PLANT GROWTH AND NUTRIENT REMOVAL IN SIMULATED SECONDARY-TREATED MUNICIPAL WASTEWATER IN WETLAND MICROCOSMS

Zhenhua Zhang
MSc National University of Ireland (Dublin)

This thesis is presented for the degree of
Doctor of Philosophy
of
The University of Western Australia
School of Earth and Geographical Sciences
Faculty of Natural and agricultural Sciences

2008

THE UNIVERSITY OF
WESTERN AUSTRALIA

DECLARATION FOR THESES CONTAINING PUBLISHED WORK AND/OR WORK PREPARED FOR PUBLICATION

This thesis contains four published papers, one paper accepted and one paper prepared for publication, all of which has been co-authored.

The bibliographic details of the works and where they appear in the thesis are set out in the next page.

I contributed all the work such as experimental designs, greenhouse labour, laboratory analyses, statistical work, draft writing and final paper corrections for all published and prepared for publication papers.

The co-authors, Professor Zed Rengel provided overall supervision and advice with experimental designs and critical comments on the papers and subsequent manuscripts, and Dr. Kathy Meney assisted in the overall supervision of the papers and thesis.

Zhenhua Zhang (MSc)	Professor Zed Rengel
Signature……………………………	Signature…………………………
Candidate	Coordinating Supervisor

PUBLICATIONS ARISING FROM THIS THESIS

1. Zhang, Z., Rengel, Z., Meney, K., 2007. Removal of nutrients from secondary-treated municipal wastewater in wetland microcosms using ornamental plant species. **International Journal of Environment and Waste Management** 1, 363-375 (Chapter 3).
2. Zhang, Z., Rengel, Z., Meney, K., 2007. Growth and resource allocation of *Canna indica* and *Schoenoplectus validus* as affected by interspecific competition and nutrient availability. **Hydrobiologia** 589, 235-248 (part of Chapter 4).
3. Zhang, Z., Rengel, Z., Meney, K., 2007. Nutrient removal from simulated wastewater using *Canna indica* and *Schoenoplectus validus* in mono- and mixed-culture in wetland microcosms. **Water, Air and Soil Pollution** 183, 95-105 (part of Chapter 4).
4. Zhang, Z., Rengel, Z., Meney, K., 2008. Interactive effects of N and P on growth but not on resource allocation of *Canna indica* in wetland microcosms. **Aquatic Botany** doi:10.1016/j.aquabot.2008.03.007 (part of Chapter 5).
5. Zhang, Z., Rengel, Z., Meney, K., 2008. Interactive effects of N and P on nutrient removal from simulated wastewater using *Schoenoplectus validus* in wetland microcosms. **Chemosphere** (accepted) (part of Chapter 5).
6. Zhang, Z., Rengel, Z., Meney, K., 2008. Differences in kinetics of ammonium, nitrate and phosphorus uptake between *Canna indica* and *Schoenoplectus validus*. **Hydrobiologia** (submitted) (Chapter 6).

ABSTRACT

The use of constructed wetlands for tertiary purification of municipal wastewater has received increasing attention around the world because direct discharge of secondary-treated municipal wastewater to water bodies has caused eutrophication. Plant species selection and vegetation management may enhance nutrient removal efficiency in constructed wetlands. However, there is a lack of knowledge on the relations between plant growth and nutrient removal efficiency in constructed wetlands. The objective of this study is to better understand how plant growth and resource allocation are influenced by nutrients in wastewater and how nutrient removal efficiencies are affected by plant species and vegetation management.

The preliminary experiment was conducted to select macrophytes, especially ornamental species, to grow in the wastewater in the wetland microcosms. Ten plant species, comprising six ornamental species: *Alocasia macrorrhiza*, *Canna indica*, *Iris louisiana*, *Lythrum sp.*, *Zantedeschia aethiopica*, *Zantedeschia sp.*, and four sedge species: *Baumea articulate*, *Baumea juncea*, *Carex tereticaulis* and *Schoenoplectus validus*, were planted in the wetland microcosms and fed a simulated wastewater solution in the concentrations similar to the secondary-treated municipal wastewater. *C. indica* has shown vigorous and healthy growth, and a relatively high potential of rooting-zone aeration and nutrient removal efficiency. *B. articulata* and *S. validus* also showed relatively high nutrient removal efficiency. It might be better to grow species in mixed culture of *C. indica* and *S. validus* in constructed wetlands for enhanced efficiency of nutrient removal from the wastewater.

An intensive study was carried out to compare plant growth and nutrient removal efficiency between *C. indica* and *S. validus* under mono- and mixed culture. The growth and resource allocation of *S. validus* were significantly influenced by mixed culture. Nutrient removal efficiencies were significant higher in planted than non-planted microcosms. The significant difference in nutrient removal efficiency was not observed between the monoculture and mixture, due to interspecies competition. Nutrient uptake by plants was the major factor responsible for nutrient removal in wetland microcosms.

The interactive effects of nitrogen (N) and phosphorus (P) on plant growth and nutrient removal efficiency were studied. The nutrient uptake by plants and nutrient removal efficiency (except for NO_x-N removal by *S. validus*) were significantly influenced by interaction of N and P. The high nutrient availability and optimum N/P ratio were required for stimulating plant growth, resulting in allocation of more resources to above-ground tissues compared to below-ground parts, and enhancing nutrient removal efficiency. Nutrient removal efficiencies were significantly influenced by growth of *C. indica* and *S. validus*, nutrient loading rates and N/P ratios in the wastewater.

The nutrient uptake kinetics of *C. indica* and *S. validus* were investigated to elucidate the differences in nutrient uptake between species. Wetland plant species have shown differential nutrient uptake efficiency and different preferences for inorganic N source, with *C. indica* preferring NO_3-N and *S. validus* preferring NH_4-N. *C. indica* had greater capacity than *S. validus* to take up PO_4-P when the concentration of PO_4-P in the solution was relatively low, whereas *S. validus* was more capable than *C. indica* to take up NO_3-N when the concentration of NO_3-N in the solution was relatively low. The PO_4-P uptake capacity was higher in younger than older plants.

Overall, the study has suggested that different plant species have differential capacity to take up nutrients. In addition to nutrient uptake, plants have significant other roles in terms of nutrient removal from the wastewater (such as leaking oxygen into the rhizosphere in which oxidation of substances like ammonia can occur). The properly high nutrient availability and optimum N/P ratio are required to stimulate the plant growth, resulting in enhancing the treatment performance in the wetlands. These findings have important implications for improving our ability to engineer ecological solutions to the problems associated with nutrient-rich wastewater.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

The work presented in this thesis was completed between June 2004 and December 2007 at School of Earth and Geographical Sciences, Faculty of Natural and Agricultural Sciences, the University of Western Australia. I gratefully acknowledge the financial support of International Postgraduate Research Scholarship (IPRS) and University Postgraduate Award (International Student) from the University of Western Australia.

I would like to take this opportunity to thank the people who have helped me over the past three and a half years. First, I would like to express my sincerest gratitude to my supervisors Professor Zed Rengel and Dr. Kathy Meney for their continuous guidance, assistance, teaching and encouragement, and for the numerous instructive and enlightening discussions of my work. Their supervision has made a significant contribution to the execution and writing of this thesis.

I gratefully give my appreciation to Michael Smirk and Paul Damon for analytical and technical assistance. I also thank my colleagues, visitors and students in the Nutrient Utilisation and Toxic Stress Laboratory who have been a constant source of help during completion of my PhD study.

I sincerely thank my employer, Jiangsu Academy of Agricultural Sciences, in China for giving me this opportunity to take study leave.

In particular, I appreciate the patience, encouragement and support given by my wife, Ping, in many ways during my PhD study, and my daughter, Minjie, for accompanying me and giving me lots of fun during my study.

Last but not least, I thank my parents and other family members in China for their encouragement and support both emotionally and financially throughout my long education.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Fresh water is vital to sustaining human life; however, only 3% of total water on earth is fresh and two-thirds of that is in frozen forms such as the polar ice caps, glaciers and icebergs. The remaining 1% of the total fresh water is either surface or ground water (USGS, 2004). Hence, water resources are not unlimited, whilst demands for water are increasing as population and living standards increase around the world.

Australia is the driest of the world's inhabited continents, with lowest percentage of rainfall as run-off, the lowest amount of water in rivers and the smallest area of permanent wetland. Most rainfall in Australia evaporates and soaks into the ground, with only 12% running off into rivers. Much of the runoff is in tropical monsoon areas with sparse communities and little development. The National Land and Water Resources Audit (NLWRA, 2002) showed that water resources from 26% of Australia's surface management areas and 31% of its groundwater management units were fully allocated or even over-allocated. In 1996-7, Australia used 26,000 GL of water: 75% for irrigation, 20% for urban and industrial purposes and 5% for stock and domestic use.

Water is valuable resource in Australia, but in short supply. There is scope to make better use of recycled water as additional water resources. In the Perth metropolitan area, around 320 million litres of wastewater are produced per day (more than 100 billion litres per year). Reuse of some of that water represents tremendous resource for productive agricultural, horticultural, viticultural, turf and forestry ecosystems based on irrigation and hydroponics.

Water pollution is a subject of growing public concern. Eutrophication continues to be one of the top concerns in many water bodies. Nitrogen (N) and phosphorus (P), the primary nutrients implicated in eutrophication, enter water bodies via a variety of pathways (Davis et al., 2006). One of these is the discharge of the wastewater into water bodies. The disposal of secondary-treated wastewater is a problem facing a large number of communities in today's society. The secondary-treated wastewater contains nutrients that, although present in reduced amounts compared to untreated wastewater, can lead to eutrophication of the receiving water bodies. High concentration of the non-ionized ammonia species are toxic to fish and other aquatic life. Nitrate and nitrite nitrogen constitute a public health concern, primarily related to methemoglobinemia and carcinogenesis. Ammonia may deplete dissolved oxygen in natural water by way of microbial nitrification reactions (EPA, 2000).

As has been found elsewhere in the world, sewage pollution of coastal rivers, estuaries, and near-shore waters in Australia has been implicated in eutrophication (Davis and Koop, 2006) because little sewage effluent is reclaimed and reused; instead, secondary-treated effluent is discharged into rivers, estuaries or oceans (Greenway, 2005). In 2001 to 2002, Australia's major cities processed 1824 GL of sewage effluent, the majority (over 90%) of which was discharged to water bodies (Radcliffe, 2004). Appropriately treated, this effluent represents valuable resource.

There is evidence that wastewater was reused as a source of irrigation for agriculture more than 5,000 years ago. Wastewater irrigation can supply almost all N, P and potassium (K) required by many crops, as well as important micronutrients (Adin, 1986; Azov and Shelef, 1991). Although there is an economic benefit based on the fertilizer value of nutrients in the wastewater, there is a high risk of transmission of water-borne diseases when raw sewage or semi-treated sewage is used (Krishnan and Smith, 1987). Epidemiological evidences exist for water-borne disease transmission by food stuffs irrigated with untreated sewage or by fish cultured in poorly treated effluent from stabilization ponds in some developing countries (Shuval et al., 1986;

Stott et al., 1999). Hence, there is a need for adequate treatment of wastewater prior to reuse and re-distribution into the environment. Despite development and implementation of advanced wastewater treatment technologies producing high quality water, there have been concerns about long-term safety of reclaimed water persist. When treated municipal wastewater is used in the urban environments, considerable health concerns need to be addressed when there is strong likelihood of direct human contact. The control of enteric viruses is a major health concern, particularly in industrialised countries with high health standards, whereas helminthes and bacteria may be a significant problem in developing regions (Shuval, 1987). It is therefore imperative that some sort of tertiary treatment of wastewater be employed. The techniques for further treatments include disinfection to remove pathogens and chemical processes for removal of N and P. However, the construction of equipment to conduct such tertiary treatments can be very expensive. Considerations such as these have led to the examination of wetlands as natural ecosystem treatment for removal of pathogens and nutrients (Kadlec and Tilton, 1979).

Wetlands have been recognized as natural resource throughout human history. Their importance is appreciated in their natural state by such people as the Marsh Arabs around the confluence of the rivers Tigris and Euphrates in southern Iraq, as well as in managed forms, for example rice paddies, particularly in South East Asia (Mitsch and Gosselink, 2000). Natural wetlands are called “natural purifiers of water”, and their capacity for water purification is now being recognized as an attractive option in wastewater treatment. Such a role of natural wetlands in water quality improvements has offered a compelling argument for wetland preservation (Kivaisi, 2001). Although studies have shown that natural wetlands are able to provide high levels of wastewater treatment (Kadlec and Tilton, 1979; Nichols, 1983; Knight et al., 1987; Kadlec and Knight, 1996; Mander and Mauring, 1997), there has been concerns over (1) possible harmful effects of toxic materials and pathogens in wastewaters; and (2) long-term degradation of wetlands due to additional nutrient and hydraulic loadings from wastewater. As a result of the exponentially increasing demands for water caused by human expansion and resource exploitation, it has been recognized that natural wetland ecosystems can not always function efficiently for desired objectives and stringent water quality standards. These and many other factors have resulted in using constructed wetlands for wastewater treatment (Hammer and Bastian, 1989).

Constructed wetlands offer effective, reliable treatment of wastewater in a simple and inexpensive manner (Kadlec & Knight, 1996). The use of constructed wetlands to treat wastewater from agricultural, mining, municipal and industrial sources has received increasing attention in recent years (Scholz and Lee, 2005). Although constructed wetlands are being developed in many parts of the world for various functions, there have been widespread problems in their performance with respect to N transformations and removal as well as P removal (EPA, 2000). Despite several pilot projects in Australia in the 1990s, this wastewater treatment technology has not been widely adopted in Australia. Interest in constructed wetlands for the treatment of municipal wastewater diminished in the late 1990s. This may have been due to relatively poor nutrient removal efficiency (especially for P) and government pressure to upgrade and augment sewage treatment plants to produce high quality tertiary effluent (Greenway, 2005). Therefore, it is imperative to improve performance and enhance nutrient removal efficiency of constructed wetlands in Australia.

1.2 Literature review

1.2.1 Constructed wetlands for wastewater treatment

Constructed wetlands, as the term suggests, are man-made wetlands artificially developed in areas where they do not occur naturally. One of the pioneers of using macrophytes to treat wastewater was Dr. Seidel of Max Planck Institute in Plon, Germany, who first reported in 1953 about the possibility to lessen the over-fertilization, pollution, and silting up of inland waters through appropriate plants (Brix, 1994b). Since its initial “discovery”, efforts to harness and develop the natural treatment ability of wetland systems have been undertaken by both government and private research entities around the world. This appears to be, at least in part, due to the growing interest in eco-technologies that support more resource conservation and environmental protection and ensure greater reliance on natural ecological processes and systems in preference to the more energy and chemically intensive mechanical or conventional waste management systems.

Today, due to the increased awareness about natural processes, the use of constructed wetlands for wastewater management and water pollution control is becoming more popular and effective in many parts of the world. Hundreds of wetlands have been constructed around the world to treat various types of wastewaters, including domestic sewage (Hammer, 1989; Kadlec and Knight, 1996; Ayaz and Akça, 2001; Karathanasis et al., 2003; Ran et al., 2004; Samecka-Cymerman et al., 2004; Solano et al., 2004), livestock wastewater (Hammer, 1994; Cronk, 1996; Lee et al., 2004; Sooknah and Wilkie, 2004), non-point source pollution (Hammer, 1992; Mitsch and Cronk, 1992), landfill leachate (Mulamoottil et al., 1999; Chang et al., 2004), stormwater runoff (Livingston, 1989), mine drainage (Fennessy and Mitsch, 1989; Wieder, 1989; Batty and Younger, 2004; Overall and Parry, 2004), aquaculture wastewater (Lin et al., 2002; Michael, 2003; Schulz et al., 2003; Lymbery et al., 2006), and other industrial discharges (Kadlec and Knight, 1996; Odum et al., 2000; Ye et al., 2003).

The advantages and limitations of the constructed wetlands have been summarized in many reports (eg. Haberl, 1999; Sundaravadivel and Vigneswaran, 2001; Scholz and Lee, 2005). Major advantages of the constructed wetlands over traditional treatment plants are as follows:

(1) Operate on ambient solar energy and require low external energy input;

(2) Achieve high levels of treatment with little or no maintenance, making them especially appropriate in locations where no infrastructure support exists;

(3) Relatively tolerant to shock hydraulic and pollutant loads, thus ensuring the

reliability of treated wastewater quality;

(4) Unlike the conventional treatment systems, no specific design life period is generally prescribed for constructed wetlands and as such they tend to have increased treatment capacity over time due to feedback loops that result in self-repairing systems;

(5) Generate oxygen and consume carbon dioxide, thereby helping improve air quality and fight global warming;

(6) Harvested aquatic plants can be used for a variety of purposes (biomass, biogas, animal feed, fertilizer, etc.); and

(7) Provide indirect benefits such as green space, wildlife habitats, and recreational and educational areas.

1.2.2 Types of constructed wetlands

Constructed wetlands for wastewater treatment involve the use of engineered systems that are designed and constructed to utilize natural processes. The systems are designed to mimic natural wetland systems, utilizing wetland plants, soil, and associated microorganisms to remove contaminants from wastewater effluents (EPA, 1993). There are various design configurations of constructed wetlands (Haberl, 1999) that can be classified according to the following:

(1) Life form of the dominating macrophytes (free-floating, emergent, submerged);

(2) Flow pattern in the wetland systems (free water surface flow, subsurface flow, horizontal and vertical);

(3) Type of configurations of the wetland cells (hybrid system, one-stage, multi-stage systems);

(4) Type of wastewater to be treated;

(5) Treatment level of wastewater (primary, secondary or tertiary);

(6) Type of pretreatment;

(7) Influent and effluent structures;

(8) Type of substrate (gravel, soil, sand, etc.); and

(9) Type of loading (continuous or intermittent).

The basic classification of constructed wetland is based on the type of macrophytic growth; further classification is usually based on the water flow regime. For example, the classifications of constructed wetlands (Figures 1.1) were provided by Vymazal (2001).

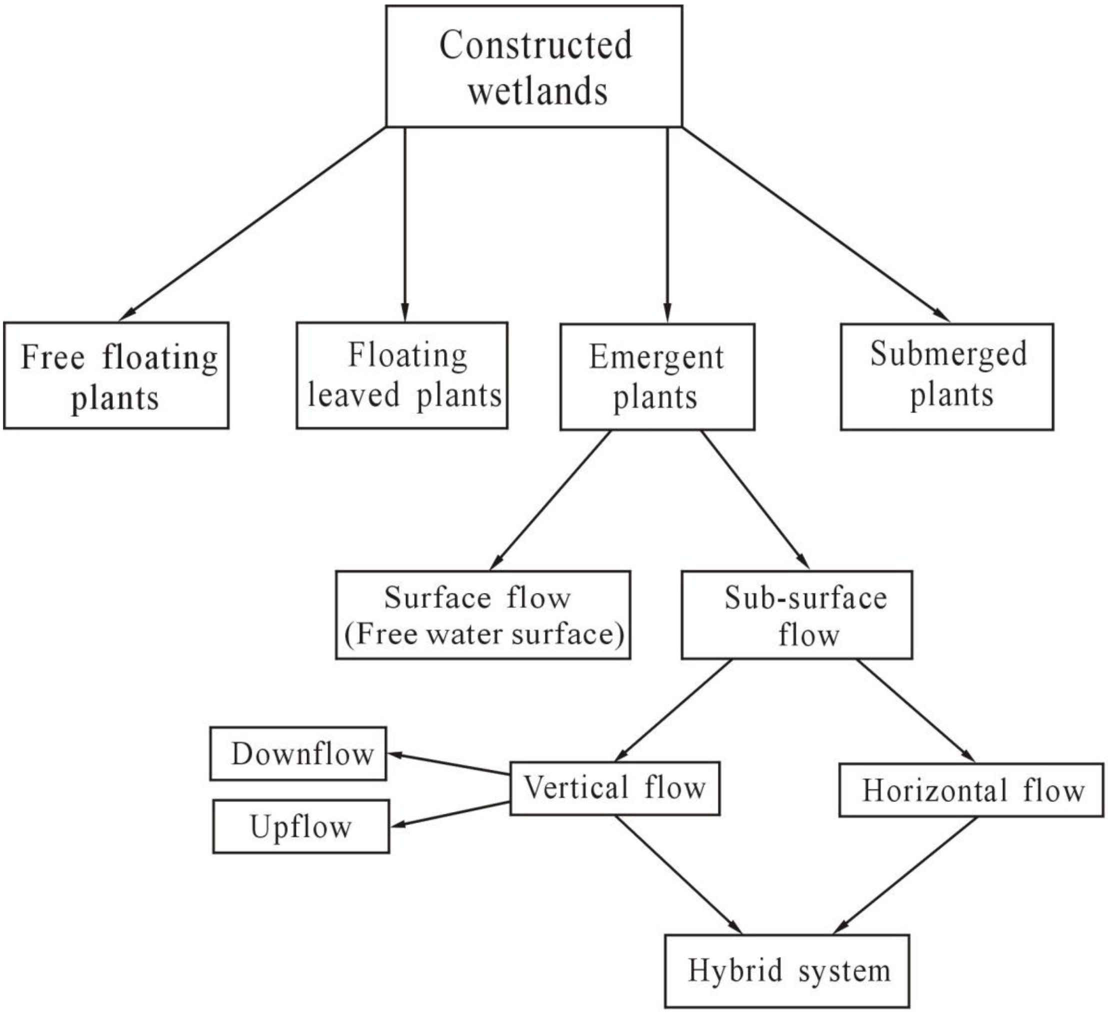

Figure 1.1 Classification of constructed wetlands for wastewater treatment (based on Vymazal, 2001)

1.2.3 Plants in constructed wetlands

1.2.3.1 Plant species

A wide variety of aquatic plants can be used in constructed wetlands designed for wastewater treatment. Commonly, however, constructed wetlands are planned as marsh-type wetlands and are planted with emergent macrophytes (rooted plants that anchor to the substrate media) adapted to water-dominated environment. Frequently used macrophytes species are cattails (*Typha sp.*), reeds (*Phragmites sp*.), bulrushes *(Scirpus sp*.) and sedges (*Carex sp.*) (Sundaravadivel and Vigneswaran, 2001).

The appropriate species for wastewater treatment wetlands depend on local conditions, the water depth, the design (surface or subsurface flow), and characteristics of the wastewater. Studies of wetland plant survival and effectiveness in constructed wetlands (Reddy and DeBusk, 1987; Hammer, 1994) have led to a list of general requirements for suitable plants (Tanner, 1996):

(1) Ecological acceptability, that is, no significant weed or disease risk or danger to the ecological or genetic integrity of surrounding natural ecosystems;

(2) Tolerance of local climatic conditions, pests and diseases;

(3) Tolerance of pollutants and hypertrophic waterlogged conditions;

(4) Ready propagation, and rapid establishment, spread and growth (perennial habit); and

(5) High pollutant removal capacity, either through direct assimilation and storage, or indirectly by enhancement of microbial transformations, such as nitrification (via root-zone oxygen release) and denitrification (via production of carbon substrates).

Therefore, it is best to use plant species that are found in nearby natural wetlands. However, in areas where some of the commonly used species are not locally found, local species should be tested for survivability and effectiveness and used in preference to non-indigenous species (Maschinski et al., 1999). Examples of plant species used in constructed wetlands with high-nutrient loads (Table 1.1) are summarized by Cronk and Fennessy (2001).

Table 1.1 Plant species used in constructed wetlands with high-nutrient loads (Cronk and Fennessy, 2001)

Latin name	Common name	Latin name	Common name
Emergent		**Submerged**	
Sagittaria spp.	Arrowhead	*Ceratophyllum demersum*	Hornwort
Scirpus spp.	Bulrush	*Najas spp.*	Naiad
Canna flaccida	Canna lily	*Potamogeton spp.*	Pondweed
Typha spp.	Cattail	*Elodea canadiensis*	Water weed
Colocasia esculenta	Elephant ear	*Vallisneria americana*	Wild celery
Zizaniopsis milacea	Giant cutgrass		
Iris versicolor, I. pseudacorus	Iris	**Floating**	
Panicum hemitomon	Maidencane	*Spirodela spp.*	Big duckweed
Pontederia cordata	Pickerelweed	*Lemna spp.*	Duckweed
Alisma spp.	Plantain	*Eichhornia crassipes*	Water hyacinth
Phragmites australis	Common reed	*Pistia stratiotes*	Water lettuce
Juncus spp.	Rush	*Salvinia spp.*	Water fern
Cyperus spp.	Sedges		
Fimbristylis spp.		**Rooted floating-leaved**	
Eleocharis spp.		*Nelumbo lutea*	American water-lily
E. dulcis	Water chestnut	*Nymphoides spp.*	Gentian
Zizania latifolia	Wild rice	*Nymphaea spp.*	Water lily
Baumea articulata			
Hydrocotyle umbellata			
Glyceria maxima	Manna grass		

It is known that ornamental plants such as canna lily (*Canna flaccida*), calla lily (*Zantedeschia aethiopica*), elephant ear (*Colocasia esculenta*), ginger lily (*Hedychium coronarium*), and yellow iris (*Iris pseudacorus*) can be used in rock/plant filters to treat septic tank effluents (Wolverton, 1989). Belmont et al. (2004) reported that ornamental flowers (calla lilies and canna lilies) with high economic value planted in the laboratory and field constructed wetland performed as well as cattail (*Typha angustifolia*) in removing N. However, although there are a variety of floriculture plants that can be grown in wetland areas of Australia, little information can be found on the nutrient removal efficiency in constructed wetlands using ornamental species in Australia. Therefore, adapting mixed culture between ornamental species and other macrophytes in constructed wetlands may have benefits in terms of increasing plant growth and purifying the wastewater through nutrient bioaccumulation. In addition, planted ornamental species improve aesthetic view and can have a substantial economic value upon harvesting.

Mixed culture is an agricultural technique of utilizing a single unit of land to plant multiple species. Mixed culture is commonly practiced in Asia and South America today. Throughout time and around the world, mixed cultures have been used to better match crop demands to available resources (sunlight, water, nutrients and labour). One of the most important reasons to grow two or more crops together is the increase in productivity per unit of land. Although numerous plant species have been tested in various constructed wetlands, few studies have given comparative data for evaluating the relative effectiveness of different plant species in improving effluent quality, and for testing whether species mixtures may be superior to monocultures in terms of nutrient removal in constructed wetlands (Coleman et al., 2001).

1.2.3.2 The role of vegetation

Plants have vital roles in constructed wetlands for the maintenance of the system. The most visible role of plants in a wetland is their impact on the aesthetics of the area and on the quality of wildlife habitat. However, it has been widely demonstrated that plants are also involved in almost every major function within wetland treatment systems (Thullen et al., 2005). The main roles are:

(1) Providing the conditions for physical filtration of wastewater and a large surface area for microbial growth, as well as a source of carbohydrates for microbes (Brix, 1997);

(2) Taking up nutrients and incorporating them into plant tissues. Although some of these nutrients are released when plants senesce and decompose, some nutrients remain in the un-decomposed litter that accumulates in wetlands, building organic sediments (Kadlec, 1995);

(3) Leaking oxygen into the sediments and creating a zone in which aerobic microbes persist and chemical oxidation can occur (Armstrong, 1978); and

(4) Having additional site-specific values by providing habitat for wildlife and making wastewater treatment systems aesthetically pleasing (Knight, 1997).

A review by Brix (1997) details the role macrophytes play in a constructed wetland system and how they are an integral part in nutrient cycling. The plants encourage the assimilation and release of nutrients within constructed wetlands. They have the ability not only to bind high amounts of nutrients in their tissues, but also to create an environment conducive to decreasing the nutrients load in the water. For example,

(1) They provide surface area on their roots/rhizome, stems and leaves, which is necessary for microbial growth and nutrient assimilation/transformation; and

(2) The roots also serve to decrease erosion and increase the oxygen concentration, which provides conditions for oxidation of toxic substances like ammonia and nitrites.

1.2.3.3 Plant nutrition and growth

Wetland plants require optimum environmental conditions in each phase of their life cycles, including germination and initial plant growth, normal seasonal growth patterns and rates of plant senescence and decay. Like all plants, wetland plants require many macro- and micronutrients in proper proportions for healthy growth. N and P are key nutrients in the life cycles of wetland plants (EPA, 2000), but the relationships between N or P supply and uptake and wetland plant growth are not fully understood.

Of all the mineral nutrients, N is required in the largest quantities. The two forms of N generally used for assimilation are ammonium and nitrate N. Plant species differ in their preferred forms of N absorbed, depending on the forms available in the soils (Lambers et al., 1998). Most plants are capable of absorbing any form of soluble nitrogen, especially if acclimated to its presence (Atkin, 1996). Wetland plants are suggested to favour NH_4-N rather than NO_3-N (Kadlec and Knight, 1996). The assimilation of NH_4-N has low energy cost; however, wetland plant species may have reduced growth under the high concentrations of NH_4-N (Hill et al., 1997; Clarke and Baldwin, 2002), and develop ammonium toxicity syndrome, which is associated with accumulation of NH_4-N in tissues or diminished cation (such as K^+, Mg^{2+} or Ca^{2+}) uptake (Mehrer and Mohr, 1989). Even species whose tolerance to NH_4-N is pronounced can suffer toxicity symptoms, given a high enough application of ammonium (Britto and Kronzucker, 2002). By contrast, NO_3-N participates in osmoregulation and can be stored in vacuoles without detrimental effects (Marschner, 1995). A widely grown variety of lowland rice was found to be exceptionally efficient in absorbing and assimilating NO_3-N in contrast to NH_4-N compared with other plant species (Kronzucker et al., 1999, 2000). This raises the possibility that NO_3-N uptake by wetland plants is more important than generally thought (Kirk and Kronzucker, 2005).

After N, P is the second most frequently limiting macronutrient for plant growth, but many aspects of P uptake and transport in wetland plants are not thoroughly understood (Schachtman et al., 1998). The different N/P supply ratio can influence plant growth and nutrient uptake, but few studies on differential supply of N and P on wetland plant growth and nutrient uptake have been reported in the literature (Romero et al., 1999).

Although there is considerable information on plant productivity, biomass and nutrient dynamics in natural and fertilized wetlands (Mitsch and Gosselink, 2000; Cronk and Fennessy, 2001), most studies on constructed wetlands receiving wastewaters have only addressed general aspects of plant growth and nutrient content (Tanner, 2001). Plants are an integral part of constructed wetland and investigation of factors affecting growth are needed to produce the healthiest systems. By isolating growth factors in bench-scale studies, a more complete understanding of plant growth may help reduce performance variability and enable scientists to better predict treatment capability of the systems (Hunter et al., 2000). Intensive studies of the growth and nutrients have predominantly been short-term and small scale (Edwards et al., 1993; Tanner, 1994, 1996; DeBusk et al., 1995; Hunter et al., 2000). Only a few studies have investigated plant growth in constructed wetlands on the field scale, eg. the growth characteristics and nutritional status of *Schoenoplectus validus* have been investigated by Tanner (2001), and the growth of *Phragmites australis* and *Phalaris arundinacea* has been compared by Vymazal and Krőpfelová (2005) in constructed wetlands for wastewater treatment. Hence, both short-term and long-term studies on plant growth, development and management of various species in constructed wetlands receiving different sources of wastewater are needed in laboratory and field conditions.

1.2.3.4 Nutrient uptake by plants

Wetland plants are able to tolerate high concentrations of nutrients and in some cases even to accumulate more nutrients than are needed for growth when supplemental nutrients are available (luxury uptake). Therefore, plant nutrient content is greater under high nutrient loads than under natural or background levels of nutrients. Greenway (1997) analysed eight common wetland plant species (emergent and floating-leaved) from both high-nutrient load and control wetlands. Plant N and P levels in the treatment wetlands averaged 7 g N kg^{-1} and 2 g P kg^{-1} dry weight more than in the control wetlands. The concentrations of N and P in plant tissues in constructed wetlands under the high nutrient loads (Table 1.2) were summarised by Cronk and Fennessy (2001).

Table 1.2 Range of N and P concentrations (g kg^{-1}) in tissues of emergent plant species in constructed wetlands under the high nutrient loads (Cronk and Fennessy, 2001)

Species	N			P		
	Leaf	Rhizome	Root	Leaf	Rhizome	Root
Cyperus involucratus	15-43	5-21	11-45	2-5	2-7	1-7
Phragmites australis	10-40	5-31	15-31	2-4	1-3	1-3
Typha spp.	5-32	2-40	4-52	1-5	1-7	2-7
Scirpus tabernaemontani	6-25	9-18	4-21	2-4	2-7	2-8
Bolboschoenus spp.	2-15	13-19	2-15	1-5	4-6	2-7
Baumea articulata	11-18	8-19	8-25	1-9	2-7	2-8

Nutrient uptake by plants varies by season, latitude and certain attributes of each species, such as growth rate and maximum biomass. Wetland plants are seasonally effective at incorporating nutrients into biomass. Most temperate herbaceous species show a maximum rate of uptake early in the growing season, slowing down considerably after flowering (Boyd, 1970; 1978) or peak biomass (Peverly, 1985). Concentrations of N and P in wetland vegetation at peak biomass range from 10 to 30 g N kg^{-1} and 1 to 3 g P kg^{-1} in emergent species and the leaves of woody vegetation (Peverly, 1985).

The potential for nutrient removal by plants is limited by their net growth rate and the concentration of nutrients in tissues. Therefore, the desirable trait of a plant used for nutrient removal through assimilation and storage would include rapid growth, high tissue nutrient concentration and the large biomass (Reddy and DeBusk, 1987). Nutrient removal capacities of commonly used macrophytes in constructed wetlands (Table 1.3) were summarised by Brix (1994a).

Table 1.3 Nutrient removal capacities of some macrophytes (based on Brix, 1994a)

Species	N (kg ha^{-1} yr^{-1})	P (g ha^{-1} yr^{-1})
Cyperus papyrus	1.1	50
Phragmites australis	2.5	120
Typha latifolia	1.0	180
Eichhornia crassipes	2.4	350
Pistia stratiodes	0.9	40
Potamogeton pectinatus	0.5	40
Ceratophylum demersum	0.1	10

The longest-term nutrient storage associated with wetland plant growth is the process of organic soil development (Nichols, 1983; Kadlec and Knight, 1996). The un-decomposed fraction of the litter that remains within the wetland accumulates in the sediment resulting in the long-term nutrient storage. The amount of nutrients that remains in the litter depends on how much is released during plant senescence. In general, the death of wetland vegetation is typically followed by the rapid release to the water of 35 to 75% of plant tissue P and smaller but still substantial amounts of N (Nichols, 1983). The nutrient content of mixed litter (composed primarily of *Phragmites australis, Typha orientalis* and *Echinochloa crus-galli*) in a constructed, clay-based, surface flow wetland in Byron Bay, New South Wales, Australia, was found to be almost as high as the nutrient content of live tissues for these species, containing from 11.4 to 13.2 g N kg^{-1} and from 1.6 to 3.0 g P kg^{-1} (Adcock et al., 1995).

1.2.4 Nutrient removal mechanisms

Removal of N and P by constructed wetlands is a complex cyclic process involving a number of conceptual compartments, including the water column, sediments, plant roots, biofilms, plant stems and leaves. The processes may be physical, chemical or biological (Tchobanoglous, 1993).

1.2.4.1 Nitrogen

The mechanisms involved in N removal in constructed wetlands are manifold and include volatilization, ammonification, nitrification/denitrification, plant uptake, and matrix adsorption.

1.2.4.1.1 Ammonification and volatilization

Nitrogen enters constructed wetlands in either an organic or inorganic form. Ammonification (as the first stage of mineralization) is the process that transforms organic matter to its inorganic constituents and results in the release of the ammonium ions. This process can occur in both aerobic and anaerobic conditions, but under anaerobic conditions is much slower than in aerobic conditions (Mitsch and Gosselink, 2000). Hence, it is predominantly aerobic process, thus competing with nitrification (as the second stage of mineralization) for oxygen. According to Kadlec and Knight (1996), ammonification proceeds more rapidly than nitrification. Furthermore, the rate of ammonification is also dependent on pH and temperature. Vymazal (1999a) suggested that ammonification may be the most temperature-dependent process of all those involved in N transformations in horizontal subsurface flow constructed wetlands.

Ammonification as such does not remove N from the wastewater in constructed wetlands — it just converts organic nitrogen to ammonium which is then available for other processes (e.g., nitrification, volatilization, adsorption, plant uptake). As organic nitrogen is mineralized, it enters the inorganic cycle. The inorganic forms are nitrate, nitrite, ammonia, and ammonium. Most of the inorganic N in wastewater entering constructed wetlands is in the form of ammonia and ammonium. Ammonia may be volatilized or taken up by plants or microbes. Under aerobic conditions, it may be transformed into nitrate in the nitrification process. Similarly, ammonium may be taken up by biota or transformed into nitrite. In addition, because of its positive charge, ammonium can be adsorbed onto negatively charged soil particles that can be deposited as sediment, but may eventually be solubilized and returned to the water column (Kadlec and Knight, 1996).

Ammonia volatilization is a physicochemical process where ammonium-N is in equilibrium between gaseous (NH_3) and hydroxyl ($NH_4^+ + OH^-$) forms. It has been shown in the literature that significant loses may occur through volatilization in open water areas, where surface turbulence is high as a result of wind action. Furthermore, in open water zones of free surface wetlands, elevated water temperature and pH may enhance ammonia volatilization to a degree that it becomes a significant N removal mechanism (EPA, 2000). Stowell et al. (1981) reported that volatilization of ammonia can result in N removal rates as high as 2.2 g N m^{-2} d^{-1}.

1.2.4.1.2 Nitrification and denitrification

In wetlands, nitrification (the oxidation of ammonia and ammonium to nitrite and nitrate) occurs in oxidized areas of the substrate or water column. Oxygen is present at the soil surface and in the root zone, where it enters the soil via diffusion from plant roots. Nitrification, similar to ammonification, does not remove nitrogen from wastewaters. However, nitrification coupled with denitrification seems to be the major N removal process in many treatment wetlands (Vymazal, 2007).

As nitrate diffuses into anaerobic areas in the soil, it is reduced by bacteria to nitrous oxide (N_2O) or dinitrogen gas (N_2) in a process called denitrification. Both N_2O and N_2 are released to the atmosphere (Gambrell and Patrick, 1978). Denitrification is an anaerobic dissimilative pathway in which nitrate is used as an electron acceptor for anaerobic respiration to generate energy (Madigan et al., 1997). The first step of denitrification involves the reduction of nitrate to nitrite. Then, nitrite can either be reduced to ammonia or sequentially reduced to nitric oxide, nitrous oxide, and dinitrogen. The synthesis of the enzymes involved in each step is repressed by the presence of O_2 (Madigan et al., 1997). Therefore, the process of denitrification is strictly an anoxic process. The occurrence of both aerobic and anaerobic soil and water layers in wetland provides ideal conditions for N conversions.

Denitrification is the most important removal pathway for N in most wetlands (Faulkner and Richardson, 1989). Some of the major environmental factors that affect denitrification include oxygen level, temperature, pH, soil moisture, and availability of carbon sources. Because the transformations of N involve microbial processes, N removal is enhanced during the growing season when high temperatures stimulate microbial population growth (Gambrell and Patrick, 1978). Low temperatures or acidic soil conditions inhibit denitrification (Engler and Patrick, 1974; Schipper et al., 1993). Although some researchers report that denitrification ceases below 5 ^{o}C (Stanford et al., 1975), others have measured some denitrification activity at 4 ^{o}C (Limmer and Steele, 1982; Pfenning and McMahon, 1996).

Detailed studies (Vymazal, 1999b) at several Czech constructed wetlands with horizontal subsurface flow revealed that removal of N is affected by temperature only slightly and, in some cases, there is no significant difference between summer and winter removal rates. The conditions in vegetated beds of constructed wetlands with horizontal subsurface flow are usually hypoxic and/or anoxic so that the major obstacle to higher removal of N is low rate of nitrification. As ammoniacal-N is the prevailing form of N in sewage wastewater, the removal of N in horizontal subsurface flow constructed wetlands should be very low. However, the results from existing systems show that N is removed in all systems to some extent and in some systems even to a high level (Vymazal, 2002).

1.2.4.1.3 Plant uptake and assimilation

Apart from bacterial activities, N removal can also occur due to uptake by plants. Wetland plants assimilate N and reduce inorganic N to organic N used for plant structure and function.

Plant uptake does not represent permanent removal of N unless plants are routinely harvested. While uptake rates of nutrients are potentially high, harvesting plant biomass to remove these nutrients has been limited to floating aquatic plant communities, in which the plants can be harvested with only brief alteration of the system performance. In tropical regions where seasonal translocation is minimal and multiple harvesting is possible, harvesting of emergent plants could play a significant removal route especially in the lightly loaded systems.

However, some reports stated that harvesting of the plant material from a constructed wetland provides only a minor N removal pathway compared to biological activity in the wetland (Reed et al., 1995). The harvested amount is usually around 10% of the total removed N and P in the subsurface flow constructed wetlands (Vymazal, 1999a and b; Tanner, 2001; Stottmeister et al., 2003).

1.2.4.1.4 Alternative routes of ammonium transformations

In aqueous environment, ammonium can be removed via various pathways, depending on a combination of factors, such as the characteristics of wastewater and availability of oxygen. However, some routes may not remove N from the system. For example, in a reduced state ammonium-N is stable and can be adsorbed onto active cation exchange sites of the substrate. However, the binding of NH_4-N on cation exchange sites is not considered to be a long-term sink for NH_4-N removal because such binding is rapidly reversible. When the ammonium concentration in the water column is reduced, such as a result of nitrification and/or plant uptake, some ammonium will be desorbed from the soil (sediment) particles to regain the equilibrium. The Freundlich equation can be used to model NH_4-N sorption (Cooper et al., 1996a).

Sun and Austin (2007) summarized alternative routes currently known for ammonium transformations (Table 1.4). Nevertheless, the method of mass balance analysis remains a fundamental tool for revealing mechanisms of N removal in constructed wetlands.

Table 1.4 Alternative routes of ammonium transformations in constructed wetlands (based on Sun and Austin, 2007)

	Brief process description	N removal	References
Adsorption	Transferring ammonium from water onto the sediment in wetlands, typically occurring prior to nitrification	No	Connolly et al. (2004)
Assimilation into biomass	Forming part of biomass generated by microorganisms during the removal of organic matter from wastewater	No	Sun et al. (2005)
Autotrophic denitrification	Anaerobic ammonia oxidation (ANAMMOX) bacteria. Under anaerobic conditions carbonate ion serves as the carbon source, nitrite as the terminal electron acceptor, to transform nitrite and ammonium into nitrogen gas	Yes	Strous et al. (1999) and Jetten (2001)
Completely autotrophic nitrogen-removal over nitrite (CANON)	In a single treatment step under hypoxic conditions, ammonia is first oxidized into nitrite; nitrite is then reacted with remaining ammonium into dinitrogen	Yes	Sun and Austin (2007)
Heterotrophic nitrification/aerobic denitrification	Direct transformation of NH_4–N to N_2 or NO_x species without the production of NO_2 or NO_3. Heterotrophic nitrifiers are also aerobic denitrifiers	Yes	Robertson and Kuenen (1990)
Methane oxidation to denitrification	Under anaerobic conditions, prokaryotes convert methane with nitrite and nitrate into CO_2 and N_2	Yes	Raghoebarsing et al. (2006)

1.2.4.2 Phosphorus

Phosphorus removal mechanisms in constructed wetlands include adsorption, complexation, sedimentation, precipitation and plant absorption (Watson et al., 1990).

