REPORT

Effects of Vegetation and Glyphosate on Denitrification in Constructed Wetlands (Work Package 3)
Project acronym: AQUISAFE 1

by
P.-A. Jacinthe, P. Vidon, L. P. Tedesco
IUPUI Center for Earth and Environmental Science
and Department of Earth Sciences
&
B. Krause, A. Weigert, N. Litz
Department for Water Treatment, German Federal Environmental Agency
for
Kompetenzzentrum Wasser Berlin gGmbH

IUPUI INDIANA UNIVERSITY PURDUE UNIVERSITY INDIANAPOLIS

Umwelt Bundes Amt Für Mensch und Umwelt

KOMPETENZZENTRUM Wasser Berlin

.

Berlin, Germany, 2009

Important Legal Notice
Disclaimer: The information in this publication was considered technically sound by the consensus of persons engaged in the development and approval of the document at the time it was developed. KWB disclaims liability to the full extent for any personal injury, property, or other damages of any nature whatsoever, whether special, indirect, consequential, or compensatory, directly or indirectly resulting from the publication, use of application, or reliance on this document. KWB disclaims and makes no guaranty or warranty, expressed or implied, as to the accuracy or completeness of any information published herein. It is expressly pointed out that the information and results given in this publication may be out of date due to subsequent modifications. In addition, KWB disclaims and makes no warranty that the information in this document will fulfil any of your particular purposes or needs. The disclaimer on hand neither seeks to restrict nor to exclude KWB's liability against all relevant national statutory provisions.

Wichtiger rechtlicher Hinweis
Haftungsausschluss: Die in dieser Publikation bereitgestellte Information wurde zum Zeitpunkt der Erstellung im Konsens mit den bei Entwicklung und Anfertigung des Dokumentes beteiligten Personen als technisch einwandfrei befunden. KWB schließt vollumfänglich die Haftung für jegliche Personen-, Sach- oder sonstige Schäden aus, ungeachtet ob diese speziell, indirekt, nachfolgend oder kompensatorisch, mittelbar oder unmittelbar sind oder direkt oder indirekt von dieser Publikation, einer Anwendung oder dem Vertrauen in dieses Dokument herrühren. KWB übernimmt keine Garantie und macht keine Zusicherungen ausdrücklicher oder stillschweigender Art bezüglich der Richtigkeit oder Vollständigkeit jeglicher Information hierin. Es wird ausdrücklich darauf hingewiesen, dass die in der Publikation gegebenen Informationen und Ergebnisse aufgrund nachfolgender Änderungen nicht mehr aktuell sein können. Weiterhin lehnt KWB die Haftung ab und übernimmt keine Garantie, dass die in diesem Dokument enthaltenen Informationen der Erfüllung Ihrer besonderen Zwecke oder Ansprüche dienlich sind. Mit der vorliegenden Haftungsausschlussklausel wird weder bezweckt, die Haftung der KWB entgegen den einschlägigen nationalen Rechtsvorschriften einzuschränken noch sie in Fällen auszuschließen, in denen ein Ausschluss nach diesen Rechtsvorschriften nicht möglich ist.

Colophon

Title

Effects of Vegetation and Glyphosate on Denitrification in Constructed Wetlands

Authors

Pierre-Andre Jacinthe, Philippe Vidon, Lenore P. Tedesco
Center for Earth and Environmental Science, Department of Earth Sciences,
Indiana University – Purdue University, Indianapolis, USA

Björn Krause, Astrid Weigert, Norbert Litz
Umweltbundesamt, Berlin, Germany

Abstract

The Aquisafe project aims at mitigation of diffuse pollution from agricultural sources to protect surface water resources. The first project phase (2007-2009) focused on the review of available information and preliminary tests regarding

(i)   most relevant contaminants,

(ii)   system-analytical tools to assess sources and pathways of diffuse agricultural pollution,

(iii)   the potential of mitigation zones, such as wetlands or riparian buffers, to reduce diffuse agricultural pollution of surface waters and

(iv)   experimental setups to simulate mitigation zones under controlled conditions.

The present report deals with (iv) and evaluates the suitability of the technical scale experimental site at the UBA in Berlin, Marienfelde for simulating processes that impact the fate and transformation of nutrients in wetlands / riparian zones. A 3-month pilot investigation (Sep. to mid Nov. 2007) was conducted in order to assess the impact of vegetation on nitrate (NO_3^-) removal in slow-sand filters (SSFs) and identifying possible interference of glyphosate with N and C cycling processes in these systems. SSFs are engineered bio-reactors that can mitigate the transfer of a wide range of pollutants including nutrients and organic contaminants to water bodies.

Two vertical-flow experimental SSFs (average area: 60 and 68 m^2, depth: 0.8 and 1.2 m, respectively) at the UBA facilities in Berlin were used in this study: one unplanted and the other vegetated with *Phragmites australis*. The SSFs received water amended with nitrate (NO_3^-) and phosphate (PO_4^-) without and with glyphosate (added for 2 weeks). Mineral N concentration at the mixing cell, SSF surface, 40 cm depth and at the SSF outlet was measured at least twice per week to calculate N removal rates. Physical water properties (pH, redox potential, temperature) and greenhouse gas emission (CO_2, CH_4 and N_2O) were also monitored to gain insights into controlling processes.

Results showed that N removal rates were several-fold higher in the vegetated than in the non-vegetated SSFs averaging 663 mg N m^{-2} d^{-1} (57 % of input) and 114 mg N m^{-2} d^{-1} (14 % of input), respectively. In both systems, most of the N removal occurred in the top 40 cm of the SSFs. Marked temporal variation in N removal rates was also detected with rates in general 3 times higher in late summer compared to mid/late autumn. In the latter period, a net release of N was observed in the non-vegetated SSF. The seasonal variation in N removal could be related to a lack of vegetation growth and thus plant N uptake, and may also reflect of the sensitivity of denitrification to climatic factors as suggested by strong ($r^2 > 0.77$) linear relationships between weekly N removal rates and SSF water temperature.

A clear impact of glyphosate addition on nitrate concentrations could not be observed. Denitrification, the process most responsible for the removal of nitrogen from waters and soils seems to be unaffected by the addition of glyphosate under the conditions in the experiment. The impact of glyphosate, if any, was probably much smaller compared to the strong influence of temperature on N dynamics in the SSFs. Difficulty of maintaining a constant concentration of glyphosate during dosing may have also contributed to this outcome.

Nitrous oxide emission accounted for < 3 % of the total N removed was always lower in the vegetated (< 0.1 - 0.3 mg N_2O-N m^{-2} d^{-1}) than in the non-vegetated SSF (0.2 - 3.8 mg N_2O-N m^{-2} d^{-1}). Conversely, CH_4 emission was always higher in the vegetated (range: +0.4 to +49.5 mg CH_4-C m^{-2} d^{-1}) than in the non-vegetated SSF (range: -2.1 to +1.32 mg

CH$_4$-C d^{-1}). These results, in connection with much lower oxidation reduction potential readings in the vegetated filter, suggest that the reduction of N$_2$O to N$_2$ was important in the SSF systems and that N$_2$ was the dominant N gas produced. Thus, N$_2$ production must be quantified in order to establish N mass balance of SSF systems.

The results show that technical-scale experiments can realistically simulate mitigation systems, while having control over contaminant loading, flow conditions and monitoring. Important lessons learnt for future applications are the following

- Denitrifying conditions can be established in both SSF of the experimental site by adjusting to low flow conditions (0.23 m³/h) and dosing nitrate.

- Dosing of trace contaminants (in this case glyphosate) needs to be improved, but will remain difficult for the large amounts of water involved. The results underline the importance of measurements in the mixing cell.

- Since seasonal effects play an important role in mitigation zone performance, any experiments need to be done in parallel, rather than in succession to be able to compare the results.

Acknowledgements

Table of Contents

List of Figures

List of Tables

List of Equations

$$N_r = \frac{(C_{in} - C_{out})(Q)}{A}$$

$$\%R = \frac{(C_{in} - C_{out})}{C_{in}} 100$$

Chapter 1

Introduction

1.1 Context

The purpose of this document is to describe and summarize the results of a pilot study conducted under WP3 of AQUISAFE 1. The overall aim of this research program was to assess the efficiency of constructed wetlands and riparian corridors for the removal of water pollutants. A pilot study was conducted to determine the suitability of the technical scale experimental site at the UBA in Berlin, Marienfelde for simulating processes that impact nutrient removal in wetlands / riparian zones. The experiments carried out, focussed on nitrate and glyphosate and were conducted between July and December 2007 at the UBA Slow Sand Filters (SSF) in Berlin.

1.2 Background

1.2.1 Nitrate removal and trace gas emission from constructed wetlands and

riparian zones

The export of nitrogen (N) from terrestrial landscapes affects aquatic ecosystems worldwide. While progress has been made in controlling point-source pollution, diffuse pollution (also known as non-point source) continues to pose a major challenge to water quality management. This challenge arises from, among other things, the diversity of the chemicals involved and the multitude of pathways by which these compounds enter water resources. Agriculture and urban runoff are major contributors to diffuse water pollution. This pollution is associated with nitrate (NO_3-) - a regulated and the most widely distributed water pollutant- but also involves the discharge of phosphorus, numerous pesticides and a wide range of so-called emerging contaminants (pharmaceuticals, personal care products).

Constructed wetlands and riparian zones have received a great deal of attention as a natural, cost-effective and low maintenance approach to deal with diffuse pollution. These systems are designed with the goal of providing optimum conditions for the biotransformation of pollutants into products that are environmentally benign and harmless to human health. The removal of NO_3- in constructed wetlands has been the focus of numerous studies and has been ascribed to denitrification - a process whereby NO_3- is successively converted into the N gases nitrous oxide (N_2O) and elemental nitrogen (N_2). The intensity of the denitrification process in these engineered systems depends on the availability of organic carbon provided via addition organic substrates (e.g. carbohydrates, alcohol…) or C originated from endogenous sources (e.g. vegetation). In addition to providing organic C to support the activity of denitrifiers, vegetation also contributes to N removal via incorporation into their biomass. Unlike plant uptake and microbial immobilization (transient NO_3- sinks), denitrification represents a permanent N removal mechanism as the N gases produced escape into the atmosphere. However, since N_2O is a greenhouse and ozone-deleting gas, limiting N_2O production in these engineered systems is a much desirable outcome. By restricting gaseous diffusion and thus increasing the residence time of N_2O in the wetland soil, the reduction of N_2O into N_2 could be favored and the emission of N_2O minimized. Low concentration of O_2 and NO_3- and alkaline pH are also known to be favorable to that conversion. Thus, constructed wetlands can be designed and operated in ways to achieve these results. Further, anaerobic conditions in constructed wetlands could also be conductive to the

production of methane (CH4), an atmospheric trace gas that also contributes to the greenhouse effect and stratospheric ozone depletion.

Wetland microbes are often concomitantly exposed to NO3- and a wide array of organic pollutants which could alter the diversity, status and functionality of the microbial community. At the present, there is limited information regarding the impact of organic pollutants (e.g. herbicides) on important wetland functions. In the present study, glyphosate was used in field experimentations as a model organic contaminant and its effect on important N and C cycling processes in riparian soils was evaluated.

1.2.2 <u>Glyphosate</u>

Glyphosate is one of the most and widely used herbicides in the world [De Jonge et al. 2001; Vereecken 2005]. It is used in agriculture, forestry and water management, as well as in urban environments [Giesy et al. 2000]. Given this universality of use and dispersion the environment, it is appropriate that intensive research be carried out into the effects glyphosate on biological processes in ecosystems that may not be specifically treated by this agrochemical.

Glyphosate is a phosphoric acid ester with the structural name N-(Phosphonomethyl) Glycine and the sum formula C3H8NO5P. It is a polar compound and highly soluble in water. According to the published data volatilization is negligible—. Glyphosate is removed from the aqueous phase primarily through adsorption onto solid surfaces.

