FINAL REPORT

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D 5.3 Extension: Report on efficient design of mitigation zones for pesticide removal

Project acronym: Aquisafe 2

by

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for

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Abstract

The wash out of agricultural auxiliary chemicals like fertilizer and pesticides via surface run-off or subsurface leaching into drainage systems or ground waters, which discharge into surface waters, presents an increasing risk for drinking water production and biodiversity in rivers and lakes. Mitigation zones are important measures to attenuate contamination at the source and relieve surface waters downstream. Under high flow conditions, as they occur during rainy seasons and snow melt, the effectiveness of such facilities is restricted due to bypass of untreated waters or very short contact times. This study of the Aquisafe 2 project focus on drainage water decontamination and examines mitigation zone designs with organic substrates for their potential to reduce a set of herbicides and nitrate (NO_3^-), concurrently and efficiently, at short hydraulic residence times (0.2 to 2.5 days) to prepare their implementation in contamination hot spots. The herbicides bentazone, atrazine and isoproturon were classified as most relevant for drinking water production.

On the basis of comprehensive literature studies the organic substrates bark mulch and straw and the design of bioretention swales emerged to be of high potential for decontamination of drainage waters in mitigation zones.

In **laboratory scale** studies the substrates were tested in degradation-, sorption- and leaching-experiments at temperatures around 21 °C for their potential to ensure long-lasting hydraulic permeability, denitrification and attenuation of the selected herbicides.

The selected organic substrates provide a high and long term stable permeable conductivity to realize and maintain high flow. The effective porosity yielded around 0.45 and reduced within 1.5 years by only 25 %. Straw is a readily available organic carbon source, which can support effective and efficient denitrification at short hydraulic residence times. Bark mulch contains more resistant carbon species, but contributes also to NO_3^- removal. In mixture with straw the performance of bark mulch as organic carbon source for denitrification increases (co-metabolic decomposition). Organic substrates are characterized by strong wash out of dissolved organic carbon (DOC) and high denitrification rates (15 to 45 g-N m^{-3} d^{-1}) in the start phase and successive decrease of denitrification performance due to loss of readily available organic carbon. Despite decline of performance, denitrification rates stabilized after one year of operation at constant conditions at a level of 4 to 10 g-N m^{-3} d^{-1} (10 to 25 % of input).

The potential of the organic substrate to retain the selected herbicides is very different for each compound and bases on different dissipation paths. Denitrifying conditions are in general disadvantageous for retention of the selected herbicides. Bentazone is too persistent and mobile to be considerably retained under high flow conditions. Atrazine can be substantially removed from drainage waters. It is suspected to be attenuated predominantly by formation of bound residues at the organic substrate, especially bark mulch, and partially by degradation to hydroxy-atrazine. Isoproturon seems to be effectively retained under suboxic conditions by degradation to metabolites.

At **technical scale** parallel retention of NO_3^- and atrazine and NO_3^- and isoproturon was investigated. The potential of the organic carbon source (mixture of bark mulch and

straw) was tested under more realistic conditions at macro scale, under the influence of seasonally changing temperatures at short HRT (0.4 to 2.5 days).

The high and stable hydraulic permeability of the substrates could be confirmed. At a bulk density that is half of the value which was observed at the laboratory scale, the effective porosity was double as high and still increased marginally over time (1.5 years).

Denitrification correlates positively with temperature. At HRT of 0.4 days, denitrification rates between 8 g-N m^{-3} d^{-1} (17%) at 9°C (after 17 months) and 30 g-N m^{-3} d^{-1} (82%) at 21°C (after 12 months) could be observed. The substrate age (availability of organic carbon) had a high influence on denitrification. In 3 month old substrate denitrification rate was 21 g-N m^{-3} d^{-1} (44%) at 5°C. At HRT higher than 1.0 days denitrification was limited by NO_3^- input in the first year.

The technical scale experiments reinforce assumptions from laboratory results about responsible retention processes of atrazine and isoproturon. At HRT of 0.4 days the formation of bound residues of atrazine correlates positively with temperature (10 mg-ATR m^{-3} d^{-1} (15%) at 9°C to 22 mg-ATR m^{-3} d^{-1} (52%) at 21°C). Substrate age seems to have low effect on atrazine retention during the experiment. At HRT higher than 1.0 days atrazine removal was limited by atrazine input in the first year.

Isoproturon seems to be sustainably retained by microbial degradation under suboxic conditions. No significant degradation is assumed at anaerobic redox conditions. The isoproturon degraders need time to adapt and their performance can be positively correlated with temperature, under the premise suboxic conditions are established (11 mg-IPU m^{-3} d^{-1} (35%) at 5°C to 21 mg-IPU m^{-3} d^{-1} (75%) at 17°C at HRT of 0.5 days).

The herbicides did not interfere with denitrification in the range of tested concentrations. Investigations regarding the effect of a dry phase on NO_3^- and atrazine retention show that denitrification can be improved for a short period, while atrazine attenuation is barely influenced. Instead, dry phases carry the risk of increased wash out of dissolved and particular organic carbon.

The performance of the organic substrate is not stable. It decreases gradually since its effect is based on depletion of the organic carbon by degradation processes (NO_3^-, isoproturon) or linked to the presence of organic carbon as adsorber (atrazine). The organic substrate has to be frequently exchanged, while intervals depend on contaminant loads in the drainage water and temperature. In case NO_3^- is completely removed, the carbon source is used for competitive respiratory processes and the efficiency of the DOC usage for denitrification decreases (70% at HRT of 0.4 days and 35% at HRT of 2.5 days).

In conclusion, the studied design presents a valuable and effective tool in the set of best management practices to reduce loads of nitrate and atrazine as well as isoproturon in drainage waters. System sizing needs to be adapted to conditions present at desired site.

Chapter 1

Introduction

The objective of this study, which is one part of the Aquisafe 2 project, is to extend the process understanding of pesticide and nitrate (NO_3^-) removal from drainage waters in selected near-natural mitigation zone designs to prepare implementation of such systems. Relevant indicator substances, potential simple and efficient reactive substrates and influencing conditions (e.g., flow rates, temperature) were tested in laboratory and technical scale experiments at UBA. The focus was on (i) the analysis of three herbicides: bentazone (BENT), atrazine (ATR) and isoproturon (IPU), all of high relevance for drinking water production due to wide use in USA and Europe (toxicity, frequent occurrence in raw waters a.s.o.), (ii) investigations of a set of organic substrates, which promise the best reduction performance and (iii) testing next to the effect of temperature and substrate age, the influence of short hydraulic residence times (HRT), which are, aside from low temperatures, most critical, but realistic for the retention potential of small mitigation zones for drainage water.

Since the drinking water production in some regions in Europe (e.g. Ic river catchment in Brittany) is endangered because of NO_3^- concentration that exceed the legal threshold, herbicide removal was studied under conditions of similar denitrification settings. According to a comprehensive literature review about NO_3^- removal (Perillon and Matzinger 2010), the addition of organic carbon sources enhance microbial activity and with that the effectiveness of mitigation zones. Reactive swales filled with organic material emerged to be one of the most efficient designs for denitrification at short hydraulic residence times (Schipper et al. 2010).

Different organic filling materials, serving as organic carbon sources for microbial processes, were investigated for their potential to enhance attenuation of pesticides under suboxic to anaerobic conditions. Suboxic conditions establish, when oxygen (O_2) is depleted and NO_3^- is used for microbial respiration and anaerobic conditions are set, when NO_3^-, iron (Fe^{3+}) and manganese (Mn^{4+}) are reduced and sulphate (SO_4^{2-}) is becoming the major electron acceptor. From aerobic to anaerobic conditions the energy yield of respiration is decreasing and reaction speed slows down. In a bioreactor the transition between redox reactions is gradual and several types can occur in parallel.

HRT is defined by flow rate and reactor size. In the experiment different HRT were set by adjusting different flow rates at equal sized reactors at each scale (laboratory and technical).

Objectives of this work were

(i) to study

- denitrification
- the mobility and persistence of selected pesticides in presence of different organic substrates at laboratory and technical scale and
- the influence of pesticides on nutrient transformation,

(ii) to investigate

- effect of a selected experimental design on removal of selected pesticides from agricultural runoff in mitigation zones

and (iii) to provide

- recommendations on efficient design parameters for mitigation systems with low HRT

Chapter 2

Overview of experiments

The overall objective of the experiments is to test reasonable organic substrates for their potential to retain NO_3^- and different herbicides in drainage waters, concurrently and efficiently, to propose sustainable mitigation zone designs for high flow conditions. At laboratory scale single retention processes and influencing factors can be investigated to assess their contribution to and their effect on contaminant attenuation in real adapted systems. Technical scale experiments aim at simulating the operation of designed medium and high flow mitigation zones (worst case) under controlled conditions and at investigating their response to seasonally changing temperatures. In Table 2-1 an overview of the main experiments with objectives and settings is presented. The detailed description of the experimental design is given in chapters 4 and 5.

Table 2-1: Overview of the laboratory and technical scale experiments in this study

Experiments	Objective	Substrates	Substances	Measurements	Remarks
Adsorption / desorption (batch-) experiment at laboratory scale	Investigation of adsorption coefficients and desorption behavior of herbicides in bioreactors with organic substrate	mixture of straw and bark mulch (vol%: 50/50)	Bentazone (80 µg L^{-1} to 6 mg L^{-1}), atrazine and isoproturon (30 µg L^{-1} to 3 mg L^{-1})	Herbicides in solution	One 24 h adsorption step and two 24 h desorption steps water temp.: 21°C
Dissipation (batch-) experiment at laboratory scale	Investigation of dissipation rate and "half life" of herbicides in bioreactors with organic substrate under suboxic to anaerobic conditions	mixture of straw and bark mulch (vol%: 50/50)	Nitrate (100 mg L^{-1}), phosphate (5 mg L^{-1}) and bentazone (50 µg L^{-1}) or atrazine (30 µg L^{-1}) or isoproturon (30 µg L^{-1})	Nutrients and herbicides + selected metabolites in solution and in extract of the substrate, physico-chemical parameters	Duration: 3.5 months water temp.: 21 °C

Experiments	Objective	Substrates	Substances	Measurements	Remarks
Leaching (column-) experiments at laboratory scale	Investigation of breakthrough behavior of nitrate and herbicides in bioreactors with different organic substrate in dependence on HRT under conditions of constant flow, dosing, suboxic milieu and temperature	Pure straw, pure bark mulch, mixture of straw and bark mulch (vol%: 50/50), mixture of straw and sand (vol%: 50/50)	Nitrate (100 mg L^{-1}), phosphate (5 mg L^{-1}) with and without bentazone (50 µg L^{-1}) or atrazine (30 µg L^{-1}) or isoproturon (30 µg L^{-1})	Nutrients and herbicides + selected metabolites in solution and in extract of the substrate, physico-chemical parameters, hydraulic permeability	Bentazone: Hydraulic residence time of 0.2 days for 1 year Atrazine: HRT of 0.2 days for 1.5 years and 0.4 days for 0.5 years Isoproturon: HRT of 0.4 days for 1 year Short term experiments with HRT of 0.4 and 2.0 days for 2 to 3 weeks water temp.: 21 °C
Leaching (swale-) experiment at technical scale	Investigation of breakthrough behavior of nitrate and herbicides in bioreactors (bioretention swales) with organic substrate in dependence on HRT and seasonal temperature change under conditions of constant flow, dosing, suboxic milieu	mixture of straw and bark mulch (vol%: 50/50)	Nitrate (100 mg L^{-1}), phosphate (5 mg L^{-1}) and atrazine (30 µg L^{-1}) or isoproturon (30 µg L^{-1})	Nutrients and herbicides + selected metabolites, physico-chemical parameters, hydraulic permeability	HRT: 0.4-0.5 days and 2.0-2.5 days (for 1-2 years), phases of saturated stagnation during winter and unsaturated stagnation during late summer water temp.: 4 - 23°C

Chapter 3

Laboratory scale

3.1 Set up of laboratory scale experiments

The organic substrates were chosen based on a comprehensive literature review. Final candidates were selected based on a set of criteria that included wide availability, cost effectiveness, and reported efficiency. Bark mulch and straw were selected. Bark mulch is known for its high potential to adsorb organic contaminants. It also provides a long-term source of organic carbon and a stable and highly conductive structure to maintain hydraulic permeability and thus substrate longevity. Straw is reported to be a more readily accessible organic carbon source than bark mulch and is expected to ensure high biological activity and efficient denitrification at short hydraulic residence times (Witz 2005, Rolf 2002).

Highly polluted drainage water was simulated by spiking bank filtered pond water {NO_3^-: < 1 mg L^{-1}, phosphate (PO_4^{3-}): < 1 mg L^{-1}, sulphate (SO_4^{2-}): 180-260 mg L^{-1}, ammonium (NH_4^+): < 0.1 mg L^{-1}, nitrite (NO_2^-): < 0.1 mg L^{-1}, dissolved organic carbon (DOC): 2-4 mg L^{-1}} with nutrients (100 mg-NO_3 L^{-1} $^-$, 5 mg-PO_4^{3-} L^{-1}) and high but realistic herbicide concentrations (30 µg-ATR L^{-1}, 50 µg-BENT L^{-1}, 30 µg-IPU L^{-1}; technically pure). The physico-chemical parameters of the pond water, which is further referred to as process water did not vary significantly during the experiment (pH value: 6.5-7.5, dissolved oxygen (DO): 5-10 mg L^{-1}, redox potential (Eh): 300-350 mV, temperature: 21±1 °C, electrical conductivity: 850-950 µS cm^{-1}).

