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Performance of wetland systems in reducing agricultural nitrogen pollution

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Michela Salvato 01/02/10

A copy of the thesis will be available at *http://paduaresearch.cab.unipd.it/*

With love to my family and in particular my grandmother Cea….

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Riassunto

Il lavoro è consistito nello studio dell'efficienza delle zone umide costruite nel ridurre l'azoto proveniente dall'inquinamento di origine agricola. La tesi prende in considerazione due prove sperimentali a diversa scala nel triennio 2007-2009: una zona umida a flusso superficiale di 3200 m^2 e una prova a scala di mesocosmo.

Nel primo caso l'obiettivo era di valutare l'efficienza della zona umida sita presso l'azienda agraria sperimentale dell'Università degli Studi di Padova, nell'abbattere azoto proveniente da 5 ha di circostante terreno coltivato. Alla fine del periodo considerato (2.5 anni) il bacino ha dimostrato di abbattere molto bene sia l'azoto nitrico che totale con efficienze di rimozione introno al 90%. La maggior parte dell'azoto in entrata è stata immagazzinata nel comprato suolo/vegetazione mentre la denitrificazione è stata stimata pari al 6%.

Nella prova a scala di mesocosmo l'obiettivo era di comparare l'efficienza di rimozione dell'azoto di cinque diverse specie. L'impianto sperimentale era costituito da vasche in plastica riempite di ghiaia e vegetate con *Carex elata* All., *Juncus effusus* L., *Typhoides arundinacea* (L.) Moench var. picta, *Phragmites australis* (Cav.) Trin., and *Typha latifolia* L.. Era presente anche un controllo non vegetato. Una soluzione con concentrazioni crescenti di NH_4-N and NO_3-N è stata applicata alle vasche effettuando 31 cicli di diversa durata. Alla fine di ogni ciclo l'acqua in uscita dalle vasche è stata analizzata per azoto nitrico e ammoniacale. I volumi di acqua in entrata e uscita sono stati misurati al fine di calcolare l'evapotraspirazione. Considerando tutti e tre gli anni di prova, *Typha latifolia* L. ha rimosso l'82% dell'azoto in entrata seguita da *Typhoides arundinacea* (L.) Moench (76%), var. picta, *Carex elata* All. (75%), *Phragmites australis* (Cav.) Trin., (72%) e *Juncus effusus* L. (64%). Il controllo non vegetato ha rimosso invece il 45%.

Azoto ammoniacale e nitrico hanno presentato diverse dinamiche di scomparsa. Il primo è stato abbattuto quasi completamente e in breve tempo in tutti i trattamenti, il secondo invece è stato rimosso in maniera diversa a seconda della stagione e della specie considerata.

La maggior quantità di azoto in entrata è stata immagazzinata nei tessuti con percentuali diverse secondo le specie. La denitrificazione calcolata varia dal 18% in *Juncus effusus* L. al 37% in *Typhoides arundinacea* (L.) Moench var.picta.

Summary

The thesis is about the performance of wetland systems in reducing agricultural nitrogen pollution. It takes into account two experiments at different scale: a 3200 m^2 pilot surface wetland and a mesocosm trial.

In the first case the aim was to evaluate the performance of the wetland, located at the experimental farm of the University of Padova (Italy), in abating nitrogen coming from 5 ha of surrounding fields. Overall, for the entire period (2.5 years), the basin performed well in abating both nitrate and total nitrogen with a removal efficiency of about 90%.

The major quantity of removed nitrogen was stored in vegetation and soil and only 6% was estimated as being lost with denitrification.

In the mesocosm experiment the aim was to compare five different species in abating nitrogen coming from a reconstructed wastewater. The experimental setup consisted of plastic tanks, filled with gravel and vegetated with *Carex elata* All., *Juncus effusus* L., *Typhoides arundinacea* (L.) Moench var. picta, *Phragmites australis* (Cav.) Trin. and *Typha latifolia* L.. There was also a control without vegetation. A solution of increasing concentrations of NH_4-N and NO_3-N was applied to the tanks, over 31 cycles of differing lengths. At the end of each cycle exiting water was analysed to determine the two nitrogen forms. All water volumes entering and exiting the tanks were measured in order to evaluate evapotranspiration. At the end of the trials *T. latifolia* had removed 82% of the entering nitrogen, followed by *T. arundinacea* (76%), *C. elata* (75%), *Ph. australis* (72%) and *J. effusus* (64%). The control removed 45% of the entering nitrogen. The fate of the two forms of nitrogen differed as NH_4-N disappeared almost completely and in short time in all the treatments while NO_3-N showed different removal efficiency depending on season and considered species.

The major quantity of entering nitrogen was stored in plant tissues with different percentages depending on species. The calculated denitrification varied from 18% for *J. effusus* to 37% for *T. arundinacea*.

CHAPTER I General background

Introduction

Agricultural nitrogen pollution

Without water, there is no life on the Earth. Water is a limited resource but there is increasing demand in many fields (food requirements, agriculture, industry, ecosystem conservation etc.) and a progressive decline in its quality due to pollution processes.

Water pollution is any chemical, physical or biological change in the quality of water that has a harmful effect on any living being that drinks or uses or lives in it. Water pollution can make water unsuited to desired use, even if the quality required changes with different uses. For example, waters with an excess of nutrients that are not of biological or drinking quality may be suitable for other uses such as irrigation of crops.

Water pollution is usually caused by human activities. Sources of water pollution are generally grouped into two categories based on their type of origin: point and nonpoint sources.

Point sources discharge pollutants in a specific location through well-defined pipelines or sewers into the surface water. Nonpoint sources are sources that cannot be traced to a single site of discharge and come from a wide surface area in a variable way in space and time. Examples of point sources are: industries, factories, sewage treatments works, isolated houses etc…Examples of nonpoint sources are: diffuse agricultural runoff, urban runoff, acid deposition from the air, traffic etc…

Agricultural production has been recognized as affecting aboveground and surface water quality adversely from both point and nonpoint sources (Follett et al., 1991; Gilliam et al., 1996; Novotny and Olem, 1994). Almost all agricultural point sources are related to animal husbandry and the non pint to field's practices.

In particular, losses of nitrogen due to intensive filed management practices, especially in Europe and the USA, have been an international concern for many years.

Nitrogen is one of the most important plant nutrients and determinant of plant growth and crop yield. The primary source of nitrogen is fertilization but the consequently nitrogen losses may vary widely. A study conducted in Denmark (Andersen et al., 1999) considering the losses of total nitrogen from six agricultural areas, typical of common local farming practices, found a range on average from 7.7 to 34.1 kg/ha/year. Annual nitrogen leaching losses from arable land in south Sweden usually amount to 15–45 kg/ha (SNV, 1997).

In Veneto region nitrogen losses from five pipe drained plots were estimated from 1985 to 1988. They showed high variability, ranging from 7.9 kg/ha/year to 158 kg/ha/year for NO_3-N (Giupponi et al., 1990).

The amount of nitrogen leached largely depends on the presence or absence of livestock, agronomic management, climate, soil properties, etc...

For example Sacco et al. (2003) pointed out that pig farms showed a surplus of nitrogen with excesses greater than 270 kg/ha; cattle farms showed a lower surplus, compared to the other livestock farms, with values close to 190 kg/ha and farms without livestock showed the closest equilibrium between fertilization and offtake, with a surplus in N equal to 40 kg/ha.

Agronomic management is surely a key factor in controlling nitrogen leaching. In a study conducted in Canada on the effect of long-term conventional tillage and no-tillage systems on soil and water quality at the field scale, it was pointed out that the total NO_3-N lost in tile drainage water over the 5 years period (1995–1999) was 82.3 kg/ha for the long-term no tillage site and 63.7 kg/ha for the long-term conventional tillage (Tan et al., 2002). Moreover in the Veneto plain (NE Italy) with rational fertilization management, potential losses can be contained below 30 kg/ha/year in 80% of the cultivated land in the region, but this figure may locally rise to more than 90 kg/ha/year if liquid manure is available and fertilization techniques are not managed correctly (Giardini and Giupponi, 1989). In another experiment, organized to simulate a typical lowland agricultural subbasin conducted at the University of Padua (NE Italy) that aimed to study two land drainage systems with two water table managements, the calculated NO_3-N losses were from 3 kg/ha/year to 47 kg/ha/year (Bonaiti and Borin, 2010).

There are several studies on the effect of climate on drainage and nitrogen leaching. For example in a study on annual tile drainage in Minnesota from 1986 to 1992 with continuous corn, drainage ranged from 26 to 618 mm/year. Drainage was minimum in 1989 with a nitrate loss of only 2 kg/ha when the growing season precipitation was 35% below normal. The highest value (139 kg/ha of nitrate lost) was recorded in 1991 when the growing season precipitation was 51% above normal (Randall and Mulla, 2001).

Regarding the influence of soil, an experiment was conducted to measure nitrate leaching in field lysimeters containing undisturbed soils of different texture and organic matter content (Bergstöm and Johansson, 1991). Spring barley was sown in each lysimeter and fertilized with 100 kg N/ha. The largest leaching losses of NO_3-N, about 65 kg/ha, occurred in a sandy soil that contained little organic matter and in a peat soil. Two loamy soils lost between 25 and 40 kg/ha. Smallest leaching losses, about 20 kg /ha or less, occurred in a clay soil and another sandy soil rich in organic matter. The difference in leaching between the two sandy soils was explained by differences in crop growth, whereas leaching differences between soil types were mainly considered to be due to different textural and structural properties.

Although the effect of high leached nitrate amounts on human health is a controversial issue (Addiscott et al., 1991; Apfelbaum, 1998), several environmental policies have been defined in order to control agricultural nitrate pollution. These policies rely mainly on legal instruments (command and control approach), such as limitation on the authorized level of pollutants or the designation of vulnerable areas (Lacroix et al, 2005).

Due to Nitrates Directive (91/676 EEC) and the connected Water Framework (2000/60 EC) agricultural has placed an increased emphasis on discovering new and innovative best management practices to mitigate the problem that are summarized in Table 1.

One possibility, as already mentioned, is to adopt best management practices at field level, such as crop rotation, correct fertilization management, conservation tillage, cover crops. But other techniques could be applied when the nitrogen leaves the field, for example the discharge of agricultural drainage water into natural or constructed wetlands before being routed into water bodies (Chescheir et al., 1991; Olson and Marshall, 1992; Gilliam et al., 1999; Kadlec and Wallace, 2008).

Table 1–Strategies and actions to control nitrogen pollution coming from farm land (Borin and Abud, 2009).

Strategy	Action	Site	Conditions
pollutant reduction	BMP	field	all
transport reduction	irrigation efficiency	field	all
	controlled drainage	field	shallow water table
pollutants transformation	controlled drainage	field	shallow water table
	buffer strips	field border	horizontal flow
	wetlands	field/stream	horizontal flow
pollutants block	buffer strips	field border	horizontal flow
	wetlands	field/stream	horizontal flow

Wetlands

Natural wetlands are areas that are wet during part or all of the year because of their location in the landscape. Wetlands are called swamps, marshes, bogs, fens, or sloughs, depending on existing plant and water conditions, and on geographical setting. Wetlands are among the most biologically productive ecosystems on the planet. As such, they are frequently inhabited by jungle-like growths of plants and are home to a multitude of animals including mammals, birds, reptiles, amphibians, and fishes that are uncommon in other ecosystems. Wetlands have a higher rate of biological activity than most ecosystems; they can transform many of the common pollutants that occur in conventional wastewaters into harmless by-products or essential nutrients that can be used for additional biological productivity (Kadlec and Wallace, 2008).

Constructed wetlands (CWs) are engineered systems that are planned and designed to emphasize specific characteristics of natural wetland ecosystems to improve pollutant removal processes.

The classification of CWs is based on the type of macrophytes growth, further classification is usually based on the water flow regime (Figure 1).

Macrophyte is not a botanical term but literally means “big plant” and it is used to describe water plants large enough to be visible to the naked eye.

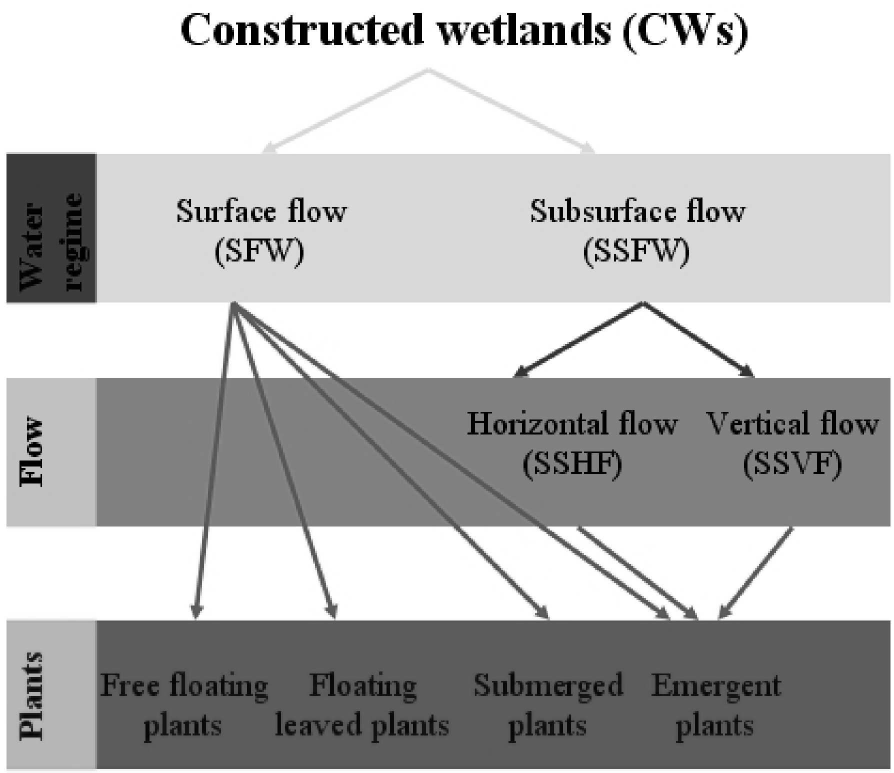


Figure 1 - Classification of constructed wetlands for wastewater treatment (modified from Vymazal, 2001).

Considering macrophytes growth, they are divided in four groups: free floating plants, floating leaved plants, submerged plants and emergent plants.

Free floating plants have leaves and stems freely floating on water surface. If roots are present, they hang free in the water and are not anchored in the sediments. Floating plants move on the water's surface with winds and water currents (Cronk and Fennessy, 2001). A widespread family of floating plants is the *Lemnaceae*, which includes the genera *Lemna* (duckweed) with some of the smallest known angiosperms; other important species are *Eichhornia crassipes* (Mart.) Solms (water hyacinth) and *Pistia stratiotes* L. (water lettuce). Some floating plants may become invasive especially in tropical and subtropical wetlands and the main characteristic that limits their widespread use is their temperature sensitivity.

Floating leaved plants, also known as floating attached plants, are plants that are rooted to sediments while leaves are floating on the water's surface. The most well-known family is the *Nymphaeaceae.*

Submerged plants are generally rooted plants (there are some rootless species that float free in the water column) with most of their vegetative mass normally below the water surface. One discerning characteristic of submerged plants are their soft (lacking lignin) tissues, which is the reason why they are flexible enough to withstand water movement without damage. Examples of families in which most of the species are submerged are the *Ceratophyllaceae* (hornwort), *Potamogenaceae* (pondweeds), *Hydrocharitaceae* (frogbit), *Haloragaceae* (water milfoil) and *Callitrichaceae* (water starwort).

Emergent plants are plants rooted in the soil with basal portions that typically stay beneath the surface of the water but whose stems, leaves and reproductive organs are above the water level. Most of the emergent plants are herbaceous but there are also some woody species such as *Taxodium distichum* (L.) Rich., *Salix* and *Populus* genera.

The most common emergent species belong to the monocotyledons and are for example *Poaceae* (grasses), in which the most widely-used species in CWs is *Phragmites australis* (Cav). Trin., *Cyperacea*e (sedges) for example *Carex* spp, *Juncaceae* (rushes) for example *Juncus spp*, *Thypaceae* in which the most widely-used species in CWs is *Typha latifolia* L. (cattail), and others such as *Sparganiaceae* (bur reed), *Alismataceae* (water plantain) etc…

Passing to the classification of CWs on the water flow regime (Figure 1) they are divided into surface flow wetlands (SFW) and subsurface flow wetlands (SSFW), these latter include, according to water flow direction, horizontal flow (SSHF) and vertical flow (SSVF).

SFW are isolated basins in which wastewater flows slowly from the inlet to the outlet and they can have areas with open water, floating vegetation, and emergent plants. As the wastewater flows through the wetland, it is treated by processes of sedimentation, filtration, oxidation, reduction, adsorption, and precipitation. The components of a typical SFW are shown in Figure 2.

Because SFW closely mimic natural wetlands, it should be no surprise that they attract a wide variety of wildlife, namely insects, molluscs, fish, amphibians, reptiles, birds, and mammals (NADB database, 1993; Kadlec and Knight, 1996).

The most common application for SFW wetlands is for treatment of agricultural wastewater because of their ability to deal with pulse flows and changing water levels.

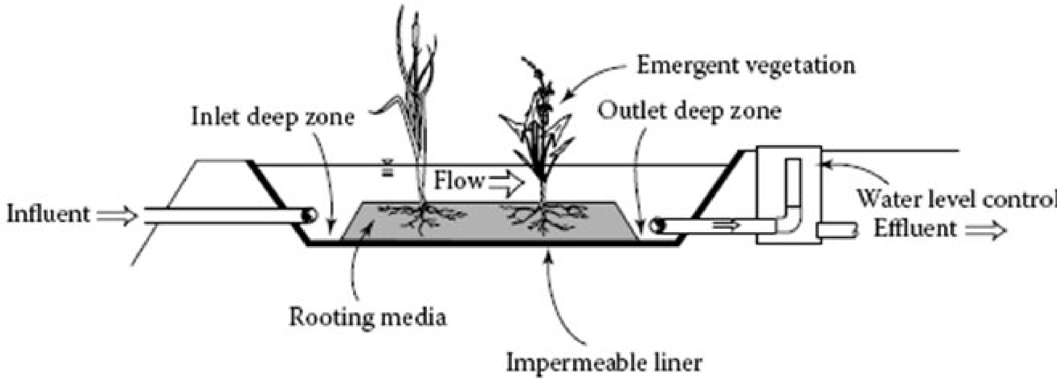


Figure 2 – Basic elements of a SFW from Kadlec and Wallace, 2008

These kinds of wetlands are not inexpensive, but are usually capital cost-competitive with alternative technologies. Operating costs are typically quite low compared to the alternatives (Kadlec and Wallace, 2008).

A few studies have been conducted in wetlands treating agricultural runoff, giving nitrogen removal efficiency that ranged from 5 to 91%.

For example in Texas, an experimental plant was constructed next to Stephenville (about 90 km south-west of Dallas city) to treat agricultural wastewaters coming from a hydrological basin of about 700 ha characterized by intensive agricultural activities. The wastewaters are collected and discharged into the plant, which is formed of a series of 3 basins of 2, 1.5 and 1.5 ha respectively, with small banks and suitable vegetation (Borin, 1997).

A small wetland about 3 m wide and 150 m long in Kiewa Valley (Australia) was studied by Raisin et al. (1997). The basin received runoff from a 90 ha agricultural catchment and the nitrogen removal efficiency was very variable from 5 to 55% depending on the intensity of rainfall events.

Four restored wetlands, with areas of 9313, 10328, 10351, and 5456 m^2, respectively, dominated by *Phragmites australis* (Cav.) Trin., *Typha latifolia* L. and *Scirpus lacustris* L., were used to improve the quality of agricultural runoff in the delta of the Ebro River (NE Spain) in 1993 (Romero et al., 1999). They removed 30-91% of the total nitrogen.

Kovacic et al. (2000) studied three treatment wetlands (0.3 to 0.8 ha in surface area) that intercepted subsurface tile drainage water coming from soybean and maize cultivations in Illinois. The basins were constructed in 1994 and received 4639 kg total N during a 3-year period (96% as NO_3-N) and removed 1697 kg N (37% of inputs).

In Veneto Region (NE Italy) in 1999 the Ca' di Mezzo wetland was constructed by the Land Reclamation Consortium Adige Bacchiglione to reduce the amount of nutrients and suspended solids driven by the Altipiano channel into the Venice lagoon (Bixio, 2000). It has a maximum depth of 1.8 m and the surface is about 30 ha: 10 ha of open water, 10 ha, that may be submerged in case of necessity, vegetated with *Phragmites australis* (Cav.) Trin. and 10 ha with local trees.

A wetland of 7822 m^2 located in the Catawba River Basin, along with the nearby Broad River basin in North Carolina, USA, was studied by Kao and Wu (2001). The basin receives water mainly from agricultural lands and was able to reduce more than 80% of entering nitrogen.

A surface flow wetland on the Chesapeake Bay shore (Maryland), receives cropland runoff from the surrounding land cultivated primarily with maize and soybean. Over the two years of the study it removed 25% of the NH_4-N and 52% of the NO_3-N (Jordan et al., 2003).

A study examined the effectiveness of a 1.2 ha created/restored emergent marsh in reducing nutrients from a 17 ha agricultural and forested watershed in the Ohio River Basin in west central Ohio, USA. Concentration of NO_3-N was 40% lower at the outflow over the 2 years of the study (Fink and Mitsch, 2004).

A pilot-scale wetland was constructed along Steamboat Creek (a river that drains urban and agricultural waters) at the Truckee Meadows Water Reclamation Facility Sparks, Nevada. The results suggested that a large-scale constructed wetlands system could be expected to reduce total N loading into the river from 19 to 30% on an annual basis (Chavan et al., 2008).

Another example is in Kunming City, China, and consists of a SFW approximately 2800 m^2, with the inflow being agricultural runoff coming from the upstream farmland with a watershed area of 0.23 km^2 (Lu et al., 2009). The removal of ammonium and total nitrogen was significant, with an average value higher than 50%.

A 1.6 ha SFW treating irrigation return flows in Yakima Basin in central Washington was studied by Beutel et al. (2009). The concentration removal efficiencies for nitrate averaged from 90–93%. Total N removal efficiencies averaged from 57–63%.

SSHF are isolated basins filled with gravel or other porous media and vegetated with emergent macrophytes. The wastewater flows horizontally from the inlet to the outlet beneath the surface of the media and flows around the roots and rhizomes of the plants (Figure 3). SSHF wetland systems are generally more expensive than SFW wetlands, although maintenance costs remain low compared to the alternatives (Kadlec and Wallace 2008). SSHF constructed wetlands are commonly used to treat municipal and domestic wastewaters as both secondary and tertiary treatment but there are various examples of industrial wastewaters which have been treated with SSHF constructed wetlands: petrochemical and chemical industries, pulp and paper, textile and tannery industries, food processing, winery and distillery etc…(Vymazal, 2009). SSHF may also be used to treat agricultural wastewaters and some examples can be found in the literature (Table 2).

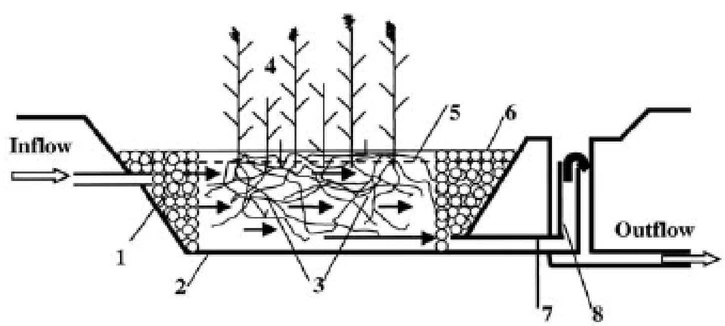


Figure 3 - Schematic representation of SSHF wetland:1, distribution zone filled with large stones; 2, impermeable liner; 3, medium (e.g., gravel, crushed stones); 4, vegetation; 5, water level in the bed; 6, collection zone filled with large stones; 7, collection drainage pipe; 8, outlet structure for maintaining of water level in the bed. The arrows indicate only a general flow pattern. From Vymazal (2001).

Table 2 - of the use of SSHF for treatment of various types of wastewater from agricultural activities. From Vymazal (2009)

Reference	Location	Type of wastewater
Finlayson et al. (1987,1990)	Australia	Pig farms
Wang et al. (1994), Junsan et al. (2000)	China	
Gray et al. (1990)	United Kingdom	
Kantawanichkul and Somprasert (2005)	Thailand	
Struseviˇcius and Struseviˇciene (2003)	Lithuania	
Lee et al. (2004)	Taiwan	
Zachritz and Jacquez (1993)	USA	Fish farm effluent
Comeau et al. (2001), Naylor et al. (2003), Chazarenc et al. (2007)	Canada	
Schulz et al. (2003)	Germany	
Mantovi et al. (2002, 2003)	Italy	Dairy
Kern and Brettar (2002)	Germany	
Hill et al. (2003), Chen et al. (1995), Drizo et al. (2006)	USA	
Tanner (1992)	New Zealand	
Schierup et al. (1990)	Denmark	
Gasiunas et al. (2005)	Lithuania	
Gray et al. (1990)	United Kingdom	

Moreover taking into account the Livestock Wastewater Treatment Database developed by CH2M HILL and Payne Engineering (1997) regarding the use of North American wetlands for treatment of high strength livestock wastewaters (Table 3), their nitrogen removal efficiency ranged from 20 to 60% for NH_4-N and from 22 to 51% for Total nitrogen. These statistics are global average values and do not necessarily reflect the performance capability of any single system. Carefully designed and operated treatment wetlands would be expected to exceed these performance expectations, while systems with less than optimal plant communities, flow distribution, or water depth control might perform at lower levels (Knight et al., 2000).

Table 3 – Average treatment wetland performance for removal of NH_4-N and TN. Source: Data from NADB database (1998) North American Treatment Wetland Database (NADB), Version 2.0. Compiled by CH2MHill. Gainesville, Florida; and Knight et al. (2000)

wastewater type	number of systems	average IN (mg/L)	average OUT (mg/L)	average reduction
NH_4-N				
cattle feeding	12	5.1	2.2	57%
dairy	351	105	42	60%
poultry	80	74	59	20%
swine	183	366	221	40%
TOT N				
dairy	32	103	51	51%
poultry	80	89	70	22%
swine	164	407	248	39%

SSVF are isolated basins in which wastewater is distributed from the top of the basins across the surface of a sand or gravel bed planted with emergent macrophytes. The water percolates through the plant root zone (Figure 4).

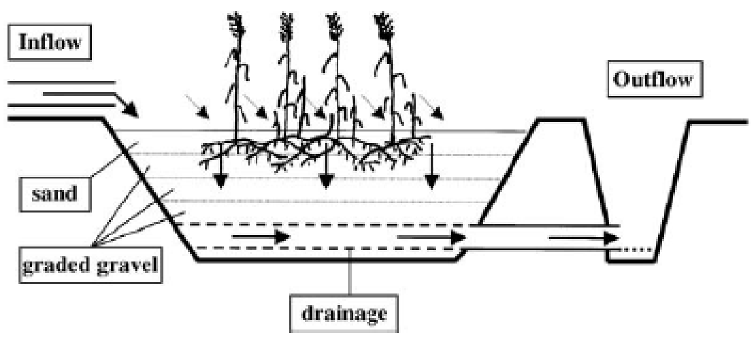


Figure 4 - Schematic representation of SSVF wetland. From Vymazal 2001.

All these type of CWs may be combined together to maximize the advantages associated with each one and creating hybrid systems.

The most important forms of nitrogen coming from agricultural wastewater are NO_3-N and organic nitrogen. When nitrogen enters CWs its various forms are involved in biological, chemical and physical transformations. The most studied processes, nitrification, denitrification, volatilization of ammonium and plant uptake are schematized in Figure 5.
Other important processes are ammonification of organic nitrogen, fixation, ammonium adsorption, organic nitrogen burial and anammox.

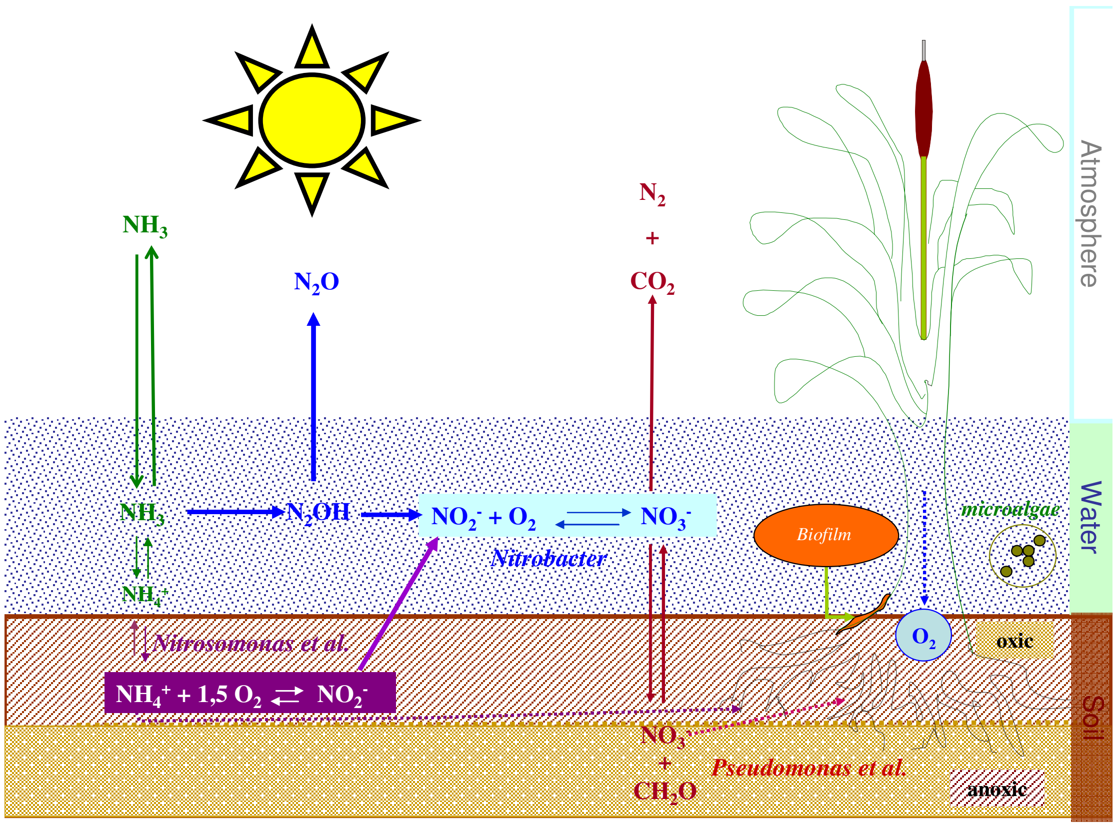


Figure 5 – Scheme of the most studied processes of nitrogen transformations in CWs.

Nitrification is the main transformation that, under aerobic conditions in many CWs, converts NH_4-N into NO_3-N, with NO_2-N as an intermediate in the reaction, but this last form of nitrogen can rarely be accumulated in terrestrial and aquatic environments. Nitrification has been typically associated with the chemoautotrophic bacteria, although it is now recognized that heterotrophic nitrification occurs and can be of significance (Keeney, 1973; Paul and Clark, 1996). Two different groups of bacteria play a role in the nitrification steps: ammonium oxidizers and nitrite oxidizers. The ammonium oxidizers include species belonging to the genera *Nitrosospira*, *Nitrosovibrio*, *Nitrosolobus*, *Nitrosococcus* and *Nitrosomonas*. The chemoautotrophic nitrifiers are generally aerobes that derive their C largely from CO_2 or carbonates (Vymazal, 2007). The second step in the nitrification process, the oxidation of NO_2-N to NO_3-N, is performed by *Nitrospira* as well as *Nitrobacter*, and the former was found to be much more prevalent in a treatment wetland (Austin et al., 2003).

A number of environmental factors influence nitrification as pH value, alkalinity of the water, inorganic C source, moisture, microbial population, concentrations of NH_4-N and dissolved oxygen (Vymazal, 1995).

Denitrification is defined as the process in which nitrate is converted into gaseous N via intermediates nitrite, nitric oxide and nitrous oxide (Hauck, 1984; Paul and Clark, 1996; Jetten et al., 1997). Denitrification is carried out by facultative heterotrophs, organisms that can use either oxygen or nitrate as terminal electron acceptors. Many organisms are capable of denitrification. They are organotrophs (*Pseudomonas, Alcaligenes, Bacillus, Agrobacterium, Flavobacterium, Propionibacterium, Vibrio* etc...), chemolithotrophs (*Thiobacillus, Thiomicrospira, Nitrosomona* etc...), photolithotrophs (*Rhodopseudomona* etc..), diazotrophs (*Rhizobium*, *Azospirillum* etc..), archaea (*Halobacterium* etc...), and others such as *Paracoccus* or *Neisseria* (Focht and Verstraete, 1977; Killham, 1994; Paul and Clark, 1996).

Ammonium volatilization is a physicochemical process where NH_4-N becomes volatile ammonium. The conversion between volatile ammonium and ammonium ions strongly depends on pH and temperature (Anthonisen, et al., 1976). At lower pH and temperature levels, the conversion decreases significantly. For a normal condition of 25 °C and pH 7, nonionized ammonium amounts to only 0.6% of the total ammonium present. At pH 9.5 and a temperature of 30 °C, the percentage of total ammonium present in the non-ionized form increases to 72%.

Plants are able to directly absorb inorganic nitrogen in the forms of NH_4-N and NO_3-N. Nitrate uptake by wetland plants is presumed to be less favoured than ammonium uptake but nitrate may become a more important source of nutrient nitrogen in nitrate rich waters, (Tanner, 1996). N is absorbed by the roots through assimilating enzymes or depending mainly on pH through free diffusion. N reaches the subsurface root system via diffusion under appropriate circumstances, but more importantly via transpiration flux, the vertical water flow driven by the transpiration requirement of the plant (Tanner, 1996).

After assimilation N is stored in above and below ground plant tissues but this storage is mostly temporary because it is subsequently released to the wetland as plant tissues die and decompose.

Ammonification of organic nitrogen is the biological process in which organic nitrogen is converted to ammonium nitrogen by hydrolysis and mineralization.

Fixation is a biological process by which nitrogen gas in the atmosphere diffuses into solution and is reduced to ammonium nitrogen by autotrophic and heterotrophic bacteria, cyanobacteria and higher plants.

$NH4+$ is generally adsorbed as an exchangeable ion in the substrate and can be released easily when water chemistry conditions change.

Some fractions of the organic nitrogen may eventually become unavailable for nutrient cycling through the process of peat formation and burial.

There is today evidence of anaerobic removal of ammonium called anammox in a number of wastewater treatments (van de Graaf et al., 1995; Mulder et al., 1995; van Loosdrecht and Jetten, 1998). The process is autotrophic and doesn't require organic carbon.