Glyphosate is a total herbicide i.e. it does not distinguish between different types of plants. The substance works systemically. It only makes its way into the organism via the green parts of the plant [Giesy et al. 2000]. Glyphosate works by inhibiting the synthesis of (Acetyl Co-A carboxylase) - an enzyme that occurs in all plants and is responsible for the production of vital amino acids (e.g. phenyl alanine, tyrosine and tryptophane). Without this enzyme, the plant withers and dies [Alibhai & Stalling 2001].

Glyphosate is not genotoxic; there is no evidence of carcinogenic or neurotoxic effect. Observed toxicity has generally been associated with the heavy doses used in some experiments; such high concentrations do not normally occur in natural ecosystems. Glyphosate has a low octanol - water coefficient, meaning that, unlike several other organic chemicals, it has a low propensity towards bioaccumulation. For this reason, the risk of food chain contamination can be considered negligible.

The herbicidal activity of glyphosate is strongly reduced when it comes into contact with soil. This effect has been ascribed to the strong adsorption of glyphosate onto the solid matrix [Hance 1976]. In the slightly acidic environment of most soils, glyphosate occurs with a surplus of negative charges (anion). Since anionic sorption capacity of soils depends on aluminium- and iron oxides [Scheffer & Schachtschabel 2002], it can be assumed, that the concentration of these oxides will determine immobilisation of glyphosate through adsorption [Sheals et al. 2002]. Due to structural similarity with glyphosate, phosphate could be a competing ion for sorption sites. It has been proposed that that glyphosate and phosphate adsorb onto solid phases using a similar mechanism [Hance 1976]. The effect of phosphate of the behavior and mobility of glyphosate is quite relevant given the natural occurrence of phosphate in soil, either due to fertilizer application or as a result of organic matter mineralization. Although less important than oxides, organic soil components could also play a role in glyphosate adsorption [Glass 1987, Dion et al. 2001 & Piccolo et al. 1994], and this probably metal ion bridges between glyphosate and humic acids [Mensink & Janssen 1994].

A wide range of soil microorganisms, including bacteria, actinomycetes, fungi can degrade glyphosate, but bacteria seem to play the leading role [Forlani et al. 1999]. Chemical hydrolysis, thermal decomposition, and photolysis have only very little influence on the degradation of glyphosate [Nomura & Hilton 1977, Sprankle et al. 1975], and this seems to be associated with the very stable C-P bonds in glyphosate [Pessagno et al. 2005]. However, photolysis could become important in aquatic ecosystems [Brønstad and Friestad 1985].

1.3 Objectives

Thus, the objectives of the experiment described in this report were to:

1. Determine the suitability of the technical scale experimental site at the UBA in Berlin, Marienfelde for simulating processes that impact nutrient removal in wetlands / riparian zones

2. Assess the impact of vegetation on the nitrate removal efficiency and trace gas emission from constructed wetlands (vegetated vs non-vegetated).

3. Evaluate interactions (synergistic or inhibitory) of glyphosate on N and C cycling processes in constructed wetlands.

Chapter 2

Materials and Methods

2.1 Study Site

This study was conducted at two experimental Slow Sand Filters (SSF) at the UBA research facilities in Berlin (Centre of Aquatic Simulations). One of the SSFs was vegetated with Phragmites australis and the other was non-vegetated. The SSFs were slightly slanted on the side (Fig. 1). Dimensions of the SSFs are given in Table 1.
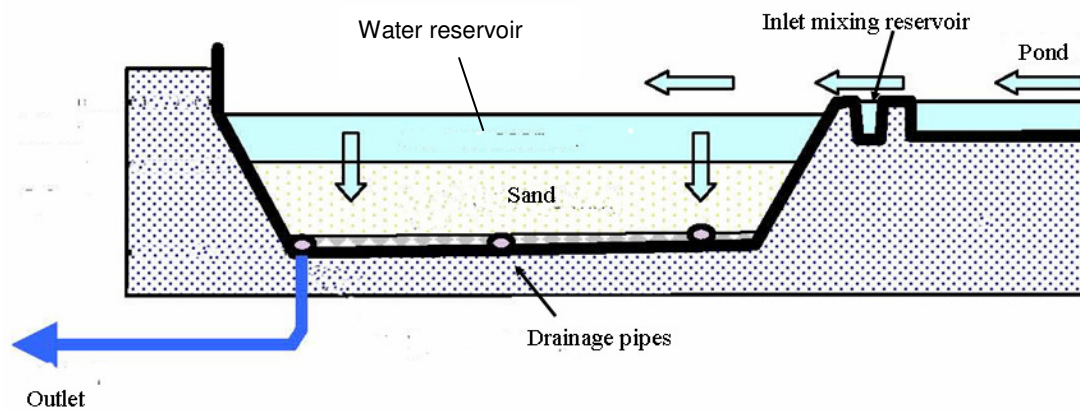
Fig. 1: General sketch of the Slow Sand filter (SSF) showing the inlet reservoir and general water flow path.

Tab. 1: Characteristic dimensions of the SSFs (without the water reservoir).

Value	Non-vegetated	vegetated
Surface [m²]	90	90
Bottom [m²]	56	56
Average [m²]	60	68
Depth [m]	0.8	1.2
Volume [m³]	48	81.6

The SSFs were continuously fed with pond water through an inlet mixing reservoir (or mixing cell) into which nutrients and organic contaminants were added during the various phases of the experiment. Water flow through the SSFs was predominantly vertical. The SSFs are sealed at the bottom, and drainage pipes collect and discharge water at a single outlet. The water flux of both SSFs was regulated at the inlet and controlled by measurements at the outlet. During the study, each SSF was instrumented (Fig. 2) with 4 static chambers to monitor trace gas fluxes, and 4 multi-parameter probes (YSI) installed at the surface of the SSF and at 40 cm depth for continuous measurement of pH, temperature, dissolved oxygen and redox potential (ORP). Two additional YSI probes were also installed at the mixing cell and outlet of each SSF. Also at the outlet,

an equilibrium headspace device was installed to determine the concentration of dissolved gases in the SSF effluent using Henry's law.

Planar view of equipment installed in EACH cell (no vegetation and vegetation)

Cross section view of sampling location in each corner or each cell (e.g. corner # 1)

Input

Mixing cell (YSI, WQ)

WQ   YSI, WQ

1   4

2   3

YSI, WQ   WQ

Combined drainage output (YSI, WQ, GS)

Water surface
(WQ, GS)
Sediment surface

(WQ) at 40 cm depth

Combined drainage output

— Sampling port at 40 cm depth (4 per cell – Total: 8)

YSI   Multiparameter probe (4 per cell (1 in mixing cell, 2 at 40 cm, 1 at combined drainage outlet) – Total: 8)

WQ   Water quality measurements (nitrate, glyphosate, …) (1 in mixing cell, 4 at the surface, 4 at 40 cm, 1 in combined outlet – Total: 20)

GS   Gas sample (N2O) (5 per cell (4 at the surface, 1 at the combined outlet) – Total = 5 per cell)

O   Surface Air chamber

+   Additional sampling port for water quality at 10 cm, 30 cm and 60 cm depth

Fig. 2: Experimental setup showing the location of sampling points and monitoring devices installed during the study.

A unique aspect of these SSFs is that they provide a setting to study contaminant transport and biotransformation at scales closely similar to field conditions. The SSFs are also engineered in ways that make it possible to monitor water discharge and chemistry, establish mass balance of nutrients and ultimately assess the impact of organic contaminants on N and C cycling processes in wetland and riparian ecosystems. During construction in 2002, the SSFs were filled with coarse sandy material containing low amount of organic carbon (0.02 %). However, after several years of operation a thin mat of biological material (probably from algal growth) has developed on the surface of the non-vegetated filter. Likewise, a layer of litter was also present on the surface on the vegetated SSF.

To assess the hydraulic characteristics of the SSF in more detail, a tracer test with chloride was conducted from July 25, 2007 to August 1, 2007 with a flow rate of 0.23 m³ h⁻¹. Electrical conductivity (EC) at the SSF outlet was monitored and breakthrough times were determined.

In addition to characterization of water flow condition in each SSF, background water quality parameters and trace gas fluxes were also collected during the pre-experiment period.

2.2 Study Phases and Dosing

The pilot study was conducted in three phases referred to phase A, phase B and phase A2. In order to simulate eutrophic conditions in SSFs, a stock solution amended with NO^{3-} and PO_4^{3-} was dosed into the inlet reservoir to obtain targeted concentrations of 10 mg N L^{-1} and 1 mg P L^{-1}, respectively. Exact concentration in the influent was monitored daily, with exception of the weekend. Each SSF was amended with the dosing solution for 1-2 weeks prior to initiating measurement in order allow the wetland soil microbial community to adjust to changes in water quality.

Phase A (Sep. 3 - Oct 8): This phase was designed to equilibrate the filters and test for the effect of vegetation on nutrient retention. Therefore, the influent water was amended only with NO_{3-} and PO_4^{3-}.

Phase B (Oct 8 - Oct 30): In this phase, the effect of glyphosate on nutrient retention was tested. Thus, in addition to NO_{3-} and PO_4^{3-}, influent water was amended with glyphosate (20 µg L-1).

Phase A2 (Oct 31 - Nov 12): This was a repetition of phase A, and was conducted with the objective of assessing the effect of season. For this assessment, data from phase A and phase A2 were compared.

Water samples were collected daily in the inlet reservoir, with exception of the weekend, and twice every week on the surface and in 40 cm depth for analysis of NO^{3-}, NH_4^+, DOC, and PO_4^{3-} at UBA lab. Gas fluxes were also monitored at the same frequency. Air samples were taken at 0, 30 and 60 min in chamber headspace and stored in evacuated crimp-sealed glass vials. Air samples were shipped to Indianapolis for CO_2, N_2O and CH_4 analysis via gas chromatography.

2.3 Computational Approach

During all phases of the experiment, a consistent decrease in NO_3^- concentration was observed as the influent water travelled between the inlet mixing reservoir and the SSF surface (Fig. 3).

Since this lowering (12-19 % decrease on average) in concentration took place outside the SSF proper, the concentration of NO_3^- at the SSF surface was taken as input in our computation of N removal rates. In addition, given the 83 h breakthrough time and our sampling schedule (Thursday and Tuesday), NO_3^- input and output measured on the same sampling date were not compared. Instead, a lag time of 5 days was included in our computation. In other words, NO_3^- input measured on Thursday was compared to NO_3^- output measured the following Tuesday. It follows that our computed N removal rates represent daily rates integrated over a 1-week period.