Column experiments (Figure 3-1) with continuous and constant nutrient dosing were conducted with pure straw, pure bark mulch and a mixture of straw and bark mulch (vol-%:50/50) in two parallels for each substrate. Hydraulic residence times (HRT) of the atrazine and bentazone experiments were set to approximately 0.2 days (2.88 L d^{-1}) to simulate high flow conditions as they occur during rainy periods and snow melts. Column experiments with a fourth substrate combination, a mixture of straw and sand (vol-%:50/50), were discontinued 4 months after start, following a rapid substantial decrease of porosity with the result of reduced denitrification performance. After 4, 5 and 11 months bentazone and atrazine were added each in one parallel column of pure straw, the mixture of bark mulch and straw and pure bark mulch, respectively, to test their potential to interfere with denitrification and the substrate's potential to attenuate the herbicides. The constant dosing of bentazone was stopped after 3 months in straw and in the mixture and after one month in bark mulch. For observation of the effect of flow rate changes on NO_3^- and herbicide removal, HRT was adjusted for short time intervals (2-3 weeks) to approximately 0.4 days (1.44 L d^{-1}) and 2.0 days (0.288 L d^{-1}).

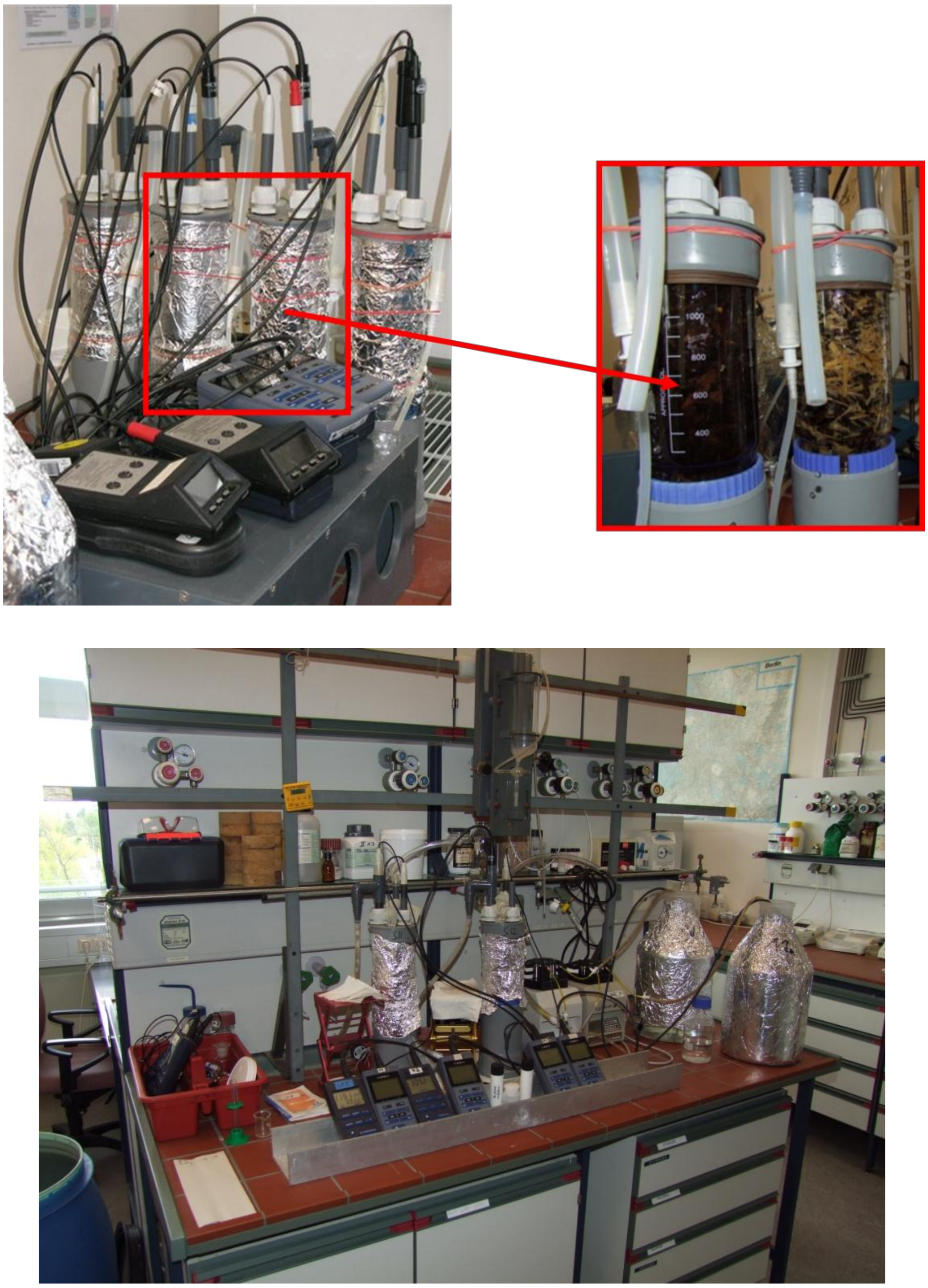


Figure 3-1: Columns of the leaching experiment in the laboratory

After one year of operation the parallels with bentazone contact were stopped. The atrazine parallels were continued for additional 0.5 years at HRT of 0.2 days and subsequently for 0.5 years at HRT of 0.4 days to observe the attenuation potential of the substrates and the effect of HRT more precisely.

Short before the end of the experiment the atrazine parallel of the mixed column was let run "dry" (unsaturated, but wet) for 4 weeks, to observe the effect of a drying period on herbicide retention. After the unsaturated phase and at the end of the main experiments the atrazine parallels of the mixed and the pure bark mulch columns were flushed for 3 weeks with un-amended process water to observe leaching of retained herbicides.

The column experiment with isoproturon was carried out using the bark mulch and straw mixture for 1.5 years, because this substrate showed best performance for concurrent NO_3^- and herbicide attenuation at low HRT. Continuous and constant nutrient- and isoproturon dosing was established from the start. In a parallel control column, only nutrients were added to describe the effect of the herbicide on denitrification.

The HRT in the isoproturon long term experiment was set to around 0.4 days, while the short term effect of decreased flow rate was tested only at HRT of 2.0 days. The change of flow conditions for observation of herbicide removal was done with respect to experiences with atrazine and bentazone in the laboratory and technical scale experiments. At technical scale basis HRTs were set higher than in the laboratory long term experiment with atrazine and bentazone to lower the risk for overlay of temperature- and HRT effects, especially at low temperatures and low HRT and to verify the results of short term HRT experiments in laboratory on long term conditions. At the end of the laboratory experiment leaching of isoproturon was evaluated by washing the organic substrate with un-amended process water and observing the release of retarded substance fractions.

The solution in the inlet and in the outlet of the columns was sampled on a one week basis and analyzed for herbicides, metabolites of atrazine and nutrients. Substrate samples of the mixed atrazine column were taken before and after the dry phase and at the end of the main experiment to analyze the extract for herbicides.

To observe single retention processes and to weigh their contribution to herbicide attenuation, dissipation and adsorption experiments were set up with the mixture of bark mulch and straw, which promised the best performance for concurrent NO_3^- and herbicide removal. In batch experiments 1.1 L glass vessels were filled with 1 L process water amended with 30 µg-ATR L^{-1}, 30 µg-IPU L^{-1} or 50 µg-Bent L^{-1} and 42 g organic substrate (30 g bark mulch and 12 g straw), covered with aluminum foil and sealed with glass stoppers. Dissipation rates and half lives of the herbicides under suboxic to anaerobic conditions were investigated over 3.5 month-period with exponentially increasing sampling intervals (days 1, 2, 4, 7, 12, 29, 56, 92 and 106). The solution and the extract of the substrate were analyzed for herbicides and metabolites of atrazine to distinguish between degradation and adsorption.

Adsorption coefficients and desorption behavior of the herbicides were evaluated with batch experiments according to OECD 106 for an equilibration time of 24 hours for adsorption followed by desorption experiments, during which the substrate was agitated with pure process water in two 24 hour lasting desorption steps. Herbicide concentrations ranged in logarithmical spacing between 80 µg L^{-1} and 6 mg L^{-1} for bentazone and between 30 µg L^{-1} and 3 mg L^{-1} for atrazine and isoproturon. The evacuated solutions of the adsorption and the two desorption steps were analyzed for herbicides.

The dissolved and adsorbed fractions of atrazine and bentazone and the dissolved fractions of isoproturon were analyzed by GC/MS. The dissolved fractions of the major metabolites of atrazine (hydroxy-atrazine (HA), desethyl-atrazine (DEA) and desisopropyl-atrazine (DIA)) and the adsorbed fraction of isoproturon were analysed by LC/MS. Recovery was considered for all measurements in comparison with internal standards, which were added to each batch of samples (terbuthylazine for atrazine and bentazone; chlortoluron for isoproturon) before preparation. In LC/MS no preparation of liquid samples was necessary. Recovery for adsorbed fraction of isoproturon was

estimated by comparison with external standards (IPU). To quantify the recovery of the substances in the organic substrate, the standards were first added to dry straw and bark mulch and extracted after volatilization of the solvent ethyl acetate (2 to 3 days).
The inorganic ions NO_3^-, SO_4^{2-}, NO_2^- were measured with ion chromatography. PO_4^{3-} and NH_4^+ were analyzed photometrically. DOC was measured after combustion as carbon dioxide. All measurements were performed in duplicates.

In Table 3-1 quantification limits and recovery rates in different media are presented.

Table 3-1: Quantification limits and recovery rates

Method	Substance	Media	Quantification limit	Recovery rate
Gas Chromatography/ Mass Spectrometry	Bentazon	Process water	2.18 µg L^{-1}	76±5%
		Straw	0.2 µg g^{-1}	80±9%
		Bark mulch	1.0 µg g^{-1}	
	Atrazin	Process water	0.17 µg L^{-1}	86±1%
		Straw	0.3 µg g^{-}	80±8%
		Bark mulch	1.1 µg g^{-1}	
	Isoproturon	Process water	0.17 µg L^{-1}	121±2%
Liquid Chromatography/ Mass Spectrometry	Isoproturon	Mixture of straw and bark mulch	0.2 ng g^{-1}	104±15%
	DIA	Process water	0.13 µg L^{-1}	-
	DEA	Process water	0.14 µg L^{-1}	-
	HA	Process water	0.05 µg L^{-1}	-
Ion Chromatography	NO_3^-	Process water	1 mg L^{-1}	-
	SO_4^{2-}	Process water	5-10 mg L^{-1}	-
	NO_2^-	Process water	0.1 mg L^{-1}	-
Photometry	PO_4^{3-}	Process water	0.1 mg L^{-1}	-
	NH_4^+	Process water	0.1 mg L^{-1}	-
Non Perchable Organic Carbon	DOC	Process water	0.1 mg L^{-1}	-

3.2 Results of laboratory experiments

3.2.1 Hydraulic conductivity of selected organic substrates

Hydraulic properties of the substrates in the three duplicate column pairs are summarized in Table 3-2. Tests indicate extremely high saturated hydraulic conductivity beyond values of coarse sand and gravel. Effective porosity is higher than in sand and gravel (15-35%). After one year of operation, effective porosity decreased in average by 25% of starting values in straw, bark mulch and the mixture of these substrates, indicating that there is stability of hydraulic conductivity over time.

Table 3-2: Hydraulic parameters of laboratory columns filled with organic substrate (this study). For each substrate two columns were operated.

Parameter	Unit	Straw-bark mulch		Bark mulch		Straw	
Saturated hydraulic conductivity (K_S-value) [a]	x 10^{-1} cm s^{-1}	1.4 ± 0.1		1.6 ±0.1		1.10± 0.05	
Bulk density [a]	g cm^{-3}	0.19		0.27		0.11	
Porosity [a]	%	59.5		48.5		82.5	
Effective porosity (at start / after one year)	%	39 / 30	51 / 37	46 / 32	48 / 39	37 / 31	56 / 37

[a] Single values are the average for the column duplicates.

3.2.2 Nitrate removal

The process, mainly accountable for NO_3^- removal in the bioreactors of this study, is expected to be denitrification. Studies under comparable conditions have shown that competitive NO_3^- transforming processes, like immobilization into biomass or DNRA (dissimilatory nitrate reduction to ammonium) are negligible in relation to reduction of NO_3^- to N_2O and N_2 (Greenan et al. 2006, Gibert et al. 2008). Schipper et al. (2010) cited NO_3^- removal studies with wooden substrates in laboratory and field, where no considerable DNRA was observed, but remained concerned about more labile carbonaceous substrates. In this study with bark mulch and straw in general ammonium concentrations lower than 0.5 mg L^{-1} (data not shown) were observed in the outlet, including potential NH_4+ formed by degradation of organic material (ammonification). In the following text the terms NO_3^- retention and denitrification are used synonymously.

Effect of the organic substrate on denitrification

Denitrification results of the column experiments on the basis of organic carbon supply by the substrates are shown in Figure 3-2 and Figure 3-3. The reduction of NO_3^- concentrations decreased within 1.5 years gradually from 30 to 8 % in pure bark mulch, 85 to 18% in the mixture and 95 to 25 % in pure straw (Figure 3-2) and stabilized at this level at the end. The increase of HRT to 0.4 days at same input concentrations resulted in doubling of relative denitrification and remained without changes for further 6 months in all three substrates (Figure 3-2).