Role of wetland plants

The plants have many important functions in CWs such as direct uptake of nitrogen for their growth, providing a surface and a carbon source for the growth of microbial communities, transferring oxygen from air to the medium, decreasing water speed and reducing its volume, stabilising the bed, isolating the surface against frost in winter (Vymazal, 2002).

In the literature only a few works have compared the removal efficiency of different species under the same conditions and over a prolonged period. For example, 35 experimental studies on the effect of macrophyte species on pollutant removal were reviewed by Brisson and Chazarenc (2009). These trials covered a wide range of species, experimental approaches (from well-replicated microcosm experiments to comparison between full-size CWs), climatic conditions (from tropical to cold-temperate) and types of effluent (domestic, industrial, etc.). In most of them macrophytes of comparable size and life form showed evident differences in pollutants removal (Coleman, 2001; Akratos and Tsihrintzis, 2007).

The nitrogen uptake from different wetland plants varies widely depending on plant specie and age, growing season, type of applied wastewater, environmental conditions etc. Examples of nitrogen stored in plant tissues ranges from 19.8 to 111 g/m^2 considering both above and belowground biomass (Table 4). Nitrogen removal is possible by harvesting the aerial part of plants and their associated nitrogen content. This amount of harvestable nitrogen ranges from 0.7 to 51 g/m^2 in the literature (Table 4). Harvesting typically requires adequate mechanical equipment for large systems. The problem of biomass disposal is often not easily resolved. Harvested biomass may either be composted or digested to form a biogas product. Both require transportation costs, a dedicated land area and processing plants. As a consequence of these problems, plant harvesting and utilization need specific studies and plans to become effective processes for nitrogen removal from wetlands.

The percentage of applied nitrogen removed by plants uptake is also widely variable ranging for instance from 0.7 % (Gottschall et al., 2007) to 89% (Bachand and Horne, 2000) and consequently playing an important or negligible role in nitrogen balance.

Table 4 – Examples of nitrogen stored in plant tissues. X indicates data not available.

Reference	plant specie	N stored in aboveground tissues g/m^2	N stored in below ground tissues g/m^2	N in plants g/m^2
Landry et al., 2009	*Typha latifolia*	X	X	48-54
	Phragmites australis	X	X	19.8-26.8
	Phalaris arundinacea	X	X	27.7
Maddison et al., 2009	*Typha latifolia*	17-32.3	11.6-19.4	28.6-51.7
Chung et al., 2008	*Typha latifolia*	7.76-8.23	2.65 - 6.25	10.51- 14.55
Sun and Liu, 2007	*Calamagrostis angustifolia*	13.59-22.56	12.36-27.94	34.92-41.43
Borin and Tocchetto, 2007	*Phragmites australis*	X	X	111
	Typha latifolia	X	X	74
Klomjek and Nitisoravut, 2005	*Typha angustifolia*	4.7	X	X
	Cyperus corymbosus	3.5	X	X
	Brachiaria mutica	1	X	X
	Digitaria bicornis	2	X	X
	Spartina patents	0.7	X	X
	Leptochloa fusca	1.8	X	X
Hunt et al., 2002	*Typha spp*	20-32	X	X
	Scirpus spp	35	X	X
Tanner, 2001	*Schoenoplectus* spp	23 -40	X	X
Vymazal et al., 1999	*Phragmites australis*	20-51	X	X
	Phalaris arundinacea	26.5	X	X
Hurry and Bellinger, 1990	*Phalaris arundinacea*	49	X	X

It is now known that microorganisms are attached on different parts of plants and media forming a biofilm layer, which plays an important role in nitrogen removal from wastewater (Thammarat and Polprasert, 1997). The plants are not only a physical support for microorganisms but are also an important source of degradable organic carbon that drives heterotrophic bacterial activity (Thullen et al., 2005; Greenway, 2007; Kadlec, 2008;). For example dissolved organic carbon useful for bacteria utilization from leachate of *Juncus effusus* L. ranged from 1.9 to 10.6 mg/L/ cm^2 (Mann and Wetzel, 1996).

Different wetland plants may supply a different quality and quantity of C, so different plant species can affect removal rates of nitrogen (Horne and Fleming-Singer, 2005; Alvarez and Becares, 2006; Corstanje et al., 2006).

Wetland plants transfer oxygen to their root system and release a fraction of this oxygen into the rhizosphere (Brix, 1997) promoting the formation of an oxidized layer around the root and creating a redox gradient ranging from +500 mV very near the root surface to -250 mV at a distance of 1–20mm from the root surface (Wiessner et al., 2002; Bezbaruah and Zhang, 2004; Münch et al., 2005). As a result, the rhizosphere exhibits a mosaic of strong redox gradients enabling the formation of many ecological niches that promote a multitude of microbial processes (Faulwetter et al., 2009).

The water speed is reduced by the development of roots, which creates a sort of permeable barrier that intercepts and slows down the water flux; the volume of water is reduced because of evapotranspiration (ET), the process by which water moves from wetlands into the atmosphere through plants and medium. ET decreases the volume of wastewater reducing the outflow and concentrating the pollutants but increases the retention time which allows more interaction time with the wetland ecosystem (Kadlec and Wallace, 2008).

Roots systems are so well-developed and well-integrated in the substrate that they play an important role also stabilizing the bed against disturbances that may come from water flux or environmental conditions.

Finally plants have a vegetative cycle that in winter season passes through a period of quiescence associated with senescence of culms and consequent formation of litter. This material may be useful also to isolate the wetland surface against frost.

This brief overview evidences the multifunctions of macrophytes and the variability of information coming from the literature, suggesting that the selection of macrophytes needs to be studied at local scale.

Research structure and objectives

The aims of this work were to evaluate the efficiency of constructed wetlands in abating nitrogen coming from agricultural wastewater and define the direct role of vegetation.

The thesis is based on two experimental activities conducted at different scale:

- a pilot surface flow wetland;
- a mesocosm experiment.

The first activity evaluated the functions of the surface wetland during the period from January 2007 to April 2009 taking into account water balance, NO_3-N and TOT N concentrations in inflow and outflow, water mass balance of NO_3-N and TOT N, nitrogen balance considering also plants and soil.

The mesocosm experiment evaluated five species of common wetland plants: *Carex elata* All., *Juncus effusus* L., *Phragmites australis* (Cav). Trin., *Typhoides arundinacea* (L.) Moench (syn. *Phalaris arundinacea* L.) var. picta, and *Typha latifolia* L.. The aim was to compare, for three years (2007-2009), the nitrogen removal efficiency under increasing input loads of a reconstructed wastewater with NO_3-N and NH_4-N. The fate of the nitrogen removed was studied in order to quantify the amount stored in above and belowground biomass and denitrified.

The common results of the two facilities were:

- calculation of nitrogen removal efficiency;
- quantification of nitrogen stored in plants biomass;
- calculation of nitrogen balance.

With respect to the pilot experiment the mesocosm allowed:

- comparison of nitrogen removal efficiency of different wetland plants;
- evaluation of tolerance of different species to increasing nitrogen load;
- study of NH_4-N for possible applications of constructed wetlands, for example to animal rearing facilities;
- a preliminary indication on plants/microorganisms interaction.

CHAPTER II Pilot surface flow wetland

Materials and methods

Site description and management

The experiment began in 1996 on the "L. Toniolo" Experimental Farm of Padova University at Legnaro (near Padova, 45° 21' N; 11° 58' E; 6 m a.s.l.) and is still ongoing. The climate of the site is sub-humid, with a mean annual rainfall of about 850 mm fairly uniformly distributed throughout the year. The temperature increases from January (average minimum value: -1.5 °C) to July (average maximum value: 27.2 °C). According to the FAO-UNESCO classification, the soil is a fulvi-calcaric Cambisol, with loamy texture in the upper 80 cm; the percentage of silt gradually increases with depth, reaching values of 68-75% at 2-2.4 m deep. Soil infiltration rate, evaluated with the double cylinder infiltrometer (Kessler and Oosterbaan, 1972), is 0.1 cm/h, and average lateral hydraulic conductivity measured by the auger hole method (Van Beers, 1958) is 4.2 cm/h. An upper layer of reduced permeability is located at 1.5-1.8 m, and there is an impervious layer at about 3 m; these involve the whole experiment area.

The experimental site extends over five hectares of land divided into 12 plots, which have different drainage systems installed to simulate the features of a typical agricultural basin on the low-lying Veneto plain. An experiment was conducted in the plots deriving from the factorial combination of two drainage systems (surface by ditches and subsurface by plastic pipes) and two management criteria (controlled and conventional drainage), with three replicates. The drainage water leaving all the plots is collected by subsurface pipes and conveyed to a manhole. Here, a pump is installed to discharge the water into a surface flow constructed wetland (SFW) before final delivery into a stream. The drainage experiment and SFW were installed and managed at the same time, to study an integrated sustainable system to reduce agricultural pollution (Figure 6).

In this work data regarding the period January 2007- April 2009 are taken into account.

During the monitoring period, the fields around the wetland were cultivated with maize (2007), sugar beet (2008) and winter wheat (2008-2009), following standard agricultural practices. Nitrogen fertilization was applied to crops according to plant requirements (about 500 kg/ha in the considered period), using only chemical fertilizers. Fertilization dates and rates are reported in Table 5.

Table 5 – Fertilization dates and rates in the cultivated plots discharging in the SFW

Year	date	kg/ha N
2007	30/3	32
	21/5	202
2008	12/2	69
	6/5	54
	27/10	18
2009	23/2	54
	8/4	69
Total		**498**

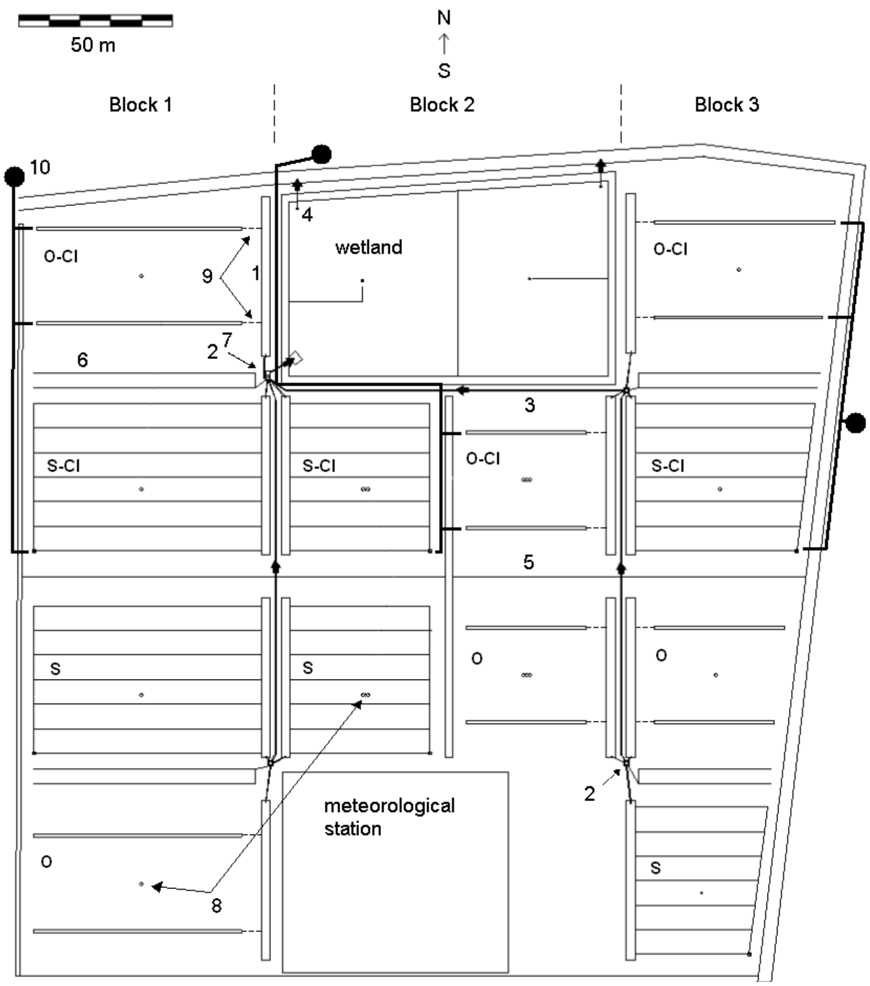


Figure 6 – Map of the experiment of controlled drainage and SFW. Letters indicate different drainage systems in the plots

Wetland features and design criteria

The SFW was excavated in summer 1996 as a single treatment cell and covers an almost square area of 3200 m^2. It receives via a pump the drainage waters coming from the surrounding plots (Figure 7). The wetland base was excavated 0.4 m below the field surface and is surrounded by banks raised above field level. The reduced depth of the basin was justified by the necessity to discharge into the stream by gravity otherwise the basin might be deeper but smaller in surface. The size of the SFW was calculated to guarantee a retention time of at least 7 days, taking into account a cumulative three-day discharge volume coming from the catchments with a return period of one year in four. At the outlet, an upward curving pipe, placed in a manhole, allows space for other pipes of various lengths to be inserted to regulate the desired depth of water in the basin. To limit lateral subsurface water flow to and from the SFW, a plastic sheeting was installed vertically, to a depth of 1.5 m along the cell perimeter. In 1996, a small bank (25 cm high) was raised within the basin to force water to follow a precise route from inlet to outlet

Wetland management

The SFW was vegetated in spring 1997, dividing the area into 4 sectors: *Typha latifolia* L. (cattail), was planted in one sector near the inlet, a mixture of cattail and *Phragmites asutralis* (Cav.) Trin., (common reed), in the second, common reed in the third and the fourth, next to the outlet, was left without vegetation. Cattail was planted as clumps with some developed plants, and common reed as rhizome cuttings with 1-3 buds. No vegetation management was done in order to study its ecological succession over the years till 2007. After the first year, common reed covered the entire area replacing most of the cattail. Nowadays there are only some plants of cattail and some plants of *Carex elata* All. (spontaneously arrivals) next to the outlet.

In 2007 some structural works were done in the basin in order to improve its functionality 10 years after the excavation.

From 1996 to 2007 there were some water losses from the basin due to the presence of sub-surface pipes in the south-north direction. These pipes had been used during a previous experimentation and not completely removed at the time of wetland installation. As a consequence they collected water form the SFW and discharged it directly into the stream, bypassing normal SFW route and without passing into the flow meter. In 2007 the pipes were precisely located, isolated and removed to guarantee an improvement in the hydraulics of the basin. In 2007, two further banks were raised to improve water routing inside the basin (Figure 7) and the banks of the basin's perimeter were reinforced.

The aerial part of the vegetation was harvested for the first time in February 2007 and on this occasion some willow and poplar trees and also some brambles that had colonized the basin were uprooted to avoid competition to common reed growth.

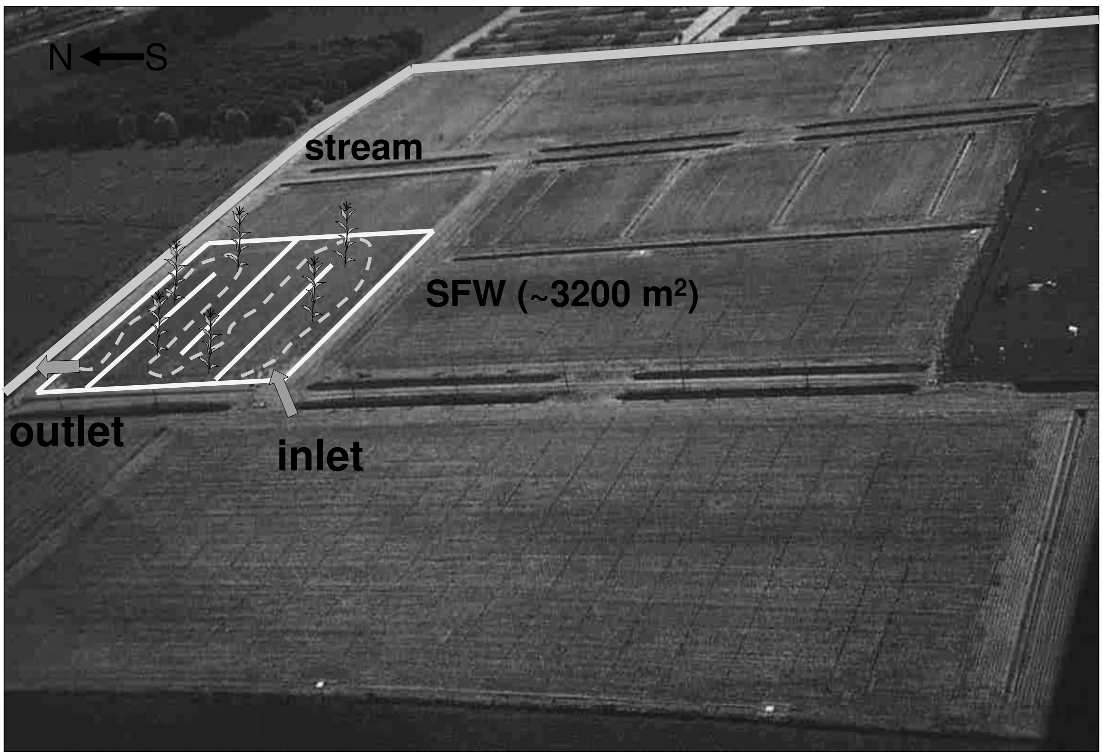


Figure 7 - View of the experimental site, on the left water flow (broken line), the perimeter of the SFW with the internal banks (solid line), surrounded by fields draining into the wetland

Water balance

To calculate a water balance inflow and outflow were measured from January 2007 to April 2009. The water inputs into the SFW were from field drainage and direct rainfall. As field drainage was discharged into the basin by a pump, a flow meter was installed at the inlet to record volumes (Figure 8). Another flow meter was installed at the end of the outlet pipe to measure the volumes discharged from the wetland. Readings of the two flow meters were taken daily.

Figure 8 – Flow meter at the inlet and at the outlet of the SFW

Rainfall was measured at the weather station on the experimental farm, at about 500 m distance from the SFW. Flooding depth was measured every time submersion occurred. The water table level was also measured in phreatimeters installed inside and outside the SFW. To estimate subsurface water losses from the wetland, the Darcy equation was used considering the hydraulic head from inside to outside the basin and previously determined soil lateral permeability (Borin et al., 2000). This estimation was calculated as follow, according to Darcy's formula:

$$Q = (\Delta h/D)PS$$

where Δh was the difference between water table depth inside and outside the SFW (Figure 9), D was the distance between the two considered phreatimeters in the calculation (inside and outside the basin,), S was the surface involved in the loss and, P is the soil lateral permeability.

The water table depth inside and outside the SFW was measured about 100 times during the monitored period and the average daily value of lateral loss for each year was used to calculate the total yearly lateral loss. This calculation probably underestimates the lateral losses and as a consequence the impermeability of the basin will be improved and the number of measurements of water table depth, inside and outside the basin, will be increased.

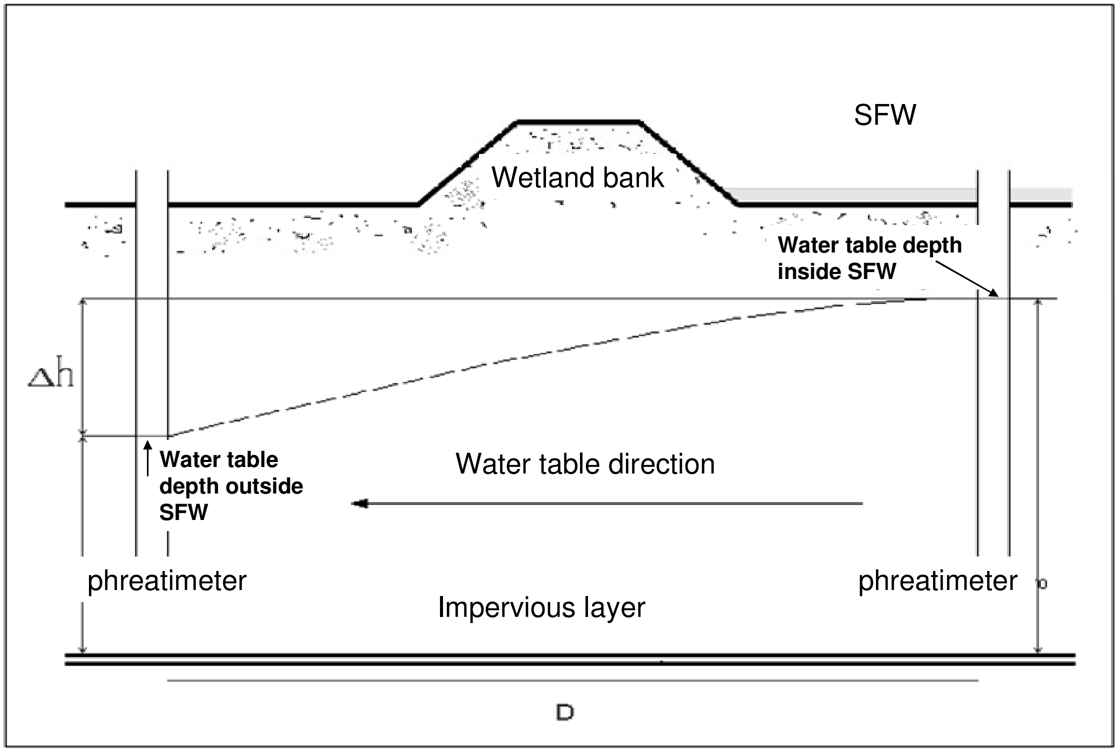


Figure 9 - Scheme used for the calculation of lateral losses

Water sampling and analysis

Samples of input and output water were taken daily when water entered or was discharged from the SFW. Samples were collected by hand, taking water directly from the input pipe and from the flow meter at the outlet. Samples of water table, inside and outside the basin, were also taken in order to estimate subsurface lateral losses for the nitrogen balance.

A total of 134 water samples were collected and analyzed to detect NO_3-N and Total N (TOT N). All samples were frozen immediately after collection and stored until laboratory analysis.

NO_3-N was determined using the modified Cataldo method (Cataldo et al., 1975) and TOT N using the Valderrama method (Valderrama, 1981). At the beginning of the experiment

NH_4-N was also determined, but the concentration values often did not even reach the instrumental detection threshold and for this reason the analysis was stopped.

Soil and vegetation sampling and analysis

Soil samples were taken before the beginning of the monitoring period, at the end of 2006, and at the end of the monitoring period in April 2009. Samples were taken following a regular grid of 6 points (2006) and 11 points (2009) along the water route and geo-referencing the sampling points with a Differential Global Positioning System (Figure 10). Sampling involved the top 0–20 cm soil layer and a deeper soil layer at 20-50 cm. After collection soil samples were air-dried, crushed using a rolling pin, sieved at 2 mm and stored at low humidity. TOT N was measured by the total Kjeldahl method (Kjeldahl, 1883).

Samples of the wetland vegetation biomass were taken in the same dates as soil samples and also in February 2008 removing both above- and below-ground parts next to the soil sample points, considering 0.25 m^2 sampling areas and two depths: 0-20 and 20-50 cm (Figure 11). To measure the dry weight, the collected biomass was first carefully washed then dried at 65° C in a forced draught oven for 36 h, then 1 g powered samples were dried at 130 °C to measure the residual moisture content.

Samples were then analyzed to determine nitrogen content using the AOAC official method (AOAC, 2002).

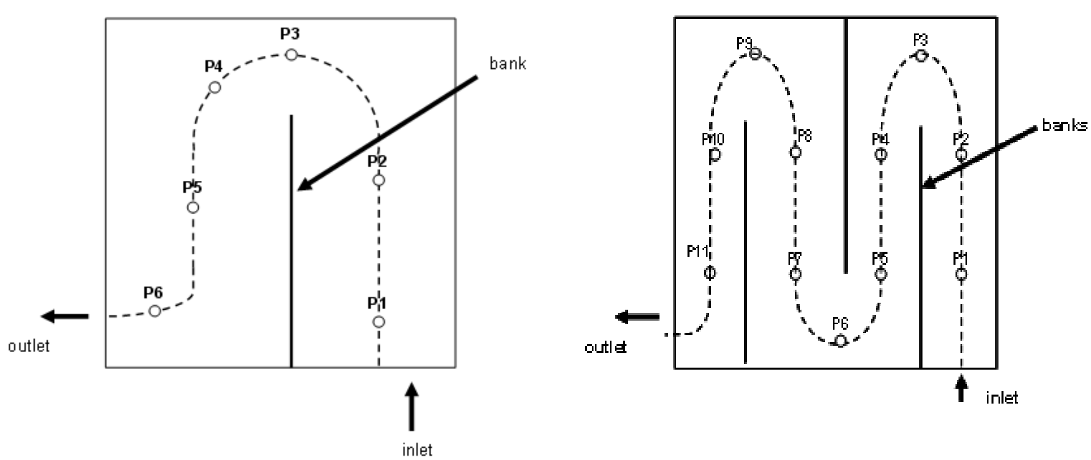


Figure 10 - Scheme of the regular grid of 6 points in 2006 and 11points in 2008 and 2009 followed for sampling soil and vegetation.

Figure 11 – Operations of belowground biomass sampling and preparation for analyses

Results

Water balance

The volumes of water entering the wetland from surrounding fields were discontinuous over the monitoring period, with the main drainage events occurring in spring in particular the spring 2007 and 2009 (Figure 12). From mid February 2007 to the end of May 2007 about 3000 mm of water entered the basin against 320 mm of rainfall in the same period. Then from June 2007 till the end of January 2008 only about 630 mm entered the basin against 380 mm of rain underlining the effect of ET in the surrounding fields. Another period of slight but prolonged water entering the basin was recorded from the end of January 2008 to the end of June 2008 when the SFW received around 2300 mm of water against 360 mm of rain. There were only a few events when water entered form the end of June 2008 to the end of October 2008, giving an input of around 300 mm against 250 mm of rain confirming the intense ET of sugarbeet in the plots outside the basin. The main entrance peak, with more than 7300 mm, was recorded from the end of October 2008 to the end of the monitored period (April 2009) corresponding to about 520 mm of rain.

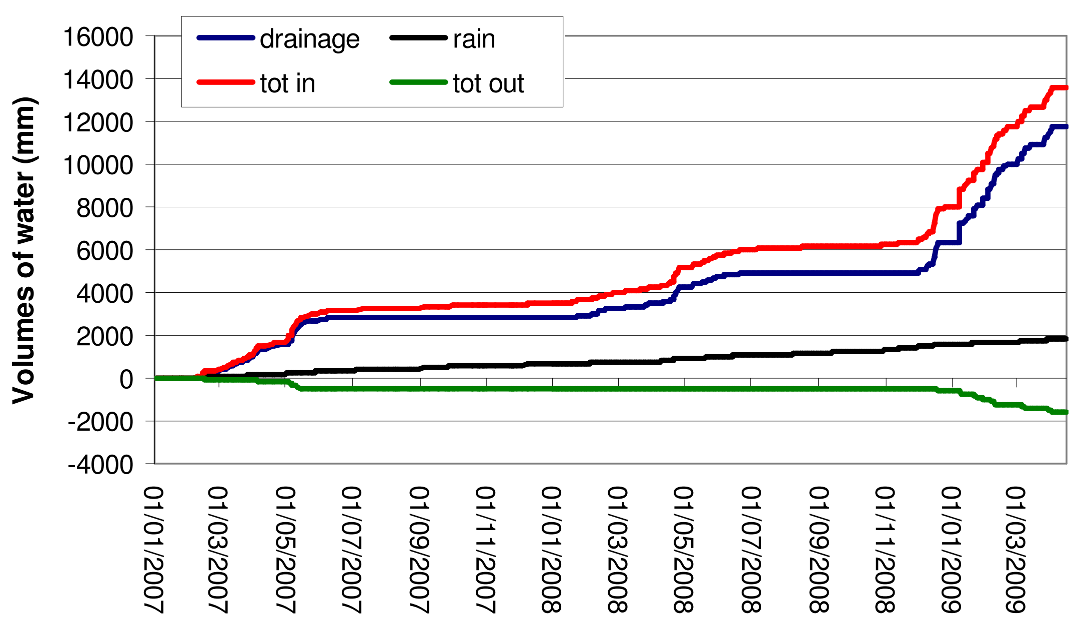


Figure 12 - Cumulative water volumes entering and exiting the SFW during the monitoring period

The maximum daily input was 849 mm in January 2009 followed by other important events around 350 mm recorded in December 2008, January and February 2009.

The time pattern distribution of wetland discharges followed the main input events. The maximum daily discharge of 159 mm was recorded in January 2009, followed by an event of 132 mm in May 2007 and few output events were recorded from the end of May 2007 till the end of November 2008.

From January 2007 to April 2009, about 13620 mm of water entered the SFW (drainage and rain), this meant that the basin received a volume of water about six and an half times the environmental rainfall in the same period (Table 6). Only 1605 mm of water were discharged and the subsurface lateral losses were estimated as around 840 mm.

From the water balance (Table 6) it is possible to estimate that a significant part of the difference between water input and output would have been lost through evapotranspiration (ET); flooding occurred on 99 days (12% of the total period) with discontinuity over time, from a minimum of 27 days in 2007 to a maximum of 39 days considering only the first half of 2009 (Table 6). Flooding depth had an average value of 18 cm.

Table 6 - Annual inlet (from drainage and rainfall), outlet, estimated lateral losses and days of flooding during the monitoring period

	2007	**2008**	**apr-09**	**TOT**
Drainage water (mm)	2831	3538	5415	11784
Rain (mm)	640	958	244	1842
TOT IN (mm)	**3471**	**4496**	**5659**	**13626**
Outlet (mm)	473	116	857	1446
Lateral Losses (mm)	353	320	166	839
TOT OUT (mm)	826	436	1023	2285
Flooding days	27	33	39	99

The water table inside the wetland basin always dropped rapidly to 1 m depth as soon as water input stopped (Figure 13). It fell below 2 m in autumn 2007 and 2008. The external water table, measured from January 2007, was almost always deeper than that in the wetland. There was therefore a hydraulic gradient that originated lateral losses.

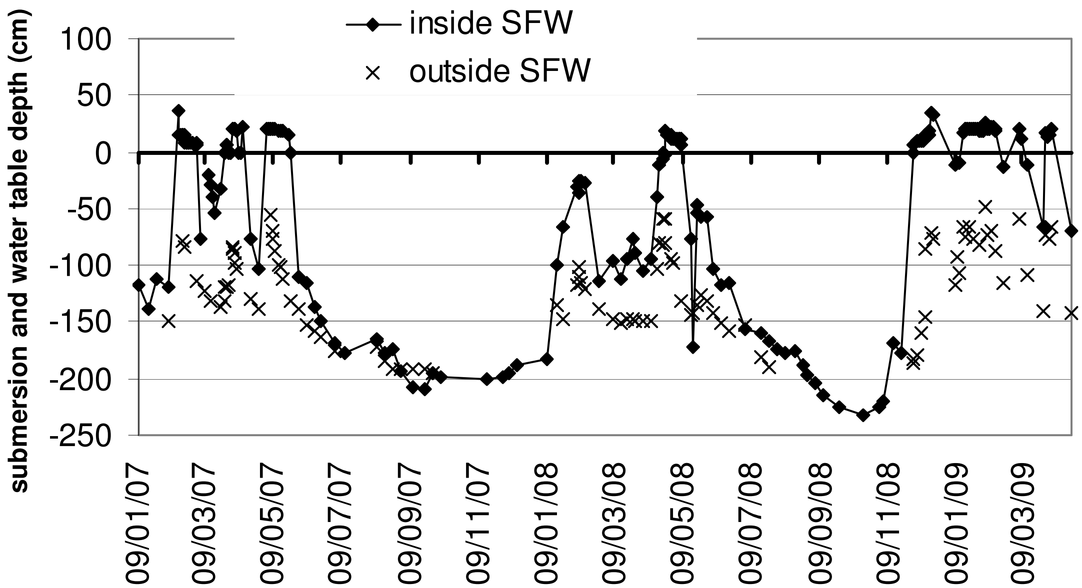


Figure 13 - Water table depth inside and outside SFW and submersion during the monitoring period

NO_3-N concentration and balance 2007-April 2009

NO_3-N concentration

Concentration inputs of nitric nitrogen entering the basin were variable but generally lower than 5 ppm (Figure 14). Two peaks were evident, the first on 14th and 15th February 2007, the second from 22nd to 24th April 2008. The high values detected on these days were probably connected with high precipitation volume (52 mm in the week in February and a total amount of 82 mm from intense rainfall events for 9 consecutive days in April). In both cases the peaks were delayed respect to the nitrogen fertilization applied to fields suggesting that the element didn't reach the surface water body in short time.

Outflow concentrations were always lower than inputs, except for two high values in February 2007 connected with the above-mentioned intense rainfall and probably also with the fact that the aerial part of common reed was harvested in this period for the first time and decomposition processes of the remaining straw could have enriched nitrogen in the exiting water.

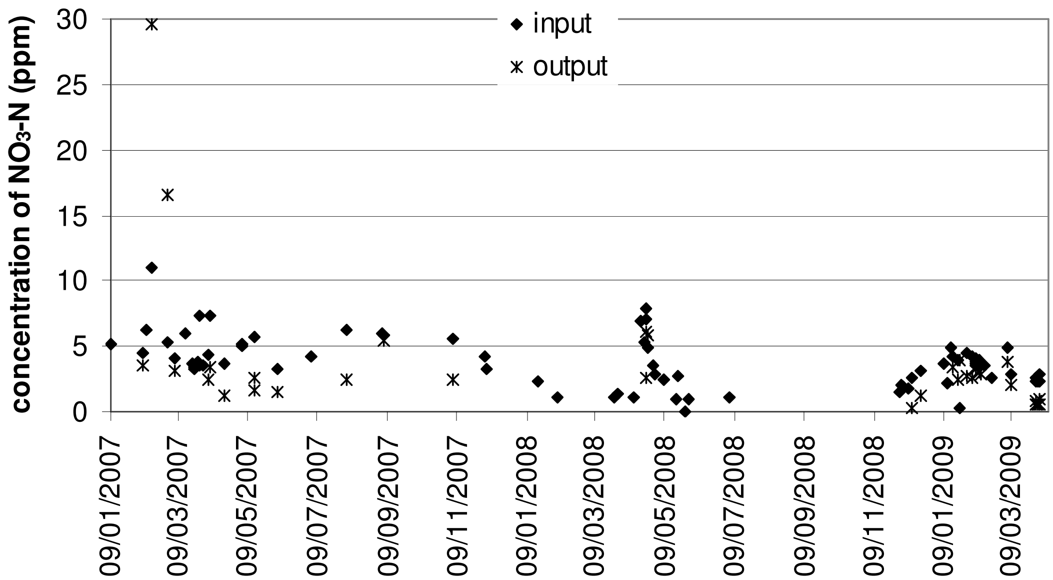


Figure 14 - Daily values of NO_3-N detected in the input and output events

Generally speaking passing through the SFW, a reduction in median values took place in 2007 and 2009 while this didn't happened in 2008 probably because most of the output events in that year happened during the *Ph. australis* growing season when the ET reaches its maximum values and, as a consequence, the volumes of output water were very low and therefore concentrated (Figure 15). In general, the wetland not only decreased NO_3-N concentration, but also reduced the variability of values at the outlet.