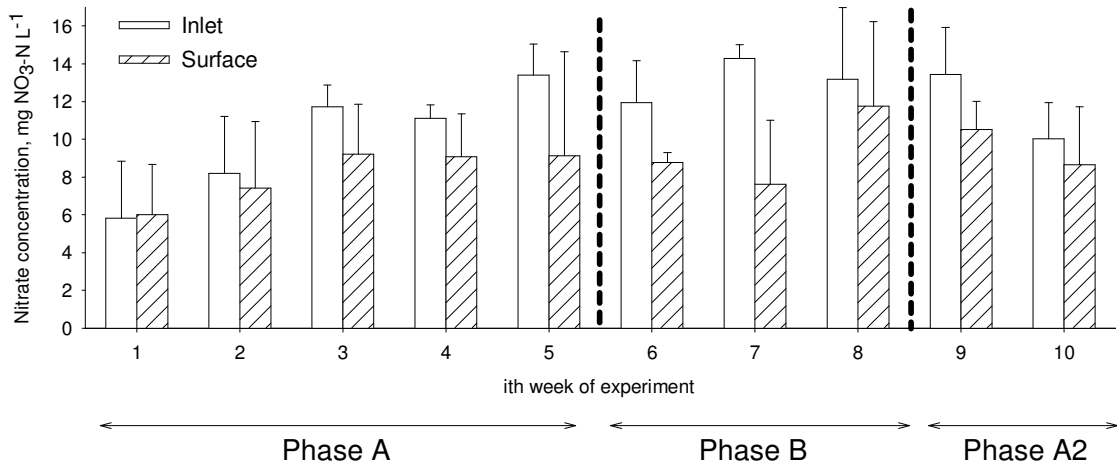Daily rate of N removal (N_r, mg N m^{-2} d^{-1}) was computed as:

$$N_r = \frac{(C_{in} - C_{out})(Q)}{A}$$

Equation 1

(A)
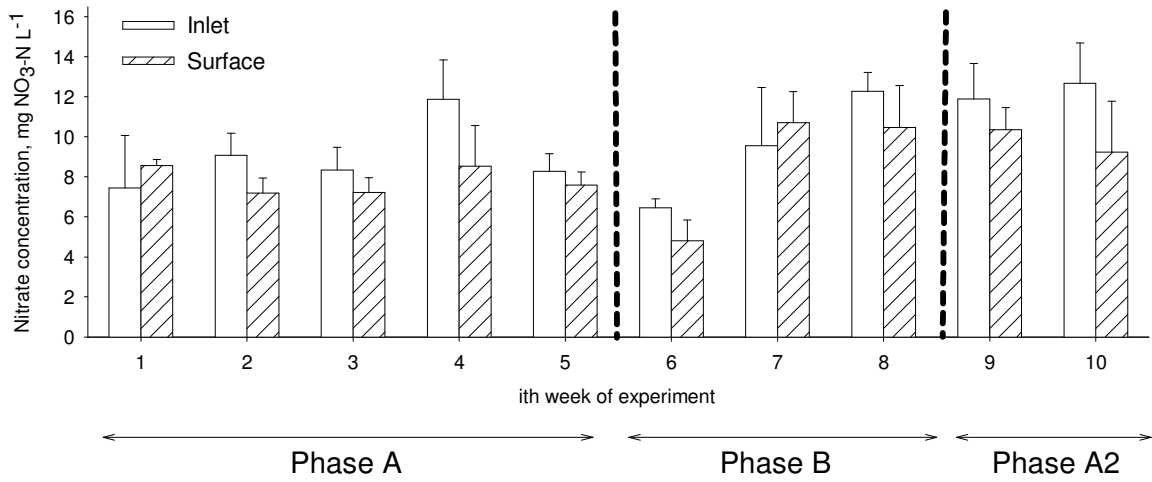
(B)

Fig. 3: Weekly nitrate concentration in the inlet mixing reservoir and at the surface of the Slow Sand Filters (SSF). Panel A = non-vegetated SSF; panel B = vegetated SSF.

with Q: discharge (m^3 d^{-1}); A: surface area (90 m^2) of the SSF; C_{in} and C_{out}: mean NO_3^- concentration (mg N L^{-1}) at SSF surface and outlet, respectively. Nitrogen removal was also expressed as a percent (% R) of input as:

$$\%R = \frac{(C_{in} - C_{out})}{C_{in}} 100$$

Equation 2

7

Chapter 3

Results

3.1 Flow Conditions

3.1.1 Hydraulically effective surface areas

The arrival of 50 % of the tracer was observed after 83.5 h and after 83 h at the outlet of the vegetated SSF and the non-vegetated SSF, respectively (Fig. 4). Taking the SSF depths into account (see Table 1) this yielded average pore velocities of 0.01437 m h^{-1} or 34 cm d^{-1} in the vegetated and 0.00964 m h^{-1} or 23 cm d^{-1} in the non-vegetated SSF. Assuming an effective pore volume (n_e) of 37 % (Grützmacher 2006) the Darcy velocity (v_f) can be calculated to be 0.00532 m h^{-1} or 12.77 cm d^{-1} in the vegetated and 0.00357 m h^{-1} or 8.57 cm d^{-1} in the non-vegetated SSF.
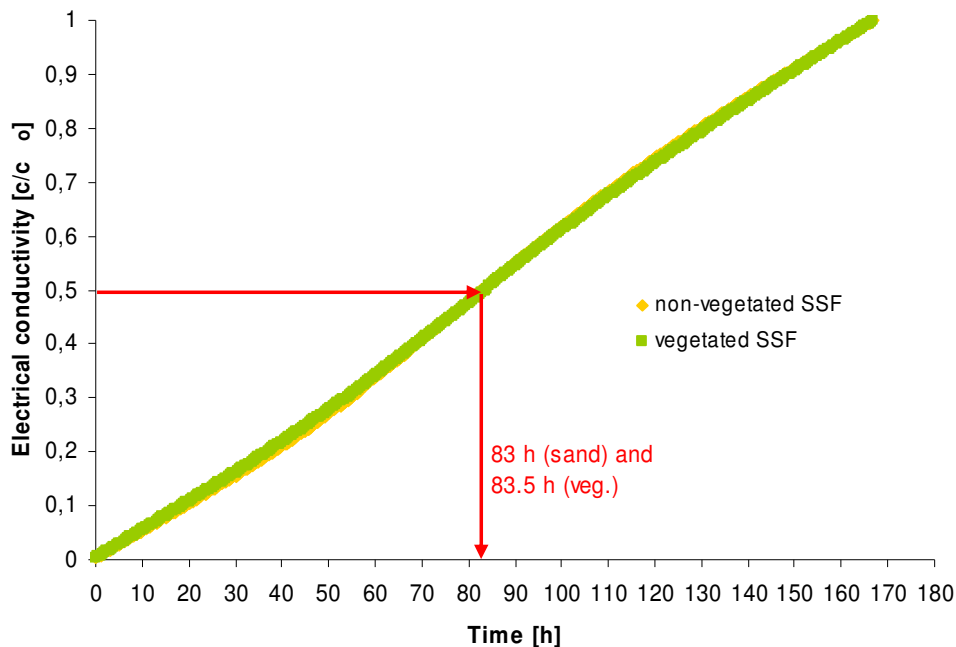
Fig. 4: Cumulative breakthrough curves of the tracer in the vegetated and the non-vegetated SSF

The hydro-chemical analyses (see chapter 3.1.3.) suggested that flow condition was not homogeneous across the vegetated SSF. Therefore, an estimation of the hydraulically effective surface areas was carried out taking into account a flow rate of 0.23 m³ h^{-1}. With regard to the continuity law ($A_e = q / v_f$), that translates into an average hydraulically effective surface area of 43.23 m² for the vegetated SSF and 64.43 m² for the non-vegetated SSF.

The average surfaces areas of the SSF are 68.06 m² and 60.37 m² for the vegetated and the non-vegetated, respectively. It can be concluded that flow completely passed through the non-vegetated SSF while the flux in the vegetated SSF was partly impeded. The low (67 % of total surface) effective surface area could be explained by clogging due to high

8

production of biomass and the extensive root system of *Phragmites* resulting in decreased pore velocities.

Discharge and Darcy velocities

During the experiment the water discharge in the vegetated filter was on average higher (0.54 ± 0.14 m^3 h^{-1}) whereas that in the non-vegetated SSF was lower (0.43 ± 0.12 m^3 h^{-1}) than the targeted 0.5 m^3 h^{-1} (Fig. 5).
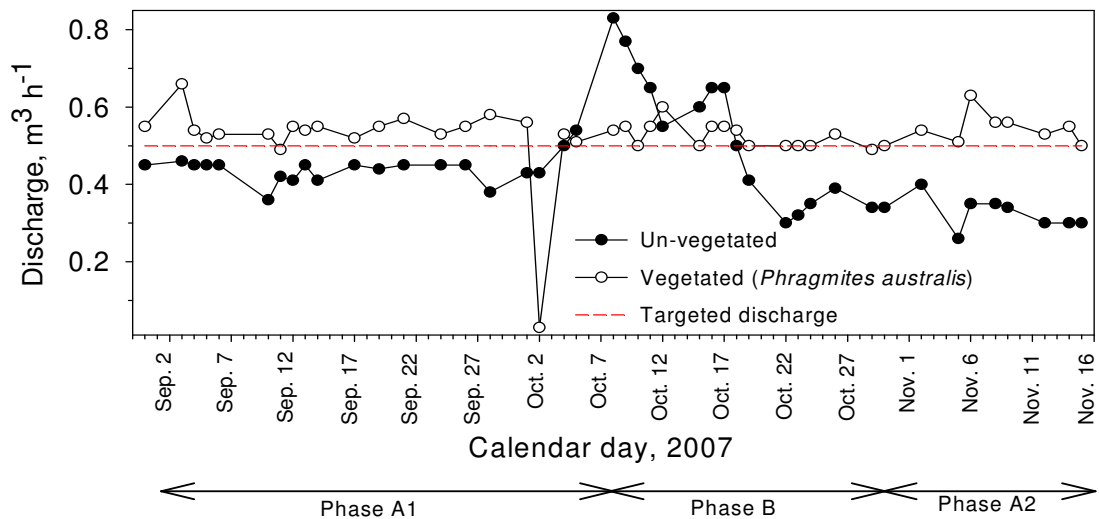
Fig. 5: Daily water discharge measured at the outlet of the slow sand filters (SSF).

Given the hydraulically effective surface areas, these discharge values corresponded to hydraulic loadings (Darcy velocities) of 30 cm d^{-1} and 16 cm d^{-1}, in the vegetated and non-vegetated SSF, respectively.

3.1.2 Flow paths

The breakthrough curves of nitrate, which provide the most comprehensive data, show significant differences in concentration at different points across the vegetated SSF (sampling points 1 to 4 on the surface and at 40 cm depth; Fig. 6 and 7).

9
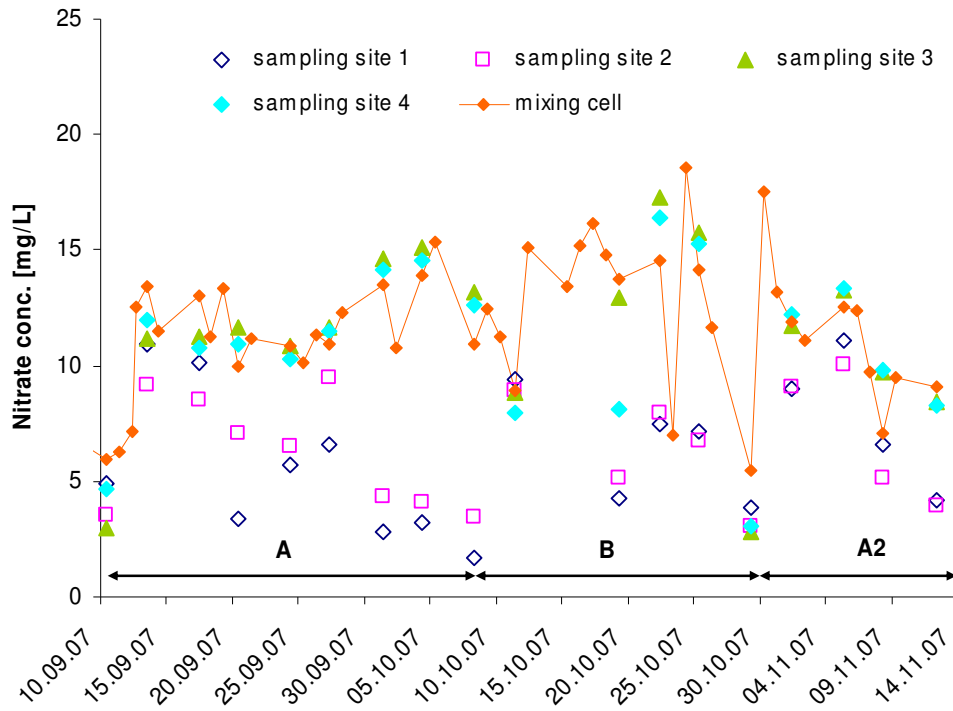
Fig. 6: Nitrate distribution on the surface of the vegetated SSF