Aslan and Türkman (2005) and Soares and Abeliovich (1998) observed equally high start rates of denitrification (40 g-N m^{-3} d^{-1} and 50 g-N m^{-3} d^{-1}, respectively) in laboratory straw columns with input concentrations of 100 mg-NO_3^- L^{-1}, at room temperatures between 25 and 30°C and at HRT between 0.1 and 0.2 d (compare with Figure 3-3). In these studies denitrification was kept stable by substrate addition as soon as rates started to drop.

Stable denitification rates of 3 to 10 g-N m^{-3} d^{-1} after 1.5 years (Figure 3-3) are in the range of average denitrification rates observed in wood-filled bioreactors between 2 and 22 g-N m^{-3} d^{-1} at temperatures between 2 and 22 °C (Schipper 2010).

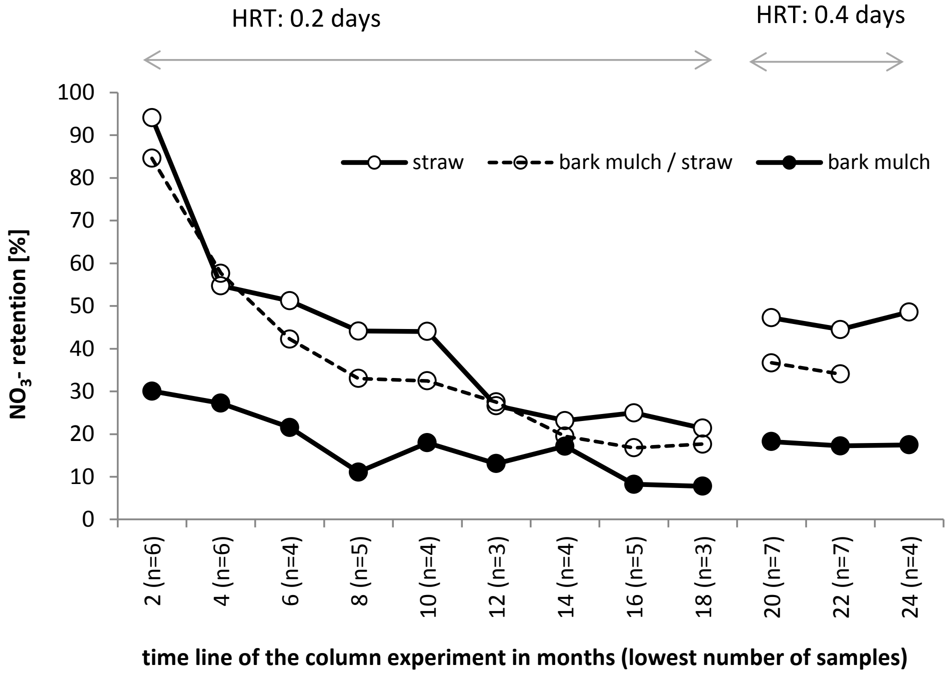
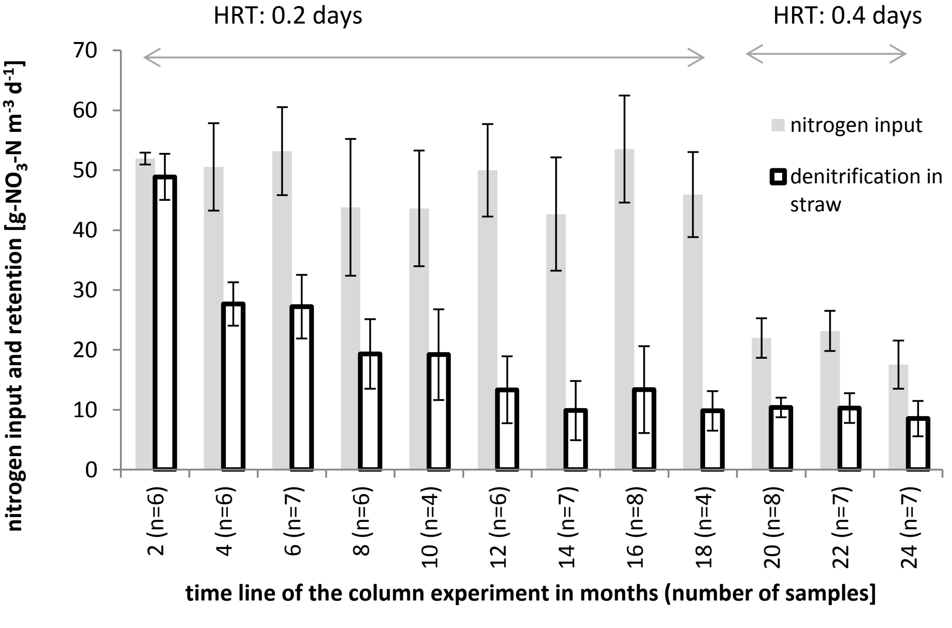
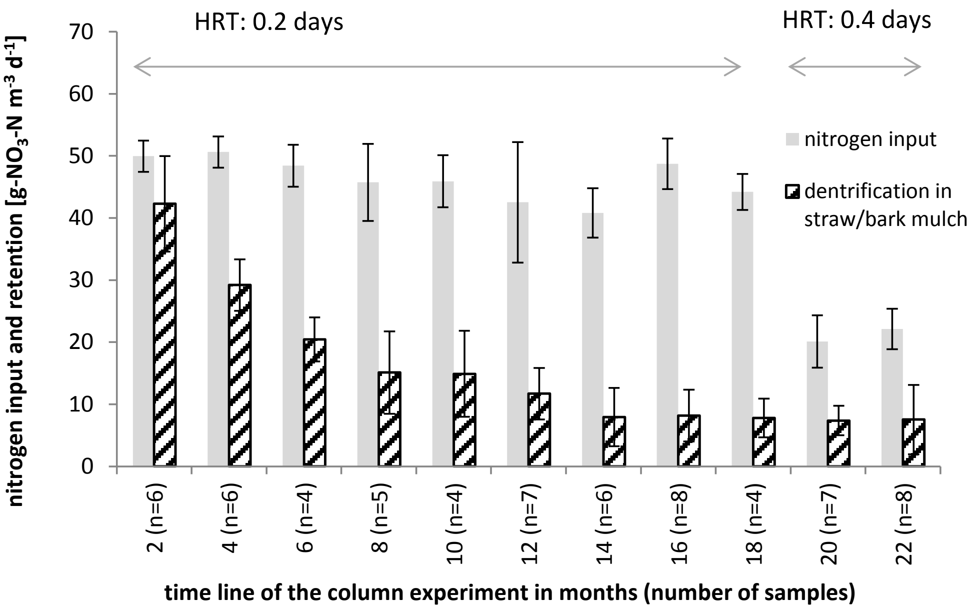


Figure 3-2: Development of NO_3^- retention (denitrification) in different substrates over 1.5 years at hydraulic residence time (HRT) of 0.2 days and over following 0.5 years at HRT of 0.4 days. The positive effect of external organic carbon sources on denitrification is excluded. The values are averages of n measurements during two month periods. The lowest number of samples (n) in the different substrates is presented at the x-axis.





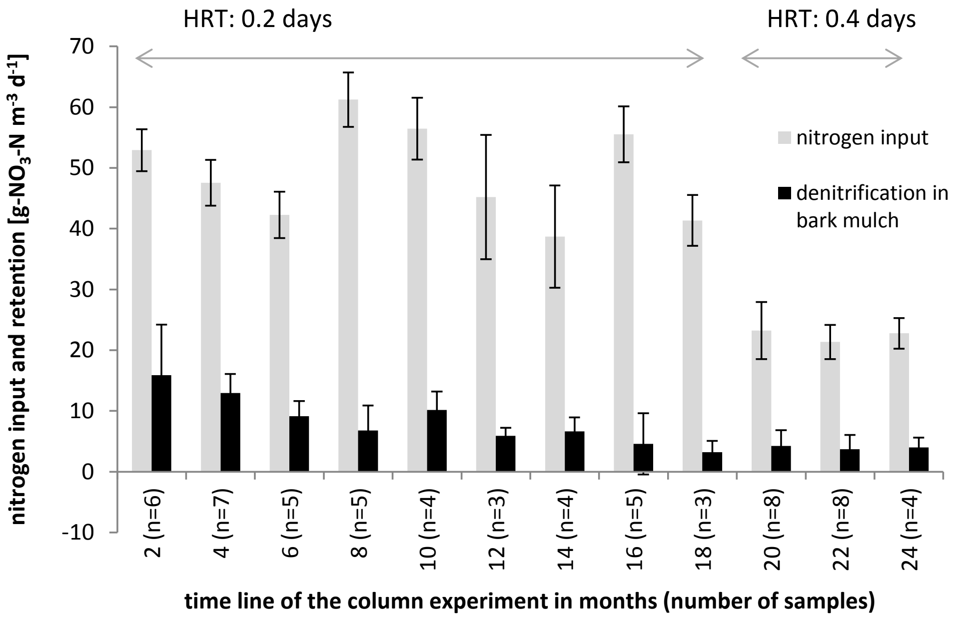


Figure 3-3: Development of nitrogen input and NO$_3^-$N retention (denitrification) in different substrates (top down: pure straw, a mix of bark mulch and straw, pure bark mulch) over 1.5 years at hydraulic residence time (HRT) of 0.2 days and over following 0.5 years at HRT of 0.4 days. The positive effect of external organic carbon sources on denitrification is excluded. The values are averages of n measurements during two month periods, the variance of the values is depicted as double standard deviation.

To evaluate, how efficient the organic carbon sources were used for denitrification, in Figure 3-4, the ratio between the wash out of internal DOC (substrate) and DOC used for denitrification on the one hand (negative values) and the ratio of the use of external DOC (inflow) and DOC used for denitrification on the other hand (positive values) in the different substrate are presented.

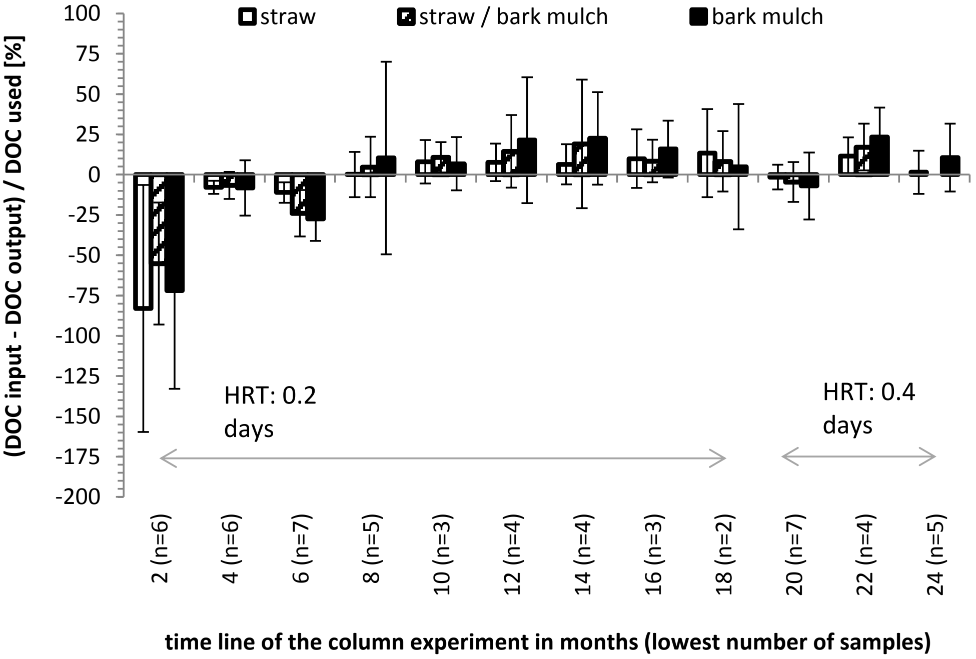


Figure 3-4: Effectiveness of different substrates as organic carbon sources for denitrification during 1.5 years at hydraulic residence time (HRT) of 0.2 days and during following 0.5 years at HRT of 0.4 days. Negative values present wash out of not used carbon in relation to carbon used for denitrification; positive values show contribution to denitrification by external carbon (inflow) in relation to carbon used for denitrification. Values are averages of n measurements during two month periods. The variance of values is depicted as ± standard deviation. The lowest number of samples (n) in the different substrates is presented at the x-axis.

The laboratory scale experiments at room temperature (21±1°C) reveal that straw and bark mulch enable efficient denitrification at short hydraulic residence times (0.2 days). With respect to results of related studies (Figure 7-1 in appendix 1), which prove the positive effect of organic substrates on denitrification in mitigation systems, straw and bark mulch show high potential as effective sources of organic carbon.

A drawback of straw as organic carbon source is high DOC leaching at the beginning (Figure 3-4). In the first two months nearly the same amount of DOC, which is used for denitrification, is washed out (80%). Start concentrations of DOC in the outlet were around 250 mg L^{-1}, decreasing gradually to 10 mg L^{-1} during the first 1.5 months (data not shown). Bark mulch is a more resistant organic carbon source, proved by the facts that observed NO_3^- removal was ~ 3 times lower than in straw throughout the experiment (Figure 3-2, Figure 3-3), the wash out of DOC in the beginning was lower (negative values in Figure 3-4) and the contribution of the external carbon source to denitrification to the end was higher (positive values in Figure 3-4) than in straw. Both substrates are characterized by unstable denitrification performance especially during the first year due to progressive decomposition of the substrate and increase of the ratio of resistant and readily available organic carbon species.