The extreme values had a similar general trend, with the exception of 2007, when there were the two previously-mentioned high values in February.

Considering the median value of NO_3-N in all the input and output events input is significantly higher than output, underlining the positive effect in the water flowing through the basin (Figure 16).

Taking into account the concentration detected in the water table inside the basin (WT inside) against the input water, it is interesting to note that the former is significantly lower suggesting that there were negligible losses due to percolation.

As regards the concentration in the water table outside the basin (WT outside) against the WT inside the former is significantly higher suggesting that no NO_3-N losses took place with subsurface lateral losses of water (Figure 16).

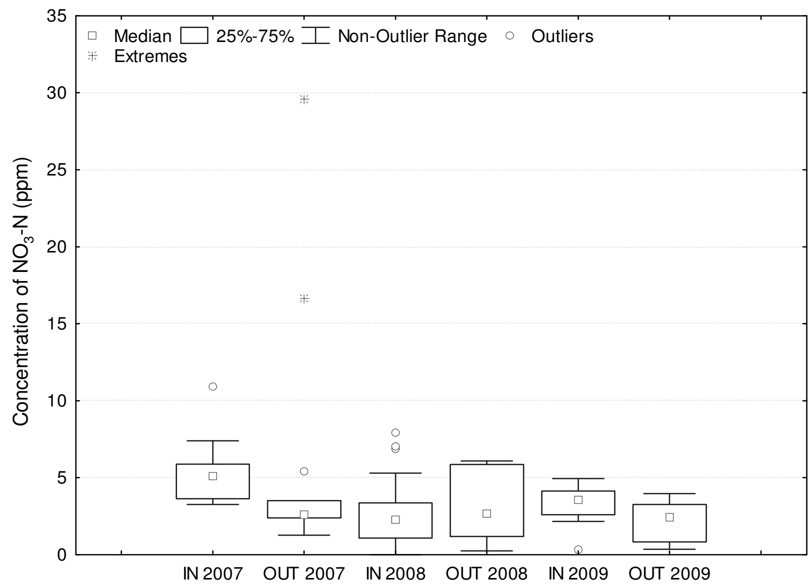
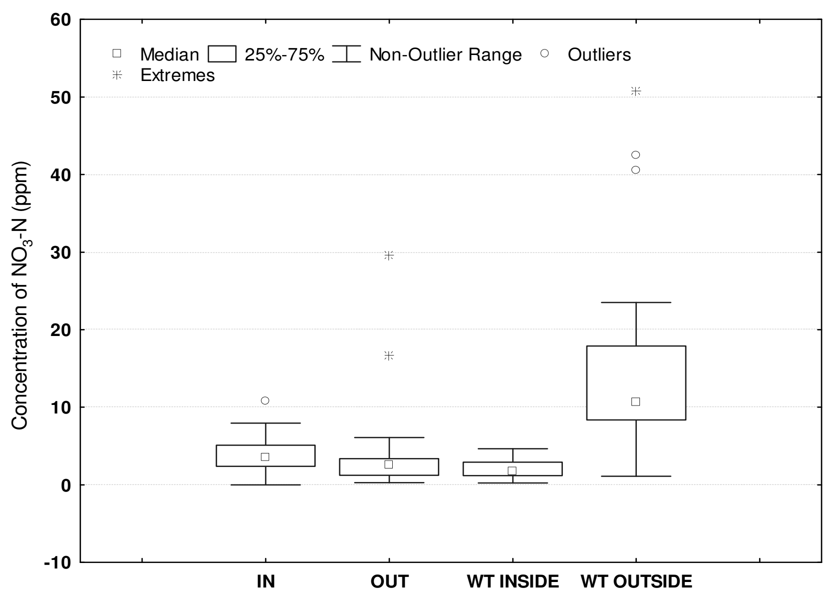


Figure 15 - Box and whiskers of NO_3-N concentration in water inflow and outflow



COMPARISONS	SIGNIFICANT DIFFERENCES
IN-OUT	YES
WT INSIDE-IN	YES
WT INSIDE-WT OUTSIDE	YES

Figure 16 - Box and whiskers of NO_3-N concentration in water input and output and in water table inside and outside the basin in the monitoring period. Significant differences on the table at 0.05 P by Krukal-Wallis

NO3-N loads and discharge

According to the water input time pattern, nitric nitrogen entered the wetland discontinuously (Figure 17) and the peaks corresponded to fertilization of crops (represented with dots in Figure 17) and major rainfall events. The median daily loads were 1.6 kg/ha with a maximum value of 28 kg/ha registered in January 2009. The entering loads originate a cumulative NO_3-N load versus time displayed as a step-shaped trend, in which an intensive load of almost 150 kg/ha is evident in spring 2007, afterwards there is another important peak at the beginning of 2009 and another smaller peak is detectable in spring 2008. During the monitoring period the wetland received a total of more than 420 kg/ha of NO_3-N and discharged only about 42 kg/ha with an apparent removal rate of more than 90%.

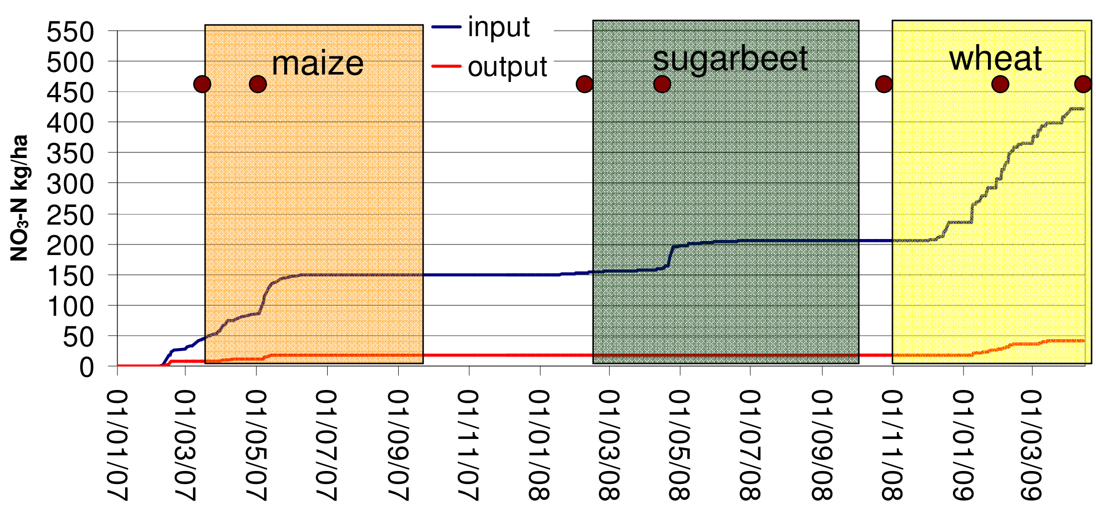


Figure 17 - Cumulative NO_3-N input and output in the SFW; the dots represent the fertilizations

TOT N concentration and balance 2007-April 2009

TOT N concentration

Input concentration values varied from 24 ppm (24th April 2009) to practically 0 at the end of March and at the beginning of April 2008 (Figure 18). Three peaks were detectable, the first in mid-May 2007, the second at the end of April 2008 and the third at the end of January 2009 connected with intense rainfall.

The output values were sometimes higher than the input, especially outside the growing season when litter degradation probably contributes to nitrogen enrichment of water output.

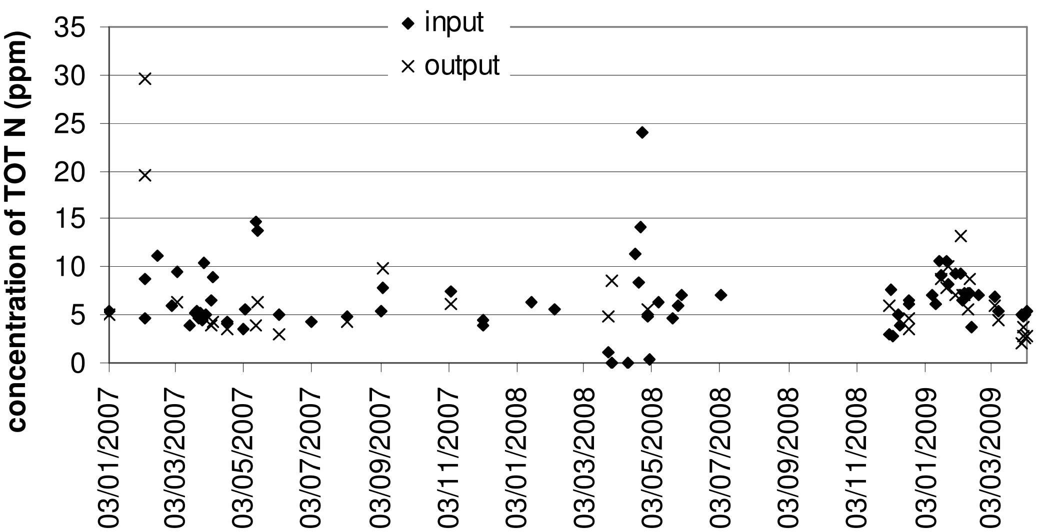


Figure 18 - Daily values of NO_3-N detected in the input and output events

The concentrations of TOT N were variable and characterized by frequent medium–low values with median values always lower than 7 ppm (Figure 19). Passing through the SFW a slight reduction in median values took place every year, but considering the whole dataset the median input and output value remained about the same and no statistical differences were found by the Kruskal-Wallis test. The median value of the water table concentration was significantly lower than the surface water and this suggests again that there were negligible losses due to percolation (Figure 20) TOT N was not measured in the water table outside the wetland but we can assume that as for NO_3-N no losses took place with subsurface lateral losses of water.

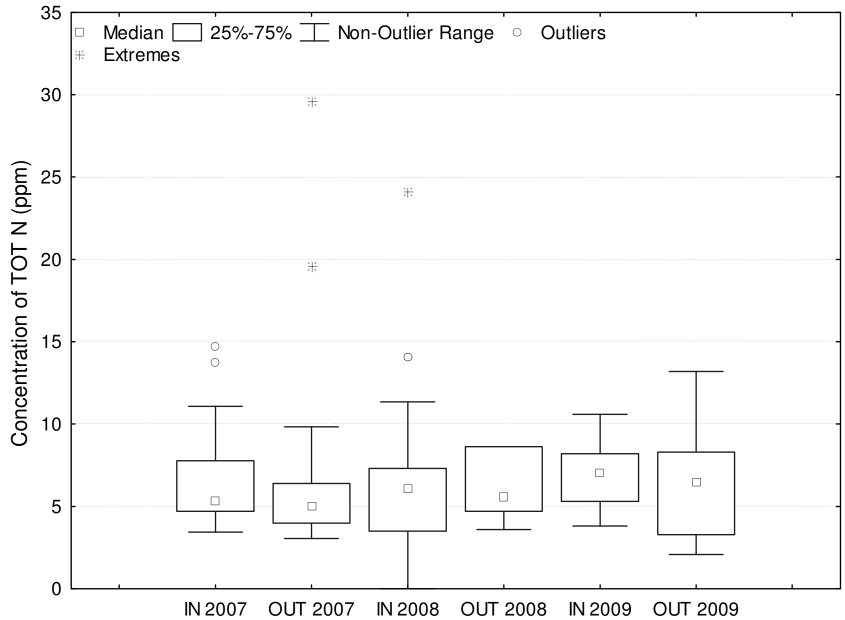


Figure 19 - Box and whiskers of TOT N concentration in water inflow and outflow in the monitoring period

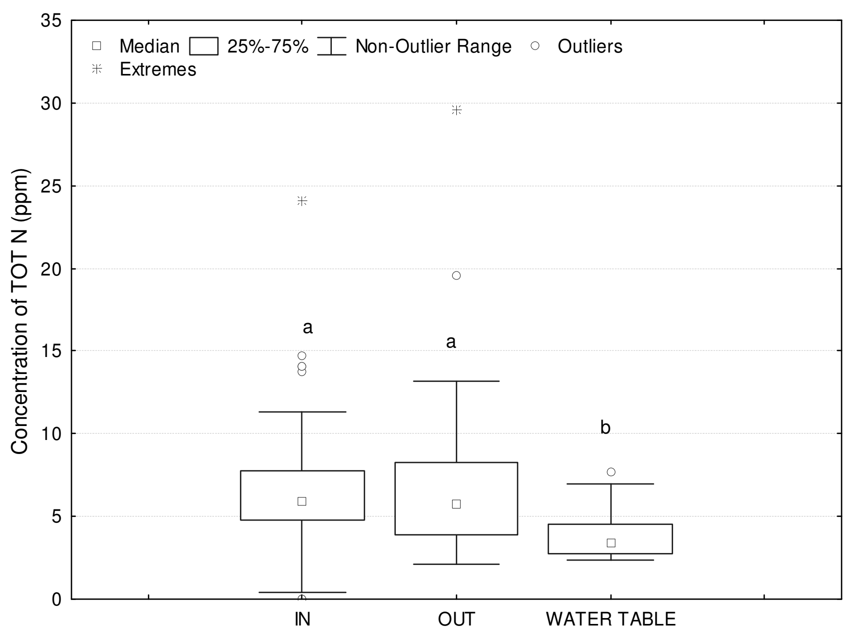


Figure 20 - Box and whiskers of TOT N concentration in water inflow and outflow and water table in the monitoring period, different letters indicate statistically significant difference by the Kruskal-Wallis test

TOT N loads and discharge

As happened for NO_3-N, TOT N entered the wetland discontinuously (Figure 21) and also in this case the peaks corresponded to fertilization of crops and major rainfall events. The median daily loads were 6.6 kg/ha with a maximum value of 57 kg/ha registered in January 2009. The entering loads originate a stepped cumulative TOT N curve, in which an intensive load of more than 200 kg/ha is evident in spring 2007, then there is another peak in spring 2008 and a big input at the beginning of 2009. In 2.5 years of monitoring, the wetland received a total of about 820 kg/ha of TOT N and discharged only about 80 kg/ha with an apparent removal rate of more than 90%.

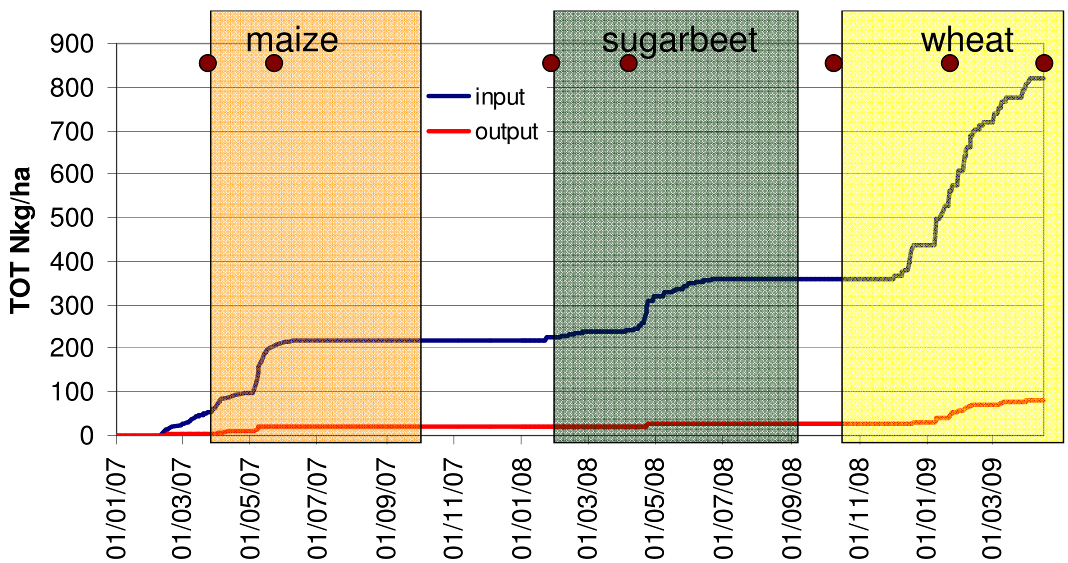


Figure 21 - Cumulative TOT N input and output in the SFW; the dots represent the fertilizations

Biomass production and nitrogen uptake

The results of vegetation sampling in terms of biomass are summarized in Table 7. In February 2008 there was a decrease in total dry matter with respect to 2006 due in particular to a decrease in the aerial biomass and straw, surely in consequence of the harvest at the beginning of 2007. The common reed had only one vegetative season to grow and probably was not able to reach its full development. Every year the belowground biomass in the 0-20 cm layer represented about or more than an half of the total dry matter.

Table 7 - Repartition of dry matter and % of each plant part on the total

dry matter	2006		2008		2009	
	t/ha ± std	%	t/ha ± std	%	t/ha ± std	%
aerial part	18.5 ± 6.2	31%	8.4 ± 3.5	19%	8.9 ± 4.1	15%
straw	9.7 ± 2.5	16%	3.6 ± 2.3	8%	4.4 ± 2.6	7%
belowground biomass 0-20 cm	27.1 ± 8.9	46%	25.7 ± 9.3	57%	39.3 ± 12.9	66%
belowground biomass 20 -50 cm	3.6 ± 3.0	6%	7.6 ± 6.1	17%	6.9 ± 2.4	12%
total	58.9 ± 19.7	100%	45.3 ± 16.2	100%	59.5 ± 16.5	100%

Considering the % of nitrogen detected in plant tissues, the straw had the high value every year (Table 8). The highest value in the aerial part and in belowground biomass (20-50 cm) was detected in 2006. The belowground biomass (0-20 cm) reached the highest value in 2008.

Table 8 - N % detected in different plant parts

% nitrogen	2006	2008	2009
	% ± std	% ± std	% ± std
aerial part	0.84 ± 0.06	0.52 ± 0.21	0.71 ± 0.20
straw	1.01 ± 0.22	0.90 ± 0.11	1.16 ± 0.18
belowground biomass 0-20 cm	0.64 ± 0.10	0.81 ± 0.31	0.74 ± 0.26
belowground biomass 20 -50 cm	0.63 ± 0.18	0.53 ± 0.14	0.51 ± 0.11

Considering the nitrogen stored in the tissues a pattern similar to dry matter was found: nitrogen stored in the aerial part and straw decreased and the most important plant part in nitrogen accumulation was the belowground biomass layer 0-20 (Table 9).

Table 9 – Repartition of N and % of each plant part on the total

nitrogen stored	2006		2008		2009	
	kg/ha ± std	%	k/ha ± std	%	k/ha ± std	%
aerial part	154 ± 55	35%	44 ± 26	14%	65 ± 38	15%
straw	100 ± 34	22%	31 ± 18	10%	52 ± 32	12%
belowground biomass 0-20 cm	172 ± 56	39%	192 ± 83	63%	273 ± 87	64%
belowground biomass 20 -50 cm	19 ± 14	4%	38 ± 31	12%	34 ± 11	8%
total	**445 ± 117**	**100%**	**305 ± 118**	**100%**	**424 ± 113**	**100%**

The vegetation was sampled following a regular grid so it was also possible to analyze the pattern of repartition of dry matter and nitrogen considering also the distance from the inlet. In 2006 there was a positive significant correlation in dry matter between the belowground biomass 0-20 cm layer and the distance from inlet, not detected for other plant parts (Figure 22).

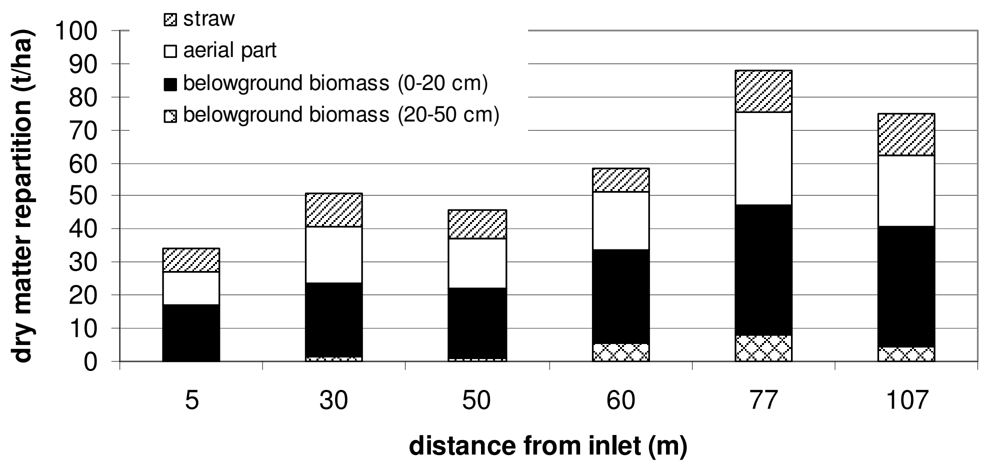


Figure 22 - Repartition of dry matter in different plants part with respect to the distance from inlet in 2006

Considering nitrogen, a positive significant correlation was found only between aerial part and distance from the inlet (Figure 23).

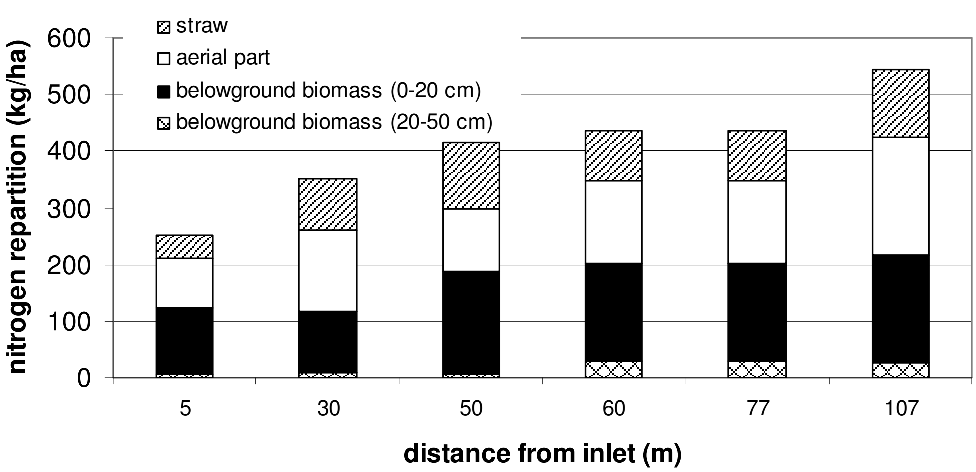


Figure 23 - Repartition of nitrogen stored in plant parts with respect to distance from the inlet in 2006

In 2008 the sampling grid was modified because of the presence of new banks and the repartition of dry matter considering the distance from the inlet is shown in

Figure **24**. No significant correlations were found between the dry matter of different plants parts and distance from the inlet, but the point with the highest dry matter was P 10 located at 180 m from the inlet with a total amount of 87.6 t/ha.

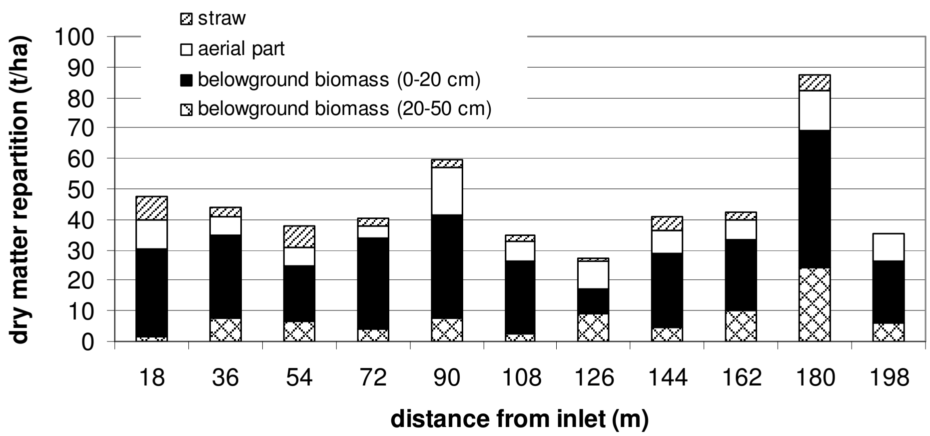


Figure 24 - Repartition of dry matter in different plant parts with respect to distance from the inlet in 2008

Also considering nitrogen in 2008 no significant correlations were found between nitrogen content of different plants parts and distance from the inlet and again the point with the largest quantity of nitrogen stored in tissues was P 10 located at 180 m from the inlet which reached a total amount of 611 kg/ha (Figure 25).

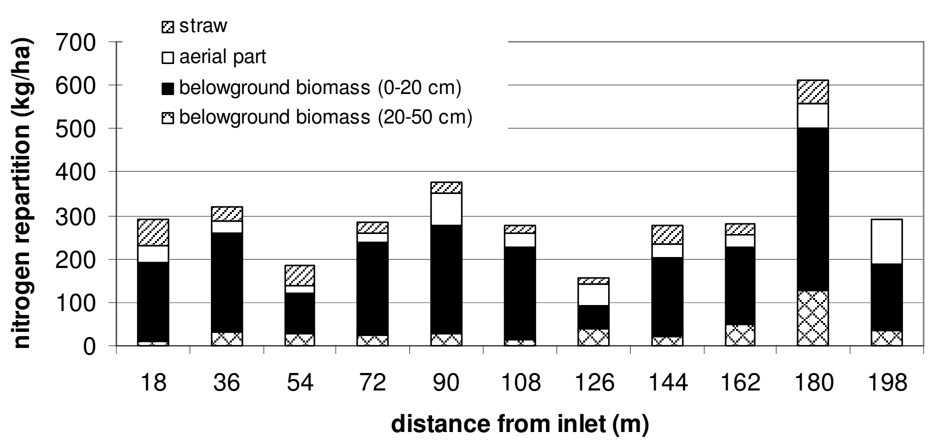


Figure 25- Repartition of nitrogen stored in plant parts with respect to distance from the inlet in 2008

The results on dry matter repartition in 2009 are shown in Figure 26. Also this year no significant correlations were found between the dry matter of different plants parts and distance from the inlet but the point with the highest dry matter was P 7, located at 126 m from the inlet with a total amount of 93.5 t/ha.

No significant correlations were found between nitrogen content of different plant parts (Figure 27) and distance from the inlet and again the point with the largest quantity of nitrogen stored in tissues was P 7 located at 126 m from the inlet that reached a total amount of 646 kg/ha.

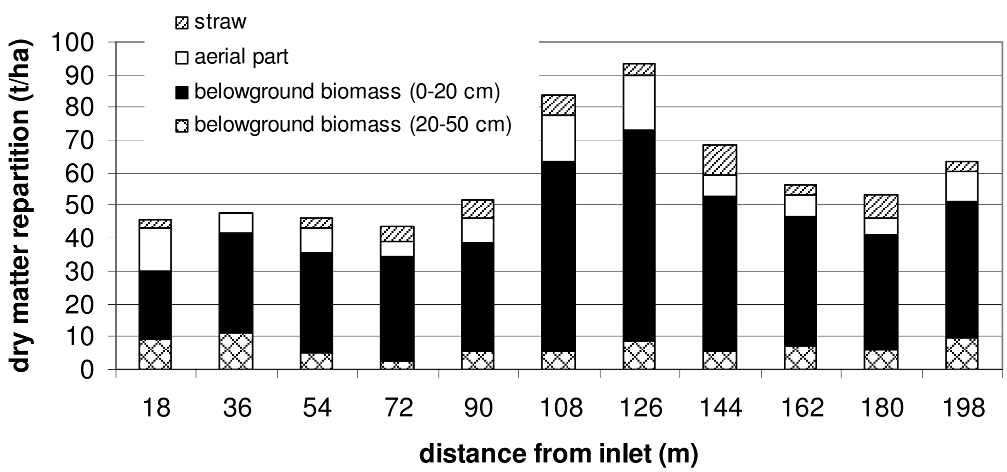


Figure 26 - Repartition of dry matter in different plant parts with respect to distance from the inlet in 2009

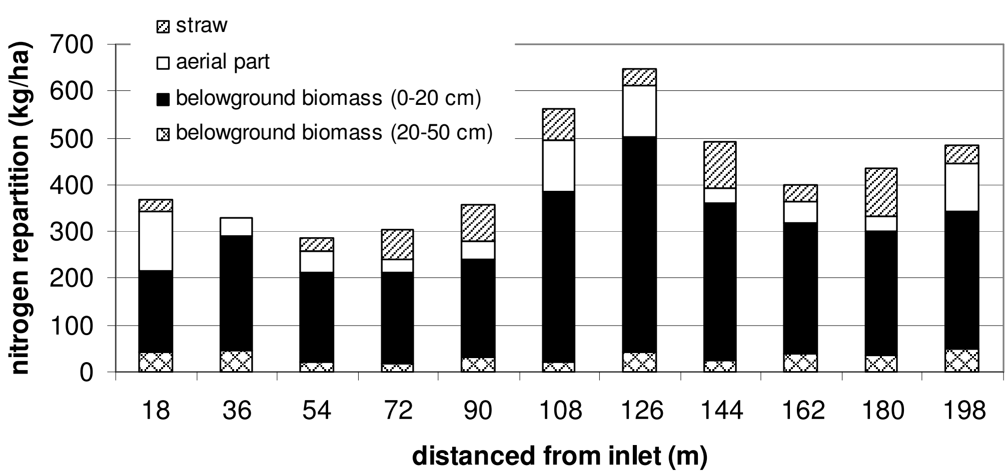


Figure 27 - Repartition of nitrogen stored in plant parts with respect to distance from the inlet in 2009

Nitrogen accumulation in the soil

Considering the nitrogen % detected in the soil and the bulk density of the soil, in 2006 and 2009 the total amount of nitrogen stored in the soil (depth 0-50) was 5099 kg/ha and 5660 kg/ha respectively, giving an accumulation of 561 kg/ha. The soil nitrogen accumulation took place in the top soil (0-20 cm) while a decrease was recorded in the 20-50 layer cm (Table 10). Taking the soil and vegetation as a unique compartment, the vegetation showed a decrease of 21 kg/ha from 2006 to 2009 (Table 9), the soil an increase, giving an accumulation of 540 kg/ha of TOT N for the whole compartment in the monitoring period.

Table 10 - Total nitrogen stored in the soil at the beginning and at the end of the monitoring period

Depth (cm)	**2006**		**2009**	
	% ± std	kg/ha	% ± std	kg/ha
soil 0-20	0.09 ± 0.03	2531	0.11 ± 0.02	3318
soil 20-50	0.05 ± 0.07	2568	0.05 ± 0.02	2342
Tot		**5099**		**5660**

Overall TOT N balance

Taking into account all the components analyzed in the monitoring period for TOT N balance, it is interesting to note that: 820 kg/ha entered the wetland, 80 kg/ha were discharged with the output water, 540 kg/ha were accumulated in the soil-vegetation compartment and 154 kg/ha were removed with the aerial part harvested in 2007. The missing amount of TOT N, about 46 kg/ha, might be removed via denitrification, that with this calculation accounted only for 6% of TOT N removal (Figure 28).

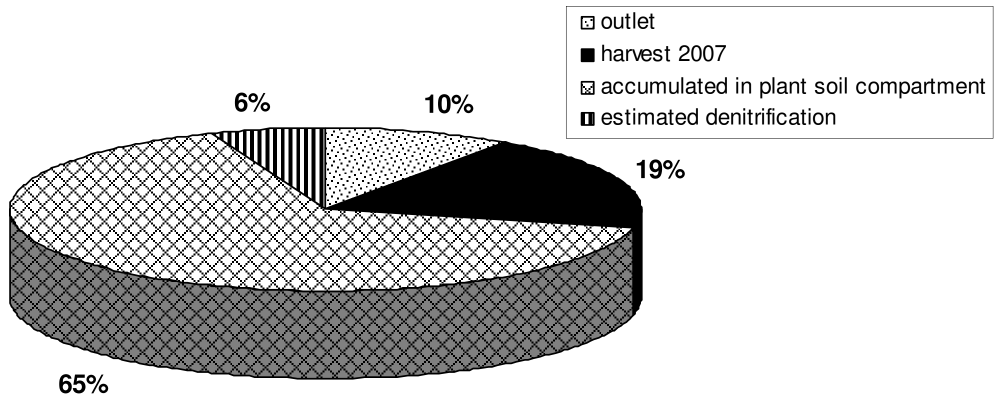


Figure 28 - TOT N balance in the monitoring period

CHAPTER III Mesocosm experiment

Materials and methods

Experimental set up and management

The five tested species were *Carex elata* All., (*Cae*), *Juncus effusus* L. (*Jue*), *Typhoides arundinacea* (L.) Moench (syn. *Phalaris arundinacea* L.) (*Pha*) var. picta, *Phragmites australis* (Cav.) Trin. (*Phr*), and *Typha latifolia* L.(*Ty*) (Figure 29).

Carex is the most species-rich genus in the family of *Cyperaceae*, commonly known as sedge. Most (but not all) sedges are found in wetlands (e.g. marshes, calcareous fens, bogs and other peat lands, pond edges, and even ditches), where they are often the dominant vegetation. *Carex elata* All., in natural conditions, it is one of the most diffused species on the Veneto plain but its presence is nowadays greatly reduced due to human activities.

Juncus effusus L. is a member of the *Juncus* genus (family *Juncaceae*) found growing in wet areas and commonly known as soft rush. It is a common plant native to most temperate countries. It grows in large clumps about 1 m tall. The stems are smooth cylinders with light pith filling.

Typhoides arundinacea (L.) Moench is a perennial grass of the *Poaceae* family, commonly known as reed canary grass. This wetland plant is a circumboreal species that grows so vigorously that it is able to inhibit and eliminate competing species. Some varieties have been selected for use as ornamental plants, such as (var. picta) the one in this study that has variegated leaves.

Phragmites australis (Cav.) Trin. is the most common species used to vegetate constructed wetlands and is commonly called common reed. It is a tall perennial grass of the *Poaceae* family found in natural wetlands throughout temperate and tropical regions of the world. It commonly forms extensive stands (known as reed beds) and is capable of reproduction by seeds, but primarily does so asexually by means of rhizomes.

Typha latifolia L., commonly known as cattail, is a perennial herbaceous plant in the genus *Typha*, (*Typhaceae* family) which grows in temperate, subtropical and tropical areas in shallow waters, at low to mid elevations. It flowers from mid to late summer and like common reed it is capable of reproduction by seeds, but primarily does so asexually thanks to belowground rhizomes.

Carex elata All. (*Cae*)

Juncus effusus L. (*Jue*)

Typhoides arundinacea (L.) Moench (*Pha*)

Phragmites australis (Cav.) Trin. (*Phr*),

Typha latifolia L.(*Ty*)

Figure 29 – Species planted in the mesocosm experiment

The experiment was conducted for three years considering different nitrogen input concentrations and different of cycles length: monthly the first year and weekly the second and third.