1.2.4.2.1 Substrate adsorption and sedimentation

Adsorption is considered the most significant mechanism of P removal, and accretion into the sediments is a long-term P removal process. The adsorption of P occurs mainly because of complexation and precipitation reactions with iron (Fe), calcium (Ca), magnesium (Mg) and aluminium (Al) minerals in the sediment (Moshiri, 1993). Adsorption and precipitation can initially only be considered as short-term storage. However, once this material is buried with a layer of peat, it becomes part of the long-term sink storage (Richardson and Craft, 1993). Sedimentation is a physical process whereby particulate matter containing P in the water column settles and accumulates on the wetland floor. If the particulate matters remain insoluble, P removal by this mechanism is permanent. However, if the particulate materials are biodegradable organics, after degradation, P will be released back to the water column.

Most wetland studies have shown that the soil/litter sedimentation is the major long-term P storage pool and that wetlands are not particularly effective as a P sink when compared with terrestrial ecosystems (Richardson, 1985; Faulkner and Richardson, 1989). However, reviews of P uptake in a wide variety of constructed wetlands in different climates receiving different loadings reveal that most function as net P sinks (Kadlec and Knight, 1996; Reddy et al., 1999). The adsorption and retention of P in wetlands is controlled by the interaction of redox potential, pH, and Fe, Ca and Al minerals. The most important retention mechanisms are claimed to be ligand exchange reactions, where phosphate displaces water or hydroxyl from the surface of Fe and Al hydrous oxides to form monodentate and binuclear complexes within the coordination sphere of the hydrous oxide (Faulkner and Richardson, 1989).

1.2.4.2.2 Plant and microbial uptake

Phosphorus is taken up by macrophytes, algae and microbes. Plants are able to rapidly assimilate P and often respond to new inputs with rapid growth. Greater P retention during the growing season in constructed wetlands has been attributed to biotic uptake (Gearhart et al., 1989). At the start of a growing season, plants take up some nutrients that are contained in the plant litter after the vegetation died (Verhoeven and Meuleman, 1999). If not harvested, most of what was taken up is released back into the wetland during the decay of the plant matter, which can provide a source of P in the wetland. Plants only contain a small amount of the total P that occurs in wetlands. Thus, the P uptake capacity of macrophytes in constructed wetlands is limited (Brix, 1994a; Vymazal, 1999b), but could be important in constructed wetlands that have low inflow loading. Plant uptake is a more important route of P removal in systems with free-floating macrophytes (Vymazal, 2007).

1.2.4.2.3 Phosphine emission

It is believed that P has no atmospheric flux and has a much longer temporal biogeochemical cycle than N (Froelich, 1988). However, thanks to the development of extremely sensitive analytical tools (gas chromatography with cryo-trapping), the ubiquitous presence of phosphine (PH_3) gas has been identified in the environment in a variety of locations (Dévai et al., 1988; Gassmann, 1994; Roels and Verstraete, 2001). There are two hypotheses for biological phosphine formation. The microbial cells benefit from the formation of phosphine, or, alternatively, phosphine is formed as the result of a metabolic energy-consuming side-reaction. Other processes could also contribute to the global presence of phosphine (Roels and Verstraete, 2001). Although the magnitude of phosphine losses in the constructed wetlands was still unknown (Tanner et al., 1998), a review by Glindemann et al. (2005) supported the existence of small gaseous (PH_3) link in the P cycle in wetlands, which could become important over the long term.

1.2.5 Nutrient removal efficiency

A survey of literature on the nutrient removal efficiencies achieved in constructed wetlands indicates extreme variations. In general, the reported results indicate that the nutrient removal was higher in small-scale experiments (microcosms or mesocosms) than large field-scale constructed wetlands. Although microcosms have limitations, they are extremely useful for controlled, mechanistic investigations to elucidate the fundamentally complex mechanisms of nutrient removal in constructed wetlands (Fraser and Keddy, 1997). However, Ahn and Mitsch (2002) pointed out that scale of experiments must be considered before the results from microcosm or mesocosm studies are generalized to large field-scale wetlands.

The simple comparison of nutrient removal efficiency by various constructed wetlands in different studies is difficult due to the variable conditions. Variation among studies is influenced by differences in vegetation type and density, media, retention times, loading rates, temperature, size of the systems, etc. (Reddy, 1983b). Nevertheless, Vymazal (2007) has summarized and compared the removal efficiencies of nutrients in published studies with various types of constructed wetlands around the world. Removal of total N in studied types of constructed wetlands varied between 40 and 55% (with the removal load ranging between 250 and 630 g N m^{-2} $year^{-1}$) and for P between 40 and 60% (with removal load ranging between 45 and 75 g P m^{-2} $year^{-1}$), depending on constructed wetland type and inflow loading. Although a slightly higher removal found for constructed wetlands with free-floating plants than with free water surface flow, horizontal sub-surface flow and vertical sub-surface flow constructed wetlands, the N removal efficiency is similar in all systems. The vertical flow constructed wetlands remove more NH_4-N than free water surface flow and horizontal flow constructed wetlands, but the potential to remove NO_3-N is very low; in most cases, the concentrations of NO_3-N increase in the outflow. Therefore, hybrid constructed wetlands (comprising vertical flow and horizontal flow) may be the best solution when total N removal is the main target (Vymazal, 2007).

1.2.6 Factors affecting nutrient removal efficiency

Nutrient removal efficiency is influenced by the physico-chemical conditions that exist in the overlying water and in the underlying sediment, such as pH, dissolved oxygen, temperature, hydraulic loading, presence of aquatic macrophytes, properties of sediments and so on.

1.2.6.1 Plant species

Plants play a significant role in constructed wetlands, especially in N and P removal (Akratos and Tsihrintzis, 2007). When compared to unplanted controls, planted constructed wetlands commonly show enhanced removal of nutrients. A number of studies on constructed wetlands confirmed that unplanted treatments had lower N and P removal compared with planted treatments (Gersberg et al., 1986; Tanner et al., 1995; Hunter et al., 2001, Lim et al., 2001; Yang et al., 2001; Fraser et al., 2004; Akratos and Tsihrintzis, 2007; Iamchaturapatr et al., 2007; Zhang et al., 2007b). Aquatic plants enhance nutrient removal in constructed wetlands through nutrient uptake, filtration of inorganic and organic particulates and creation of oxidized rhizospheres (Burgoon et al., 1991; Brix, 1994a). However, some researchers did not detect any significant difference between planted and unplanted systems (Balizon et al., 2002; Calheiros et al., 2007). The beneficial role of plants in constructed wetlands is not always evident, depending on several parameters, such as the duration of operation, type of vegetation and characteristics of the wastewater (Calheiros et al., 2007).

The results on nutrient removal efficiency for various plant species in the constructed wetlands are inconsistent in the literature. Most researchers have shown that wetland plants in the constructed wetlands could have a positive influence on nutrient removal, and the choice of species can be important for the nutrient removal efficiency (Gersberg et al., 1986; Coleman et al., 2001; Fraser et al., 2004; Kyambadde et al., 2004; Akratos and Tsihrintzis, 2007; Calheiros et al., 2007; Iamchaturapatr et al., 2007; Sim et al., 2007; Yang et al., 2007). A comparative study of constructed wetlands for wastewater treatment in a tropical climate showed the plant uptake and storage contributed more to N and P removal in *Cyperus papyrus* wetlands than in *Miscanthidium violaceum* wetlands (Kyambadde et al., 2004). Similarly, Yang et al. (2007) observed that a wetland planted with *Canna indica* had generally higher N and P removal rates than one planted with other species. In contrast, some researchers have found that the choice of wetland species did not have a major impact on nutrient removal efficiency (Thomas et al., 1995; da Motta Marques et al., 2000; Huang et al., 2000; Jing et al., 2002; Zhang et al., 2007b). Thomas et al. (1995) observed no significant difference in nutrient removal from the secondary-treated sewage effluent in a pilot constructed wetland planted with either *Schoenoplectus validus* or *Juncus ingens*. Jing et al. (2002) also found that the type of macrophytes (*Phragmites australis*, *Ludwigia octovalvis* and *Commelina communis*) in small-scale constructed wetlands did not make a major difference in nutrient removal. Similarly, in two free-water surface treatment cells at the Iron Bridge Wetland in Florida, *Scirpus californicus* removed total N and P to a similar extent as *Typha latifolia* (EPA, 2000).

When compared to mono-culture, the results on nutrient removal efficiency using mixed cultures were inconclusive. Some studies showed that mixed culture performed better than monoculture (Coleman et al., 2001), but others reported that mixed cultures were not significantly different from the monocultures in terms of nutrient removal (Thomas et al., 1995; Fraser et al., 2004; Zhang et al., 2007). However, the mixed culture may provide other benefits for the performance of constructed wetland. The monoculture systems are more susceptible to plant death due to predation or disease. Therefore, it is generally assumed that mixed cultures are more resilient than monocultures (EPA, 2000).

Plants make a greater contribution to the percentage nutrient removal in constructed wetlands under low load compared with high load conditions. In systems with high load, the plants may take up higher amounts of nutrients, but as a percentage of the incoming loads, uptake is small. Peterson and Teal (1996) compared plant uptake in constructed wetlands with high loads of N (3.2 to 15.6 g N m^{-2} d^{-1}) to wetlands with lower loads (0.4 to 2.0 g N m^{-2} d^{-1}). The plants assimilated only 1 to 4% of the nitrogen in the heavily loaded system, whereas plant uptake accounted for 18 to 30% of nitrogen removal in the lightly loaded system.

1.2.6.2 Temperature

Nutrient removal in constructed wetlands has been shown to be temperature dependent. Many reports suggested that the nutrient removal efficiency of constructed wetlands decreased sharply with decreased temperature. This reasoning was based on the well-documented fact that microbial growth rates and rates of treatment processes assayed in vitro decreased sharply with a temperature decrease (Stein and Hook, 2005). There is extensive literature supporting the strong effect of temperature on microbial N processing, with doubling of rates over a temperature range of about 10 ºC (Kadlec, 2006). The optimum temperature for nitrification in pure cultures ranges from 25 to 35 ºC and in soils from 30 to 40 ºC. The minimum temperatures for growth of *Nitrosomonas* and *Nitrobacter* are 5 and 4 ºC, respectively (Cooper et al., 1996b). Seasonal patterns of nitrification and denitrification in a natural and a restored salt mash wetland have been reported by Thompson et al. (1995). Kuschk et al. (2003) have observed that annual cycle of N removal by a pilot-scale subsurface horizontal flow constructed wetland under moderate climate. However, according to the study by Lee et al. (1999), the low ammonium removal in winter is not only the result of cessation or slowing of the nitrification process; in addition to reduced nitrifier growth and metabolism, it is more likely due to a combination of many factors, including plant death, that result in less oxygen being transferred to the root zone.

The nutrient removal is often a primary factor to be considered when designing constructed wetlands in cold climates (Werker et al., 2002). There is little research comparing the relative nutrient removal efficiency of different plant species in constructed wetlands in cold climates. Most research involves just a few species, and direct comparisons among species have focused mostly on growing season at relatively warm temperature. However, the results from Stein and Hook (2005) suggested that the overall influence of plants and differences among species might be greater during periods of low temperature and plant dormancy. The seasonal variation can be minimized through selecting plant species (Picard et al., 2005).

The nutrient removal performance of constructed wetlands in tropical climates presents a vastly different picture. Tropical climatic conditions are conducive to rapid plant growth due to a continuous growing season and higher biological activity that result in more efficient removal of nutrients than under temperate conditions (Jinadasa et al., 2006). The N and P removal efficiencies of a full-scale constructed wetland treating domestic wastewater in Bhubaneshwar, India, have been found to be around 70% and 43%, respectively (Juwarkar et al., 1995). However, the adaptation of wetland technology in tropical and sub-tropic climates was surprisingly slow (Jinadasa et al., 2006). Fewer studies have been conducted in tropical conditions compared to temperate climates. The design parameters need to be modified significantly in tropical conditions, and the reliance on the guidelines derived from temperate climates would not be feasible for tropical conditions.

1.2.6.3 Oxygen

Nitrogen removal efficiency is dependent on sequential mineralization of organic N to ammonium N, followed by nitrification of the ammonium to nitrite and/or nitrate, and denitrification of nitrite or nitrate to gaseous N form. Oxygen is required for nitrification. Two dominant routes have been documented for transfer of oxygen from air to constructed wetlands: (1) oxygen release from plant roots, and (2) atmospheric oxygen diffusion (Kadlec and Knight, 1996). Typically, oxygen release rates from the roots of a number of aquatic plants were reported to range from 0.02 to 12 g m^{-2} d^{-1} (Wu et al., 2001). The variation may have been caused by species differences (Allen et al., 2002; Wießner et al. 2002), daily and seasonal changes (Reddy, 1981; Brix, 1997) or the techniques used in the studies (Sorrell and Armstrong, 1994; Wu et al., 2001). Early publications estimated high rate of plant-mediated oxygen transport. However, further research indicated that oxygen transfer to wetlands is dominated by atmosphere oxygen diffusion with little oxygen actually escaping from plant roots to the rhizosphere (Bezbaruah and Zhang, 2004). Nevertheless, plant-mediated oxygen transport to the rhizosphere varies among species and seasons.

The interaction among plants and seasonal variations in temperature and other factors can strongly influence nutrient removal processes in constructed wetlands, especially in winter (Stein and Hook, 2005). Recently, an attempt was made to use artificial aeration to enhance ammonium N removal in constructed wetland in cold climates (Jamieson et al., 2003; Ouellet-Plamondon et al., 2006). The results indicated that the artificial aeration has great potential to enhance nitrification and improve N removal efficiency in constructed wetlands receiving agricultural and aquacultural wastewater in cold climate.

1.2.6.4 pH

pH can affect the biological activity in the constructed wetlands, resulting in variation of nutrient removal in the systems. Plant growth and nutrient uptake are affected by pH (Rengel, 2002). The growth of *Typha latifolia* almost completely stopped at pH 3.5 in the solution culture experiments (Brix et al., 2002). The growth inhibition at low pH was probably due to a reduced nutrient uptake and a consequential limitation of growth by nutrient stress. The uptake of NH_4-N generally decreased with decreasing external pH, but the uptake of NO_3-N is largely unaffected or may even increase at slightly acidic pH levels (Brix et al., 2002). When the soil pH drops below 5, aluminum (Al^{3+}) is solubilized and becomes the most important rhizotoxic Al species (Kinraide, 1991). The primary symptom of Al toxicity is a rapid inhibition of root growth, resulting in a reduced and damaged root system and limited water and mineral nutrient uptake, but some plant species have developed Al tolerance (Barcelo and Poschenrieder, 2002).

Nitrification and denitrification are influenced by pH. Paul and Clark (1996) reported that the optimum pH for nitrification may vary from 6.6 to 8.0; however, acclimatized systems can nitrify at a much lower pH (Cooper et al., 1996b). The optimum pH for denitrification is between 6 and 8. Denitrification becomes slow, but still remains significant below 5 and is negligible or absent below 4 (Paul and Cark, 1996).

The ammonia volatilization is regulated by pH. Reddy and Patrick (1984) pointed out that losses of NH_3 through volatilization from flooded soils and sediments are insignificant if the pH value is below 7.5 and very often losses are not serious if the pH is below 8.0. At pH of 9.3, the ratio between ammonia and ammonium ions is 1:1 and the losses via volatilization are significant.

The P precipitation is related to pH in the substrate of constructed wetlands. There are three general conclusions about the tendency of P to precipitate with selected ions (Reddy et al., 1999):
(1) In acid soils, P is complexed as Al-P and Fe-P. P sorption to clay particles is greatest under strongly to slightly acidic conditions;
(2) In alkaline soils, P is bound by Ca and Mg; and
(3) The bioavailability of P is greatest at neutral to slightly acid pH.

Under anaerobic wetland soil conditions, P may also be released into solution by a pH change brought about by organic, nitric or sulphuric acids produced by chemosynthetic bacteria (Stumm and Morgan, 1996).

1.2.6.5 Substrate

The different substrates have different P sorption capacity, which is a crucial parameter for the P removal (Richardson, 1985; Drizo et al., 2002). For example, Drizo et al. (1999) have measured P adsorption capacity of seven substrates: bauxite, shale, burnt oil shale, limestone, zeolite, light expanded clay aggregates (LECA) and fly ash. Fly ash and shale had the highest P adsorption values, followed by bauxite, limestone and LECA. Xu et al. (2006) have found that the furnace slag had the highest P sorption capacity, followed by the fly ash, with sand being the lowest, even though different kinds of sands showed varying P sorption capacity. The P sorption capacity is influenced by physico-chemical characteristic of substrate, and by added organic matter that changes pH of the substrate and affects the P removal efficiency. Although the P sorption isotherms may provide a quick screening tool to select potential substrate, when obtained under laboratory conditions, they can not be used to determine the longevity of P retention in constructed wetlands (Korkusuz et al., 2007).

A large number of different substrates for potential use for P removal from wastewater have been reviewed by Westholm (2006). The majority of these substrates have been tested in laboratory experiments, but the data from the laboratory are difficult to compare with those from the field. For example, Brix et al. (2001) showed that the P sorption by a calcite was ten times higher in laboratory experiments than in the field. Therefore, further research is needed to learn more about the substrate behaviour under realistic field conditions.

Currently, clay soil which provides many exchange sites to adsorb ammonium is usually not used in constructed wetlands due to potential clogging. Instead, sand and gravel that have fewer exchange sites compared with the clay soil are mostly used as substrate in constructed wetlands. Therefore, ammonium adsorption by substrate is limited in constructed wetlands (Vymazal, 2007). The system with substrate containing a soil-to-sand ratio of 75:25 had the highest nutrient removal efficiencies in a small-scale experiment (Sirianuntapiboon et al., 2006). The mixtures of different substrates may enhance the adsorption of N in constructed wetlands.

An attempt was made by Wu et al. (2006a) to investigate the simultaneous removal of ammonium and P by zeolite synthesized from fly ash saturated with different cations. The results showed that zeolite synthesized from fly ash could be used in simultaneous removal of ammonium and P at low concentrations with pre-saturation by an appropriate cation such as Al^{3+} through salt treatment (Wu et al., 2006a).

1.2.6.6 Hydraulic retention time

The nutrient removal efficiency of a constructed wetland is directly related to the hydraulics of the wetland system (Mitsch and Gosselink, 2000; Martinez and Wise, 2003). Shilton and Prasad (1996) have stated that performance of many constructed wetlands systems varied due to a lack of understanding of the system hydraulics. Majority of studies showed that a longer hydraulic retention time could increase nutrient removal (Hunter et al., 2001; Jing et al., 2002; Mayo and Mutamba, 2004; Sirianuntapiboon et al., 2006; Akratos and Tsihrintzis, 2007; Iamchaturapatr et al., 2007). As theoretical wastewater retention times increased from 2 to 7 days, mean reduction of total N increased from 12 to 41% and 48 to 75% in the unplanted and planted wetlands, respectively, and total P removal increased from 12 to 36% and 37 to 74%, respectively (Tanner et al., 1995). However, the optimum retention time is depended on other factors, such as temperature, loading rate and plant growth. For instance, an 8-day retention time is adequate for an acceptable N removal at temperatures above 15 ºC, while longer retention time is needed at temperatures below 15 ºC (Akratos and Tsihrintzis, 2007).

1.2.6.7 Nutrient loading rate

Nutrient removal efficiency is strongly dependent on nutrient loading rate in the constructed wetlands. The relationship between mass loading and removal rates of total N and P can be observed. In the planted wetlands, mean annual removal rates of total N (0.15-1.4 g m^{-2} d^{-1}) and total P (0.13-0.32 g m^{-2} d^{-1}) increased gradually with mass loading rates. The unplanted wetlands showed a marked decline in percentage of total N and P removal at the high loads (Tanner et al., 1995). Experience with gravel bed treatment wetlands for treating domestic wastewater has shown that removal of nutrients in the longer term can be optimized up to about 50% for N and 40% for P in systems with low loading rates (Brix, 1994c).

1.2.7 Summary

Constructed wetlands provide a useful complement to traditional sewage treatment systems. They are often a cheap alternative to expensive wastewater treatment technologies for purification of tertiary wastewater. However, nutrient removal efficiencies vary widely among constructed wetlands, and appear to be affected by many factors. Hence, the process-based laboratory and long-term investigations are required to evaluate sustainable nutrient removal rates and identify the key factors regulating performance.

Wetland plants are often central to wastewater treatment in constructed wetlands (Scholz and Lee, 2005), in addition to the other design factors such as hydraulics, substrate, etc. Macrophytes take up and assimilate nutrients in their tissue. This attribute relates to the rapid growth rates of species in resource-rich environments, and ability to concentrate luxury amounts of nutrients in their above- and below-ground biomass. The partitioning of nutrients between shoots and roots/rhizomes varies between species and seasons. In small, lightly loaded constructed wetlands, plant uptake can be the principal form of nutrient removal, often accounting for up to 80% of the nutrient pool (Kadlec and Knight 1996). Plants are also the principal nutrient sinks during the initial years of establishment in the wetland. The removal of plant material through a harvest reduces the potential for biologically-assimilated nutrients being remobilised into the wetland system. Harvesting can also encourage large nutrient uptake by the plants during the rapid growth and recovery of the harvested plants (Kim and Geary, 2001; Toet et al., 2005). Therefore, the function of plants in nutrient stripping is a dynamic one, and requires a better understanding of plant tolerances and nutrient requirements to optimize performance in terms of uptake.

Different wetland plant species have different capacity to take up nutrients (Tylova-Munzarova et al., 2005), and require different forms of N (Fang et al., 2007a and b). Improvements in plant selection and management may make the constructed wetlands more efficient for nutrient removal from the wastewater. However, there is a lack of knowledge on (i) how plants respond to nutrient availability in the supplied wastewater, (ii) whether species and mixtures of species affect nutrient uptake and removal efficiency in constructed wetland, (iii) to what extent the plants respond and acclimate to the interactive effects of N and P supply, and (iv) if these effects influence the nutrient removal from the wastewater.

1.3 Outline of the study

1.3.1 General

The purpose of this study was to provide a better understanding of using macrophytes to purify secondary-treated municipal wastewater in terms of nutrient removal in constructed wetlands, thus making a contribution to the waste management and environmental improvement, especially in wastewater disposal and reuse. The general approach was to conduct a series of studies of plant growth in, and nutrient removal from, simulated secondary-treated municipal wastewater in the wetland microcosms using emergent wetland macrophytes.

1.3.2 Aims

A. To characterise the potential of various species of macrophytes, especially ornamental species, to grow in the wastewater in the wetland microcosms;
B. To compare the plant growth and nutrient uptake and distribution between the mono-cultures and the mixed culture, and elucidate mechanisms behind the difference in effectiveness of single species vs. multiple species in removing nutrients from the wastewater;
C. To investigate the interactive effects of differential N and P supply on plant growth, nutrient uptake, biomass and nutrient allocation, and elucidate how the nutrient availability and the ratio of N and P in wastewater affect the plant growth and eventually influence the nutrient removal efficiency in the constructed wetland; and
D. To investigate the kinetics of plant nutrient uptake and elucidate mechanisms behind the difference in nutrient uptake efficiency between plant species.

1.4 Structure of the thesis

A general introduction and literature review that embraces all aspects of the study have been given in Chapter 1. General details of experimental procedures, sample preparation, analytical instruments and techniques, statistical analysis and descriptions of *Canna indica* and *Schoenoplectus validus* used intensively in this study are contained in Chapter 2. Identification of suitable ornamental plant species for the wetland microcosms is given in Chapter 3. A comparative study of plant growth and nutrient removal efficiency in simulated secondary-treated wastewater using *C. indica* and *S. validus* between the mono- and mixed culture is presented in Chapter 4. The interactive effects of differential N and P loading rates on plant growth and nutrient removal efficiency of the above two species in the wetland microcosms are given in

Chapter 5. The kinetics of nutrient uptake by the above two species is presented in Chapter 6. General conclusions and discussion, limitations of this work and suggestions for further work are contained in Chapter 7.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 General

This chapter provides a general explanation of the various analytical and experimental procedures employed in Chapters 3, 4, 5 and 6, and the descriptions of *Canna indica* and *Schoenoplectus validus* used intensively in this study (Chapters 3, 4, 5 and 6). Further details on materials and methods specific to individual experiments are included in specific chapters (Chapters 3, 4, 5 and 6).

2.2 Concentrations of N and P in the secondary-treated municipal wastewater effluent

The data on quality of discharge water from secondary-treated municipal wastewater effluent collected at Subiaco Wastewater Treatment Plant in Shenton Park, Perth, Western Australia, from July 2002 to November 2004 were obtained from Water Corporation, Western Australia. The yearly ranges of the total N and P concentrations in the wastewater effluent were 8.2 to 28 mg N L^{-1} and 5.6 to 17 mg P L^{-1}; the average concentrations between 1 November 2003 to 31 October 2004 were 17 mg N L^{-1} and 10 mg P L^{-1}. These N and P concentrations are used to guide the choice of treatment N and P concentrations throughout the study. The average concentrations of biochemical oxygen demand (BOD), suspended solids (SS) and total dissolved solids (TDS) were 9, 25 and 750 mg L^{-1}, respectively, in the effluent of Subiaco Wastewater Treatment Plant from 1 November 2003 to 31 October 2004.

2.3 Design of wetland microcosm

A wetland microcosm was established in 33-L plastic containers (0.39 m × 0.29 m × 0.30 m) with a hole fitted with a plastic tube close to the base to facilitate drainage of water

(Figure 2.1). A mesh covering the hole was fastened on the inside of the microcosm to prevent loss of sand during the water drainage. Approximately 25 kg of river sand was added to each container, giving a sand depth of about 0.15 m. Some properties of the sand were presented in Table 2.1.

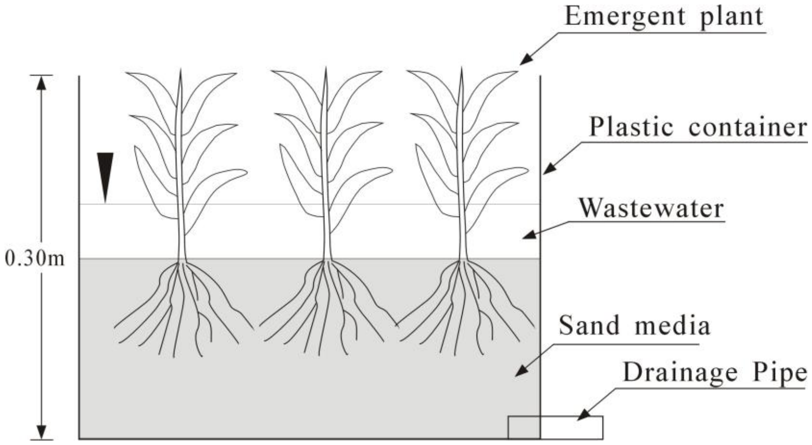

Figure 2.1 The design of the wetland microcosm

Table 2.1 Some properties of the sand used in microcosms

Parameter	Sand size (mm)	pH (H_2O)	pH ($CaCl_2$)	Conductivity (dS m^{-1})	Organic carbon (g kg^{-1})	Sulphur (mg kg^{-1})	Iron (mg kg^{-1})
Value	1-4	7.1	6.3	0.022	0.80	5.2	187

2.4 Experimental setup

All experiments in Chapters 3, 4, 5 and 6 were conducted in a phytotron chamber at the University of Western Australia (31°58’ S, 115°49’ E) with controlled day/night temperatures under natural light conditions. All experiments were established in the designed wetland microcosms, except kinetics of nutrient uptake in hydroponics (Chapter 6). More details of experimental setup could be found in each chapter (Chapters 3, 4, 5 and 6).

All seedlings of wetland plant species were obtained from the local plant nursery. Healthy plants of relatively the same size were transplanted into the microcosms.

In order to minimize variables within the experiment, a simulated nutrient solution was used in the microcosms. Except N, P and K that varied depending on experimental objectives, the solution contained other macro- and micronutrients (mg L^{-1}): 10 Ca, 5 Mg, 7 S, 0.04 Zn, 0.01 Cu, 0.34 Fe, 0.05 Mn, 0.26 B and 0.05 Mo.

2.5 Sampling and analytical methods

2.5.1 Water samples

Influent samples were taken just before the microcosms were filled with simulated wastewater. Effluent samples were taken immediately after solution was drained. The pH and dissolved oxygen were measured immediately after the samples were taken. Water samples of water were frozen before analysis of nutrients.

The pH and dissolved oxygen in the effluent were measured using a combination glass membrane electrode with a Calomel internal reference (Cyberscan 20 pH meter, Eutech Instruments, Singapore) and a membrane electrode with galvanic probe (OAKTON DO 300 waterproof portable meter, Eutech Instruments, Singapore), respectively. Ammonium, nitrate, and nitrite in water were measured by an automated Skalar segmented-flow analyzer. The method for the determination of NH_4-N is based on the modified Berthelot reaction (Searle, 1984), and for NO_x-N on the hydrazinium reduction reaction (Kempers and Luft, 1988). Orthophosphate in water was determined by colourimetry (HITACHI U-1100 spectrophotometer) using either ascorbic acid or vanado-molybdate method (Clesceri et al., 1998).

2.5.2 Plant and sand samples

At the harvest, shoots were cut at the sand surface and their base washed to remove any adhering sediments. Each microcosm was then excavated and hand sorted into above-ground plant parts (stems, leaves, inflorescences and flowers), rhizomes (including stem base) and roots. The plants grown in mixed culture were separated by species. Samples of sand were taken from each microcosm for nutrient analysis. The rhizomes and roots were separated from the sand by washing away the sand using tap water and collecting roots onto a mesh. All plant samples were dried to constant weight at 70 ºC for 5 days, weighed and ground to pass a 0.75-mm mesh. All sand samples were dried and ground to pass a 0.15-mm mesh.

Total nitrogen and carbon in plant and soil samples were determined by the Dumas combustion method using an automated CN analyser (LECO CHN-1000, LECO Company, St Joseph, Michigan, USA). Total phosphorus in the plant material was determined by colourimetry (HITACHI U-1100 spectrophotometer) using the vanado-molybdate method after digesting material in mixture of concentrated nitric and perchloric acids (Bassett et al., 1978). Total phosphorus in sand was determined by colourimetry (HITACHI U-1100 spectrophotometer) using the ascorbic acid-molybdate method (Murphy and Riley, 1962) after digesting the material in concentrated perchloric acid (Jackson, 1958).

2.6 Calculations

Dry biomass production was estimated from the total biomass of each microcosm divided by the area of the microcosm. Plant dry weights were used for the calculation of relative growth rate [RGR = (ln final weight – ln initial weight)/days] (Coombs et al., 1985). The biomass allocation was characterized using the ratio of root-supported tissue (above-ground and rhizomes) to root biomass (S/R) and the ratio of above-ground to below-ground biomass (A/B) (Lorenzen et al., 2001). Resource allocation ratio was defined as the ratio of a certain tissue biomass or N or P content to total plant biomass or total plant N or P content. The allocation into above-ground parts, rhizomes and roots was calculated as the ratio between the biomass or nutrient content of the relevant parts and the total biomass or nutrient content of the plants. Nutrient use efficiency was calculated as the total dry biomass divided by total N or P content. The following biometric characteristics were estimated: relative shoot growth rate = (ln final shoot length – ln initial shoot length)/days, and relative increase in shoot number = (ln final shoot number – ln initial shoot number)/days (Tylova-Munzarova et al., 2005).

Nutrient removal efficiency was calculated using the equation (1) (Burgoon et al., 1991; Abe and Ozaki, 1998; Ge et al., 2000):

$$\text{Removal efficiency (\%)} = [1 - (Ce \times Ve)/ (Ci \times Vi)] \times 100 \qquad (1)$$

Where Ce = nutrient concentration in the effluent; Ve = water volume in the effluent; Ci = nutrient concentration in the influent; Vi = water volume in the influent.

The mass balance approach was used to acquire an integrated measure of partitioning (% of input) of the added N and P after the days of the nutrient or/and plant treatments. The factors estimated were total mass of N and P by considering (1) amounts added N and P to the microcosms, and amounts released N (eg. NH_4-N desorption) from substrate (the difference in amounts determined in substrate at the beginning and the end treatments when amounts of N storages in substrate decreased after the treatments); (2) amounts exported from the microcosms; (3) amounts accumulated by the macrophytes (the difference in amounts determined in plants at the beginning and the end treatments); (4) amounts stored N and P (eg. adsorbed, precipitated etc) in the substrate (the difference in amounts determined in substrate at the beginning and the end treatments when amounts of N or P storages in substrate increased after the treatments); and (5) unaccounted for (eg. losses due to denitrification and periphyton growth) (Breen, 1990; Newman and Pietro, 2001; Pant et al., 2001; Braskerud, 2002; Chung et al., 2008).

2.7 Statistical analyses

All statistical tests were performed using SPSS for windows. Procedures employed in the statistical analysis of data are described separately in each chapter.

2.8 Descriptions for *Canna indica and Schoenoplectus validus*

2.8.1 *C. indica*

Canna indica L. is an upright perennial rhizomatous herb, belonging to the family *Cannaceae* (see plate 2.1). It is not usually over 1.5 m high, leaves elliptic, rather fleshy, with thin margins, usually not more than 30 cm long and half as broad, lanceolate to sub-orbicular, veins arching-parallel, flowers either single or in pairs, perianth tube c. 10 cm long, lobes 3-4 cm long, unequal, margins in curved, staminodes 4; outer staminodes 3 to 5 cm long and 1.5 cm wide, often unequal, red to yellow or variegated, showy; inner staminode to 4-5 cm long and 0.8 cm wide, recurved at apex; single petaloid filament 3-4 mm wide; anther about 10 mm long. It is a native plant of tropical America, and very popular ornamental plant throughout tropical and subtropical regions around the world. It grows in thickets, crowding out other plants and is difficult to remove due to its spread by rhizomes (Bourne et al., 1988; National Herbarium of New South Wales, 2007).

2.8.2 *S. validus*

Schoenoplectus validus (Vahl) A. Löve & D. Löve is tall, perennial, herbaceous sedge, belonging to the family *Cyperaceae* (see plate 2.2). It grows up to 3 m tall. The underground parts of a hard rhizome with loose papery scales are approximately 2 cm long. The stems are erect and rush-like (0.3-1.5 cm in diameter near the base). The stem is rounded, light blue-green colour; soft and easily crushed. The leaves are reduced to sheaths at the base of the stems, and are up to 30 cm long and sometimes with a short blade. The flowers are borne in an open inflorescence of many stalked, budlike spikelets (5-12 mm long) covered by reddish brown scales below the top of the stem. The spikelets are egg-shaped. Flowering is from spring to summer. The fruit is a brownish gray achene. The seed are between 2-2.5 cm long, egg-shaped and end in a short sharp point. The seed body is smooth, shiny and grey-black in colour. It occurs in deep or shallow water, or in muddy or marshy ground around lakes, ponds, streams and wooded wetlands. This species usually occurs in poorly drained soil and tolerates a wide range of salinity in all of the Australia States and New Zealand (Chambers et al., 1995; Tasmanian Public Land Use Commission, 1996; National Herbarium of New South Wales, 2007).

Plate 2.1 ***Canna indica***

Plate 2.2 ***Schoenoplectus validus***

2.8.3 Cross section and root porosity for *C. indica and S. validus*

The cross sections of *C. indica* and *S. validus* roots made by hand were examined under a compound microscope. In addition, root porosity was measured using the method described by Raskin (1983) with equations as modified by Thomson et al. (1990). Roots were cut into 50-mm segments and a sub-sample of 1-2 g fresh weight was used for measurements.

The root cross sections of *C. indica* and *S. validus* showed the different root structure of the species (Figure 2.2). Both species have morphological adaptations to waterlogging with extensive aerenchyma present. The root porosities [% gas volume (root volume)$^{-1}$] of *C. indica* and *S. validus* were 6.28 and 6.33 %, respectively. No significant difference in root porosity was detected between the two species.

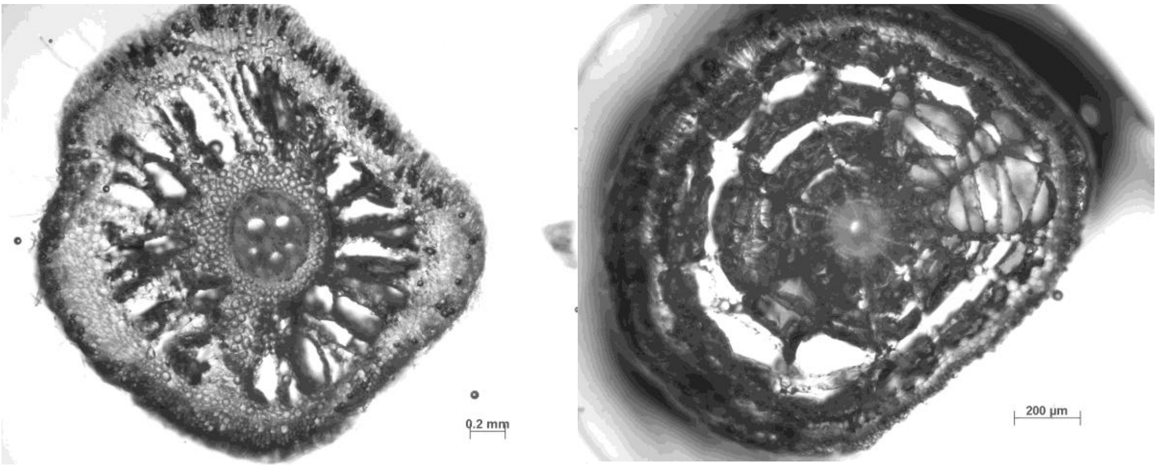

Figure 2.2 The root cross sections of *C. indica* (left) and *S. validus* (right) showing structural difference between species.

Acknowledgement

The data of water quality for the wastewater at Subiaco Wastewater Treatment Plant in Shenton Park, Perth, Western Australia were provided by Ms. Roni Oma (Water Corporation, Western Australia).

CHAPTER 3

REMOVAL OF NUTRIENTS FROM SIMULATED WASTEWATER IN WETLAND MICROCOSMS USING ORNAMENTAL PLANT SPECIES

Abstract

Different wetland species may have a differential capacity to take up nutrients from wastewater in the process of tertiary water purification. Ornamental hydrophytes may function as an important part of a wastewater treatment system and also provide economic benefits to the community. Ten emergent plant species, comprising six ornamental species: *Alocasia macrorrhiza*, *Canna indica*, *Iris louisiana*, *Lythrum sp.*, *Zantedeschia aethiopica*, *Zantedeschia sp.*, and four sedge species: *Baumea articulate*, *Baumea juncea*, *Carex tereticaulis* and *Schoenoplectus validus*, were planted in the wetland microcosms and fed a simulated wastewater solution containing 17.5 mg N L^{-1} in the 1:1 proportion of NH_4-N and NO_3-N, and 10 mg P L^{-1} in the concentrations similar to the secondary-treated municipal wastewater. Different growth rates of ornamental species were observed, with *C. indica* showing the most vigorous and healthy growth in the microcosms. Significant differences in both above-ground and below-ground biomass were found among plant species. Significant differences in the removal efficiencies of NH_4-N, NO_x-N and PO_4-P were detected among different species, with *C. indica* achieving the relatively high nutrient removal efficiency. Although biomass of *C. indica* was not the highest among the ornamental species, it has shown vigorous and healthy growth, and a relatively high potential of rooting-zone aeration and nutrient removal efficiency in the wetland microcosms. The sedge species such as *S. validus* showed relatively high nutrient removal efficiency. It might be better to grow in mixed culture of *C. indica* and *S. validus* in constructed wetlands for enhanced efficiency of nutrient removal from the secondary-treated municipal wastewater.

Key words: constructed wetland; nitrogen; ornamental species; phosphorus; wastewater

3.1 Introduction

Nutrients that are most limiting in pristine water systems are phosphorus (P) and nitrogen (N). Increased loading of these two nutrients causes eutrophication of surface waters, which is a serious environmental problem around the world. Generally, N and P are present in the secondary-treated wastewater in average concentrations of 40 mg N L^{-1} and 12 mg P L^{-1} (Water Corporation of Western Australia, 2000). It is important to note that N and P concentrations even lower than those above can result in eutrophication of the receiving water bodies (Kadlec and Tilton, 1979). In fact, discharges to coastal rivers and estuaries have been contributing to significant eutrophication problems in Australia for many years (Davis and Koop, 2006).

The use of constructed wetlands for tertiary water purification of municipal wastewater has received an increasing attention recently. Constructed wetlands offer effective, reliable treatment of wastewater in a simple and inexpensive manner (Sundaravadivel and Vigneswaran, 2001). Wetland plants play an important role in removing nutrients in constructed wetlands (Mitsch and Cronk, 1992; Brix, 1997; Stottmeister et al., 2003; Gottschall et al., 2007). Plants not only take up nutrients such as N and P from the wastewater, but also transport oxygen to the root zone to enable aerobic microbes to decompose the pollutants and aid in the settling of suspended materials by reducing the rate of wastewater flow (Brix, 1997). Essential characteristics of plant species suited for use in constructed wetlands are that they grow vigorously in the wetland, with high evapotranspiration, and most importantly, improve the water quality (Neralla et al., 1999). The selection of plant species should be based on aesthetics, impacts on wetland operation, and long-term plant health and viability in a given area (EPA, 2000).

At present, the most common aquatic plants used in constructed wetlands are bulrush (*Scirpus* spp.), cattail (*Typha* spp.) and reeds (*Phragmites* spp.). However, there is potential to use other types of waterlogging- and inundation-tolerant plants in constructed wetlands (Belmont et al., 2004).

Ornamental hydrophytes may function as an important part of a wastewater treatment system. Wolverton (1989) reported that *Zantedeschia aethiopica*, *Canna flaccida*, and three other ornamental plant species planted in a rock filter used to treat septic tank effluents added oxygen and increased biological activity in the septic bed. Neralla et al. (1999) concluded from greenhouse experiments that ornamental plants, including *Z. aethiopica*, were as effective as *Typha angustifolia* in improving the quality of effluents from septic tanks. High removal efficiency of N was observed in the laboratory-scale subsurface-flow wetlands using *Z. aethiopica* (Belmont and Metcalfe, 2003). Belmont et al. (2004) reported that ornamental flowers (*C. flaccida* and *Z. aethiopica*) with high economic value planted in the laboratory- and field-constructed wetlands performed as well as *T. angustifolia*. Zurita et al. (2006) studied five ornamental species (*Anthurium andreanum, Canna hybrids, Hemerocallis dumortieri, Strelitzia reginae* and *Z. aethiopica*) in laboratory-scale subsurface-flow constructed wetlands for domestic wastewater treatment and observed good quality of the effluent as well as good development of the plants. The effect of *C. indica* on domestic sewage was better than *Phragmites communis* and the root system biomass of *C. indica* was the best among the five tested species (Zhao et al., 2003).

Ornamental hydrophytes may provide economic benefits to the communities upon harvesting in addition to the efficiency of the wastewater treatment. They may have more economical value than sedge plants if harvested and utilized as cut flowers after harvesting. However, there still have been few reports using ornamental species in wastewater treatment despite the fact that these plants can be harvested and sold for economic benefits (Zhang et al., 2007).

It is important to develop a low-cost and energy-saving technique for nutrient removal from wastewater in the process of tertiary water purification. The mixed culture of different species such as ornamental and sedge species may enhance the nutrient treatment and provide economic return. However, there is little information on the efficiency of different ornamental species in removing nutrients from secondary-treated municipal wastewater in constructed wetlands in Australia. The objective of this study was to test the feasibility of using ornamental species in wetland microcosms to remove N and P from simulated secondary-treated municipal wastewater in order to select the suitable candidate species to be tested in mono- or mixed culture for efficient removal of N and P.

3.2 Materials and Methods

3.2.1 Wetland microcosm

The design of the wetland microcosm was described in Chapter 2. Thirty microcosms were used as experimental units in this study.