The combination of both substrates seems to reduce individual negative side effects, while denitrification is nearly as high as in straw (Figure 3-2, Figure 3-3).The comparison of denitrification in the straw-, the bark mulch- and the mixed-column shows that in the presence of straw, denitrification on the basis of bark mulch as carbon source is higher (+23 %) than in the pure bark mulch column. The availability of organic carbon from bark mulch seems to be enhanced, because overall microbial activity is increased due to access of readily available organic carbon from straw. The more resistant carbon species like lignin can be degraded by co-metabolic reactions (Kögel-Knabener 2010, p.61). According to estimations by Schipper et al. (2010) the high denitrification rates indicate that DOC from straw and bark mulch is predominantly used for denitrification. Since NO_3^- is, with exception of the start phase, no limiting factor, and SO_4^{2-} concentrations are barely reduced (data not shown), desulfurication seems to be an insignificant competing organic carbon consumer, at least at HRT lower than 0.4 days.

Effect of HRT on denitrification

The HRT is defined by the volume of the reactor and the flow rate. It can be modified by changing the volume of the reactor, the flow rate or both (under the premise the ratio of both values is changing). In our attempt the HRT was increased by lowering the flow rate. To avoid disrupting the primary experiment, the effect of changing HRT was examined in embedded short duration experiments. For two to three weeks HRT was increased via changes in flow to the twofold and to the tenfold value to learn more about the behavior and the potential of the carbon sources. The changes in HRT were adjusted between the 100th and the 300th day of the column experiment (parallels with atrazine) when denitrification performance was less influenced by wash out of DOC and contribution of the external carbon source to denitrification (Figure 3-2, Figure 3-3, Figure 3-4). The observed values during these phases of higher HRT {0.34 days (8 months after start) and 1.7 days (4 months after start)} were compared to the average of values for low HRT of the main experiment (0.17 days) between the 100th and the 300th day.

Figure 3-5 compares 23 weeks with HRT of 0.17 days (19 samples) with 2 weeks with HRT of 0.34 days (3 samples) and 3 weeks with HRT of 1.7 days (1 sample). The resulting uncertainty is shown in the standard deviation. Despite the different duration of the three experiments, results show with increasing HRT a trend of increasing DOC concentrations, decreasing redox potential and decreasing NO_3^- concentrations in the outlet.

At HRT of 0.34 d relative denitrification (reduction of concentrations) nearly doubles with double HRT. As the input load of NO_3^- is half of the load of NO_3^- at HRT of 0.17 days, loads of reduced NO_3^- are about the same. It is possible that the reduced input loads prevailing at higher HRT contributed to the observed increased relative denitrification; however, this cannot be evaluated with experimental design. At HRT of 1.7 days DOC production is sufficient for complete denitrification of 100 mg NO_3^- L^{-1} in all substrates. With NO_3- concentration becoming the limiting factor, unused DOC is washed out and the potential steps down to less energetically effective redox reactions. While reduction of NO_3^- concentrations is improving with an increase in HRT, the straw column could become a source of DOC. In contrast, bark mulch alone becomes more potent as a carbon source for denitrification at increased HRT, showing the same denitrification potential as straw but without the associated DOC release.

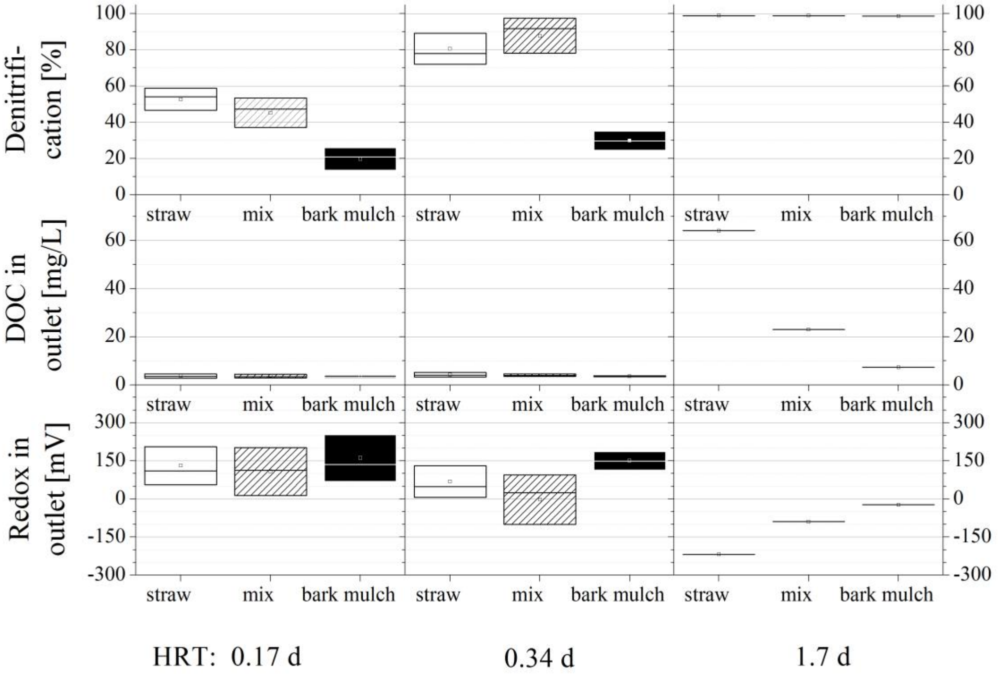


Figure 3-5: Effect of hydraulic residence time on denitrification (ratio of reduced nitrate concentrations and input concentrations), dissolved organic carbon (DOC) and redox conditions for three substrates straw, bark mulch and a mix of straw and bark mulch. The box plots show mean, median and standard deviation of measurements between the 100th and the 300th day of the experiment. HRT was increased for short term periods (2 to 3 weeks). At HRT of 0.17, 0.34 and 1.7 days the numbers of samples for all substrates were 19, 3 and 1, respectively.

Long term studies with increased HRT (0.4 days) at the end of the column experiments with atrazine and bentazone and throughout the column experiments with isoproturon put the results of the short term experiments into perspective. Increased DOC wash out cannot be confirmed, neither the difference of wash out between straw and bark mulch. It seems to be a temporal effect of the change of HRT on the microbiological equilibrium with higher impact in straw, which decreases and relativizes with time. On the other hand, it can be confirmed that relative denitrification (reduction of concentrations) increases with increasing HRT (Figure 3-2). Similar behaviour of organic carbon sources (sugar cane and reed) were observed by Wang and Wang (2012) for lower temperatures (12°C).

The increase of HRT could also be responsible for the stabilization of denitrification rates to the end of the experiment, but this is still uncertain. To understand the HRT effect conclusively, the influence of HRT on NO_3^- loads has to be evaluated in further studies.

Effect of herbicides on denitrification

To observe possible interactions with herbicides, denitrification was observed before and after the start of atrazine and bentazone dosing in the duplicates of the straw-bark mulch column. In Figure 3-6 it is shown that the denitrification performance did not change significantly after herbicide dosing (188th day of the column experiment). This is a strong indication that herbicides in the range of applied concentrations do not interfere with denitrification. Ilhan et al. (2011) used 100-fold higher concentrations of atrazine in wood chips and did not observe negative effects on denitrification. Jørgensen et al. (2004) found in columns with till that was degraded to natural DOC that bentazone rather improved denitrification, acting as a source of organic carbon.

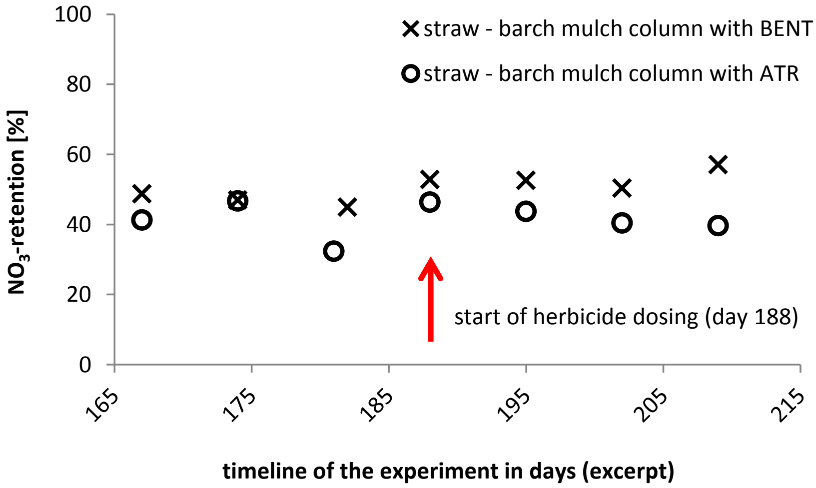


Figure 3-6: Denitrification performance in the straw-bark mulch columns before and after dosing of herbicides on the 188th day of the experiment – values are ratio of difference between input- and output-concentration and input concentration (single measurements).

In Figure 3-7 the denitrification performance over time in the straw-bark mulch parallels with and without isoproturon are presented. Differences in denitrification during the experiment are low and can be explained rather with differences in access to DOC than effect of the herbicide. Higher denitrification in the column without isoproturon compared to the column with isoproturon during the first half year of the experiment correlates with higher DOC concentrations in the outlet of the column without isoproturon (data not shown) and seems to contribute to higher content of immediately available organic carbon, which is provided by enzymatic decomposition of the substrate after death of the plant (senescence). The organic substrates are highly heterogenic materials and variations in the columns due to composition of the substrate and packing have to be expected. Higher denitrification in the column with isoproturon than in the column without isoproturon after 6 months can be explained with (i) the decrease of the effect of immediately available organic carbon due to use up of its reservoir and (ii) the increase of the effect of higher DOC input concentrations due to continuous dosing of isoproturon in an organic dissolving agent. It can be stated, that isoproturon did not interfere with microbiological NO_3^- reduction through the range of concentrations evaluated. Widenfalk et al. (2004) observed no effect of isoproturon (30 µg L^{-1}) on denitrification rates in freshwater sediment.

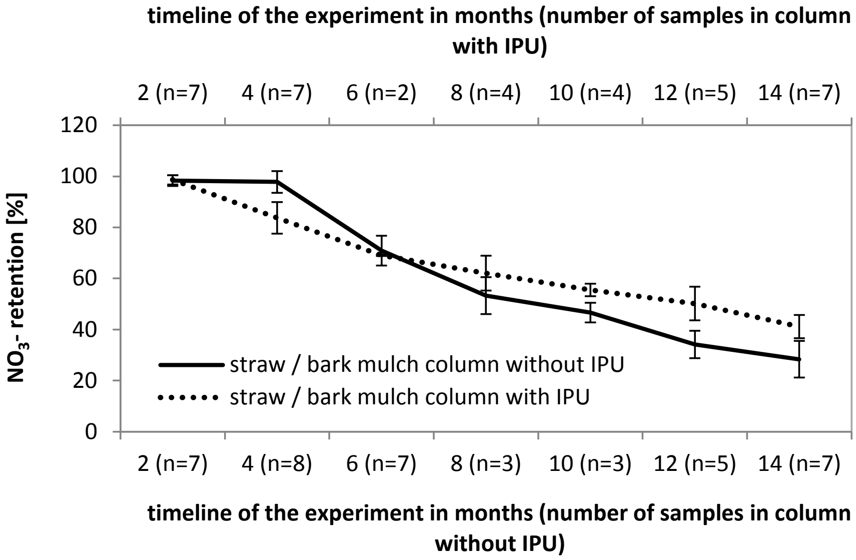


Figure 3-7: Nitrate retention in straw-bark mulch columns with and without addition of isoproturon

Effect of unsaturated conditions on denitrification

In addition to the effect of changes in HRT on denitrification, the impact of unsaturated phases on the availability of DOC and on the activity of denitrifying bacteria was studied in the straw-bark mulch column shortly before end of the main experiment.

Figure 3-8 shows that after 3 weeks of drying (unsaturated, wet conditions from day 13 to day 34) and 4 weeks of rewetting (saturated conditions without addition of nutrients and herbicides from day 34 to day 63) denitrification reconstitute immediately, which can be noted from lowered NO_3^- output concentrations with the restart of dosing. The increase of NO_3^- concentrations shows that denitrification performance decreases gradually back to the level before start of dry phase within 1 month. Improved NO_3^- removal can be explained by stronger depletion of the organic substrate during dry phase under wet but aerobic conditions which resulted in formation of a reservoir of readily available carbon, which is then reduced under re-established saturated conditions. The enhanced DOC production under aerobic conditions carries along the risk of turning the organic substrate into a source of nutrients (wash out of DOC), when no or not sufficient electron acceptors, like NO_3^- are present.