On some occasions the general protocol of analyses was intensified to better understand the processes involved in nitrogen fate. Periods of intensive monitoring were carried out in July and December 2007, July 2008 and February 2009.

October 2006-March 2008 (First year)

The study was conducted in a cold glasshouse and consisted in a combination of 10 vegetated and 2 non-vegetated plastic tanks filled with gravel grain size of 0.1-10 mm (d_{10}= 3.9 mm; d_{60} = 5.2 mm) with porosity of 25% (Figure 30).

The tanks were 50x40x29 cm in length, width, height respectively with a tap at the base to collect water samples. A vertical perforated PVC pipe was installed in the centre of each tank in order to measure dissolved oxygen (DO), conductivity, pH and redox potential (Eh) with the portable digital instrument HQD 40d by Hach Lange according to standards methods (APHA, 1992).

There were two tanks for each species (Figure 30) *Cae, Jue, Pha*, *Phr* and *Ty,* with 30 plants/m^2 planted in October 2006.

The tanks were managed with monthly cycles of load and discharge of a reconstructed wastewater. At the beginning of the cycle, 60 L/m^2 of wastewater were applied to each tank. Every week each tank was emptied from the tap, the collected water was measured to determine the losses due to evapotranspiration (ET) by difference, and reapplied adding clean water to replace the initial volume of 60 L/m^2. At the end of every monthly cycle the residual water was discharged, measured, sampled and disposed of. Samples were analyzed to detect the concentration of NO_3-N and NH_4-N, Total N (TOT N) was derived as the sum of the previous two forms.

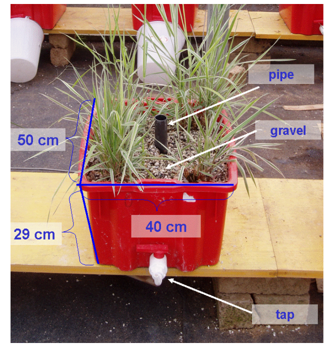


Figure 30 - General view of the experimental set up and detail of a tank

From January to July 2007 seven cycles with an average input concentration of 55 ppm of NO_3-N and 55 ppm of NH_4-N (low load) were carried out using a solution of water and ammonium-nitrate. The concentration was then almost doubled (high load: average input concentration of 112 ppm of NO_3-N and 105 ppm of NH_4-N) from August 2007 to February 2008 for other seven cycles. The cumulative total load of nitrogen was 46.5 g/m^2 in the first period and 91.2 in the second.

After vegetation establishment, plant height (all species) and stems density (*Jue, Phr,* and *Ty*) were measured weekly. Plant height was taken considering the highest green leaf of the plant. Observations on height and stems were interrupted at the quiescence of the plants and restarted after the winter with the emission of new stems. On January 10th 2008 the aerial part of all the plants was harvested in order to measure the biomass productivity and determine the % of nitrogen. This allowed the amount of nitrogen taken up by the plants and stored in the above-ground tissues to be calculated. Temperature of the air and the water in one vegetated tank were recorded every two hours from March 2007 to March 2008 using a data logger (FT 2300, Econorma).

July and December 2007 (First year)

Chemical analyses were conducted weekly in July 2007 in order better study the pollutants removal dynamics. In December 2007, the chemical analyses were done one week and, as usual, one month after the load of ammonium-nitrate.

April 2008-December 2008 (Second year)

For the second year of experimentation, on March 2008 the mesocosms were doubled by dividing each tank into two parts, one part (vegetation and gravel) was used to set up a new tank and the other was refilled with new gravel. So the entire trial consisted of 24 tanks, 4 tank per species and 4 non vegetated tanks as control. The tanks were moved outside (Figure 31) arranged with a completely randomized scheme and equipped with a lateral pipe and a graduated container to collect excess water in the case of heavy rainfall events.
In March the tanks were fed with a nutrient solution with potassium and microelements.

Figure 31 - View of the experimental set up in June 2008

The tanks were filled with wastewater once a month and the output water was analyzed after one week. At the beginning of the cycle 60 L/m^2 of wastewater were applied to each tank. Every week, and every 2-3 days during the summer months, each tank was emptied from the tap, the collected water was measured and reapplied adding clean water, to replace the initial volume of 60 L/m^2. In the case of heavy rainfall events, the water collected in the graduated container under each tank was taken into account in the calculation of ET and removal efficiency. At the end of the weekly cycle the residual water was discharged, measured, sampled and disposed of. Samples were analyzed to detect the concentrations of NO_3-N and NH_4-N.

From May to November 2008 seven weekly cycles with an average input concentration of 105 ppm of NO_3-N and 100 ppm of NH_4-N were carried out using a solution of water and ammonium-nitrate. The cumulative total load of nitrogen was 85.9 g/m^2. Starting from this second year the tanks were also fed with phosphorus at an input concentration of 50 ppm at the beginning of each cycle, giving a total P load of around 21 g/m^2.

After vegetation establishment, plant height (all species) and stems density (*Jue, Phr,* and *Ty*) were measured weekly.

On December 4th 2008 the aerial part of all the plants was harvested in order to measure the biomass productivity and determine the % of nitrogen.

Temperature, humidity, solar radiation, rain volumes, wind speed and direction were recorded every two hours from June 2008 using a meteorological station (CR 800 series, Campbell Scientific).

July 2008 (Second year)

In July 2008, during the weekly cycle, chemical analyses were performed every day in order to better describe the pollutants' fate. Four beakers of 2L volume were also set up; they were sterilized in an autoclave at 120 °C for three hours and filled with sterilized gravel and covered with a cotton lint, to create a situation without any bacterial activity. The four beakers (SC) were managed as all the other tanks.

December 2008-March 2009 (Second year)

At the beginning of December 2008, in order to preserve the tanks from the ice, 18 mesocosms were put to rest, emptied and covered with polystyrene. The 6 remaining tanks, one per species and one control, were left uncovered and in operation.

February 2009 (Second year)

In February 2009 the six tanks left outside, and one beaker set up as in July 2008, were filled with 110 ppm of NH_4-N and 115 of NO_3-N and the chemical analyses were done 12 times in a week (4, 9, 16, 23, 39, 48, 62, 71, 95, 119, 143 and 167 hours after application). The aim was to more thoroughly study the dynamics of the pollutants variation in the cold season.

April 2009-September 2009 (Third year)

At the beginning of April all the tanks were put in operation again and fed with the same nutrient solution as the previous year. They were managed as in 2008: filled with wastewater, once a month in the first two cycles and twice in the others, the output water was analyzed after one week. At the beginning of the cycle 60 L/m^2 of wastewater were applied to each tank. In April and May the loads were of 104 ppm of NO_3-N and 119 ppm NH_4-N, then for another 8 cycles the input concentration was doubled (average NO_3-N 200 ppm and 207 ppm NH_4-N). The cumulative total load of nitrogen was 222 g/m^2.

Also this year the tanks were fed with phosphorus at an input concentration of 50 ppm, at the beginning of each cycle, giving a total P load of 30 g/m^2.

Water management and chemical analyses were done as in 2008.

Dates, number and length of cycles and nitrogen concentrations input in the three years of experimentation are summarized in Table 11. By the end of three years, considering also the periods of intensive monitoring, more than 2200 water chemical analyses had been conducted.

Table 11 – Dates, number, length and nitrogen forms input concentration in the three years of mesocosm experiment

period	number of cycles	length of cycle	average N input concentration (ppm)	
			NO_3-N	NH_4-N
18/01/07 31/07/07	7	monthly	55	55
31/07/07 06/03/07	7	monthly	112	105
09/05/08 06/11/08	7	weekly	105	95
17/04/09 22/05/09	2	weekly	104	119
29/05/09 17/05/09	8	weekly	200	207

Final observations

On September 28th the experiment was stopped. The aerial part of vegetation was harvested and the content of the tanks was carefully separated into roots, rhizomes and gravel. This work was very laborious because it was necessary to wash small quantities of gravel each time, using a particular method based on a circular movement of water in a suitable container in order to separate the gravel onto the base of the container and make the roots float so they could be manually collected (Figure 32).

The above and below ground biomass of the vegetation was analyzed for nitrogen using the total Kjeldahl method (Kjeldahl, 1883) and AOAC official method (2002).

Approximately 500 g of gravel and 500 g of belowground biomass were sampled from each tank in order to do microbiological analyses and to investigate conductivity (EC), pH, water soluble carbon (WSC) and ammonium and nitrate contained in biofilm that developed on the gravel and root systems. This (nitrate or ammonium gravel and roots biofilm) can provide information on degradation of organic compounds in the presence of available nitrogenous nutrients with and without root activity.

The following protocol was used:

-gravel: an extraction was prepared using 100 g of gravel and 100 ml of distilled water and this sample was left for three hours at room temperature in a mechanical shaker then filtered through filter paper;

-roots: the process was the same but the initial quantity was 20 g of roots and 100 ml of distilled water because the roots were too voluminous.

The obtained extraction was used to measure: 1) pH, using a Titroproprocessor 672 of Methron (Switzerland), 2) electrical conductivity (EC), using Conmet 2 Hanna Instruments Italia, 3) NO_3-N and NH_4-N, using a selective electrode (Sevenmulti Mettler Toledo) and 4) water soluble carbon (WSC) was determined by acid digestion with $K_2Cr_2O_7$ and H_2SO_4 at 140 °C for 2 h. A spectrophotometric method was used to quantify the Cr^{3+} produced by the reduction of Cr^{6+} (λ 590 nm) (Yeomans et al., 1988).

Figure 32 – Steps of belowground organs separation: opening of the tanks, first separation of roots and gravel, washing operations and final samples.

Water chemical analysis

The water analyses were conducted with a portable spectrophotometer (DR2800 by Hach Lange) immediately after the water sampling; ammonium was determined colorimetrically based on the indophenol blue method, nitrate was determined colorimetrically based on the 2,6.dimethylphenol method and nitrite was determined colorimetrically based on diazotization method (LCK standard test kits, Hach Lange). This last analysis was stopped because the concentrations detected during the initial phases were negligible and preformed only in the intensive monitoring periods of July 2008 and February 2009. The ISO 7150-1 method was used for ammonium analysis and the ISO 7890-1-2-1986 method for nitrate analysis (Hach, 1989).

Data elaboration

For each cycle the removal efficiency (R.E.) was calculated as follows:

R.E. = $[(CiVi - CeVe)/CiVi] \times 100$

where Ci and Vi are respectively the concentration and volume (60 L/m^2) of applied solution at the beginning, Ce and Ve are the concentration and volume detected in the residual water at the end.

Statistical analyses for concentration data were performed using Box and Whiskers and the non parametric test of Kruskal-Wallis because of the non normality of the dataset also after transformations.

Removal efficiencies data, thanks to the normal distribution, were instead analyzed with ANOVA and significantly different means were differentiated with the Student-Newman Keuls test.

Microbiological analysis

These analyses were conducted in collaboration with CNR Institute of Ecosystem Studies (ISE) of Pisa to investigate the aerobic microbial populations and *Pseudomonas* genus of gravel and roots biofilm.

Colony forming units (CFU) were determined based on the surface- plate counting procedure (Jayasekara et al., 1998).

This technique is a well established method and does not require complex instrumentations and, by using different media, is applicable to a wide range of bacteria types.

The execration of samples for microbiological analyses was carried out placing one sample of roots and one gravel for each tank in sterile tubes with 10 ml of water (1:10 w/v for roots and 1:2 w/v for gravel) and vortexed for 2 minutes at room temperature.

Serial dilutions at the levels reported in Table 12 were made for the extracts using distilled water. A volume of 0.2 ml was spread onto plates prepared with adequate growing medium (agar for PCA and *Pseudomonas* cetrimide agar for *Pseudomonas*) and producing two plates for lower dilutions and one for the higher that was used to check the reliability of the former. In total 240 plates coming form vegetated tanks and 12 coming from unvegetated control were prepared and analysed.

Table 12 – Dilutions applied to roots and gravel extracts for PCA and *Pseudomonas* counting

	roots	**gravel**
PCA	10^{-7} - 10^{-8}	10^{-4} - 10^{-5}
Pseudomonas	10^{-4} - 10^{-5}	10^{-3} - 10^{-4}

Plates were incubated at 29 °C for 24-48 (PCA) and 72 hours (*Pseudomonas*) and then CFU were counted.

The formula used to count the CFU (Picci and Nannipieri, 2002) is the following:

$CFU = (\sum C)/(\sum n * z)$

C = number of colonies counted in different plates

z = dilution factor (10^{-x})

n = number of plates for each dilution

Results

First year

As just stated, the first year of experimentation was conducted inside an unheated glass house. The period considered was from January 2007 to March 2008.

Environmental conditions

Air and water temperature were recorded from March 2007. Air temperature rose from the start of monitoring till July 2007, then decreased till the end of the year and then began to slowly rise again during the first months of 2008 (Figure 33). There were wide daily fluctuations in both minimum and maximum values. Maximum values were above 35 °C from the end of May 2007 till the end of August 2007, with values over 40 °C in the period from July 15th to 23rd. Minimum values fell below 0 °C in the second half of December 2007 and first half of February 2008, when the temperature reached the lowest value of -3.1 °C. In spring and summer there was a daily average range of 17.1 °C, in autumn and winter this decreased to 10.7 °C. Water temperature followed the same time pattern, with lower maximum (absolute max 36.6 °C) and higher minimum (absolute minimum 0.1 °C).

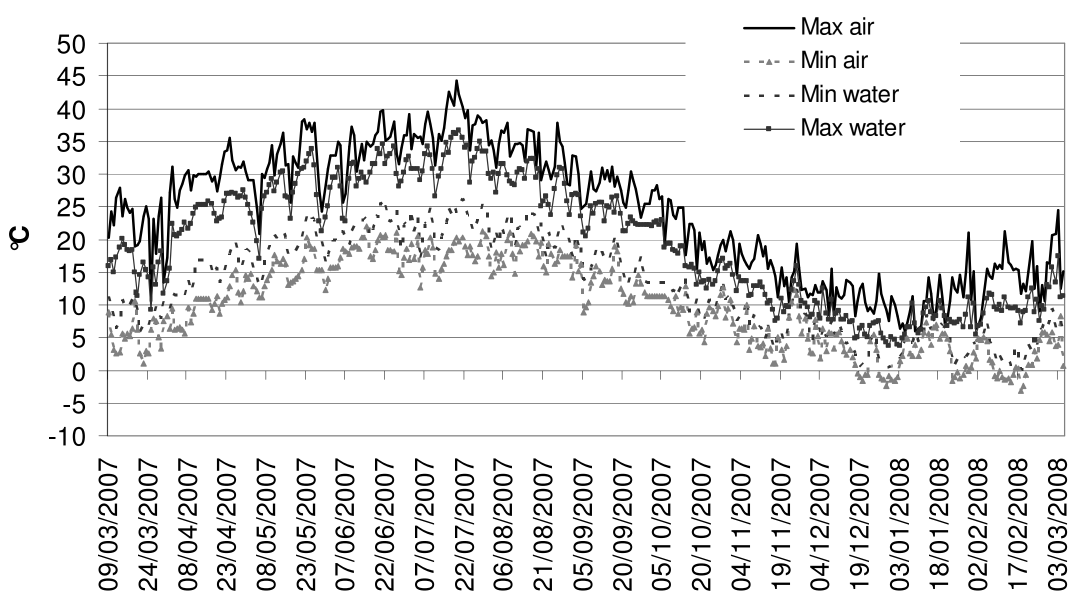


Figure 33 - Air and water temperature from March 2007 to March 2008

The water temperature ranges were also narrower than the air ones, being 8.2 °C in spring and summer and 4.7 °C in autumn and winter (Figure 33). Taking the whole period, the average maximum air temperature was 24.1 °C and minimum was 10.0 °C; the values in water were 19.6 °C and 13.0 °C, respectively.

Plant growth

Ty was the fastest growing species and reached a maximum height of 126 cm on August 21st 2007. *Cae* and *Phr* grew with similar dynamics, and *Cae* reached a maximum height of 92 cm in mid-July and *Phr* 100 cm at the end of July (Figure 34).

Pha grew more slowly but continuously, reaching a maximum height of 79 cm in mid-October. *Jue* reached the maximum height of 68 cm in early April then maintained an average value of 55 cm. *Jue* was the first to restart growth after the winter quiescence, while *Ty* was the last.

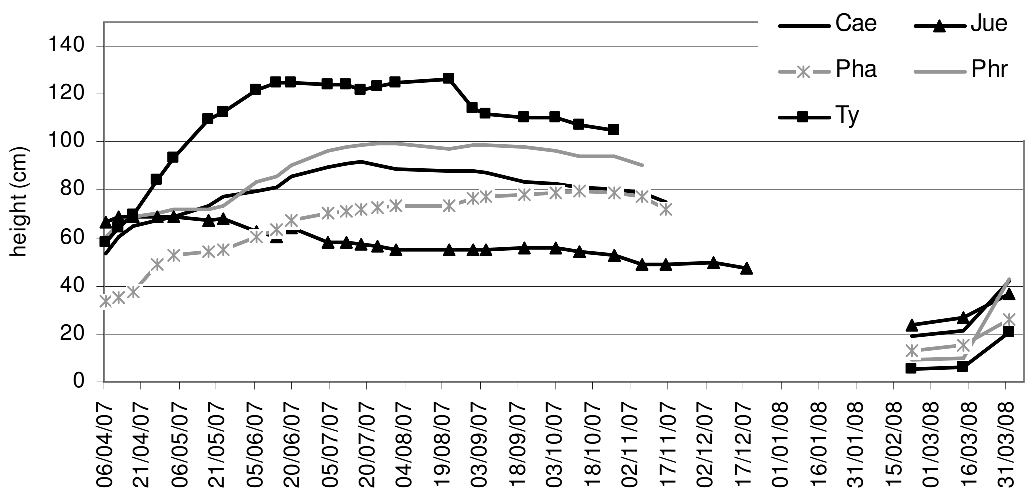


Figure 34 - Height of plants during the first year of experimentation

Phr and *Ty* emitted stems regularly from the end of winter 2007 till the end of August, reaching a stems density of 212/m^2 and 116/m^2, respectively. *Jue* instead emitted more stems with high intensity, reaching the density of 855/m^2 followed by a reduction to about 700 stems/m^2 in mid-June (Figure 35). After this the plants again started to emit new stems till the end of September, reaching a final density of about 850/m^2.

The aerial parts of all plants dried out during autumn and re-growth started at the beginning of February.

Stems density and growth of *Ty* and *Phr* showed a similar time pattern to that observed in the open field during the first growing season (Borin et al., 2001).

Considering plant growth and stems density *Jue* showed the longest vegetative season.

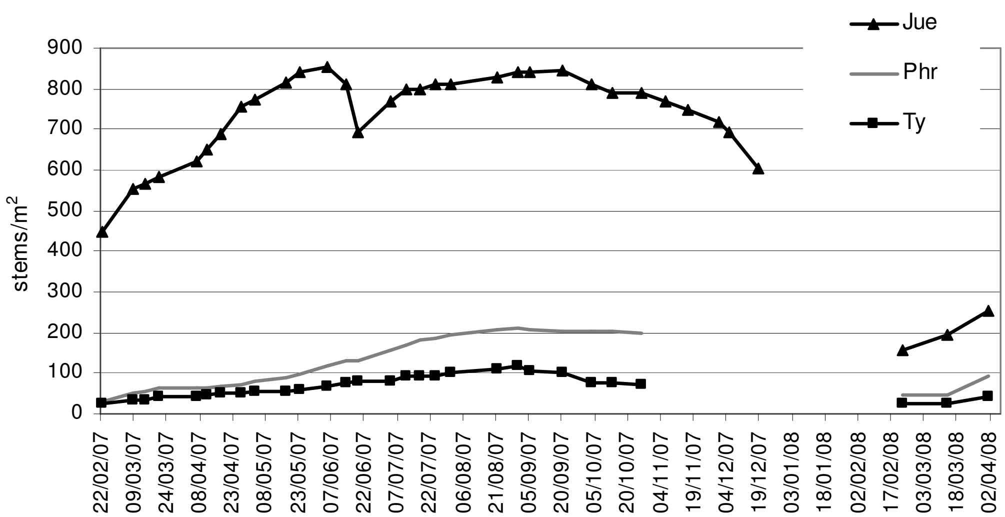


Figure 35 - Stems density during the first year of experimentation

Evapotranspiration

The time pattern of the cumulative ET allows three sub-phases to be distinguished: initial, in late winter-early spring, characterised by low temperatures and reduced plant growth; central, during late spring-summer, with high temperatures and intensive plant growth; late (autumn-winter) with low temperatures and quiescent plants (Figure 36). In the first phase, average daily ET was 1.61 mm in the vegetated tanks (without relevant differences among species) and 0.88 mm in the control. In the second, plants consumed 4 mm per day on average, more than twice that of the control (1.79 mm/day). *Ty*, with a daily ET of 5.6 mm consumed much more than the other species (3.3-3.9 mm). In the autumn-winter phase, daily ET was quite low, but again vegetated tanks, without relevant differences among species, consumed twice that of the control (0.75 and 0.37 mm/day respectively).

Over the whole period, the water consumption of *Ty* was the highest among the studied macrophytes, reaching a cumulative value of over 1000 mm. *Pha*, *Phr* and *Jue* had similar ET, with a final value around 800 mm. *Cae* consumed a little less water (730 mm) and the control lost only 400 mm.

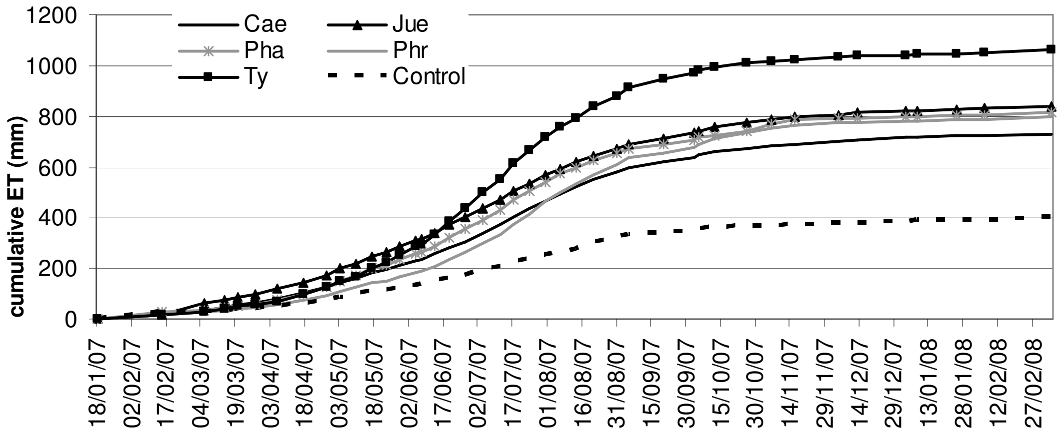


Figure 36 - Cumulative ET of treatments during the first year of experimentation

Nitrogen concentration

Considering all the values collected over the entire period, NH_4-N in water discharged from the tanks almost completely disappeared in all treatments, in particular during the first period with 50 ppm load (Figure 37), in agreement with the findings of Moreno et al. (2002). These data confirm the findings of Hill (1997) on the tolerance of all the species to the applied ammonium concentrations. The disappearance of NH_4-N suggests possible nitrification (Bastviken et al., 2003), which is confirmed by the median concentration values of NO_3-N that ranged between 60 and 95 ppm during the first period when the median applied concentration was 55 ppm, and between 136 and 204 ppm during the second when the median applied concentration was 114 ppm (Figure 38). Among treatments, *Ty* and *Phr* had the lowest median value in the first period (83 ppm) and *Ty* and *Pha* had the lowest in the second period (136 and 170 ppm respectively). The only significant difference detected was in the concentration of NO_3-N during the high load period between *Jue* (with the highest median value) and *Ty* (with the lowest median value).

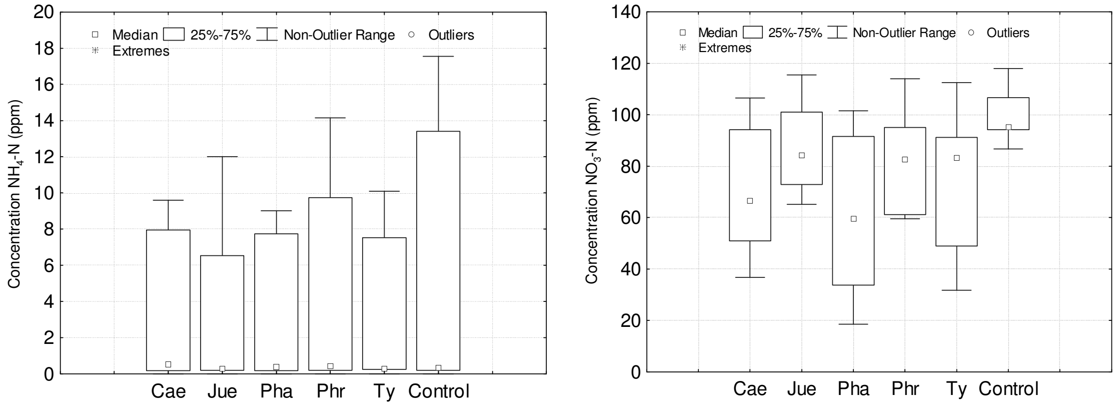


Figure 37 - Box and whiskers of NH_4-N and NO_3-N concentrations in the discharged water in the period of low input concentration (50 ppm)

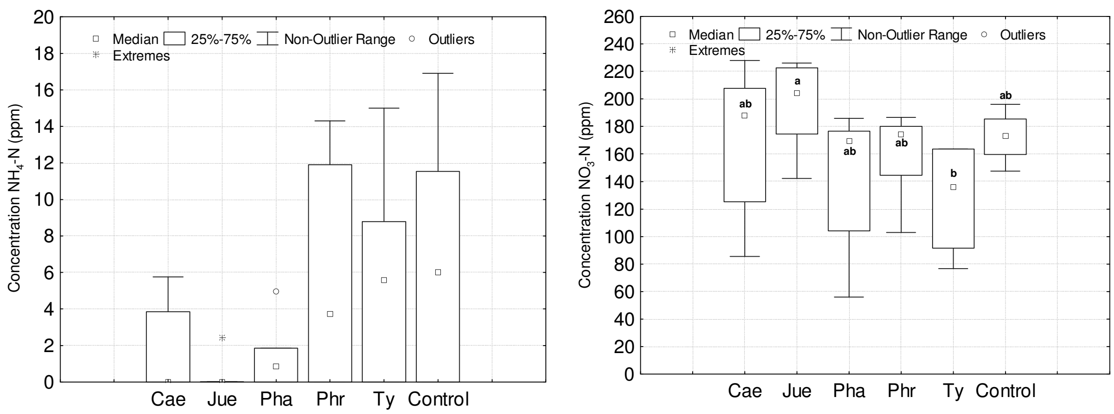


Figure 38 - Box and whiskers of NH_4-N and NO_3-N concentrations in the discharged water in the period of high input concentration (100 ppm). Different letters indicate significant differences at P = 0.05 by Kruskal-Wallis test

Nitrogen mass removal

Concentration data are not enough to obtain complete information on nitrogen fate because of the varying amounts of water discharged from the tanks due to different ET.

Indeed taking the whole monitored period, the removal efficiency calculated on the mass balance for TOT N was 43% for vegetated tanks and 24% for the control (Table 13). Abatement was higher during the period with lower nitrogen load (18/01/07-31/07/07), with 54% for planted treatments and 23% in the control.

Table 13 - Removal efficiency of N-NO_3, N-NH_4 and TOT N during the first year of experimentation. Different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test

		First period (low load)						
	removed (%)	*18/01/07 15/02/07*	*15/02/07 05/03/07*	*05/03/07 06/04/07*	*06/04/07 03/05/07*	*03/05/07 07/06/07*	*07/06/07 04/07/07*	*04/07/07 31/07/07*
Cae	NH4-N	87% a	88% ab	99% a	99% a	100% a	100% a	100% a
	NO3-N	-17% b	-30% a	-49% a	18% b	14% ab	56% b	71% a
	TOT N	**32% b**	**28% a**	**23% a**	**59% b**	**58% ab**	**79% b**	**86% a**
Jue	NH4-N	91% a	92% a	100% a	99% a	100% a	100% a	100% a
	NO3-N	41% a	-24% a	-40% a	16% b	-1% ab	43% b	30% b
	TOT N	**65% a**	**33% a**	**28% a**	**58% b**	**50% ab**	**74% b**	**66% b**
Pha	NH4-N	89% a	88% ab	99% a	99% a	100% a	100% a	100% a
	NO3-N	2% b	-32% a	-38% a	34% a	30% a	75% ab	86% a
	TOT N	**44% b**	**27% a**	**28% a**	**67% a**	**65% a**	**88% ab**	**93% a**
Phr	NH4-N	80% b	84% ab	99% a	99% a	100% a	100% a	100% a
	NO3-N	-5% b	-49% a	-70% ab	4% b	-10% ab	60% b	80% a
	TOT N	**35% b**	**16%** a	**12%** ab	**52%** b	**46%** ab	**81%** b	**90%** a
Ty	NH4-N	90% a	84% ab	100% a	99% a	100% a	100% a	100% a
	NO3-N	-14% b	-40% a	-53% a	12% b	26% a	98% a	93% a
	TOT N	**35% b**	**21% a**	**21% a**	**55% b**	**63% a**	**99% a**	**97% a**
Control	NH4-N	79% b	71% b	99% a	99% a	100% a	100% a	100% a
	NO3-N	-24% b	-51% a	-85% b	-44% c	-68% b	-26% c	-24% c
	TOT N	**25% b**	**9% a**	**4% b**	**27% c**	**17% b**	**41% c**	**38% c**

		Second period (high load)						
	removed (%)	*31/07/07 31/08/07*	*31/08/07 05/10/07*	*05/10/07 06/11/07*	*06/11/07 05/12/07*	*05/12/07 08/01/08*	*08/01/07 06/02/08*	*06/02/07 06/03/08*
Cae	NH4-N	100% a	100% a	100% a	100% a	100% a	96% a	96% a
	NO3-N	63% a	2% ab	-32% a	-50% a	-70% b	-69% c	-58% a
	TOT N	**80% a**	**46% ab**	**31% a**	**25% a**	**14% a**	**9% c**	**20% a**
Jue	NH4-N	100% a	100% a	100% a	98% abc	100% a	100% a	100% a
	NO3-N	34% b	-41% bc	-30% a	-62% a	-58% ab	-73% c	-78% a
	TOT N	**65% b**	**21% bc**	**32% a**	**19% a**	**20% a**	**9% c**	**13% a**
Pha	NH4-N	100% a	100% a	100% a	99% ab	99% ab	95% b	99% a
	NO3-N	74% a	20% a	1% a	-30% a	-41% ab	-46% b	-43% a
	TOT N	**86% a**	**55% a**	**49% a**	**35% a**	**29% a**	**21% b**	**29% a**
Phr	NH4-N	100% a	100% a	100% a	97% abc	95% abc	86% b	91% a
	NO3-N	72% a	-7% ab	0% a	-22% a	-46% ab	-48% b	-42% a
	TOT N	**85%** a	**40%** ab	**48%** a	**38%** a	**24%** a	**16%** bc	**26%** a
Ty	NH4-N	100% a	100% a	100% a	96% bc	94% bc	87% b	93% a
	NO3-N	84% a	23% a	34% a	-8% a	-26% a	-21% a	-29% a
	TOT N	**91% a**	**57% a**	**66% a**	**44% a**	**33% a**	**30% a**	**34% a**
Control	NH4-N	100% a	100% a	100% a	95% c	90% c	84% b	95% a
	NO3-N	2% c	-60% c	-64% a	-41% a	-41% ab	-39% b	-43% a
	TOT N	**48% c**	**11% c**	**15% a**	**27% a**	**24% a**	**19% bc**	**27% a**

	removed (%)	*tot 50 ppm*	*tot 100 ppm*	*tot*
Cae	NH4-N	96% ab	99% a	98% b
	NO3-N	8% a	-31% b	-20% abc
	TOT N	**52% ab**	**32% ab**	**39% ab**
Jue	NH4-N	97% a	100% a	99% a
	NO3-N	9% a	-44% b	-29% bc
	TOT N	**54% ab**	**26% b**	**35% b**
Pha	NH4-N	97% ab	99% a	98% b
	NO3-N	21% a	-9% ab	1% ab
	TOT N	**59% a**	**43% ab**	**48% ab**
Phr	NH4-N	95% b	96% b	95% c
	NO3-N	1% a	-13% ab	-1% ab
	TOT N	48% b	39% ab	42% ab
Ty	NH4-N	96% ab	96% b	96% c
	NO3-N	17% a	8% a	12% a
	TOT N	**56% ab**	**50% a**	**52% a**
Control	NH4-N	93% c	95% b	94% d
	NO3-N	-46% b	-41% b	-47% c
	TOT N	**23% c**	**25% b**	**24% c**

During the second period (31/07/07-06/03/08) with doubled nitrogen load, the abatement was 38% for plants and 25% in the control. The lower average removal of the vegetated treatments could be connected not only to the doubled load of nitrogen but also to the period of winter quiescence in accordance with the seasonal nutrient removal found by Picard et al. (2005).

Taking into account the removal calculated for TOT N, it is possible to distinguish some general tendencies every month (Table 13):

- the differences among treatments were more evident from March to September; in this period there was often a significant difference between planted treatments on the one side and unplanted control on the other;
- different performances among species started in April, when *Pha* gave the best removal and the other species had similar behaviour;
- there was an increasing differentiation among species from May to September, *Ty* and *Pha* being the best performing with abatements of around 90% in June-August; *Jue* was the worst (74-65% of removal from June to August) and the other species were intermediate.
- in the first cycle of doubled N load (August), the vegetated treatments showed a similar performance to the previous load cycle; removal dramatically reduced in the second cycle (September), probably also due to decreased vegetative activity;
- during the autumn-winter period, although differences among treatments were only statistically significant once, *Ty* appeared to maintain the best performance, followed by *Pha*.

In almost all the load cycles and treatments, NH_4-N loads were almost completely removed at the end of the month. The disappearance was 99-100% from March to October.

The removal of NH_4-N was accompanied by generation and accumulation of NO_3-N in early spring 2007 and autumn-winter 2007-08, as suggested by the negative values in Table 13. Nevertheless, during late spring and summer the vegetated tanks were also able to abate NO_3-N, *Ty* and *Pha* being the best performing (63% and 60% average removal from April to August respectively), followed by *Cae* and *Phr* (45% and 41%) and lastly *Jue* with 25%.

In the same period the unplanted control had an average negative removal of 32%, highlighting the importance of plants in nitrogen abatement.