Whereas nitrate concentrations on the surface of the vegetated SSF at sampling points 3 and 4 corresponded well to each other and to concentrations measured in the mixing cell (Phase A: 11 mg L^{-1}), values obtained at sampling points 1 and 2 (Phase A: 6 mg L^{-1}) were markedly lower (by 45 %)
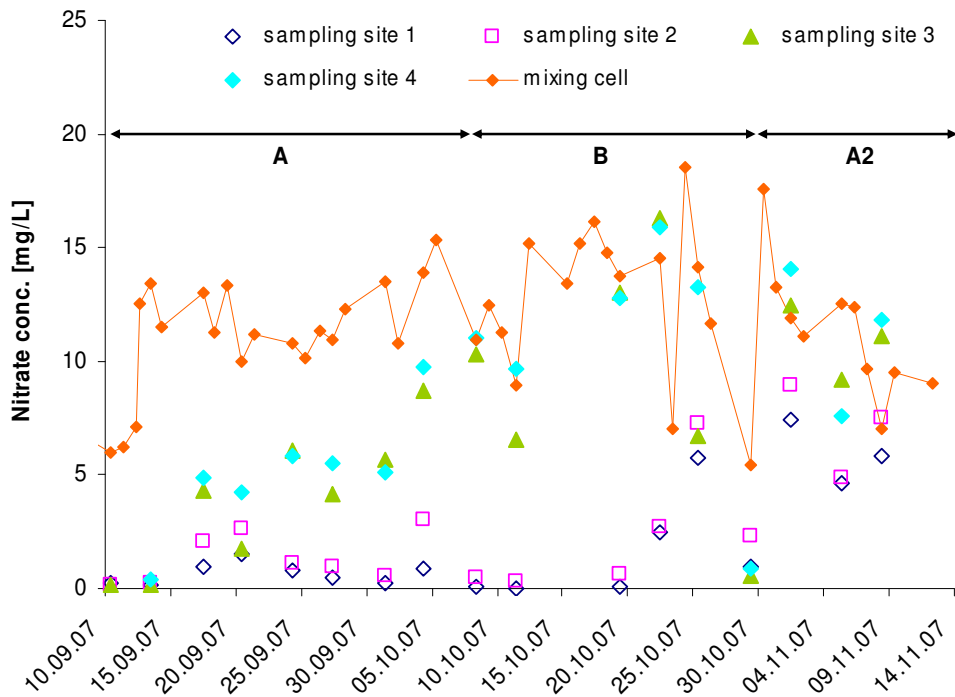
Fig. 7: Nitrate distribution in 40 cm depth of the vegetated SSF

Similarly, nitrate concentrations in the 40 cm depth layer were higher at sampling points 3 and 4 (phase A: 4.5 mg L^{-1}) than at sampling points 1 and 2 (phase A: 0.9 mg L^{-1}).

Lower Darcy velocities could cause higher reduction rates. This gives rise to the previously mentioned assumption that there is a distinct difference in flow between the left (sampling points 3 and 4) and the right (sampling points 1 and 2) side of the vegetated SSF. This behaviour is probably the reason for the observation that the average NO_3^- concentrations from all sampling points in the 40 cm depth were lower than concentration in the effluent (see Appendix A: Fig. 23)

In contrast, the non-vegetated SSF showed only small variations in concentration and therefore more homogenous flow conditions prevailed across the area on the surface and in 40 cm depth (see Appendix A Fig. 21 and 22).

3.2 Nitrogen Removal in SSF

The concentration of NO_3^- generally declined between SSF surface and 40 cm depth (Fig.8 and 9).
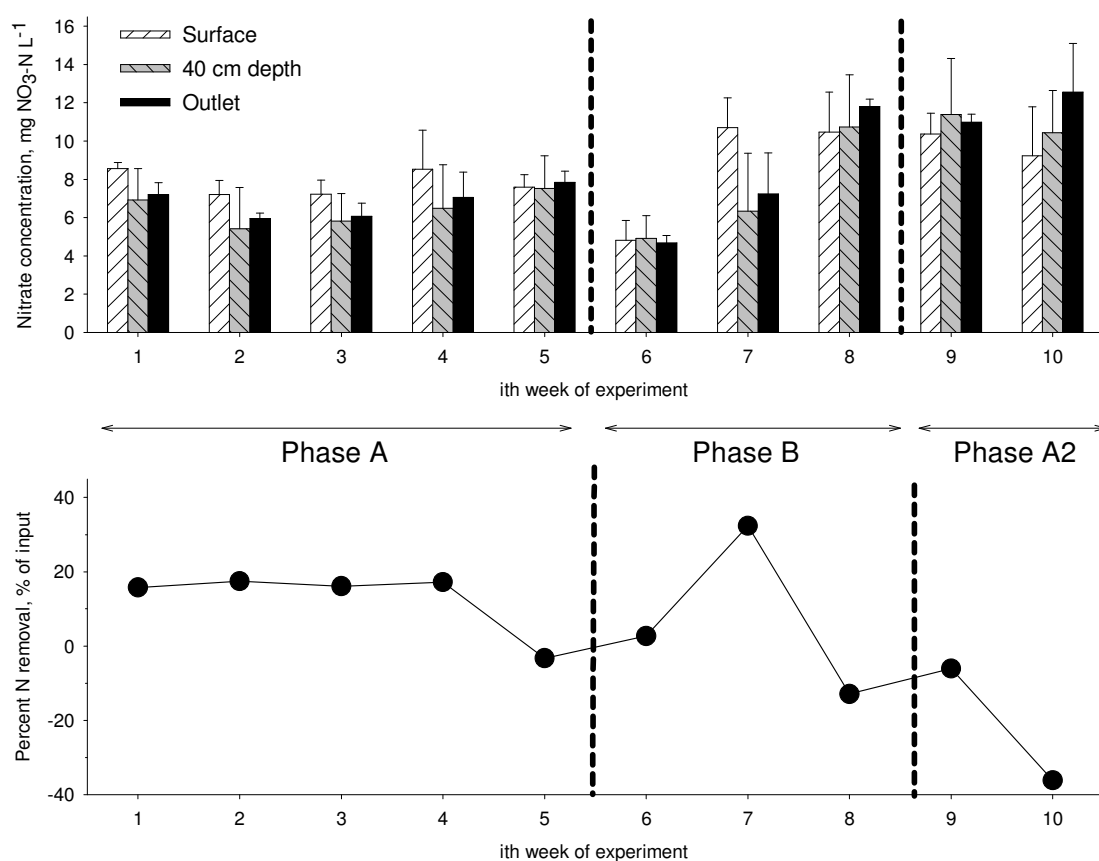
Fig. 8: Daily rates of N removal during various phases of the experiment in non-vegetated SSF. Error bars are standard deviations of the mean.
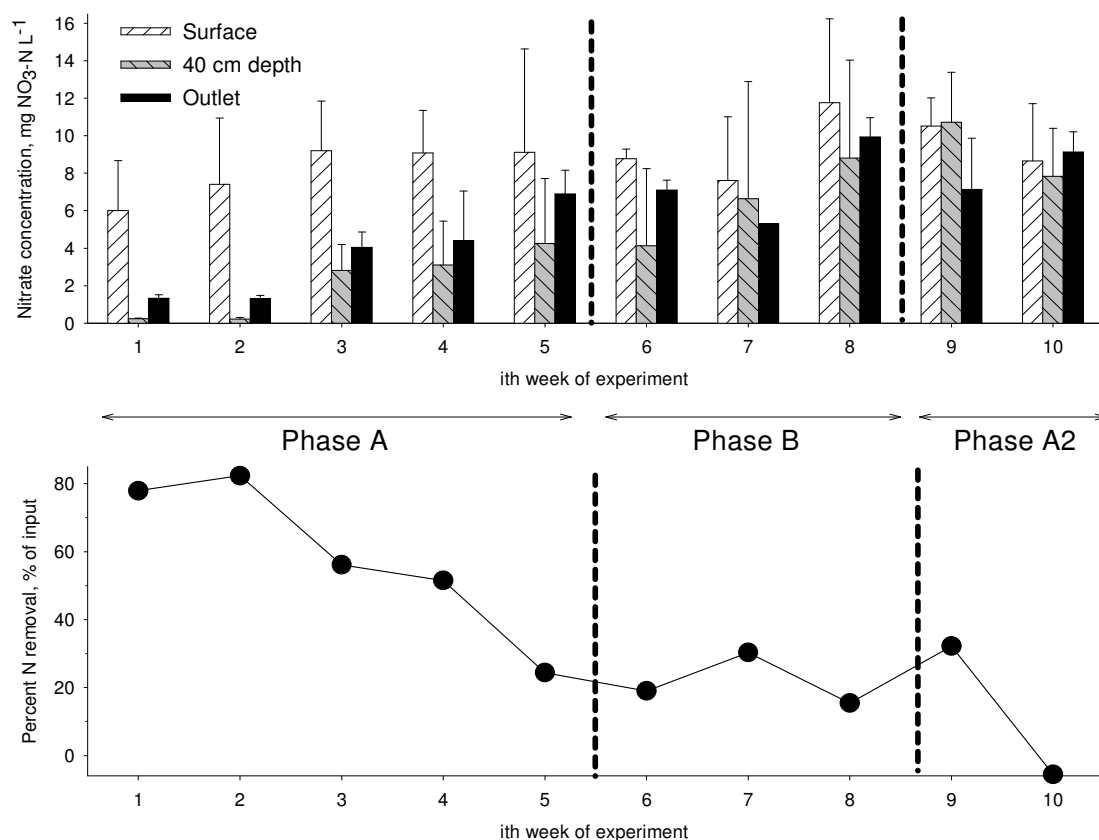
Fig. 9: Daily rates of N removal during various phases of the experiment in vegetated SSF. Error bars are standard deviations of the mean.

In a few instances, a slight increase in concentration was noted between the 40 cm depth and the outlet due to the above mentioned irregularities in flow paths. Since no systematic variation in NH_4^+ concentration (< 0.3 mg N L^{-1}, not shown) and given the low ORP values, it is unlikely that this increase was due to nitrification.

The two SSFs exhibited distinct differences in N removal capacity with rates obtained in the vegetated unit 2-5 times greater than in the non-vegetated SSF (Figs. 8 and 9). Rates of NO_3^- removal also varied among the phases of the experiment. During phases A, B and A2, removal rates averaged 114, 141 and -173 mg N m^{-2} d^{-1}, respectively. Corresponding rates in the vegetated filter were respectively 663, 272 and 199 mg N m^{-2} d^{-1} (Fig. 10).
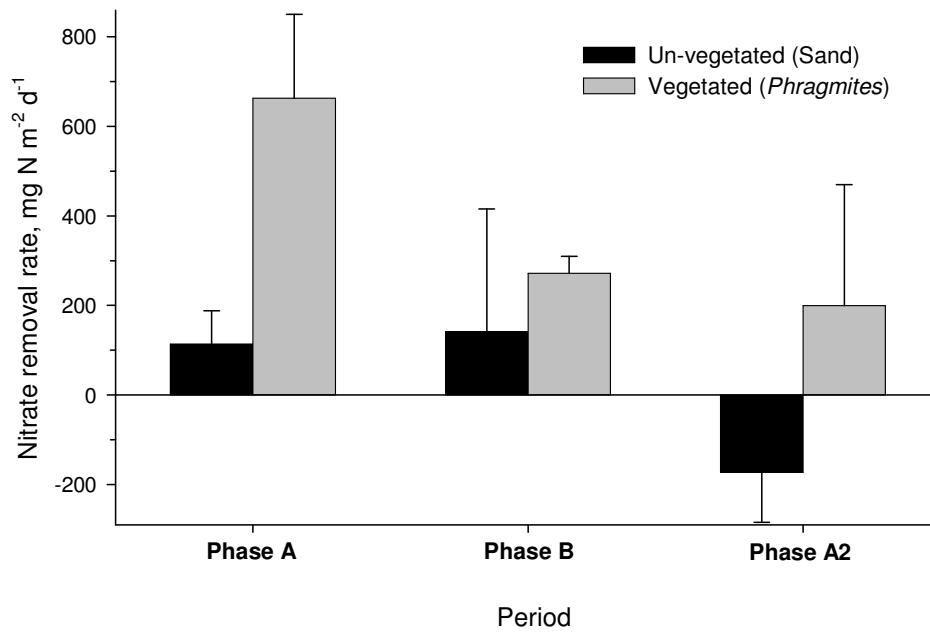
Fig. 10: Average rate of nitrate removal in the slow sand filters (SSFs) during various phases of the experiment.