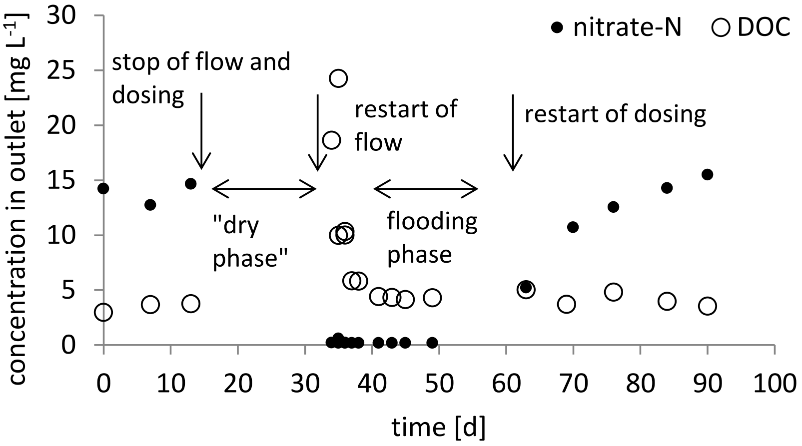


Figure 3-8: Impact of “dry phase” (unsaturated, wet) on denitrification potential. During flooding phase no nutrients or herbicide have been dosed. Nitrate-N stands for nitrate nitrogen and DOC stands for dissolved organic carbon.

3.2.3 Mobility and persistence of bentazone, atrazine and isoproturon in presence of organic substrates

3.2.3.1 Bentazone

Under the column experimental conditions (suboxic conditions: ~ +150 mV, HRT: 0.2 days, room temperature: 21±1°C), in all three substrates no notable reduction of bentazone was observed. In Figure 3-9 the straw-bark mulch column is shown as an example. Increasing the HRT to 0.4 days did not change this behaviour (Figure 7-1 in appendix 1). Dissipation (data not shown) and adsorption experiments (Figure 7-4 and Table 7-3 in appendix 4) confirmed high mobility of bentazone. This is believed to be related to its low adsorptive behaviour, its high solubility in water and its high persistence under suboxic conditions (Krause et al. 2010).

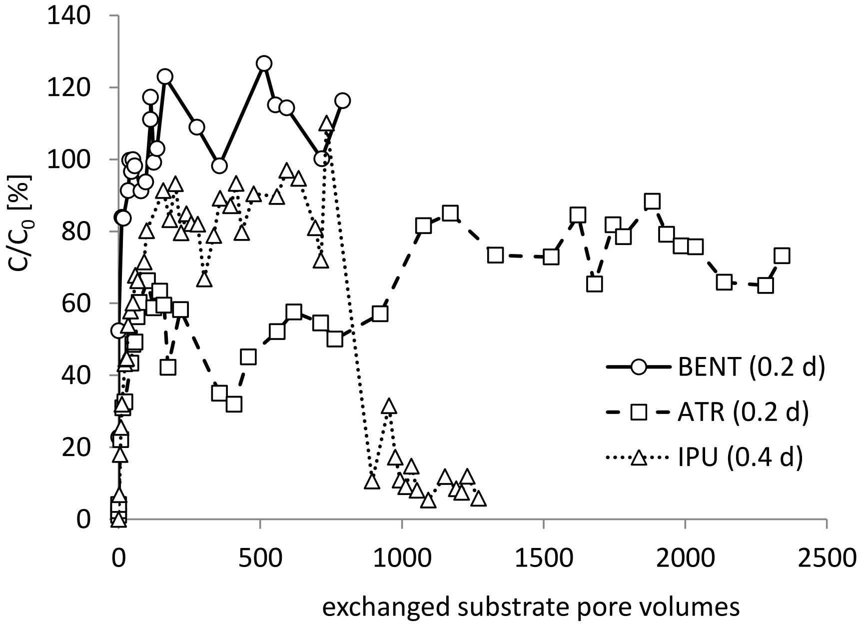


Figure 3-9: Breakthrough curves of herbicides in experiment with straw-bark mulch columns. Variances due to short-term experiments with altered flow are excluded. Values in legend are hydraulic residence time (HRT).

3.2.3.2 Atrazine

From the start of atrazine dosing in the column experiment at HRT of 0.2 days the atrazine concentration in the aqueous solution was substantially lowered after passage through the straw-bark mulch column. Initial values of relative retention (60%) decreased within 4 months to a level of approximately 20%, at which they remain for 0.5 years, before they increase again to about 45%, due to increase of HRT from 0.2 to 0.4 days (Figure 3-10).

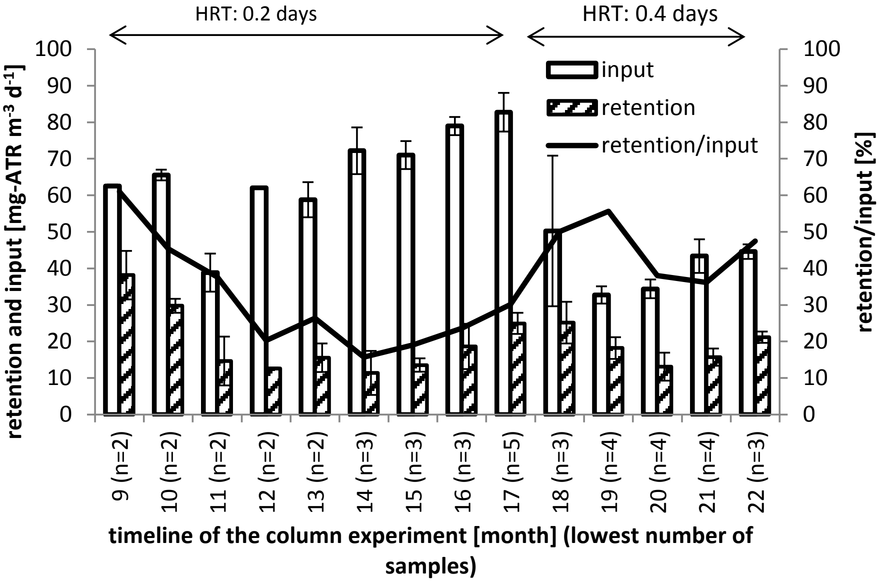


Figure 3-10: Development of atrazine retention in the straw-bark mulch column at HRT of 0.2 and 0.4 days.

The results of the batch experiments for investigation of dissipation (Figure 7-3 and Table 7-2 in appendix 3) and adsorption (Figure 7-4, Table 7-3 and Table 7-4 in appendix 4) reflect these observations of fast and substantial removal from solution.

Increased HRT at same input concentrations results in increased relative retention (reduction of concentrations) of atrazine in the mixture of straw and bark mulch (HRT of 0.4 days: 50 % of inflow (8th month and 19th to 22nd month); HRT of 2.0 days: 80% of inflow (17th month)) (Figure 3-11). The absolute retention of atrazine (reduction of loads) is not changing with increasing HRT (Figure 3-10).

But, according to Lesan and Bhandari (2004), longer herbicide-soil contact times result in attenuated water extractability and enhanced non-extractable residue formation. Also, with increasing HRT the contribution of degradation to atrazine removal from solution is expected to be increasing, as indicated by increased concentrations of the metabolite HA (Figure 3-11).

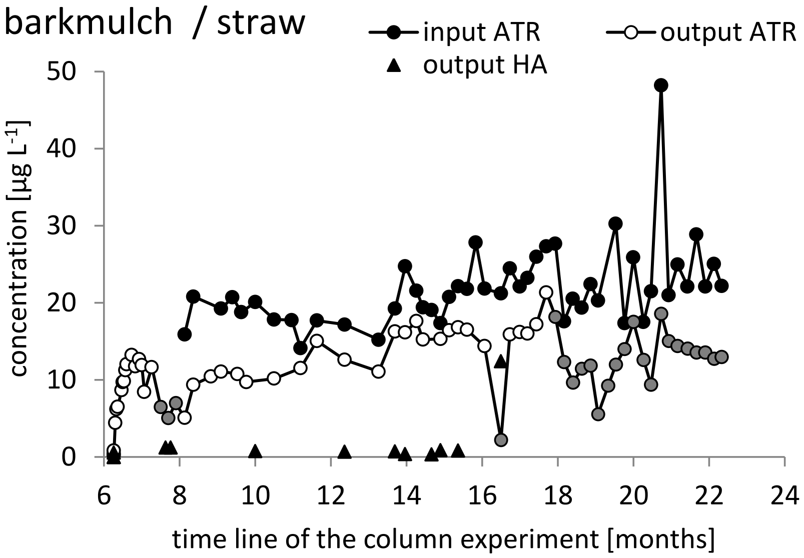


Figure 3-11: Input and output concentrations of atrazine (ATR) and output concentrations of hydroxyl-atrazine (HA) at hydraulic residence time (HRT) of 0.2 days in the straw-bark mulch column. Grey symbols present output concentrations during short term experiments with increased HRT (0.4 days in month 8 and from month 19 to 22 and 2.0 days in month 17).

Although degradation can be proven by metabolite analysis, its contribution to atrazine dissipation in solution is of minor relevance (< 15%) at HRT of 0.2 to 0.4 days. HA seems to be the major metabolite. The findings of DEA and DIA analysis were in all columns below 0.2 µg L^{-1}. At short HRT adsorption seems to be the major retention process. At increased HRT of 2.0 days the relative atrazine retention and thereby the contribution of degradation on atrazine retention increases to 50 %. The formation of HA loads is about the same at all HRT.

In Figure 3-12 analogue values of atrazine retention in pure bark mulch are presented. Atrazine retention is more effective than in the straw-bark mulch mixture, due to higher content of bark mulch in the column, which indicate the importance of bark mulch as adsorber and the contribution of adsorption to atrazine removal. Other than in the straw-bark mulch mixture HA formation in bark mulch is barely increased at HRT of 2.0 days. The microbiologically more active environment of straw could favour co-metabolic atrazine degradation. This observation has to be confirmed with technical scale experiments.

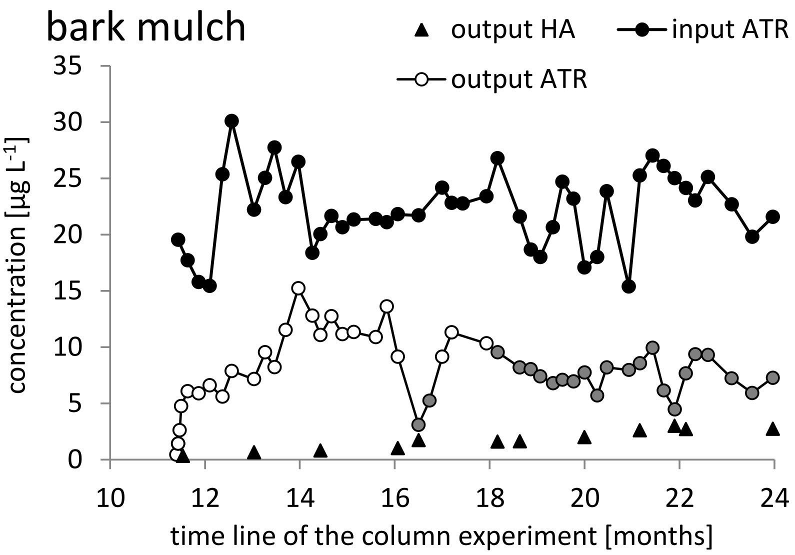


Figure 3-12: Input and output concentrations of atrazine (ATR) and output concentrations of hydroxy-atrazine (HA) in the bark mulch column at hydraulic residence time (HRT) of 0.2 days. Grey symbols present output concentrations during periods with increased HRT (2.0 days in 17th month and 0.4 days from 19th to 24th month).

Effective adsorption leads to high retention but also to high persistence against microbial degradation of the adsorbed fraction, because access of microorganisms is restricted. Even so, direct microbial degradation of atrazine on the matrix may be possible (Abdelhafid et al., 2000). Trigo et al. (2010) found that atrazine is bioaccessible in organoclay complexes, Park et al. (2003) observed mineralization of soil-sorbed atrazine and proclaimed that high concentration on the matrix and the attachment of microorganisms onto soil particles seem to contribute to its bioavailability and Jablonowski et al. (2008) showed that long-term aged bound residues of atrazine are still partly accessible to the degrading microorganism Pseudomonas sp. strain adp. Herzberg et al. (2004) observed high degradation of atrazine adsorbed to granulated activated carbon [70 mg-ATR L^{-1} d^{-1}] by isolated Pseudomonas sp. strain adp., using the herbicide as N source.

However, adsorption experiments, literature studies (Gendrault 2004), low metabolite concentrations and the comparison of atrazine breakthrough in pure straw and in pure bark mulch (Figure 3-13) indicate predominantly adsorption to bark mulch and formation of bound residues. The important role of organic matter (humins, humic and fulvic acids) in soils for atrazine retention and formation of bound residues is emphasised in several studies, e.g. Loiseau et al. (2000), Lesan and Bhandari (2004). Under suboxic conditions as established in the experiment the formation of humic substances is expected, e.g. by lignin modification.