By the end of the experimental period, *Ty* treatment had removed more than 72 g/m^2 (52% of the cumulative load that was 137.7 g/m^2) of total N, followed by *Pha* (48%), *Phr* (42%), *Cae* (39%), *Jue* (35%) and the control (24%) (Figure 39). There were slight differences among vegetated treatments in the first period of nitrogen load, while there was a progressive difference between plants and the control. After this, a clearer differentiation among vegetated treatments took place. During winter, the control performed similarly to *Jue* and *Cae*.

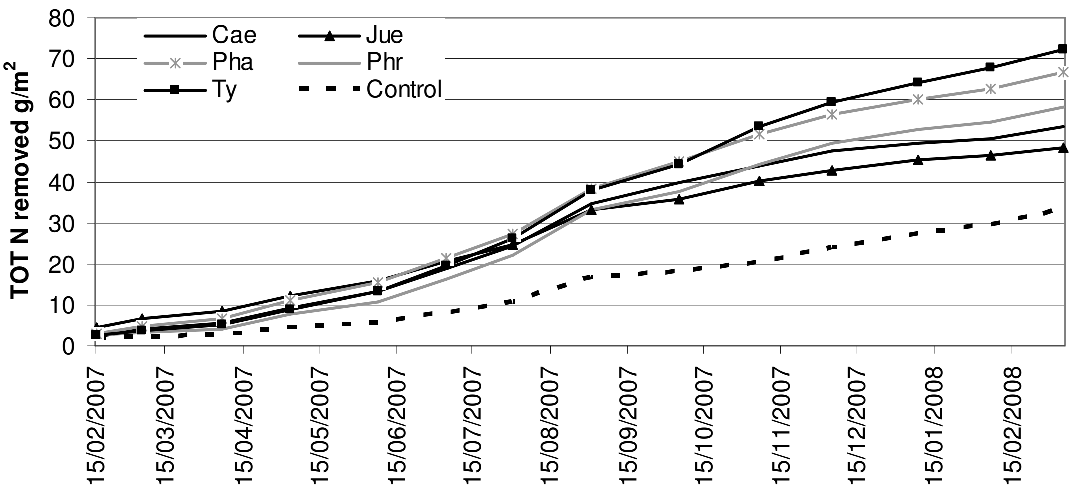


Figure 39 - Cumulative total N removed in the treatments during the first year of experimentation

Removal efficiency and temperature

R.E. of NO_3-N and TOT N were significantly correlated with monthly average maximum and minimum air temperatures for every monthly cycle among all vegetated treatments, with correlation coefficients ranging from 0.80 to 0.97 for air and from 0.83 to 0.98 for water (Table 14). Considering R.E. of NH_4-N the correlation with temperature was not significant for *Cae* and *Jue* while for other vegetated treatments the correlation coefficient ranges from 0.58 to 0.79 for air and from 0.59 to 0.77 for water. Taking into account all the vegetated treatments together the correlation coefficients, all significant, range from 0.72 to 0.95.

Table 14 – Correlation coefficients among R.E. of all the forms of nitrogen and maximum, minimum and average air and water temperature in all the treatments during the first year of experimentation. In bold significant coefficients

Treatments	R.E	max air	min air	average air	max water	min water	average water
Cae	NH_4-N	0.49	0.57	0.53	0.50	0.55	0.52
	NO_3-N	**0.96**	**0.97**	**0.97**	**0.97**	**0.98**	**0.98**
	TOTN	**0.95**	**0.96**	**0.97**	**0.97**	**0.97**	**0.97**
Jue	NH_4-N	0.17	0.17	0.17	0.18	0.16	0.17
	NO_3-N	**0.92**	**0.92**	**0.93**	**0.93**	**0.93**	**0.93**
	TOTN	**0.90**	**0.89**	**0.91**	**0.90**	**0.91**	**0.91**
Pha	NH_4-N	**0.64**	**0.58**	**0.62**	**0.61**	**0.59**	**0.60**
	NO_3-N	**0.94**	**0.97**	**0.96**	**0.96**	**0.97**	**0.96**
	TOTN	**0.93**	**0.96**	**0.96**	**0.95**	**0.96**	**0.96**
Phr	NH_4-N	**0.74**	**0.71**	**0.74**	**0.72**	**0.73**	**0.72**
	NO_3-N	**0.80**	**0.88**	**0.85**	**0.83**	**0.86**	**0.85**
	TOTN	**0.82**	**0.88**	**0.86**	**0.84**	**0.87**	**0.86**
Ty	NH_4-N	**0.79**	**0.73**	**0.78**	**0.77**	**0.75**	**0.76**
	NO_3-N	**0.80**	**0.91**	**0.85**	**0.84**	**0.88**	**0.86**
	TOTN	**0.82**	**0.91**	**0.87**	**0.85**	**0.89**	**0.87**
Control	NH_4-N	**0.78**	**0.69**	**0.76**	**0.75**	**0.72**	**0.74**
	NO_3-N	0.26	0.38	0.31	0.31	0.35	0.32
	TOTN	0.42	0.50	0.46	0.45	0.48	0.47
Vegetated Treatments	NH_4-N	**0.75**	**0.72**	**0.74**	**0.73**	**0.73**	**0.73**
	NO_3-N	**0.91**	**0.95**	**0.94**	**0.93**	**0.95**	**0.94**
	TOTN	**0.91**	**0.94**	**0.93**	**0.93**	**0.94**	**0.94**

According to Picard et al. (2005) and Akratos and Tsihrintzis (2007), nitrogen removal processes are positively influenced by increasing temperature. This parameter can hence be an indicator of the expected abatement performance, as exemplified by the regression in Figure 40, where R.E of TOT N increases by 17% every 5 °C in the range of 5-30 °C.

A significant correlation between removal efficiency and temperature was not evident in the control for NO_3-N and TOT N but there was a significant correlation between R.E of NH_4-N and temperature (Table 14).

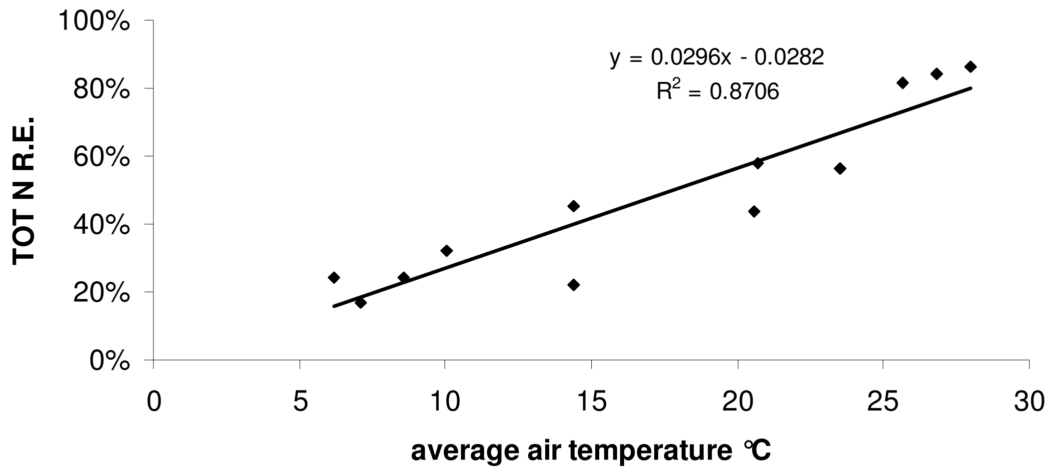


Figure 40 - Regression between average air temperature and TOTN R.E. of vegetated treatments during the first year of experimentation

Removal efficiency, O_2 concentration and pH

NH_4-N R.E. was positively and significantly correlated with monthly average O_2 concentrations recorded at least once a month for *Cae*, *Phr*, *Ty* and considering the vegetated treatments together, but with correlation coefficient always lower than 0.69. This kind of correlation was not detected in the control (Table 15).

The significant correlation between NH_4-N R.E. and O_2 concentrations is probably due to the fact that macrophytes transfer oxygen from the air to the rhizosphere and then to the medium, increasing aerobic conditions and so nitrification as found by many authors (Zhu and Sikora, 1995; Bodelier et al., 1996; Brix, 1997 and Vymazal, 2007).

The median O_2 values detected for the vegetated treatments in this first year ranged from 4.38 mg/L in *Cae* to 2.45 mg/L in *Ty* against 2.15 of the control.

On the other hand, NH_4-N R.E. was positively and significantly correlated with pH monthly average values in all treatments apart from *Pha* (Table 15). Giving that NH_4-N was abated almost completely in all treatments the regression between NH_4-N R.E. and pH values for vegetated treatments is limited in R.E values from 93% to 100% and points out that for every 0.1 variation in pH there is a variation of 1% in the R.E (Figure 41).

The correlation between pH and NH_4-N R.E can be also connected with the process of nitrification. It is known that optimum pH values for nitrification may vary from 6.6 to 8.0, (Paul and Clark, 1996) however, acclimatized systems can be operated to nitrify at a much lower pH value (Cooper et al., 1996)

Table 15 - Correlation coefficients among R.E. of all the forms of nitrogen and O2 mg/L and pH in measured in the water inside the tanks in all the treatments during the first year of experimentation. In bold significant coefficients

Treatments	R.E	O_2 mg/L	pH
Cae	NO_3-N	**0.58**	0.71
	NH_4-N	**0.59**	**0.90**
	TOT N	0.56	0.71
Jue	NO_3-N	**0.59**	**0.80**
	NH_4-N	0.02	-0.28
	TOT N	0.57	**0.79**
Pha	NO_3-N	0.40	0.73
	NH_4-N	0.53	0.72
	TOT N	0.39	0.72
Phr	NO_3-N	0.31	0.39
	NH_4-N	**0.69**	**0.82**
	TOT N	0.32	0.43
Ty	NO_3-N	0.15	0.22
	NH_4-N	**0.61**	**0.79**
	TOT N	0.17	0.27
Control	NO_3-N	**0.69**	**0.82**
	NH_4-N	-0.28	-0.05
	TOT N	-0.17	0.22
Vegetated treatments	NO_3-N	0.43	0.62
	NH_4-N	**0.72**	**0.86**
	TOT N	0.42	0.63

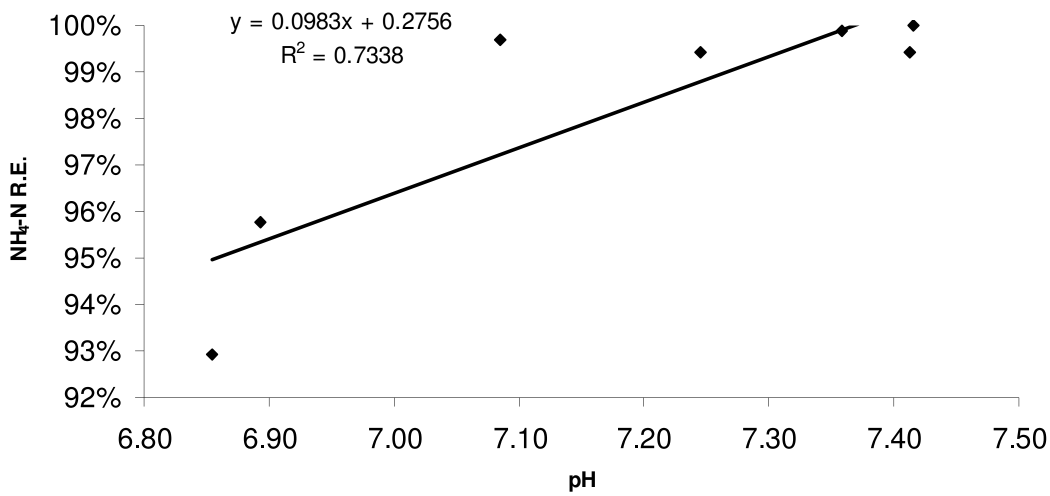


Figure 41 - Regression between NH_4-N R.E. and average pH of vegetated treatments during the first year of experimentation

Weekly analysis

The weekly analysis conducted in July 2007 showed that NH_4-N disappeared almost completely in all the treatments after two weeks, but was strongly reduced after just one week (Figure 42). In the same period, there was also a reduction of NO_3-N in all the vegetated treatments. This was not detected in the control, where the amount increased due to transformation of NH_4-N not compensated by uptake or denitrification. In the following weeks, different behaviour became evident among treatments: minimal presence of NO_3-N was detected in *Ty*; there was a progressive decline in *Pha*, *Phr* and *Cae*; it remained constant in *Jue* and the control.

Similarly to July, in December, NH_4-N almost completely disappeared one week after the application, while the amount of NO_3-N increased in all treatments. *Ty* again showed the best performance, although without any significant difference. Total N was reduced in all treatments but without any significant difference (Table 16).

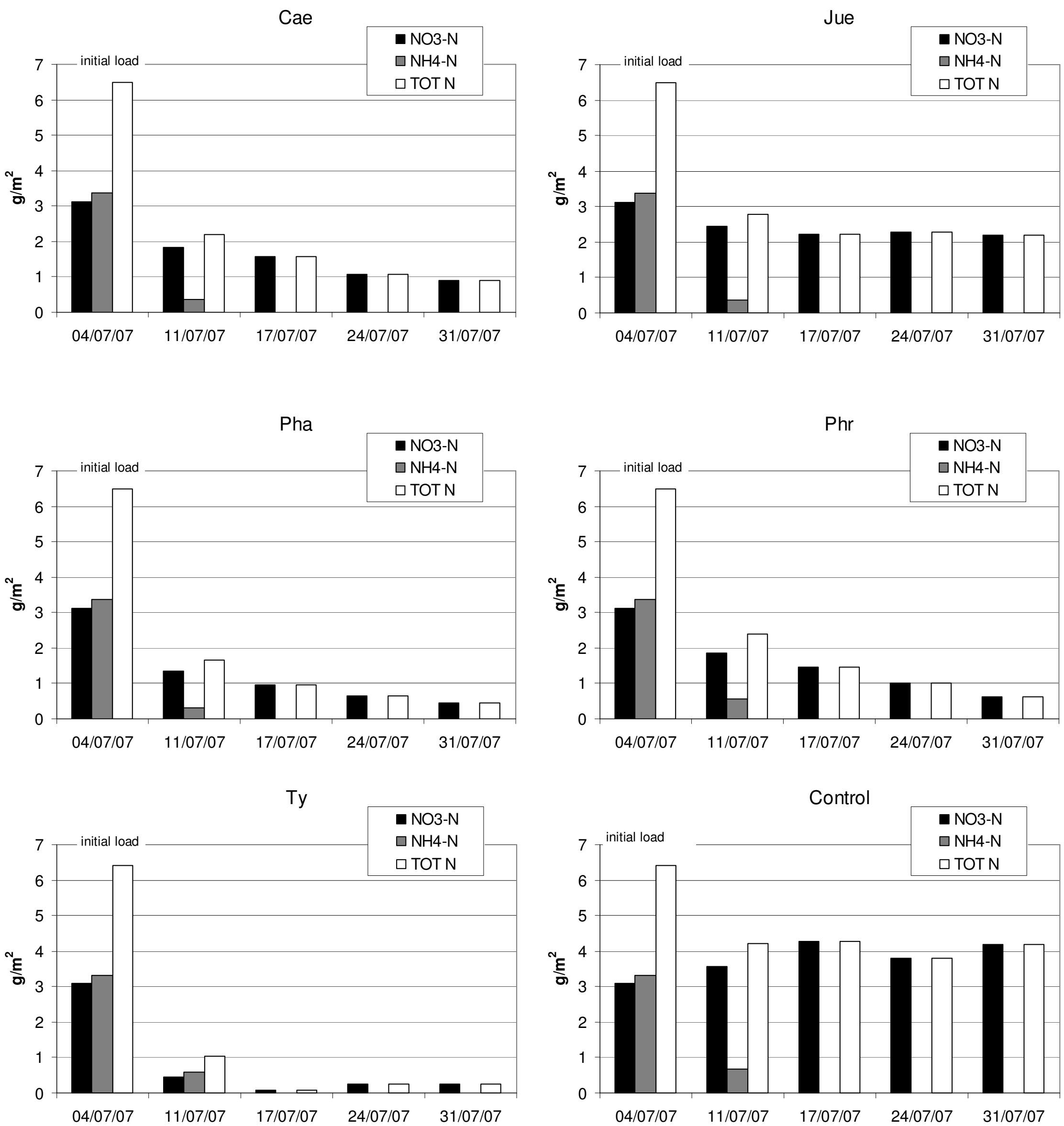


Figure 42 - Presence of NO_3-N NH_4-N and TOT N detected weekly after the application on 04/07/07.

Table 16 - R.E of the different forms of nitrogen one week after the application on 05/12/07. Different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test

	variation%					
	NO_3-N		NH_4-N		TOT N	
Cae	-33%	a	98%	a	32%	a
Jue	-37%	a	99%	a	31%	a
Pha	-19%	a	98%	a	39%	a
Phr	-12%	a	96%	b	42%	a
Ty	-6%	a	96%	b	45%	a
Control	-23%	a	96%	b	36%	a

Aerial biomass production and nitrogen uptake

Dry matter production of the aerial parts harvested on January 10th 2008 was similar among species, but the % of nitrogen detected was significantly different (Table 17). *Jue* presented the highest % and consequently highest amount of nitrogen in the aerial parts and *Ty* the lowest. In general the values of biomass production and nitrogen concentrations were low compared to those reported in the literature (Kadlec and Knight, 1996; Tanner, 1996; Greenway, 1997; Barbera et al., 2009), probably because the mesocosms were supplied only with NO_3-N and NH_4-N and the lack of other elements was a limiting factor, and because the experiment was conducted in the first growing season, as in Morari and Giardini (2009).

The contribution of harvestable nitrogen to the removal was scarce in all treatments (3-12%) but this is not surprising because similar data are reported regarding the first year of vegetation planting (Lin et al., 2002; Chung et al., 2008; Kantawanichkul et al., 2009).

The ratio between disappeared total nitrogen and that stored in the aerial parts was highest in *Jue* (12%) and lowest in *Ty* (3%). Hence in the treatment with the highest R.E (*Ty*) the contribution of the harvestable part was the lowest; the contrary happened in *Jue*, which was the treatment with the lowest R.E. This behaviour may be related to different accumulation in the belowground tissues, as suggested by Maddison et al. (2009), and/or different microbial activities associated with different species.

Table 17 - Dry matter and nitrogen in the aerial parts of plants; different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test

species	dry matter (aerial part) (g/m^2)	% nitrogen	nitrogen in aerial part (g/m^2)	Stored in aerial part/disappeared
Cae	349 a	1.00 c	3.49 ab	7% b
Jue	358 a	1.58 a	5.63 a	12% a
Pha	307 a	1.32 b	4.05 ab	6% b
Phr	318 a	0.77 d	2.47 ab	4% b
Ty	323 a	0.54 e	1.92 b	3% b

Second year

In March 2008 the mesocosms were doubled and moved outside. This second year considers the period from May to November.

Environmental conditions

Temperature rose from the beginning of the recorded values (beginning of June 2008) till the end of June 2008 reaching the maximum value of 36.4 °C for maximum daily temperature on June 27th afterwards decreased slightly at the beginning of July and rose again reaching another high value in maximum temperature of 35.5 °C at the beginning of August (Figure 43). From August onwards temperature decreased till the end of the monitoring period reaching the minimum value of -4.7 °C on November 24th. In this general trend of decrease there was an important decreasing event on 14th September connected with intense rainfall.

The average value in the considered period was 18.9 °C.

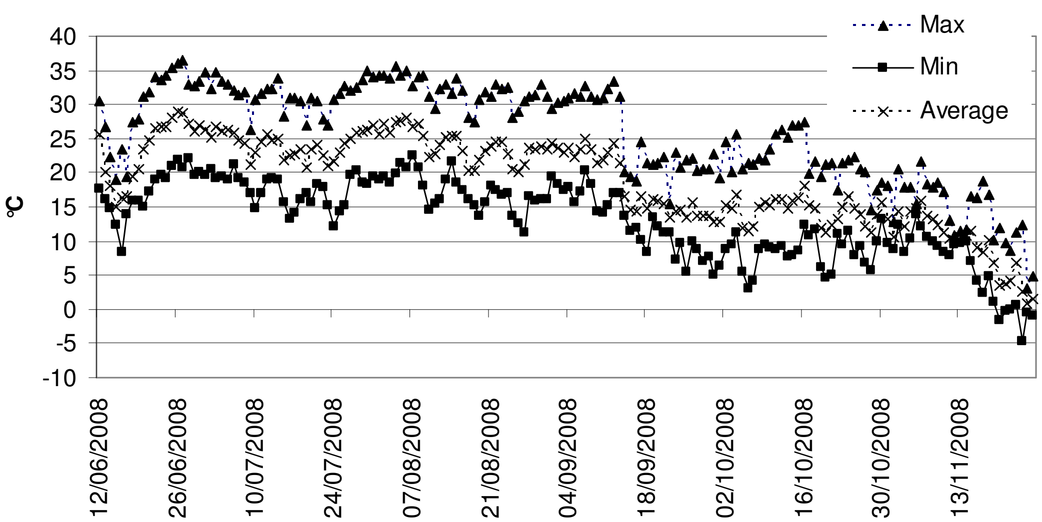


Figure 43 - Maximum, average and minimum daily temperature from June to November 2008

Apart from a prolonged dry period from 17th September to 30th October rainfall was rather uniformly distributed in the monitoring period with a cumulated value of 496 mm. The most important event was on September 14th, with more than 50 mm (Figure 44).

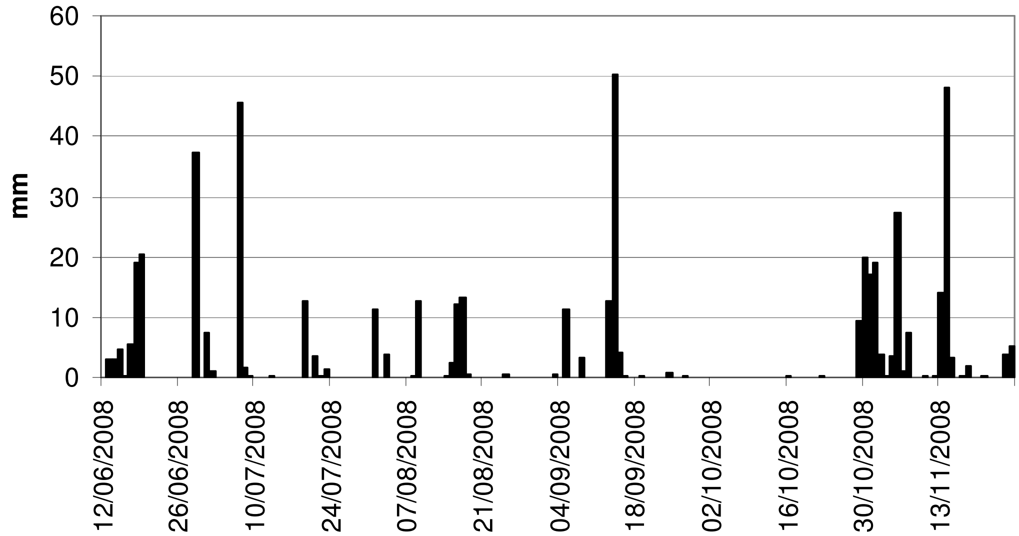


Figure 44 - Rainfall distribution in the monitoring period

Other meteorological values useful for calculation of ET_0 are reported in Table 18.

Table 18 - Monthly average variables for calculation of ET_0

	June*	**July**	**August**	**September**	**October**	**November**
T Max (°C)	29.9	31.5	32.0	25.4	21.7	13.4
T Min (°C)	17.4	17.6	17.6	12.9	8.5	5.0
RH Max (%)	95.2	95.3	95.8	95.5	96.8	97.1
RH Min (%)	43.8	39.7	38.9	42.2	49.9	65.7
Rs Tot (MJ/m^2)	22.4	23.7	20.6	13.0	8.3	4.5
Ws (m/s)	0.6	0.5	0.5	0.5	0.4	0.6

* values available after 15^{th} June

Plant growth

Ty was again the fastest growing species and reached a maximum height of 154 cm on July 14^{th} 2008. *Cae*, *Phr*, *Pha* grew with similar dynamics reaching a maximum value respectively of 124 cm, 132 cm, 76 cm and 101 cm respectively at the end of August. *Jue* reached the maximum value of 101 cm in mid July, then plant height remained about stable till the end of August when started a slight but constant decrease began (Figure 45). In this second year all the species reached taller height respect with the previous one. The aerial part was harvested on 4th December 2008.

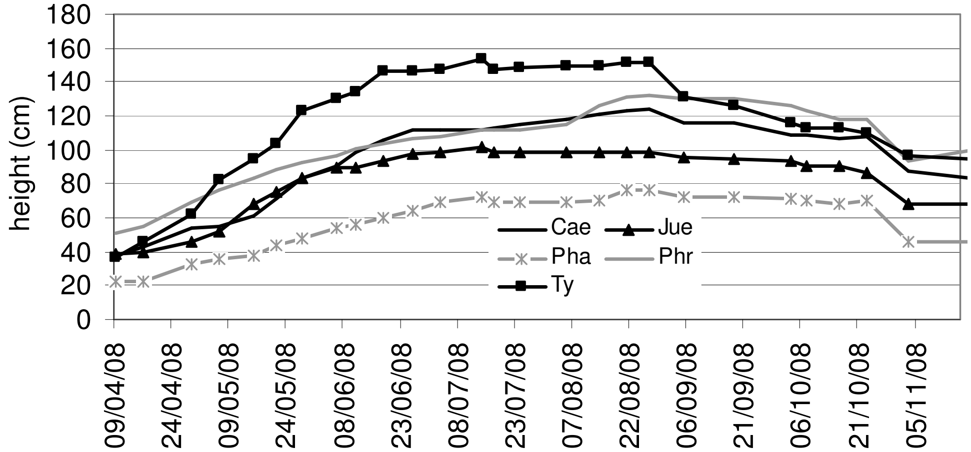


Figure 45 - Height of plants during the second year of experimentation

Phr and *Ty* emitted stems regularly from the beginning of April till the end of October, reaching a stems density of 756/m^2 and 301/m^2, respectively. *Jue* emitted more stems with high intensity, reaching the enormous density of 5823/m^2 at the end of October (Figure 46). The final quantity of stems was absolutely higher with respect to the previous year underlining that the plants were colonizing the tanks well.

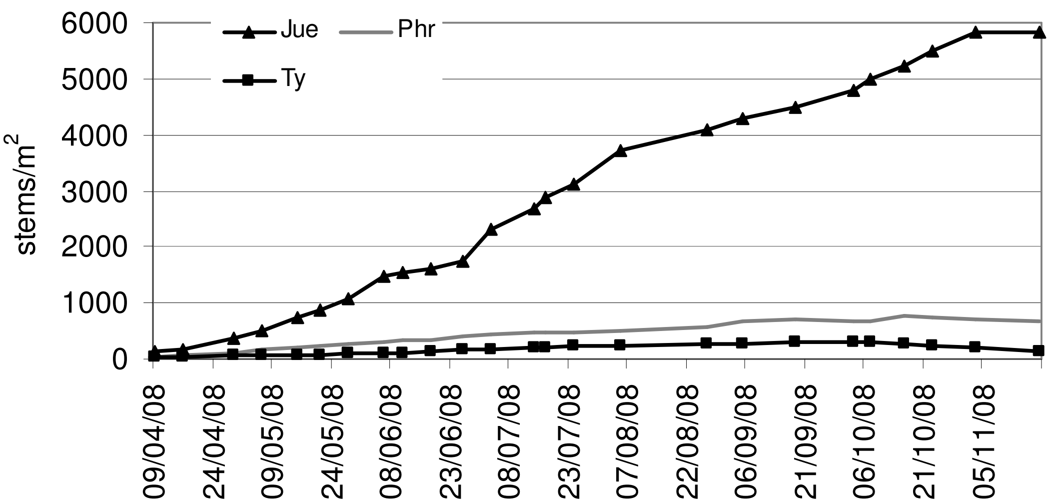


Figure 46 - Stems density during the second year of experimentation

Evapotranspiration

The time pattern of the cumulative ET allows two sub-phases to be distinguished (Figure 47), the first from June to the beginning of September in which there was a progressive differentiation among treatments (average daily ET of vegetated treatments =13.7 mm) and the second from September to the beginning of December in which the differences among treatments were less evident (average daily ET of vegetated treatments = 7.3 mm).

Over the whole period, the water consumption of *Cae* was the highest among the studied macrophytes, reaching a cumulative value of over 2278 mm (daily ET rate of 13.3 mm). *Ty* and *Jue* had similar ET, with a final value around 1800 (daily ET rate of 10 mm). *Phr* consumed 1587 mm (daily Et rate of 9.2 mm) and *Pha* 1405 mm (daily ET rate of 8.2 mm). It is interesting to note that the control lost only 520 mm (daily ET rate of 3 mm) a value very similar to the one calculated for ET_0, 455 mm (daily ET rate of 2.7 mm).

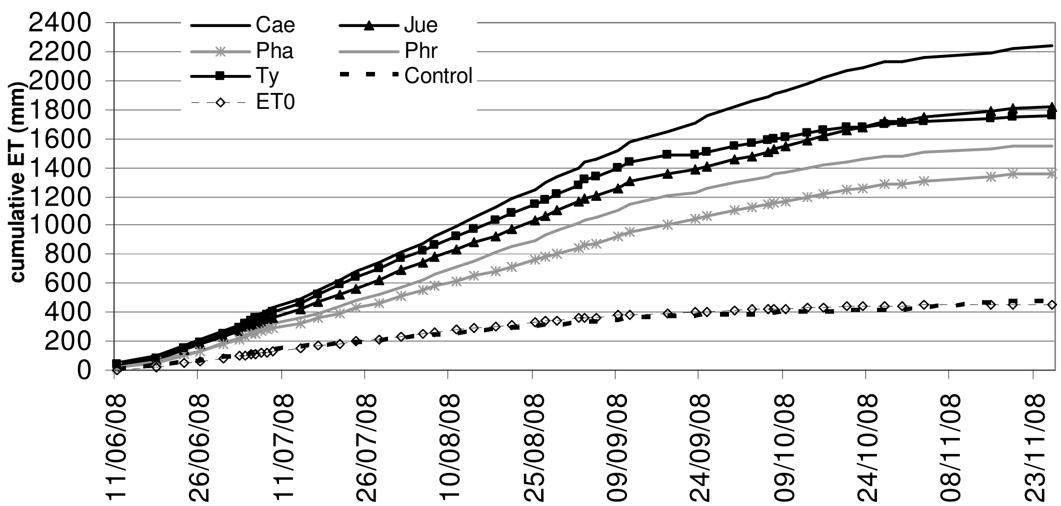


Figure 47 - Cumulative ET of treatments and ET_0 during 2008

Knowing ET for each species and the ET_0 it was possible to calculate the K_c with a similar meaning of K_c as that for agricultural crops (Allen et al., 1998). However, considering the entire period the K_c of all treatments increased month by month, unlike in agricultural crops. The K_c of *Cae* was always the highest with an average of 6.6. *Pha* always had the lowest K_c apart from the last two months with an average value of 4.2 *Jue* showed intermediate behaviour with an average value of 5.5 and *Phr* and *Ty* had similar trends with average values of 4.4 and 4.2 respectively. In November the K_c values were very high but also high for the control because calculated ET_0 resulted as very low.

Table 19 Monthly K_c of all the treatments

	Cae	*Jue*	*Pha*	*Phr*	*Ty*	*Control*
June	3.2	2.8	2.1	2.2	3.0	1.0
July	3.6	3.0	2.2	2.6	3.5	0.9
August	4.6	3.7	2.6	3.4	4.0	0.9
September	6.8	5.0	4.2	4.8	4.6	1.0
October	10.1	8.5	5.9	6.0	5.2	1.0
November	11.0	10.1	7.9	7.4	5.0	5.8
average	6.6	5.5	4.2	4.4	4.2	1.8

Nitrogen concentration

Considering all the values of the second year NH_4-N in water discharged from the tanks again almost completely disappeared in all treatments (Figure 48). These data suggest the tolerance of all the species to the applied ammonium concentrations (median value of 93 ppm) always for a prolonged period. The disappearance of NH_4-N again suggests possible nitrification, but in this case it wasn't very evident in the median concentration values of NO_3-N, which ranged between 2.28 detected in *Ty* to 90.2 in the control. This highlights the role of the plants whose intensive growth might have assimilated more nitrogen in the tissues respect to the previous year. At the same time plant biomass and activity might have supported higher microbial denitrification respect to the control. There were significant differences among treatments especially in NO_3-N concentration and in particular the vegetated treatment with the lowest value was *Ty* and the highest was detected in *Jue.* All the vegetated treatments performed better than the control in abating both NO_3-N and NH_4-N concentration.

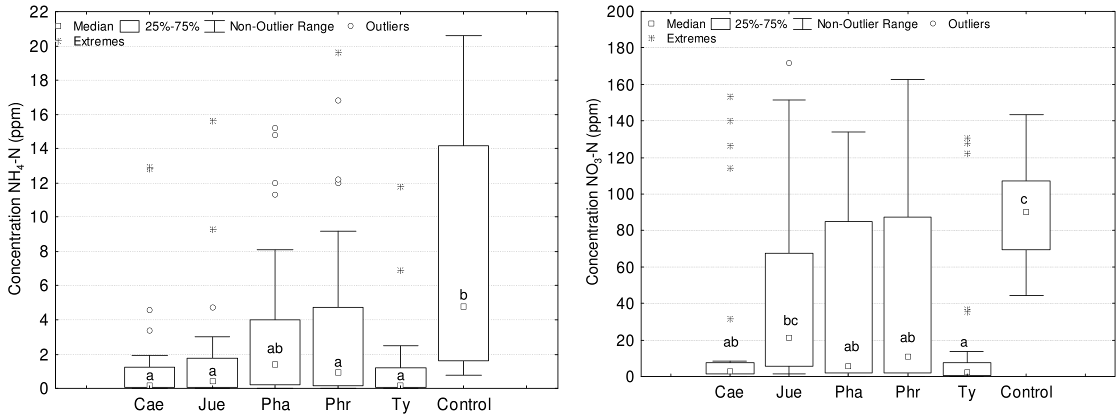


Figure 48 - Box and whiskers of NH4-N and NO3-N concentrations in 2008. Different letters indicate significant differences at P = 0.05 by Kruskal-Wallis test

Nitrogen mass removal

The removal efficiency calculated on the mass balance for TOT N was 85% for vegetated treatments and 48% for the control (Table 20). Abatement was higher for all the treatments starting from June, with 77% for planted treatments and 41% in the control. In particular R.E was higher than 90% for *Ty* and *Cae.* In July *Ty* and *Cae* again had a good R.E of higher than 95% but other treatments also improved their R.E in particular *Pha* that reached 88%.

From August till the end of the monitored period the vegetated treatments showed an average R.E. of 92% with the best performances being *Cae* and *Ty* (99% and 96% respectively).

Significant differences in the R.E. among treatments were not highlighted but all the vegetated treatments performed better than the control from August till November.