3.2.2 Experimental setup

The experiment was conducted in a phytotron chamber with controlled day/night temperatures of 20/15 ºC from the late December, 2004 to the late March, 2005.

A completely randomized design with three replicates was used. Ten emergent species, including six ornamental: *Alocasia macrorrhiza*, *Canna indica*, *Iris louisiana*, *Lythrum sp.*, *Zantedeschia aethiopica*, *Zantedeschia sp.*, and four sedge species: *Baumea articulate*, *Baumea juncea*, *Carex tereticaulis* and *Schoenoplectus validus*, were selected initially for investigation. The seedlings of each species from the local nursery were selected for uniformity of size and condition, and planted in each microcosm. Each microcosm was planted with 6 seedlings of ornamental species or 6 clumps of sedge species that contained three or four shoots for sedge species depending on growth characteristics of different species. The initial biomass of similar seedlings was recorded for each species. A simulated nutrient solution was used in the microcosms at the average concentrations of total N and P in the secondary-treated wastewater at Subiaco Wastewater Treatment Plant. The solution contained 17.5 mg N L^{-1} (1:1 NH_4-N and NO_3-N), 10 mg P L^{-1} and 13 mg K L^{-1}. Other nutrients were added as described in Chapter 2. Microcosms were filled with the nutrient solution to achieve the water depth of about

0.02 m above the sand surface and refilled fortnightly. After 10 weeks of plant growth, microcosms were filled with 8 L simulated nutrient solution to achieve the water depth of about 0.05 m above the sand surface. Each microcosm was drained and refilled weekly. The hydraulic loading rate for designed treatment of each microcom was 73 L m^{-2} from week 11 to week 13 of the plant growth. The vitality (weak, medium or vigorous), maturity (early or late) and mortality (low or high) of the plant growth were observed and recorded during the experimental period.

3.2.3 Sampling and analytical methods

After 10 weeks of plant growth, volumes of influent and effluent solution were recorded, and samples collected once per week over 3 weeks. The plants were harvested at week 14.

The methods for harvesting, sampling, measuring and calculations of water, plant and soil samples were described in Chapter 2.

3.2.4 Statistical analyses

A one-way ANOVA was used to determine significance of the plant species effect on relative growth rate, biomass, nutrient concentration and accumulation in plants, together with pH, dissolved oxygen (DO) in the effluents and nutrient removal. Least significant difference (LSD) was applied to test for significance between treatment means. Linear regressions were performed for each plant species to determine the possible effect of biomass on N and P removal, and the relationships between nutrient accumulation or biomass or concentrations in plant tissues, and pH or DO in the effluents.

3.3 Results

3.3.1 Plant growth

All test species showed positive growth during the recovery and early growing stages in the microcosms, but the different growth vitalities and relative growth rate of species were observed (Table 3.1). The relative whole plant growth rate (RGR) extended from 0.016 to 0.022 d^{-1} among ornamental species and 0.019 to 0.030 d^{-1} among sedge species. *L. sp., A. macrorrhiza* and *C. indica* were more vigorous than other ornamental species. *A. macrorrhiza* and *L. sp.* had early maturity. The high mortality was only observed for *L. sp.* among the tested species, whereas *C. indica* showed vigorous and health growth in the microcosms. *B. articulata* and *S. validus* were more vigorous than other sedge species.

Table 3.1 Growth vitality and whole plant relative growth rate (RGR) of tested species grown in the microcosms

Species	Common name	RGR (d^{-1})	Growth vitality
Ornamental			
Alocasia macrorrhiza	Elephant ears	0.021abc	Vigorous
Canna indica	Queensland arrowroot	0.019abc	Vigorous
Iris louisiana	Iris	0.018bc	Medium
Lythrum sp.		0.022abc	Vigorous
Zantedeschia aethiopica	Green goddess	0.016c	Weak
Zantedeschia sp.		0.016c	Weak
Sedge			
Baumea articulata	Jointed twig rush	0.030a	Vigorous
Baumea juncea	Bare twig rush	0.019abc	Vigorous
Carex tereticaulis	Tube Sedge	0.024abc	Vigorous
Schoenoplectus validus	Lake club rush	0.029ab	Vigorous

Means with different letters within column is significantly different based on LSD ($P \leq 0.05$).

3.3.2 Biomass

After 14 weeks of plant growth, significant difference in both above-ground and below-ground biomass was observed, with mean total biomass ranging between 208 and 806 g m^{-2} and mean biomass ratios of above-ground (AG)/ below-ground (BG) between 0.10 and 3.08 (Table 3.2). The highest total biomass was in *L. sp.*, due to its relatively early maturity, whereas the smallest total biomass was that of *B. juncea.*

Above-ground biomass ranged from 34 to 257 g m^{-2}. The highest above-ground biomass was reached by *A. macrorrhiza,* whereas *Z. aethiopica* and *Z. sp.* accumulated a relatively small amount of above-ground biomass.

Below-ground biomass ranged from 51 to 626 g m^{-2}. Maximum below-ground biomass was recorded for *L. sp.* (3.5 times greater than the above-ground biomass), with only 16% of below-ground biomass being roots. Rhizomes (including stem bases) accounted for 27-76% of the below-ground biomass of the other species. The ornamental species showed a relatively high rhizome biomass compared with the sedge species. The rhizome biomass of the ornamental species was more than 50% of the below-ground biomass. The below-ground biomass was usually higher in the ornamental than sedge species.

Table 3.2 Biomass, percentage of below-ground biomass comprising root biomass and ratio of above-ground (AG)/below-ground (BG) of each species grown in the microcosms

Species	Biomass (g m^{-2})			Proportion of root in BG (%)	AG:BG ratio
	Total	AG	BG		
Ornamental					
Alocasia macrorrhiza	633ab	257a	376bc	43	0.68
Canna indica	548bc	118bc	430b	35	0.27
Iris louisiana	445bc	100bc	345bc	24	0.29
Lythrum sp.	806a	180ab	626a	16	0.29
Zantedeschia aethiopica	385cd	34c	351bc	33	0.10
Zantedeschia sp.	298d	52c	246cd	47	0.21
Sedge					
Baumea articulata	293d	159b	134de	73	1.19
Baumea juncea	208d	157b	51e	54	3.08
Carex tereticaulis	392cd	155b	236cd	52	0.66
Schoenoplectus validus	248d	100bc	148de	53	0.68

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

3.3.3 Concentrations of nutrients in plant tissues

The concentrations of N and P in above-ground plant tissues ranged from 5.0 to 18.4 g N kg^{-1} and 0.66 to 2.9 g P kg^{-1}. The levels of N and of P ranged from 2.9 to 5.3 g N kg^{-1} and 0.74 to 4.2 g P kg^{-1} in rhizomes, and from 4.5 to 11.3 g N kg^{-1} and 0.87 to 3.0 g P kg^{-1} in roots (Table 3.3). The concentrations of N (except N in rhizomes) and P in plant tissues differed significantly among the tested species. The concentrations of N were significantly higher in above-ground than below-ground tissues, but no significant difference was observed in the concentrations of P. The highest concentrations of N and P were in the tissues of *Z. aethiopica* and the lowest were in the tissues of *L. sp.*

Table 3.3 Concentrations of N and P in above-ground and below-ground tissues for the ten test species grown in the microcosms

Species	N (g kg^{-1})			P (g kg^{-1})		
	Above-ground	Rhizome	Root	Above-ground	Rhizome	Root
Ornamental						
Alocasia macrorrhiza	9.4bcd	3.5a	8.4abc	2.3abc	1.2cd	1.7ab
Canna indica	10.4bc	2.9a	5.2c	1.8abcd	1.2cd	1.3bc
Iris louisiana	7.7cd	2.9a	6.3bc	1.1cd	0.74d	1.3bc
Lythrum sp.	6.5d	5.3a	5.6c	0.66d	2.2bc	0.87c
Zantedeschia ethiopicia	18.4a	4.1a	10.6ab	2.9a	1.1cd	2.3ab
Zantedeschia sp.	13b	3.1a	11.3a	2.6ab	1.7cd	2.9a
Sedge						
Baumea articulata	5.6d	4.9a	4.5c	0.89d	3.5ab	3.0a
Baumea juncea	5.0d	5.2a	6.6bc	1.5bcd	4.2a	1.6ab
Carex tereticaulis	7.2cd	3.6a	4.6c	1.6bcd	1.6cd	1.1bc
Schoenoplectus validus	8.5cd	4.0a	5.3c	2.5ab	1.9c	1.2bc

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

3.3.4 Accumulations of N and P by plants

Accumulation of N and P in above-ground and below-ground tissues largely reflected patterns of biomass allocation, with total accumulation of up to 4.8 g N m^{-2} and 1.1 g P

m^{-2}. Significant differences in accumulation of N and P were detected among species in both above-ground and below-ground tissues. Due to its relative large biomass, the highest uptake rate of N and P in above-ground tissues occurred in *A. macrorrhiza*, with 24.5 mg N m^{-2} d^{-1} and 5.9 mg P m^{-2} d^{-1}, but no significant difference was observed among other plant species. The highest uptake rate of N and P in below-ground tissues was recorded for *L. sp.*, with 35.7 mg N m^{-2} d^{-1} and 6.9 mg P m^{-2} d^{-1} (Table 3.4).

Table 3.4 Uptake of N and P in above-ground and below-ground tissues for the ten test species grown in the microcosms

Species	N uptake (mg m^{-2} d^{-1})			P uptake (mg m^{-2} d^{-1})		
	Above-ground	Rhizome	Root	Above-ground	Rhizome	Root
Ornamental						
Alocasia macrorrhiza	24.5a	5.9a	18.4bc	5.9a	2.0b	3.7b
Canna indica	12.5b	4.5abc	14.8c	2.2b	1.9b	3.8b
Iris louisiana	7.8b	2.4cd	16.7c	1.1b	0.6c	3.3b
Lythrum sp.	11.9b	5.3ab	30.4c	1.2b	2.2b	4.7ab
Zantedeschia aethiopica	6.4b	4.8ab	25.4ab	1.0b	1.3bc	5.4a
Zantedeschia sp.	6.9b	3.6abcd	15.1c	1.4b	2.0b	3.8b
Sedge						
Baumea articulata	9.1b	4.9ab	1.7d	1.4b	3.5a	1.1c
Baumea juncea	8.1b	1.4d	1.5d	2.4b	1.2bc	0.4c
Carex tereticaulis	11.4b	4.6abc	5.3d	2.6b	2.0b	1.3c
Schoenoplectus validus	8.7b	3.1bcd	3.8d	2.6b	1.5bc	0.8c

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

3.3.5 Nutrient removal efficiency

Average removal efficiencies by the microcosms were 82-93% for NH_4-N, 62-87% for NO_x-N and 71-85% for PO_4-P from week 11 to 13 (Table 3.5). Significant differences in the removal efficiencies of NH_4-N, NO_x-N and PO_4-P were detected among different species. The relatively high removal efficiencies of nutrients were observed in the microcosms with *A. macrorrhiza* and *C. indica*. The removal efficiencies of both NO_x-N and PO_4-P showed a significant ($P < 0.01$) positive linear correlation with plant total biomass.

Table 3.5 Average nutrient removal efficiency by the microcosms from week 11 to week 13 experimental periods

Species	Removal efficiency (%)		
	NH_4-N	NO_x-N	PO_4-P
Ornamental			
Alocasia macrorrhiza	90a	87a	83a
Canna indica	93a	86ab	85a
Iris louisiana	86b	82b	81ab
Lythrum sp.	83bc	87a	81ab
Zantedeschia aethiopica	82c	74c	75c
Zantedeschia sp.	83bc	75c	73c
Sedge			
Baumea articulata	86b	75c	75c
Baumea juncea	82c	62d	71c
Carex tereticaulis	86b	76c	78bc
Schoenoplectus validus	84bc	74c	75c

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

3.3.6 pH and Dissolved oxygen (DO) in the effluents

The significant differences of pH and dissolved oxygen (DO) were found in the effluents (Figure 3.1). The average values of pH in drained effluent at the end of experiment ranged from 6.07 to 6.79, and the concentrations of the dissolved oxygen were between 1.46 and 4.15 mg L^{-1}. The highest pH was recorded in the effluent from microcosms containing *S. validus*, and the lowest in the effluent from microcosms with *C. indica*. The highest DO was also found in the effluent from microcosms containing *C. indica*. *C. indica* effluent always showed relatively high DO and low pH compared with the other effluents during the experimental period. No significant relationship was detected between pH and DO in the effluents.

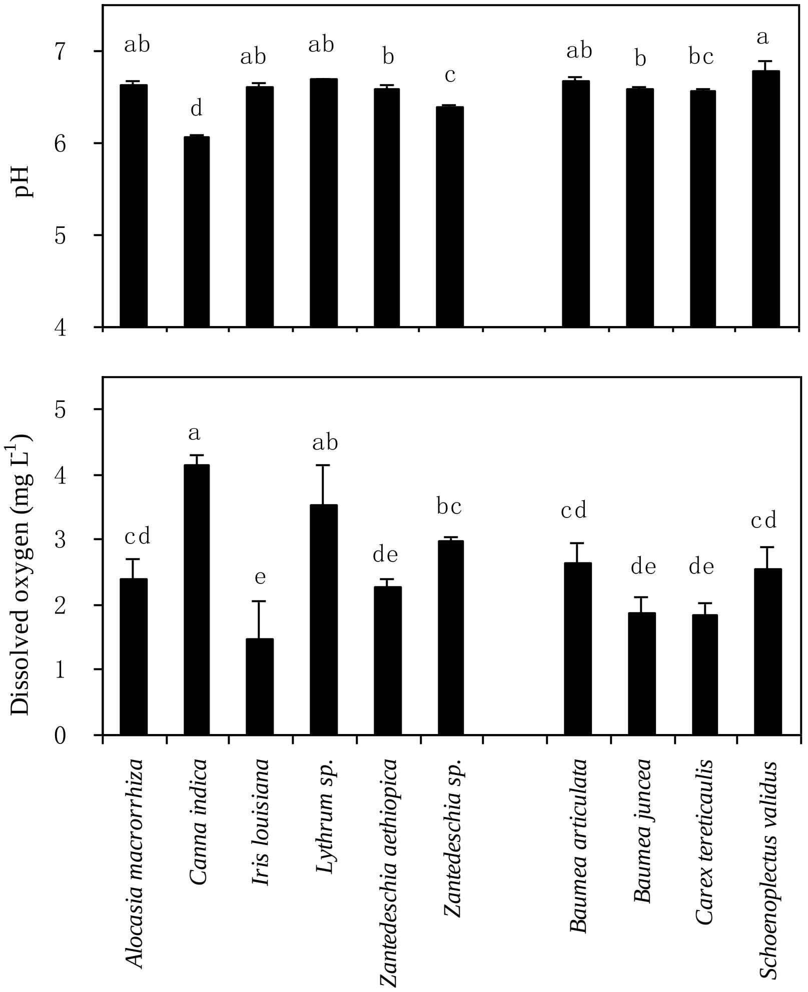

Figure 3.1 The pH and concentrations of dissolved oxygen in the effluents. Bars (means + SE, n = 9) with different letters are significantly different based on LSD (*P* ≤ 0.05).

3.4 Discussion

3.4.1 Plant growth

Wetland plant species differ substantially in their growth rate, morphology, physiology and size. Therefore, apart from general appearance, relative growth rate (RGR) was used to confirm the growth vitality of plants (Table 3.1). These RGR values were found to be similar to those found in the literature. For example, the values of RGR for *Phragmites mauritianus* were 0.02-0.03 d^{-1} in the mesocosm (Sekiranda and Kiwanuka, 1998). The values of RGR for eight emergent plants were 0.002-0.028 d^{-1} in constructed wetlands under saline conditions (Klomjek and Nitisoravut, 2005).

3.4.2 Nutrient accumulation by plants

Differences in species N and P accumulation are likely to reflect species and developmental stage differences in efficiency of nutrient uptake and use. In the present study, the concentrations of N and P in the plant tissues (Table 3.3) were in the ranges of the other reported studies. For example, McJannet et al. (1995) measured concentrations of N and P in above-ground biomass for 41 wetland plant species after one season of growth with excess nutrition. Tissue nutrient concentrations ranged from 2.5 to 21.4 g kg^{-1} for N and from 1.3 to 10.7 g kg^{-1} for P.

Differences in nutrient uptake among species and allocation of elements among plant tissues depend partly on the plant life form, absorption site and physiological function of the element in the plant tissues (Sculthorpe, 1967; Bosserman, 1981). In the present study (Table 3.4), the uptake of N and P by plants was relatively low due to a low loading rate, but still comparable to the study reported by Klomjek and Nitisoravut (2005). They reported that the nutrient uptake rates of eight plant species were in the range of 6-61 mg N m^{-2} d^{-1} and 0.2-2.4 mg P m^{-2} d^{-1}.

3.4.3 Nutrient removal efficiency

The high N and P removal efficiencies of small scale batch-fed wetlands during their active plant grown phase have been investigated by several researchers (Breen, 1990; Huett et al., 2005; Zurita et al., 2006; Iamchaturapatr et al., 2007). In the present study (Table 3.5), the removal efficiencies of N and P were comparable to the reported study in the literature. For example, about 80-99% of N and P could be removed by 21 aquatic plants in planted vertical free surface-flow microcosms after a 14-day retention time (Iamchaturapatr et al., 2007). The use of five ornamental species with commercial interest (*Anthurium andreanum, Canna hybrids, Hemerocallis dumortieri, Strelitzia reginae* and *Z. aethiopica*) in laboratory-scale subsurface-flow constructed wetlands for domestic wastewater treatment was investigated and observed that total N removal efficiencies were above 70% in each planted cell, except for the cell planted with *A. andreanum.* The P removal efficiency was above 66% in all plant cells. Even though no significant difference between the performances of plants was observed, *C. hybrids* and *Z. aethiopica* adapted better to the subsurface-flow constructed wetlands and maximum performance of the treatment was reached faster (Zurita et al., 2006). In the microcosm, the greater ratio of plant biomass to wetland volume is likely to have enhanced contact between plant roots and wastewaters, and provided a greater relative plant growth sink for nutrients. However, the high N and P removal efficiencies recorded by Headley et al. (2001) and Headley (2004) in much larger scale wetlands during the establishment phase indicated that scale was not the sole factor.

Nutrient removal efficiency of a system containing plants will depend on the type of aquatic plant, growth rates of plants, nutrient composition of the water, and physico-chemical environment in the water (Reddy, 1983b). Many researchers have indicated that the efficiency of nutrient removal with different plant species was significantly different in the microcosms and mesocosms (Gersberg et al., 1986; Burgoon et al., 1991; Coleman et al., 2001; Fraser et al., 2004). Fraser et al. (2004) tested four wetland species (*Scirpus validus, Carex lacustris, Phalaris arundinacea* and *Typha latifolia*) and found that *S. validus* was the most effective and *T. latifolia* the least effective at reducing N and P in subsurface wetland microcosms. Contrary to the above findings, Coleman et al. (2001) found that *T. latifolia* was very efficient at removal of nutrients. In the present study (Table 3.5), significant differences in the removal efficiencies of nutrients were observed among the tested species, and removal efficiency was positively correlated with plant biomass. Such findings further supported a suggestion that plant uptake had a great influence on nutrient removal at low loading rates or during initial establishment in the field (Tanner, 1996). However, because of the relatively short duration of the growth trials in relation to the life cycles of these clonal species, care must be exercised in attempting to generalize the results from the microcosm and mesocosm experiments to field-scale wetlands, especially with regard to the quantitative role of plant uptake as a nutrient removal mechanism (Busnardo et al., 1992).

3.5 Conclusion

Among the tested species, some ornamental species had the similar capability of growth and efficiency of nutrient removal as sedge species in the microcosms. Therefore, ornamental species could probably be used together with other species in the wetland to removal nutrients from wastewater. The biomass of *C. indica* was not the highest among the ornamental species at the present study, but it has showed vigorous and healthy growth, and relatively high potential of rooting-zone aeration (relatively higher DO) and nutrient removal efficiency in microcosms. In addition, the aesthetic value of *C. indica* is unique in the constructed wetlands. Overall, the preliminary results suggested that *C. indica* together with *B. articulata* and/or *S. validus* might be a suitable candidate species to test in mixed culture in the constructed wetlands for more efficient removal of nutrients from the secondary-treated wastewater.

CHAPTER 4

EFFECTS OF NUTRIENT AVAILABILITY AND MIXED CULTURE ON GROWTH AND NUTRIENT REMOVAL EFFICIENCY USING *CANNA INDICA* AND *SCHOENOPLECTUS VALIDUS* IN WETLAND MICROCOSMS

Abstract

Nutrient availability and species competition may affect emergent wetland plant growth and resource allocation, resulting in different nutrient removal efficiencies in monoculture and mixed stands in constructed wetlands for tertiary purification of wastewater. A glasshouse study was conducted to investigate the influence of mono- and mixed culture of *Canna indica* and *Schoenoplectus validus* on their growth in, and nutrient removal from, simulated wastewater in the wetland microcosms. Plants were grown for 50 days before imposing nutrient treatments that simulated secondary-treated municipal wastewater effluent with either low (17.5 mg N and 10 mg P per litre) or high (35 mg N and 20 mg P per litre) nutrient concentrations. Treatment solutions were renewed in weekly intervals. After 65 days, the high nutrient treatment stimulated plant growth and resulted in allocation of more resources to the above-ground tissues compared to below-ground ones. The concentrations of N and P in the plant tissues (except P in above-ground tissues) were significantly higher, whereas N and P use efficiencies were significantly lower in the high than the low nutrient treatment. The total biomass for *C. indica* in the mixture increased significantly in the high nutrient treatment, but that for *S. validus* was significantly lower in the mixture than in the monoculture. Relative yield (RY) indicated that there was significant species competition between *S. validus* and *C. indica* in mixtures, with *C. indica* being the superior competitor. The growth of *S. validus* was significantly inhibited by the presence of *C. indica*. Compared with monoculture, *S. validus* in the mixture had significantly higher percentages of root biomass and allocations of N and P to roots, whereas *C. indica* was not significantly affected by the mixed culture. The accumulation of N and P in above- and below-ground tissues largely reflected patterns of biomass allocation. Significant differences in nutrient removal efficiencies were observed between the planted and non-plant treatments and among the retention times, but no significant difference was detected between the nutrient treatments. Plant uptake was the major nutrient removal pathway in the wetland microcosms. Nutrient removal from simulated wastewater in mixed culture was not greater than in monocultures, due to species competition. The results suggested that the growth and resource allocation of *C. indica* and *S. validus* could be altered by differential nutrient availability and species competition in constructed wetlands.

Key words: *Canna indica*; constructed wetland; interspecific competition; nutrient removal; *Schoenoplectus validus*; wastewater

4.1 Introduction

The use of constructed wetlands for municipal wastewater treatment has undergone dramatic development since the 1990s (Kadlec and Knight, 1996). Constructed wetlands are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils and their associated microbial assemblages to assist in treating wastewater. The operational and maintenance costs may be affordable, even in developing countries (Nyakang'O and van Bruggen, 1999; Kurniadie and Kunze, 2000). Research has shown that constructed wetlands can significantly decrease nutrient concentrations in secondary-treated municipal wastewater. In published studies with constructed wetlands, however, a wide range of nutrient removal efficiencies have been reported, with many wetlands failing to meet relevant government standards, especially for total N and P discharge concentrations (Fraser et al., 2004; Greenway, 2005).

Improvements in plant selection and cultivation might make the constructed wetlands more efficient for nutrient removal from the wastewater. It is not clear if it is desirable to maintain a single plant species, or a mix of plant species, in constructed wetland. However, single plant (monoculture) systems are more susceptible to plant death due to predation or disease. Therefore, it is generally assumed that multiple plant (mixture) and native plant systems are more resilient than monocultures (EPA, 2000). For organic carbon–limited free-surface wetlands, Bachand & Horne (2000) recommended a mixture of labile (submergent, floating) and more recalcitrant (emergent, grasses) plants as a reasonable approach to improving denitrification rates.

Overall, the polyculture systems seemed to provide the best and most consistent treatment for fecal bacteria, biological oxygen demand (BOD) and total suspended solids (TSS), while being least susceptible to seasonal variation (Karathanasis et al., 2003). The presence of diverse species may have provided a more effective distribution of the rooting biomass and a habitat for more diverse microbial populations than the monoculture systems.

However, few reports on the nutrient removal efficiency by various plant species in mixed culture in the constructed wetlands are inconsistent. Coleman et al. (2001) found that mixed culture of *Juncus effusus*, *Typha latifolia* and *Scirpus cyperinus* was effective at reducing nutrient level in small-scale constructed wetlands receiving primary-treated wastewater. However, mixed culture of four wetland plant species (*Scirpus validus, Carex lacustris, Phalaris arundinacea* and *Typha latifolia*) in subsurface wetland microcosms did not increase the potential for N and P removal from mimicked domestic effluent compared with monocultures (Fraser et al., 2004).

It is reported that positive, negative or indifferent interrelationship may occur between plants of different species. The results of such competition might cause the preferential establishment and growth of certain species, and/or the suppression and extinction of other species (Agami & Reddy, 1990). In recent years, several studies have been reported on the interspecies competition for emergent wetland species. For example, Wetzel and van der Valk (1998) found that *Phalaris arundinacea* is an inherently better competitor than *Carex stricta* or *Typha latifolia*. Coleman et al. (2001) observed that *Typha latifolia* was the superior competitor among a three-species (*Juncus effusus*, *Typha latifolia* and *Scirpus cyperinus*) mixture in small-scale constructed wetlands. In plant mixtures consisting of *Carex flava, Centaurea angustifolia, Lycopus europaeus* and *Selinum carcifolia* grown in sand culture with different N:P supply ratios and different total supplies of N and P, *Lycopus europaeus* performed best at low and intermediate N:P ratios, and *Carex flava* at high N:P ratio (Güsewell & Bollens, 2003). However, few studies have investigated the competitive impact between species with different growth forms or significantly different morphologies.

Schoenoplectus validus and closely related species *S. lacustris*, *S. acutus* and *S.*

californicus have been used widely in constructed wetlands around the world (Tanner, 2001). Similarly, ornamental species *Canna indica* has been used to treat (i) septic tank effluent in a simulated vertical-flow constructed wetlands (Zhu et al., 2004); (ii) domestic wastewater in a medium-scale vertical/reverse-vertical flow constructed wetland (Yue et al., 2004); and (iii) municipal wastewater in a full-scale subsurface-flow constructed wetland (Shi et al., 2004). Although *C. indica* and *S. validus* and their closely related species have been planted in the various constructed wetlands for the improvement of water quality and landscape restoration under mono- or mixed culture conditions due to their relatively high nutrient removal efficiency and aesthetic value (Grosse et al., 2001; Fu et al., 2006; Wu and Ding, 2006; Wu et al., 2006; Calheiros et al., 2007), there is no knowledge on the effects of mixed culture using these two species on plant growth and nutrient removal. The objective of this study was to investigate the effectiveness of *C. indica* and *S. validus* in mono- and mixed culture on their growth and resource allocation, and in removing N and P from simulated secondary-treated municipal wastewater in wetland microcosms.

4.2 Materials and methods

4.2.1 Wetland microcosm

The design of the wetland microcosm was described in Chapter 2. Thirty-six microcosms were used as experimental units in this study.

4.2.2 Experimental setup

The experiment was conducted in a phytotron chamber with controlled day/night temperatures of 25/20 ºC from the beginning of June to the late September in 2005.

At the establishment of plant treatment, the four plant culture treatments with nine replicates were: (A) control (with no plants added); (B) monoculture of *C. indica*; (C) monoculture of *S. validus*; and (D) mixture between *C. indica* and *S. validus*. Each microcosm contained six plants in two rows of three: six *C. indica*, six *S. validus* or three of each species (Figure 4.1). At the planting time, seedlings of *C. indica* were approximately 8 cm tall with 1-2 leaves, and *S. validus* were approximately 10 cm tall with 4-6 ramifications. Microcosms were filled with the nutrient solution to achieve the water depth of 0.02 m above the sand surface and renewed weekly. The solution contained 17.5 mg N L^{-1} (1:1 NH_4-N and NO_3-N), 10 mg P L^{-1} and 13 mg K L^{-1}. Other nutrients were added as described in Chapter 2.

After plants were grown in the microcosms for 50 days, three replicates of each plant culture treatment (including control) were harvested, and plant (shoot, rhizome and root) and substrate samples were taken. A complete randomized block factorial design (four plant culture treatments × two nutrient concentration treatments) with three replicates was employed. The remaining microcosms were initialized with two nutrient treatments each in three replicates: (1) low level containing nutrients as in the starting solution described above, and (2) high level with double concentrations of N and P. The concentrations of other nutrients were kept the same as in the starting solution described above, except that concentrations of K were doubled. Microcosms were filled with 10 L of either low or high nutrient solution to achieve the water depth of about 0.08 m above the sand surface. Each microcosm was drained and refilled every 7 days. The hydraulic loading rate for designed treatment was 91 L m^{-2}.

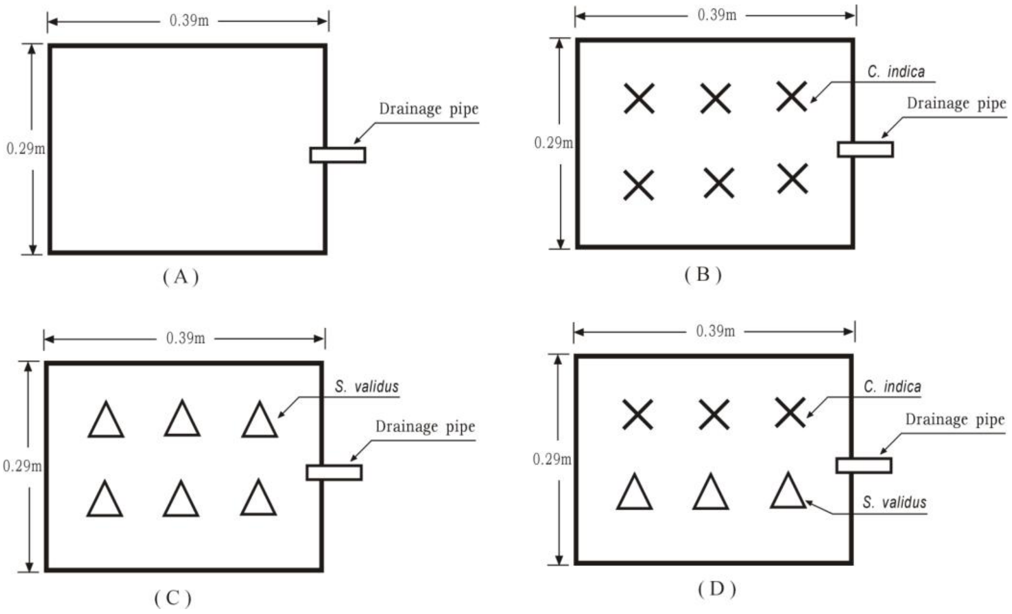

Figure 4.1 The vertical view of the design plant treatment in the microcosms. (A) control (with no plants added); (B) monoculture of *C. indica*; (C) monoculture of *S. validus*; and (D) mixture between *C. indica* and *S. validus*

4.2.3 Sampling and measurements

After nutrient treatments were imposed, volumes of influent and effluent solution were recorded, and samples collected once per week during 9 weeks. Additional solution samples were taken from the microcosms at 1.5- and 3.5-day retention times for the last three weeks.

Total shoot numbers and shoot heights in each microcosm were measured at approximately weekly intervals from the day after imposing nutrient treatments until the plants were harvested at the end of the experiment (65 days later). The shoot height was measured from the base of the plants to the top of the longest leaves for *C. indica* or the top of culms for *S. validus*. The plants were harvested 2 days after last solution renewal (65 days of nutrient treatment).

All data for calculation and analysis were obtained from the commencement of nutrient treatments to the end of the experiment (65 days of plant growth). The relevant methods for harvesting, sampling, measuring and calculation of pH, DO, nutrients and biomass in water, plant and soil samples were described in Chapter 2.

To estimate above-ground biomass of plants prior to the destructive sampling for *S. validus*, a regression relationship between total shoot length and dry above-ground biomass was developed at the end of the experiment. For *C. indica*, no significant regression between the shoot number and above-ground biomass was detected, so shoot number was not included in the regression. These equations were used to estimate above-ground biomass from weekly shoot height and number measurements. The regression equation for each species was:

S. validus

$$\ln \text{above-ground biomass (g)} = 1.421 \ln \text{length of total shoot (cm)} - 9.077, \quad (r^2 = 0.993, P < 0.001)$$

C. indica

$$\ln \text{above-ground biomass (g)} = 2.566 \ln \text{shoot height (cm)} - 7.135, \quad (r^2 = 0.882, P < 0.001)$$

The competitive effects of species were examined by calculating the relative yield of individuals of a species when grown with another species (interspecies competition) compared to the relative yield of the species grown alone (intraspecies competition) while maintaining the same overall density. Relative yield (RY) of above-ground biomass was calculated as:

RY of species A = (yield of species A in mixture)/ (yield of species A in monoculture)

A RY of 1 indicates that plants were the same size in mixed culture as in monoculture. RY < 1 indicates that plants were smaller in mixed culture than monoculture and RY >1 indicates that plants were larger in mixed culture than monoculture (Harper, 1977).

The approaches of nutrient removal efficiency and mass balance were described in Chapter 2.

4.2.4 Statistical analyses

Two-way ANOVA was used to determine significance of nutrient and plant treatment effects on plant biomass and characteristics, plant concentrations of N and P, resource allocation (the percentage after log transformation), accumulation of nutrients in the plant tissues, and contents of total N and P in various components of the microcosms. Percentage nutrient removal values were normalized using an arcsine transformation. Measurements of each parameter in water samples at different sampling date were not independent; therefore, difference in pH, concentration of dissolved oxygen, and nutrient removal efficiency (the percentage after arcsine transformation) were tested using a repeated measures two-way ANOVA. The sampling date was set as the repeated measure. Three-way ANOVA was used to determine significance of retention time, and nutrient and plant treatments on nutrient removal efficiency (the percentage after arcsine transformation). Least significant difference (LSD) was applied to test for significance between treatment means.

4.3 Results

4.3.1 Plant biomass

Significant differences in the above-ground and total biomass were observed, but not in the below-ground, rhizome and root biomass between the nutrient treatments. Significant differences in the biomass of various plant parts were observed among the plant treatments. The significant interactions between nutrient and plant treatments were observed in the above-ground and total biomass (Table 4.1). After 65 days of nutrient and plant treatments, the total biomass for *C. indica* in the mixture was significantly higher in the high than in the low nutrient treatment, but that for *S. validus* was not significantly affected by the nutrient treatments. The above-ground biomass for *C. indica* in both monoculture and mixture, and that for *S. validus* in monoculture, were significantly increased by the high nutrient treatments. The below-ground biomass for *C. indica* was significantly higher, whereas that for *S. validus* was significantly lower in mixture than in the monoculture treatment (Table 4.1).

Table 4.1 Mean (±SE, n=3) dry weight (kg m^{-2}) in different parts of plants influenced by nutrient and plant treatments after 65 days of growth

Treatment	Total	Above-ground	Below-ground	Rhizome	Root
Low nutrient					
Mono-*C. indica*	1.77±0.10bc	0.48±0.06d	1.29±0.06c	1.16±0.06c	0.13±0.01c
Mixed-*C. indica*	2.23±0.10b	0.59±0.04cd	1.64±0.06b	1.48±0.03b	0.16±0.05c
Mono-*S. validus*	1.60±0.10c	0.94±0.02bd	0.66±0.12d	0.40±0.09de	0.26±0.03a
Mixed-*S. validus*	0.74±0.07d	0.36±0.05d	0.38±0.02e	0.18±0.02e	0.20±0.01abc
High nutrient					
Mono-*C. indica*	2.18±0.15b	0.86±0.08bc	1.32±0.07c	1.17±0.07c	0.15±0.01c
Mixed-*C. indica*	3.51±0.35a	1.59±0.19a	1.92±0.20a	1.77±0.19a	0.15±0.01c
Mono-*S. validus*	2.09±0.16b	1.34±0.11a	0.75±0.15d	0.51±0.12d	0.24±0.05ab
Mixed-*S. validus*	0.97±0.08d	0.59±0.05cd	0.39±0.04e	0.22±0.03e	0.17±0.02bc
Source of variation					
Nutrient treatment	***	***	NS	NS	NS
Plant treatment	***	***	***	***	**
Nutrient × plant	*	**	NS	NS	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

Species mixture influenced above-ground plant growth before imposition of nutrient treatments. For both species, these early effects followed the same trends as those at harvesting time. After imposing nutrient treatments, the relative growth of *C. indica* in the mixture increased in the high nutrient treatment, but the effect disappeared after day 40. In contrast, the relative growth of *S. validus* in the mixture was not enhanced by the high nutrient treatment. Those effects in 65 days of plant growth were shown using the relative yield (RY) of above-ground biomass for *C. indica* and *S. validus* under the low and high nutrient treatments (Figure 4.2). The relative yield (RY) indicated that there was significant interspecies competition between *S. validus* and *C. indica* in the mixture, with *C. indica* being the superior competitor.

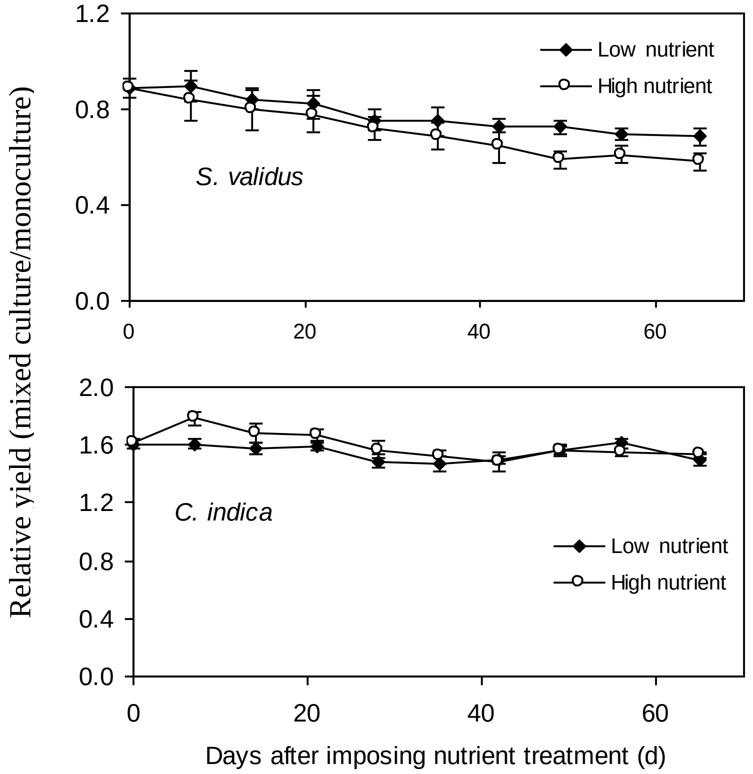

Figure 4.2 Relative yield (means ± SE, n = 3) of above-ground biomass for *C. indica* and *S. validus* under the low and high nutrient treatments. The nutrient treatments were imposed after 50 days of growth.

4.3.2 Biomass allocation and biometric characteristics

Biomass allocation and plant characteristics (except relative rate of shoot growth among the plant treatments) were significantly affected by the nutrient and plant treatments, but no significant interaction between nutrient and plant treatments was detected (Table 4.2). Plants had significantly higher percentage of above-ground biomass, relative rate of shoot growth and relative increase in numbers of shoots in the high than the low nutrient treatment. Compared with monoculture, *S. validus* in the mixture had significantly lower A/B ratio (ratio of above-ground biomass to below-ground biomass), percentage of above-ground biomass and relative increase in numbers of shoots under the low nutrient treatment, and S/R ratio [(ratio of the biomass of root-supported tissue (above-ground, rhizomes) to root biomass] and relative increase in numbers of shoots under the high nutrient treatment, whereas *C. indica* was not significantly affected by mixtures. Compared with *C. indica, S. validus* had more biomass allocated into above-ground tissues (Table 4.3).

Table 4.2 Analysis of variance for biomass allocation to different tissues and biometric characteristics of plants between nutrient and plant treatments after 65 days of growth

Variable	Source of variability		
	Nutrient treatment	Plant treatment	Interaction
Biomass allocation			
A/B ratio	**	***	NS
S/R ratio	***	***	NS
Above-ground (%)	***	***	NS
Rhizomes (%)	**	***	NS
Roots (%)	***	***	NS
Biometric characteristics			
Relative rate of shoot growth	*	NS	NS
Relative increase in shoot number	**	***	NS

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$. A/B — ratio of above-ground biomass to below-ground (rhizome and root) biomass; S/R — ratio of the biomass of root-supported tissue (above-ground, rhizomes) to root biomass.

Table 4.3 Mean (±SE, n=3) biomass allocation and biometric characteristics of plants influenced by nutrient and plant treatments after 65 days of growth

Treatment	Low nutrient				High nutrient			
	Mono-*C. indica*	Mixed-*C. indica*	Mono-*S. validus*	Mixed-*S.validus*	Mono-*C. indica*	Mixed-*C. indica*	Mono-*S. validus*	Mixed-*S.validus*
Biomass allocation								
A/B ratio	0.5±0.1d	0.5±0.04d	1.5±0.2b	1.0±0.1c	0.7±0.03cd	0.8±0.1cd	2.0±0.4a	1.6±0.1ab
S/R ratio	9.3±0.4b	9.4±1.3b	5.2±0.3c	3.1±0.3c	10.4±0.7a	13.6±0.9a	10.2±2.4b	4.8±0.1c
Above-ground %	33±2e	32±0.3e	59±4b	51±2c	41±1d	46±2cd	66±5a	61±2a
Rhizomes %	57±2a	58±2a	25±3c	24±0.3c	50±0.3b	47±2b	25±4c	22±2c
Roots %	10±0.4c	10±2c	16±1b	25±2a	9±0.4cd	7±0.6d	10±2c	17±0.4b
Biometric characteristics								
Relative rate of shoot growth (mm cm^{-1} d^{-1})	0.023±0.018c	0.030±0.017bc	0.048±0.023abc	0.037±0.015abc	0.053±0.008abc	0.074±0.008a	0.068±0.011ab	0.047±0.005abc
Relative increase in shoot number (No. d^{-1})	0.014±0.001a	0.012±0.002a	0.009±0.001b	0.005±0.001c	0.015±0.001a	0.014±0.001a	0.013±0.001a	0.007±0.002bc

Means with different letters within rows are significantly different based on LSD ($P \leq 0.05$)

A/B — ratio of above-ground biomass to below-ground (rhizome and root) biomass; S/R — ratio of the biomass of root-supported tissue (above-ground, rhizomes) to root biomass.

4.3.3 Concentrations of N and P in plant tissues

The concentrations of N and P in the plant tissues were significantly influenced by nutrient and plant treatments after 65 days of growth. The concentrations of N and P (except P in above-ground tissues) were significantly higher in the high nutrient than in the low nutrient treatment. The concentrations of N and P in plant tissues (except rhizome) were significantly different in plant treatments. The concentrations of N and P in the roots of *S. validus* were significantly lower in mixed culture than in monoculture in the high nutrient treatment (Table 4.4).