During the first phase of the experiment, 57 and 14 % of the N that entered the non-vegetated and the vegetated SSF was removed (Figs. 8 and 9). The same trend was observed during other phases of the experiment. Strong relationships were also observed between N removal rates and water temperature (Fig. 11).
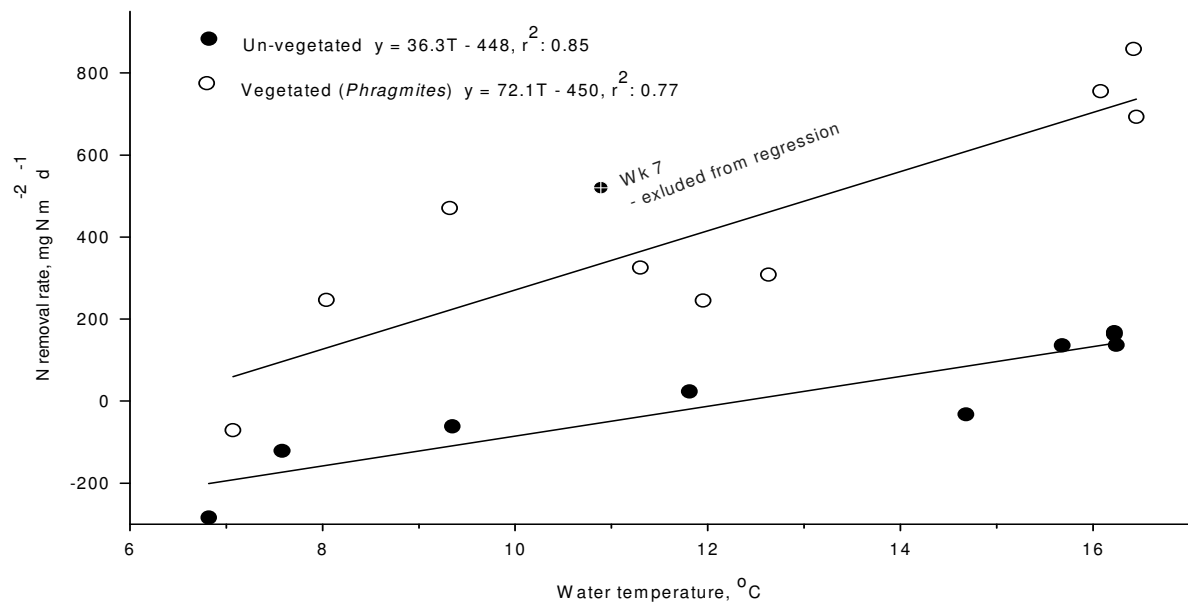
Fig. 11: Relationships between N removal rates in the slow sand filters and water temperature.

These results suggest that the *Phragmites* vegetation significantly contributes to the NO_3^- sink capacity of the SSFs. This contribution could involve the supply of organic substrates to support the activity of denitrifiers and incorporation of N into vegetation biomass. Information in literature regarding N uptake by *Phragmites* varies. In a recently

13

constructed wetland (2-yr old, hydraulic loading rate: 3.9 - 6.5 cm d^{-1}), Healy and Cawley (2002) reported N uptake by *Phragmites* ranging between 48-83 mg N m^{-2} d^{-1}. In the SSF systems investigated, plant uptake was probably lower because of the shorter water residence time.

3.3 Interactions of glyphosate in constructed wetlands

3.3.1 Mobility and persistence of glyphosate in the SSFs

Before assessing and comparing the effects of glyphosate on the N and C cycling processes on non-vegetated and vegetated constructed wetlands or riparian zones, respectively, it is necessary to assess the mobility of glyphosate in the SSFs.

The concentrations of glyphosate measured in the mixing cell, on the surface, in 40 cm depth and in the outlet of the vegetated SSF are given in figure 12 and 13. With respect to the flow conditions, the vegetated SSF is divided in two zones with different hydraulic and subsequently hydro-chemical characteristics.

In the inlet of the vegetated SSF, the glyphosate concentration overstepped the planed level of 20 µg L^{-1} slightly (21.2 µg L^{-1} in average). In sampling sites 3 and 4 only low reduction was observed in the water reservoir above the SSF surface (19 µg L^{-1} in average). In 40 cm depth the maximum concentration of glyphosate was retarded by 11 days and reduced to approximately 7 µg/L.
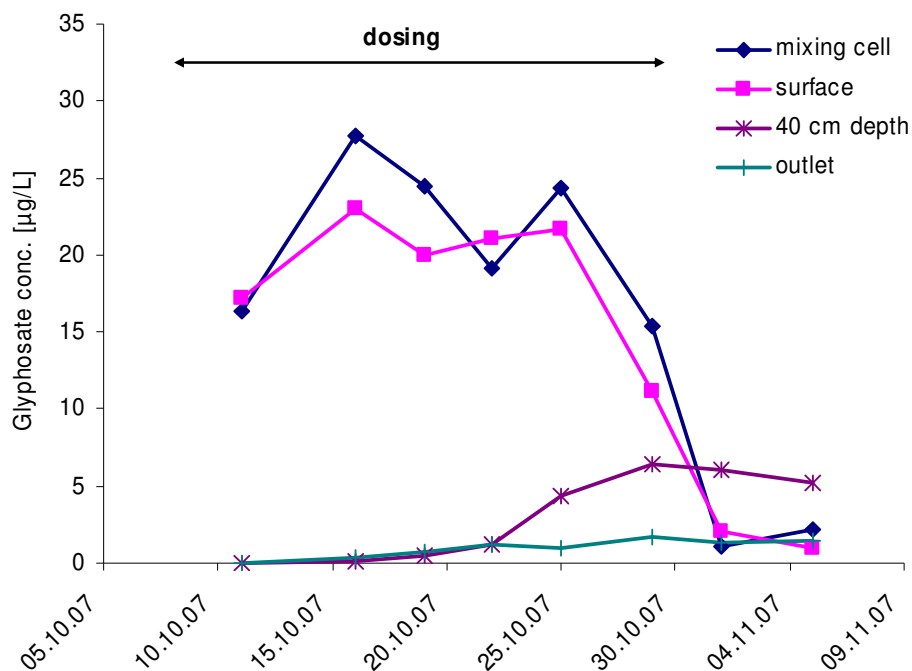
Fig. 12: Glyphosate distribution in the sampling sites 3 and 4 of the vegetated SSF

In sampling site 1 and 2 the concentrations decreased by more than 50 % between mixing cell and surface of the SSF. Glyphosate was completely removed from solution in 40 cm depth, which seems to be due to stagnation of flow, long residence times, and therefore higher efficiency of reduction.
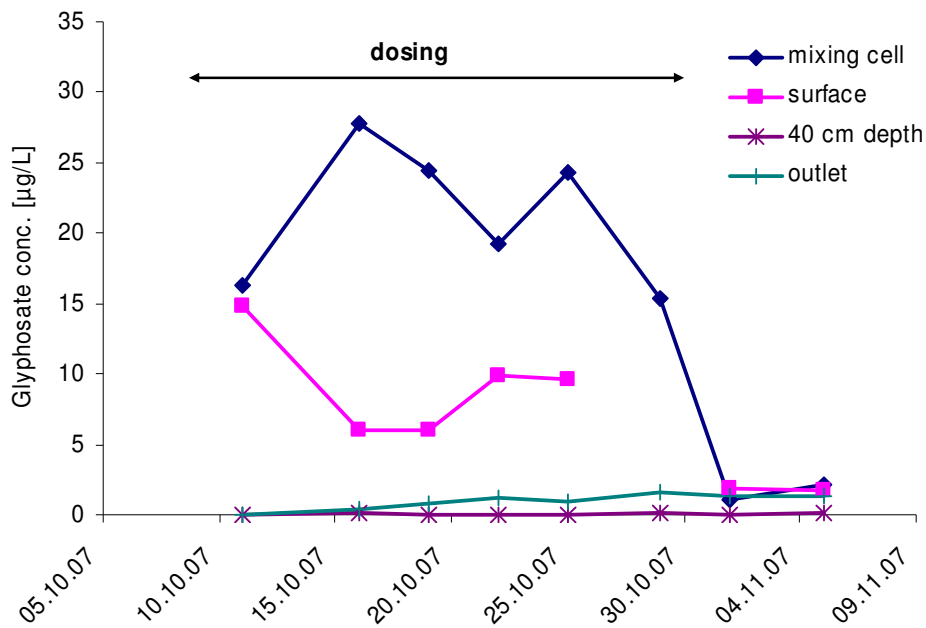
Fig. 13: Glyphosate distribution in the sampling sites 1 and 2 of the vegetated SSF

While the vegetated SSF has to be divided in two zones, the non-vegetated SSF can be seen as homogenous. Therefore, the glyphosate concentrations in each depth of the non-vegetated SSF can be considered equally by creating an average concentration (Fig. 14).
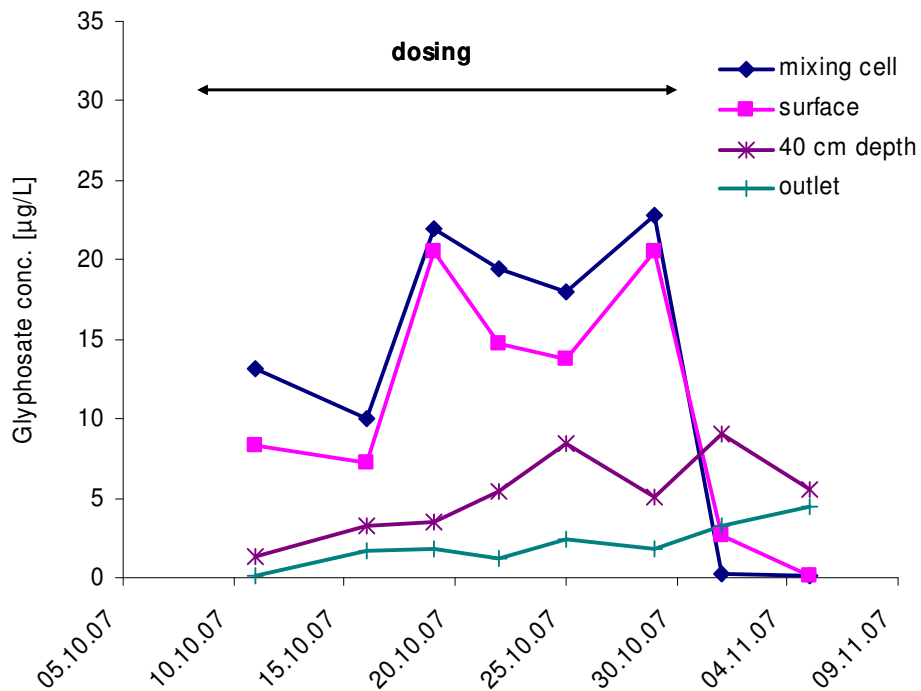
Fig. 14: Glyphosate distribution in the non-vegetated SSF

The inlet concentrations of the non-vegetated SSF did not reach the planed level of 20 µg/L. In average it was lower and characterized by strong fluctuations probably due to degradation processes in the stock solution (17.6 µg L^{-1} in average). Maximum glyphosate concentration decrease to 9 µg/L after 40 cm of filter passage. The concentration in the outlet did not reach the climax of the breakthrough curve. The maximum concentration which could be detected was 4.5 µg/L.

Comparing the concentrations in 40 cm depth and in the effluent of the non-vegetated SSF with those of the sampling sites 3 and 4 as representatives for the vegetated SSF, the glyphosate concentrations were reduced to a lower level in the vegetated SSF, although the inlet concentrations were higher and the residence time were lower.

The higher reduction rate of glyphosate in the vegetated SSF could be due to the strong biological activity, which was observed (lower oxygen contents and subsequently lower redox potential). The redox potential at 40 cm depth was highly variable in both SSFs. Redox potential averaged -200 mV at sampling points 3 and 4 (left side of the SSF, as representative for the vegetated) and 235 mV in the non-vegetated SSF (see Appendix A: Fig. 24 and 25). The decisive factor seems to be the availability of high organic carbon content, due to vegetation growth and litter deposition.

3.3.2 Effects on nitrate concentration by glyphosate

The nitrate and glyphosate concentrations in the vegetated and the non-vegetated SSF are shown comparatively in figure 15 and 16.