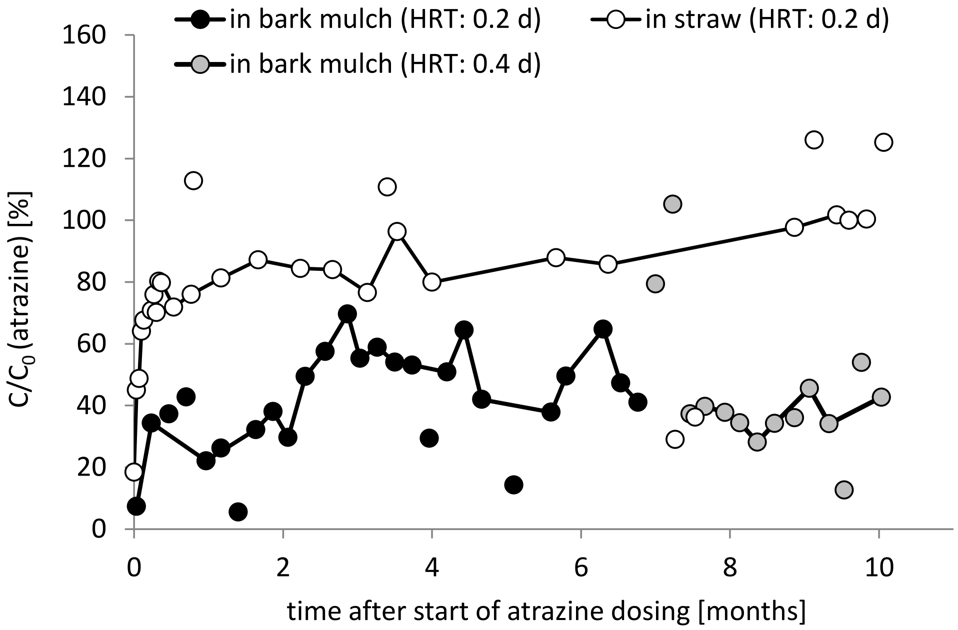


Figure 3-13: Breakthrough curves of atrazine (ATR) in pure straw and in pure bark mulch. Single symbols are outliers. After 7 months of atrazine dosing the flow in the bark mulch column was reduced by half (grey symbols).

Positively in the scope of sustainable attenuation of atrazine is that according to de Andrea et al. (1997) the main part of the bound residues is expected to be HA, which is predominantly formed under denitrifying conditions (Crawford et al., 1998) and in anaerobic sediments with carbon addition (Chung et al., 1996) by chemical degradation (Ma and Selim 1996). The hydrolysis of atrazine is soil-surface catalyzed, i.e. adsorption favours degradation to HA (Armstrong et al. 1967). The metabolite shows a more intense binding behaviour than atrazine (Bouwer et al. 1990, Lerch et al. 1998) and is less toxic in case it is released by desorption from the matrix or decomposition and dissolution of the organic substrate.

Although atrazine can be successfully retained under high flow and suboxic conditions by adsorption to the studied substrates, the fate of atrazine in the studied substrates and with that the risk of its revolving into the environment remains uncertain, since the expected comprehensive transformation to HA cannot be proven in this study.

In addition to the effects of temperature and changes in HRT, the impact of unsaturated phases on atrazine retention and the leaching potential of atrazine was studied in the straw-bark mulch column.

Analysis of atrazine bound to the matrix before and after the unsaturated phase (3 weeks) showed a decrease of 75% of the herbicide fraction, which was extractable with methanol and water. It has to be kept in mind, that the extracted fraction of adsorbed atrazine is marginal in comparison to the retained fraction which was not degraded to HA, DEA or DIA during the experiment (<1 %). It seems that the extraction method was not adequate to determine aged bound residues of atrazine.

Leaching studies after the unsaturated phase and after the regular flow phase at the end of the experiment have shown that if the reactor is flushed with un-spiked process water, the atrazine concentrations in the outlet decrease progressively within 2 to 3 weeks to 5-10% of former input concentrations. The thereby washed out fraction of atrazine is marginal in comparison with the retained fraction during the experiment, which suggests stable binding of atrazine to the organic matrix.

3.2.3.3 Isoproturon

The mechanism of isoproturon dissipation is different from that of atrazine. In sorption experiments the adsorption potential of the straw-bark mulch mixture is identical for atrazine and isoproturon (Figure 7-4 in appendix 4). But, the stability of the bindings between isoproturon and the matrix of the substrate seems to be lower (Table 7-3 in appendix 4). In accordance to these observations, reduction of isoproturon in the column experiment is less efficient at the onset compared to atrazine, although contact times are twice as high (Figure 3-9, Figure 7-2 in appendix 2).

The short term increase of HRT to 2.0 days provoked even lower retention. After change back to standard flow (HRT: 0.4 days) a modification in the mechanism of retention processes was observed. The reduction of isoproturon concentrations increased drastically and remained stable at this level (Figure 3-9, Figure 7-2 in appendix 2). The reason is not clear, yet. One explanation could be the establishment of efficient isoproturon metabolism as result of increased HRT and nearly complete NO_3^- reduction. It is possible that the low flow, NO_3^- poor, anaerobic phase was necessary for the adaptation of the microbial population to use isoproturon as a source of nutrients. After re-establishment of high flow and suboxic conditions, the isoproturon metabolism was not suppressed and the energetically more efficient milieu resulted in efficient isoproturon degradation, at least to metabolites which are less relevant for drinking water production (Tedesco et al. 2009). According to the Directive 91/414 EEC, Isoproturon has no known relevant metabolites; this signifies that (i) the known metabolites have no comparable intrinsic properties to the parent chemical in terms of its biological target activity, and (ii) the known metabolites have no toxicological properties that are considered severe, and (iii) the known metabolites do not pose a higher or comparable risk to organisms than the parent substance. Chemical degradation of isoproturon in aqueous solution under moderate temperature and within a pH range of 4 to 10 is of minor importance in most agricultural soils (Sørensen et al. 2003).

3.3 Summary of laboratory scale results

In laboratory experiments straw and bark mulch were found to be suitable organic carbon sources, supporting instantaneous and efficient denitrification at high temperatures (21±1°C) and at low HRT (0.2 days). Relative denitrification increases with increasing HRT as outflow concentrations decrease (to 0 at HRT of 2.0 days). Hydraulic permeability is assured for long term period. The combination of both substrates diminishes negative side effects such as DOC wash out in straw and the resistance of organic carbon species in bark mulch against decomposition. With exception of the start phase, when denitrification performance gradually decreases, the organic substrates are stable carbon sources for constantly high and potentially long-lasting denitrification (compare Figure 3-3). Periodical contact with oxygen as in unsaturated phases improves degradation of the substrates and the availability of organic carbon on the upside but increases the risk for DOC wash out and reduces longevity of the carbon source on the downside.

The suitability of the substrates for herbicide retention under denitrifying conditions depends on specific herbicide characteristics. Atrazine and bentazone are highly persistent against biotic degradation under suboxic conditions at low HRT. While bentazone is barely retained due to high solubility in water, dissolved atrazine is partly degraded to metabolites, but predominantly adsorbed on the substrate. Atrazine attenuation is substantial and bindings are supposedly irreversible in form of HA. Increasing HRT positively affects the stability of bindings and the contribution of degradation to atrazine retention. The effect of the substrates on isoproturon retention is still uncertain. Straw and bark mulch contribute to retardation by adsorption, but other than atrazine, the behavior of isoproturon in the column indicates effective degradation after adaption of microorganisms to the herbicide, perhaps as source of nutrients.

Chapter 4

Technical scale

4.1 Set up of technical scale experiments

There are many potential experimental design configurations possible in laboratory experiments. However, conditions are artificial and experimental design has a strong influence on experimental outcomes. Laboratory experiments alone are not suitable for reproducing complex natural processes, but designed well, they give a good first approximation of processes and can help to narrow down the variability of possible solutions. Technical scale experiments provide another level of information because they enable to simulate near natural conditions. The variability of influencing factors can still be limited to distinguish between single processes and environmental effects.

Laboratory results show, that bentazone is too mobile and persistent to be reduced under denitrifying conditions at short HRT and therefore was not applied in technical scale experiments. Furthermore, the mixture of bark mulch and straw is favored for parallel and efficient NO_3^- and atrazine or isoproturon retention and was chosen as substrate for all technical scale experiments. In the laboratory HRT was shown to be an influencing factor for relative retention of NO_3^- and herbicides. Since temperatures at technical scale would be lower, higher HRTs in the range from 0.4 to 2.5 days were realized to make sure effects of HRT and temperature on retention could be observed and distinguished.

The inflow water was of the same origin and equally spiked with contaminant concentrations as in the laboratory column experiments, except bentazone was not further considered. As systems were equally sized, HRT of approximately 0.5 and 2.0 days were established by different flow rates (about 500 L h^{-1} and 100 L h^{-1}, respectively), resulting in decreased input loads at higher HRT

To measure impact of temperature variation and HRT on NO_3^- and atrazine retention, two parallel swales (8x2x0.9 m) were manually filled each to a height of 0.8 m with a mixture of straw (550 kg) and bark mulch (1050 kg) and operated at water level of 0.4 m at HRT of about 0.4 and 2.5 days, respectively (Figure 4-1).

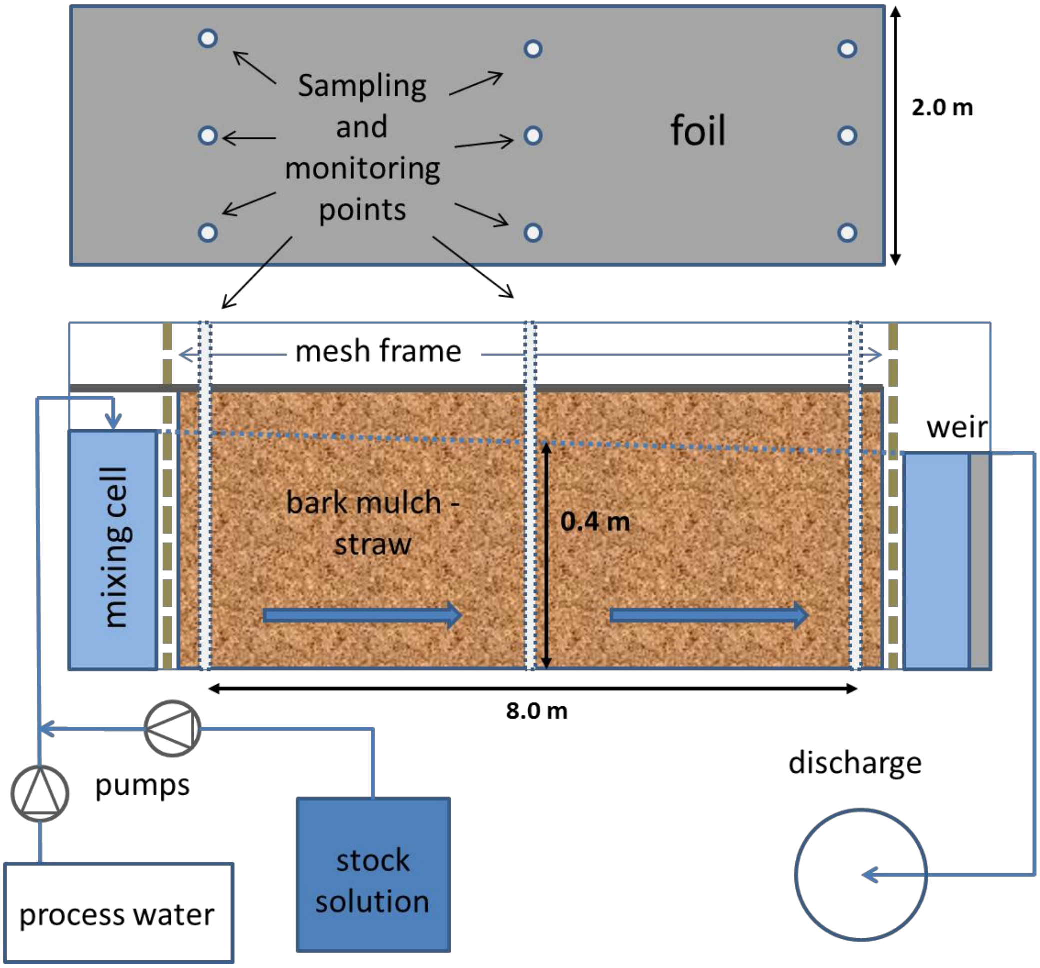


Figure 4-1: Plan view and side view (in section) of reactive swale at technical scale (sketch not true to scale)

The substrates were alternately added in several layers, stepwise mixed and compressed by foot to prevent preferential flow (Figure 7-5 in appendix 5). The swale was covered with a foil to prevent influence of precipitation. The layer of dry organic substrate between the water level and the cover foil was reserved to replace depleted substrate below and to buffer thermal radiation from the foil. An identically constructed third swale with HRT of about 2.0 days and without atrazine added was prepared as a control to be able to compare denitrification in the swales. The control swale was run for one year while the two atrazine swales were run for 1.5 years.

To test isoproturon retention, two reactive swales were prepared in the same manner as for atrazine. HRT's were set to around 0.5 and 2.0 days, respectively, isoproturon and nutrients were added analogue to the column experiment and duration was one year.

Herbicides, nutrients and chemical as well as physical parameters were analysed and measured analogue to the laboratory experiments.

In table 5-1 the experimental plan of the technical scale experiments is presented.

Table 4-1: Time scheme of technical scale experiments in 5 swales: all swales were filled with the same substrate (mixture of bark mulch and straw); swales were run with hydraulic residence time (HRT) of around 0.5 days (light grey) or HRT of around 2.0 days (dark grey); all swales were run with addition of nitrate, phosphate and herbicide with exception of the control, which was run with nitrate and phosphate only.