Table 4.4 Mean (±SE, n=3) concentrations (g kg^{-1}) of N and P in various plant tissues influenced by nutrient and plant treatments after 65 days of growth

Treatment	N concentration			P concentration		
	Above-ground	Rhizome	Root	Above-ground	Rhizome	Root
Low nutrient						
Mono-*C. indica*	10.2±0.5de	10.1±1.2c	8.7±0.4cd	3.8±0.1c	3.7±0.1d	2.8±0.1b
Mixed-*C. indica*	10.5±0.1d	9.7±0.3c	8.0±0.4d	4.3±0.1bc	4.0±0.1bcd	2.9±0.1b
Mono-*S. validus*	8.4±0.8e	11.6±1.7c	8.3±0.2d	4.6±0.1ab	3.8±0.1cd	1.4±0.1d
Mixed-*S. validus*	8.4±0.5e	10.9±1.3c	8.7±0.4cd	5.0±0.3a	4.2±0.2abcd	1.6±0.1cd
High nutrient						
Mono-*C. indica*	20.5±1.2ab	17.1±0.8ab	14.2±0.5ab	4.6±0.1ab	4.3±0.2abc	3.7±0.2a
Mixed-*C. indica*	21.3±0.8a	18.3±0.7a	14.5±0.9a	4.4±0.2abc	4.3±0.2abc	3.4±0.1a
Mono-*S. validus*	19.0±0.4bc	14.9±0.4b	12.7±1.0b	4.6±0.2ab	4.6±0.3a	2.5±0.2b
Mixed-*S. validus*	17.0±0.4c	15.4±0.3ab	10.1±0.4c	4.9±0.3a	4.5±0.1ab	1.9±0.1c
Source of variation						
Nutrient treatment	***	***	***	NS	***	***
Plant treatment	***	NS	**	**	NS	***
Nutrient × plant	NS	NS	**	NS	NS	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$)

NS: not significant. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$

4.3.4 Nutrient allocation

Plant nutrient (N and P) allocations and N/P ratios (except N/P ratios in roots between nutrient treatments) were significantly affected by nutrient and plant treatments.

Significant interactions between nutrient and plant treatments were detected in N allocation to rhizome and P allocation to above-ground organs and rhizomes (Table 4.5). Plants had significantly higher relative allocation of N and P to above-ground organs in the high than the low nutrient treatment. Compared with monoculture, *S. validus* in the mixture had significantly higher relative allocation of N and P to roots (except P allocation to roots under the high nutrient treatment) and significantly lower N/P ratios in above-ground tissues and rhizomes, whereas *C. indica* was not significantly affected by mixtures (Table 4.6).

Table 4.5 Analysis of variance for nutrient allocation to different tissues of plants between nutrient and plant treatments after 65 days of growth

Variable	Source of variability		
	Nutrient treatment	Plant treatment	Interaction
N/P ratio			
Above-ground	**	***	NS
Rhizome	***	***	NS
Root	NS	***	NS
N allocation ratio			
Above-ground (%)	***	***	NS
Rhizome (%)	*	***	*
Root (%)	**	***	NS
P allocation ratio			
Above-ground (%)	**	***	*
Rhizome (%)	**	***	*
Root (%)	*	**	NS

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

Table 4.6 Mean (±SE, n=3) N/P ratio, N and P allocation to different tissues of plants influenced by nutrient treatments and plant treatments after 65 days of growth

Treatment	Low nutrient				High nutrient			
	Mono-*C. indica*	Mixed-*C. indica*	Mono-*S. validus*	Mixed-*S.validus*	Mono-*C. indica*	Mixed-*C. indica*	Mono-*S. validus*	Mixed-*S.validus*
N/P ratio								
Above-ground	4.8±0.2ab	4.5±0.2ab	3.4±0.2c	3.3±0.2c	4.7±0.1ab	5.1±0.1a	4.4±0.3b	3.6±0.2c
Rhizome	2.9±0.1b	2.7±0.1bc	2.3±0.2c	2.1±0.2c	3.5±0.1a	3.5±0.1a	2.9±0.2b	2.3±0.1c
Root	3.3±0.1b	3.0±0.03b	7.0±1.0a	6.4±0.6a	2.9±0.3b	3.0±0.1b	5.3±0.9a	6.5±0.9a
N allocation ratio								
Above-ground (%)	45±1d	47±1cd	72±3ab	66±1b	52±1c	56±1bc	75±3a	72±2ab
Rhizome (%)	48±1a	47±1a	17±2c	16±1c	43±1ab	40±1b	18±3c	15±1c
Root (%)	7±0.2cd	6±1d	11±1bc	18±2a	5±1d	4±0.04d	7±2cd	13±2b
P allocation ratio								
Above-ground (%)	34±2c	35±1c	71±3a	66±1a	44±1b	47±2b	69±3a	70±2a
Rhizome (%)	59±2a	58±1a	24±3c	25±0.4c	49±1b	48±2b	26±4c	23±2c
Root (%)	7±0.3ab	7±1ab	5±1b	9±1a	7±0.6ab	5±0.2b	5±1b	7±1ab

Means with different letters within rows are significantly different based on LSD ($P \leq 0.05$).

4.3.5 Nutrient use efficiency

N and P use efficiency of *C. indica* and *S. validus* were significantly affected by the nutrient treatments, but not by the plant treatments. No significant interaction was detected between nutrient and plant treatments (Table 4.7). N and P use efficiency were significantly higher in the low nutrient treatment (86 g dry weight g^{-1} N and 474 g dry weight g^{-1} P, averaged over the plant treatments) than in the high nutrient treatment (61 g dry weight g^{-1} N and 404 g dry weight g^{-1} P, averaged over the plant treatments).

Table 4.7 Mean (±SE, n=3) N and P use efficiency (g dry weight g^{-1} N or P) of *C. indica* and *S. validus* influenced by nutrient and plant treatments after 65 days of growth

Treatment	N use efficiency	P use efficiency
Low nutrient		
Mono-*C. indica*	86±6a	276±5a
Mixed-*C. indica*	85±1a	246±3ab
Mono-*S. validus*	87±5a	278±4a
Mixed-*S. validus*	92±6a	281±21a
High nutrient		
Mono-*C. indica*	58±3b	222±10b
Mixed-*C. indica*	56±3b	230±14b
Mono-*S. validus*	61±3b	243±10b
Mixed-*S. validus*	71±1b	245±13b
Source of variation		
Nutrient treatment	***	***
Plant treatment	NS	NS
Nutrient × plant	NS	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$)

NS: not significant. *** Significant at $P < 0.001$

4.3.6 Accumulation of N and P by plants

Accumulation of N and P in above- and below-ground tissues largely reflected patterns of biomass allocation. The accumulation of N and P (except accumulation of N by roots) was significantly (*P*<0.001) higher in the high than the low nutrient treatment. Significant difference in accumulation of N and P by above-ground tissues and rhizome was observed among the plant treatments. The accumulation of N by the whole plant was significantly higher in monoculture of *C. indica* than in monoculture of *S. validus,* but no significant difference was observed in the accumulation of P by the whole plant. The accumulation of N and P in the mixed culture was between the two monoculture treatments (Table 4.8).

Table 4.8 Mean accumulation (g m^{-2}) of N and P averaged over nutrient treatments by various parts of plants grown for 65 days in the microcosms

Plant treatment	Total		Above-ground		Below-ground		Rhizome		Root	
	N	P	N	P	N	P	N	P	N	P
Mono-*C. indica*	27.3a	8.0a	11.9c	3.0b	15.4a	5.0a	14.2a	4.5a	1.2a	0.5a
Mixed-culture	25.8ab	7.6a	13.9b	3.5b	11.9b	4.1b	10.5b	3.7b	1.4a	0.4a
Mono-*S. validus*	24.3b	7.0a	18.0a	4.9a	6.3c	2.1c	4.3c	1.8c	2.0a	0.3a

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

4.3.7 Nutrient removal efficiency

The average removal efficiencies of NH_4-N, NO_x-N and PO_4-P were presented in Table 4.9. Significant difference in removal efficiency was observed among plant treatments, but no significant difference was detected between nutrient treatments. The interaction was only found in NO_x-N removal efficiency. The removal efficiencies of NH_4-N, NO_x-N and PO_4-P were significantly lower in non-planted than in treatments with plants. Percentage of NO_x-N removal was significantly greater in the low than the high nutrient treatment in non-plant microcosms. The removal efficiencies were significantly (*P*<0.01) influenced by the sampling date, suggesting influence by plant growth and/or other biota in the microcosms. Nitrogen removal was more variable in the non-plant than in the planted treatments. There was a decreasing trend for P removal in non-plant microcosms (data not shown).

Significant differences in NO_x-N and PO_4-P (but not NH_4-N) removal efficiencies were found among the retention times (Figure 4.3). Percentage of NO_x-N removal with a 7-day retention time was 16% greater than that with a 3.5-day and 45% higher than that with a 1.5-day retention time. PO_4-P removal with a 7-day retention time was 15% greater than that with a 3.5-day and 40% higher than that with a 1.5-day retention time.

Table 4.9 Mean (±SE, n=27) nutrient removal efficiency (%) averaged over sampling times influenced by 65-day nutrient and plant treatments in the microcosms

Treatment	NH_4-N removal	NO_x-N removal	PO_4-P removal
Low nutrient			
Non-planted	70±10b	76±5b	56±5b
Mono-*C. indica*	98±1a	93±1a	85±2a
Mixed-culture	94±4a	90±2a	82±2a
Mono-*S. validus*	98±2a	93±2a	77±3a
High nutrient			
Non-planted	77±5b	55±7c	42±5b
Mono-*C. indica*	99±0.3a	98±0.4a	88±2a
Mixed-culture	99±0.3a	96±1a	83±2a
Mono-*S. validus*	99±1a	94±1a	76±3a

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

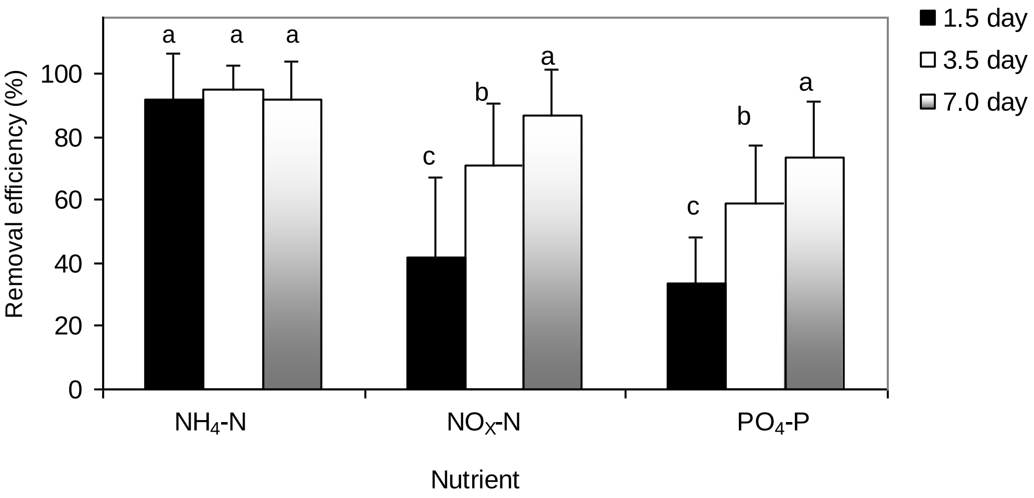

Figure 4.3 Nutrient removal efficiency at 1.5-, 3.5- and 7.0-day retention times averaged over nutrient and plant treatments during the last 3 weeks in the microcosms. Bars (means + SE, n = 9) with different letters are significantly different based on LSD (*P* ≤ 0.05).

4.3.8 pH and dissolved oxygen in effluents

Significant differences in pH of the effluents were found among treatments, but no significant interaction between nutrient and plant treatments was observed. Significant differences in the concentrations of dissolved oxygen in the effluents were found between plant, but not nutrient treatments. There was a significant interaction between sampling date and plant treatments. The pH of the effluents was significantly ($P<0.01$) lower in the high than the low nutrient treatment. The lowest effluent pH was detected in monoculture of *C. indica*, followed by mixed culture, but no significant difference was found between monoculture of *S. validus* and the treatment without plants. The lowest concentration of dissolved oxygen in the effluents was detected in monoculture of *S. validus*, but no significant difference was found among non-planted, monoculture of *C. indica* and mixed-culture treatments (Figure 4.4).

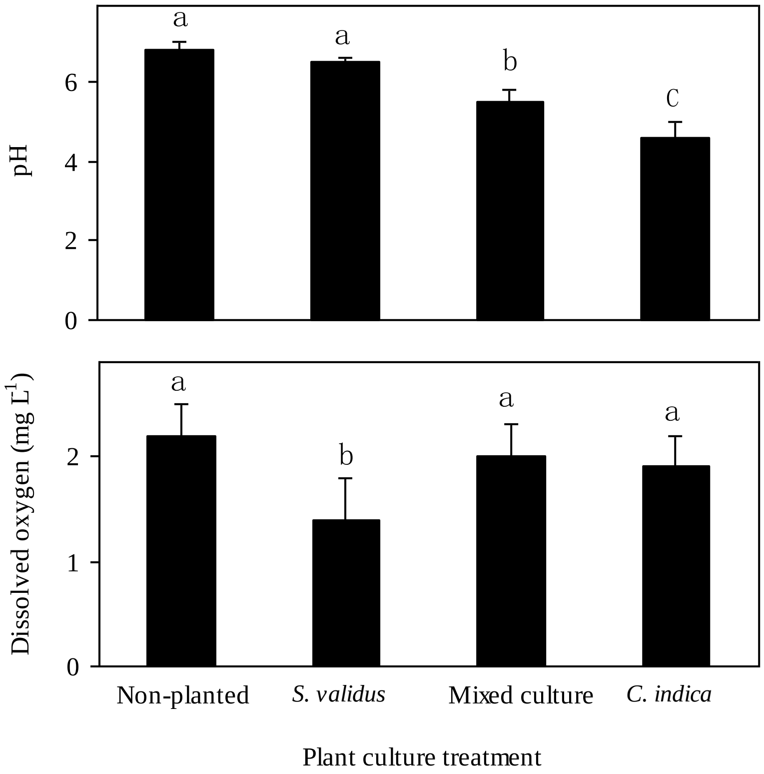

Figure 4.4 The pH and concentrations of dissolved oxygen averaged over nutrient concentrations and sampling times in the effluents in different plant treatments. Bars (means + SE, n = 27) with different letters are significantly different based on LSD ($P \leq 0.05$).

4.3.9 Nutrient partitioning in the water-substratum-plant continuum

4.3.9.1 N and P in the effluents

The recovery percentages of N and P in the effluents of the planted treatments were 5-12% and 27-35%, respectively (Tables 4.10 and 4.11). Compared with any other plant treatments, the recoveries in the effluent of non-plant treatments were relatively high, ranging from 34-48% of the influent N and 56-69% of the influent P. The recovery percentages of P among the planted treatments were similar in the low and the high nutrient treatments.

4.3.9.2 N and P uptake by plants

N and P removals by plant uptake were relatively high, with 62-87% and 35-55% of the N and P removed, respectively (Tables 4.10 and 4.11). The total nutrient accumulation by *C. indica* was relatively high, but the above-ground nutrient accumulation was relatively low compared with *S. validus*. In comparison with monoculture, the N accumulation by plants in mixed culture was relatively low in the low nutrient treatment, but was similar to *C. indica* in the high nutrient treatment. The P accumulation by plants in mixed culture was between the two monoculture treatments.

4.3.9.3 N and P in the substrata

Significant differences in the contents of total P were found between the nutrient treatments, but no significant difference in the contents of total N and P was observed among the plant treatments. The average contents of total P were significantly higher after imposing nutrient treatments than before, with the highest P content in the high nutrient treatment (Figure 4.5). Although no significant difference in the content of total N was detected, the average contents of total N in the substrate were relatively low after completion of nutrient treatments (0.199 and 0.215 g kg^{-1} in the low and the high nutrient treatment, respectively) compared to the commencement of nutrient treatments (0.225 g kg^{-1}) (Figure 4.5). The same amounts of N release from the substrate were calculated in each nutrient treatment.

Table 4.10 Nitrogen mass balance (% of input) influenced by 65-day nutrient and plant treatments in the microcosms (The inputs of influent N were 2.21 and 4.26 g in the low and high nutrient treatments, respectively)

Treatment	Input		Output						
	Influent	Sand release	Effluent	Plant uptake					Other losses
				Above-ground	Rhizome	Root	Below-ground	Total	
Low nutrient									
Non-planted	77	23	34	0	0	0	0	0	66
Mono-*C. indica*	77	23	12	27	44	4	48	75	13
Mixed culture	77	23	12	27	30	5	35	62	26
Mono-*S. validus*	77	23	11	48	11	7	18	66	23
High nutrient									
Non-planted	94	6	48	0	0	0	0	0	52
Mono-*C. indica*	94	6	5	41	42	3	45	86	9
Mixed culture	94	6	6	50	32	4	36	86	8
Mono-*S. validus*	94	6	7	58	14	5	19	77	16

Table 4.11 Phosphorus mass balance (% of input) influenced by 65-day nutrient and plant treatments in the microcosms (The inputs of influent P were 1.21 and 2.47 g in the low and high nutrient treatments, respectively)

Treatment	Input	Output							
	Influent	Effluent	Plant uptake					Sand storage	Other losses
			Above-ground	Rhizome	Root	Below-ground	Total		
Low nutrient									
Non-planted	100	56	0	0	0	0	0	33	11
Mono-*C. indica*	100	28	16	36	3	39	55	15	2
Mixed culture	100	32	19	28	3	31	50	12	6
Mono-*S. validus*	100	31	35	12	2	14	49	17	3
High nutrient									
Non-planted	100	69	0	0	0	0	0	19	12
Mono-*C. indica*	100	27	18	21	2	23	41	23	9
Mixed culture	100	30	20	18	2	20	40	16	14
Mono-S. *validus*	100	35	24	9	2	11	35	21	9

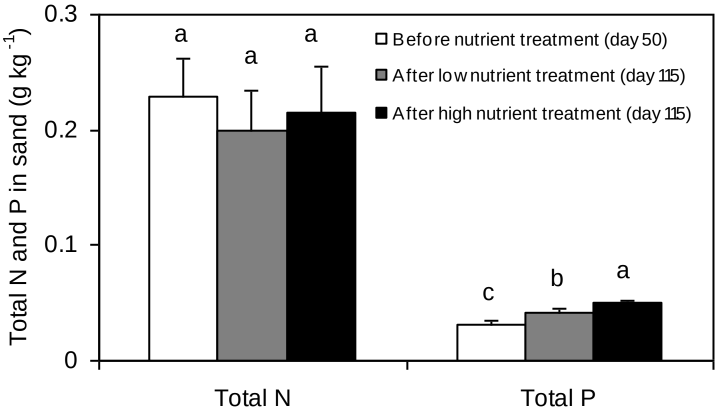

Figure 4.5 Mean contents of total N and P averaged over plant treatments in the substrate before and after nutrient treatments. Bars (means + SE, n = 3) with different letters are significantly different based on LSD ($P \leq 0.05$).

4.4 Discussion

4.4.1 Resource allocation

Aquatic plants can take up large quantities of nutrients, and even assimilate them luxuriously (Cronk and Fennessy, 2001). The present results showed the plants were capable of taking up more N and P (Table 4.4) and producing more biomass (Table 4.1) in the high than the low nutrient treatment. Differences between species in biomass accumulation, and tissue N and P concentrations are likely to reflect species and developmental stage differences in efficiency of nutrient uptake and use (Tanner, 1996; Güsewell and Bollens, 2003). In the present study, significant differences in the nutrient use efficiency were not detected among the plant treatments (Table 4.7). Therefore, the differences in biomass and nutrient concentration might be related to the nutrient uptake efficiency. Although the below-ground biomass was relatively high in the high nutrient treatment compared with the low nutrient treatment, significant difference in the below-ground biomass was not observed between the nutrient treatments. This might be due to the relatively high plant density and small size of the microcosms, which limited below-ground plant growth in the high nutrient treatment. Güsewell and Bollens (2003) also pointed out that the total below-ground biomass was less responsive to nutrient treatments than the above-ground biomass, with an inconsistent effect of the N/P supply ratio in the pot experiments. The total below-ground biomass was higher in the intermediate nutrient supply than the high nutrient supply at 15 N/P supply ratio.

Plant productivity and resource allocation varied widely between *C. indica* and *S. validus* (Tables 4.1, 4.3 and 4.6). This variation is likely to arise from relative differences in initial propagule vigor, as well as from intrinsic species and possibly ecotype growth characteristics (Daniels, 1991). However, factors such as the physiological and developmental state of the propagules are likely to have been of more importance than the biomass of the propagules per se (Tanner, 1996).

The above-ground/below-ground ratios of the species in the present study ranged from 0.6 to 1.7. These values were in the ranges of other aquatic plants (Hogetu, 1984). Plants alter their resource allocation to above-ground and below-ground tissues along environmental gradients of disturbance and resource availability (Kirkman and Sharitz, 1993; Blanch et al., 1999). In the present study, the resource allocations were altered by nutrient availability, and also changed for *S. validus* in the mixture. The growth of *S. validus* in the mixture was strongly inhibited by the presence of *C. indica*, resulting in allocation of a greater of percentage biomass to its below-ground tissues. Wu and Yu (2004) found that *Nymphoides peltata* decreased the ratios of above-ground to below-ground biomass with increasing density of *Zizania latifolia* in mixture.

Resource allocation is known to change during the growing season for most plant species (Aerts et al., 1992). The resource allocation patterns observed in the present experiment may only reflect the short length of the experiment. It is important to note that while a microcosm trial enables more control over experimental conditions than field trials, there are significant differences in the temporal and spatial aspects of such studies. Thus, the present results of the species combination, and the relatively short duration of the growth trials in relation to the life cycles of these clonal species, should be used with care in attempting to generalize the results from the microcosm to field-scale constructed wetland.

4.4.2 Competition in mixture

There was a significant interspecies competition between *C. indica* and *S. validus* in the mixed-culture treatment, with *C. indica* being the superior competitor. The interspecies competition for emergent wetland species has been reported by several researchers (Coleman et al., 2001; Güsewell and Bollens, 2003), but no research has been done for *C. indica* and *S. validus*.

Grime and Hodgson (1987) listed characteristics of species with high competitive ability: (1) a robust perennial life form with a strong capacity to ramify vegetatively, (2) the rapid commitment of captured resources to the construction of new leaves and roots, (3) high morphological plasticity during the differentiation of leaves and roots, and (4) short life spans of individual leaves and roots. Both *C. indica* and *S. validus* are robust perennials, which rapidly produce ramets and have high growth rates. In addition, *C. indica* produces large storage rhizomes and has high growth rates (Zhao et al., 2003).

Wetzel and van der Valk (1998) pointed out that rapid growth was not the only factor, and suggested that plant architecture played a significant role in competition between *Carex stricta*, *Phalaris arundinacea* and *Typha latifolia*. Morphological characteristics of a plant affecting competition for light have been reported in agricultural and woody plants (McLachlan et al., 1993; Sipe and Bazzaz, 1994; Webster et al., 1994). Species with different morphologies showed large differences in canopy structure. A grass, having a more open canopy, was consistently a weak competitor when grown with forbs (Tremmel and Bazzaz, 1993). The morphological characteristics, such as tall shoot, leaf shape and large canopy diameter were significantly correlated with increased competitive ability in wetland plants (Gaudet and Keddy, 1988). Changes of water levels, and the presence/absence of competitor species, produced significant morphological responses in mature individuals of five freshwater wetland plant species: *Agrostis stolonifera* and *Carex rostrata*, *Deschampsia caespitosa*, *Filipendula ulmaria* and *Phalaris arundinacea*. These responses provide evidence for potential advantages in survival and ability to spread vegetatively (Kennedy et al., 2003). In the present study, *C. indica* and *S. validus* differ substantially in their growth rate, morphology, physiology and size. It is possible that *C. indica*, having large leaf areas and canopy diameter, maximized the capture of light and nutrient resources by maximizing vegetative growth under both nutrient availabilities and out-competed *S. validus* in the mixture.

Interspecies competition is often regarded as being caused by mutual exploitation of limiting resources (resource consumption, including light interception by plants and space occupancy by space-limited sessile organisms), by the production of toxins, and by various combinations of these mechanisms (Tilman, 1987). In the present study, however, it is unknown whether interspecies competition between *C. indica* and *S. validus* was caused by a single mechanism or combinations of above mentioned mechanisms. It is worth mentioning that there were significant differences in pH of the effluents among the plant treatments, with the lowest effluent pH in monoculture of *C. indica*, followed by mixed culture and monoculture of *S. validus* (Figure 4.4).

4.4.3 Nutrient removal efficiency

In the present study, the removal efficiencies of N and P were comparable to the reported studies on the same or different water flow in the microcosms with other plant species (Breen, 1990; Huett et al., 2005; Iamchaturapatr et al., 2007). The removals of total N and P in an up-flow artificial wetland with *Typha orientalis* were 95 and 99% of the input, respectively (Breen, 1990). Huett et al. (2005) reported that >96% of added N and P was removed from simulated nursery runoff by the planted *Phragmites australis* in small-scale subsurface flow wetlands during the 19-month study period. Ayaz and Akça (2001) also reported that the removal was 90% of total added N and 55% of added P in pilot-scale constructed wetlands.

Most researchers have observed that wetland plant species in the constructed wetlands could show significant differences in nutrient removal efficiency (Gersberg et al., 1986; Coleman et al., 2001; Fraser et al., 2004; Kyambadde et al., 2004; Akratos and Tsihrintzis, 2007; Calheiros et al., 2007; Iamchaturapatr et al., 2007; Sim et al., 2007; Yang et al., 2007). For instance, Gersberg et al. (1986) investigated constructed wetlands that were planted to *Scirpus validus*, *Phragmites communis*, *Typha latifolia* or were left unplanted. *Scirpus validus* was the most effective and *Typha latifolia* the least effective in removing N. However, Huang et al. (2000) found that two macrophytes (*Scirpus cyperinus* and *Typha latifolia*) in small-scale constructed wetlands did not make a major difference in nutrient removal. Our results agreed with their findings, with no significant difference in nutrient removal efficiency between *C. indica* and *S. validus*.

In the present study, significant difference in nutrient removal efficiency was found between non-planted and planted treatments. A majority of experimental studies on N and P removal in constructed wetlands confirmed that unplanted treatment had a lower N and P removal compared with planted treatment (Hunter et al., 2001, Lim et al., 2001; Yang et al., 2001; Fraser et al., 2004; Iamchaturapatr et al., 2007). The greater nutrient removal efficiency in planted microcosms over non-plant microcosms is likely due to plant uptake (Brix, 1993). The water movement due to plant uptake contributes to N removal and offers a partial explanation for increased N removal in planted wetland treatment systems (Martin et al., 2003). Tanner (1996) suggested that a relationship between total biomass and nutrient removal could be described by a linear regression equation.

Our results confirmed that a longer retention time increased nutrient removal efficiency in constructed wetland and the majority of studies show that a longer hydraulic retention time could increase nutrient removal (Hunter et al., 2001; Jing et al., 2002; Mayo and Mutamba, 2004; Sirianuntapiboon et al., 2006; Akratos and Tsihrintzis, 2007; Iamchaturapatr et al., 2007). For example, Iamchaturapatr et al. (2007) have observed that the losses of N and P concentrations in the solutions have an exponential regression with the retention times in the vertical free surface-flow wetland.

The nutrient removal efficiency was not greater in mixed culture of *C. indica* and *S. validus* compared with monocultures. The results agreed with the finding of Fraser et al. (2004). One of the reasons was due to interspecies competition between *C. indica* and *S. validus*, the former out-competing the latter. The growth of *S. validus* in the mixture was inhibited significantly by *C. indica*, but if the effect of interspecies competition could be reduced, planting different species in mixed culture may provide other benefits over monocultures, such as balanced pH and DO (cf. Figure 4.4), improved aesthetic view, and an added economic value through harvesting ornamental species. It might also enhance tolerance to abiotic stress or enhance treatment efficiency of decomposing toxins or removing nutrients (Fraser et al., 2004).

4.4.4 pH and dissolved oxygen (DO)

In the present study, the pH in the effluents was decreased by plants. Fu et al. (2005) also found that the pH of effluent in the system with *C. indica* for salty wastewater treatment was smaller than that in non-plant control. Iamchaturapatr et al. (2007) reported that rapid reduction of pH at an early stage of the experiment was found in the planted reactors. The reason for this might be the formation of dissolved carbon dioxide (CO_2) and carbonic acid (H_2CO_3) in water by the degradation of organic compounds (residual organic matters in media beds and planted materials) of aerobic organisms resulting in pH reduction (Kyambadde et al. 2004). Plants take up significant amounts of sparingly soluble nutrients from the rhizosphere by using their ability to acidify the rhizosphere (Rao et al., 2002). The abilities of plants to induce the decrease of soil pH stem from H^+ excretion during the root uptake of cations coupled with root exudation of organic acids and release of CO_2 from the root respiration (Hinsinger et al., 2003). Nitrification possibly reduces the pH in the rhizosphere (Bezbaruah and Zhang, 2004).

Significantly lower pH was observed in the microcosms containing *C. indica* (Figure 4.4). Iamchaturapatr et al. (2007) reported that there was insignificant change of mean pH values among planted reactors, but Kyambadde et al. (2004) found that water pH was significantly lower in *Cyperus papyrus* than *Miscanthidium violaceum*-based constructed wetlands compared to the non-planted controls. Strong respiratory CO_2 release to the rhizosphere may cause the pH values to decrease. CO_2 release was dependent on species and vegetation biomass (Salhani and Stengel, 2001). Zhu and Sikora (1995) found that the higher concentrations of organic carbon were released from the canarygrass (*Phalaris arundinacea*) roots than bulrush (*Scirpus atrovirens georgianus*), typha (*Typha latifolia*) or reed (*Phragmites communis*).

In the present study, the concentrations of DO were lower after the treatment in the microcosms compared with initial DO. The decrease of DO concentrations in unplanted and planted sand/gravel beds can be generally explained by the lower oxygen (O_2) transfer (diffusion) through the water–soil medium compared with single water medium (Iamchaturapatr et al., 2007). Mitsch and Gosselink (2000) explained that this phenomenon can occur either after several hours or a few days, when an undrained condition is developed.

The DO was significantly lower in the microcosms containing *S. validus* than *C. indica* in the present study. The influence of species on effluent DO was also found in the literature. For example, DO was lower in *Miscanthidium violaceum* than *Cyperus papyrus*-based constructed wetlands compared to the non-planted controls (Kyambadde et al., 2004). The lowest records of DO concentrations were found in the microcosms containing *Scirpus triqueter, Iris pseudacorus*, *Alisma plantago-aquatica*, and *Plantago asiatica* (Iamchaturapatr et al., 2007). Wetland plants are reported to transfer photosynthetic oxygen to the rhizosphere, thus boosting oxygen concentration in the water (Brix, 1994a). The fact that plant roots release oxygen to their immediate environment has been confirmed by Bezbaruah and Zhang (2004); the amount of oxygen release by the roots varies with the amount of oxygen stress (e.g., biochemical oxygen demand) present in the immediate rhizosphere environment (Sorrel and Armstrong, 1994). The oxygen release intensities were found to vary between *Typha latifolia* (cattail) and *Juncus effusus* (rush) and also to depend on the redox state of the rhizosphere. The total size of the root system does not significantly affect the intensity of oxygen release; instead, the oxygen release was governed by the size of the above-ground biomass (Wießner et al., 2002).

4.4.5 Nutrient mass balance

A major advantage of using a mass balance approach for assessing the wastewater dynamics is that the removal of nutrients can be accurately measured. Breen (1990) stated that the plants were the major nutrient sinks for N and P, with 51 and 67% of the influent N and P, respectively, removed by the plants in microcosms. Busnardo et al. (1992) found that removal by emergent macrophytes in mesocosms removed 50% and 52% of the influent N and P, respectively. The laterite gravel-rooted *Phragmites mauritianus* removed 29% and 54% of the influent N and P, respectively (Sekiranda and Kiwanuka, 1998). Nutrient removal from simulated nursery runoff by *Phragmites* *australis* in the subsurface flow wetlands accounted for 76% and 86% of total input N and P, respectively (Huett et al., 2005). The results of the present study (Tables 4.10 and 4.11) showed that N and P removals by plants were high (62-87% and 35-55% of the influent N and P, respectively). The higher percentage of N compared with P removal in our study was possibly due to low N:P ratio in the influents compared to above mentioned references.

In addition to plant uptake, N removal can also occur by (i) NH_3 volatilisation favoured by high pH, (ii) nitrification under aerobic conditions, and (iii) denitrification under anaerobic conditions (Sekiranda and Kiwanuka, 1998). In the present study, N removal by volatilisation was unlikely, due to the pH of the solution being less than 7 (Figure 4.4). The other losses of N in the present study (Table 4.10) were likely due to denitrification. Breen (1990) stated that while the plants represent a major sink for N, denitrification was a significant removal mechanism in both planted and unplanted systems. This removal mechanism has the advantage of requiring no additional operational procedures such as harvesting.

The substrate of the sands can adsorb N to a certain degree. However, N release from substrate was found in the present study (Figure 4.5). It was likely that N was adsorbed to sand during the 50-day pre-treatment and was subsequently desorbed during the 65-day treatment period, due to relatively high plant growth rate and a relatively low N loading rate compared to P loading rate.

Phosphorus removal from wastewater by vertical-flow wetlands occurs through three parallel mechanisms: bed/soil sorption, plant uptake and microbial assimilation (Lantzke et al., 1998; Luederitz et al., 2001). Given that the medium used in this study was river sand that contained oxides of Fe and Al, it could enhance the effect of P retention by chemical adsorption and precipitation in constructed wetlands (Arias et al., 2001).

Losses of 2-14% of P were recorded (Table 4.11), possibly due to formation of organic film that was not quantified in the present study. Breen (1990) found that organic film represented a total of 11 and 10% of the influent N and P, respectively. Although comparatively small, the complex films of microbial cells and organic materials form the interface between plant and substratum surfaces and the interstitial water and influent load. As such, this component is directly involved in the transformation and availability of nutrients.

Phosphine (PH_3) gas has been identified in the environment in a variety of locations (Dévai et al., 1988; Gassmann, 1994), but P removal by PH_3 is unlikely in the present study due to a relatively short duration of the experiment.

4.5 Conclusions

The high nutrient availability could stimulate plant growth and accumulation of nutrients by plants, resulting in allocation of more resources to the above-ground tissues compared to below-ground ones. Due to interspecies competition, the growth and resource allocation of *S. validus* were significantly influenced by mixture, but *C. indica* was less affected. Significant removal of nutrients was found in the microcosms planted with aquatic plants compared with the non-planted, but not among the planted treatments (mono-*C. indica*, mono-*S. validus* and mixture). The N and P removal efficiencies were within the ranges reported in the literature. Plant uptake and incorporation into tissue was the major factor responsible for N and P removal. Species had an influence on effluent pH and dissolved oxygen. The nutrient removal was not greater in mixed culture than in monocultures between *C. indica* and *S. validus*, but mixed culture may provide other benefits such as improving plant growth conditions (pH and dissolved oxygen). To enhance the aesthetic appeal of constructed wetlands, but avoid the interspecies competition, the intensive studies on nutrient uptake for each plant species and their mixtures at various nutrient concentrations and at various planting densities are needed in both laboratory and field conditions.

CHAPTER 5

INTERACTIVE EFFECTS OF NITROGEN AND PHOSPHORUS ON GROWTH AND NUTRIENT REMOVAL EFFICIENCY USING C*ANNA INDICA* AND S*CHOENOPLECTUS VALIDUS* IN WETLAND MICROCOSMS

Abstract

The concentrations of N and P in the wastewater may affect emergent wetland plant growth and nutrient removal efficiency in constructed wetlands. The interactive effects of three levels of nitrogen (mg N L^{-1}) (low 5, medium 30 and high 90 in 1:1 NH_4-N and NO_3-N) and two levels of phosphorus (mg P L^{-1}) (low 3 and high 15) on growth and nutrient removal efficiency using *Canna indica* and *Schoenoplectus validus* were studied in the wetland microcosms. After nutrient treatments, the growth of plants in the high nutrients outperformed that in the low nutrients, growing taller, producing more stems, leaves and flowers, but the performance of growth was not significantly different between the medium N-low P and high N-low P. The total biomass of the plants and concentrations of N and P in the plant tissues were significantly influenced by interaction of N and P treatments. For *C. indica*, the growth performance was related to the physiological responses. The photochemical efficiency (F_v/F_m) significantly increased from 0.84 to 0.85 with an increase in N additions. The photosynthetic rate increased from 13 to16 μmol m^{-2} s^{-1} in the low P levels and from 14 to 20 μmol m^{-2} s^{-1} in the high P levels with an increase in N applications, but significant difference was only from low to medium N levels at the high P levels. The tissue concentrations of N increased with an increase in N application and decreased with an increase in P addition. For *C. indica*, the tissue concentrations of P increased with an increase in P additions and decreased with an increase in N applications, whereas for *S. validus*, the tissue concentrations of P decreased with an increase in N applications at the low P treatment, but increased at the high P treatment. The biomass allocation of plants did not totally reflect the nutrient allocation to different plant tissues. There was a significant interactive effect of N and P treatments on the removal efficiencies of NH_4-N, NO_x-N and PO_4-P (except for the removal of NO_x-N by *S. validus*). On average, more than 56% of the nutrients accumulated by plants was allocated to the above-ground tissues, and therefore, could be removed by harvesting. For *C. indica*, plant was the major nutrient (N and P) removal pathway in the wetland microcosms (except P removal in the low N-high P treatments), whereas for *S. validus*, the plant uptake, substrate storage and other losses (such as denitrification) had similar contribution to N removal when N loading rates were relatively low (the low N treatments), but other losses contributed more to N removal in the medium N-high P treatments. The P storage by substrate was the main contribution to P removal when P loading was relatively high or N and P loading rates were relatively low and plants were not intensively growing, but plant uptake was the major factor responsible for P removal when N loading was relatively high and plants were vigorously growing. The properly high nutrient availability and optimum ratio of N and P are needed to stimulate the growth of wetland plants, resulting in preferential allocation of resources to the above-ground tissues, and enhancing the nutrient removal in constructed wetlands.

Key words: *Canna indica*; constructed wetland; N and P interaction; nutrient removal; plant growth; *Schoenoplectus validus*

5.1 Introduction

In constructed wetlands, plants not only take up nutrients from wastewater and substrates, but also act as catalysts for purification reactions by increasing the environmental diversity in the rhizosphere, and promoting a variety of chemical and biological reactions that enhance nutrient removal (Jenssen et al., 1993; Brix, 1997). A well-developed vegetation also shades out algae that cause a clogging effect at the surface of constructed wetlands (Cheng et al., 2002).

The concentrations of nutrients, most importantly N and P, in the wastewater and loading rate to the constructed wetlands vary depending on the quality of wastewater, type of wastewater treatment facilities and the season. These changes to nutrient supply possibly affect plant growth responses and nutrient removal efficiency in constructed wetlands

(Tanner, 1994, 1996, 2001). Plants not only grow at a slow rate at low nutrient supply compared with high nutrient supply, but also increase their biomass allocation to roots (Poorter and Nagel, 2000) and reduce the nutrient concentrations in the biomass (Aerts and Chapin, 2000). Therefore, the optimum N and P availability is of principal concern in the growth of wetland plants in constructed wetlands.

Few existing reports on the interactive effects of N and P on plant growth, biomass allocation, nutrient uptake and distribution in wetland plants are inconsistent (Cary and Weerts, 1984a and b; Ulrich; 1985; Ulrich and Burton, 1988; Romero et al., 1999). For example, Ulrich and Burton (1988) found that N (NO_3-N) and P treatments and their interaction strongly affected plant growth and biomass of *Typha latifolia*, *T. angustifolia*, *Sparganium eurycarpum* and *Phragmites australis*. However, Cary and Weerts (1984a) and Romero et al. (1999) did not observe an interactive effect of N and P for either *Typha orientalis* or *Phragmites australis*.

The tropical and subtropical plant species *Canna indica* is often grown in urban parks because of its easy growth and reproduction, long flowering duration, and ornamental value. *C. indica* has also been cultivated in constructed wetlands to improve the quality of lakes and rivers polluted by wastewater due to its relative high nutrient removal efficiency and aesthetic value (Grosse et al., 2001; Shi et al., 2004; Yue et al., 2004; Fu et al., 2006; Wu and Ding, 2006; Wu et al., 2006b; Calheiros et al., 2007; Yang et al., 2007). Similarly, *Schoenoplectus validus* and closely related species *S. lacustris*, *S. acutus* and *S. californicus* have been used widely in constructed wetlands around the world (Tanner, 2001). However, there is a lack of knowledge on the influence of different N and P loading rates on growth and nutrient removal efficiencies using *C. indica* and *S. validus* in constructed wetlands. The objectives of this study were: (1) to investigate interactive effect of N and P on plant growth, physiological responses and resource allocation; and (2) to elucidate the relationships between the growth and physiological responses as well as between the biomass and nutrient allocation.

5.2 Materials and Methods

5.2.1. Wetland microcosms

The design of the wetland microcosm was described in Chapter 2. Thirty-six microcosms were used as experimental units in this study.

5.2.2 Experimental setup

The experiment was conducted in a phytotron chamber with controlled day/night temperatures of 25/20 ºC from the beginning of May to the early July in 2006 for *S. validus* and from the beginning of May to the late July in 2006 for *C. indica*.

Plants were grown in a factorial experiment with six treatments: 3 levels of N (low 5, medium 30 and high 90 mg L^{-1} in 1:1 NH_4-N and NO_3-N) and 2 levels of P (low 3 and high 15 mg L^{-1}) with three replicates. Potassium was 25 mg K L^{-1} in all treatments, with part of this amount provided as KH_2PO_4 and the rest by adding K_2SO_4. All other macro- and micronutrients were kept at the same level in all treatments as described in Chapter 2.

At the transplanting time, seedlings of *C. indica* had 3-4 leaves approximately 150 mm in length. Seedlings of *S. validus* had 3-4 shoots per clumps approximately 100 mm in length. Each microcosm was planted with 6 seedlings of *C. indica* or 6 clumps of *S. validus*. The initial biomass of similar seedlings was recorded for each species. Microcosms were filled with the treatment nutrient solution to achieve a water depth of 0.02 m above the sand surface and the solution was renewed weekly. After the plant acclimation (2 weeks for *S. validus* and 3 weeks for *C. indica*), microcosms were filled with 8 L nutrient solution of each treatment to achieve a water depth of 0.05 m above the sand surface. Each microcosm was drained and refilled weekly. The hydraulic loading rate for designed treatment was 73 L m^{-2}.

5.2.3 Sampling and measurements

After nutrient treatments were imposed, volumes of influent and effluent solution were recorded, and samples were collected once per week.

Total shoot numbers and shoot heights were measured in each microcosm at approximately weekly intervals after plant acclimation. The shoot height was measured for *C. indica* from the base of the plants to the top of the longest leaf, and for *S. validus* from the base of the plants to the top of culms.

At day 72 after transplanting of *C. indica*, the photochemical efficiency (F_v/F_m) was measured on the recently developed leaves with a portable chlorophyll fluorometer (HANSATECH Instruments LTD, Kings Lynn, England). The photosynthetic rate, stomatal conductance and transpiration rate were measured with a portable photosynthesis system (LI-6400; LI-Cor, Lincoln, NE, USA). A young fully expanded leaf was inserted into the leaf chamber and three consecutive measurements were taken in the phytotron chamber condition at 25 °C, 80% relative humidity. The settings were: 1000 mL min^{-1} flow rate, 400 μmol mol^{-1} concentration of CO_2 and 1500 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR). The lamina length and maximum width were also recorded.

The plants were harvested at the beginning of the flowering after 10 (*S. validus*) and 13 weeks (*C. indica*) of nutrient treatments. The relevant methods for harvesting, sampling, measuring and calculating for pH, DO, nutrients and biomass in water, plant and soil samples, and the approaches of nutrient removal efficiency and mass balance were described in Chapter 2.