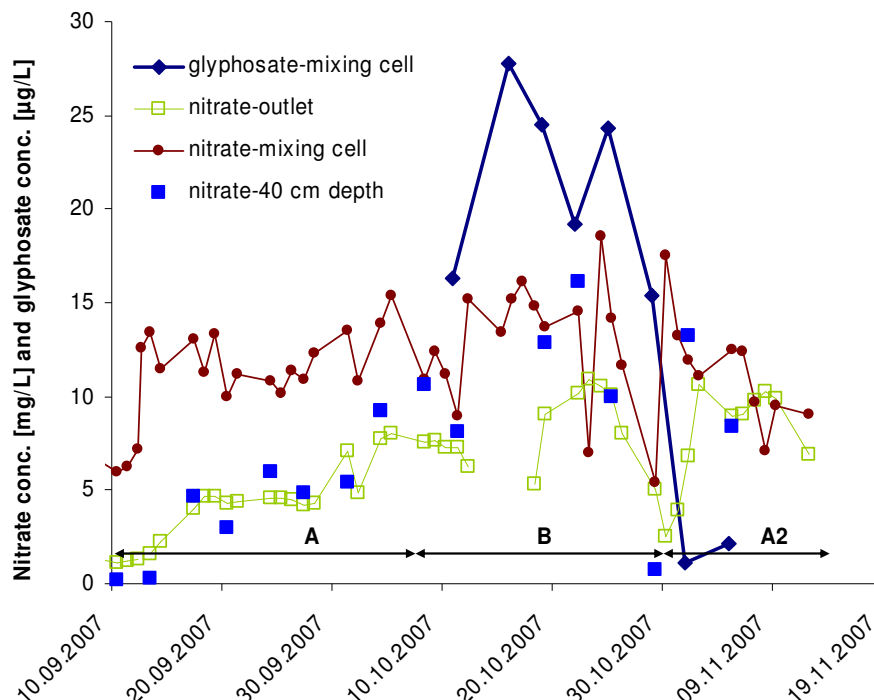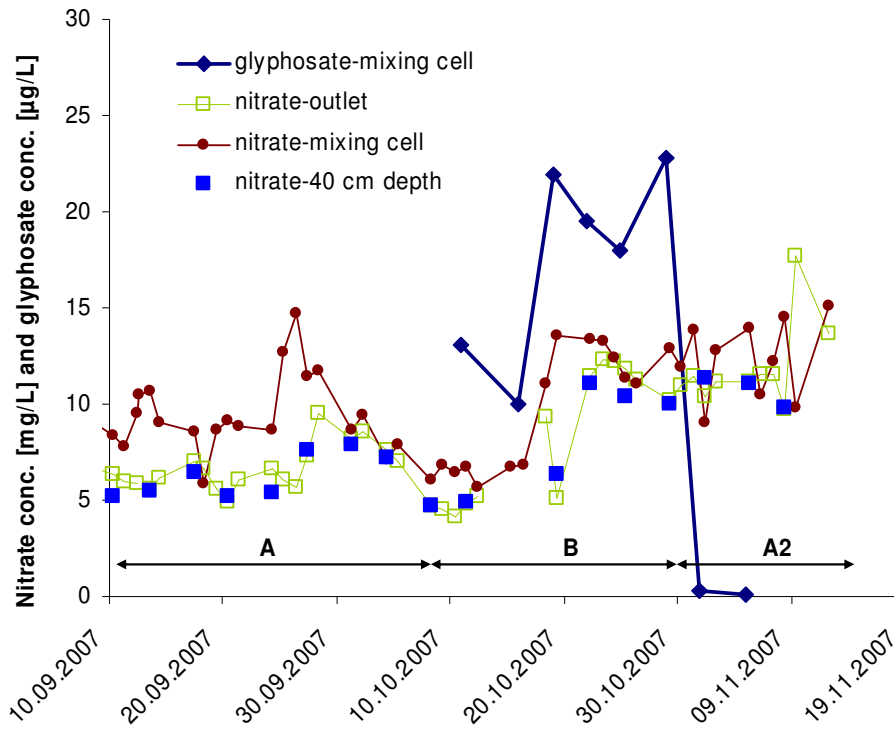**Fig. 15: Nitrate and glyphosate concentration distribution in the sampling sites 3 and 4 of the vegetated SSF**

Fig. 16: Nitrate and glyphosate concentration distribution in the non-vegetated SSF

An effect of glyphosate addition on nitrate transformations could not be documented, neither in the non-vegetated nor in the vegetated SSF. The figures 8 and 9 show the rates of nitrate removal. It can be seen that the decrease of performance started before the dosing of glyphosate. The deviations in nitrate concentration seem to be more dependent on oscillation and decrease of the temperature (see fig. 17) and variations of the starting concentration in the mixing cell due to difficulties of maintaining constant glyphosate dosing.
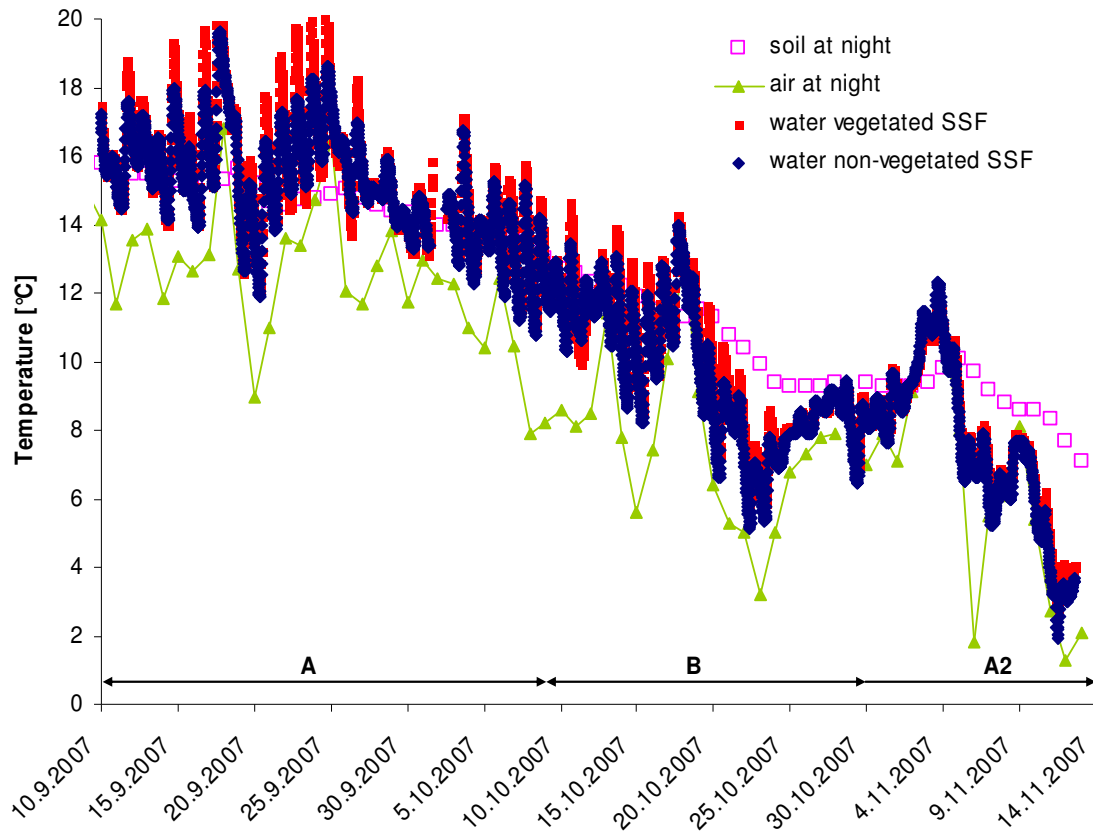
Fig. 17: Temperature during the experiment

3.4 Trace Gas Fluxes and N Balance

SSF effluent was super-saturated with respect to dissolved trace gases. Mean concentrations were 4-145 times greater than equilibrium concentration. Under the experimental conditions (7-18 $^{\circ}$C), CO_2, CH_4 and N_2O concentrations in equilibrium with the atmosphere are 0.84 mg CO_2 L^{-1}, 0.05 µg CH_4 L^{-1} and 0.2 µg N_2O L^{-1}, respectively. Dissolved CO_2 and N_2O in the vegetated SSF outlet was consistently greater that in the non-vegetated SSF probably due to greater availability of organic C (Fig. 18).
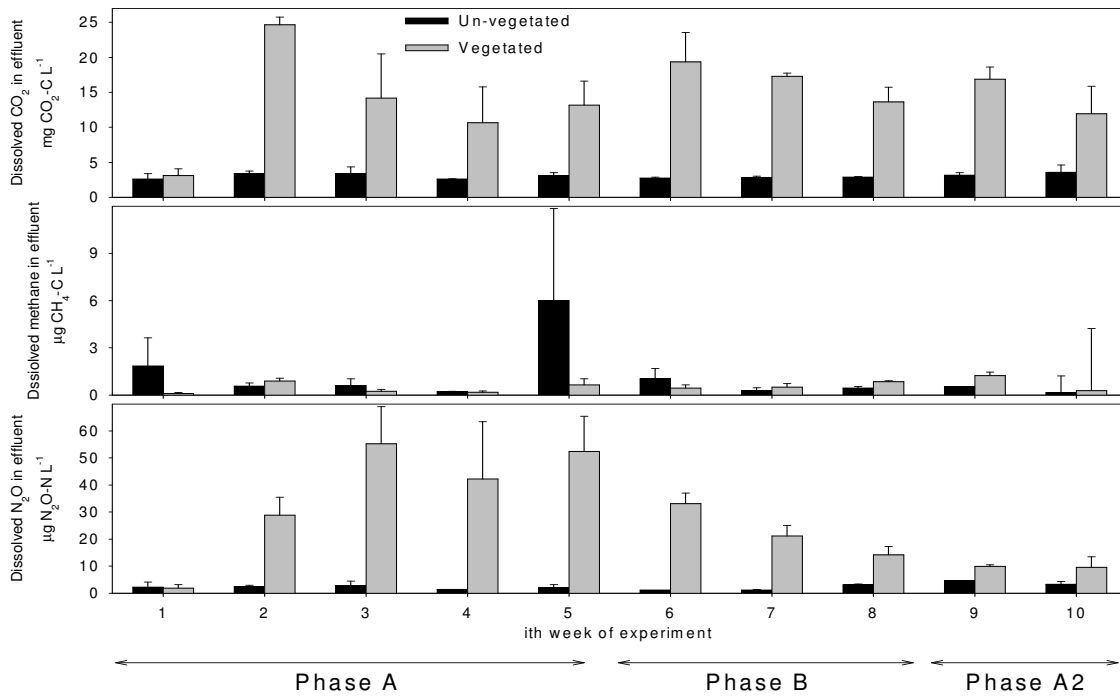
Fig. 18: Weekly mean concentration of trace gases dissolved in SSF effluent.

The trend was less consistent with regard to CH_4; especially during the 5th week of the experiment when flow adjustment difficulties (see Fig. 5) may have led to a rapid export of CH_4 to the non-vegetated SSF outlet.