The R.E of NH_4-N was always close to 100% in the vegetated treatments that performed slightly but significantly better than control (91%).

NO_3-N was also removed well from vegetated treatments (average of 73% in the total period) conversely to the control (6%). Even though there weren't statistical differences among vegetated treatments, the best performing specie were apparently *Cae* and *Ty* that removed almost completely the NO_3-N load in the cycles from August to October.

It should be pointed out that the presence of algae was detected very often in the control and this may contribute to the disappearance of nitrogen.

By the end of 2008, *Ty* treatment had removed 82.5 g/m^2 (96%) of the cumulative load that was 85.9 g/m^2) of TOT N, followed by *Cae* (93%), *Jue* (82%), *Pha* (81%), *Phr* (77%) and the control (48%) (Figure 49). There were slight differences in the first nitrogen load among vegetated treatments. After this, a clearer differentiation among treatments took place, grouping *Ty* and *Cae* with the highest R.E, *Jue*, *Pha* and *Phr* with intermediate R.E. and the control.

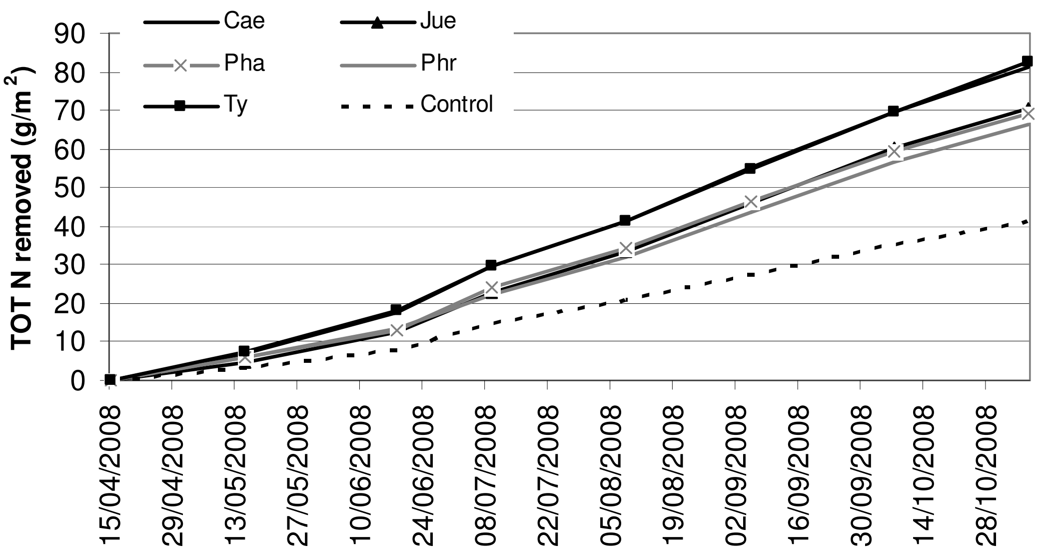


Figure 49 - Cumulative TOT N removed in the treatments during 2008

Table 20 - Removal efficiency of N-NO_3, N-NH_4 and TOT N during the first year of experimentation. Different letters indicate significant differences at P = 0.05 on the same date by Student-Newman Keuls test

	removed %	09/05/2008 15/05/2008	11/06/2008 18/06/2008	03/07/2008 09/07/2008	01/08/2008 08/08/2008	29/08/2008 05/09/2008	30/09/2008 07/10/2008	30/10/2008 06/11/2008	tot
	NH4-N	96% a	99% a	99% a	100% a	100% a	100% a	100% a	99% a
Cae	NO3-N	45% a	87% a	97% a	98%	98% a	100% a	97% a	90% a
	TOT N	**68% a**	**92% a**	**98% a**	**99% a**	**99% a**	**100% a**	**98% a**	**94% a**
	NH4-N	94% a	96% a	99% a	100% a	100% a	100% a	99% a	99% a
Jue	NO3-N	4% ab	46% ab	69% ab	84% a	84% a	91% a	74% a	66% a
	TOT N	**45% ab**	**68% ab**	**84% ab**	**92% a**	**92% a**	**96% a**	**85% a**	**82% a**
	NH4-N	95% a	89% ab	99% a	100% a	99% a	98% a	92% a	96% a
Pha	NO3-N	24% ab	44% ab	78% a	80% a	72% a	76% a	77% a	65% a
	TOT N	**57% ab**	**64% ab**	**88% a**	**89% a**	**86% a**	**88% a**	**84% a**	**81% a**
	NH4-N	91% a	92% ab	99% a	100% a	99% a	98% a	91% a	97% a
Phr	NO3-N	31% a	39% ab	49% ab	67% a	69% a	76% a	78% a	59% a
	TOT N	**58% ab**	**63% ab**	**74% ab**	**82% a**	**85% a**	**88% a**	**84% a**	**77% a**
	NH4-N	97% a	99% a	100% a	100% a	100% a	100% a	88% a	98% a
Ty	NO3-N	47% a	91% a	91% a	98% a	98% a	100% a	86% a	88% a
	TOT N	**69% a**	**95% a**	**95% a**	**99% a**	**99% a**	**100% a**	**87% a**	**96% a**
	NH4-N	92% a	83% b	98% a	99% a	98% b	88% b	74% b	91% b
Control	NO3-N	-18% b	7% b	13% b	8% b	-9% b	15% b	23% b	6% b
	TOT N	**32% b**	**41% b**	**56% b**	**51% b**	**47% b**	**55% b**	**45% b**	**48% b**

Removal efficiency and temperature

Neither NH_4-N R.E nor NO_3-N R.E., and consequently TOT N R.E., of vegetated treatments were significantly correlated with maximum and minimum temperature recorded during the weekly cycle. The only positive and significant correlation was found between NH_4-N R.E. and maximum air temperature and average air temperature for the control (Table 21). Temperature seemed to be less important for R.E. than in the previous year, but this is probably due to the fact that cold months were not taken into account in 2008. The correlation with the control pointed out that probably microbial activity not associated with plants was more affected by temperature.

Table 21– Correlation coefficients among R.E. of all forms of nitrogen and maximum, minimum and average air temperature in all treatments during 2008. Significant coefficients in bold

Treatments	R.E	max air	min air	average air
Cae	NH_4-N	-0.22	-0.36	-0.30
	NO_3-N	0.19	-0.03	0.09
	TOTN	0.18	-0.05	0.08
Jue	NH_4-N	0.18	-0.04	0.07
	NO_3-N	0.13	-0.15	0.00
	TOTN	0.18	-0.11	0.04
Pha	NH_4-N	0.67	0.39	0.55
	NO_3-N	0.18	0.05	0.13
	TOTN	0.30	0.11	0.22
Phr	NH_4-N	0.80	0.48	0.67
	NO_3-N	-0.32	-0.43	-0.39
	TOTN	-0.08	-0.29	-0.19
Ty	NH_4-N	0.70	0.39	0.57
	NO_3-N	0.50	0.10	0.32
	TOTN	0.61	0.23	0.45
Control	NH_4-N	**0.94**	0.71	**0.86**
	NO_3-N	-0.62	-0.51	-0.57
	TOTN	0.33	0.02	0.20
Vegetated Treatments	NH_4-N	0.76	0.42	0.62
	NO_3-N	0.06	-0.16	-0.04
	TOTN	0.23	-0.06	0.10

Removal efficiency and O_2 concentration

The median O_2 values detected in vegetated treatments ranged from 3.7 mg/L in *Jue* to 2.67 in *Pha* against 1.7 in the control.

There was a positive and significant correlation between O_2 concentration and R.E of all the nitrogen forms in *Jue*. Other positive and significant correlations were found in *Phr* between R.E of NO_3-N and TOT N and O_2 concentration (Table 22).

Because of technical problems with the pH probe during the second year of experimentation it was not possible to measure pH and thus to make correlation between R.E. and pH.

Table 22 - Correlation coefficients among R.E. of all forms of nitrogen and dissolved oxygen (mg/L) during 2008. Significant coefficients in bold

R.E	**Cae**	**Jue**	**Pha**	**Phr**	**Ty**	**Control**	**Vegetated treatments**
NH_4-N	0.05	**0.81**	0.42	0.53	0.04	-0.04	0.49
NO_3-N	0.07	**0.81**	0.47	**0.76**	0.66	0.44	0.67
TOTN	0.07	**0.81**	0.52	**0.81**	0.63	0.42	0.68

Daily analyses in July 2008

As mentioned above, from 3rd to 9th July 2008 chemical analyses were performed every day including for NO_2-N; four completely sterilized beakers (SC) with volumes of 2L were set up and managed as all the other tanks.

The most important TOT N removal took place just after the first day and there are no statistical differences among treatments but only with respect to SC (Table 23). From the second day there was a differentiation among vegetated treatments, control and SC that continued in the following days with a progressive decrease in differences between control and SC.

The main differences were in R.E. of NH_4-N and NO_2-N, with vegetated treatments showing a higher R.E. than the control and SC.

By the end of the week the treatment with the best performance was *Cae* (TOT N R.E. 98%) followed by *Ty* (95%).

Table 23 - R.E of N-NO_3, N-NH_4, NO_2-N and TOT N during the daily analysis in the first week of July 2008. Different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test in the same date

	removed %	04/07/2009	05/07/2009	06/07/2009	07/07/2009	08/07/2009	09/07/2009
Cae	NH_4-N	85% a	95% a	99% a	100% a	100% a	99% a
	NO_3-N	66% a	74% a	79% a	91% a	95% a	97% a
	NO_2-N	-690% a	-784% a	13% a	37% a	53% a	51% a
	TOT N	75% **a**	84% **a**	89% **a**	95% **a**	97% **a**	98% **a**
Jue	NH_4-N	81% ab	92% a	98% a	98% a	99% a	99% a
	NO_3-N	53% ab	54% a	47% a	63% a	60% ab	69% ab
	NO_2-N	-104% a	-161% a	-361% a	-266% a	-221% a	-75% a
	TOT N	67% **a**	72% **ab**	72% **ab**	81% **ab**	80% **ab**	84% **a**
Pha	NH_4-N	76% ab	83% ab	94% a	99% a	99% a	99% a
	NO_3-N	53% a	56% a	66% a	66% a	73% ab	78% a
	NO_2-N	-5061% a	-3391% a	-565% a	-441% a	-368% a	60% a
	TOT N	62% **a**	68% **ab**	80% **ab**	82% **ab**	85% **a**	88% **a**
Phr	NH_4-N	76% ab	90% a	93% a	98% a	98% a	99% a
	NO_3-N	49% a	55% a	42% a	49% a	45% ab	49% ab
	NO_2-N	-1130% a	-209% a	0% a	20% a	26% a	44% a
	TOT N	62% **a**	73% **ab**	68% **ab**	74% **ab**	71% **ab**	74% **ab**
Ty	NH_4-N	81% ab	95% a	99% a	100% a	100% a	99% a
	NO_3-N	63% a	76% a	82% a	86% a	88% a	91% a
	NO_2-N	-399% a	-492% a	34% a	47% a	57% a	37% a
	TOT N	72% **a**	85% **a**	91% **a**	93% **a**	94% **a**	95% **a**
Control	NH_4-N	62% b	76% b	84% b	92% a	97% a	98% a
	NO_3-N	27% a	27% a	11% a	37% a	11% b	13% b
	NO_2-N	-209% a	-82% a	-986% a	-916% a	-877% a	-166% a
	TOT N	45% **a**	51% **b**	47% **b**	64% **b**	53% **b**	55% **bc**
SC	NH_4-N	37% c	42% c	23% c	52% b	57% b	60% b
	NO_3-N	44% a	58% a	42% a	62% a	51% ab	53% ab
	NO_2-N	-110963% b	-91531% b	-117772% b	-60126% b	-60225% b	-27375% b
	TOT N	-3% **b**	14% **c**	-14% **c**	34% **c**	30% **c**	46% **c**

Looking at dynamics of the different nitrogen forms expressed in g/m^2 (Figure 50- Figure 56) it is clear that in vegetated treatments the higher quotas of disappearance of NH_4-N and NO_3-N took place just after the first day in particular in *Cae* and *Ty*. In the following two days NH_4-N decreased in all the vegetated treatments but NO_3-N decreased only in *Cae*, *Pha* and *Ty*. In the last three days there was a decrease of NH_4-N and NO_3-N in all the vegetated treatments, but *Phr* and *Jue* showed a slower disappearance especially on the fifth day. Practically no NO_2-N was detected in the vegetated treatments except for a small quantity in *Pha* during the second and third day, confirming that it had rapidly been transformed into NO_3-N. This is the reason why this form of nitrogen can rarely be accumulated in terrestrial and aquatic environments.

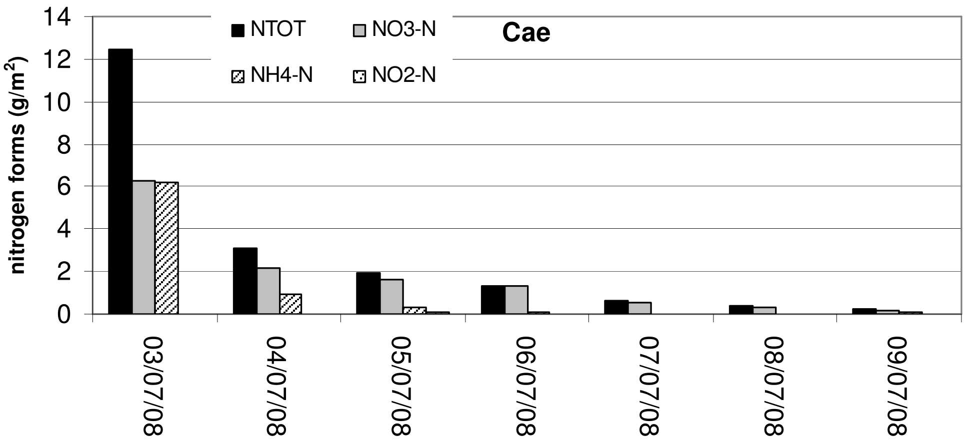


Figure 50- Daily presence in *Cae* of different nitrogen forms in the first week of July 2008

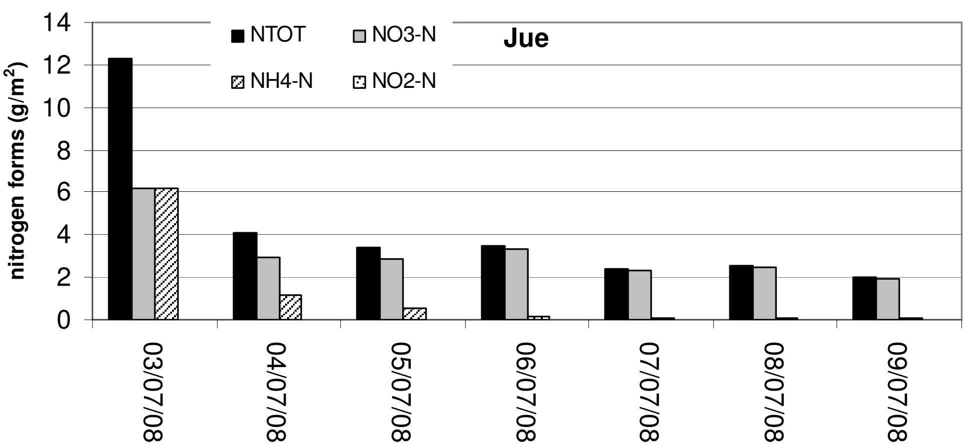


Figure 51 - Daily presence in *Jue* of different nitrogen forms in the first week of July 2008

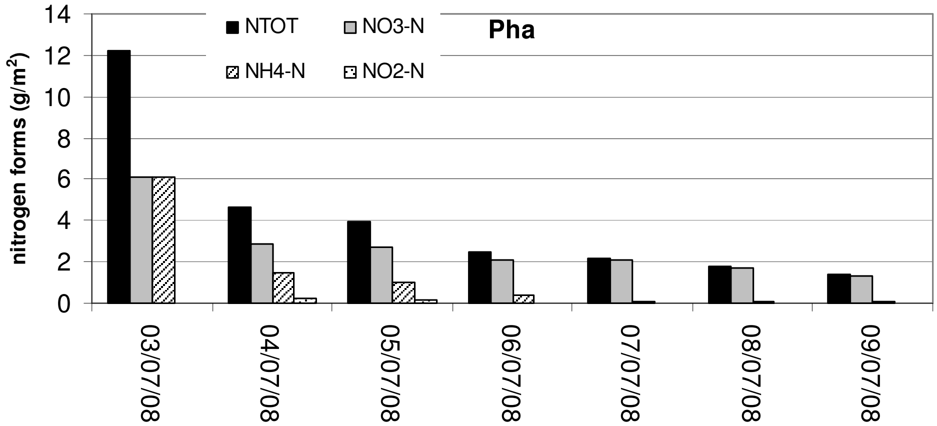


Figure 52 - Daily presence in *Pha* of different nitrogen forms in the first week of July 2008

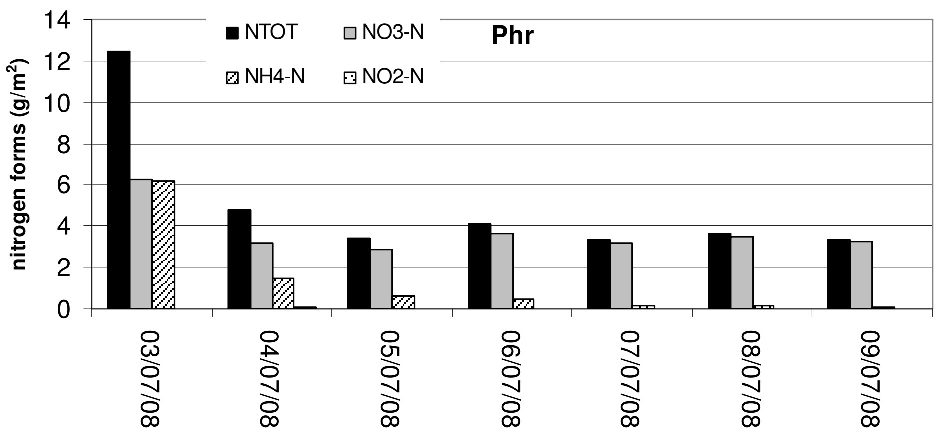


Figure 53 - Daily presence in *Phr* of different nitrogen forms in the first week of July 2008

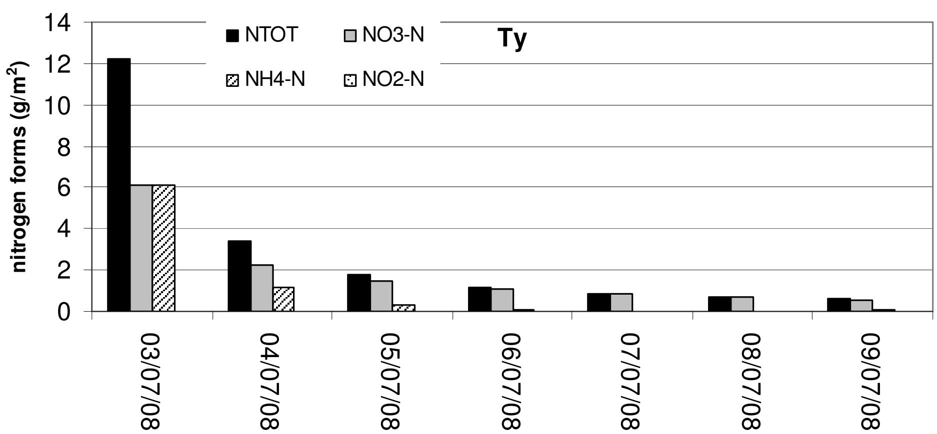


Figure 54- Daily presence in *Ty* of different nitrogen forms in the first week of July 2008

In the control (Figure 55) the disappearance dynamics of nitrogen was very different: the predominant disappearance happened the first day, this was followed by a slight decrease the second day and then the situation remained more or less constant till the end of the week with some increases probably due to an inhomogeneous sample. Again there was no NO_2-N in the control.

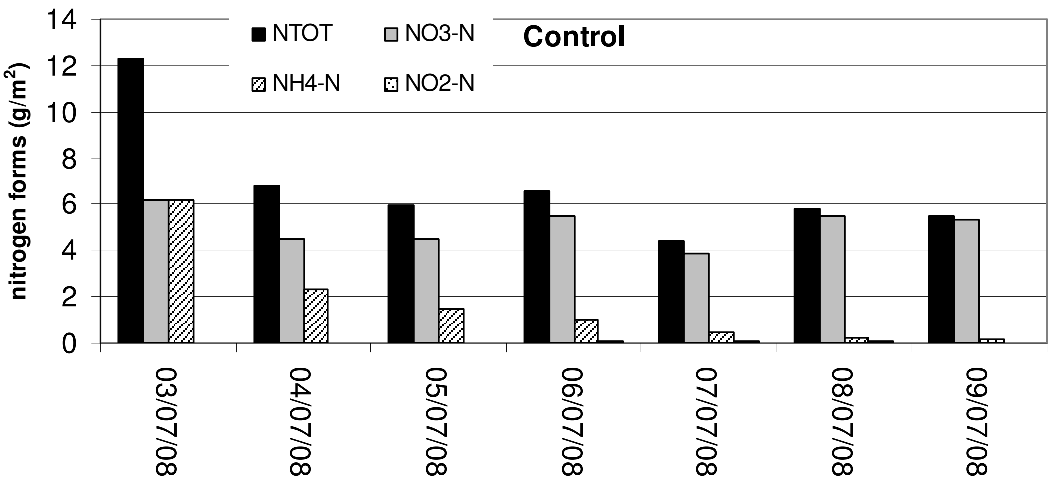


Figure 55 - Daily presence in the control of different nitrogen forms in the first week of July 2008

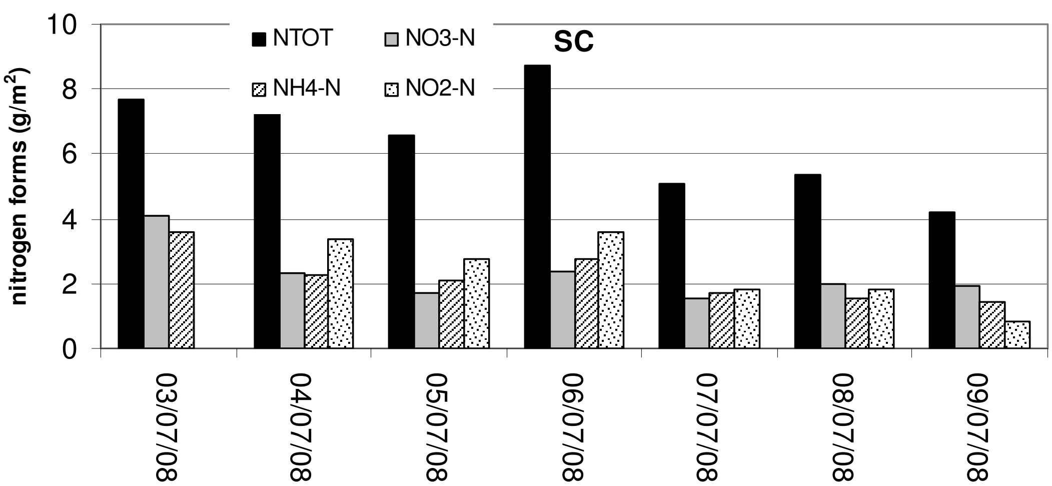


Figure 56 – Daily presence in SC of different nitrogen forms in the first week of July 2008

In SC (Figure 56) the dynamics were again different. TOT N did not decrease in the first three days but only changed the oxidation forms, showing a big increase of NO_2-N.

From the third day the situation changed and nitrogen started to disappear but there was a heavy rainfall event on 6th July that probably contaminated the sterilized SC.

Intensive monitoring in February 2009

For one tank per species chemical analyses were performed 12 times from 17th to 24th February 2009, also considering NO_2-N. One completely sterilized beaker (SC) with a volume of 2L was also set up and managed as in July.
Average air temperature was around 2.1 °C, with a minimum of -6 °C and a maximum of 13.3°C.
Input concentrations were 115, 100 and 0 ppm for NO_3-N, NH_4-N and NO_2-N respectively. Again the majority of nitrogen disappearance took place just after the first 4 hours following the application in all the treatments except for SC (Figure 57- Figure 63). There was then a continuous decrease of all the nitrogen forms detected in *Cae* (Figure 57) but for the other vegetated treatments there was an evident decrease only after 39-48 hours. By the end of the 167 hours of monitoring *Cae* showed the best R.E reaching 100%. This was followed by *Pha*, *Ty* and *Jue* with around 78% and *Phr* and the control with 61%. Again there was no NO_2-N and the NH_4-N was also very low in this season. SC also showed no NO_2-N (Figure 63), but this may be because of contamination due to frequent rainfall events in the period or perhaps because of the environmental conditions. The R.E. of TOT N detected in the SC at the end of the period was 41%.

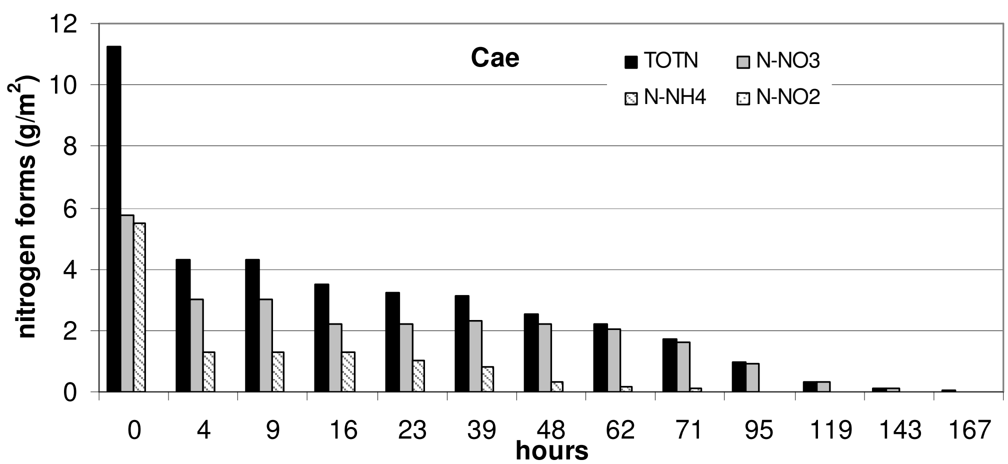


Figure 57 - Hourly presence of different nitrogen forms in February 2009 in *Cae*

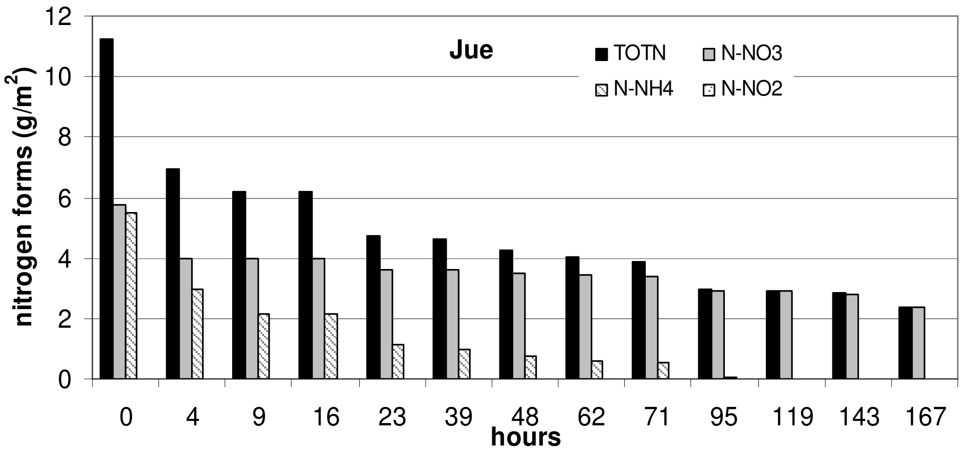


Figure 58 - Hourly presence of different nitrogen forms in February 2009 in *Jue*

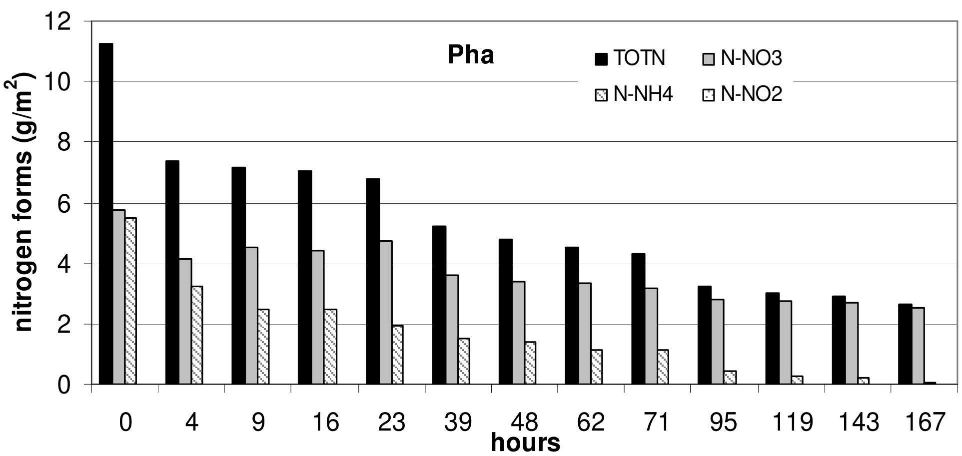


Figure 59- Hourly presence of different nitrogen forms in February 2009 in *Pha*

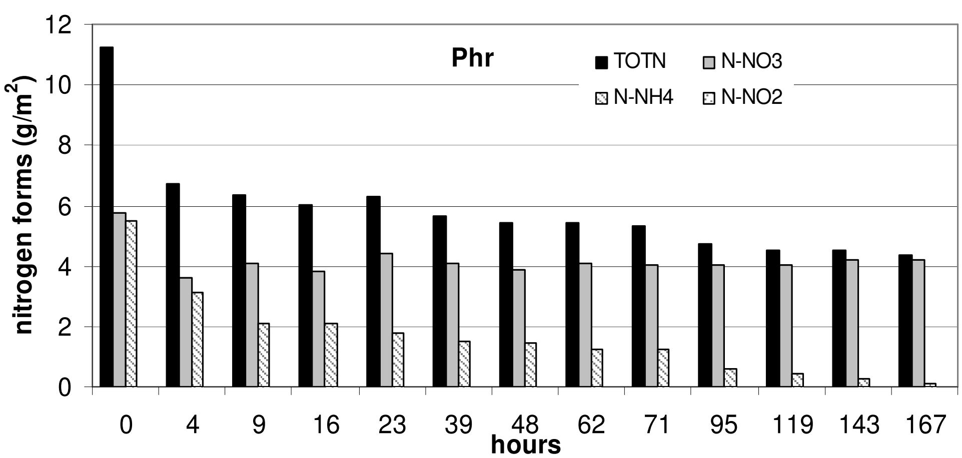


Figure 60 - Hourly presence of different nitrogen forms in February 2009 in *Phr*

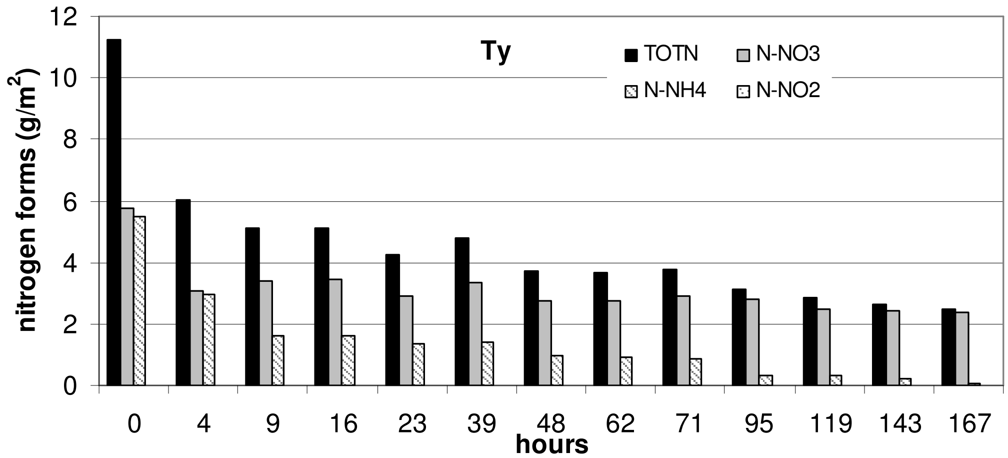


Figure 61- Hourly presence of different nitrogen forms in February 2009 in *Ty*

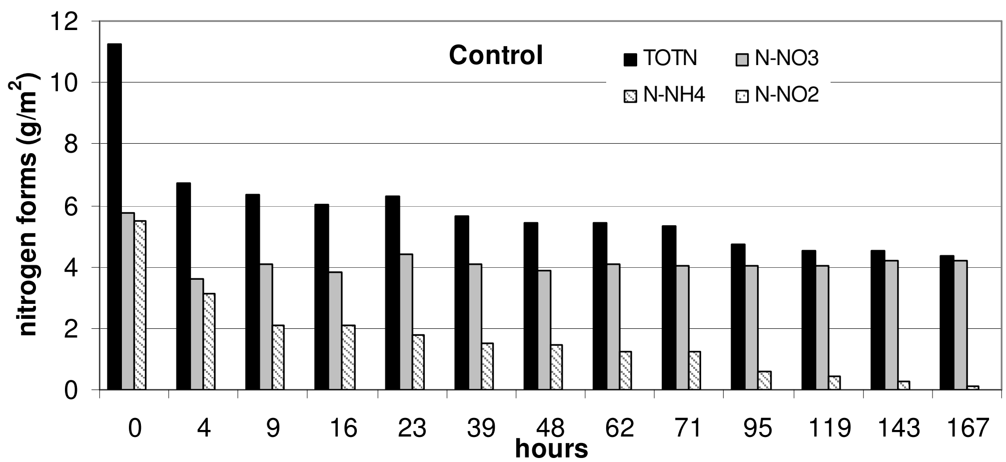


Figure 62- Hourly presence of different nitrogen forms in February 2009 in control

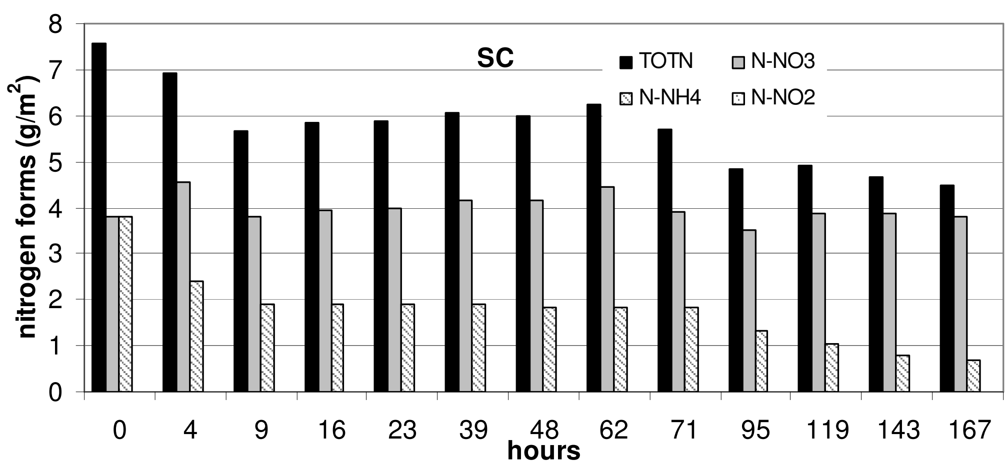


Figure 63 - Hourly presence of different nitrogen forms in February 2009 in SC

Aerial biomass production and nitrogen uptake

Dry matter production of the aerial parts harvested on December 4th 2008 was significantly different among species, with the highest production detected in *Jue* and the lowest in *Pha* (Table 24). The % of nitrogen detected was not significantly different among species but the lowest value was detected in *Ty* as in the previous years. These results of the second growing season, in particular for *Ty* and *Phr*, were lower with respect to those of Morari and Giardini (2009), highlighting that the plants hadn't yet reached the maturity stage probably also because of being transplanted at the beginning of the growing season.