5.2.4 Statistical analyses

Two-way ANOVA was used to determine significance of nutrient treatment effects on relative growth rate, biometric characteristics, photochemical efficiency, photosynthetic rate, stomatal conductance, transpiration rate, biomass and resource allocation (percentage after log transformation). Differences in the proportions of shoots in each length category were analysed after data transformation into cumulative functions, using curve estimation with a logistic model. Three-way ANOVA was used to test significance of N and P treatments and different tissues on concentrations of N and P and N/P ratios. Percentage nutrient removal values were normalized using an arcsine transformation. Measurements of each parameter in water samples at different sampling date were not independent; therefore, difference in pH, concentration of dissolved oxygen, and nutrient removal efficiency were tested using a repeated measures two-way ANOVA. The sampling date was set as the repeated measure. Two-way ANOVA was used to determine significance of nutrient effects on biomass and total contents of N and P in various components of the microcosms. Least significant difference (LSD) was applied to test for significance between treatment means.

5.3 Results

5.3.1 Plant growth

The relative growth rate (RGR) for whole plant was significantly influenced by interaction between N and P treatments for *C. indica* (Figure 5.1), but not for *S. validus* (Figure 5.2). For *C. indica*, the lowest RGR was at low N treatment, regardless of P level, while the highest was at high N-high P treatment. The RGR was not significantly different among the high N-low P, medium N-low P and medium N-high P treatments. For *S. validus*, the lowest RGR was in the low N treatment and no significant difference in RGR was detected between the medium N and high N treatments. The RGR was significantly higher in the high P than in the low P treatment.

The tallest shoot height of *C. indica* increased with an increase in N and P additions (Figure 5.3), whereas the tallest shoot height of *S. validus* was influenced by N treatments, but no significant N × P interaction was detected (Figure 5.4). At harvest, the shoot height for *C. indica* and *S. validus* increased by 65% and 78% in medium N, and 104% and 90% in high N, respectively, compared with the low N treatment. The total number of green leaves, number and length of shoots, and number of flowering shoots of *C.*

indica were significantly affected by the interaction between N and P (Table 5.1). The highest number of green leaves, number and length of shoots, and number of flowering shoots were in the high N-high P treatment. The total number of shoots and flowering shoots of *S. validus* were significantly affected by N treatments. The total shoot number increased with an increase in N additions, whereas the highest total flowering shoot number was in the medium N treatments. The total shoot length was significantly affected by N and P interaction (Table 5.2). The proportion of shoots in each length category for *C. indica* was significantly influenced by interaction between N and P treatments (Figure 5.5). The proportion of shoots in each length category for *S. validus* was significantly influenced by N treatments (Figure 5.6). A significantly different pattern of length category was detected in the low N compared with the medium N and high N treatments, but not between the medium N and high N treatments.

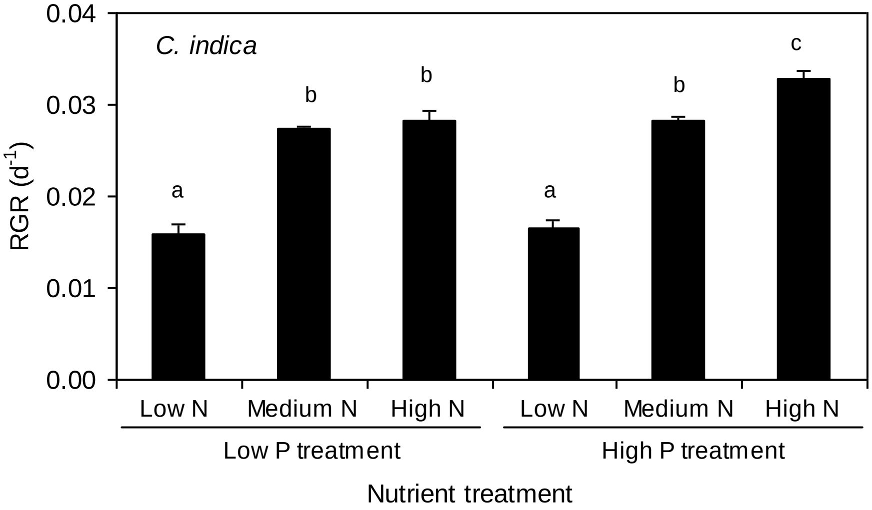

Figure 5.1 Relative growth rate (RGR) of *C. indica* influenced by interaction of N and P during 91 days of growth in the wetland microcosms. Bars (means + SE, n = 3) with different letters are significantly different based on LSD ($P \leq 0.05$).

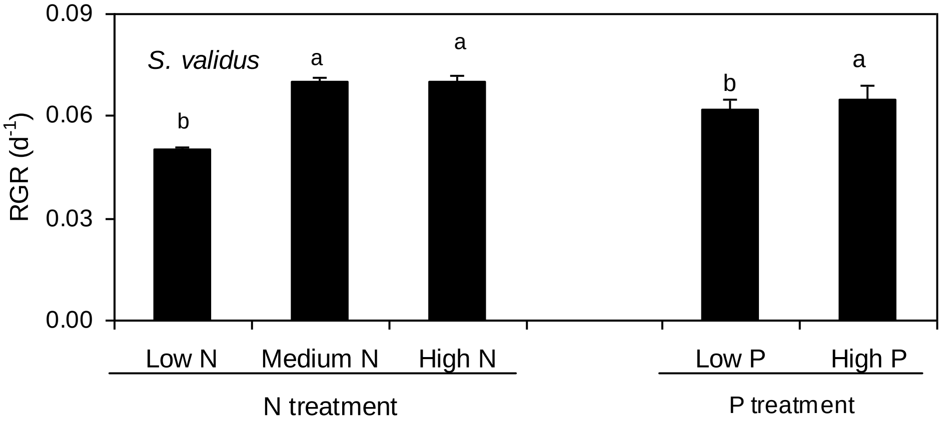

Figure 5.2 Relative growth rate (RGR) of *S. validus* influenced by N and P (N × P interaction was not significant) during 70 days of growth in the wetland microcosms. Bars (means + SE, n = 6, averaged over P treatments in the left 3 bars) with different letters are significantly different based on LSD ($P \leq 0.05$) and those (means + SE, n = 9, averaged over N treatments in the right 2 bars) with different letters are significantly different based on LSD ($P \leq 0.05$).

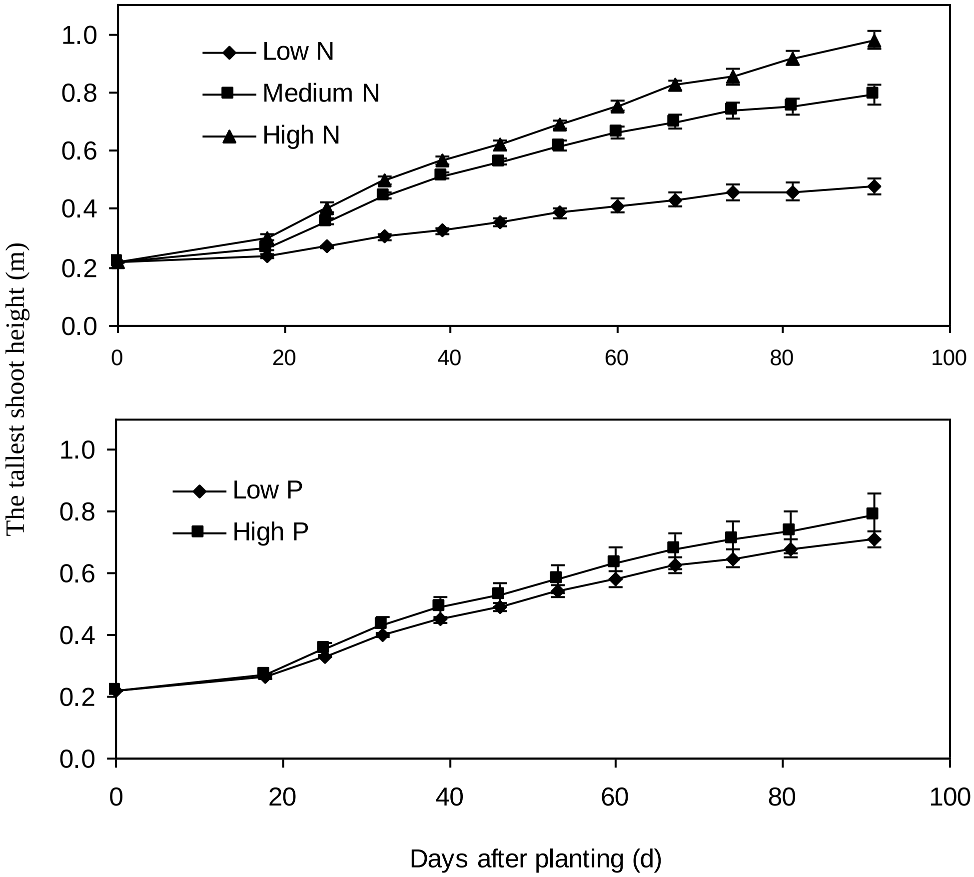

Figure 5.3 The height of the tallest shoot of *C. indica* influenced by N and P treatments during 91 days of growth in the wetland microcosms. No significant interaction was observed. Data (means ± SE, n = 6) in the top graph were averaged over P treatments and those (means ± SE, n = 9) in the bottom graph were averaged over N treatments

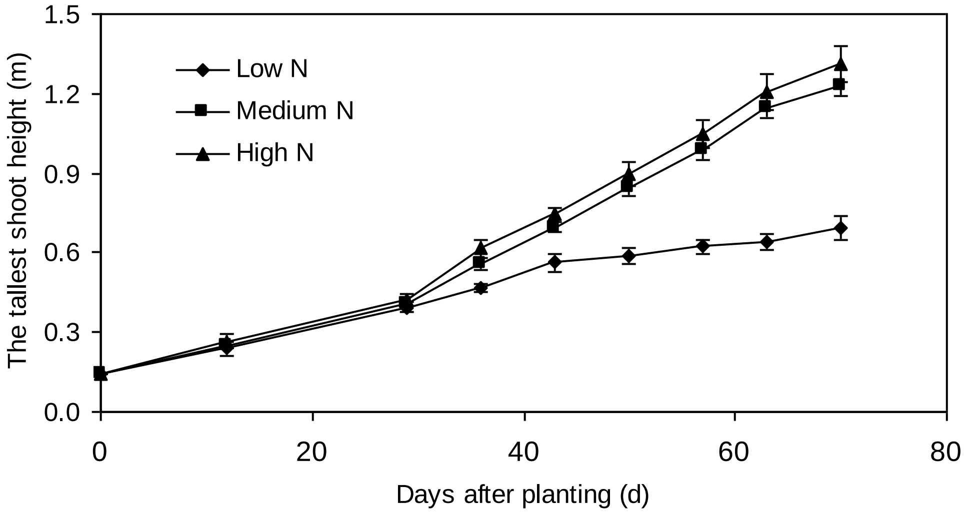

Figure 5.4 The height of the tallest shoot of *S. validus* influenced by N treatments during 70 days of growth in the wetland microcosms. Neither P effect nor N × P interaction was significant. Data (means ± SE, n = 6) were averaged over P treatments

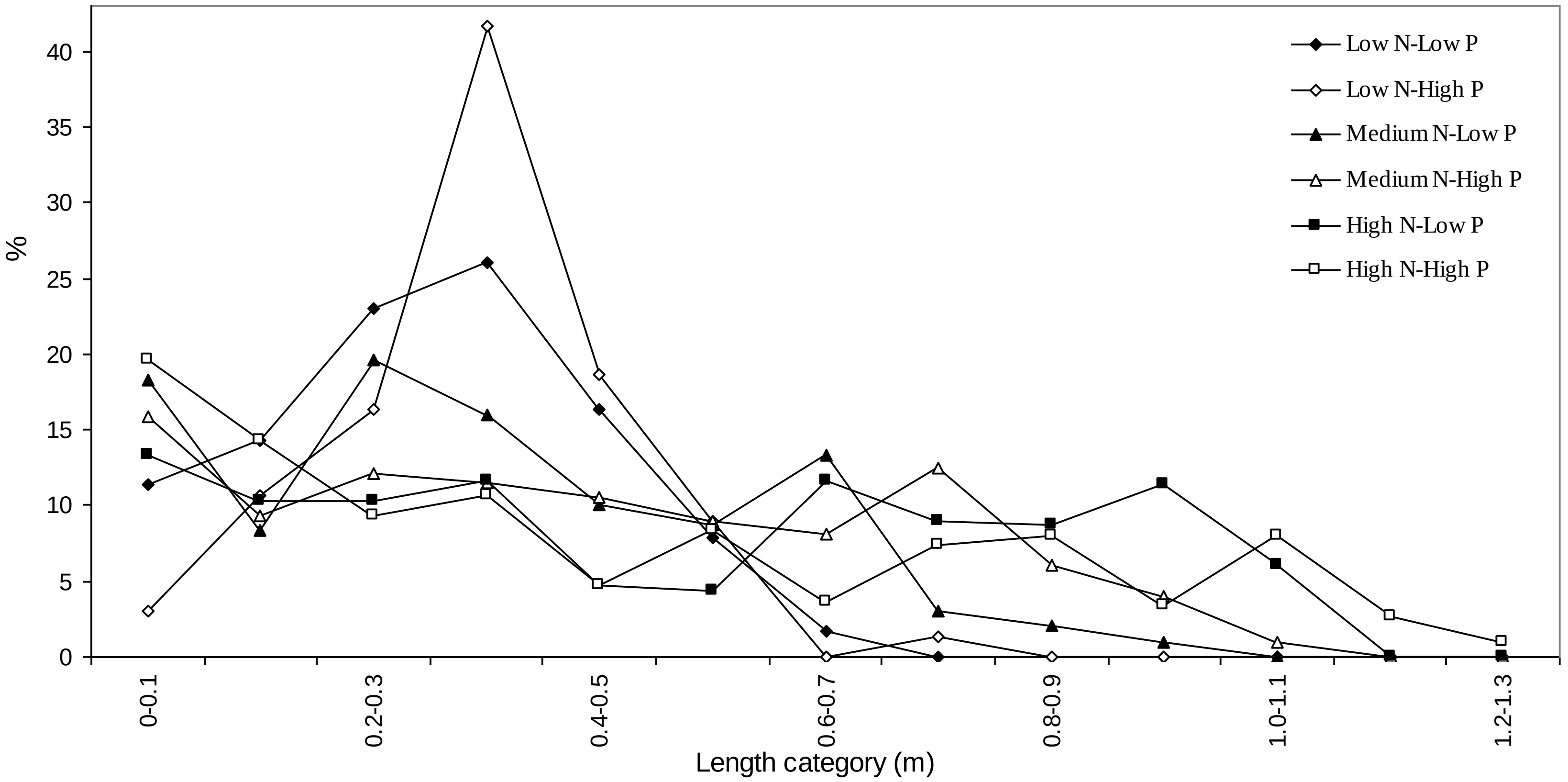

Figure 5.5 Mean proportion of *C. indica* shoots in each length category influenced by N and P treatments after 91 days of growth. Statistical analysis after the data transformation into cumulative functions, using curve estimation with a logistic model, revealed significant interaction of N and P treatments

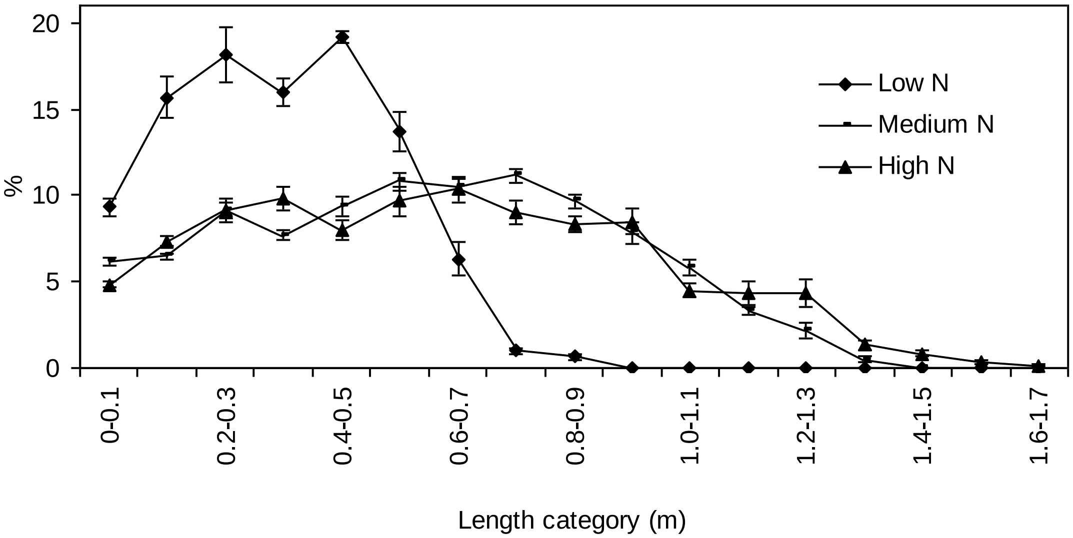

Figure 5.6 Mean proportion of *S. validus* shoots in each length category influenced by N treatments after 70 days of growth in the wetland microcosms. Statistical analysis after the data transformation into cumulative functions, using curve fitting with a logistic model, revealed significant difference among N treatments, but not between P treatments and no significant interaction was observed. Data (means ± SE, n = 6) in the graph were averaged over P treatments.

Table 5.1 The total number of green leaves, number and length of shoots, and number of flowering shoots of *C. indica* influenced by N and P treatments after 91 days of growth in the wetland microcosms

Treatment	Green leaf number (leaves m^{-2})	Total shoot number (shoots m^{-2})	Total shoot length (m m^{-2})	Flowering shoot number (shoots m^{-2})
Low P				
Low N	506d	209c	64d	0c
Medium N	639b	297b	109c	0c
High N	600bc	209c	109c	3b
High P				
Low N	512cd	203c	70d	0c
Medium N	697b	321b	139b	3b
High N	906a	382a	168a	30a
Source of variation				
N	***	***	***	***
P	***	**	***	***
N × P	**	**	**	***

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

Table 5.2 The total number and length of shoots, and number of flowering shoots of *S. validus* influenced by N and P levels after 70 days of growth in the wetland microcosms

Treatment	Total shoot number (shoots m^{-2})	Total shoot length (m m^{-2})	Total flowering shoot (shoots m^{-2})
Low P			
Low N	561c	192c	9b
Medium N	985b	616b	100a
High N	1006b	550b	9b
High P			
Low N	564c	198c	9b
Medium N	1097ab	631b	109a
High N	1203a	863a	73a
Source of variation			
N	***	***	**
P	NS	*	NS
N×P	NS	**	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

5.3.2 Photochemical efficiency and photosynthetic rate for *C. indica*

For *C. indica*, the photochemical efficiency as well as lamina length and width significantly increased with an increase in N application. The N and P treatments significantly influenced the photosynthetic rate, but not the stomatal conductance and transpiration rate. No significant interaction between N and P treatments was detected (Table 5.3). The photochemical efficiency (F_v/F_m) significantly increased from 0.84 to 0.85 with an increase in N additions. The photosynthetic rate increased from 13 to16 μmol m^{-2} s^{-1} in the low P levels and from 14 to 20 μmol m^{-2} s^{-1} in the high P levels with an increase in N applications, but significant difference was only from low to medium N levels at the high P levels. The stomatal conductances ranged between 0.68 and 1.05 mol m^{-2} s^{-1}. The transpiration rates varied from 6.49 to 7.54 mmol m^{-2} s^{-1}.

Table 5.3 Effects of N and P treatments on photochemical efficiency, photosynthetic rate, lamina length and maximum width of *C. indica* at day 72 after transplanting plants in the wetland microcosms

Treatment	Photochemical efficiency (Fv/Fm)	Photosynthetic rate (μmol m^{-2} s^{-1})	Lamina length (m $leaf^{-1}$)	Lamina width (m $leaf^{-1}$)
Low P				
Low N	0.844b	13.2b	0.22c	0.06c
Medium N	0.847ab	15.5b	0.32b	0.12b
High N	0.855a	15.9b	0.38a	0.14a
High P				
Low N	0.843b	14.0b	0.22c	0.07c
Medium N	0.851a	20.2a	0.34b	0.12b
High N	0.853a	20.2a	0.39a	0.15a
Source of variation				
N	***	*	***	***
P	NS	*	NS	NS
N×P	NS	NS	NS	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. *** Significant at $P < 0.001$.

5.3.3 Biomass

The biomass was significantly influenced by interaction between N and P treatments, except for flowers of *C. indica* (Tables 5.4) and rhizome of *S. validus* (Tables 5.5). The highest total biomass was in the high N-high P treatment, whereas the lowest in the low N treatment, regardless of P level. The below-ground biomass was negatively influenced by the high N-low P treatment, and *S. validus* was more affected than *C. indica*.

Table 5.4 Effects of N and P treatments on biomass (g m^{-2}) in different parts of *C. indica* after 91 days of growth in the wetland microcosms

Treatment	Total	Leaves	Stems	Flowers	Rhizomes	Roots
Low P						
Low N	551c	111d	74d	0b	255c	111d
Medium N	1599b	427c	267c	0b	657a	248b
High N	1724b	623b	421b	13a	460b	207c
High P						
Low N	578c	121d	84d	0b	251c	121d
Medium N	1712b	488c	340bc	2b	622a	261b
High N	2590a	937a	720a	25a	606a	310a
Source of variation						
N	***	***	***	**	***	***
P	***	***	***	NS	NS	***
N × P	***	***	**	NS	*	***

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

Table 5.5 Effects of N and P treatments on biomass (g m^{-2}) of different parts of *S. validus* after 70 days of plant growth in the wetland microcosms

Treatment	Total	Above-ground	Below-ground	Rhizome	Root
Low P					
Low N	108d	55c	52c	23b	30b
Medium N	422bc	296b	126ab	76a	50b
High N	345c	247b	98bc	58ab	40b
High P					
Low N	112d	58c	53c	22b	31b
Medium N	483ab	309b	175a	92a	82a
High N	581a	455a	126ab	89a	37b
Source of variation					
N	***	***	***	***	***
P	*	**	NS	NS	NS
N×P	*	**	NS	NS	*

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

5.3.4 Biomass allocation

The ratio of above-ground biomass to below-ground (rhizome and root) biomass (A/B), ratio of the biomass of root-supported tissue (above-ground and rhizomes) to root biomass (S/R) and percent biomass allocation to different plant tissues (except biomass allocation to rhizomes) for *S. validus* (Tables 5.7) were significantly influenced by interaction between N and P, but not for *C. indica* (Tables 5.6). The allocation to above-ground tissues increased with an increase in N applications, while allocation to below-ground decreased with an increase in N additions, regardless of P level. For *C. indica*, the highest percent biomass was allocated to leaves (36%) in the high N treatment, and to rhizomes in the low N (45%) and medium N (39%) treatments. The percent biomass allocation to above-ground tissues in the high N treatment was twice that in the low N treatment. For *S. validus*, over 52% biomass was allocated to above-ground tissues.

Table 5.6 Ratio of above-ground biomass to below-ground (rhizome and root) biomass (A/B), ratio of the biomass of root-supported tissue (above-ground and rhizomes) to root biomass (S/R) and percent biomass allocation to different tissues of *C. indica* influenced by N and P treatments after 91 days of growth in the wetland microcosms

Treatment	A/B	S/R	Leaf %	Stem %	Rhizome %	Root %	Flower %
Low P							
Low N	0.51e	4.0c	20.3c	13.6c	46.2a	19.9a	0b
Medium N	0.77d	5.5b	26.7b	16.8b	41.0b	15.5b	0b
High N	1.59b	7.4a	36.4a	24.5a	26.8d	12.0c	0.28b
High P							
Low N	0.55e	3.8c	21.0c	14.6c	43.5ab	21.0a	0b
Medium N	0.94c	5.6b	28.5b	19.9b	36.3c	15.2b	0.11b
High N	1.83a	7.4a	36.1a	27.6a	23.4d	12.0c	0.92a
Source of variation							
N	***	***	***	***	***	***	*
P	*	NS	NS	***	**	NS	NS
N × P	NS	NS	NS	NS	NS	NS	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

Table 5.7 Ratio of above-ground biomass to below-ground (rhizome and root) biomass (A/B), ratio of the biomass of root-supported tissue (above-ground and rhizomes) to root biomass (S/R) and percent biomass allocation of *S. validus* to different components influenced by N and P treatments after 70 days of growth in the wetland microcosms

Treatment	A/B	S/R	Shoot %	Rhizome %	Root %
Low P					
Low N	1.05c	2.7c	51.6d	21.1a	27.3a
Medium N	2.34bc	7.8a	70.8bc	17.6ab	11.6c
High N	2.52b	7.6a	71.7b	16.7ab	11.6c
High P					
Low N	1.10c	2.6c	52.0d	20.2a	27.8a
Medium N	1.77c	4.9b	64.2c	18.8ab	17.0b
High N	3.62a	7.3a	78.4a	15.1b	6.5d
Source of variation					
N	***	***	***	*	***
P	NS	**	NS	NS	NS
N×P	**	***	*	NS	***

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

5.3.5 Tissue concentrations of C, N and P

The concentrations of C, N and P in the plant tissues and N/P ratios were significantly affected by interaction between N and P treatments (Tables 5.8 and 5.9). In general, the N/P ratios increased significantly with an increase in N addition and decreased with an increase in P addition. For *C. indica*, the highest concentration of N was in green leaves, followed by roots, rhizomes and stems. The highest concentration of P was in green leaves when the applied N was high and in stems when the N addition was low. The highest concentrations of N occurred in high N-low P treatment. Similarly, the highest concentrations of P occurred in low N-high P treatment. For *S. validus*, the highest concentrations of N and P were in the shoots, whereas that of C was in the roots among the different plant tissues. The concentrations of N and P markedly increased with an increase in N and P applications. The highest concentration of N occurred in the high N and low P treatment, whereas the highest concentrations of P occurred in the high N and high P treatment.

Table 5.8 The concentrations (g kg^{-1}) of N, P and C, and N/P ratios in the various plant tissues of *C. indica* influenced by N and P treatments after 91 days of growth in the wetland microcosms

Element	Tissue	Low P			High P		
		Low N	Medium N	High N	Low N	Medium N	High N
N	Stem	8.1d	10.9c	26.2a	8.3d	9.7c	18.3b
	leaf	17.3d	22.0c	30.8a	18.8d	22.7c	28.8b
	Rhizome	6.0d	12.3c	27.0a	5.9d	10.4c	23.8b
	Root	8.3e	13.1c	25.7a	7.8e	11.4d	23.4b
P	Stem	4.8b	1.3d	1.0d	7.1a	4.9b	3.2c
	leaf	3.0c	1.6d	1.4d	4.9a	4.2b	4.2b
	Rhizome	3.0c	1.2e	1.0e	4.5a	3.6b	2.5d
	Root	2.1c	1.2d	1.3d	3.8a	3.8a	3.3b
C	Stem	366c	392b	393a	355d	391b	401a
	leaf	393c	408b	413ab	402b	409b	420a
	Rhizome	368b	388a	387a	362b	388a	391a
	Root	381c	401a	400a	371d	393b	405a
N/P ratio	Stem	1.7d	8.2b	26.7a	1.2d	2.0d	5.7c
	leaf	5.8cd	14.2b	22.0a	3.9d	5.5cd	7.0c
	Rhizome	2.0c	10.3b	28.3a	1.3c	2.9c	9.5b
	Root	4.0d	11.0b	19.6a	2.1f	3.0e	7.2c

Source of variation

	N treatment (N)	P treatment (P)	Tissue (T)	N×P	N×T	P×T	N×P×T
N	***	***	***	***	***	***	***
P	***	***	***	***	***	***	***
C	***	NS	***	***	***	**	*
N/P ratio	***	***	***	***	***	*	***

Means with different letters within rows are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

Table 5.9 The concentrations (g kg^{-1}) of N, P and C, and N/P ratios in the various plant tissues of *S. validus* influenced by N and P treatments after 70 days of growth in the wetland microcosms

Element	Tissue	Low P			High P		
		Low N	Medium N	High N	Low N	Medium N	High N
N	Shoot	12.4e	21.7c	32.2a	10.9e	15.5d	29.2b
	Rhizome	6.9d	16.0b	29.6a	6.4d	10.1c	27.0a
	Root	7.3d	13.7b	21.5a	7.1d	10.0c	22.1a
P	Shoot	3.2b	2.3c	2.1c	3.9b	3.6b	4.5a
	Rhizome	2.9c	2.2d	2.1d	3.4bc	3.7ab	4.3a
	Root	1.6c	1.6c	1.7c	2.1c	2.9b	3.5a
C	Shoot	364c	399a	400a	362c	383b	402a
	Rhizome	388b	405a	410a	388b	393b	403a
	Root	391b	409a	415a	403ab	400ab	413a
N/P	Shoot	3.8d	9.4b	15.5a	2.8e	4.3d	6.5c
	Rhizome	2.4d	7.4b	14.2a	1.9d	2.7d	6.3c
	Root	4.6d	8.6b	12.9a	3.5e	3.4e	6.3c

Source of variation

	N treatment (N)	P treatment (P)	Tissue (T)	N×P	N×T	P×T	N×P×T
N	***	***	***	***	***	*	NS
P	*	***	***	***	**	NS	NS
C	***	*	***	**	***	NS	NS
N/P	***	***	***	***	***	*	*

Means with different letters within rows are significantly different based on LSD ($P \le 0.05$).

NS: not significant. * Significant at $P \le 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

5.3.6. Nutrient accumulation and use efficiency

The nutrient accumulation in tissues and use efficiency were significantly affected by interaction of N and P, except for N-use efficiency (Tables 5.10 and 5.11). In general, the nutrient accumulation increased with an increase in N and P applications, but N use efficiency decreased with an increase in N additions and increased with an increase in P applications. Phosphorus use efficiency for *C. indica* increased with the increasing N levels and decreased with the increasing P levels, and for *S. validus* increased with an increase in N additions at the low P treatment, but decreased with an increase in N applications at the high P treatment.

Table 5.10 Effects of N and P treatments on nutrient accumulation in plant tissues and nutrient use efficiency of *C. indica* after 91 days of growth in the wetland microcosms

Treatment	Nutrient uptake ($mg\ m^{-2}\ d^{-1}$)		Nutrient accumulation ($g\ m^{-2}$)		Nutrient use efficiency (g dry weight g^{-1} N or P)	
	N	P	N	P	N	P
Low P						
Low N	41d	16e	3.7d	1.46e	148a	367c
Medium N	230c	21d	20.9c	1.88d	76b	851b
High N	502b	20d	45.6b	1.78de	38c	963a
High P						
Low N	43d	28c	3.9d	2.53c	148a	229e
Medium N	246c	74b	22.4c	6.69b	77b	256de
High N	659a	92a	60.0a	8.42a	43c	308cd
Source of variation						
N	***	***	***	***	***	***
P	***	***	***	***	NS	***
N×P	***	***	***	***	NS	***

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. *** Significant at $P < 0.001$.

Table 5.11 Effects of N and P treatments on nutrient accumulation in plant tissues and nutrient use efficiency of *S. validus* after 70 days of growth in the wetland microcosms

Treatment	Nutrient uptake ($mg\ m^{-2}\ d^{-1}$)		Nutrient accumulation ($g\ m^{-2}$)		Nutrient use efficiency (g dry weight g^{-1} N or P)	
	N	P	N	P	N	P
Low P						
Low N	14d	4e	1.0d	0.28d	113a	379b
Medium N	114bc	13c	8.0bc	0.89c	52c	467a
High N	149b	10cd	10.4b	0.69c	33d	498a
High P						
Low N	12d	5de	0.9d	0.36d	125a	314bc
Medium N	91c	24b	6.4c	1.69b	75b	285c
High N	234a	36a	16.4a	2.54a	36d	228c
Source of variation						
N	***	***	***	***	***	NS
P	NS	***	NS	***	**	***
N×P	**	***	**	***	NS	**

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. . ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

5.3.7 Nutrient allocation

For *C. indica*, the allocation of N to the different plant tissues (except to flowers) was significantly affected by N treatments, and the allocation of N to leaves and rhizomes was significantly affected by P treatments. The significant interaction between N and P treatments was detected in the allocation of N to leaves (Table 5.12). The pattern of increasing N allocation to stems corresponded to increasing N availability, whereas a decreasing pattern was revealed in roots, regardless of P levels. The allocation of P to leaves was significantly affected by N and P treatments; in contrast, the allocation of P to stems was significantly affected by P treatments, and the allocation of P to rhizomes was significantly affected by N treatments. No significant difference was found in the allocation of P to roots and flowers. The significant interaction between N and P treatments was detected in the allocation of P to stems (Table 5.13). The pattern of increasing P allocation to leaves corresponded to increasing N availability, whereas a decreasing pattern was observed in rhizomes, regardless of P levels.

For *S. validus*, the significant interactive effect of N and P treatments on nutrient allocation to the plant tissues (except allocation of N or P to rhizomes) was detected (Table 5.14). The patterns of increasing N and P allocation to above-ground tissues corresponded to increasing N availability under the high P levels, whereas a decreasing pattern was observed in rhizomes and root.

Table 5.12 Effects of N and P treatments on the allocation of N to different tissues of *C. indica* after 91 days of growth in the wetland microcosms

Treatment	Stem %	Leaf %	Rhizome %	Root %	Flower %
Low P					
Low N	11b	51a	16c	22a	0a
Medium N	13b	38b	34a	15b	0a
High N	24a	40b	25b	11b	0.3a
High P					
Low N	13b	52a	13c	22a	0a
Medium N	14b	48a	25b	13b	0.3a
High N	22a	43ab	22b	12b	1.0a
Source of variation					
N	***	***	***	***	NS
P	NS	**	**	NS	NS
N×P	NS	*	NS	NS	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

Table 5.13 Effects of N and P treatments on the allocation of P to different tissues of *C. indica* after 91 days of growth in the wetland microcosms

Treatment	Stem %	Leaf %	Rhizome %	Root %	Flower %
Low P					
Low N	22b	21d	42a	15a	0a
Medium N	17c	35b	33bd	15a	0a
High N	21bc	48a	16e	14a	1.1a
High P					
Low N	22b	22d	38ab	18a	0a
Medium N	25ab	29c	31d	15a	0.1a
High N	27a	44a	16e	12a	1.2a
Source of variation					
N	NS	***	***	NS	NS
P	***	*	NS	NS	NS
N×P	*	NS	NS	NS	NS

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. *** Significant at $P < 0.001$.

Table 5.14 Effects of N and P treatments on the allocations of N and P to plant tissues of *S. validus* after 70 days of growth in the wetland microcosms

Treatment	N allocation			P allocation		
	Shoot %	Rhizome %	Root %	Shoot %	Rhizome %	Root %
Low P						
Low N	62c	17a	21b	61c	23a	16ab
Medium N	78ab	14a	8.0d	74b	17bc	8.4c
High N	76ab	16a	8.2d	73b	17bc	9.5c
High P						
Low N	60c	16a	24a	61c	22a	17a
Medium N	73b	14a	13c	66c	20ab	14b
High N	81a	14a	5.0e	80a	15c	5.1d
Source of variation						
N	***	NS	***	***	**	***
P	NS	NS	NS	NS	NS	NS
N×P	*	NS	**	*	NS	***

Means with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

5.3.8 Nutrient removal efficiency

There was a significant interactive effect of N and P treatments on the removal efficiencies of NH_4-N, NO_x-N and PO_4-P in the wetland microcosms, except on the removal of NO_x-N with *S. validus* (Table 5.15). The lowest removal efficiency of NH_4-N and NO_x-N was in the high N-low P treatments, whereas the lowest removal efficiency of PO_4-P was in the low N-high P treatment. The removal efficiencies of nutrients (except NO_x-N removal with *C. indica*) were significantly influenced by plant growth in the microcosms during the experiment. Variations of nutrient removal efficiencies from week 4 to 13 after planting as influenced by the high N-low P and high N-high P treatments in the wetland microcosms were presented in Figures 5.7 and 5.8. For *S. validus*, the removal efficiency of NH_4-N was relatively higher compared with that of NO_x-N, except in the low N treatments. A negative removal of NO_x-N was observed in the high N-low P treatment at the last week of sampling (Figure 5.8). The lowest removal efficiency of PO_4-P was at week 7 after nutrient treatments (except in the high N and high P treatment) compared to the other weeks.

Table 5.15 Nutrient removal efficiency (%) influenced by N and P treatments in wetland microcosms planted with *C. indica* and *S. validus*, with two-way repeated measures ANOVA

Treatment	*C. indica*			*S. validus*		
	NH_4-N	NO_x-N	PO_4-P	NH_4-N	NO_x-N	PO_4-P
Low P						
Low N	98.7±0.5ab	98.2±0.2a	88.7±1.8c	97.3±1.2ab	97.3±0.5a	73.5±3.0a
Medium N	99.9±0.0a	99.9±0.0a	94.7±1.2b	92.2±3.2b	66.1±6.6b	80.3±3.5a
High N	89.8±0.4c	69.9±1.4b	95.9±1.0ab	58.7±3.3d	21.3±6.1c	77.4±4.4a
High P						
Low N	99.9±0.1a	99.4±0.2a	63.2±0.9d	98.6±0.4ab	99.4±0.5a	48.3±1.8b
Medium N	99.9±0.0a	90.5±9.2a	92.8±1.1b	98.7±0.7a	86.4±7.5a	70.8±2.2a
High N	98.3±0.7b	88.9±1.2a	98.5±0.4a	72.3±1.7c	30.7±3.0c	76.2±3.1a
Source of variation						
Between-subject						
N	***	***	***	***	***	***
P	***	NS	***	**	*	***
N ×P	***	**	***	*	NS	*
Within-subject						
Date	***	NS	***	***	***	***
Date ×N	***	NS	***	***	***	***
Date ×P	***	NS	***	NS	NS	***
Date ×N ×P	***	NS	NS	***	NS	NS

Means (± SE, for *C. indica*, n = 30; for *S. validus*, n = 24) with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

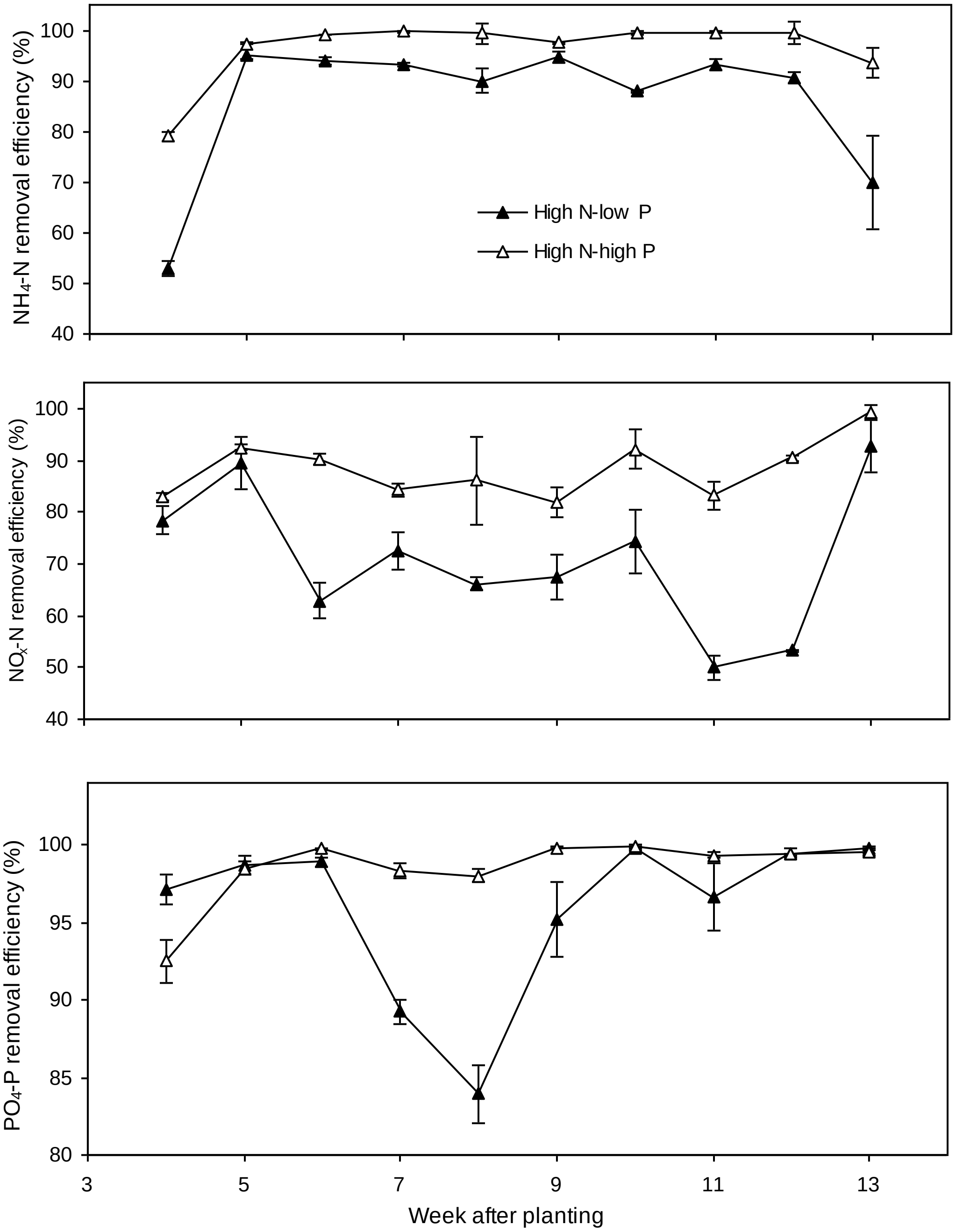

Figure 5.7 Variations in nutrient removal efficiencies (means ± SE, n = 3) from week 4 to 13 after planting as influenced by the high N-low P and high N-high P treatments in the wetland microcosms planted with *C. indica*

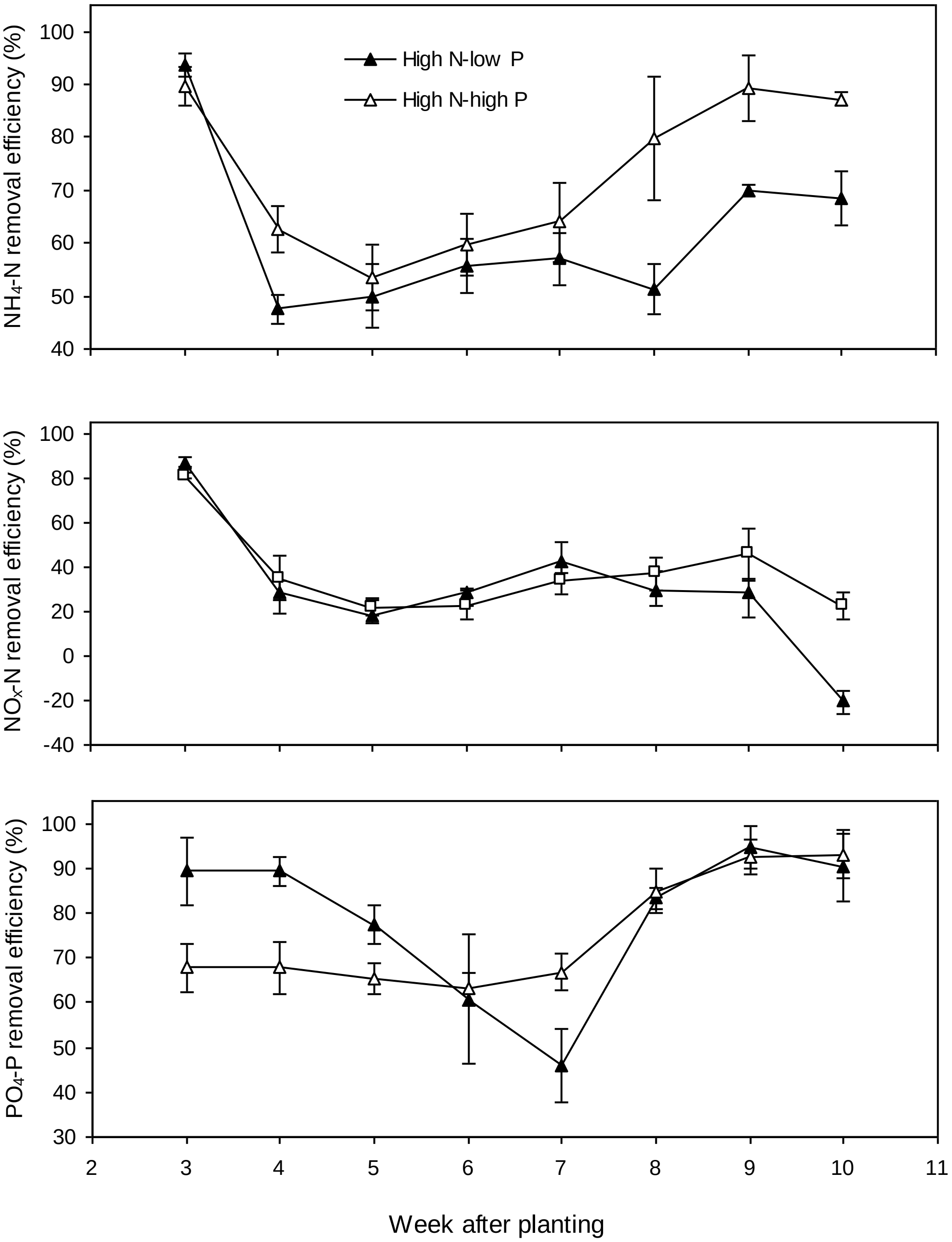

Figure 5.8 Variations in nutrient removal efficiencies (means ± SE, n = 3) from week 3 to 10 after planting as influenced by the high N-low P and high N-high P treatments in the wetland microcosms planted with *S. validus*

5.3.9 pH and dissolved oxygen (DO) in effluents

The pH and concentrations of dissolved oxygen (DO) in the effluents were not influenced by the interaction, except DO with *S. validus* (Table 5.16). In general, the values of pH and concentrations of dissolved oxygen (DO) decreased with an increase in the N additions.

