., Clayton, J.S., Upsdell, M.P., 1995. Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands−II. Removal of nitrogen and phosphorus. Water Research 29: 27−34.

Tanner, C.C., Sukias, J.P.S., Upsdell, M.P., 1998. Organic matter accumulation during maturation of gravel−bed constructed wetlands treating farm dairy wastewaters, Water Research 32: 3046–3054.

Throbäck, N.I., Enwall, K., Jarvis, Á., Hallin, S., 2004. Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbial Ecology 49: 401−417.

Tokida, T., Miyazaki, T., Mizoguchi, M., Seki, K., 2005. In situ accumulation of methane bubbles in a natural wetland soil. European Journal of Soil Science 56: 389−395.

Tseng, K.−F. and Wu, K.−L., 2004. The ammonia removal cycle for a submerged biofilter used in a recirculating eel culture system. Aquacultural Engineering 31: 17−30.

Tunney, H., Breeuwsma, A., Withers, P.J.A., Ehlert, P.A.I., 1997. Phosphorus fertiliser strategies: past, present and future. In: Tunney, H., Carton, O.T., Brookes, P.C., Johnson, A.E. (eds.), Phosphorus Losses from Soil toWater. CAB International, New York, USA, pp. 358–361.

US EPA, 2000. Constructed Wetlands Treatment of Municipal Wastewater. United States (US) Environmental Protection Agency (EPA), Office of Research and Development, Cincinnati, Ohio, USA.

US EPA, 2004. Guidelines for water reuse. EPA/-625/R-04-108, U.S. Environmental Protection Agency and U.S. Agency for International Development, Washington, D.C.

Uygur, A. and Kargi, F., 2004. Salt inhibition on biological nutrient removal from saline wastewater in a sequencing batch reactor. Enzyme and Microbial Technology 34: 313−318.

Vaccari, D.A., Strom, P.F. and Alleman, J.E., 2006. Environmental biology for engineers and scientists. John Wiley & Sons, New Jersey, USA.

van der Gast, C.J., Jefferson, B., Reid, E., Robinson, T., Bailey, M.J., Judd, S.J., Thompson, I.P. (2006) Bacterial diversity is determined by volume in membrane bioreactors. Environmental Microbiology 8(6), 1048−1055.

van Verseveld H.W., Röling, W.F.M., 2004. Cluster analysis and statistical comparison of molecular community profile data. In: Kowalchuk, G.A., de Bruijn, F.J., Head, I.M., Akkermans, A.D.L., van Elsas, J.D. (eds). Molecular Microbial Ecology Manual, 2nd edn, Vol. 2, Section 7.04. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 1373–1397.

Vesanto, J., Himberg, J., Alhoniemi, E., Parhankangas, J., 1999. Self-organizing map in Matlab: the SOM toolbox. In: Proceedings of the Matlab DSP Conference (16−17/11/1999), Espoo, Finland, 34−40. Available online at http://www.cis.hut.fi/projects/ide/publications/papers/matlab99.doc. Software available online at http://www.cis.hut.fi/projects/somtoolbox/.

Vohla, C., Alas R., Nurk, K., Baatz, S., Mander, Ü., 2007. Dynamics of phosphorus, nitrogen and carbon removal in a horizontal subsurface flow constructed wetland. Science of the Total Environment 380, 66–74.

Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Science of the Total Environment 380: 48−65.

Wallace, S.D., Knight, R.L., 2006. Small−scale constructed wetland treatment systems. Water Environment Research Foundation, IWA Publishing, London, UK.

Wallace, W., Nicholas, D.J.D., 1969. The biochemistry of nitrifying microorganisms. Biological Review 44: 359−402.

Wang, D., Li, X., Yang, Q., Zeng, G., Liao, D., Zhang, J., 2008. Biological phosphorus removal in sequencing batch reactor with single−stage oxic process. Bioresource Technology 99: 5466−5473

Wetzel, R.G., 2001. Limnology – Lake and River Ecosystems. 3rd edition, Academic Press, San Diego, USA, 1006 p.

Winter, K.-J., Goetz, D., 2003. The impact of sewage composition on the soil clogging phenomena of vertical flow constructed wetlands. Water Science and Technology 48: 9−14

Woese, C.R., Fox, G.E., 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proccedings of National Academy of Sciences, USA. 74: 5088–5090.

Woese, C. R., 1994. There must be a prokaryote somewhere: microbiology's search for itself. Microbiology Review 58: 1–9.

Zhao, Y.Q., Sun, G., Allen, S.J., 2004. Anti-sized reed bed system for animal wastewater treatment: a comparative study. Water Research 38: 2907−2917.

Zhu, T., Sikora, F.J., 1994. Ammonium and nitrate removal in vegetated and unvegetated gravel bed microcosm wetlands. Water Science and Technology 32: 219–228.

Zumft, W. G. 1992. The denitrifying prokaryotes, p. 554−582. In A. Balows (ed.), The prokaryotes: a handbook of the biology of bacteria: ecophysiology, isolation, identification, app.lications. Springer−Verlag, New York, N.Y. 34

Appendix

Selected Publications

Scholz, M., Harrington, R., Carroll, P., Mustafa, A., 2007. The Integrated Constructed Wetlands (ICW) Concept. Wetlands 27: 337─354.

Zhang, L., Scholz, M., Mustafa, A. and Harrington, R., 2008. Assessment of the Nutrient Removal Performance in Integrated Constructed Wetlands with the Self-organizing Map. Water Research 42: 3519─3527.

Mustafa, A., Scholz M., Harrington, R. and Carroll, P., 2009. Long-term Performance of a Representative Integrated Constructed Wetland Treating Farmyard Runoff. Ecological Engineering 35: 779─790.