Higher rates of CO_2 and CH_4 emission were also recorded in the vegetated SSF underscoring the organic C contribution of growing *Phragmites* to biogeochemical processes in the SSFs (Fig. 19).
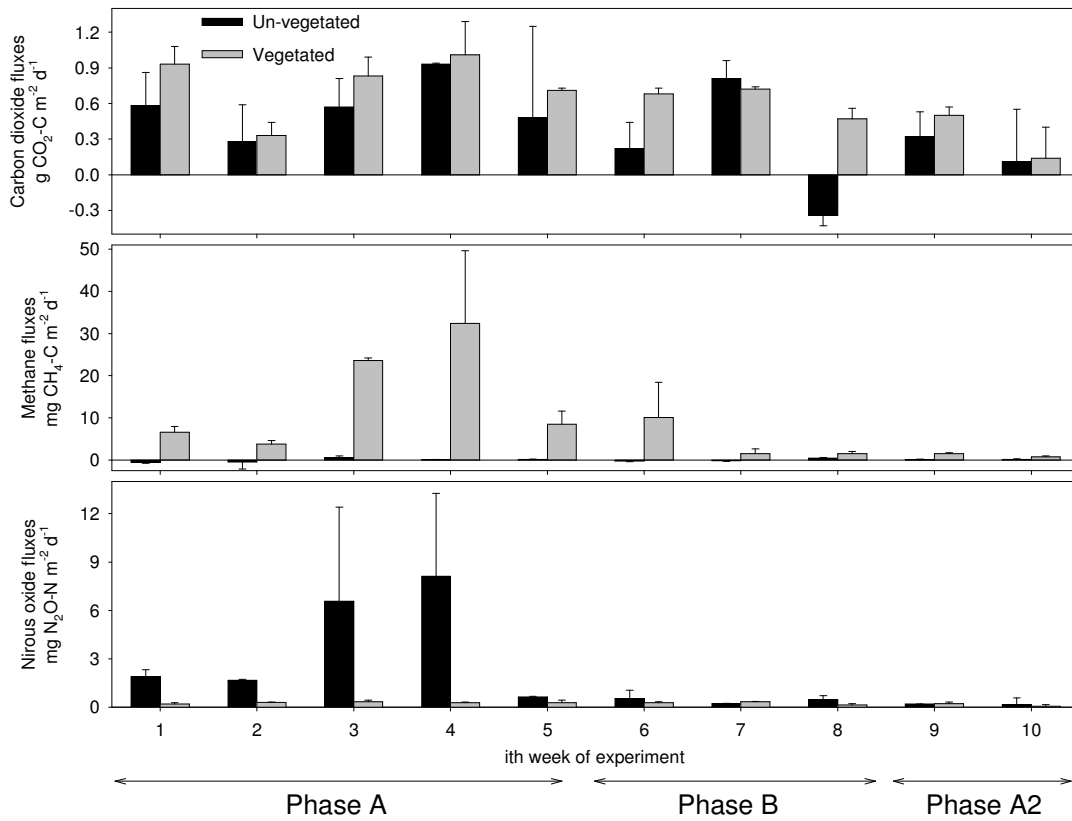
Fig. 19: Weekly GHG fluxes at the slow sand filter surface.

More reductive conditions in the vegetated SSFs resulted in greater production and emission of CH_4, but in lower N_2O emission compared to the non-vegetated unit. Consequently, CH_4 emission and ORP were negatively related (r^2: 0.49; $P < 0.004$). Low redox is conducive to the conversion of N_2O into N_2, thus mitigating the potential air quality impact of SSFs. Reductive conditions prevailed in the vegetated SSF through the end of phase A (Fig. 20).
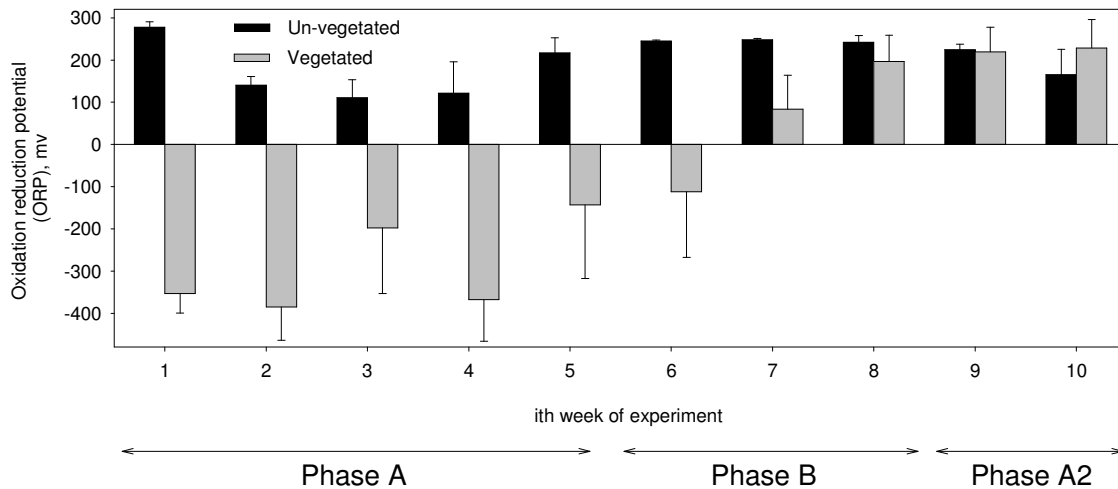
Fig. 20: Variations in oxidation reduction potential in the slow sand filters (SSF) during the course of the experiment.

As vegetation senesced, no difference in ORP was detected between the vegetated and non-vegetated SSF suggesting a link between *Phragmites* growth, root exudation and O_2 depletion in the SSF.

During all phases of the experiment, and irrespective of SSF, the amount of N_2O produced (emission from SSF surface plus export as effluent, Table 2) never exceeded 6 mg N m^{-2} d^{-1}.

Tab. 2: Nitrogen fluxes in the slow sand filters.

	Phase	N removal	N_2O emission	Dissolved N_2O in outlet
		_____ mg N m^{-2} d^{-1} _____		
Non-vegetated SSF	A	113.9	3.8	0.3
	B	140.6	0.4	0.2
	A2	-173.2	0.2	0.4
Vegetated (*Phragmites*) SSF	A	663.1	0.3	5.2
	B	272.2	0.2	3.2
	A2	199.3	0.1	1.4

Thus, production of N_2O represented a minor fraction of the total N removal (up to 750 mg N m^{-2} d^{-1}). These results compared favorably with those of other studies (Mander et al. 2003, 2005; Picek et al. 2007) that have also reported negligible amounts of N_2O produced in constructed wetlands designed for the treatment of wastewaters.

Chapter 4
Summary and Conclusions

Technical scale feasibility experiments were conducted at the UBA research facilities in Berlin, Marienfelde to evaluate processes and simulating conditions that impact the nutrient removal capacity of wetlands / riparian zones. Results are summarized below:

Assessment of the impact of vegetation on the nitrate removal efficiency and trace gas emission from constructed wetlands (vegetated vs non-vegetated).

- Despite variability in the data, the overall trend suggests a positive effect of *Phragmites* on N removal in the SSF. N removal rates were seasonally variable being on average 3 times lower in the fall than in late summer. The effect of *Phragmites* was likely due to the release of easily decomposable organic substrates (litter and root exudates) to support the activity of denitrifying microorganisms. Vegetation uptake is an additional N removal mechanism, but that was not quantified given the experimental set up.

- The effect of vegetation was detectable only during phase A of the experiment. Later the influence of vegetation was overshadowed by steep decline in temperature, vegetation senescence and dosing inconsistency.

- Overall, the SSFs were net sources of methane. Emission rates were much greater in the vegetated than in the non-vegetated SSF, and showed a strong decline from summer to fall. Conversely, emission of N_2O was higher in the non-vegetated filter and exhibited similar seasonal variation. When compared to N removal rates, however, N_2O emission accounted for a very small portion of the N removed. At best, N_2O accounted for 3 % of N removed. Thus, one may conclude that the SSFs will not aggravate the atmospheric N_2O burden and elemental nitrogen (N_2) is probably the dominant end-product of denitrification in these engineered systems.

Evaluation of interactions (synergistic or inhibitory) of glyphosate on N and C cycling processes in constructed wetlands.

- The concentration of glyphosate applied (although in accordance with studies that have detected glyphosate in environment samples) was probably too low to significantly affect the nitrate removal capacity of the SSF. Toxic effects on vegetation and degradation processes (denitrification) were not detectable. Additionally, it could be argued that the vegetated SSF maintained higher microbiological activity possibly resulting in higher reduction of glyphosate.

Recommendations

- Based on the pilot study, it can be stated that the SSFs are adequate to simulate constructed wetlands with vertical flow component. Denitrifying conditions were successfully established by adjusting to low flow rates and dosing with nitrate. Additional dosing with dissolved organic carbon was not necessary. In Aquisafe 2,

the experimental settings for the technical scale study need to be adjusted to address these questions.

- The analysis of the hydraulic measurements showed potential drawbacks of the system and helped identify improvements that will be needed with regard to experimental set-up and procedure. To obtain a more constant discharge, modifications have already been made. The design of dosing installations will be adapted to guarantee higher stability of influent concentrations.

- Since in the vegetated SSF, flow tends to be non-homogenous , its construction has to be improved, so that multiple sampling could be avoided. If homogeneity is not achievable due to strong influence of natural variability, it will be necessary to conduct more detailed tracer tests during the experiment (measurements at all multiple sampling points) to better understand and characterize flow conditions.

- The YSI probes deliver high resolution measurements (daytime dependent fluctuations visible) and reduce sampling effort. For long time experiments, however, hand measurements would be sufficient.

- For the examination of temperature-sensitive processes, the seasonal variations are too variable to simulate the effect of different dosing conditions in subsequent phases of the experiment . In order to separate seasonal effects from other factors, long term experiments or parallel experiments would be more suitable.