Table 5.16 The values of pH and concentrations (mg L^{-1}) of dissolved oxygen (DO) in the effluents influenced by N and P treatments in wetland microcosms planted with *C. indica* and *S. validus*

Treatment	*C. indica*		*S. validus*	
	pH	DO	pH	DO
Low P				
Low N	4.9ab	5.5a	5.7ab	4.7a
Medium N	4.5bc	4.6ab	5.5b	4.1ab
High N	3.6d	4.3b	3.9c	3.6bc
High P				
Low N	5.1a	5.6a	5.9a	4.2a
Medium N	4.3c	5.0ab	6.0a	2.6c
High N	3.7d	4.6ab	3.8c	3.9ab
Source of variation				
N	***	**	***	*
P	NS	NS	*	NS
N×P	NS	NS	NS	*

Values with different letters within columns are significantly different based on LSD ($P \leq 0.05$).

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.

5.3.10 Nutrient partitioning in the water-substratum-plant continuum

5.3.10.1 N and P in the effluents

The recovery percentages of N in the effluents increased with an increase in the N applications and decreased with the P additions, whereas that of P in the effluents decreased with an increase in N applications and increased with an increase in the P additions (except in the high N-high P treatment for *C. indica* and in the medium N-low P treatment for *S. validus*). Compared with other treatments, the recovery of N in the effluent was relatively high in the high N-low P treatment, whereas that of P was relatively high in the low N-high P treatment (Tables 5.17, 5.18, 5.19 and 5.20).

5.3.10.2 N and P uptake by plants

N removal by plant uptake was relatively high; in contrast, P removal was low. For *C. indica*, the highest percentage of N (94%) and P (77%) removal by plants was in the medium N-high P and the medium N-low P treatments, respectively, whereas the lowest percentage of N (61%) and P (21%) removal by plants was in the high N-low P and low N-high P treatments, respectively (Tables 5.17 and 5.18). For *S. validus*, the highest percentage of N (42%) and P (50%) removal by plants was in the medium N-low P treatments, whereas the lowest percentage of N (30%) and P (4%) removal by plants was in the high N-high P and low N-high P treatments, respectively (Tables 5.19 and 5.20).

5.3.10.3 N and P in the substrate

There was a significant interactive effect of N and P treatments on the contents of total N and P in the substrate for *C. indica* (Figure 5.9), but not for *S. validus* (data not shown). The percentage of P storage was relatively high compared with N storage by the substrate. For *C. indica*, the highest contents of total N and P in the substrate were in the high N-low P and low N-high P treatments, respectively. The N release from the substrate was observed in the low N treatments during the experiment because the total N content in the substrate was lower than initial. The percentage of P storage by substrate (46%) was higher than P uptake by plants (21%) in the low N-high P treatment (Tables 5.17 and 5.18). For *S. validus*, the content of total P in the substrate was significantly higher in the high P (0.0458 g kg^{-1}) than in the low P (0.0316 g kg^{-1}) treatments, but no significant difference in the content of total N in the substrate was detected. The percentage of P storage (25-54%) was relatively high compared with N storage (1-31%) by the substrate. The percentage of P storage by substrate was higher compared with P uptake by plants, except in the medium- and high N-low P treatments (Tables 5.19 and 5.20).

Table 5.17 Nitrogen mass balance (% of input) influenced by N and P treatments in the microcosms planted with *C. indica* (The inputs of influent N were 0.41, 2.61 and 8.25 g in the low, medium and high N treatments, respectively)

Treatment	Input		Output			
	Influent	Sand release	Effluent	Plant uptake	Sand storage	Other losses
Low P						
Low N	89	11	4	87		9
Medium N	100		7	88	3	1
High N	100		22	61	1	16
High P						
Low N	87	13	3	90		7
Medium N	100		6	94	2	1
High N	100		7	80	1	12

Table 5.18 Phosphorus mass balance (% of input) influenced by N and P treatments in the microcosms planted with *C. indica* (The inputs of influent P were 0.27 and 1.33 g in the low and high P treatments, respectively)

Treatment	Input	Output			
	Influent	Effluent	Plant uptake	Sand storage	Other losses
Low P					
Low N	100	9	60	21	10
Medium N	100	4	77	13	6
High N	100	3	73	21	3
High P					
Low N	100	30	21	46	3
Medium N	100	6	55	37	2
High N	100	1	69	27	2

Table 5.19 Nitrogen and phosphorus mass balance (% of input) influenced by N and P treatments in the microcosms planted with *S. validus* (The inputs of influent N were 0.30, 2.08 and 6.07 g in the low, medium and high N treatments, respectively)

Treatment	Input	Output			
	Influent	Effluent	Plant uptake	Sand storage	Other losses
Low P					
Low N	100	3	35	30	32
Medium N	100	15	42	4	39
High N	100	47	19	1	33
High P					
Low N	100	1	32	31	35
Medium N	100	3	34	4	59
High N	100	38	30	1	31

Table 5.20 Phosphorus mass balance (% of input) influenced by N and P treatments in the microcosms planted with *S. validus* (The inputs of influent P were 0.20 and 0.98 g in the low and high P treatments, respectively)

Treatment	Input	Output			
	Influent	Effluent	Plant uptake	Sand storage	Other losses
Low P					
Low N	100	20	16	54	10
Medium N	100	15	50	25	10
High N	100	17	39	39	6
High P					
Low N	100	39	4	53	4
Medium N	100	22	19	54	5
High N	100	18	28	45	9

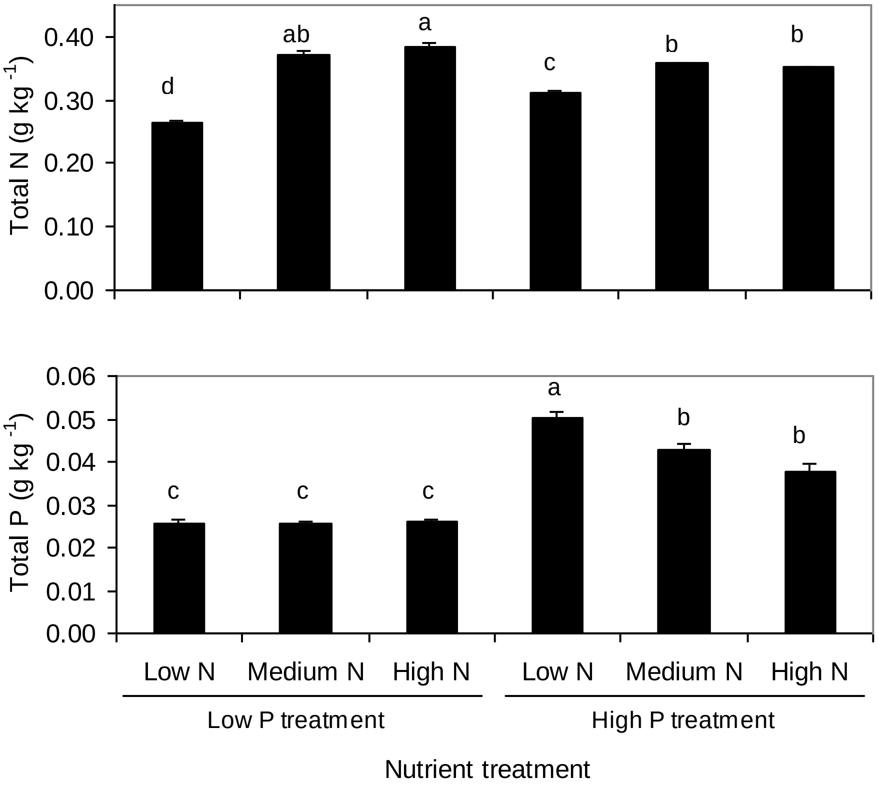

Figure 5.9 Contents of total N and P in the substrate influenced by interactions of N and P treatments in the wetland microcosms planted with *C. indica* after 91 days of growth. Bars (means + SE, n = 3) with different letters are significantly different based on LSD ($P \leq 0.05$)

5.4 Discussion

5.4.1 Plant growth and physiological response

The present results indicated that proper N and P supply was required for the intensive growth of plants, whereas imbalanced nutrient supply could not enhance the plant growth. Similarly, Ulrich and Burton (1988) also found that interaction between N (NO_3-N) and P supply strongly affected growth of the wetland plants in sand culture experiments. However, that interactive effect between N and P supply was not observed by Cary and Weerts (1984a) and Romero et al. (1999) in hydroponic culture experiments. Vojtíšková et al. (2004) pointed out that the simple comparison of growth reactions of wetland plants under nutrient enrichment in different studies is difficult, because of different nutritional conditions. Not only the total amount of added nutrient, but also the N/P ratio, the proportion of NH_4-N versus NO_3-N, and the presence or absence of organic matter in the substrate, which dramatically change the flooded substrate properties, should be considered (Barko and Smart, 1983; Barko et al., 1991; Johnson, 1991).

For *S. validus*, although the significant difference was not observed between medium N and high N treatments (except for root biomass between the medium N-high P and the high N-high P treatments), the below-ground, rhizome and root biomass were, to some extent, negatively influenced by the high N treatment. In addition, the above-ground biomass was also negatively affected by the high N-low P treatment. The lower pH (Table 5.16) in the solution could have influenced plant growth. Brix et al. (2002) have observed that the growth of *Typha latifolia* almost completely stopped at pH 3.5 in the solution culture experiments. The growth inhibition at low pH was probably due to a reduced nutrient uptake and a consequential limitation of growth by nutrient stress. In the present study, pH in the solution with *C. indica* was lower compared to *S. validus,* but only affected the below-ground growth in the high N-low P treatments. Therefore, *C. indica* could have greater capacity to tolerate acidic conditions compared with *S. validus*. Several species of wetland plants grow on the margins of lakes with an open water pH of 3 and below, and are obviously exposed to extremely acidic conditions around the roots

(Fyson, 2000). *Typha latifolia* could grow in wetlands receiving acid mine drainage with pH levels at 3.4-3.5 (Wieder et al., 1990). These species must possess a tolerance or an avoidance strategy for low pH conditions.

Many wetland plants growing in soil are able to create large gradients in oxygen concentration, pH and nutrient concentrations around the roots in the rhizosphere (Sorrell and Orr, 1993). The common occurrence of *Typha latifolia* in very acidic areas was likely due to the plant's ability to modify pH conditions in the rhizosphere (Brix et al., 2002). In another aspect, *S. validus* might have suffered the ammonia toxicity at the high N treatment (90 mg L^{-1} in 1:1 NH_4-N and NO_3-N). Ammonia has been shown to be toxic to a variety of wetland plant species. For instance, Hill et al. (1997) found that the growth of *Schoenoplectus acutus* var. *acutus* was negatively affected by ammonia. Clarke and Baldwin (2002) also observed that the growth of *Schoenoplectus tabernaemontani* was negatively affected by ammonium in the concentrations of 45 mg NH_4-N L^{-1}.

For *C. indica*, the photochemical efficiency significantly increased with an increase in N additions in the present study. Some studies have shown that N deficiency decreases the photochemical efficiency (Nunes et al., 1993; Verhoeven et al., 1997), whereas others have demonstrated no substantial changes in the photochemical efficiency, suggesting that N deficiency had little effect on the photochemistry (Khamis et al., 1990; Lu and Zhang, 2000). A significantly lower photosynthetic rate was observed in the low N treatments compared with the other treatments. Similarly, Longstreth and Nobel (1980) observed that the net rate of CO_2 uptake for leaves of *Gossypium hirsutum* L. was reduced when the plants were grown at low concentrations of nitrate, phosphate and potassium. There was little effect on stomatal conductance. Loustalot et al. (1950) found that the N concentration of 1.98 g kg^{-1} in leaves was close to the critical N level for photosynthesis in the tung seedlings. In the present study, the concentrations of N in the leaves of *C. indica* at the low N treatments were lower than the above mentioned critical N concentration. The photosynthetic capacity of leaves is related to the N concentration primarily because the majority of leaf N are proteins of the Calvin cycle and thylakoids (Evans, 1989). However, the photosynthetic capacity and leaf N concentration should only be closely correlated when other nutrients were available in non-limiting quantities (Reich and Schoettle, 1988). Plants grown at a low nutrient supply have a decreased rate of photosynthesis per unit leaf mass, with growth being hampered more than photosynthesis (Pooter and Nagel, 2000). In the present study, the growth performance was related to the physiological responses. The significantly lower growth rate was correlated to the significantly lower photosynthetic rate in the low nutrient availability, resulting in allocation of more biomass to the below-ground tissues.

5.4.2 N and P in plant tissues

In general, the concentrations of N and P in the plant tissues increased with an increase in N and P supply. The N/P ratios increased significantly with the increasing N and decreased with the increasing P supply in the present study (Tables 5.8 and 5.9). Koerselman and Meuleman (1996) studied the N/P ratios of various types of wetland vegetation known to be limited by either N or P supply. They suggested that the N/P ratio of the vegetation directly indicates which of the two nutrients, N or P, is limiting. An N/P ratio greater than 16 on a weight basis should indicate P limitation on a community level, whereas an N/P ratio less than 14 should be indicative of N limitation. In the present study, the N/P ratios in plant tissues for *C. indica* were greater than 16 in the high N-low P treatment, indicating P limitation. For *S. validus*, the N/P ratios were between 13 and 16 in the high N-low P treatment, indicating an optimum N/P ratio in supplied nutrient, but these were less than 14 in the rest of treatments, indicating N limitation.

When plants are grown experimentally with N and P supplied in different proportion, the tissue concentrations of N and P tend to correlate negatively (Güsewell, 2004). Plants have the capacity to regulate N/P ratios: 10-fold variation in N/P supply ratios caused only two- to three-fold variation in biomass N/P ratios (Güsewell and Koerselman, 2002). Our results on the concentrations of N and P and N/P ratios supported these relationships.

The present results indicated that wetland plants could accumulate high concentrations of N and P in above-ground tissues under conditions of abundant supply. This capacity in addition to its high growth rate is desirable characteristics for any aquatic plant used in constructed wetlands for wastewater treatment. The nutrient concentrations in plant tissues were comparable to those in the literature (Pompêo et al. 1999; Cronk and Fennessy, 2001). On average, more than 56% of the N and P taken up by plants was located in the above-ground tissues; therefore, harvesting the shoots would remove most of these nutrients accumulated by the plants used in constructed wetlands for wastewater treatment.

5.4.3 Resource allocation

When nutrients are scarce, plants often allocate a greater proportion of their biomass to the root system. This acclimation response is a consequence of metabolic changes in the shoot and an adjustment of carbohydrate transport to the root (Hermans et al., 2006). In the present study, the biomass allocation into the roots decreased markedly with increasing N availability. As a consequence, a unit of root biomass gradually supported greater amounts of root-supported tissues (shoot and rhizome) with increasing N availability. In contrast, shoots supported lower amounts of heterotrophic tissues (rhizomes and roots) under high N compared with both medium and low N treatments. Such changes in biomass allocation are common for plants under high N (Ericsson, 1995; Müller et al., 2000) in contrast to low N availability. In our previous study, the resource allocation of plants was also altered by the nutrient availability (Zhang et al., 2007a).

Most studies on resource allocation in plants have concentrated on the allocation of biomass. Biomass is easy to measure, and the distribution of biomass is thought to reflect the distribution of other resources, such as N (Reekie and Bazzaz, 1987). The patterns of biomass and nutrient allocation in the present study were not absolutely clear in all tissues, but some correlations could be found. For instance, the similar pattern for *C. indica* in increasing biomass as well as N allocation to stems were observed, while decreasing biomass allocation to roots was revealed under increasing N availability. Increasing biomass allocation to leaves also corresponded to increasing P allocation to that tissue under increasing N availability, whereas a decreasing pattern was found in rhizomes. Nevertheless, the patterns of N and P allocation were not totally the same as those of biomass allocation. Thus, our data indicated that it would not be prudent to measure biomass allocation and assume that it reflected nutrient allocation. Similar results for *Verbasscum thapsus* and five *Solidago* species were observed by Abrahamson and Hal (1982). They indicated that assessing nutrients is more important for evaluating resource allocation than assessing biomass, and that the allocation pattern of critical resources reflects the ecological strategy of the plant.

5.4.4 Nutrient removal efficiency

In the present study, the removal efficiencies of N and P were comparable to other reported studies in literature (Busnardo et al., 1992; Greenway and Woolley, 2001; Sekiranda and Kiwanuka, 1998; Huett et al., 2005; Sim et al., 2007; Yang et al., 2007). For example, the removal efficiencies were 95 of N and 99% of P in the laboratory artificial wetland with *Typha orientalis* (Breen, 1990). The high N and P removal was also recorded in large-scale wetlands. For instance, Yang et al. (2007) reported that the removal was 76% of total added N and 85% of added N in the pilot-scale constructed wetlands planted with *C. indica*. A mass reduction of 85% N and 21% P from secondary-treated municipal wastewater was found in surface-flow constructed wetland in Cairns, Australia (Greenway and Woolley, 2001). Sim et al. (2007) also reported that the nutrient removal from storm-water was 82% for total N, 71% for NO_3-N and 84% for P in the full-scale constructed wetland in Malaysia.

In the present study, the removal efficiency of NH_4-N with *S. validus* was relatively high compared with that of NO_x-N (Table 5.15). The higher concentrations of NO_x-N in the effluents was found in the high N-low P treatment compared to that in the influents at the last week of the present study (Figure 5.8). This might be caused by (1) *S. validus* preferring NH_4-N than NO_X-N in N uptake; (2) nitrification; and (3) the sand substrata also adsorbing NH_4-N to a certain degree. On average, about 1.5 mg N kg^{-1} was stored to the sand in the present study.

5.4.5 pH and dissolved oxygen (DO)

In general, the pH in the effluents was decreased with an increase in N applications in the present study. Plant uptake and assimilation of NH_4-N is a proton-generating process and usually leads to a decrease in the external pH and in the contents of carboxylates in the roots (Marschner, 1995; Hinsinger et al., 2003; Bezbaruah and Zhang, 2004).

In the present study, the concentrations of DO were higher in the low N treatment compared with the medium and high N treatments. Plant shading in the medium and high N treatments inhibited the growth of suspended photosynthetic organisms resulting in decreased O_2 production and the higher plant above-ground biomass limited O_2 transfer from air to the water (Iamchaturapatr et al., 2007).

5.4.6 Nutrient mass balance

The results of the present study (Tables 5.17 and 5.18) showed that N and P removals by *C. indica* uptake (61-94% and 21-77% of the influent N and P, respectively) were comparable to the reports in the literature (Breen, 1990; Busnardo et al., 1992; Sekiranda and Kiwanuka, 1998; Huett et al., 2005), whereas N and P removals by *S. validus* uptake (19-42% and 4-39% of the influent N and P, respectively) were relatively low compared with those in the small-scale constructed wetland, but were still comparable to the pilot studies (Greenway and Woolley, 2001; Browning and Greenway, 2003; Sim et al., 2007). Sim et al. (2007) reported that the uptake of nutrients from the pilot tank system was 42% N and 29% P for *Phragmites karka* and 17% N and 26% P for *Lepironia articulata*. Nutrient removals from secondary-treated municipal wastewater by plants accounted for 15-80% N and 24-80% P in surface-flow constructed wetland in Cairns, Australia (Greenway and Woolley, 2001). The highest nutrient uptake by *Baumea articulata* accounted for 11% of N and 3% of P removal (Browning and Greenway, 2003). The different nutrient loading rates were responsible for the different nutrient uptake by plants in the constructed wetlands.

In the present study, N removal by volatilisation was unlikely due to pH in the solution being less than 7 (Table 5.16). The other losses of N in the present study (Tables 5.17 and 5.19) were likely due to denitrification. Vymazal (2007) stated denitrification is considered as a major removal mechanism for N in most types of constructed wetlands. The sand substrate can adsorb NH_4-N. For *C. indica*, however, the N release from substrate (Table 5.17) was found in the low N treatments. This phenomenon was also observed in our previous study (Zhang et al., 2007b). For *S. validus*, the plant uptake, substrate storage and other losses had similar contribution to N removal when N loading rates were relatively low (the low N treatments). However, other losses contributed more to N removal in the medium N-high P treatments (Table 5.19). The results suggested that the properly high nutrient availability and optimum N/P ratio could stimulate plant growth, and enhance N removal efficiency in constructed wetlands.

Sorption of P to the substrate has been recognized as one of the most important removal mechanisms (Richardson, 1985). Given that the substrate used in this study was river sand that contained oxides of Fe and Al, it could enhance the effect of P retention by chemical adsorption and precipitation in constructed wetlands (Arias et al., 2001). On average, the total amounts of P storage in the substrate were about 11.8 mg P kg^{-1} in the present study. The lowest removal efficiency of PO_4-P for *S. validus* was observed at week 7 after nutrient treatments (Figure 5.8). This possibly suggested that the adsorption of PO_4-P by the sand might decrease, and the plant uptake might contribute more to removal of PO_4-P after 7 weeks growth.

Losses of 2-10% of P were recorded (Tables 5.17 and 5.19), possibly due to formation of organic film that was not quantified in the present study. The percentages of P losses were similar to our previous study (Zhang et al., 2007b).

5. 5 Conclusions

The wetland plant growth, biomass and concentrations of N and P in plant tissues were significantly affected by interaction of N and P treatments. The patterns of percentage biomass allocation were different from those of percentage nutrient (N and P) allocation to the different plant tissues in the wetland microcosms. The significant interactive effects of N and P treatments on the nutrient removal efficiencies were observed in the present study. The different nutrient loading rates were responsible for the different nutrient uptake by plants in the wetlands. However, the high concentrations of N (NH_4-N) in supplied nutrients might affect the growth of wetland plants, resulting in decreasing the pH in the solutions, inhibiting the plant growth and reducing nutrient removal efficiency in the wetlands. Therefore, the properly high nutrient availability and optimum N/P ratio are required to stimulate plant growth, resulting in allocating more resource to the above-ground tissues and improving nutrient removal efficiency in constructed wetlands. In order to improve understanding of plant responses to nutrient availability in constructed wetlands, the intensive studies on the effects of various concentrations of nutrients, different forms of N and ratios of N to P on the wetland plant growth and nutrient removal efficiency are needed in both laboratory and field conditions in the future.

CHAPTER 6

THE KINETICS OF AMMONIUM, NITRATE AND PHOSPHORUS UPTAKE BY *CANNA INDICA* AND *SCHOENOPLECTUS VALIDUS*

Abstract

Emergent wetland plant species may have variable nutrient uptake efficiency when grown in constructed wetlands for tertiary purification of wastewater. The kinetics of nutrient uptake by *Canna indica* and *Schoenoplectus validus* were investigated. The maximum uptake rate I_{max} was significantly lower for NH_4-N than NO_3-N in *C. indica*, whereas the reverse was true in *S. validus*. The I_{max} for NH_4-N was significantly lower in *C. indica* than *S. validus,* but no significant difference in I_{max} for NO_3-N uptake was observed. The significantly lower K_m for NO_3-N uptake was detected in *S. validus* compared to *C. indica* and NH_4-N uptake in both species. The I_{max} for PO_4-P uptake was significantly higher after 4 weeks than 6 weeks plant growth, but no significant difference was observed between the species, whereas the K_m for PO_4-P uptake was significantly lower in *C. indica* than in *S. validus*, but significant difference was not detected between the younger plants and older ones. The results indicate that wetland plant species could have different nutrient uptake efficiency and different preferences for inorganic nitrogen source, with *C. indica* preferring NO_3-N and *S. validus* preferring NH_4-N. *C. indica* had greater capacity than *S. validus* to take up PO_4-P when the concentration of PO_4-P in the solution was relatively low, whereas *S. validus* was more capable than *C. indica* to take up NO_3-N when the concentration of NO_3-N in the solution was relatively low. The capacities of P uptake were significantly different between the younger plants and older ones. These results have implications for the selection of wetland plants for the wastewater treatment in constructed wetlands.

Key words: *Canna indica*; constructed wetland; nutrient; *Schoenoplectus validus*; uptake kinetics; wastewater

6.1 Introduction

Different wetland species have a differential capacity to take up nitrogen (N) and phosphorus (P) from wastewater, with some showing impressive abilities to assimilate nutrients (Kadlec and Knight, 1996). These may reflect species and developmental stage differences in efficiency of nutrient uptake and use (Tanner, 1996; Güsewell and Bollens, 2003).

Most plants get N from the soil as either NH_4-N or NO_3-N, with some species showing a strong preference for one ionic form over the other (Kronzucker et al., 1997; Forde and Clarkson, 1999). Plant species differ greatly in their capacities to utilize particular N forms and these adaptations may contribute to the unique spatial and/or temporal distributions of these species (Bledsoe and Rygiewicz, 1986; Chapin et al., 1993; Kronzucker et al., 1997; Min et al., 1998, 1999). Wetland plants are suggested to favour NH_4-N rather than NO_3-N (Tylova-Munzarova et al., 2005). However, it was found that a widely grown variety of lowland rice was exceptionally efficient in absorbing and assimilating NO_3-N in contrast to NH_4-N compared with other plant species (Kronzucker et al., 1999, 2000). From four wetland plants studied, two (*Bacopa monnieri* and *Azolla* spp.) had preference for NO_3-N, whereas both N forms were required by *Ludwigia repens* for N uptake (Fang et al. 2007b). This raises the possibility that NO_3-N uptake by wetland plants is more important than generally thought.

Plant species differ in P uptake efficiency; some plants can grow well under low-P conditions because they are able to take up enough P for optimum growth. Differences in influx at the same concentration are related to uptake kinetic parameters, which results from plant adaptation to low nutrient concentrations (Bhadoria et al., 2004).

Uptake kinetics of many nutrients (including N and P) can be described by the Michaelis-Menten equation (Barber, 1984). The maximal uptake rate or influx ($V_{\max}$ or $I_{\max}$) was obtained when all the available carrier sites are loaded. During the uptake process of a nutrient, only the net uptake of ions is determined, the result of the inflow and outflow of ions at root surface. The Michaelis-Menten constant, K_m, is equal to the substrate ion concentration that gives half of the maximal transport rate; the lower this constant, the higher the affinity between the carrier sites and ions.

The knowledge regarding nutrient uptake kinetics by plants allows comparisons of uptake efficiency among and within species, provides insight into the function of uptake mechanisms, and facilitates predictive modelling of nutrient uptake. However, the relationship between growth of aquatic plants and their nutrient supply is poorly understood although such information would be useful in the clarification of the functions of aquatic macrophytes in constructed wetlands. To our knowledge, few studies of nutrient uptake kinetic have been reported on fresh water emergent macrophytes (Brix et al., 1994; Dyhr-Jensen and Brix, 1996; Romero et al., 1999; Tylova-Munzarova et al., 2005). Although *Canna indica* and *Schoenoplectus validus* have been used in constructed wetlands for improvement of water quality and landscape restoration due to relatively high nutrient removal efficiency and aesthetic value (Thomas et al., 1995; Grosse et al., 2001; Tanner, 2001, 2005; Yue et al., 2004; Zhu et al., 2004; Fu et al., 2006; Wu and Ding, 2006; Wu et al., 2006b; Calheiros et al., 2007; Yang et al., 2007), no report can be found on the nutrient uptake kinetics of *C. indica* and *S. validus*. The objective of this study was to compare the uptake kinetics of ammonium, nitrate and phosphorus between the two plants species.

6.2 Materials and Methods

6.2.1 Plant material and initial conditions

The experiment was conducted in a phytotron chamber at the University of Western Australia with controlled day/night temperatures of 20/15 ^{0}C under natural conditions between the early March and the middle April in 2007.

The seedlings of *C. indica* and *S. validus* were transplanted into a solution culture system using a 4-L plastic vessel. A simulated nutrient solution was used at the average concentrations of total N and P mimicking the secondary-treated wastewater at Subiaco Wastewater Treatment Plant as described in Chapter 2. The pH was adjusted to 6.5 and the nutrient solutions were replaced twice weekly to avoid significant changes in pH and depletion of nutrients. Solutions were vigorously aerated. After approximate 4 weeks growth, the plants of uniform size were selected and the uptake kinetics of ammonium and nitrate by plants were measured separately. The uptake of phosphorus by plants was measured after 4 and 6 weeks of plant growth.

6.2.2 Uptake kinetic measurements

The modified depletion method (Barber, 1984) was used to measure nutrient uptake kinetics. The measurements were conducted in the same phytotron chamber used for the plant establishment. The plants were rinsed with deionised water and placed into nutrient solutions as described above but without N or P for 2 days to elicit maximal uptake response during the subsequent experiment. After this starvation period, one plant of *C. indica* and 3 clumps of *S. validus* (due to their differences in morphology and size etc.) were put into a jar containing 400 mL nutrient solution with different concentrations of N or P. The concentrations (mmol L^{-1}) of N [$(NH_4)_2SO_4$ or KNO_3] were 0.05, 0.1, 0.2, 0.4, 0.6, 1.0 and 2.0. The concentrations (μmol L^{-1}) of P (KH_2PO_4) were 3, 5, 10, 30, 60, 120 and 240. The other nutrients were compensated for and kept at the same levels in all solutions. Each jar was weighed at the beginning and at the end of the experiment to calculate the water loss through evapotranspiration during the period. Each treatment had four replicates. The solution was taken and the plants were harvested after 2 h of nutrient uptake. The solution samples were frozen before N or P analysis. The roots were separated from the rhizomes. All plant samples were dried to a constant weight at 70 ºC for 5 days in a forced-air cabinet and weighed.

The relevant methods for measuring nutrients in solution samples were described in Chapter 2.

6.2.3 Calculations and statistical analyses

The uptake rates were calculated by measuring the depletion from the solution based on the root dry weights. The concentration of the external nutrient solution and the uptake rate were fitted to the Michaelis–Menten equation to obtain the kinetic parameters of I_{max} and K_m. The parameters were estimated by using a double-reciprocal Lineweaver-Burk plot (Lineweaver and Burk, 1934) and Hofstee plot (Hofstee, 1952).

The significance of the regression co-efficient (r^2) for estimating parameters of nutrient uptake kinetics was determined. Two-way ANOVA was used to determine significance of the K_m and I_{max} for NH_4-N and NO_3-N uptake between N forms and plant species. Two-way ANOVA was used to determine significance of the plant growth age and species on the K_m and I_{max} for PO_4-P uptake. Least significant difference (LSD) was applied to test for significance between the means.

6.3 Results

6.3.1 NH_4-N, NO_3-N and PO_4-P uptake by plants

The uptake of NO_3-N by *C. indica* was slightly higher than that of NH_4-N. By contrast, the uptake of NO_3-N by *S. validus* was markedly lower than that of NH_4-N (Figure 6.1). The uptake of NH_4-N was greater by *S. validus* than *C. indica* (Figure 6.1). The uptake of PO_4-P by the plants was larger after 4 than 6 weeks growth (Figure 6.2).

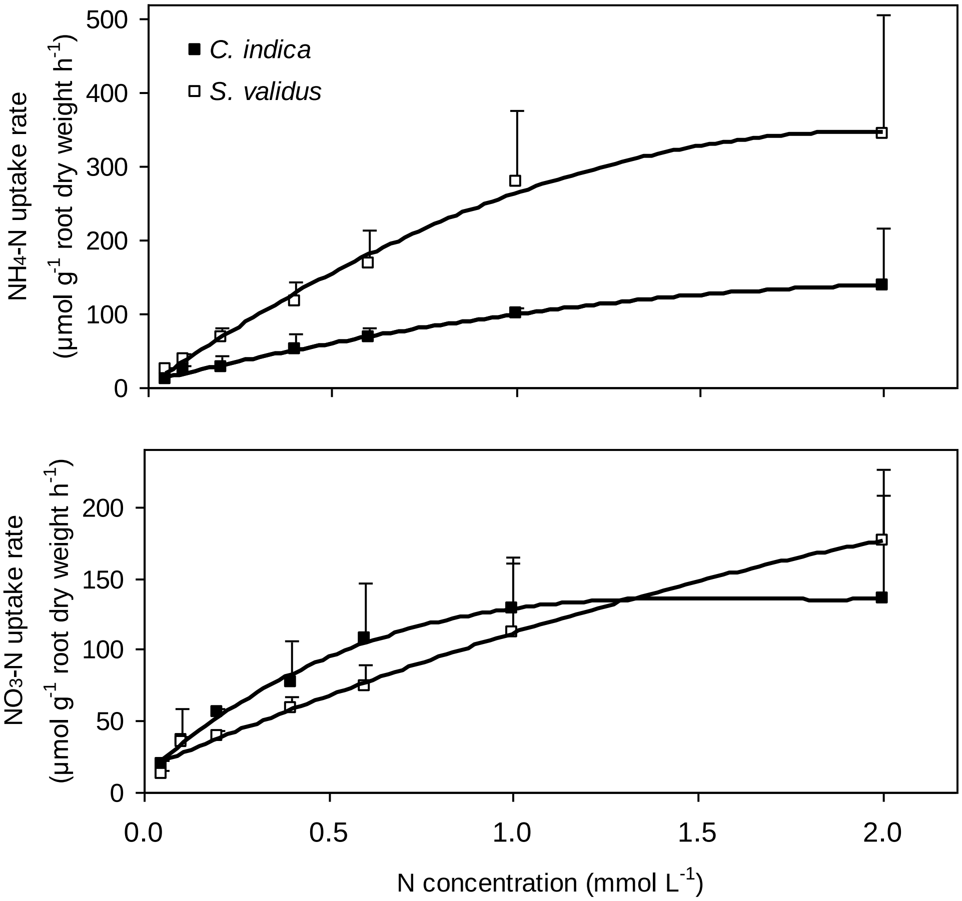

Figure 6.1 The rates (means + SE; n = 4) of NH_4-N and NO_3-N uptake by *C. indica* and *S. validus* as a function of N concentrations. The lines were drawn using a non-linear curve-fitting model.

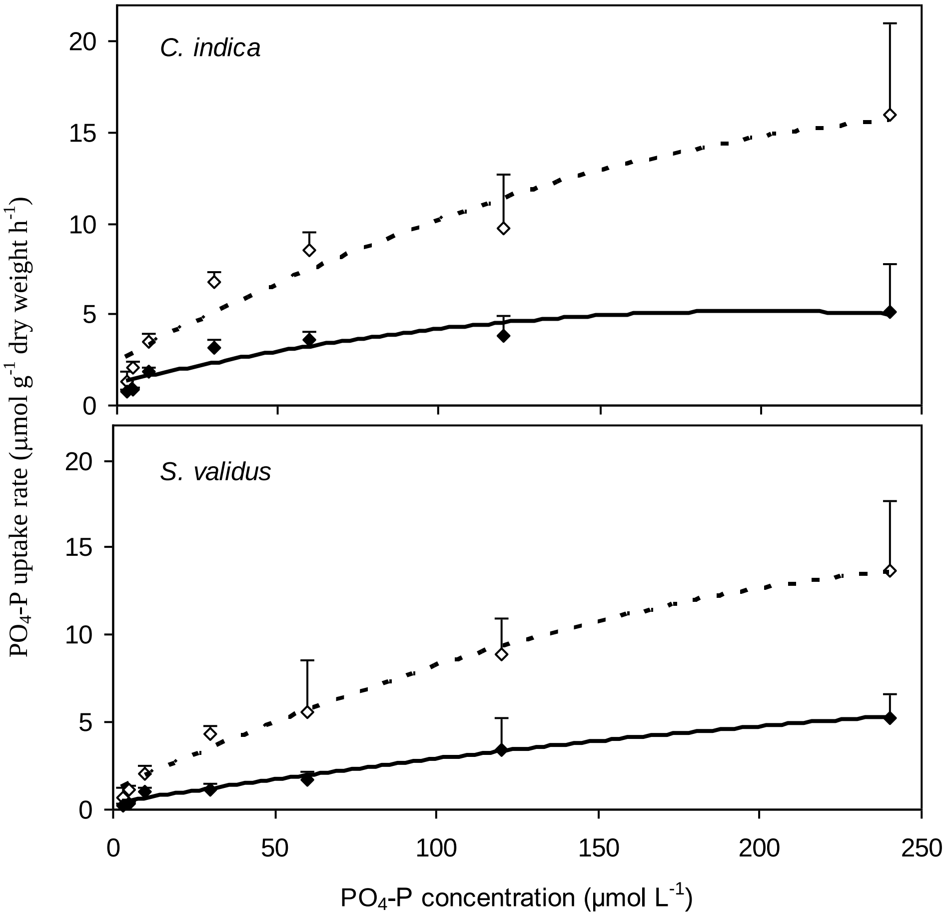

Figure 6.2 The rates (means + SE; n = 4) of PO$_4$-P uptake by *C. indica* and *S. validus* as a function of PO$_4$-P concentrations after 4 weeks of growth (open symbols and dashed lines) and 6 weeks of growth (closed symbols and solid lines). The lines were drawn using a non-linear curve-fitting model.

6.3.2 Regression co-efficient (r^2) of Lineweaver-Burk and Hofstee plot methods for estimating parameters of nutrient uptake kinetics

The significance of the regression co-efficient (r^2) for estimating parameters of nutrient uptake kinetics was greater for the Lineweaver-Burk than the Hofstee method (Table 6.1). The Lineweaver-Burk method was all significant at $P<0.001$. Therefore, the means of I_{max} (μmol g^{-1} dry root weight h^{-1}) and K_m (μmol L^{-1}) for NH_4-N, NO_3-N and PO_4-P uptake kinetics in *C. indica* and *S. validus* were obtained by the Lineweaver-Burk method.

Table 6.1 The significance of the regression co-efficient (r^2) of Lineweaver-Burk and Hofstee line plot methods for estimating parameters of nutrient uptake kinetics

Nutrient uptake	Species	Plant age	Lineweaver-Burk	Hofstee
NH_4-N	*C. indica*	after 4 weeks	0.97***	0.69*
	S. validus	after 4 weeks	0.98***	0.80**
NO_3-N	*C. indica*	after 4 weeks	0.96***	0.68*
	S. validus	after 4 weeks	0.996***	0.95***
PO_4-P	*C. indica*	after 4 weeks	0.998***	0.78**
		after 6 weeks	0.991***	0.90**
	S. validus	after 4 weeks	0.997***	0.78**
		after 6 weeks	0.98***	0.53^{NS}

NS: not significant. * Significant at $P \leq 0.05$. ** Significant at $P<0.01$. *** Significant at $P<0.001$.

6.3.3 I_{max} and K_m for NH_4-N, NO_3-N and PO_4-P uptake kinetics

The significant differences in the kinetic parameters between plant species and N forms were detected, and a significant interaction was observed (Figure 6.3). The I_{max} for NH_4-

N (149 μmol g^{-1} dry root weight h^{-1}) was significantly lower than that for NO_3-N (175 μmol g^{-1} dry root weight h^{-1}) in *C. indica*. In contrast, the I_{max} for NH_4-N (303 μmol g^{-1} dry root weight h^{-1}) was significantly higher than that for NO_3-N (169 μmol g^{-1} dry root weight h^{-1}) in *S. validus*. The I_{max} for NH_4-N was significantly lower in *C. indica* than in *S. validus*, but no significant difference between plant species was observed in I_{max} for NO_3-N. The significantly lower K_m for NO_3-N uptake in *S. validus* was detected.

The I_{max} for PO_4-P uptake was significantly higher after 4 weeks than 6 weeks plant growth, but no significant difference was observed between the species, whereas the K_m for PO_4-P uptake was significantly lower in *C. indica* than *S. validus*, but no significant difference was noted between after 4 weeks and 6 weeks plant growth (Figure 6.4).

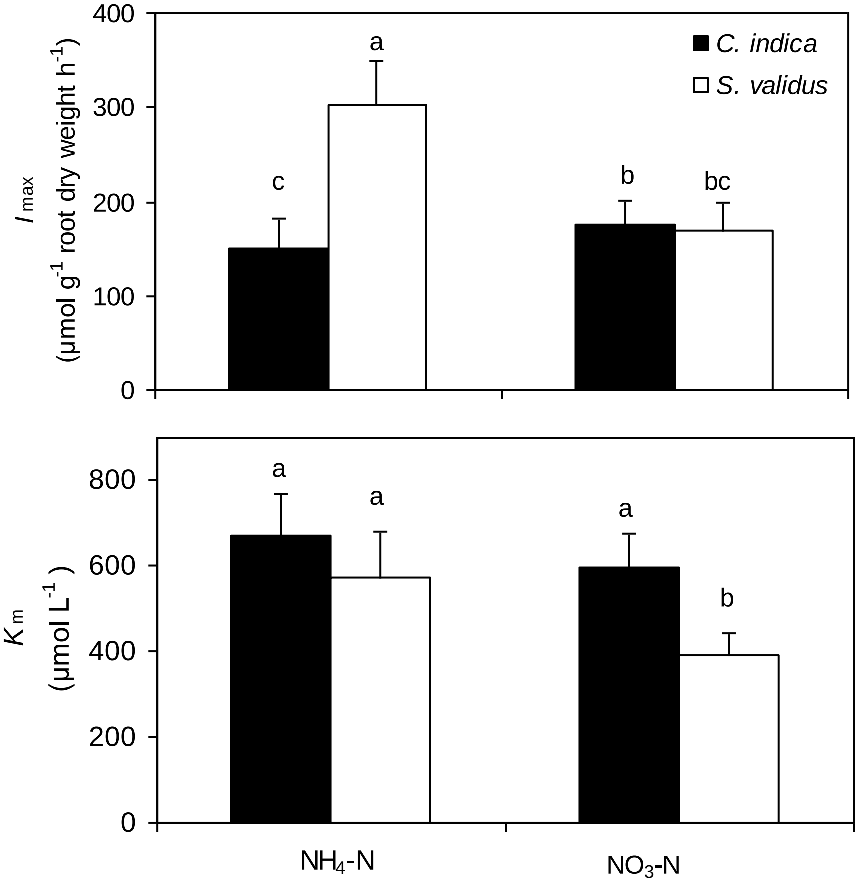

Figure 6.3 Means of I_{max} and K_m for NH_4-N and NO_3-N uptake kinetics of *C. indica* and *S. validus*. Bars (means + SE, *n* = 4) with different letters are significantly different based on LSD ($P \leq 0.05$).