<table>
<tr><th></th><th></th><th>May 2010</th><th>Jun 2010</th><th>Jul 2010</th><th>Aug 2010</th><th>Sep 2010</th><th>Oct 2010</th><th>Nov 2010</th><th>Dec 2010</th><th>Jan 2011</th><th>Feb 2011</th><th>Mar 2011</th><th>Apr 2011</th><th>May 2011</th><th>Jun 2011</th><th>Jul 2011</th><th>Aug 2011</th><th>Sep 2011</th><th>Oct 2011</th><th>Nov 2011</th><th>Dec 2011</th><th>Jan 2012</th><th>Feb 2012</th><th>Mar 2012</th><th>Apr 2012</th><th>May 2012</th><th>Jun 2012</th><th>Jul 2012</th></tr>
<tr><td rowspan="4">swales</td><td>1</td><td colspan="2">░ No additives</td><td colspan="2">░ A</td><td colspan="3">░ B</td><td colspan="4">p_s</td><td colspan="5">░ B</td><td colspan="2">p_{us}</td><td>░ B</td><td colspan="8">-</td></tr>
<tr><td>2</td><td colspan="3">-</td><td>▓ A</td><td colspan="3">▓ B</td><td colspan="4">p_s</td><td colspan="5">▓ B</td><td colspan="2">p_{us}</td><td colspan="2">▓ B</td><td colspan="7">-</td></tr>
<tr><td>3</td><td colspan="3">-</td><td colspan="4">▓ A</td><td colspan="4">p_s</td><td colspan="4">▓ A</td><td colspan="12">-</td></tr>
<tr><td>4</td><td colspan="16">-</td><td colspan="4">░ C</td><td colspan="3">p_s</td><td colspan="4">░ C</td></tr>
<tr><td></td><td>5</td><td colspan="17">-</td><td colspan="3">▓ C</td><td colspan="3">p_s</td><td colspan="4">▓ C</td></tr>
<tr><td colspan="29">Additives to process water: A = NO_3^-+PO_4^{3-}, B = NO_3^-+PO_4^{3-} + ATR, C = NO_3^-+ PO_4^{3-} + IPU
ATR = atrazine, IPU = isoproturon, NO_3^- = nitrate, PO_4^{3-} = phosphate</td></tr>
<tr><td colspan="2">░</td><td colspan="7">= hydraulic residence time (HRT): ~ 0.5 days</td><td colspan="3">▓</td><td colspan="4">= HRT: ~ 2.0 days</td><td colspan="13">p_s = paused saturated
p_{us} = 1st month paused unsaturated; 2nd month flooded without addition of ATR, NO_3^- or PO_4^{3-}</td></tr>
</table>

4.2 Results

4.2.1 Hydraulic conductivity of reactive swales

Due to the dimensions of the technical scale experiment, the compression of the filling substrate is less effective, so that the bulk density of the bark mulch straw mixture (0.1 g/cm³) is half of the value established in the laboratory scale experiments. This results in higher effective porosity in the swales. Moreover, effective porosity in the swales increased slightly over time, presumably due to decomposition of the straw that fills the pore space between bark mulch. To measure effective porosity, tracer tests were conducted at the onset and repeated at the end of the experiments after 9 to 15 months (Figure 4-2, Figure 7-6 in appendix 6).The risk of favoured flow paths is increased at higher HRT. In swale 3 at HRT of 2.0 days preferential flow was observed at the end of the experiment. On the other side in swale 5 preferential flow at the start of the experiment was levelled out with time (Figure 7-6 in appendix 6)

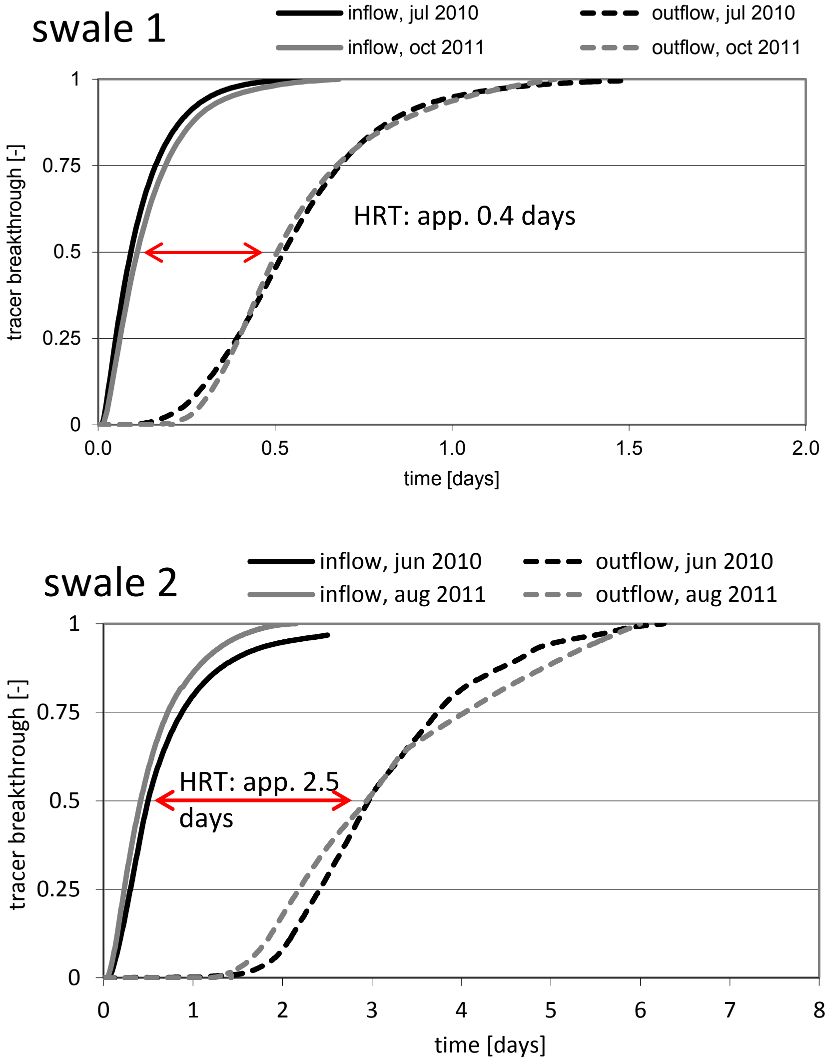


Figure 4-2: Tracer breakthrough curves at the inlet and outlet of the swales 1 and 2 at the start and at the end of the experiment.

Mean start value of effective porosity in the 5 swales that were operated in total was 80±7% (average ± standard deviation) and mean end value was 85±11%. Thus, it can be concluded that the longevity of high hydraulic conductivity in the mixture is ensured under saturated conditions. In case the substrate is filled in and packed properly, the risk of preferential flow is low.

Under long lasting unsaturated and wet conditions, and under load, the structure of straw is expected to be less resistant. However, it could be shown, that the mixture of straw and bark mulch has the potential to preserve hydraulic stability. While straw is being depleted, bark mulch gives structure to the filter and facilitates stable flow over time.

4.2.2 Nitrate removal

Temperature effect

The process, mainly accountable for NO_3^- removal in the bioreactors of this study, is expected to be denitrification (see laboratory experiments). In the following text the terms NO_3^- retention and denitrification are used synonymously.

The influence of temperature on denitrification in the range of 6 to 22°C in the technical scale experiments is presented in Figure 4-3. When denitrification is not limited (HRT: 0.4 days), denitrification correlates positively at first-order with water temperature.

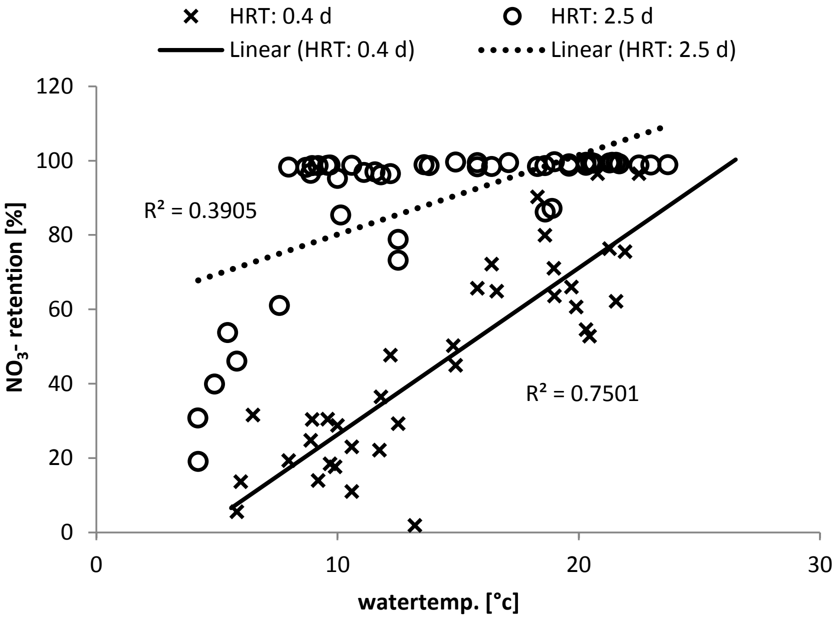


Figure 4-3: Correlation of relative denitrification and water temperature at HRT of 0.4 and 2.5 days

In Figure 4-4 and Figure 4-5 relative and absolute denitrification at HRT of 0.4 and 2.5 days are presented. Complete denitrification after start of dosing is a result of the presence of high loads of readily available organic carbon on the substrate's surface, formed during senescence of the plant. In the swales wash out of DOC was observed before dosing of NO_3^-. Concentrations start at 800 to 1100 mg L^{-1} and decrease within days to 100 mg L^{-1} and within weeks to 10 mg L^{-1}.

At HRT of 2.5 days, no temperature effect is observed in the first year due to limitations of denitrification by NO_3^- input. NO_{3-} is completely denitrified for one year. During the second winter at similar low temperatures (5 to 9°C) as in the first one, conditions in the swales change and NO_3^- concentrations in the outlet increase with decrease of temperatures. Responsible for the visibility of the temperature effect at HRT of 2.5 d on denitrification is assumed to be substrate aging (the decreasing availability of organic carbon over time), which has already shown strong influence on development of denitrification in the course of the laboratory experiment.

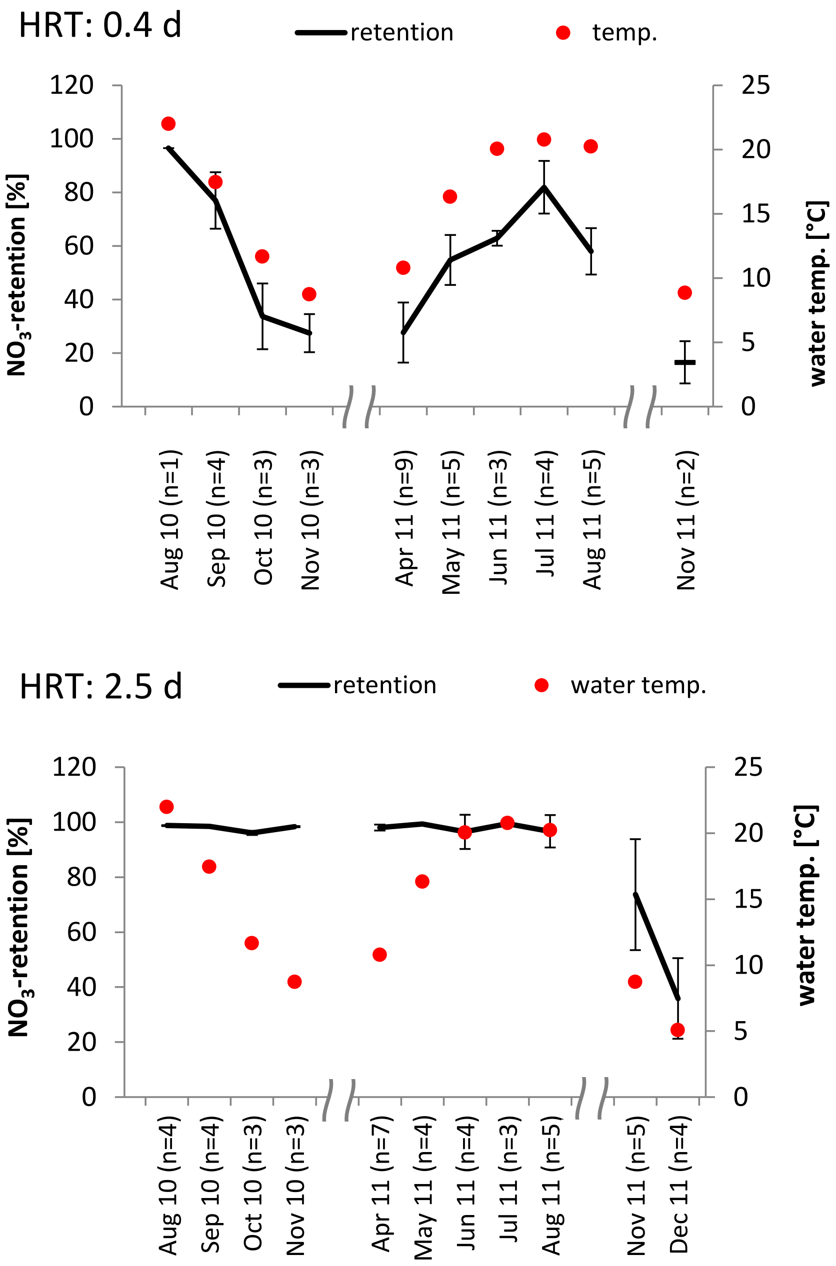


Figure 4-4: Relative denitrification (retention of concentrations) in the reactive swales 1 and 2 with HRT of 0.4 and 2.5 days, respectively.