The quantity of harvestable nitrogen was very variable in the treatments, with the highest and significantly different quantity recorded for *Jue*. The ratio between nitrogen stored in aerial parts and disappeared nitrogen varied from 21% (*Pha*) to 76% (*Jue*) and again the only significant difference was for *Jue* with respect to the other species.

Again this behaviour may be related to different accumulation in the belowground tissues, and/or different microbial activities associated with different species.

Table 24- Dry matter and nitrogen in the aerial parts of plants; different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test

species	dry matter (aerial part) (g/m^2)				% nitrogen				nitrogen in aerial part (g/m^2)				stored in aerial part/disappeared			
Cae	2772	a	±	118	1.3	a	±	0.1	35.3	b	±	2.2	43%	b	±	3%
Jue	3210	a	±	124	1.6	a	±	0.4	51.2	a	±	12.5	76%	a	±	34%
Pha	1057	b	±	648	1.7	a	±	0.6	15.7	b	±	10.0	21%	b	±	13%
Phr	2022	ab	±	1,214	1.5	a	±	0.8	22.3	b	±	11.8	32%	b	±	15%
Ty	2723	a	±	617	0.8	a	±	0.1	21.7	b	±	8.9	26%	b	±	10%

Third year

This year took into account the period from the end of April to the end of September and the same nitrogen load was left inside the tanks for one week.

Environmental conditions

Temperature rose from the beginning of the monitoring period till the end of May 2009, reaching 36.5°C on 26th May (Figure 64). It then remained more or less constant till the end of June and then rose again till mid-August when it reached the seasonal maximum of 36.8 °C and finally decreased till the end of September. The minimum value of 5.62 °C was recorded on 26th April. The average for the period was 21.7 °C.

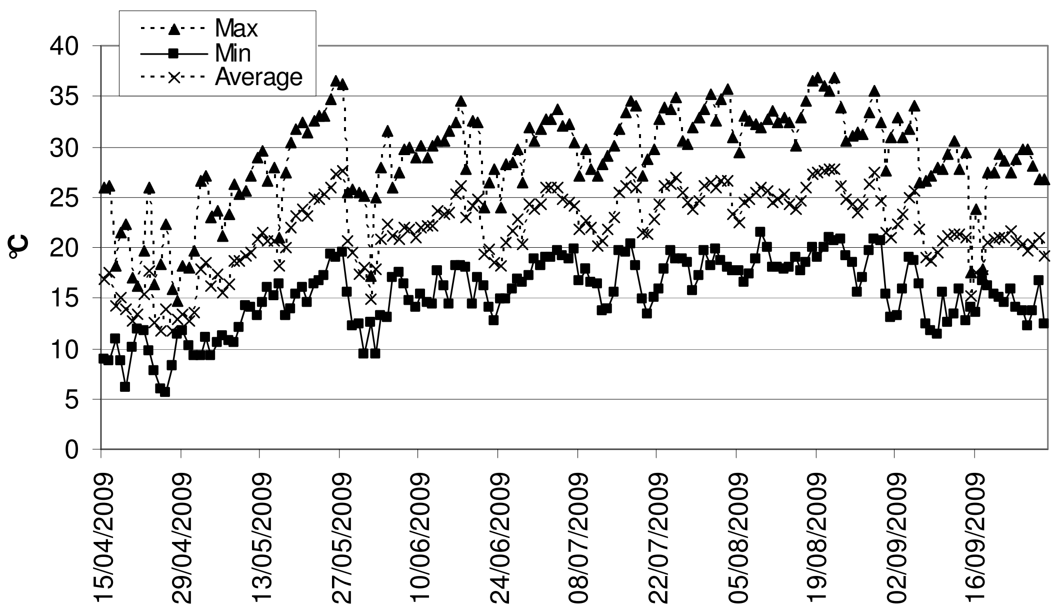


Figure 64- Maximum, average and minimum daily temperature from April to September 2009

Rainfall was uniformly distributed in the period with a cumulated value of 525 mm and the most important event with 135.8 mm was on September 17th (Figure 65).

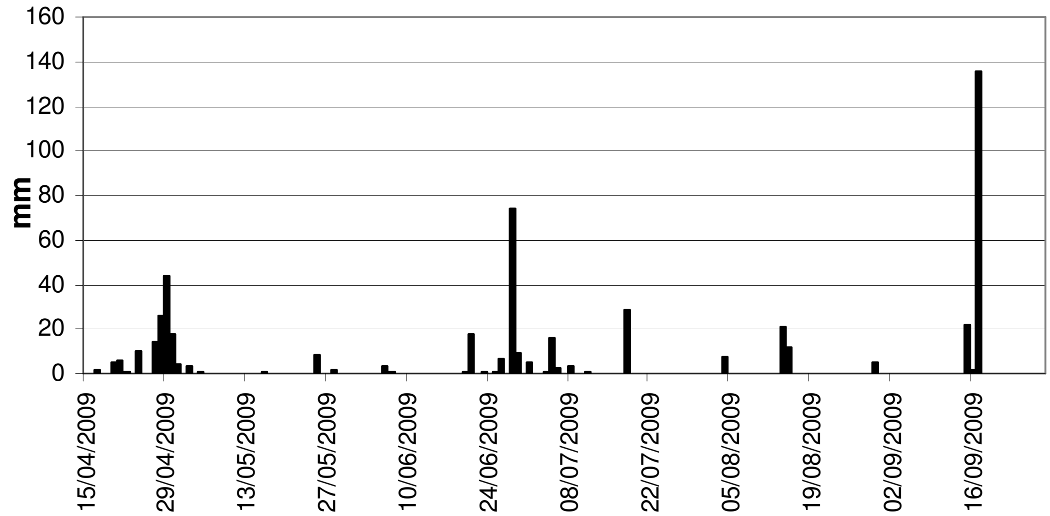


Figure 65 - Rainfall distribution in the monitoring period

Plant growth

Ty was confirmed as the fastest growing species and reached a maximum height of 142 cm on May 29th 2009. Similarly *Phr* grew in a continuous way till the end of May reaching the height of 120 cm. It maintained this value till half July then dried a dried a bit and remained at a practically constant height of around 115 cm till the end of September.

Cae and *Pha* grew with similar dynamics till mid-June when *Pha* reached the maximum height of 88 cm, *Cae* then grew a little more till mid-August reaching a maximum of 97 cm. Instead *Jue* grew till mid-July reaching the maximum value of 74 cm (Figure 66). The aerial part was harvested on 28th September 2009.

In 2009 stems of *Jue* were not counted because of the excessive number (more than 850 stems/m^2 just in May), but the monitoring was continued for *Phr* and *Ty* that emitted stems regularly till the end of September, reaching densities of 2580 and 513 stems/m^2 respectively (Figure 67).

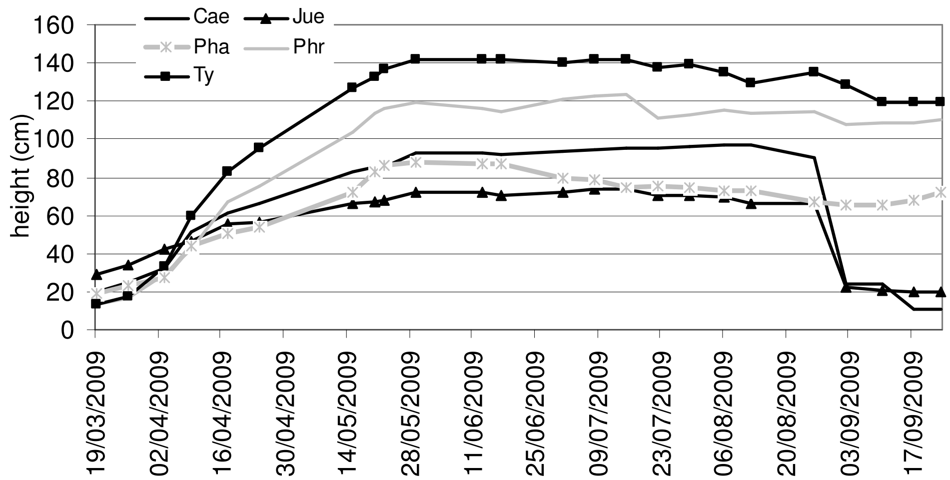


Figure 66 - Height of plants during the third year of experimentation

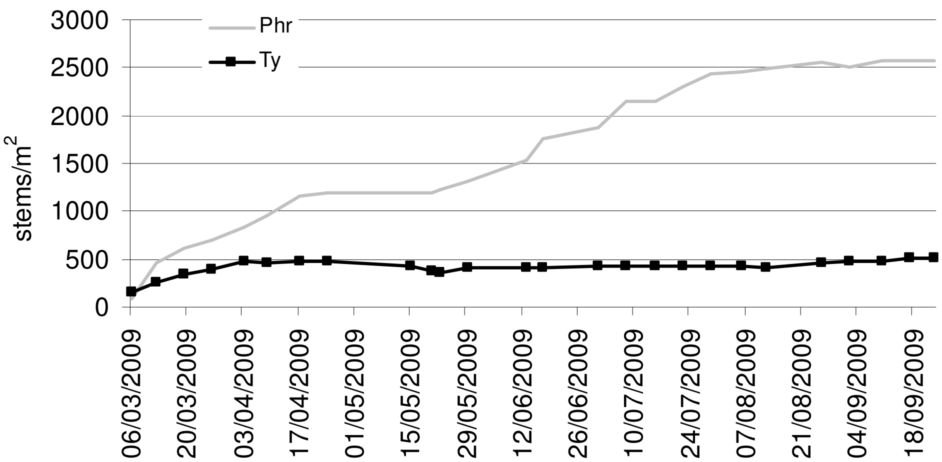


Figure 67 - Stems density during the third year of experimentation

Evapotranspiration

The time pattern of cumulative ET allows three sub-phases to be distinguished (Figure 68) the first from February to mid-May in which there was a progressive differentiation among treatments (average daily ET of vegetated treatments =7.8 mm) the second from mid-May to the beginning of September in which the differences among treatments were more evident (average daily ET of vegetated treatments = 28.7 mm) and a third in which ET started to decrease (average daily ET of vegetated treatments =14 mm).

Over the whole period, the water consumption of *Ty*, *Cae* and *Pha* were the highest reaching a cumulative value of 4294, 4254 and 4213 mm respectively (daily ET rate of 20 mm). *Phr* reached a final value of 3953 mm (daily ET rate of 18 mm) and *Jue* consumed a little less water with a final value of 3629 mm (daily ET rate of 17 mm) and showed a lower increase rate from the end of August probably connected with a certain suffering from nitrogen loads that will be discussed later.

The control lost only 787 mm (daily ET rate of 3.6 mm) a value very similar to the calculated ET_0, 667 mm (daily ET rate of 3.1 mm).

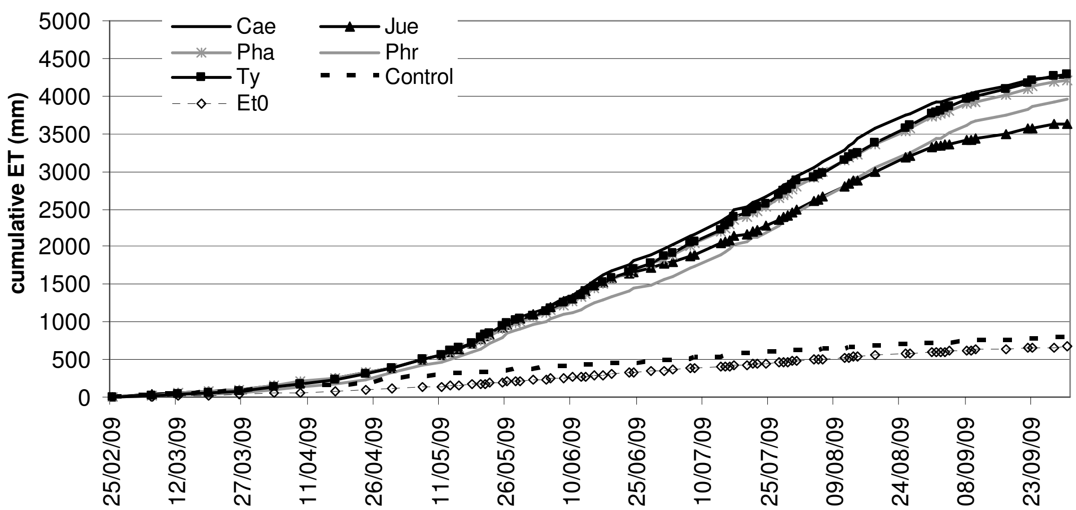


Figure 68 - Cumulative ET of treatments and ET_0 during 2009

Also for 2009, knowing the ET for each species and ET_0 it was possible to calculate the K_c (Table 25). Considering the entire period the K_c of all treatments increased month by month till August and the highest average K_c was that of *Ty* with a value of 6 followed by *Pha* with 5.8.

Looking at the same months in the previous year (Table 19) it is clear that in all species and for each month the K_c increases because of the age and development of plants. It is probable that the plants in each tank reached the maximum development for a mesocosm scale in 2009 (being at the third year of growth) and that the K_c of 2009 may be considered a parameter associated with the stage of maturity of the studied macrophytes.

Table 25 - Monthly K_c of all the treatments in 2009

	Cae	*Jue*	*Pha*	*Phr*	*Ty*	Control
March	2.4	2.6	2.7	1.6	2.0	2.1
April	4.2	4.1	4.1	3.7	4.4	2.3
May	6.2	6.0	5.6	5.4	6.0	1.1
June	6.9	5.7	6.3	4.9	6.2	0.9
July	7.3	5.3	7.2	6.7	7.6	0.9
August	6.9	7.1	7.9	8.0	8.2	0.8
September	5.3	4.5	6.8	7.8	7.6	1.2
average	5.6	5.0	5.8	5.4	6.0	1.3

Nitrogen concentration

Considering all the values of 2009, NH_4-N almost completely disappeared in water discharged from treatments (Figure 69). These data confirm the tolerance of all the species to these high applied ammonium concentrations (median value of 202 ppm), in accordance with Clarke and Baldwin (2002).

NO_3-N concentration also decreased because median concentration values ranged between 2 ppm detected in *Phr* and 132.1 in the control against an input concentration of 200 ppm, suggesting that plant uptake and/or denitrification were important processes of nitrogen removal with these input concentrations.

There were significant differences in NO_3-N concentration among treatments and in particular the vegetated treatment with the lowest value was *Phr* and the highest was detected in *Jue* that performed as the unvegetated control

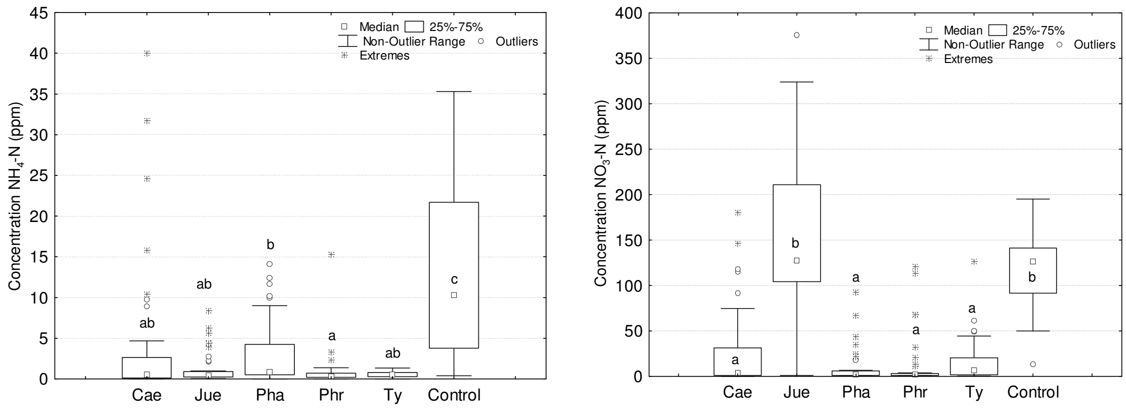


Figure 69 - Box and whiskers of NH4-N and NO3-N concentrations in 2009. Different letters indicate significant differences at P = 0.05 by Kruskal-Wallis test

Nitrogen mass removal

Taking the whole monitored period in 2009, R.E. calculated on the mass balance for TOT N was 92% for vegetated tanks and 63% for the control (Table 26). Abatement differed in the two periods of different loads, in particular *Cae*, *Ty,* and *Jue* had a higher R.E in the first period (low load median input concentration of 104 ppm of NO_3-N and 119 of NH_4-N) and the other treatments in the second (high load median input concentration of 200 ppm of NO_3-N and 203 of NH_4-N). In the first period there were no significant differences in the R.E. among treatments but all the vegetated treatments performed better than the control. In the second period there were statistical differences between *Jue* (with lower R.E.) and all the other vegetated treatments, but again all the vegetated treatments removed statistically more nitrogen with respect to the control.

It is interesting to note that *Jue* decreased its R.E. cycle by cycle, in particular in the period with high load, suggesting that the species is not adapted for high and prolonged nitrogen loads. All the treatments, but *Cae* in particular, showed a decrease in the R.E. during the last cycle, maybe because of the approach of the resting season.

By the end of 2009 *Pha* treatment had removed 216.5 g/m^2 (98% of the cumulative load that was 222 g/m^2) of TOT N, followed by *Phr* and *Ty* (97%), *Cae* (93%), *Jue* (75%) and the control (63%) (Figure 70).

Table 26 - R.E. of N-NO_3, N-NH_4 and TOT N in 2009. Different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test on the same date

		low load		high load										
	removed %	17/04/2009 24/04/2009	15/05/2009 22/05/2009	29/05/2009 05/06/2009	17/06/2009 24/06/2009	01/07/2009 08/07/2009	15/07/2009 22/07/2009	29/07/2009 06/08/2009	12/08/2009 18/08/2009	26/08/2009 02/09/2009	10/09/2009 17/09/2009	tot 100 ppm	tot 200 ppm	tot 2009
Cae	NH4-N	100% a	100% a	100% a	100% a	100% a	100% a	100% a	100% a	96% b	84% b	100% a	97% c	98% b
	NO3-N	99% a	100% a	99% a	99% a	99% a	98% a	99% a	99% a	91% a	81% b	100% a	87% a	89% a
	TOT N	**100% a**	**100% a**	**100% a**	**100% a**	**100% a**	**99% a**	**100% a**	**100% a**	**96% a**	**89% b**	**100% a**	**92% a**	**93% a**
Jue	NH4-N	100% a	100% a	100% a	100% a	100% a	100% a	100% a	100% a	99% a	97% a	100% a	99% ab	99% a
	NO3-N	88% a	85% a	83% a	82% a	55% b	65% b	56% b	50% b	51% b	50% c	87% a	45% b	50% b
	TOT N	**95% a**	**93% a**	**92% a**	**78% b**	**77% b**	**82% b**	**78% b**	**75% b**	**76% b**	**75% c**	**94% a**	**73% b**	**75% b**
Pha	NH4-N	99% a	99% a	100% a	100% a	100% a	100% a	100% a	100% a	97% ab	94% a	99% a	99% b	99% a
	NO3-N	92% a	100% a	100% a	100% a	100% a	99% a	99% a	100% a	98% a	99% a	96% a	96% a	96% a
	TOT N	**96% a**	**100% a**	**100% a**	**100% a**	**100% a**	**100% a**	**99% a**	**100% a**	**99% a**	**98% a**	**98% a**	**98% a**	**98% a**
Phr	NH4-N	97% a	100% a	100% a	100% a	100% a	100% a	100% a	100% a	100% a	99% a	98% a	100% a	100% a
	NO3-N	64% a	99% a	88% a	88% a	98% a	100% a	99% a	100% a	99% a	99% a	81% a	97% a	95% a
	TOT N	**81% a**	**99% a**	**94% a**	**99% a**	**99% a**	**100% a**	**100% a**	**100% a**	**100% a**	**100% a**	**90% a**	**99% a**	**97% a**
Ty	NH4-N	100% a	100% a	100% a	100% a	100% a	100% a	100% a	98% a	100% a	98% a	100% a	99% ab	99% a
	NO3-N	100% a	100% a	95% a	95% a	97% a	99% a	99% a	98% a	100% a	97% a	100% a	94% a	95% a
	TOT N	**100% a**	**100% a**	**98% a**	**98% a**	**98% a**	**100% a**	**100% a**	**99% a**	**99% a**	**98% a**	**100% a**	**97% a**	**97% a**
Control	NH4-N	88% b	98% b	88% b	94% b	97% b	99% b	98% b	95% b	90% c	81% b	93% b	92% d	92% c
	NO3-N	-1% b	28% b	42% b	42% b	25% c	50% b	40% c	36% c	27% c	24% d	13% b	34% b	32% c
	TOT N	**47% b**	**65% b**	**66% b**	**61% c**	**59% c**	**73% c**	**69% c**	**67% c**	**62% c**	**59% d**	**56% b**	**64% c**	**63% c**

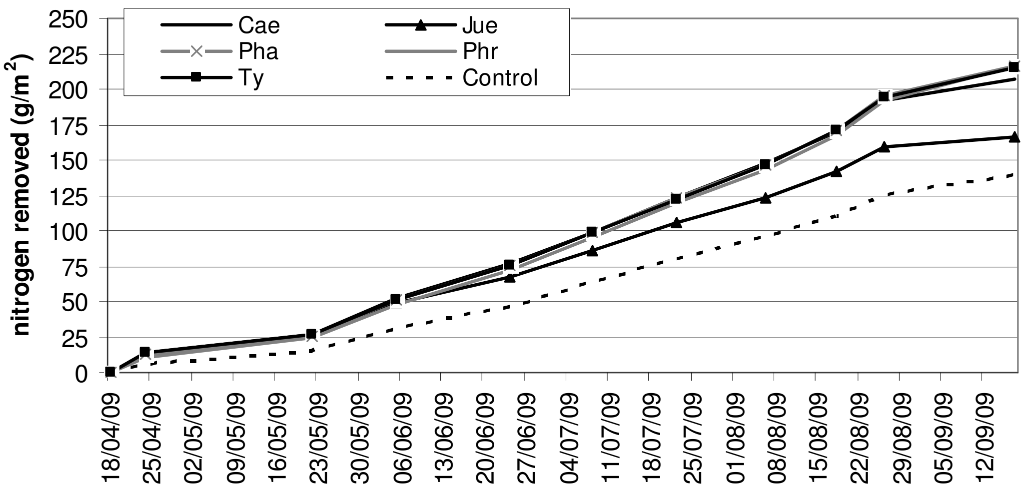


Figure 70- Cumulative TOT N removed in the treatments during 2009

Removal efficiency and environmental variables

In 2009 the correlations between nitrogen removal efficiencies and temperature were not easy to interpret because of some opposite tendencies. For example NO_3-N and TOT N R.E. in *Jue* was significantly and negatively correlated with minimum air temperature and the contrary happened for *Phr* (Table 27). Considering all the vegetated treatments together no significant correlations were found between nitrogen removal efficiency and temperature. The average temperature in the period of 21.7 °C was suitable for plant and microorganism activities.

O_2 concentration, pH and redox potential were also poorly correlated with nitrogen removal efficiency underlining that they weren't limiting factors for applied nitrogen concentrations in the considered period (Table 28).

Table 27 - Correlation coefficients among R.E. of all the forms of nitrogen and maximum, minimum and average air temperature in all the treatments during 2009. Significant coefficients in bold

Treatments	R.E	max air	min air	average air
Cae	NH_4-N	0.22	0.11	0.20
	NO_3-N	0.15	0.06	0.13
	TOTN	0.18	0.07	0.15
Jue	NH_4-N	0.10	0.02	0.09
	NO_3-N	-0.61	**-0.71**	**-0.63**
	TOTN	-0.60	**-0.73**	-0.63
Pha	NH_4-N	0.31	0.23	0.30
	NO_3-N	**0.73**	**0.75**	**0.74**
	TOTN	**0.79**	**0.78**	**0.80**
Phr	NH_4-N	**0.83**	**0.84**	**0.84**
	NO_3-N	**0.86**	**0.87**	**0.87**
	TOTN	**0.84**	**0.86**	**0.85**
Ty	NH_4-N	-0.03	-0.14	-0.05
	NO_3-N	0.21	0.06	0.17
	TOTN	0.11	-0.04	0.07
Control	NH_4-N	**0.66**	0.57	**0.65**
	NO_3-N	**0.63**	0.58	**0.64**
	TOTN	**0.78**	**0.68**	**0.78**
Vegetated Treatments	NH_4-N	0.32	0.21	0.30
	NO_3-N	0.17	0.02	0.15
	TOTN	0.23	0.06	0.20

Table 28 - Correlation coefficients among R.E. of all nitrogen forms and O_2 (mg/L) pH and redox potential in 2009. Significant coefficients in bold

	R.E	O_2 mg/L	pH	redox
Cae	NO_3-N	0.34	0.60	-0.45
	NH_4-N	0.29	0.60	-0.39
	TOT N	0.33	0.61	-0.44
Jue	NO_3-N	**0.75**	0.27	-0.45
	NH_4-N	0.60	0.54	-0.49
	TOT N	**0.74**	0.24	-0.46
Pha	NO_3-N	**-0.64**	**0.63**	-0.55
	NH_4-N	0.30	0.38	-0.01
	TOT N	-0.53	**0.72**	-0.51
Phr	NO_3-N	-0.16	0.20	-0.11
	NH_4-N	-0.19	0.50	-0.34
	TOT N	-0.14	0.26	-0.15
Ty	NO_3-N	0.49	-0.14	0.19
	NH_4-N	0.04	0.31	-0.09
	TOT N	0.57	-0.15	0.22
Control	NO_3-N	-0.53	0.54	-0.37
	NH_4-N	-0.29	0.62	-0.52
	TOT N	-0.53	0.51	-0.34
Vegetated treatments	NO_3-N	0.10	0.42	-0.35
	NH_4-N	0.24	0.58	-0.32
	TOT N	0.13	0.43	-0.35

Aerial biomass production and nitrogen uptake

Dry matter in the aerial part for all the species increased with respect to the previous years, probably because plants had reached the maximum productivity allowed by the growing conditions. There were significant differences among species, with the highest value in *Ty* (8240 g/m^2) and the lowest in *Phr* (4313 g/m^2). On the other hand *Ty* also confirmed this year the lowest and significantly different % of nitrogen (1.3%) and *Cae* the highest (1.8%).

The amount of harvestable nitrogen was significantly lower in *Phr* with respect to the other species apart from *Pha.* The % of nitrogen disappeared stored in the aerial part showed significant differences among treatments, with the lowest value detected in *Phr* (29%) and the highest in *Jue* (55%) confirming that it was the species that allocates the main amount of nitrogen removed in the aerial part (Table 29 –.

These results were very high if compared to the literature (Table 4) probably because of the intense plant feeding with easily uptaken nitrogen forms.

Table 29 – Dry matter and nitrogen in the aerial parts of plants; different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test

species	dry matter (aerial part) (g/m^2)				% nitrogen				nitrogen in aerial part (g/m^2)				Stored in aerial part/disappeared			
Cae	6021	b	±	576	1.8	a	±	0.2	107	a	±	18	52%	ab	±	8%
Jue	5271	b	±	1089	1.7	a	±	0.2	91	a	±	15	55%	a	±	12%
Pha	4824	bc	±	381	1.7	a	±	0.2	82	ab	±	7	38%	bc	±	3%
Phr	4313	bc	±	944	1.5	ab	±	0.3	63	b	±	17	29%	c	±	8%
Ty	8240	a	±	635	1.3	b	±	0.1	107	a	±	13	50%	ab	±	6%

Final observations

pH, EC, water soluble carbon and nitrogen in gravel and roots extracts

The pH values resulted very similar between root and gravel extracts, even if higher values were generally shown at root level. In the different plant species non significant differences of pH were observed among the roots and gravel (Figure 71).

The EC always resulted as being higher at root level with respect to the extracts coming from gravel, probably due to the input of salts by plant roots exudates (Figure 72); in particular, *Pha* and *Phr* seemed to have a higher effect on this parameter than the other plant species. A similar pattern was observed for water soluble carbon (WSC) concentration, confirming the production and release of roots exudates (Garcia et al., 1997). The WSC detected in gravel was, in fact, lower than those of the root extracts (Figure 73). This labile organic carbon fraction, which is considered easily degradable by soil microorganisms (Cook and Allan, 1992), is probably responsible for the activation of the resident microbial populations biofilm attached to gravel or roots.

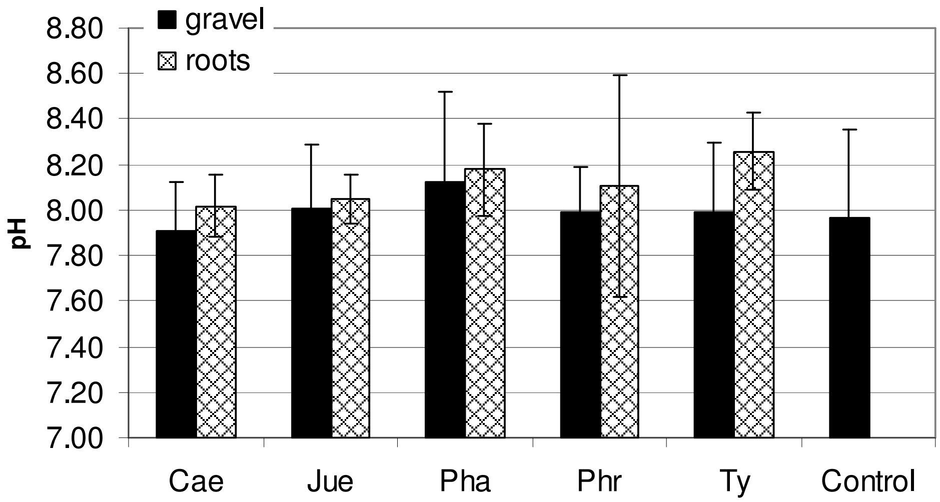


Figure 71 – pH detected in gravel and roots extracts at the end the experiment

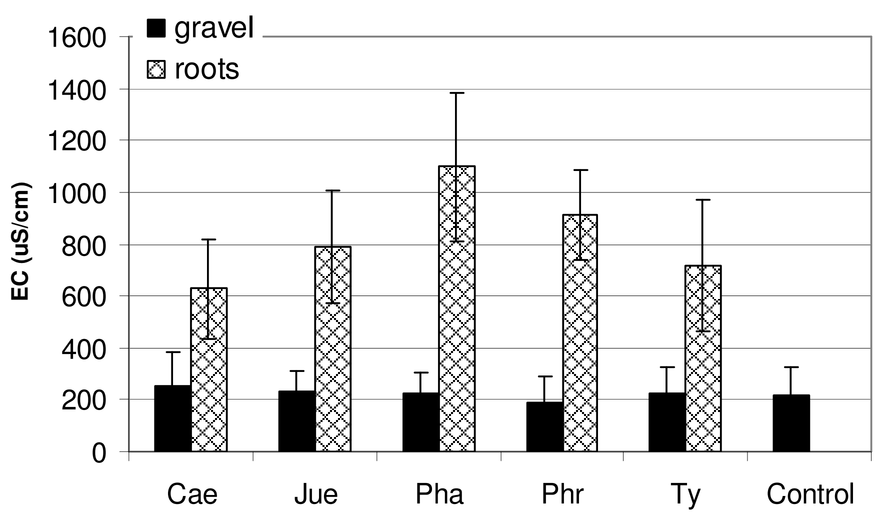


Figure 72 – Conductivity detected in gravel and roots extracts at the end the experiment

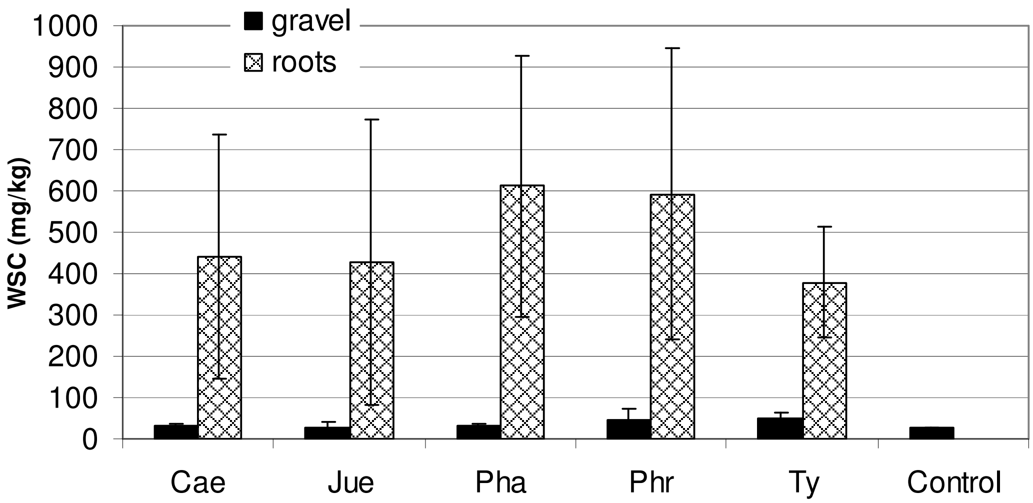


Figure 73 – Water soluble carbon (WSC) detected in gravel and roots and extracts at the end the experiment

NO_3-N roots biofilm ranged from 3.5 g/m^2 in *Cae* to 7.6 in *Jue*, NH_4-N from 0.7 in *Ty* to 2.7 in *Pha*.

NO_3-N gravel biofilm ranged from 1.5 g/m^2 in *Cae* to 5 in *Jue*.

The increase in NO_3-N in gravel treated with plants with respect to control gravel suggested that the presence of plants enhances the aeration, as also observed by Sorrell et al. (2000), thus stimulating the nitrification process.