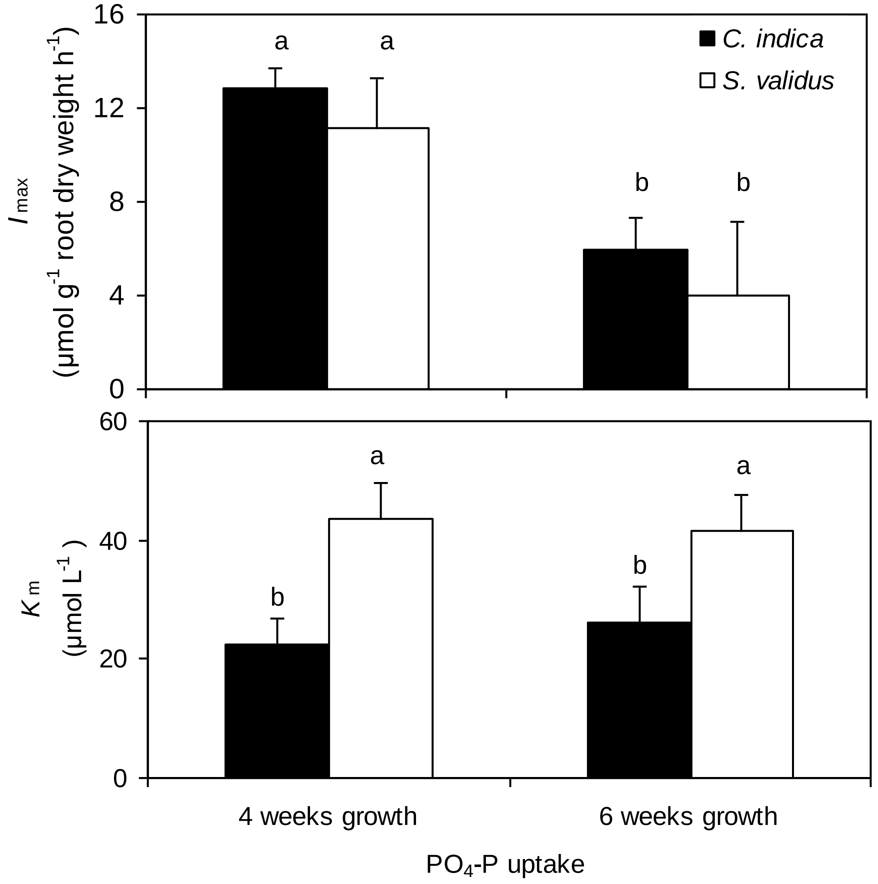

Figure 6.4 Means of I_{max} and K_m for PO$_4$-P uptake kinetics of *C. indica* and *S. validus* after 4 weeks and 6 weeks growth. Bars (means + SE, *n* = 4) with different letters are significantly different based on LSD ($P \leq 0.05$).

6.4 Discussion

6.4.1 N uptake

The present results on N uptake kinetics revealed the differences in uptake of NH_4-N versus NO_3-N between *C. indica* and *S. validus*. Both NH_4-N and NO_3-N are important nitrogen sources for plant growth and the preferences that different species may have for particular N sources can have important ecological and practical implications (Forde and Clarkson, 1999). Plant preference for different forms of N is influenced by environmental factors, such as temperature, aeration, pH and composition of nutrients in solution, water and salt stress, and also by the plant growth stage and its ability to form a symbiosis with bacteria or fungi (Brix et al., 1994; Dyhr-Jensen and Brix, 1996; Garnett and Smethurst, 1999; Brix et al., 2002). The mechanisms of the preference for different forms of N in aquatic species should be further investigated.

The present study showed high maximum uptake capacity (I_{max}) for NH_4-N compared to NO_3-N in *S. validus*. Similarly, Tylova-Munzarova et al. (2005) observed the same results in *Phragmites australis* and *Glyceria maxima*. These results indicate that wetland species may share characteristics of plants colonizing habitats with restricted nitrification (Kronzucher et al., 1997). For example, rice, ericaceous species and many conifers occur naturally on soil enriched in NH_4-N and organic N, and appear not to suffer NH_4-N toxicity. The preference for NH_4-N over NO_3-N as inorganic N source has also been observed in many coniferous tree species (Marschner et al., 1991; Peuke and Tischner, 1991; Kronzucker et al., 1997; Malagoli et al., 2000). In contrast to above, *C. indica* did not show the preference for NH_4-N. Similarly, Kronzucker et al. (2000) also found that both capacity for and efficiency of NO_3-N utilization in *Indica* rice were greater than for NH_4-N, indicating a highly specialized adaptation to the NO_3-N source. A modelling study by Kirk and Kronzucker (2005) implicated that wetland plants may be efficient in capturing NO_3-N formed in the rhizosphere. Fang et al. (2007b) observed that different wetland species require different forms of N. NO_3-N uptake by wetland plants could hence be more important than thought hitherto.

The present results showed high uptake capacity ($I_{\max}$) for NH_4-N and affinity (K_m) for NO_3-N in *S. validus* compared to *C. indica*. Many studies on N uptake have been conducted on crop species. For example, Rao et al. (1993) observed that there were significant differences in $I_{\max}$ and K_m for NH_4-N between legumes and cereals. The difference in kinetics of NO_3-N between the two groups of plants became apparent at the high concentration tested. Compared to crops, fewer studies on N uptake kinetics have been reported on wetland species, but the studies by Tylova-Munzarova et al. (2005) and Fang et al. (2007b) have the evidence that different wetland species have different efficiency for N uptake and preference for N forms. Tylova-Munzarova et al. (2005) found that *Phragmites australis* had high affinity for NH_4-N and NO_3-N compared to *Glyceria maxima*.

Reported $I_{\max}$ and K_m values are greatly variable with plant age, species or varieties and growth conditions, but also due to resolution of the measurement set-up (Bot et al., 1998). For NH_4-N and NO_3-N, most K_m values lay between 5 and 100 μmol L^{-1} (Glass and Siddiqi, 1984; Peuke and Kaiser, 1996), but values as high as 985 μmol L^{-1} have also been reported for NO_3-N (Pace and McClure, 1986). The $I_{\max}$ and K_m values in the present study were under the highest values in the literature. It is worth noting that the rhizome system of *S. validus*, which is similar to *Carex,* might contribute to N uptake, because Brooker et al. (1999) reported that the rhizome system of *Carex bigelowii* could act as a route for N uptake. The nutrient uptake by rhizome may result in the calculated uptake rate to be higher than the actual uptake by the roots of *S. validus*. In contrast, *C. indica* also has rhizome, but its rhizome plays only a storage role for nutrients and its root system is principally made of fibrous roots (Yang et al., 2007).

6.4.2 P uptake

In the present study, compared to *S. validus*, the $I_{\max}$ for PO_4-P was slightly higher and the K_m was significantly lower in *C. indica*. Therefore, the efficiency of P uptake was better in *C. indica* than *S. validus* when P concentration in the substrate was relatively low. Numerous reports in the literature stated that different species, varieties and genotypes have differential efficiency of P uptake (Chapin, 1980; Schenk and Barber, 1980; Clark, 1983; Bhadoria et al., 2004; Akhtar et al., 2007). For example, Bhadoria et al. (2004) found differences in P uptake kinetics between maize (*Zea mays*) and groundnut (*Arachis hypogaea*).

The present results showed that the younger plants had higher capacity ($I_{\max}$) of P uptake compared to older ones. Jungk and Barber (1975) investigated P influx characteristics of maize plants and found that the uptake rate of P per cm of root reached a maximum at 25 days, then declined rapidly with age thereafter. The decrease with age is similar to observations for field-grown maize by Mengel and Barber (1974). Plant age and P treatment level were also important factors affecting P uptake rates among four sorghum genotypes (Furlani et al., 1984).

6.5 Conclusions

The different N uptake efficiency and different preferences for inorganic N source between the plant species, and different P uptake efficiency between the plant species and plant growth period were observed in the present study. The results suggest that in order to enhance the nutrient removal efficiency in constructed wetlands, plant species should be selected according to their preferences for different N forms, and for having high nutrient uptake efficiency. The nutrient uptake efficiency should be tested for a variety of wetland plant species in different conditions, such as nutrient concentration levels, nutrient forms, temperature, pH, plant age and so on.

CHAPTER 7

CONCLUDING COMMENTS

7.1 Summary of key findings

The research consisted of four parts: (1) selection of suitable species from six ornamental and four sedge species; (2) comparison of plant growth and nutrient removal in mono- and mixed culture between *C. indica* and *S. validus*; (3) interaction of N and P on plant growth and nutrient removal by *C. indica* and *S. validus*; and (4) kinetics of nutrient uptake by *C. indica* and *S. validus*. The results generated from each part can be related to each other towards an understanding the plant growth affected by nutrients in the wastewater and nutrient removal from the wastewater achieved by plants in constructed wetlands. The key findings are:

(1) Different ornamental species have differential capacity to take up nutrients from the wastewater, with *C. indica* having shown a better performance in terms of the plant growth and nutrient removal efficiency in the wetland microcosms;

(2) The plant growth and resource allocation are influenced by nutrient availability and interspecies competition. Nutrient removal efficiencies were significantly affected by the presence of plants, but significant difference was not observed between the monoculture and mixture with *C. indica* and *S. validus*. Plant uptake and incorporation into tissues was the major factor responsible for N and P removal;

(3) The optimum nutrient availability and N/P ratio were required for stimulating plant growth, resulting in allocation of more resources to above-ground tissues compared to below-ground ones, and enhancing nutrient removal efficiency. The biomass allocation could not totally reflect the nutrient allocation to different tissues. Nutrient removal efficiencies were significantly influenced by interactions of N and

P loading rates in supplied wastewater, but different plant species had differential impact depending on growth of *C. indica* and *S. validus* at these loading rates; and

(4) Differences in N uptake efficiency, preferences for inorganic N source and P uptake efficiency were observed between *C. indica* and *S. validus*. The capacities of P uptake were significantly different between the younger plants and older ones. These findings may have important implications for plant selection and vegetation management in constructed wetlands.

7.2 General discussion and suggestions for further research

The nutrient removal efficiencies in constructed wetlands are influenced by many factors such as the sources of wastewater, the types of constructed wetlands and the presence of wetland plants, etc. A list of nutrient removal efficiencies in various types of experimental or pilot constructed wetlands with different sources of wastewater using *C. indica* and *S. validus* is presented in Tables 7.1. In the present study, the nutrient removal efficiencies were comparable to the literature. In general, nutrient removal efficiencies were higher in *C. indica* than *S. validus*, but they were variable depending on nutrient loading rate, retention time and plant growth stage. It could be suggested that different growth rates or phenological phases of the plants affected nutrient removal efficiency.

Although macrophytes are widely used in constructed wetlands around the world, the role of macrophytes and the effect of different plant species on the treatment performance have been controversial (Edwards et al., 2006; Lee and Scholz, 2007). Plants are an important part of constructed wetlands for wastewater treatment. Macrophytes have several important functions that will improve purification efficiency and prolong the working life of constructed wetlands (Brix, 1997). One important aspect is nutrient uptake. Plants absorb nutrients that are assimilated in the tissues of plants for their growth, maintenance and reproduction during the growing season. The nutrients can be removed by plants through harvesting or may be bound in substrates.

Majority of studies reported a considerable contribution of macrophytes to nutrient removal (Reddy and DeBusk, 1985; Peterson and Teal, 1996; Hunter et al., 2001, Lim et al., 2001; Yang et al., 2001; Fraser et al., 2004; Silvan et al., 2004; Gottschall et al., 2007; Iamchaturapatr et al., 2007; Lee and Scholz, 2007). For example, wetland plants have been reported to remove 16 to 75% of N inputs and 12 to 73% of P inputs from nutrient-enriched waters in microcosm retention ponds (Reddy and DeBusk, 1985). Our results supported these findings, with nutrient removal efficiency being significantly higher in the planted than non-plant microcosms. Nutrient uptake by different plant species in various types of experimental constructed wetland was summarized in Table 7.2. Depending on plant species used, type of wastewater treated and rates of nutrients loaded, plant nutrient uptake can account for between 17 % and 94 % of input N removal and from 4 % to 96 % of input P removal.

Different plant species had differential potential to take up nutrients. It was suggested that *C. indica* could have a higher potential to take up nutrients from the wastewater in constructed wetlands compared with *S. validus*, but the results in the present study have showed that the potential of N uptake was higher in *S. validus* than *C. indica*. However, the nutrient uptake capacity might be changed by plant growth stage and other environmental factors such as concentrations and compositions of nutrients in the wastewater. It was worth noting that the plants were fed a mixture of NH_4-N and NO_3-N, but the kinetics of nutrient uptake were conducted with either NH_4-N or NO_3-N as a sole N source.

The present study was conducted in the small-scale wetland microcosms, and the duration of experiments was relatively short. The results obtained from the present study can only be used as the reference for constructed wetland implementation in

Table 7.1 Comparison of removal efficiencies for nutrients in various types of experimental or pilot constructed wetlands with different sources of wastewater using *C. indica* and *S. validus*

Type of constructed wetland	Source of wastewater	Nutrient removal efficiency (%)				References
		NH_4-N	NO_3-N	Total N	Total P	
C. indica						
Up-flow-down-flow constructed wetland	Non-point	98	15	>73	49-71	Wang et al. (2001)
Surface-flow constructed wetland	Domestic	57		50	69	Zhao et al. (2003)
Vertical-flow microcosm	Septic	66			67	Zhu et al. (2004)
Subsurface vertical-flow constructed wetland	Industrial and domestic	75		73	90	Shi et al. (2005)
Water culture microcosm	Salty water	64	51	51	74	Fu et al. (2006)
Surface-flow constructed wetland	Eutrophic water	71				Wu and Ding (2006)
Surface-flow constructed wetland	Domestic			76	85	Yang et al. (2007)
Vertical-flow microcosm	Simulated secondary-treated	93	86		85	This study (Chapter 3)
Vertical-flow microcosm	Simulated secondary-treated	98-99	93-98		85-88	This study (Chapter 4)
Vertical-flow microcosm	Simulated secondary-treated	98-99.9	70-99.9		63-99	This study (Chapter 5)
S. validus						
Surface-flow constructed wetland	Abattoir	54		56	61	Finlayson and Chick (1983)
Surface-flow constructed wetland	Primary	94				Gersberg et al. (1986)
Subsurface flow constructed wetland	Sewage	17	65		22	Thomas et al. (1995)
Subsurface flow constructed wetland	Dairy	34-71		48-75	37-74	Tanner et al. (1995)
Vertical-flow microcosm	Simulated secondary-treated	84	74		75	This study (Chapter 3)
Vertical-flow microcosm	Simulated secondary-treated	98-99	93-94		77-76	This study (Chapter 4)
Vertical-flow microcosm	Simulated secondary-treated	59-99	21-99		48-80	This study (Chapter 5)

Table 7.2 Comparison of N and P uptake by plant species as percent input in various types of constructed wetlands with different sources of wastewater

Type of constructed wetland	Source of wastewater	Species	N uptake as % input	P uptake as % input	Reference
Free water surface microcosm	Enriched drainage	*Hydrocotyle umbellata*	NA	65	Reddy (1983a)
Free water surface microcosm	Enriched drainage	*Eichhornia crassipes*	NA	29	Reddy (1983a)
Free water surface microcosm	Enriched drainage	*Typha latifolia and Egeria densa*	NA	4	Reddy (1983a)
Vertical-flow microcosm	Secondary-treated	*Typha orientalis*	51	67	Breen (1990)
Free water surface mesocosm	Simulated secondary-treated	*Scirpus californicus*	50	52	Busnardo et al. (1992)
Free water surface microcosm	Septic	*Typha angustifolia*	43	NA	Koottatep and Polprasert (1997)
Vertical-flow mesocosm	Primary	*Phragmites mauritianus*	19-29	37-54	Sekiranda and Kiwanuka (1998)
Subsurface-flow microcosm	Simulated nursery runoff	*Phragmites australis*	48-76	86-96	Huett et al. (2005)
Surface-flow mesocosm	Simulated eutriphic	*Phragmites karka*	42	29	Sim et al. (2007)
Surface-flow mesocosm	Simulated eutriphic	*Lepironia articulata*	17	26	Sim et al. (2007)
Vertical-flow microcosm	Simulated secondary-treated	*C. indica*	75-86	41-55	This study (Chapter 4)
Vertical-flow microcosm	Simulated secondary-treated	*C. indica*	61-94	21-77	This study (Chapter 5)
Vertical-flow microcosm	Simulated secondary-treated	*S. validus*	66-77	35-49	This study (Chapter 4)
Vertical-flow microcosm	Simulated secondary-treated	*S. validus*	19-42	4-50	This study (Chapter 5)

NA: not available.

the field conditions. Examination of the combined results from the present study and literature suggests following areas for further investigation in terms of relations between plant growth and nutrient removal efficiency.

(1) Many reports have indicated that plant growth, N uptake and translocation to the shoots are superior when plants were fed with a mixture of NH_4-N and NO_3-N compared with either NH_4-N or NO_3-N as a sole N source (Cox and Reisenzuer, 1973; Heberer and Below, 1989; Ramam et al., 1995; Kronzucker et al., 1999). Hence, in order to enhance nutrient removal efficiency, a wide ranges of N and P levels, and N forms should be used to investigate the optimum N/P ratio for growth of different wetland species in constructed wetland.

(2) Most reports have suggested that wetland plants preferred NH_4-N rather than NO_3-N (Toetz, 1971, 1973; Nelson et al., 1981; Reddy, 1983a; Tylova-Munzarova et al., 2005). However, several wetland species have shown to exhibit superior uptake of NO_3-N (Kronzucker et al., 1999, 2000; Fang et al. 2007b). The differences between species in the kinetics of nutrient uptake may be important in selecting suitable species for specific applications and in adaptation to the unique spatial and/or temporal distribution of these species, but plant preference for different forms of N is influenced by many environmental factors (Tylova-Munzarova et al., 2005; Fang et al. 2007b). Hence, the precise mechanisms underpinning preference between NH_4-N and NO_3-N in wetland species should be further investigated under different conditions such as plant growth stage, source of wastewater, etc.

(3) Denitrification is a significant pathway of nitrate-N loss in wetlands (Mitsch and Gosselink, 2000). However, Kirk and Kronzucker (2005) established a model to estimate the potential for nitrification, denitrification and nitrate uptake in the rhizosphere of wetland plants. The model showed that NO_3-N uptake accounted for 34 % of total N uptake and the ratio of N denitrified to total N uptake was between 0.20 and 6.6 % of the NH_4-N initially in the soil. The model also showed that rates of denitrification and subsequent loss of N from the soil remain small even where NO_3-N production and uptake by plants were considerable. The calculation showed that

wetland plants growing in flooded soil could take up a large part of their N as NO_3-N formed from NH_4-N in the rhizosphere, without excessive losses of N through denitrification. The model is based on lowland rice (*Oryza sativa*), but whether other wetland plants are similarly efficient in capturing NO_3-N formed in the rhizosphere should be investigated to quantify role of denitrification in N removal from wastewater in constructed wetlands.

(4) High P removal rates were dependent on removing P from the system through biomass harvesting. For example, laboratory and greenhouse studies have demonstrated that harvested macrophytes have exhibited mass P removal 30- to 50-fold higher than non-harvested wetland cells (DeBusk and Dierberg, 1999). However, wetland plants have considerable differences in their efficiency to assimilate P from wastewater. Our review in literature and results in present study have revealed that wetland plants typically have P concentrations between 1 and 9 g P kg^{-1} in stems and leaves. Concentrations higher than 10 g P kg^{-1} in dry matter of some plant varieties can often be toxic (Marschner, 1995), but some plant varieties can hyper-accumulate P (defined as P concentrations between 8 and 14.5 g P kg^{-1} in dry matter). For instance, Clark and Brown (1974) reported that one genotype of maize when grown in high-P solutions was able to acquire 12.4 g P kg^{-1} in dry matter compared with 6.2 g P kg^{-1} for another maize genotype under the same conditions. It can be suggested that there is genetic diversity, and should be considered a new plant management approach to removal of P from wastewater in constructed wetland. Therefore, the study on isolating P nutritional traits from the germplasm of these P-hyper-accumulator plants and adding these traits through traditional breeding or modern transgenic techniques to wetland species is needed.

(5) An aggressive, fast-growing wetland plant species is generally consider undesirable in constructed and restored wetlands where diverse plant communities are often desired (Mitsch and Gosselink, 2000). The small-scale and short duration of the study may not represent the field situations. The interspecies competition should be investigated in size- and density-symmetric and in size- and density-asymmetric mixtures among various species under different nutrient conditions. In addition to

nutrient removal, mixtures may provide other benefits over monoculture. Hence, other benefits such as improved plant growth conditions in constructed wetland for various species in mixed culture should be further investigated.

(6) It is important to manage vegetation in constructed wetlands for optimal treatment performance (Thullen et al., 2005). Although plants can act as long-term stores of nutrients, seasonal senescence and the resultant litter decomposition can results in remobilisation of previously stored nutrients. Permanent removal of biologically stored nutrients can be achieved through the formation of anaerobic peat at the water sediment interface, a process which may take many years and is usually restricted to deep wetlands with limited mixing that allows a permanent anaerobic zone and hence limits organic decomposition. Alternatively, plant biomass can be removal from the wetland before it senesces via a process of plant harvest. Uptake rates of N and P have been shown to increase when plants are harvested at least once a year (Kim and Geary, 2001; Toet et al., 2005), whereas part of the nutrient uptake by plants can be leached out of the system. A net release of nutrients often occurs in the fall and early spring as a result of decomposition and nutrient leaching of plant litter (Mitsch and Gosselink, 2000). Therefore, a long-term study on monitoring and management is needed to investigate the effects of species on seasonal patters of nutrient uptake and release, in order to better manage vegetation to increase nutrient removal from wastewater in constructed wetlands.

(7) Although harvesting of the above-ground biomass is a potential option to remove nutrients on a permanent basis, the overall efficiency of the harvest as a long-term management option may be limited as it is labour intensive and expensive (EPA, 2000). However, the production of the biomass in constructed wetlands can provide economic returns when they is used to produce “bio-gas”, animal feed, compost and paper making etc (Lakshman, 1987). Furthermore, the ornamental plants such as *Canna spp.* can be harvested and sold for significant economic benefits if utilized as cut flowers after harvesting (Belmont et al., 2004; Zurita et al., 2006; Zhang et al., 2007). Nevertheless, intensive research on the plant production utilisation after

harvesting in constructed wetlands is of important in terms of management of constructed wetlands.

(8) The world is experiencing significant growth in human population, and fresh water is becoming a scarce resource. This is particularly the case in developing countries. Many authors suggested that the most effective long-term measure for the control of eutrophication in a water body is the reduction of the input of external nutrients (Davis and Koop, 2006; Davis et al., 2006). One way to reduce the nutrients coming from point sources such as municipal wastewater is to remove them by biological treatments. Unfortunately, the cost of installation and operation of a wastewater treatment plant does not permit the municipal governments of developing countries to invest in such technologies (Belmont et al., 2004; Zurita et al., 2006). Constructed wetlands are potentially a low-cost solution to treat wastewater in developing countries (Zhang et al., 2007). Most of the research on constructed wetlands has been conducted in northern countries. However, there is not enough information about the treatment efficacy in most developing countries located in tropical and subtropical areas. Therefore, the use of plant species, especially ornamental ones, for wastewater treatment in constructed wetlands and the utilisation of them after harvesting should be explored in tropical and subtropical areas around the world. Indeed, the model of the constructed wetlands in Australia may provide a very good example of wastewater treatment for most developing countries.

(This page is intentionally blank)

REFERENCES

Abe, K., Ozaki, Y., 1998. Comparison of useful terrestrial and aquatic plant species for removal of nitrogen and phosphorus from domestic wastewater. Soil Science and Plant Nutrition 44, 599-607.

Abrahamson, W. G., Hal, C., 1982. On the comparative allocation of biomass, energy and nutrient in plant. Ecology 63, 982-991.

Adcock, P. W., Ryan, G. L., Osborne, P. L., 1995. Nutrient partitioning in a clay-based surface flow wetland. Water Science and Technology 32, 203-209.

Adin, A., 1986. Problems associated with particulate matter in water reuse for agricultural irrigation and their prevention. Water Science and Technology 18, 185-195.

Aerts, R., Chapin, F. S., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research 30, 1-67.

Aerts, R., de Caluwe, H., Konings, H., 1992. Seasonal allocation of biomass and nitrogen in four *Carex* species from mesotrophic and eutrophic fens as affected by nitrogen supply. Journal of Ecology 80, 653-664.

Agami, M., Reddy, K. R., 1990. Competition for space between *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* L. cultured in nutrient-enriched water. Aquatic Botany 38, 195-208.

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

Akhtar, M. S., Oki, Y., Adachi, T., Murata, Y., Khan, Md. H. R., 2007. Relative phosphorus utilization efficiency, growth response, and phosphorus uptake kinetics of brassica cultivars under a phosphorus stress environment. Communications in Soil Science and Plant analysis 38, 1061-1085.

Akratos, C. S., Tsihrintzis, V. A., 2007. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Ecological Engineering 29, 173-191.

Allen, C. W., Hook, P. B., Biederman, J. A., Stein, O. R., 2002. Temperature and wetland plant species effects on wastewater treatment and root zone oxidation. Journal of Environmental Quality 31, 1010-1016.

Arias, C. A., Bubba, M. D., Brix, H., 2001. Phosphorus removal by sands for use as media in subsurface flow constructed reed beds. Water Research 35, 1159–1168.

Armstrong, W., 1978. Root aeration in the wetland condition. In: Hook, D. D., Crawford, R. M. M., Eds., Plant life in anaerobic environments. Ann Arbor, MI. Ann Arbor Science Publishers. pp. 269-297.

Atkin, O. K., 1996. Reassessing the nitrogen relations of arctic plants: a mini-review. Plant, Cell and Environment 19, 695-704.

Ayaz, S. Ç., Akça, L., 2001. Treatment of wastewater by natural systems. Environment International 26, 189-195.

Azov, Y., Shelef, G., 1991. Effluent quality along a multiple-stage wastewater reclamation system for agricultural reuse. Water Science and Technology 23, 2119-2126.

Bachand, P. A. M., Horne, A. J. 2000. Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. Ecological Engineering 14, 17–32.

Balizon, M. E., Dolmus, R., Quintana, J., Navarro, Y., Donze, M., 2002. Comparison of conventional and macrophyte-based systems for the treatment of domestic wastewater. Water Science and Technology 45, 111-116.

Barber, S. A., 1984. Soil nutrient bioavailability: a mechanistic approach. John Wiley and Sons Inc, New York, USA.

Barcelo, J., Poschenrieder, C., 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: A review. Environmental and Experimental Botany 48, 75-92.

Barko, J. W., Gunnison D, Carpenter S. R., 1991. Sediment interactions with submerged macrophyte growth and community dynamics. Aquatic Botany 41, 41-65.

Barko, J. W., Smart, R. M., 1983. Effects of organic matter additions to sediment on the growth of aquatic plants. Journal of Ecology 71, 161-175.

Bassett, J., Denney, R. C., Jeffery, G. H., Mendham, J., 1978. Vogel's textbook of quantitative inorganic analysis including elementary instrumental analysis. 4th edition. Longman, London, New York.

Batty, L. C., Younger, P. L., 2004. Growth of *Phragmites australis* (Cav.) Trin ex. Steudel in mine water treatment wetlands: effects of metal and nutrient uptake. Environmental Pollution 132, 85-93.

Belmont, M. A., Cantellano, E., Thompson, S., Williamson, M., Sánchez, A., Metcalfe, C. D., 2004. Treatment of domestic wastewater in a pilot-scale natural treatment system in central Mexico. Ecological Engineering 23, 299-311.

Belmont, M. A., Metcalfe, C. D., 2003. Feasibility of using ornamental plants (*Zantedeschia aethiopica*) in subsurface flow treatment wetlands to remove nitrogen, chemical oxygen demand and nonylphenol ethoxylate surfactants—a laboratory-scale study. Ecological Engineering 21, 233-247.

Bezbaruah, A. N., Zhang, T. C., 2004. pH, redox and oxygen micro-profiles in rhizosphere of bulrush (*Scirpus validus*) in a constructed wetland treating municipal wastewater, Biotechnology and Bioengineering 88, 60–70.

Bhadoria, P. S., Dessougi, H. EI., Liebersbach, H., Claassen, N., 2004. Phosphorus uptake kinetics, size of root system and growth of maize and groundnut in solution culture. Plant and Soil 262, 327-336.

Blanch, S. J., Ganf, G. G.., Walker, K. F., 1999. Growth and resource allocation in response to flooding in the emerged sedge *Bolboschoenus medianus*. Aquatic Botany 63, 145-160.

Bledsoe, C. S., Rygiewicz, P. T., 1986. Ecotomycorrhizas affect ionic balance during ammonium uptake by Douglas-fir roots. New Phytologist 106, 271-283.

Bosserman, R. W. 1981. Elemental composition of aquatic plants from Okefenokee Swamp. Journal of Freshwater Ecology, 1, 307--320.

Bot, J. L., Adamowicz, A., Robin, P., 1998. Modelling plant nutrition of horticultural crops: a review. Scientia Horticulturae 74, 47-82.

Bourne, M. J., Lennox, G. W., Seddon, S. A., 1988. Fruits and Vegetables of the Caribbean. Macmillan Publishers Ltd., London.

Boyd, C. E., 1970. Production, mineral accumulation and pigment concentrations in *Typha latifolia* and *Scirpus americanus*. Ecology 51, 285-290.

Boyd, C. E., 1978. Chemical composition of wetland plants. In: Goood, R. E., Whighham, D. F., Simpson, R. L., Eds., Freshwater Wetlands: Ecological Processes and Management Potential. New York. Academic Press. pp 155-167.

Braskerud, B. C., 2002. Factors affecting nitrogen retention in small constructed wetlands treating agricultural non-point souce 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.

Britto, D. T., Kronzucher, H. J., 2002. NH_4^+ toxicity in high plants: a critical review. Journal of Plant Physiology 159, 567-584.

Brix, H., 1993. Wastewater treatment in constructed wetlands: system design, removal processes, and treatment performance. In: Moshiri, G. A., Ed., Constructed Wetlands for Water Quality Improvement. Lewis, Boca Raton, Ann Arbor, London, Tokyo, pp. 9-22.

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

Brix, H., 1994b. Use of constructed wetlands in water pollution control: historical development, present status and future perspectives. Water Science and Technology 30, 209-223.

Brix, H., 1994c. Constructed wetlands for municipal wastewater treatment in Europe. In: Mitsch, W. J., Ed., Global wetlands – old world and new. Elsevier, Amsterdam, pp 325-334.

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

Brix, H., Arias, C. A., Del Bubba, M., 2001. Media selection for sustainable phosphorus removal in subsurface flow constructed wetlands. Water Science and Technology 44, 47–54.

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., Lorenzen, B., Morris, J. T., Schierup. H. H., Sorrell, B. K., 1994. Effect of oxygen and nitrate on ammonium uptake kinetics and adenylate pools in *Phalaris arundinacea* L. and *Glyceria maxima* (Hartm) Holmb. Proceedings of Royal Society Edinburgh 102B, 333-342.

Brooker, R. W., Callaghan, T. V., Jonasson, S., 1999. Nitrogen uptake by rhizomes of the clonal sedge *Carex bigelowii*: a previously overlooked nutritional benefit of rhizomatous growth. New Phytologist 142, 35–48.

Browning, K., Greenway, M., 2003. Nutrient removal and plant growth in a subsurface flow constructed wetland in Brisbane, Australia, Water Science and Technology 48, 183-190.

Burgoon, P. S., Debusk T. A., Reddy, K. R., Koopman, B., 1991. Vegetated submerged beds with artificial substrates. II: N and P removal. Journal of Environmental Engineering 117, 408-424.

Busnardo, M. J., Gersberg, R. M., Langis, R., Sinicrope, T. L., Zedler, J. B., 1992. Nitrogen and phosphorus removal by wetland mesocosms subjected to different hydroperiods. Ecological Engineering 1, 287-307.

Calheiros, C. S. C., Rangel, A. O. S. S., Castro, P. M. L., 2007. Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater. Water Research 41, 1790-1798.

Cary, P. R., Weerts, P. G. J., 1984a. Growth and nutrient composition of *Typha orientalis* as affected by water temperature and nitrogen and phosphorus supply. Aquatic Botany 19, 105-118.

Cary, P. R., Weerts, P. G. J., 1984b. Growth of *Salvinia molesta* as affected by water temperature and nutrition. III. Nitrogen-phosphorus interactions and effect of pH. Aquatic Botany 19, 171-182.

Chambers, J. M., Fletcher, N. L., McComb, A. J., 1995. A guide to emergent wetland plants of south-western Australia. Marine and Freshwater Research Laboratory, Environmental Science, Murdoch University, Perth, Western Australia.

Chang, J., Yue, C., Ge, Y., Zhu, Y., 2004. Treatment of polluted creek water by multifunctional constructed wetland in China’s subtropical region. Fresenius Evironmental Bulletin 13, 545-549.

Chapin, F. S. III, 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics, 11, 233-260

Chapin, F. S. III, Moilanen, L., Kielland, K., 1993. Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature 361, 150-153.

Cheng, S., Ren, F., Grosse W., Wu, Z., 2002. Effects of cadmium on chlorophyll content, photochemical efficiency, and photosynthetic intensity of *Canna indica* Linn. International Journal of Phytoremediation 4, 239-246.

Chung, A. K. C., Wu, Y., Tam, N. F. Y., Wong, M. H., 2008. Nitrogen and phosphate mass balance in a sub-surface flow constructed wetland for treating municipal wastewater. Ecological Engineering 32, 81-89.

Clark, R. B., 1983. Plant genotype differences in the uptake, translocation, accumulation, and use of mineral elements required for plant growth. Plant and Soil 72, 175-196.

Clark, R. L., Brown, J. C., 1974. Differential phosphorus uptake by phosphorus-stressed corn inbreds. Crop Science 14, 505-508.

Clarke, E., Baldwin, A. H., 2002. Responses of wetland plants to ammonia and water level. Ecological Engineering 18, 257–264.

Clesceri, L. S., Greenberg, A. E., Eaton, A. D., 1998. Standard Methods for the Examination of Water and Wastewater. 20th Ed., APHA, AWWA, WPCF, Washington, DC.

Coleman, J., Hench, K., Garbutt, K., Sextone, A., Bissonnette, G., Skousen, J., 2001. Treatment of domestic wastewater by three wetland plant species in constructed wetlands. Water, Air and Soil Pollution 128, 283-295.

Connolly, R., Zhao, Y., Sun, G., Allen, S., 2004. Removal of ammoniacal-nitrogen from an artificial landfill leachate in downflow reed beds. Process Biochemistry 39, 1971–1976.

Coombs, J, Hall, D. O., Long, S. P., Scurlock, J. M. O., 1985. Techniques in Bioproductivity and Photosynthesis. Pergamon Press, Oxford, England, UK. 298 pp.

Cooper, P. F., Job, G. D., Green, M. B., Shutes, R. B. E., 1996a. Reed beds and constructed wetlands for wastewater treatment. Medmenham, Marlow, UK: WRc Publications, 184 pp.

Cooper, P. F., Smith, M., Maynard, H., 1996b. The design and performance of a nitrifying vertical flow reed bed treatment system. Water Science and Technology 35, 215-221.

Cox, W. J., Reisenzuer, H. M., 1973. Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium, or both. Plant and Soil 28, 363-380.

Cronk, J. K., 1996. Constructed wetlands to treat wastewater from dairy and swine operations: a review. Agriculture, Ecosystems and Environment 58, 97-114.

Cronk, J. K., Fennessy, M. S., 2001. Wetland plants: Biology and Ecology. Lewis Publisher, Boca Raton, FL, USA. 462 pp.

da Motta Marques, D. M. L., Leite, G. R., Giovannini, S. G. T., 2000. Performance of two macrophyte species in experimental wetlands receiving variable loads of anaerobically treated municipal wastewater. Water Science and Technology 35, 27-34.

Daniels, R. E., 1991. Variation in the performance of *Phragmites australis* in experimental culture. Aquatic Botany 42, 41-48.

Davis, A. P., Shokouhian, M., Sharma, H., Minami, C., 2006. Water quality improvement through bioretention media: nitrogen and phosphorus removal. Water Environment Research 78, 284-293.

Davis, J. R., Koop, K., 2006. Eutrophication in Australian rivers, reservoirs and estuaries-a southern hemisphere perspective on the science and its implications. Hydrobiologia 559, 23-76.

DeBusk, T. A., Dierberg, F. E., 1999. Techniques for optimizing phosphorus removal in treatment wetlands. In: Reddy, K. R., O'Conner, G. A., Schelske, C. L., Eds., Phosphorus Biogeochemistry in Subtropical Ecosystems. Lewis Publication, Boca Raton, FL. pp. 467-488.

DeBusk, T. A., Peterson, J. E., Reddy, K. R., 1995. Use of aquatic and terrestrial plants for removing phosphorus from dairy wastewaters. Ecological Engineering 5, 371–390.

Dévai, I., Felfoldy, L., Wittner, I., Plósz, S., 1988. Detection of phosphine: New aspects of the phosphorus cycle in the hydrosphere. Nature (London) 333, 343-345.

Drizo, A., Comeau, Y., Forget, C., Chapuis, R. P., 2002. Phosphorus saturation potential: a parameter for estimating the longevity of constructed wetland systems. Environmental Science and Technology 36, 4642–4648.

Drizo, A., Frost, C. A. , Grace, J., Smith, K. A., 1999. Physico-chemical screening of phosphate-removing substrates for use in constructed wetland systems. Water Research 33, 3595-3602.

Dyhr-Jensen, K., Brix, H., 1996. Effects of pH on ammonium uptake by *Typha latifolia* L. Plant, Cell and Environment 19, 1431-1436.

Edwards, K. R., Čižková, H., Zemanová, K., Šantrůčková, H., 2006. Plant growth and microbial processes in a constructed wetland planted with *Phalaris arundinacea*. Ecological Engineering 27, 153-165.

Edwards, M. E., Brinkmann, K. C., Watson, J. T., 1993. Growth of soft-stem bulrush (*Scirpus validus*) plants in gravel-based subsurface flow constructed wetlands. In: Moshiri, G. A., Ed., Constructed wetlands for water quality improvement. Lewis Publishers, Boca Raton FL. pp. 415–425.

Engler, R. M, Patrick, W. H. Jr., 1974. Nitrate removal from floodwater overlying flooded soils and sediments. Journal of Environmental Quality 3, 409-413.

EPA, 1993. Constructed wetlands for wastewater treatment and wild life habitat: 17 case studies. EPA832-R-93-005. US Environmental Protection Agency, Cincinnati, OH.

EPA, 2000. Manual, Constructed Wetlands Treatment of Municipal Wastewaters. EPA/625/R-99/010. US Environmental Protection Agency, Cincinnati, OH.

Ericsson, T., 1995. Growth and shoot–root ratio of seedlings in relation to nutrient availability. Plant and Soil 169, 205–214.

Evans, J. R., 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78, 8-19.

Fang, Y. Y., Babourina, O., Rengel, Z., Yang, X. E., Pu, P. M., 2007a. Ammonium and nitrate uptake by floating plant *Landoltia punctata.* Annals of Botany 99, 365-370.

Fang, Y. Y., Babourina, O., Rengel, Z., Yang, X. E., Pu, P. M., 2007b. Spatial distribution of ammonium and nitrate fluxes along roots of wetland plants. Plant Science 173, 240-246.

Faulkner, S. P., Richardson, C. J., 1989. Physical and chemical characteristics of freshwater wetland soils. In: Hammer, D. A., Ed., Constructed wetlands for wastewater treatment. Lewis Publishers, Chelsea, Michigan, pp 41-72.

Fennessy, M. S., Mitsch, W. J., 1989. Treating coal mine drainage with an artificial wetland. Research Journal Water Pollution Control Federation 61, 1691-1701.

Finlayson, C. M., Chick. A. J. 1983. Testing the potential of aquatic plants to treat abattoir effluent. Water Research 17, 415-422.

Forde, B. G., Clarkson, D. T., 1999. Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. Advances in Botany Research 30, 1–90.

Fraser, L. H., Carty, S. M., Steer, D., 2004. A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. Bioresource Technology 94, 185-192.

Fraser, L. H., Keddy, P., 1997. The role of experimental microcosms in ecological research. Tree 12, 478-481.

Froelich, P. N., 1988. Kinetic control of dissolved phosphate in natural rivers and estuaries: a primer on the phosphate buffer mechanism. Limnology and Oceanography 33, 649-668.

Fu, C., Tang, Y., Chen, X., Xu, Y., Xing, G., 2006. Effects of purification highly salty reuse water quality by three plants in TEDA landscape river. Journal of Chongqing University (Natural Science Edition) 29, 118-121 (in Chinese, with English abstract).

Fu, C., Tang, Y., Zhang Z., Li, J., Li, J., Chen, S., Guang, D., 2005. Study on the removal efficiencies of salt water by *Canna indica* Linn. in TEDA landscape river. Journal of Irrigation and Drainage 24, 70-73 (in Chinese, with English abstract).

Furlani, A. M. C., Clark, R. B., Maranville, J. W., Ross, W. M., 1984. Sorghum genotype differences in phosphorus uptake rate and distribution in plant parts. Journal of Plant Nutrition 7, 1113-1126.

Fyson, A., 2000. Angiosperms in acidic waters at pH 3 and below. Hydrobiologia 433, 129-135.

Gambrell, R. P., Patrick, W. H., 1978. Chemical and microbiological properties of anaerobic soils and sediments. In: Hook, D. D., Crawford, R. M. M., Eds., Plant life in anaerobic environments, Ann Arbor Science Publishers, Michigan Ann Arbor. pp. 375-432.

Garnett, T. P., Smethurst, P. J., 1999. Ammonium and nitrate uptake by *Eucalyptus nitens*: effect of pH and temperature. Plant and Soil 214, 133-140.

Gassmann, G., 1994. Phosphine in the fluvial and marine hydrosphere. Marine Chemist 45, 197-205.

Gaudet, C. L., Keddy, P. A., 1988. A comparative approach to predicting competitive ability from plant traits. Nature 334: 242-243.

Ge, Y., Chang, J., Wang, X.Y., 2000. Relationship between the physiological characters and purification ability of different plants in waters with two trophic levels. *Acta Ecologica Sinica* 20, 1050-1055 (in Chinese, with English abstract).

Gearhart, R. A., Klopp, F., Allen, G., 1989. Constructed free surface wetlands to treat and receive wastewater; pilot project to full scale. In: Hammer, D. A., Ed., Constructed wetlands for wastewater treatment. Lewis Publishers, Chelsea, Michigan, pp 121-138.

Gersberg, R. M., Elkins, B. V., Lyon, S. R., Goldman, C. R., 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Research 20, 363-368.

Glass, A. D. M., Siddiqi, M. Y., 1984. The control of nutrient uptake rates in relation to the inorganic composition of plants. Advances in Plant Nutrition 1, 103-147.