- The greenhouse gas production should be taken into account if possible; therefore, gas emission should be measured to be able to balance possible impacts on climate change.
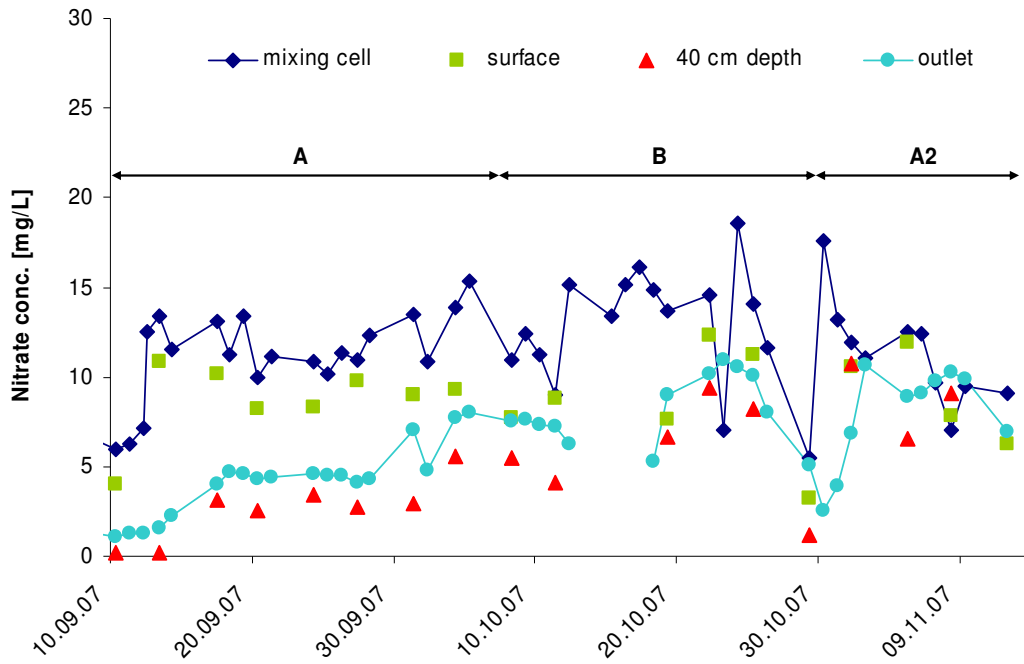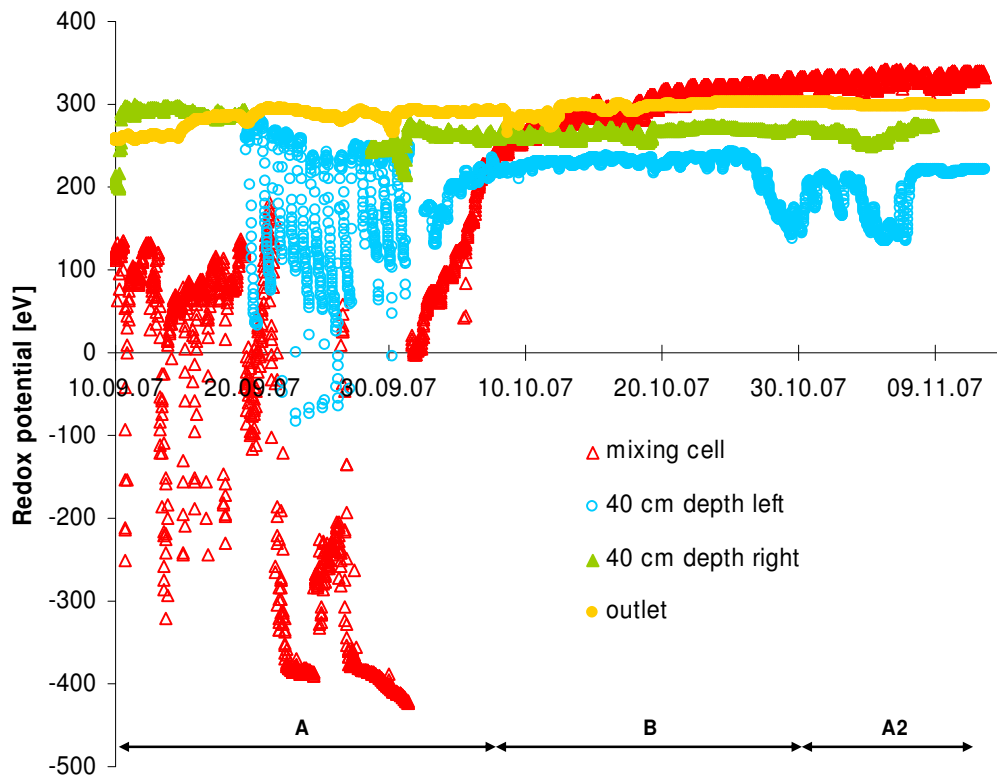
Appendix A
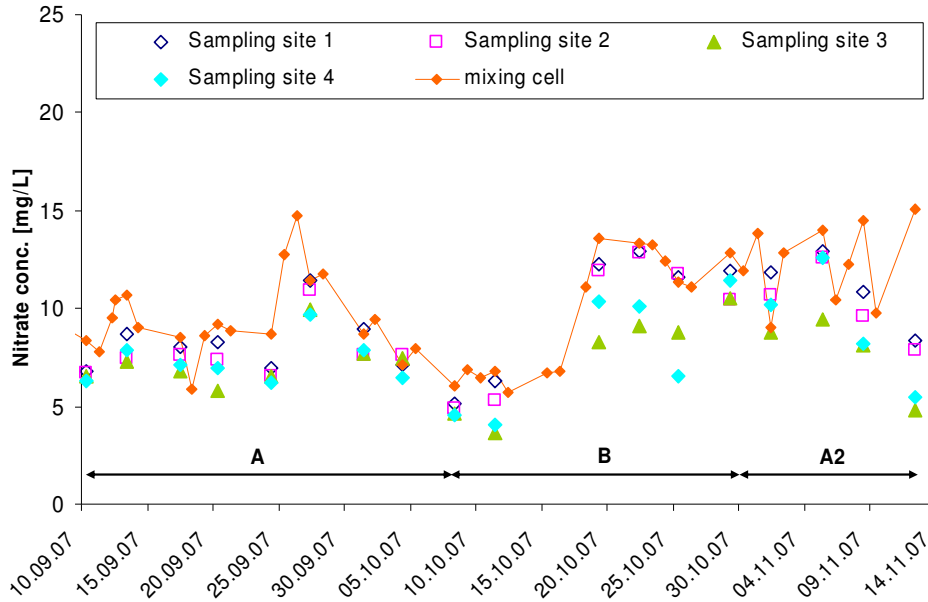
Fig. 21: Nitrate distribution on the surface of the non-vegetated SSF
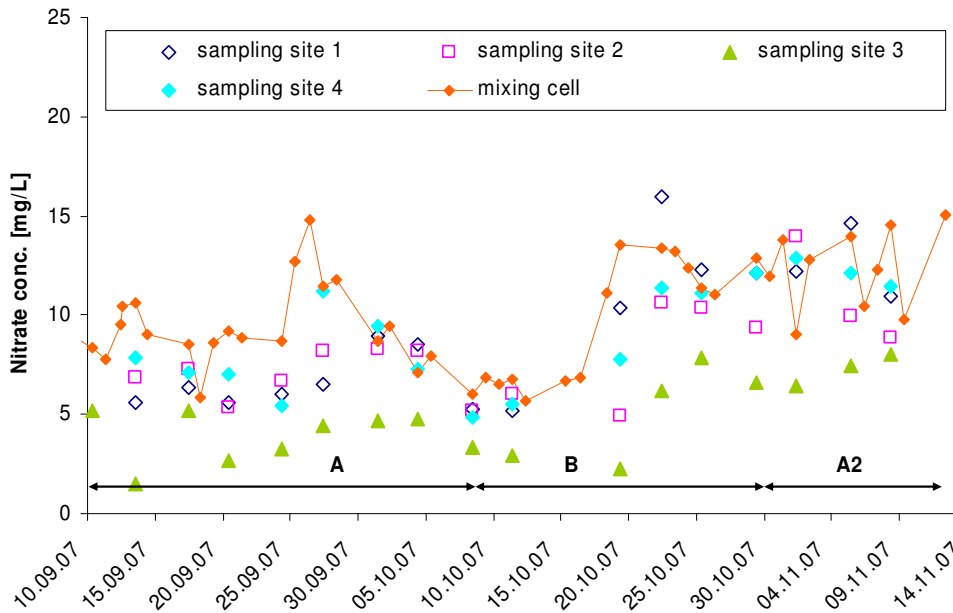
Fig. 22: Nitrate distribution in 40 cm depth of the non-vegetated SSF

Fig. 23: Nitrate distribution in the vegetated SSF (concentrations: average of all sampling sites)

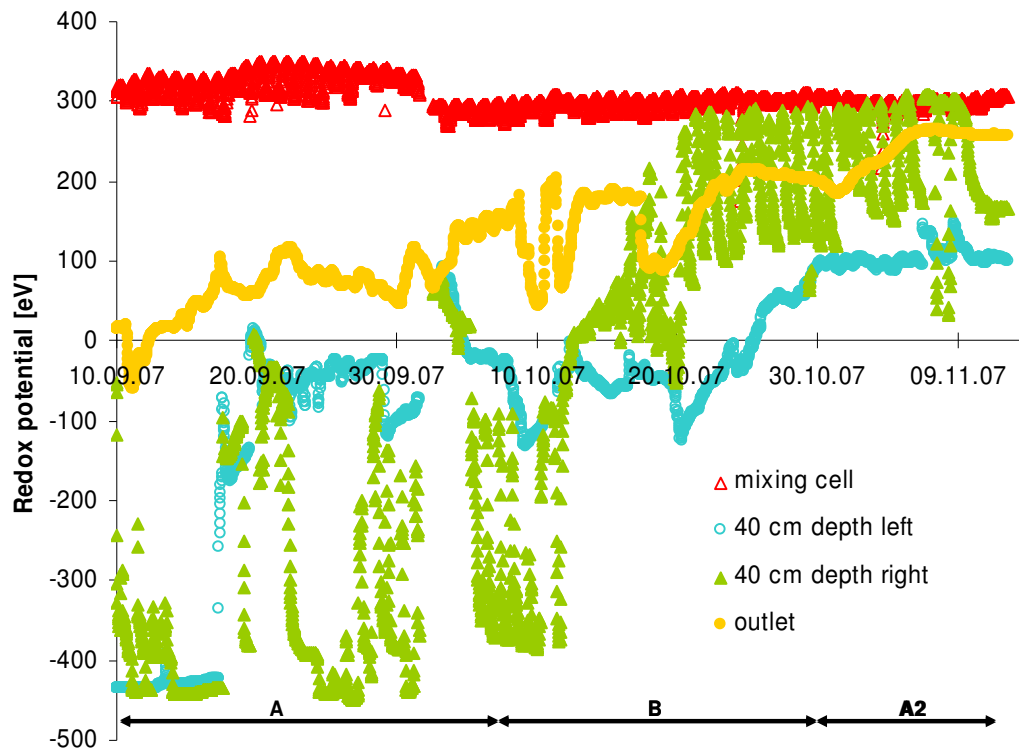**Fig. 24: Redox potential in the non-vegetated SSF**

Fig. 25: Redox potential in the vegetated SSF

Bibliography

Alibhai, Murtaza F. & Stalling, William C. 2001. Closing down on Glyphosate inhibition with a new structure for drug discovery. In Nord, B.; Sikora, M. & Norgren C. (ed.) 2006. The herbicide glyphosate and its fate in the environment. Published in Enviromental Chemistry, Umea University, Student Projects, S. 15-19.

Brønstad, J. O. & Friestad O. H. 1985. Behaviour of glyphosate in aquatic environment. In Giesy, J. P.; Dobson, S. & Solomon, K. R. 2000. Ecotoxicological Risk Assessment for Roundup® Herbicide. Published in Reviews of Environmental Contamination and Toxicology, No. 167, P. 35-120.

De Jonge, H.; De Jonge, L. W.; Jacobsen, O.H.; Yamaguchi, T. & Moldrup, P. 2001. Glyphosate sorption in soils of different ph and phosphorus content. Published in Soil Science, 166. edition, No. 4, P. 230-230.

Dion, H. M.; Harsh, J. B. & Hill Jr., H. H. 2001. Competitive sorption between glyphosate and inorganic phosphate on clay minerals and low organic matter soils. Published in Journal of Radioanalytical and Nuclear Chemistry, 249. edition, No. 2, P. 385-390.

Giesy, J. P.; Dobson, S. & Solomon, K. R. 2000. Ecotoxicological Risk Assessment for Roundup® Herbicide. Published in Reviews of Environmental Contamination and Toxicology, No. 167, P. 35-120.

Hance, R. J. 1976. Adsorption of glyphosate by soils. Published in Pesticide Science, No. 7, P. 363-366.

Healy, M. & Cawley, A.M. 2002. Nutrient processing capacity of a constructed wetland in western Ireland. Published in J. Environ. Qual. 31: 1739-1747.

Mander, U.; Kuusemets, V.; Lohmus, K.; Mauring, T.; Teiter, S. & Augustin, J. 2003. Nitrous oxide, dinitrogen and methane emission in a subsurface flow constructed wetland. Published in Water Sci. Technol. 48: 135-142.

Mander, U.; Teiter; S. & Augustin, J. 2005. Emission of greenhouse gases from constructed wetlands for wastewater treatment and from riparian buffer zones. Published in Water Sci. Technol. 52: 167-176.

Mensink, H. & Janssen, P. 1994. Glyphosate. Published in Environmental Health Criteria 159. World Health Organization, IPCS INCHEM (International Programme on Chemical Safety & Canadian Centre for Occupational Health and Safety), Geneva, P. 177p.

Normura, N. S. & Hilton, H. W. 1977. The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils. Published in Weed Research, 17. edition, P. 113-121.

Pessagno, R. C.; Dos Santos Afonso, M. & Sanchez, R. M. T. (2005): „N-(Phosphonomethyl) Glycine Interactions with Soils", in: J. Argent. Chem. Soc., Auflage 93, n.4-6, S. 97-108, ISSN 0365-0375.

Piccolo, A.; Celano, G.; Arienzo, M. & Mirabella, A. 1994. Adsorption and Desorption of Glyphosate in some European Soils. Published in Journal Environment Science Health, No. B29 (6), P. 1105-1115.

Picek, T.; Cizkova, H. & Dusek, J. 2007. Greenhouse gas emissions from a constructed wetland - Plants as important sources of carbon. Published in Ecological Engineering 31: 98-106.

Scheffer, F. & Schachtschabel, P. 2002. Lehrbuch der Bodenkunde. Published by Enke Verlag, Stuttgart.

Sheals, J.; Sjöberg, S. & Persson, P. 2002. Adsorption of Glyphosate on Goethite: Molecular Characterization of Surface Complexes. Published in Environment Science & Technology, 36. edition, No. 14, P. 3090-3095.

Sprankle, P.; Meggitt, W.F. & Penner, D. 1975. Adsorption mobility and microbial degradation of glyphosate in soil. Published in Weed Science, 23. edition, No. 3, P. 229-234.

Vereecken, H. 2005. A review - Mobility and leaching of glyphosate. Published in Pesticide Management Science, No. 61, P. 1139-1151.