The roots and gravel nitrogen biofilm accounted from 0.9% (control) to 5.7% (*Jue*) of nitrogen disappeared. Considering only vegetated treatments it was 3.7% on average of that disappeared, with the highest amount being in the roots biofilm.

Table 30 – NO_3-N and NH_4-N attached to roots and gravel

	roots		**gravel**		
	NO_3-N (g/m^2)	NH_4-N (g/m^2)	NO_3-N (g/m^2)	NH_4-N (g/m^2)	biofilm N/ disappeared N
Cae	3.5 ± 1.2	1.2 ± 1.3	1.5 ± 1.1	0.1 ± 0.2	2.4% ± 1%
Jue	7.6 ± 1.0	1.0 ± 0.8	5.0 ± 2.7	0.0 ± 0.0	5.7% ± 1%
Pha	4.0 ± 2.7	2.7 ± 2.8	2.7 ± 2.5	0.1 ± 0.1	3.4% ± 3%
Phr	5.6 ± 1.5	1.5 ± 1.3	4.0 ± 6.4	0.1 ± 0.1	3.9% ± 5%
Ty	5.6 ± 0.7	0.7 ± 0.7	2.6 ± 2.2	0.1 ± 0.1	3.0% ± 2%
Control			1.6 ± 1.2	0.1 ± 0.1	0.9% ± 1%

Nitrogen in belowground plant tissues

Dry matter of belowground biomass ranged from 2395 g/m^2 detected in *Jue* to 6373 in *Phr* and, as hypothesized in the first year, significant differences were found in particular between *Jue* and all the other species apart from *Pha* (Table 31). The % of nitrogen in roots ranged from 1.1 in *Cae* to 1.6 in *Ty* and the amount of nitrogen stored in belowground biomass ranged from 28 g/m^2 in *Jue* to 103 in *Ty*.

It is interesting to note that, except for *Ty*, the % of nitrogen in belowground tissues in all species is lower than the % detected in the aerial part.

Table 31 - Dry matter and nitrogen in the roots of plants; different letters indicate significant differences at P = 0.05 by Student-Newman Keuls test

species	dry matter (roots) (g/m^2)				% nitrogen roots				nitrogen in roots (g/m^2)			
Cae	4675	a	±	1319	1.1	b	±	0.2	48	bc	±	9
Jue	2395	b	±	225	1.2	ab	±	0.2	28	c	±	4
Pha	4229	ab	±	774	1.5	a	±	0.1	65	bc	±	12
Phr	6373	a	±	3613	1.4	ab	±	0.3	83	ab	±	39
Ty	6367	a	±	653	1.6	a	±	0.3	103	a	±	17

Microbiological results

The CFU/g detected in gravel extracts were significantly lower than those detected in roots extracts, confirming that roots offer a more attractive environment for microorganisms than the bare gravel.

The values detected in gravel ranged from 9.01E+06 in *Pha* to 2.28E+06 in the control (Figure 74). Considering the roots the values were higher than 1.00E+7 - 1.00E+8 CFU/g detected by Calheiros et al., 2009. In fact *Phr* showed apparently the highest quantity of CFU/g reaching the value of 1.08E+11, followed by *Pha* with 8.66E+10, *Jue* with 5.30E+10, *Ty* with 4.00E+10 and *Cae* with 1.035E+10 (Figure 75).

In a similar experiment, Vymazal et al. (2001) observed that there were significantly more bacteria on *Phragmites australis* (Cav.) Trin. roots than on the roots of *Phalaris arundinacea* L., confirming the trend of our results.
Data collected for the same species showed a large variability probably associated with the small sample size or with sample heterogeneity that may sometimes not be representative. The methodology should be improved for further microbiological investigations, trying, for example, to better homogenize the sample or to analyze a wider amount of samples.

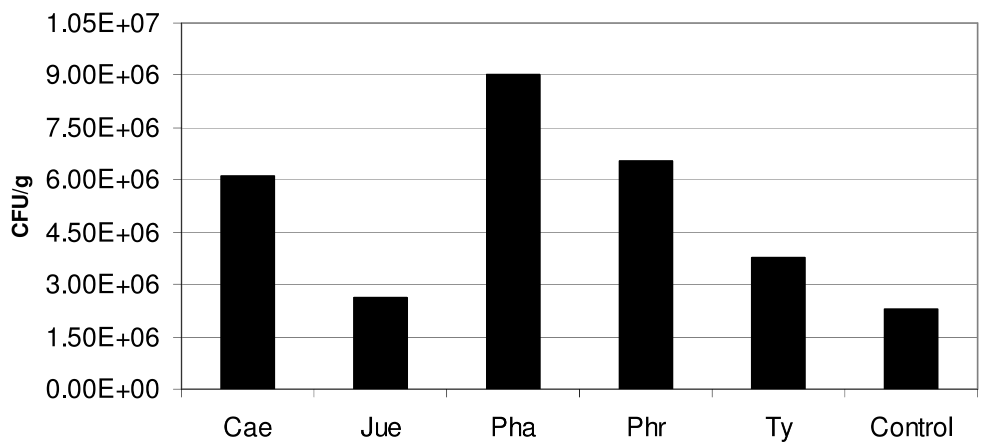


Figure 74 - CFU/g detected in gravel extracts

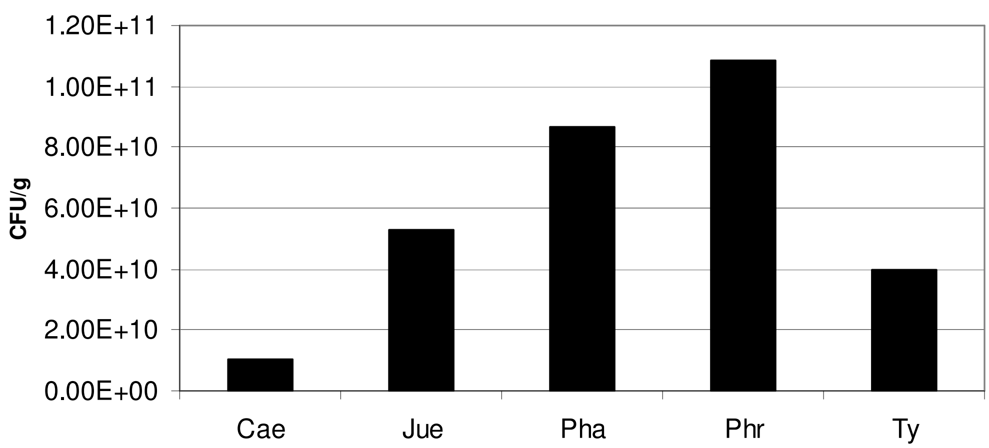


Figure 75 - CFU/g detected in roots extracts

Considering *Pseudomonas* detected in roots extracts (Figure 76), there were no significant differences in CFU/g among *Pha*, *Cae* and *Jue;* these plant species showed higher values with respect to *Phr* and *Ty*, suggesting a more intense denitrification operated by this genus of organisms in association with the former three species.

In gravel samples (Figure 77), *Ty* and *Pha* showed the higher value of *Pseudomonas* colonies with respect to the other treatments. The most suitable treatment for denitrification by *Pseudomonas,* considering both roots and gravel, seemed to be *Pha*.

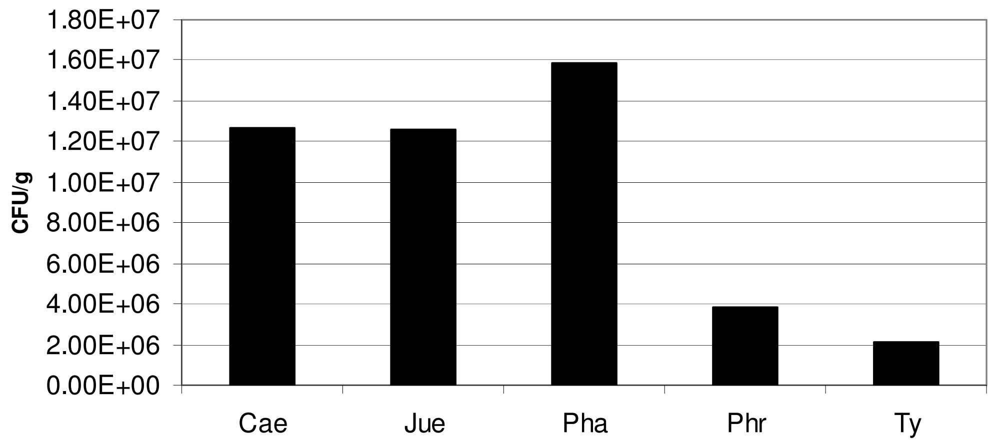


Figure 76- CFU/g of *Pseudomonas* detected in roots extracts

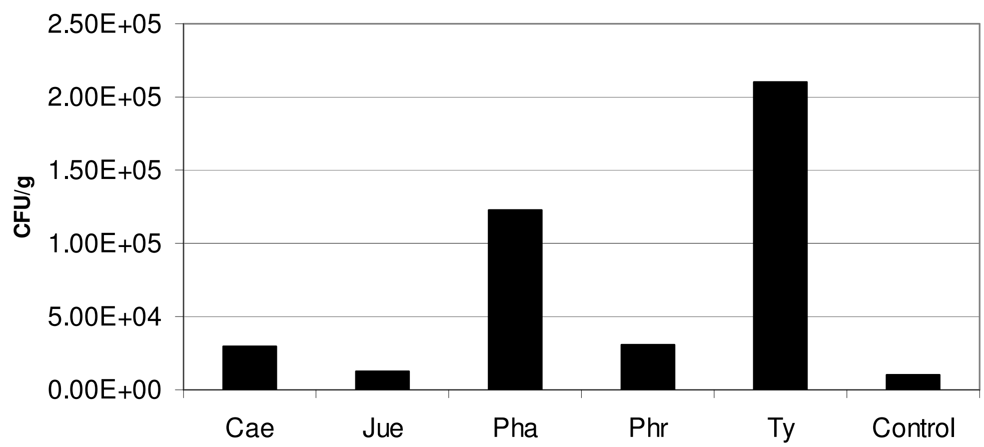


Figure 77 – CFU/g of *Pseudomonas* detected in gravel extracts

Nitrogen stored in plants

Plants played a direct very important role in nitrogen removal. Considering the aerial part harvested in 2008 and 2009 and the root compartment (nitrogen in biomass and biofilm) the amount of nitrogen stored in vegetation is reported in Figure 78. Different species showed different allocation. All the species increased the nitrogen stored in the aerial part from 2008 to 2009 highlighting that they were not well developed in 2008.

The amount of nitrogen roots biofilm was very similar for all the species.

Among species, *Cae* (together with *Ty*) showed the major quantity of nitrogen stored in the aerial part in 2009 but it had a low amount in belowground biomass. *Jue* had an intermediate quantity of nitrogen stored in the aerial part in 2009 and a very low amount in belowground biomass (it was probably suffering from high nitrogen loads in 2009). *Pha* had most of its nitrogen in the aerial part in 2009 and an intermediate amount in the belowground biomass. *Phr* had a similar quantity of nitrogen as *Pha* but most was in the belowground tissues. Lastly, *Ty* stored the highest amount of nitrogen both in belowground tissues and in the aerial part in 2009 with respect to other species, instead the harvestable part was very low in 2008.

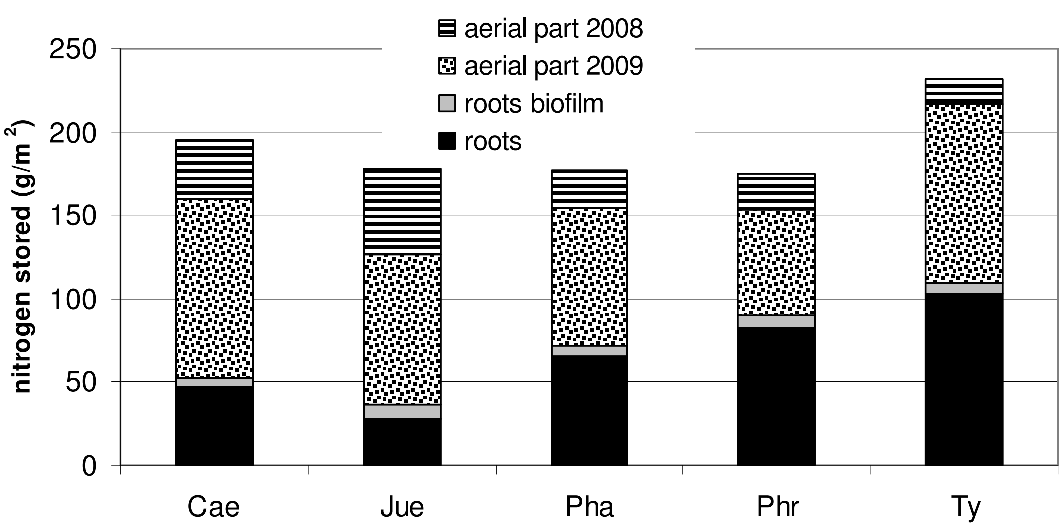


Figure 78 – Nitrogen allocation in plants

More than half of the disappeared nitrogen was stored in plants biomass (Table 32). There were significant differences among species in the amount of nitrogen removed stored in tissues and the highest value was detected in *Ty* (78%) and the lowest in *Pha* (57%).

The rate of harvestable nitrogen on removed was significantly different among species with the highest value detected in *Jue* (61%) and the lowest in *Phr* (38%).

Table 32 - % of removed N ± std stored in the entire plant and in the harvestable part.

	stored/removed				**harvestable/removed**			
Cae	66%	a	±	4%	49%	ab	±	5%
Jue	73%	a	±	17%	61%	a	±	16%
Pha	57%	a	±	7%	34%	bc	±	4%
Phr	58%	a	±	14%	30%	c	±	4%
Ty	78%	a	±	5%	43%	bc	±	6%

Nitrogen balance

The nitrogen balance was calculated taking into account the data collected in 2008 and 2009 excluding the first year because of the changes in the experimental design and in order to consider more real environmental conditions (open air).

The following equation was used:

N input = N output + N harvested in 2008 + N harvested in 2009 + N stored in roots + N gravel biofilm + N roots biofilm + N gaseous losses

All the equation's variables were measured except the gaseous losses consequently this term was calculated by the difference, giving values ranging from 54 g/m^2 in *Jue* to 113 in *Pha* (Table 33).

Table 33 – Nitrogen allocation ± std expressed in g/m^2

	input	output	harvest 2008	harvest 2009	roots	gravel biofilm	roots biofilm	gaseous losses
Cae	308	19 ± 5	35 ± 2	107 ± 18	48 ± 9	1.7 ± 1.3	5.3 ± 3.7	92 ± 14
Jue	308	71 ± 26	51 ± 12	91 ± 15	28 ± 4	5.0 ± 2.7	8.3 ± 3.7	54 ± 47
Pha	308	22 ± 17	16 ± 10	82 ± 7	65 ± 12	2.8 ± 2.4	6.9 ± 5.0	113 ± 19
Phr	308	25 ± 28	22 ± 12	63 ± 17	83 ± 39	4.1 ± 6.3	7.3 ± 7.9	103 ± 31
Ty	308	10 ± 3	22 ± 9	107 ± 13	103 ± 17	2.7 ± 2.1	6.2 ± 3.6	57 ± 15

Figure 79 represents the fate of nitrogen entering the different species.

Nitrogen that left the system with the discharged water ranged from 3% in *Ty* to 23% in *Jue*, underlining again that *Jue* was the species with the worst R.E.

Nitrogen stored in the aerial part of all species ranged from 20% in *Phr* to 35% in *Ty* and *Cae*.

Nitrogen stored in the belowground biomass ranged from 9% in *Jue* to 33% in *Ty* and there was lastly the nitrogen in roots and gravel biofilm that ranged from 3% to 5%.

The missing quantity of nitrogen was estimated as having been lost as gaseous nitrogen and ranged from 18% in *Jue* to 37% in *Pha*.

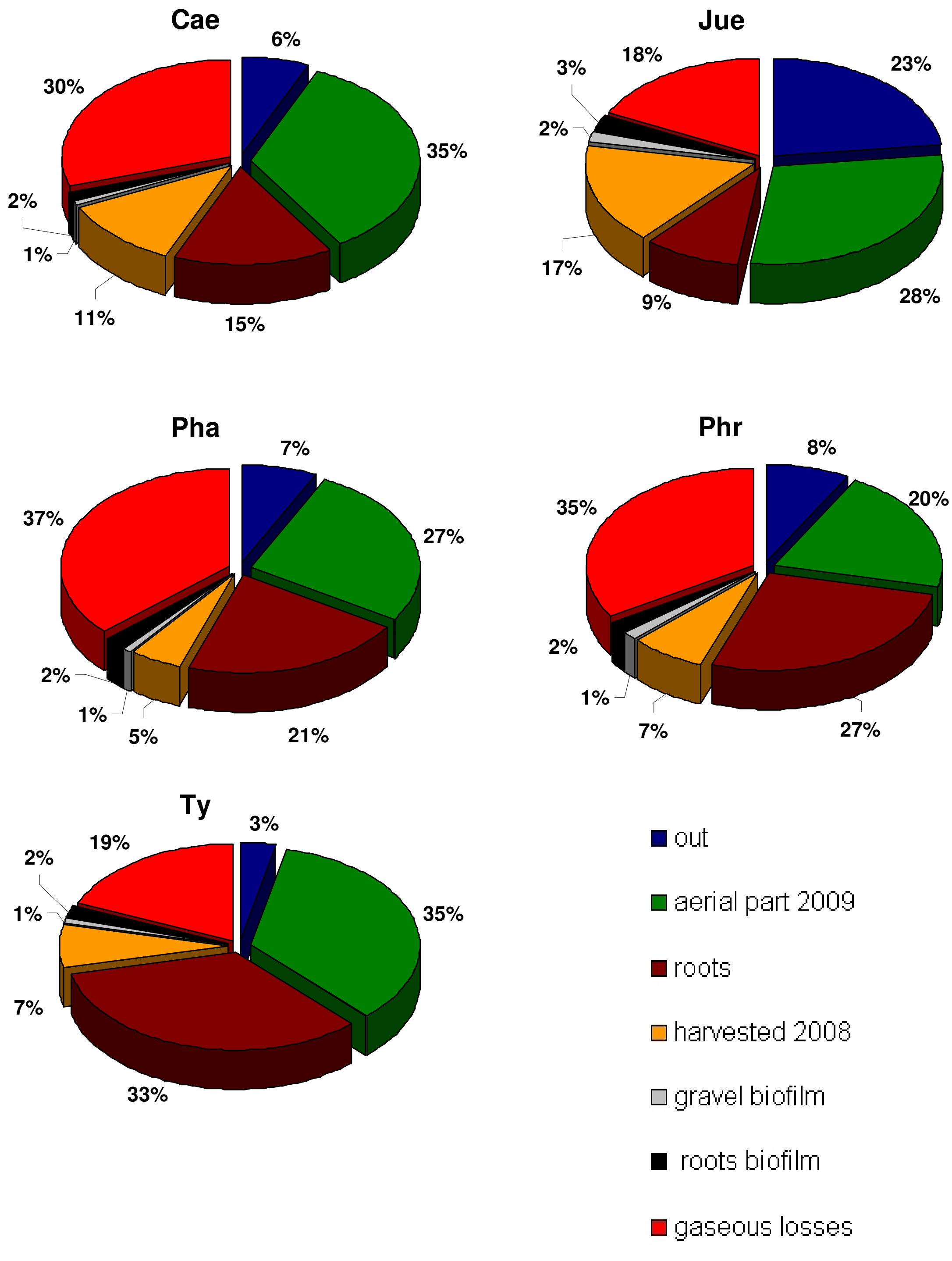


Figure 79 – Fate of entering nitrogen (%) in different species

CHAPTER IV Discussion and conclusions

The pilot scale experiment on the surface flow wetland pointed out that it was able to abate successfully NO_3-N and TOT N coming from an agricultural area in terms of both concentration and mass balance with an average reduction capacity of around 90% of input loads.

Most of the nitrogen disappearance was due to accumulation in plants and soil, with only a minor contribution that can be attributed to denitrification confirming the findings of a previous monitoring period from 1998 to 2002 (Borin and Tocchetto, 2007).

This suggests that this surface flow wetland is able to give a stable performance over the years. The stability can also be connected to the fact that surface flow constructed wetlands are not subjected to clogging processes that negatively affect the behaviour of subsurface flow wetlands (Cooper 2009).

This high efficiency of the basin over the years and the low creation and maintenance costs increase the interest in constructed wetlands as an effective and sustainable tool for reducing agricultural nitrogen loading to surface waters. In this specific case the size of the constructed wetland, about 5% of the catchment area, is relatively large if compared to other small wetlands used for treating agricultural drainage waters (e.g. Kovacic et al., 2000; Tanner et al., 2005), but it was due to the necessity of discharging into the stream only by gravity. In other conditions the same volume could be obtained by deepening the cell, allowing less farmland to be dedicated to wetland treatment. This suggests that small wetlands can be scattered over a farming area to offer treatment for diffuse pollution, provided that they are correctly sited to intercept significant amounts of agricultural drainage (Crumpton, 2001).

In the mesocosm experiment the aim was to study different species suitable for vegetated constructed wetlands, especially the role of vegetation in nitrogen removal and to add information on different nitrogen agricultural wastewaters.

By the end of three years of monitoring using a reconstructed wastewater with ammonium-nitrate the average removal efficiency of TOT N in vegetated treatments was 76% against 48% in the unvegetated control (Table 34) according to e.g. Yang et al., 2001, Fraser et al., 2004, Chung et al., 2008. The removal efficiency of the tested species increased year by year apart for *Juncus effusus* L. that is the only specie that showed to suffer high nitrogen concentration input. In particular *Carex elata* All. and *Typha latifolia* L. showed the highest increase in the removal efficiency passing from the first to the second year and then remained more or less constant. *Phragmites australis* (Cav). Trin. and *Typhoides arundinacea* (L.) Moench, instead, showed a slower increase of R.E., year by year probably connected with a delayed maturity stage.

Table 34 – TOT N input loads in different experimental periods and R.E of all the treatments

	2007	**2008**	**2009**	**Average**
TOT N input (g/m^2)	**137.7**	**85.9**	**222**	**148.5**
Cae R.E.	39%	94%	93%	**77%**
Jue R.E.	35%	82%	75%	**64%**
Pha R.E.	48%	81%	98%	**79%**
Phr R.E.	42%	77%	97%	**77%**
Ty R.E.	52%	96%	97%	**83%**
Vegetated treatments	43%	86%	92%	**76%**
Control	24%	48%	63%	**48%**

The two studied forms of nitrogen showed different disappearance dynamics.

NH_4-N removal efficiency was very high ranging from 91% to 100% for all the treatments included unvegetated control. This high removal efficiency may also be related to the water management of the tanks which were frequently (at least once a week) emptied and refilled, favouring oxygenation and nitrification in turn. This water management scheme mimics vertical subsurface flow systems, which are well known to be effective in ammonium removal.

The NO_3-N average removal efficiency of vegetated treatments was 40% while in absence of vegetation was practically 0, highlighting the contribution of plants according to many authors (e.g. Lin et al., 2002, Huett et al., 2005).

Behaviour differed among vegetated treatments depending on the applied concentration and age of the plants:

-with an input concentration of about 50 ppm there were no significant differences among species and the removal efficiency varied from 8% in *Carex elata* All. to 21% in *Typhoides arundinacea* (L.) Moench;

-with an input concentration of about 100 ppm, significant differences were detected in the first year between *Typha latifolia* L. (removal efficiency 8%) on one side and *Carex elata* All. (-31%) and *Juncus effusus* L., (-44%) on the other; conversely, removal efficiency was higher in 2008 (ranging from 59% in *Phragmites australis* (Cav). Trin. to 90% in *Carex elata* All.) without significant differences among species. The improved performance can be attributed to the bigger plants in their second growing season;

-with an input concentration of 200 ppm the removal efficiency of vegetated treatments ranged from 87% (*Carex elata* All.) to 97% (*Phragmites australis* (Cav). Trin.), with the exception of *Juncus effusus* L. (45%).

Since water chemical analyses were conducted at different times elapsed from input load it was possible to determine that the weekly cycle seemed to be adequate for a good nitrogen removal efficiency, showing different dynamics for the two studied forms of the element. In fact NH_4-N was almost completely and quickly removed in both the cold and warm season while NO_3-N removal efficiency was lower in the winter.

An important finding of this study is that, unlike the other tested species, *Juncus effusus* L. is not a suitable species for removing nitrogen, especially with high and prolonged concentration inputs.

Extending these results to other types of agricultural effluents, such as untreated dairy farm effluent, and treated meat effluent with typical NH_4-N concentrations respectively of about 170 ppm and 115 ppm (Bhandral et al., 2007) *Carex elata* All. , *Typhoides arundinacea* (L.) Moench, *Phragmites australis* (Cav). Trin. and *Typha latifolia* L. seem to be adequate species to treat these kind of wastewaters.

The most important nitrogen removal processes were plants uptake and denitrification.

Concerning plant tissues, the concentration of nitrogen in the aerial part was lower with respect to the literature in the first and second year but increased the last year fitting literature values (Kadlec and Knight, 1996). The % of nitrogen removed from different treatments (considering the 2008-2009 period) and stored in plant biomass ranged from 51% in *Juncus effusus* L. to 70% in *Typha latifolia* L. with an average value of 55%. These values are very high if compared with some results in the literature (Lin et al., 2002; Gotscahll et al., 2007; Paredes et al., 2007) but in agreement with other studies (Breen, 1990; Huett et al., 2005; Borin and Tocchetto, 2007). The harvestable part of this nitrogen stored in the aerial part represented from 30% in *Phragmites australis* (Cav). Trin. to 61% in *Juncus effusus* L., suggesting the importance of different macrophytes in the case of harvesting as nitrogen removal practice.

The calculated nitrogen gaseous losses can be a good estimate of denitrification losses; they ranged from 18% with *Juncus effusus* L. to 37% with *Typhoides arundinacea* (L.) Moench, also in this case underlining the different roles of the macrophytes.

Another important finding of the mesocosm experiment was the big influence of ET in the hydrological regimes of the different treatments. This phenomenon, reducing water volume, hardly affects the nitrogen concentration in water output underestimating the efficiency of vegetated treatments if mass balance is not taken into account. In spite of this the median ammonium concentration in the effluent was always at least 200 times lower than the input. The median output value of nitric nitrogen was instead at a maximum 1.2 times higher than input. Reduction of output water volume could be a positive effect if the constructed wetland was designed to act as a zero discharge system. On the other hand the high ET rates measured in the summer could be a problem if wetland output water has to be reused. For these reasons it was useful to calculate the K_c of different tested species. The calculated values of K_c, ranging from 2.8 for *Typhoides arundinacea* (L.) Moench in 2008 to 6 for *Typha latifolia* L. in 2009 (average of the considered periods), were very high if compared to the typical K_c for agricultural crops, which in most cases are in the range 0.9-1.2. This finding has to be considered as preliminary and requires confirmation, but agrees with the rationale that inspires the K_c for agricultural crops, which is a way to transform the reference ET into the effective crop water requirements. Indeed, according to the FAO 56 approach, the K_c for a given crop is variable over the growing season but is reasonably the same under very different environmental conditions. In our case the K_c assumes different patterns and values in relation to plant age and growth stage. In fact with younger plants, still not completely established, K_c is lower but increases continuously over the growing season, while with mature plants the time pattern of K_c is characterised by a first part with increasing values followed by a part with constant values and a final tendency to decreasing values. If the K_c approach and values calculated for the studied species were validated in other conditions, the amount of water dispersed by a wetland could easily be calculated and then taken into account in the design and in the prevision of wetland performance.

In order to study the influence of environmental variables on the nitrogen removal efficiency, temperature, dissolved oxygen, pH and redox potential were measured at least once a week.

Clear correlations between nitrogen removal efficiency and temperature were only found in the first year. This may be due to the fact that in the first year the experiment, conducted in a glasshouse, covered a wider range of temperature conditions, running from January 2007 to March 2008. As a consequence it included cold months while in the following years the study was only done during the spring-autumn period. More in detail, the average temperature of the experimental periods was 17.5 °C in the glasshouse, 18.9 °C in the open air in 2008 and 21.7 °C in the open air in 2009. In the same periods the monthly excursions were 36.4°C, 26.1°C, 24.0°C respectively and the absolutes were 47.4°C, 41.2°C, 31.2°C confirming the first year as the one with wider temperature variation.

Temperature has long been recognized as affecting plant metabolism and denitrification rates (Bremner and Shaw, 1958; Beauchamp et al., 1989) and this is also confirmed by this experiment, but only including cold months. In December 2007 the denitrification rate was lower than previous periods for all the studied species (Table 16) and this was also confirmed in February 2008 for *Phragmites australis* (Cav). Trin.. Moreover, *Phragmites australis* (Cav). Trin. and *Typhoides arundinacea* (L.) Moench, also showed correlations between nitrogen removal efficiency and temperature in 2009, pointing out that species belonging to the family of *Poaceae* may be more affected by temperature.

Similarly to temperature, the effect of other environmental conditions (O_2, pH redox) also showed some influence on nitrogen removal efficiency only during the first season, when both oxygen concentration and pH were positively correlated with NH_4-N removal efficiency of vegetated treatments.

The preliminary investigation on microbial communities associated with roots and gravel detected higher values with respect to the values found in the literature (Calheiros et al., 2009), highlighting a higher microbial presence in the tanks vegetated with *Phragmites australis* (Cav). Trin. and *Typhoides arundinacea* (L.) Moench, which were also the species with the highest estimated denitrification as resulted from mass balance (Figure 79).

On the other hand considering *Pseudomonas*, a genus highly involved in the denitrification process, it was very well represented in roots extracts of *Typhoides arundinacea* (L.)

Moench, confirming that there are microbial communities able to denitrify associated with this plant species. The lower concentration of *Pseudomonas* colonies in *Phragmites australis* (Cav.) Trin. suggested that for this species other genera of microorganisms, such as *Thiobacillus denitrificans* (Justin and Kelly, 1978) or *Hyphomicrobium* strain *X* (Meiberg et al., 1980), could be involved in the denitrification process. The microbiological analyses conducted in this study didn't take these microorganisms into account so further analyses will be necessary to support the hypothesis. High data variability found for the same species and the fact that a large quantity of microorganisms cannot be cultivated with the plate count technique suggest that more attention had to be paid in sampling and that other techniques, such as the genomic method or estimation of enzymatic activity, could be useful to study this important subject further.

The common specie of the two experiments was *Phragmites australis* (Cav.) Trin. The average yearly TOT N entering load was more than 5 times higher in the mesocosm respect with the surface flow wetland (Table 35). In spite of this the average yearly R.E. in the mesocosm remained quite high 72%. But, to represent the R.E. of plants well developed and mature, only the last year 2009 has to be taken into account. Summarizing, *Phragmites australis* (Cav.) Trin. considered in its mature growing stage, performs very well in abating both low and high total nitrogen loads both in mesocosm and in pilot surface wetland.

Table 35 – TOT N yearly load in surface constructed wetland and in mesocosm and the calculated R.E. of *Phragmites australis* (Cav.) Trin.

	SFW		**Mesocosm**	
	TOT N input kg/ha	R.E.	TOT N input kg/ha	R.E.
2007	219	90%	1377	42%
2008	219	97%	859	77%
2009	382	92%	2220	97%
average	273	93%	1485	77%

An objective of the two experiments was to identify the main removal pathways of nitrogen in constructed wetlands fed with agricultural wastewater using mass the balance approach.

In the pilot surface flow wetland both dry matter and % of nitrogen in tissues in *Phragmites australis* (Cav.) Trin. were lower with respect to the mesocosm experiment and consequently the average amount of nitrogen stored in plants biomass was 43.5 g/m^2, whereas in the mesocosm it was more than three times higher (146 g/m^2). In the mesocosms for other species it varied from 119 g/m^2 of *Juncus effusus* L. to 210 g/m^2 of *Typha latifolia* L..

This was surely the result of different entering nitrogen loads and very different conditions connected with the presence of soil in the surface flow wetland and gravel in the mesocosm.

The results of the two experiments at different scales were also quite different regarding % of nitrogen removed by denitrification.

In the pilot surface flow wetland it was estimated at around 6%, while in the mesocosm experiment it ranged from 18% in *Juncus effusus* L. to 37% detected in *Typhoides arundinacea* (L.) Moench. (*Phragmites australis* (Cav.) Trin. 35%). This finding is not surprising in particular taking into account the age of the two facilities and the different loading rates. It is already known that denitrification is greater in young constructed wetlands than in mature constructed wetlands (Sirivedhin and Gray, 2006) and that high nitrate concentrations input enhances denitrification rates in wetlands (Hanson et al., 1994; Lowrance et al., 1995; Willems et al., 1997; Sartoris et al., 2000).

Moreover the soil is a more complex system than gravel and influences nitrogen processes in a large number of pathways that are impossible to control. It supports a wide variety of oxidation and reduction reactions (e.g. ferric–ferrous iron conversion) and presents chemical and biochemical conditions that are strongly driven by redox potential; its pH influences many biochemical transformations of carbonates and ammonium, and controls the solubility of gases (Kadlec and Wallace, 2008). Finally the soil accumulate a great deal of nitrogen in organic matter (present study; Passoni et al., 2009) subtracting the element to denitrification.

Looking at other links between the two experiments it is possible to evaluate what the performance and management of the pilot surface wetland might be like if other species were used.

The first aim of constructed wetlands is to abate nitrogen loads in the discharged water. This goal could be achieved in the range from 92% (*Phragmites australis* (Cav.) Trin.) to 97% (*Typha latifolia* L.) (Figure 79) with all the tested species in the mesocosm experiment apart from *Juncus effusus* L. that showed a R.E. of 75% and is therefore not a suitable specie and will be excluded in the following considerations.

Taking into account the fate of entering nitrogen the choice of species depends on the common management of surface wetland and on the preferred process of nitrogen removal.

If harvesting is the preferred nitrogen removal process, the best species is *Carex elata* All. because 49% of entering nitrogen was taken away by harvesting. This amount of harvestable nitrogen ranged, in the other species, from 30% in *Phragmites australis* (Cav.) Trin. to 43% in *Typha latifolia* L.. On the other hand, if denitrification is the preferred process the best species are *Typhoides arundinacea* (L.) Moench and *Phragmites australis* (Cav.) Trin.) (denitrification 37% and 35% of entering nitrogen), followed by *Carex elata* All. (30%).

But in the end the more important objective is to remove nitrogen in a definitive way. This can be achieved by denitrification and harvesting. The % of entering nitrogen removed by these two processes together ranged from 60% with *Typha latifolia* L. to 76% with *Carex elata* All., identifying this latter species as the best performing.

In conclusion, the pilot surface flow wetland performs well in abating low nitrogen loads but, as suggested by the mesocosm experiment, it is reasonable to expect that it will also perform satisfactorily in abating much higher nitrogen loads.

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