Glindemann, D., Edwards, M., Liu, J., Kuschk, P., 2005. Phosphine in soils, sludges, biogases and atmospheric implications – a review. Ecological Engineering 24, 457-463.

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 agricultureal (dairy) wastewater, Ontario, Canada. Ecological Engineering 29, 154-163.

Greenway, M., 1997. Nutrient content of wetland plants in constructed wetland receiving municipal effluent in tropical Australia. Water Science and Technology 35, 135-142.

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.

Greenway, M., Woolley, A., 2001. Change in plant biomass and nutrient removal over 3 years in a constructed wetlands, Cairns, Australia. Water Science and Technology 44, 303-310.

Grime, J. P., Hodgson, J. G.., 1987. Botanical contributions to contemporary ecological theory. New Phytologist 106 (Suppl.): 283-295.

Grosse, W., Wissing, F. W., Perfler, R., Wu, Z., Chang, J., Lei, Z., 2001. Water quality improvement in tropical and subtropical areas for reuse and rehabilitation of aquatic ecosystem. In: Gawlik, B. M., Platzer, B., Muntau, H., Eds., Freshwater Contamination in China. Office for official publications of the European Communities, European Communities, pp 19-32.

Güsewell, S., 2004. N:P ratios in terrestrial plants: variation and functional significance. New Phytologist 164, 243-266.

Güsewell, S., Bollens, U., 2003. Composition of plant species mixtures grown at various N:P ratios and levels of nutrient supply. Basic and Applied Ecology 4, 453-466.

Güsewell, S., Koerselman, W., 2002. Variation in nitrogen and phosphorus concentrations of wetland plants. Perspectives in Plant Ecology Evolution and Systematics 5, 37-61.

Haberl, R., 1999. Constructed wetlands: a chance to solve wastewater problems in developing countries. Water Science and Technology 40, 11-17.

Hammer, D. A., 1989. Constructed wetlands for wastewater treatment. Chelsea, MI. Lewis Publishers. pp 831.

Hammer, D. A., 1992. Designing constructed wetland systems to treat agricultural nonpoint source pollution. Ecological Engineering 1, 49-82.

Hammer, D. A., 1994. Guidelines for design, construction and operation of constructed wetlands for livestock wastewater treatment. In: DuBowy, P. J., Reaves, R. P., Eds., Proceedings of a workshop on constructed wetlands for animal waste management. Lafayette, IN, pp. 155-181.

Hammer, D. A., Bastian, R. X., 1989. Wetlands ecosystems: Natural water purifiers. In: Hammer, D. A., Ed., Constructed Wetlands for Wastewater Treatment. Chelsea, Lewis, pp. 5-19.

Harper, J. L., 1977. Population Biology of Plants. Academic Press, New York.

Headley, T. R., 2004. Removal of nutrients and plant pathogens from plant nursery runoff using horizontal subsurface flow constructed wetlands. PhD Thesis, Southern Cross University, Lismore, NSW, Australia, 170p.

Headley, T. R., Huett, D. O., Davison, L., 2001. The removal of nutrients from plant nursery irrigation runoff in subsurface horizontal-flow wetlands. Water Science and Technology 44, 77–84.

Heberer, J. A., Below, F. E., 1989. Mixed nitrogen nutrition and productivity of wheat grown in hydroponics. Annals of Botany 63, 643-649.

Hermans, C., Hammond, J. P., White, P. J., Verbruggen, N., 2006. How do plants respond to nutrient shortage by biomass allocation? Trends in Plant Science 11, 610-617.

Hill, D. T., Payne, V. W. E., Rogers, J. W., Kown, S. R., 1997. Ammonia effects on the biomass production of five constructed wetland plant species. Bioresource Technology 62, 109-113.

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant and Soil 248, 43-59.

Hofstee, B. H. J., 1952. On the evaluation of the constants V_m and K_m in enzyme reactions. Science 116, 329-331.

Hogetu, K., 1984. Bioeconomics. Syokabo Press. Tokyo, 245 p.

Huang, J., Reneau Jr, R. B., Hagedorn, C., 2000. Nitrogen removal in constructed wetlands employed to treat domestic wastewater. Water Research 34, 2582-2588.

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

Hunter, R. G., Combs, D. L., Georage, D. B., 2000. Growth of softstem bulrush (*Scirpus validus*) in microcosms with different hydrologic regimes and media depths. Wetlands 20, 15-22.

Hunter, R. G., Combs, D. L., Georage, D. B., 2001. Nitrogen, phosphorus and orgnic carbon removal in simulated wetland treatment systems. Archives of Environmental Contamination and Toxicology 41, 274-281.

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.

Jackson, M. L., 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Jamieson, T. S., Stratton, G. W., Gordon, R. and Madani, A., 2003. The use of aeration to enhance ammonia nitrogen removal in constructed wetlands. Canadian Biosystems Engineering 45, 9-14.

Jenssen, P. D., Maehlum, T., Krogstad, T., 1993. Potential use of constructed wetlands for wastewater treatment in northern environments. Water Science and Technology 28, 149-157.

Jetten, M. S. M., 2001. New pathways for ammonia conversion in soil and aquatic systems. Plant and Soil 230, 9–19.

Jinadasa, K. B. S. N., Tanaka, N., Mowjood, M. I. M., Werellagama, D. R. I. B., 2006. Free water surface constructed wetlands for domestic wastewater treatment: A tropical case study. Chemistry and Ecology 22, 181-191.

Jing, S. H., Lin, Y. F., Wang, T. W., Lee, D. Y., 2002. Microcosm wetland for wastewater treatment with different hydraulic loading rates and macrophytes. Journal of Environmental Quality 31, 690-696.

Johnson, C. A., 1991. Sediment and nutrient retention by freshwater wetlands: Effects on surface water quality. CRC Critical Review of Environmental Control 21, 491-565.

Jungk, A., Barber, S. A., 1975. Plant age and the phosphorus uptake characteristics of trimmed and untrimmed corn root systems. Plant and Soil 42, 227-239.

Juwarkar, A. S., Oke, B., Juwarkar, A., Patnaik, S. M., 1995. Domestic wastewater treatment through constructed wetland in India. Water Science and Technology 32, 291-294.

Kadlec, R. H, 1995. Overview: surface flow constructed wetlands. Water Science and Technology 32, 1-12.

Kadlec, R. H., 2006. Water temperature and evapotranspiration in surface flow wetlands in hot arid climate. Ecological Engineering 26, 328-340.

Kadlec, R. H., Knight, R. L., 1996. Treatment Wetlands. Lewis Publisher, Boca Raton, FL, USA, 893 pp.

Kadlec, R. H., Tilton, D. L, 1979. The use of freshwater wetlands as a tertiary wastewater treatment alternative. CRC Critical Reviews in Environmental Control 9, 185-212.

Karathanasis, A. D., Potter, C. L., 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.

Karathanasis, A. D., Potter, C. L., 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.

Kempers, A. J., Luft, A. G., 1988. Re-examination of the determination of environmental nitrate as nitrite by reduction with hydrazine. Analyst 113, 1117-1120.

Kennedy, M. P., Milne, J. M., Murphy, K. J., 2003. Growth responses to groundwater level variation and competition in freshwater wetland plant species. Wetlands Ecology and Management 11: 383 – 396.

Khamis, S., Lamaze, T., Lemoine, Y., Foyer, C., 1990. Adaptation of the photosynthetic apparatus in maize leaves as a result of nitrogen limitation. Plant Physiology 94, 1436-1443.

Kim, S. Y., Geary, P. M., 2001. The impact of biomass harvesting on phosphorus uptake by wetland plants. Water Science and Technology 44, 61–67.

Kinraide, T. B., 1991. Identity of the rhizotoxic aluminum species. Plant and Soil 134, 167-178.

Kirk, G. J. D., Kronzucker, H. J., 2005. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. Annals of Botany 96, 639–646.

Kirkman, L. K., Sharitz, R. R., 1993. Growth in controlled water regimes of three grasses common in freshwater wetlands of the southeastern USA. Aquatic Botany 44: 345-359.

Kivaisi, A. K., 2001. The potential for constructed wetlands for wastewater treatment and reuse in developing countries: a review. Ecological Engineering 16, 545-560.

Klomjek, P., Nitisoravut, S. 2005. Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere 58, 585--593.

Knight, R. L., 1997. Wildlife habitat and public use benefits of treatment wetlands. Water Science and Technology 35, 35-43.

Knight, R. L., McKim, T. W., Kohl, H. R., 1987. Performance of a natural wetland treatment system for wastewater management. Journal Water Pollution Control Federation. 59, 746-754.

Koerselman, W., Meuleman, A. F. M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33, 1441-1450.

Koottatep, T., Polprasert, C., 1997. Role of plant uptake on nitrogen removal in constructed wetlands located in the tropics. Water Science and Technology 36, 1-8.

Korkusuz, E. A., Beklioğlu, M., Demirer, G. N., 2007. Use of blast furnace granulated slag as a substrate in vertical flow reed beds: Field application. Bioresource Technology 98, 2089-2101.

Krishnan, S. B., Smith, L. E., 1987. Public health issues of aquatic systems used for waste treatment. In: Reddy, K. R., Smith, W. H., Eds., Aquatic Plants for Water Treatment and Resource Recovery. Magnolia Publishing, Orlando, FL, pp. 855-878.

Kronzucher, H. J., Glass, A. D. M., Siddiqi, M. Y., Kirk, G. J. D., 2000. Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: implications for rice cultivation and yield potential New Phytologist 145, 471–476.

Kronzucker, H. J., Siddiqi, M. Y., Glass, A. D. M., 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385, 59-61.

Kronzucker, H. J., Siddiqi, M. Y., Glass, A. D. M., Kirk, G. J. D., 1999. Nitrate–ammonium synergism in rice: a subcellular flux analysis. Plant Physiology 119, 1041–1045.

Kurniadie, D., Kunze, C., 2000. Constructed wetlands to treat house wastewater in Bandung, Indonesia. Journal of Applied Botany 74, 87-91.

Kuschk, P., Wießne, A., Kappelmeyer, U., Wießbrodt, E., Kästner, M., Stottmeister, U., 2003. Annual cycle of nitrogen removal by a pilot-scale subsurface horizontal flow in a constructed wetland under moderate climate. Water Research 37, 4236-4242.

Kyambadde, J., Kansiime, F., Gumaelius, L., Dalhammer, G., 2004. A comparative study of *Cyperus papyrus* and *Miscanthidium violaceum*-based constructed wetlands for wastewater treatment in a tropical climate. Water Research 38, 475-485.

Lakshman, G., 1987. Ecotechnological opportunities for aquatic plants, a survey of utilization options. In: Reddy, K. R., Smith, W. H. eds., Aquatic plants for water treatment and resource recovery. Magnolia Publishing Inc. Orlando, FL, pp 49-68.

Lambers, H., Chapin III, F. S., Pons, L., 1998. Plant physiological ecology. New York, Springer.

Lantzke, I. R., Heritage, A. D., Pistillo, G., Mitchell, D. S., 1998. Phosphorus removal rates in bucket size planted wetlands with a vertical hydraulic flow. Water Research 32, 1280–1286**.**

Lee, B. H., Scholz, M., 2007. What is the role of *Phragmites australis* in experimental constructed wetland filters treating urban runoff? Ecological Engineering 29, 87-95.

Lee, C. Y., Lee, C. C., Lee, F. Y., Tseng, S. K., Liao, C. J., 2004. Performance of subsurface flow constructed wetland taling pretreated swine effluent under heavy loads. Bioresource Technology 92, 173-179.

Lee, M. A., Stansbury, J. S., Zhang, T. C., 1999. The effect of low temperatures on ammonia removal in laboratory-scale constructed wetland. Water Environment Research 71, 340-347.

Lim, P. E., Wong, T. F., Lim, D. V., 2001. Oxygen demand, nitrogen and copper removal by free-water-surface and subsurface-flow constructed wetlands under tropical conditions. Environment International 26, 425-431.

Limmer, A. W., Steele, K. W., 1982. denitrification potentials: measurement of seasonal variation using a short-term anaerobic incubation technique. Soil Biology and Biochemistry 14, 179-184.

Lin, Y. F., Jing, S. R., Lee, D. Y., Wang, T. W., 2002. Nutrient removal from aquaculture wastewater using a constructed wetlands system. Aquaculture 209, 169-184.

Lineweaver, H., Burk, D., 1934. The determination of enzyme dissociation constants. Journal of American Chemistry Society 56, 658-666.

Livingston, E. H., 1989. Use of wetlands for urban stormwater management. In: Hammer, D. A,, Ed., Constructed Wetlands for Wastewater Treatment. Chelsea, Lewis, pp. 253-264.

Longstreth, D. J., Nobel, P. S., 1980. Nutrient influence on leaf photosynthesis: effects of nitrogen, phosphorus, and potassium for Gossypium hirsutum L. Plant Physiology 65, 541-543.

Lorenzen, B., Brix, H., Mendelssohn, I. A., McKee, K. L., Miao, S. L., 2001. Growth, biomass allocation and nutrient use efficiency in *Cladium jamaicense* and *Typha domingensis* as affected by phosphorus and oxygen availability. Aquatic Botany 70, 117-133.

Loustalot, A. J., Gilbert, S. G., Drosdoff, M., 1950. The effect of nitrogen and potassium levels in tung seedlings on growth, apparent photosynthesis, and carbohydrate composition. Plant Physiology 25, 394-412.

Lu, C., Zhang, J., 2000. Photosynthetic CO_2 assimilation, chlorophyll fluorescence and photoinhibition as affected by nitrogen deficiency in maize plants. Plant Science 151, 135-143.

Luederitz, V., Eckert, E., Lange-Weber, M., Lange, A., Gersberg, R. M., 2001. Nutrient removal efficiency and resource economics of vertical flow and horizontal flow constructed wetlands. Ecological Engineering 18, 157–171.

Lymbery, A. J., Doupé, R. G., Bennett, T., Starcevich, M. R., 2006. Efficacy of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. Aquacultural Engineering 34, 1-7.

Madigan, M. T., Martinko, J. M., Parker, J., 1997. Brock Biology of Microorganisms, eighth ed. New Jersey, Prentice hall, 1036 p.

Malagoli, M., Dal Canal, A., Quaggiotti, S., Pegoraro, P., Bottacin, A., 2000. Differences in nitrate and ammonium uptake between Scots pine and European larch. Plant and Soil 221, 1–3.

Mander, Ü., Mauring, T., 1997. Constructed wetlands for wastewater treatment in Estonia. Water Science and Technology 35, 323-330.

Marscher, H., 1995. Mineral Nutition of High Plants. 2nd ed. Academic Press, London.

Marschner, H., Häussling, M., Georage, E., 1991. Ammonium and nitrate uptake rate and rhizoshere pH in non-mycorrhizal roots of Norway spruce [*Picea abies* (L.) Karst.]. Trees 5, 14-21.

Martin, J., Hofherr, E., Quigley, M. F., 2003. Effect of Typha latifola transpiration and harvesting on nitrate concentrations in surface water of wetland microcosms. Wetland 23, 835-844.

Martinez, C. J., Wise, W., 2003. Hydraulic analysis of Orlando easterly wetland. Journal of Environmental Engineering 129, 553-560.

Maschinski, J., Southam, G., Hines, J., Strohmeyer, S., 1999. Efficiency of a subsurface constructed wetland system using native southwestern US plant. Journal of Environmental Quality 28, 225-231.

Mayo, A. W., Mutamba, J., 2004. Effect of HRT on nitrogen removal in a coupled HRP and unplanted subsurface flow gravel bed constructed wetland. Physics and Chemistry of the Earth 29, 1253-1257.

McJannet, C. L., Keddy, P. A., Pick, F. R., 1995. Nitrogen and Phosphorus tissue concentrations in 41 wetland plants a comparison across habitats and functional groups. Functional Ecology 9, 231-238.

McLachlan, S. M., Tollenaar, M., Swanton, C. J., Weise, S. F., 1993. Effect of corn-induced shading on dry matter accumulation, distribution, and architecture of redroot pigweed (*Amaranthus retroflexus*). Weed Science 41, 568-573.

Mehrer, I., Mohr, H., 1989. Ammonium toxicity—description of the syndrome in *Synapis alba* and the search for its causation, Physiologia Plantarum 77, 545–554.

Mengel, D. B., Barber, S. A., 1974. Rate of nutrient uptake per unit of corn under field conditions. Agronomy Journal 66, 399-402.

Michael Jr., J. H., 2003. Nutrients in salmon hatchery wastewater and its removal through the use of a wetland constructed to treat off-line settling pond effluent. Aquaculture 226, 213-225.

Min, X., Siddiqi, M. Y., Guy, R. D., Glass, A. D. M., Kronzucker, H. J., 1998. Induction of nitrite uptake and nitrate reductase activity in trembling aspen and lodgepole pine. Plant, Cell and Environment 21, 1039-1046.

Min, X., Siddiqi, M. Y., Guy, R. D., Glass, A. D. M., Kronzucker, H. J., 1999. A comparative study of fluxes and compartmentation of nitrate and ammonium in early-successional tree species. Plant, Cell and Environment 22, 821-830.

Mitsch, W. J., Cronk, J. K., 1992. Creation and restoration of wetlands: some design considerations for ecological engineering. Advances in Soil Science 17, 217-259.

Mitsch, W. J., Gosselink, J. G., 2000. Wetlands. 3rd edition. John Wiley & Sons, New York. 920p.

Moshiri, G. A., 1993. Wastewater treatment in constructed wetlands: system design, removal processes and treatment performance. In: Moshiri, G. A., Ed., Constructed wetlands for wastewater quality improvement. CRC Press, Chelsea, Michigan, pp 9-22.

Mulamoottil, G., Mcbean, E. A., Rovers, F., 1999. Constructed wetlands for the treatment of landfill leachate, Boca Raton, FL. Lewis Publishers, pp. 273.

Müller, I., Schmid, B., Weiner, J., 2000. The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspectives in Plant Ecology, Evolution and Systematics 3, 115-127.

Murphy, J., Riley, J. P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31-36.

National Herbarium of New South Wales, 2007. Flora of New South Wales: A comprehensive botanical treatment in an electronic format http://plantnet.rbgsyd.nsw.gov.au.

Nelson, S. G., Smith, B. D., Best, B. R., 1981. Kinetics of nitrate and ammonium uptake by the tropical freshwater macrophyte P*istia stratiotes* L. Aquaculture 24, 11-19.

Neralla, S., Weaver, R. W., Varvel, T. W., Lesikar, B. J. 1999. Phytoremediation and on-site treatment of septic effluents in sub-surface flow constructed wetlands. Environmental Technology 20, 1139--1146.

Newman, S., Pietro, K., 2001. Phosphorus storage and release in response to flooding: implications for Everglades stormwater treatment areas. Ecological Engineering 18, 23-38.

Nichols, D. S., 1983. Capacity of natural wetlands to remove nutrients from wastewater. Journal Water Pollution Control Federation 55, 495-505.

NLWRA, 2002. Australian's natural resources 1997-2002 and beyond. National Land and Water Resources Audit, Canberra ACT.

Nunes, M. A., Ramalho, J. C., Dias, M. A., 1993. Effect of nitrogen supply on the photosynthythetic performance of leaves from coffee plants exposed to bright light. Journal of Experimental Botany 262, 893-899.

Nyakang'O, J. B., van Bruggen, J. J. A., 1999. Combination of a well functioning constructed wetland with a pleasing landscape design in Nairobi, Kenya. Water Science and Technology 40, 249-256.

Odum, H. T., Wojcik, W., Pritchard, L., Jr Ton, S., Delfino, J. J., Woojcik M, Leszczynski S, Patel JD, Doherty SJ, Stasik J. 2000. Heavy metals in the environment: using wetlands for their removal, Boca Raton, FL. Lewis Publishers, 326 pp.

Ouellet-Plamondon, C., Chazarenc, F., Comeau, Y., Brisson, J., 2006. Artificial aeration to increase pollutant removal efficiency of constructed wetlands in cold climate. Ecological Engineering 27, 258-264.

Overall, R. A., Parry, D. L., 2004. The uptake of uranium by *Eleocharis dulcis* (Chinese water chestnut) in the Ranger Uranium Mine constructed wetland filter. Environmental Pollution 132, 307-320.

Pace, G. M., McClure, P. R., 1986. Comparison of nitrite uptake kinetic parameters across maize inbred lines. Journal of Plant Nutrition 9, 1095-1111.

Pant, H. K., Reddy, K. R., Lemon, E., 2001. Phosphorus retention capacity of root bed media of sub-surface flow constructed wetlands. Ecological Engineering 17, 345-355.

Paul, E. A., Clark, F. E., 1996. Soil microbiology and biochemistry. 2nd ed. San Diego, California, Academic Press. 340 pp

Peterson, S. B., Teal, J. M., 1996. The role of plants in ecologically engineered wastewater treatment systems. Ecological Engineering 6, 137-148.

Peuke, A. D., Kaiser, W. M., 1996. VI. Nitrate or ammonium uptake and transport, and rapid regulation of nitrate reduction in higher plants. In: Behnke, H. D., Lüttge, U., Esser, K., Kadereit, J. W., Runge, M., Eds.., Progress in Botany, Vol. 57. Springer-Verlag, Berlin, pp. 93–113.

Peuke, A. D., Tischner, R., 1991. Nitrate uptake and reduction of aseptically cultivated spruce seedlings, *Picea abies* (L.) Karst. Journal of Experimental Botany 42, 723-728.

Peverly, J. H., 1985. Element accumulation and release by macrophytes in a wetland stream. Journal of Environmental Quality 14, 137-143.

Pfenning, K. S., McMahon, P. B., 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rate in nitrate-rich riverbed sediments. Journal of Hydrology 187, 283-295.

Picard, C.R., Fraser, L.H., Steer, D., 2005. The interacting effects of temperature and plant community type on nutrient removal in wetland microcosms. Bioresource Technology 96, 1039-1047.

Pompêo, M. L. M., Henry, R., Moschini-Carlos, V., 1999. Chemical composition of tropical macrophyte *Echinocloa polystachya* (H.B.K.) Hitchcock in Jurimirim reservoir (São Paulo, Brazil). Hydrobiologia 411, 1-11.

Poorter, H., Nagel, O., 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Australian Journal of Plant Physiology 27, 595-607.

Radcliffe, J. C., 2004. Water recycling in Australia. Australia Academy of Technological Sciences and Engineering, pp 233.

Raghoebarsing, A. A., Arjan, P., van de Pas-Schoonen, K. T., Smolders, A. J. P., Ettwig, K. F., Rijpstra, W. I. C., Schouten, S., Sinninghe Damste, J. S., Op den Camp, H. J. M., Jetten, M. S. M., Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440, 918–921.

Raman, D. R., Spanswich, R. M., Walker, L. P., 1995. The kinetics of nitrate uptake from flowing nutrient solutions by rice: influence of pretreatment and light. Bioresource Technology 53, 125-132.

Ran, N., Agami, M., Oron, G., 2004. A pilot study of constructed wetlands using duckweed (*Lemna gibba L.*) for treatment of domestic primary effluent in Israel. Water Research 38, 2241-2248.

Rao, T. P., Ito, O., Matsung, R., 1993. Difference in uptake kinetics of ammonium and nitrate in legumes and cereals. Plant and Soil 154, 67-72.

Rao, T. P., Yano, K., Iijima, M., Yamauchi, A., Tatsumi, J., 2002. Regulation of rhizosphere acidification by photosynthetic activity in cowpea (*Vigna unguiculata* L. Walp.), Seedl. Annals of Botany 89, 213–220.

Raskin, I., 1983 A method for measuring leaf volume, density, thickness, and internal gas volume. HortScience 18, 698-699.

Reddy, K. R., 1981. Diel variation of certain physico-chemical parameters of water in selected aquatic system. Hydrobiologia 85, 201-207.

Reddy, K. R., 1983a. Fate of nitrogen and phosphorus in a waste-water retention reservoir containing aquatic macrophytes. Journal of Environmental Quality 12, 137-141.

Reddy, K. R., 1983b. Nitrogen and phosphorus interchange between sediments and overlying water of a wastewater retention pond. Hydrobiologia 98, 237-243.

Reddy, K. R., DeBusk, W. F., 1985. Nutrient removal potential of selected aquatic macrophytes. Journal of Environmental Quality 14, 459–462.

Reddy, K. R., DeBusk, W. F., 1987. Nutrient storage capabilityes of aquatic and wetland plants. In: Reddy, K. R., Smith, W. H., Eds., Aquatic Plants for Water Treatment. Orlando, L. Magnolia Publishing. pp. 337-357.

Reddy, K. R., Kadlec, R. H., Flaig, E., Gale, P. M., 1999. Phosphorus retention in streams and wetlands: a review. Critical Reviews in Environmental Science and Technology 29, 83-146.

Reddy, K. R., Patrick, W. H., 1984. Nitrogen transformations and loss in flooded soils and sediments. Critical Review in Environmental Control 13, 273–309.

Reed, S. C., Crites, R. W., Middlebrooks, E. R. J., 1995. Natural systems for waste management and treatment, second edition, McGraw hill, New York.

Reekie, E. G., Bazzaz, F. A., 1987. Reproductive effort in plants. 2. Does carbon reflect allocation of other resources? American Naturalist 129, 897-906.

Reich, P. B., Schoettle, A. W., 1988. Role of phosphorus and nitrogen in photosynthetic and whole plant carbon gain and nutrient use efficiency in eastern white pine. Oecologia 77, 25-33.

Rengel, Z., 2002. Handbook of plant growth: pH as the master variable. New York, Marcel Dekker.

Richardson, C. J., 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. Science 228, 1424-1427.

Richardson, C. J., Craft, C. B., 1993. Effective phosphorus retention in wetlands: fact or fiction? In: Moshiri, G. A., Ed., Constructed wetlands for wastewater quality improvement. CRC Press, Chelsea, Michigan, pp 271-282.

Robertson, L. A., Kuenen, J. G., 1990. Combined heterotrophic nitrification and aerobic denitrification in thiosphaera-pantotropha and other bacteria. Antonie van Leeuwenhoek 57, 139–152.

Roels, J., Verstrarte, W., 2001. Biological formation of volatile phosphorus compounds. Bioresource Technology 79, 243-250.

Romero, J. A., Brix, H., Comín, F. A., 1999. Interactive effects of N and P on growth, nutrient allocation and NH_4 uptake kinetics by *Phragmites australis*. Aquatic Botany 64, 369-380.

Salhani, N., Stengel, E., 2001. A comparative study of the gas exchange potential between three wetland species using sulfur hexafluoride as a tracer. Ecological Engineering 18, 15-22.

Samecka-Cymweman, A., Stepien, D., Kempers, A. J., 2004. Efficiency in removing pollutants by constructed wetland purification systems in Poland. Journal of Toxicology and Environmental Heath, Part A, 67, 265-275.

Schachtman, D. P., Reid, R. J., Ayling, S. M., 1998. Phosphorus uptake by plants: from soil to cell. Plant Physiology 116, 447-453.

Schenk, M. K., Barber, S. A., 1980. Potassium and phosphorus uptake by corn genotyes grown in the field as influenced by root characteristics. Plant and Soil 54, 65-76.

Schipper, L. A., Cooper, A. B., Harfoot, C. G., Dyck, W. J., 1993. Regulators of denitrification in an organic riparian soil. Soil Biology and Biochemistry 25, 925.

Scholz M., Lee B. H., 2005. Constructed wetlands: a review. International Journal of Environmental Studies 62, 421-447.

Schulz, C., Gelbrecht, J., Rennert, B., 2003. Treatment of rainbow trout farm effluents in constructed wetland with emergent plants and subsurface horizontal water flow. Aquaculture 217, 207-221.

Sculthorpe, C. D., 1967. The biology of aquatic vascular plants. Edward Arnold, London.

Searle, P. L., 1984. The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. Analyst 109, 549-568.

Sekiranda, S. B. K., Kiwanuka, S., 1998. A study of nutrient removal efficiency of *Phragmites mauritianus* in experimental reactors in Uganda. Hydrobiologia 364, 83-91.

Shi, L., Wang, B., Cao, X., Wang, J., Lei, Z., Wang, Z., Liu, Z., Lu, B., 2004. Performance of a subsurface-flow constructed wetland in southern China. Journal of Environmental Sciences-China 16, 476-481.

Shi, L., Wang, B., Cao, X., Wang, J., Liu, Z., Lu, B., 2005. Growing characteristics of several plants and their removal ability on pollutants in Shatian constructed wetland. Journal of Agro-Environment Science 24, 98-103 (in Chinese, with English abstract).

Shilton, A. N., Prasad, J. N., 1996. Tracer studies of a gravel bed wetland. Water Science and Technology 34, 421-425.

Shuval, H. I., Adin, A., Fattal, B., Rawitz, E., Yekutiel, P., 1986. Wastewater irrigation in developing countries. World Bank Technical Paper, No. 51.

Shuval, I., 1987. Health guidelines and standards for wastewater reuse in agriculture: historical perspective. Water Science and Technology 23, 2073-2080.

Silvan, N., Vasander, H., Laine, J., 2004. Vegetation is the main factor in nutrient retention in a constructed wetland buffer. Plant and Soil 258, 179–187.

Sim, C. H., Yusoff, M. K., Shutes, B., Ho, S. C., Mansor, M., 2007. Nutrient removal in a pilot and full scale constructed wetland, Putrajaya city, Malaysia. Journal of Environmental Management, doi: 10.1016/j.jenvman.2007.03.011

Sipe, T. W., Bazzaz, F. A., 1994. Gap partitioning among Maples (*Acer*) in central New England: shoot architecture and photosynthesis. Ecology 75: 2318-2332.

Sirianuntapiboon, S., Kongchum, M., Jitmaikasem, W., 2006. Effects of hydraulic retention time and media of constructed wetland for treatment of domestic wastewater. African Journal of Agricultural Research 1, 27-37.

Solano, M. L., Soriano, P., Ciria, M. P., 2004. Constructed wetlands as a sustainable solution for wastewater treatment in small villages. Biosystems Engineering 87, 109-118.

Sooknah, R. D., Wilkie, A. C., 2004. Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. Ecological Engineering 22, 27-42.

Sorrell, B. K., Armstrong, W., 1994. On the difficulties of measuring oxygen release by root systems of wetland plants. Journal of Ecology 82, 177-183.

Sorrell, B. K., Orr, P. T., 1993. H^+ exchange and nutrient uptake by roots of the emergent hydrophytes, *Cyperus involucratus* Rottb, *Eleocharis sphacelata* R.BR. and *Juncus ingens* Wakef, N.A. New Phytologist 125, 85-92.

Stanford, G., Dzienia, S., Van der Por, R., 1975. Effect of temperature on denitrification rate in soils. Journal of Soil Science Society of American 39, 867-870.

Stein, O. R, Hook, P. B., 2005. Temperature, plants, and oxygen: how does season affect constructed wetland performance? Journal of Environmental Science and Health, Part A. 40, 1331-1342.

Stott, R., Jenkins, T., Bahgat, M., Shalaby, I., 1999. Capacity of constructed wetlands to remove parasite eggs from wastewater in Egypt. Water Science and Technology 40, 117-123.

Stottmeister, U., Wießne, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., Müller, R. A., Moormann, H., 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnology Advances 22, 93-117.

Stowell, R., Ludwig, R., Colt, J., Tchobanoglous, G., 1981. Concepts in aquatic treatment system design. Journal of Environmental Engineering Division ASCE 107, 919-940.

Strous, M., Fuerst, J. A., Kramer, E. H. M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K. T., Webb, R., Kuenen, J. G., Jetten, M. S. M., 1999. Missing lithotroph identified as new planctomycete. Nature 400, 446–449.

Stumm, W., Morgan, J. J., 1996. Aquatic chemistry: chemical equilibria and rates in natural waters. 3rd edition, New York, John Wiley and Sons.

Sun, G., Austin, D., 2007. Completely autotrophic nitrogen-removal over nitrite in lab-scale constructed wetlands: Evidence from a mass balance study. Chemosphere 68, 1120-1128.

Sun, G., Zhao, Y., Allen, S., 2005. Enhanced removal of organic matter and ammoniacal-nitrogen in a column experiment of tidal flow constructed wetland system. Journal of Biotechnology 115, 189–197.

Sundaravadivel, M., Vigneswaran, S., 2001. Constructed wetlands for wastewater treatment. Critical Reviews in Environmental Science and Technology 31, 351-409.

Tanner, C. C., 1994. Growth and nutrition of *Schoenoplectus validus* in agricultural wastewaters. Aquatic botany 47, 131-153.

Tanner, C. C., 1996. Plants for constructed wetland treatment systems—a comparison of the growth and nutrient uptake of eight emergent species. Ecological Engineering 7, 59-83.

Tanner, C. C., 2001. Growth and nutrient dynamics of soft-stem bulrush in constructed wetland treating nutrient-rich wastewaters. Wetlands Ecology and Management 9, 49-73.

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. Relationships between loading rates and pollutant removal during maturation of gravel-bed constructed wetlands. Journal of Environmental Quality 27, 448-458.

Tasmanian Public Land Use Commission, 1996. Environment and Heritage Report Vol. IV, Background Report, Part C, Tasmanian Commonwealth Regional Forest Agreement, Hobart.

Tchobanoglous, G., 1993, Constructed wetlands and aquatic plant systems: research, design, operation, and monitoring issues. In: Moshiri, G. A., Ed., Constructed wetlands for water quality improvement. CRC Press, Boca Raton, FL, pp 23-34.

Thomas, P. R., Glover, P., Kalaroopan, T., 1995. An evaluation of pollutant removal from secondary treated sewage effluent using a constructed wetland system. Water Science and Technology 32, 87-93.

Thompson, S. P., Paerl, H. W., Go, M. C., 1995. Seasonal patterns of nitrification and denitrification in a natural and a restored salt marsh. Estuaris 18, 399-408.

Thomson, C. J., Armstrong, W., Waters, I., Greenway, H., 1990. Aerenchyma formation and associated oxygen movement in seminal and nodal roots of wheat. Plant, Cell and Environment 13, 395-403.

Thullen, J. S., Sartoris, J. J., Nelson, S. M., 2005. Managing vegetation in surface-flow wastewater-treatment wetlands for optimal treatment performance. Ecological Engineering 25, 583-593.

Tilman, D., 1987. The importance of the mechanisms of interspecific competition. The American Naturalist 129, 769-774.

Toet, S., Bouwman, M., Cevaal, A., Verhoeven, J. T. A., 2005. Nutrient removal through autumn harvest of *Phragmites australis* and *Typha latifolia* shoots in relation to nutrient loading in a wetland system used for polishing sewage treatment plant effluent. Journal of Environmental Science and Health 40, 1133–1156.

Toetz, D., 1971. Diurnal uptake of NO_3 and NH_4 by a *Ceratophyllum-periphyton* community. Limnology and Oceanography 16, 819-822.

Toetz, D., 1973. The kinetics of NH_4 uptake by *Ceratophyllum*. Hydrobiologia 41, 275-290.

Tremmel, D. C., Bazzaz, F. A., 1993. How neighbour canopy architecture affects target plant performance. Ecology 74: 2114-2124.

Tylova-Munzarova, E., Lorenzen, B., Brix, H., Votrubova, O., 2005. The effects of NH_4^+ and NO_3^- on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*. Aquatic Botany 81, 326-342.

Ulrich, K. U., 1985. The effects of nitrate, phosphate and potassium fertilization on growth and nutrient uptake patterns of *Phragmites australis* (Cav.) Trin. Ex Steudel. Aquatic Botany 21, 53-62.

Ulrich, K. U., Burton, T. M., 1988. An experimental comparison of the dry matter and nutrient distribution patterns of *Typha latifolia* L., *T. angustifolia* L., *Sparganium eurycarpum* Engelm. and *Phragmites australis* (Cav.) Trin. Ex Steudel. Aquatic Botany 32, 129-139.

USGS, 2004. Where is Earth's water located? From the U.S. Geological Survey's Water Science for Schools. http://ga.water.usgs.gov/edu/waterdistribution.html

Verhoeven, A. S., Demmig-Adams, B., Adams III, W. W., 1997. Enhanced employment of the xanthophyll cycle and thermal energy dissipation in spinach exposed to high light and N stress. Plant Physiology 113, 817-824.

Verhoeven, J. T. A., Meuleman, A. F. M., 1999. Wetlands for wastewater treatment: opportunities and limitations. Ecological Engineering 12, 5-12.

Vojtíšková, L., Munzarová, E., Votrubová, O., Říhová, A., Juřicová, B., 2004. Growth and biomass allocation of sweet flag (*Acorus calamus* L.) under different nutrient conditions. Hydrobiologia 518**,** 9–22.

Vymazal, J., 1999a. Nitrogen removal in constructed wetlands with horizontal sub-surface flow-can we determine the key process? In: Vymazal J, Ed., Nutrient cycling and retention in natural and constructed wetlands. Backhuys Publishers, Leiden, The Netherlands, pp 1-17.

Vymazal, J., 1999b. Removal of phosphorus in constructed wetlands with horizontal sub-surface flow in the Czech Republic. In: Vymazal, J., Ed., Nutrient cycling and retention in natural and constructed wetlands. Backhuys Publishers, Leiden, The Netherlands, pp 73-83.

Vymazal, J., 2001. Type of constructed wetlands for wastewater treatment: their potential for nutrient removal. In: Vymazal, J., Ed., Transformations of nutrients in natural and constructed wetlands. Backhuys Publishers, Leiden, The Netherlands, pp 1-93.

Vymazal, J., 2002. The use of sub-surface constructed wetlands for wastewater treatment in the Czech Republic: 10 years experience. Ecological Engineering 18, 633-646.

Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Science of the Total Environment 380, 48-65.

Vymazal, J., Krőpfelová, L., 2005. Growth of *Phragmites australis* and *Phalaris arundinacea* in constructed wetlands for wastewater treatment in the Czech Republic. Ecological Engineering 25, 606–621.

Wang, X., Xu, Q., Ge, Y., 2001. Study on the treatment of the non-point source pollution in Westlake area through the artificial wetland. Journal of Hangzhou Teachers College (Natural Science) 18, 6-10 (in Chinese, with English abstract).

Water Corporation of Western Australia, 2000. What is wastewater? Bulletin No.1. 2p.

Watson, J. T., Reed, S. C., Kadlec, R. H., Knight, R. L., Whitehouse, A. E., 1989. Performance expectations and loading rates for constructed wetlands. In: Hammer, D. A., Ed., Constructed wetlands for wastewater treatment. Lewis Publishers, Chelsea, Michigan, pp 319-351.

Webster, T. M., Loux, M. M., Regnier., E. E., Harrison, S. K., 1994. Giant ragweed (*Ambrosia trifida*) canopy architecture and interference studies in Soybean (*Glycine max*). Weed Technology 8, 559-564.

Werker, A. G., Dougherty, J. M., McHenry, J. L., Van Loon, W.A., 2002. Treatment variability for wetland wastewater treatment design in cold climates. Ecological Engineering 19, 1–11.

Westholm, L. J., 2006. Substrates for phosphorus removal - Potential benefits for on-site wastewater treatment? Water Research 40, 23-36.

Wetzel, P. R., van der Valk, A. G. 1998. Effect of nutrient and soil moisture on competition between *Phalaris arundinacea*, *Carex stricta* and *Typha latifolia*. Plant Ecology 138, 179-190.

Wieder, R. K., 1989. A survey of constructed wetlands for acid coal mine drainage treatment in the eastern United States. Wetlands 9, 299-315.

Wieder, R. K., Linton, M. N., Heston, K. P., 1990. Laboratory mesocosm studies of Fe, Al, Mn, Ca and Mg dynamics in wetlands exposed to synthetic acid coal mine drainage. Water, Air and Soil Pollution 51, 181-196.

Wießner, A., Kuschk, P., Stottmeister, U., 2002. Oxygen release by roots of *Typha latifolia* and *Juncus effuses* in laboratory hydroponic systems. Acta Biotechnologica 22, 209-216.

Wolverton, B. C., 1989. Aquatic plant/microbial filters for treating septic tank effluent. In: Hammer, D. A., Ed., Constructed wetlands for wastewater treatment. Lewis Publishers, NY. pp. 173-178.

Wu, D., Zhang, B., Li, C., Zhang Z., Hong, H., 2006a. Simultaneous removal of ammonium and phosphate by zeolite synthesized from fly ash as influenced by salt treatment. Journal of Colloid and interface Science 304, 300-306.

Wu, J., Ding, L., 2006. Study on treatment of polluted river water using pilot-scale surface flow constructed wetlands system. Environmental Pollution and Control 28, 432-434 (in Chinese, with English abstract).

Wu, J., Huang, S., Ruan, X., Ding, L., 2006b. Treatment of polluted river water using surface flow constructed wetlands in Xinyi river floodplain, Jiangsu Province. Journal of Lake Sciences 18, 238-242 (in Chinese, with English abstract).

Wu, M., Franz, E. H., Chen, S., 2001. Oxygen fluxes and ammonium removal efficiencies in constructed treatment wetlands. Water Environment Research 73, 661-666.

Wu, Z., Yu, D., 2004. The effects of competition on growth and biomass allocation in *Nymphoides peltata* (Gmel.) O. Kuntze growing in microcosm. Hydrobiologia 527, 241-250.

Xu, D., Xu, J., Wu, J., Muhammad, A., 2006. Studies on the phosphorus sorption capacity of substrates used in constructed wetland systems. Chemosphere 63, 344-352.

Yang, L., Chang, H., Huang, M. L., 2001. Nutrient removal in gravel- and soil-based wetland microcosms with and without vegetation. Ecological Engineering 18, 91-105.

Yang, Q., Chen, Z. H., Zhao, J. G., Gu, B. H., 2007. Contaminant removal of domestic wastewater by constructed wetlands: effects of plant species. Journal of Integrative Plant Biology 49, 437–446.

Ye, Z. H., Lin, Z. Q., Whiting, S. N., de Souza, M. P., Terry, N., 2003. Possible use of constructed wetland to remove selenocyanate, arsenic, and boron from electric utility wastewater. Chemosphere 52, 1571-1579.

Yue, C. L., Chang, J., Ge, Y., Zhu, Y. M., 2004. Treatment efficiency of domestic wastewater by vertical/reverse-vertical flow constructed wetlands. Fresenius Environmental Bulletin 13, 505-507.

Zhang, X., Liu, P., Yang, Y., Chen, W., 2007. Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes. Journal of Environmental Sciences-China 19, 902-909.

Zhang, Z., Rengel, Z., Meney, K., 2007a. Growth and resource allocation of *Canna indica* and *Schoenoplectus validus* as affected by interspecific competition and nutrient availability. Hydrobiologia 589, 235-248.

Zhang, Z., Rengel, Z., Meney, K., 2007b. Nutrient removal from simulated wastewater using *Canna indica* and *Schoenoplectus validus* in mono- and mixed-culture in wetland microcosms. Water, Air and Soil Pollution 183, 95-105.

Zhao, J., Yang, Q., Chen, Z., Huang Z., 2003. Studies on root system biomass of the plants in several kinds of wetland. China Environmental Science 23, 290-294 (in Chinese, with English abstract).

Zhu, T., Sikora, F. J., 1995. Ammonium and nitrate removal in vegetated and unvageted gravel bed microcosm wetlands. Water Science and Technology 32, 219-228.

Zhu, X., Cui, L., Liu, W., Liu, Y., 2004. Removal efficiencies of septic tank effluent by simulating vertical-flow constructed *Canna indica* Linn. wetlands. Journal of Agro-Environmental Science 23, 761-765 (in Chinese, with English abstract).

Zurita, F., de Anda, J., Belmont, M. A. 2006. Performance of laboratory-scale wetlands planted with tropical ornamental plants to treat domestic wastewater. Water Quality Research Journal of Canada 41, 410-417.