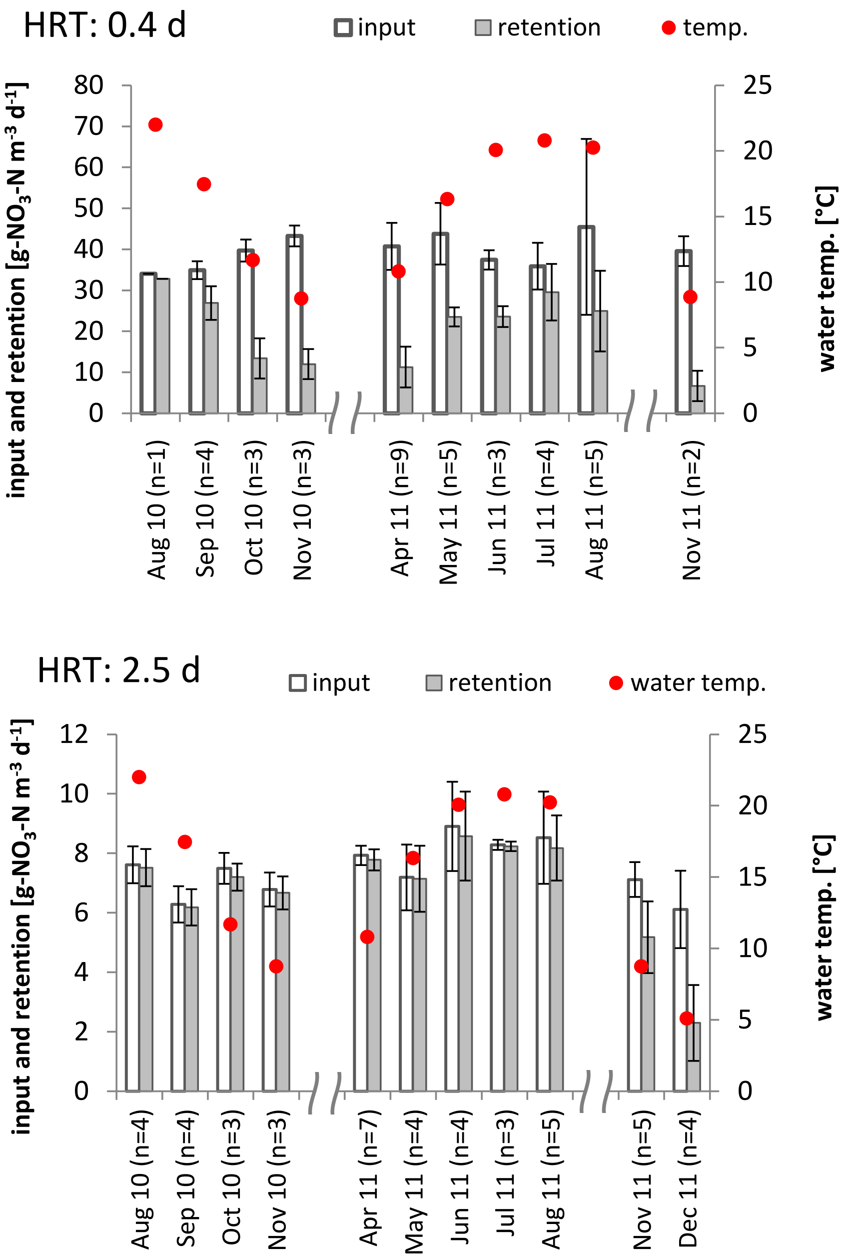


Figure 4-5: Absolute denitrification (retention of loads) in the reactive swales 1 and 2 with HRT of 0.4 and 2.5 days, respectively.

The redox potential can be used as a rough indicator for the occurrence of denitrification processes. In Figure 4-6 the development of the redox potential during the year in the swales 1 and 2 at HRT of 0.4 and 2.5 days is presented. Denitrification can be observed below + 500 mV. When the redox potential is below +220 mV, NO_3^- is completely reduced and denitrification is limited by NO_3^- input (Kretschmar 2010, p.167). Above + 220 mV the redox potential seems to correlate negatively with temperature. The increase of redox potential in swale 2 at HRT of 2.5 days in the winter 2011/12 reflects the lack of sufficient available organic carbon for total denitrification.

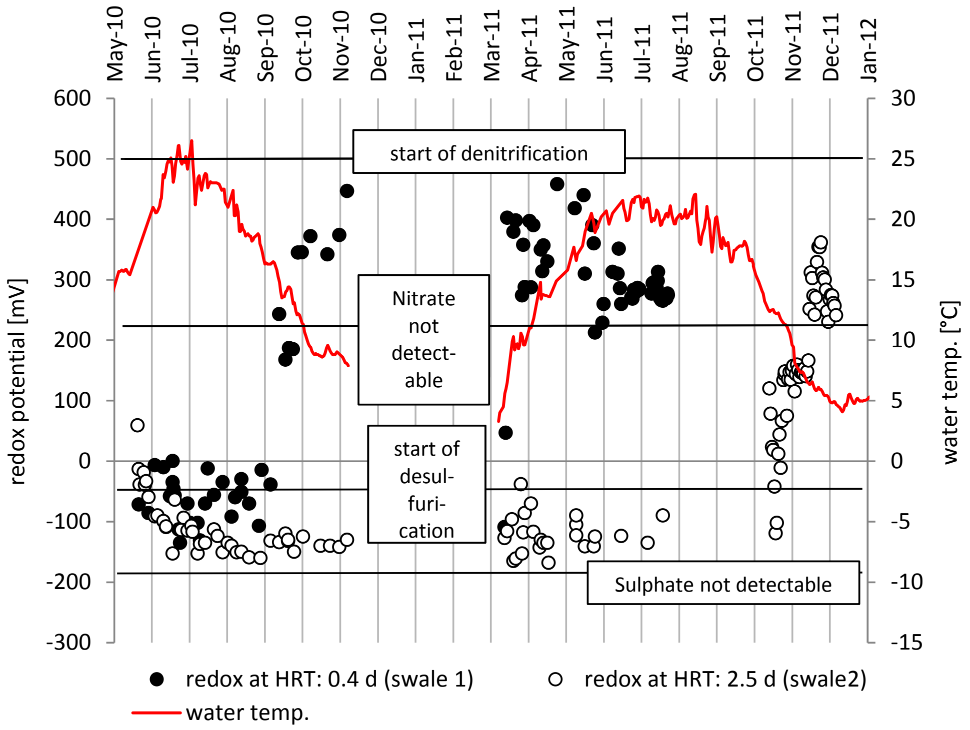
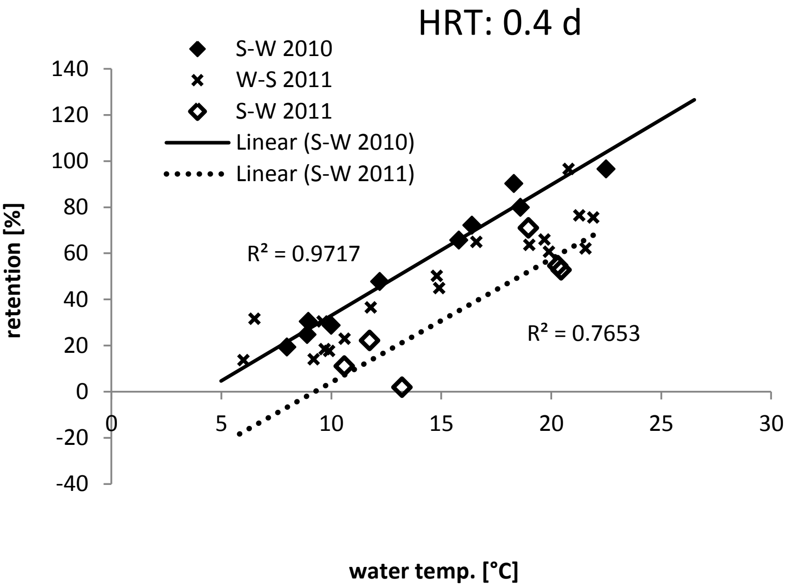


Figure 4-6: The redox potential at the outlet of the swales 1 and 2 as indicator for nitrate retention at hydraulic residence time (HRT) of 0.4 and 2.5 days. Redox potential limits for denitrification and desulfurication according to Kretschmar (2010).

<u>Substrate aging effect</u>

In Figure 4-7, the influence of substrate aging on denitrification is presented. At HRT of 0.4 days a steady decrease of relative denitrification over the complete temperature range can be observed from the second half of 2010 to the second half of 2011. In swale 2 at HRT of 2.5 days denitrification is limited by NO_3- input until denitrification becomes limited by DOC production, due to substrate aging, as observed in the second half of 2011.



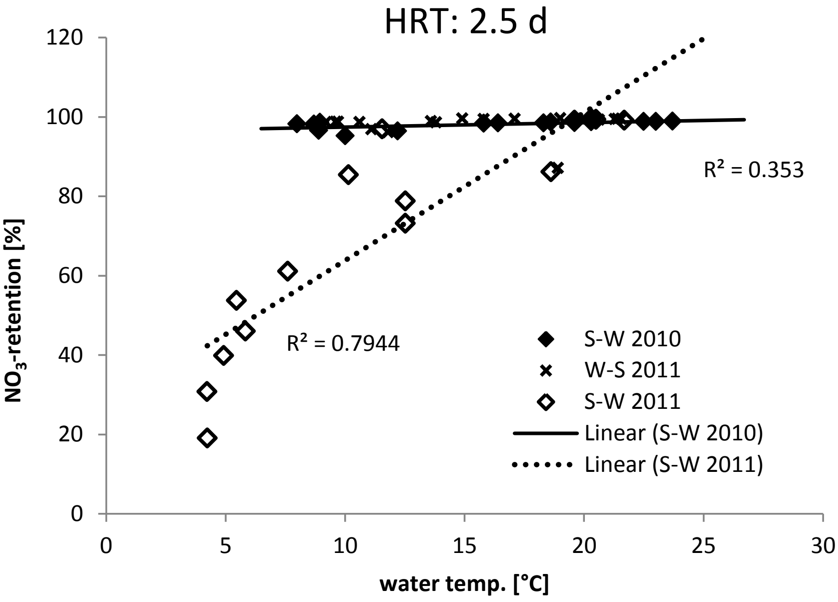


Figure 4-7: Effect of substrate aging on relative denitrification at HRT of 0.4 days and of 2.5 days.

To assess whether the influence of substrate age or temperature is stronger, denitrification in the swales 1 and 2 with atrazine was compared with denitrification in the swales 4 and 5 with isoproturon, in which the same substrate mixes were used, under similar conditions (flow rate, temperature) but with different substrate ages during winter (achieved by starting the isoproturon swales in autumn instead of summer). In general, the denitrification results of the technical scale experiments in 2011/12 confirm the results of the technical scale experiments in 2010/11. In swale 5 at HRT of 2.0 days,

NO_3^- is completely removed during the first year independent on temperature. In swale 4 at HRT of 0.5 days relative and absolute denitrification showed similar dependence on temperature as in swale 1 at HRT of 0.4 days, but comparing the results in detail (Figure 4-5, Figure 7-9 in appendix 7), it can be observed that in fresher substrate, higher denitrification performance can be observed even at lower temperatures as summarized in Table 4-2.

Table 4-2: Influence of temperature and substrate age on denitrification in swales at HRT of 0.4 to 0.5 days

swale	temperature	Substrate age	nitrate reduction of load	Nitrate reduction of conc.
	°C	months after start of dosing	$g\text{-}NO_3^-N\ m^{-3}\ d^{-1}$	% of input
4	9	2	25	60
1	9	5 (1st winter)	12	26
1	9	17 (2nd winter)	8	17
4	5	3	21	44

The interference of the different herbicides (isoproturon in the fresher and ATR in the older substrate) should be insignificant in the attempt of investigating the influence of substrate aging on denitrification in comparison to temperature, as literature studies and laboratory scale experiments at HRT between 0.2 and 2.0 days have shown. After comparison of denitrification rates in the swales at HRT of 2.0 to 2.5 days, one amended with atrazine (swale 2) and one without atrazine (swale 3), no negative effect of atrazine on denitrification was observed (Figure 7-7 in appendix 7). Increased output concentrations in swale 3 at low temperatures are expected to be the result of preferential flow and lower HRT of the matrix flow and, therefore lower HRT of flow fractions and subsequently higher NO_3^- loads, as tracer tests suggest (Figure 7-6 in appendix 6).

HRT effect

In Table 4-3 average denitrification rates at different HRT at laboratory and at technical scale in the same temperature range (9 and 21°C) were compared. In the swales at HRT of 2.0 to 2.5 days samples were taken in the middle of the reactor to study retention at intermediate HRT (1.0 to 1.25 days).

Table 4-3: Denitrification performance (relative and absolute) at different HRT and at temperatures of 9 and 21 °C.

HRT		0.2 days (lab)	0.4 days (lab)	0.4-0.5 days (tec)	1.0-1.25 days (tec)*	2.0-2.5 days (tec)*
Relative **[%]** and absolute ([g-NO_3^-N m^{-3} d^{-1}]) denitrification at	21 °C	**42** (20±5)[a]	**67** (16±2)[b]	**81** (38±9)[c] - **82** (30±7)[d]	**97** (17±1)[e] - **99** (19±2)[f]	**99** (8±1)[f] - **99** (9±1)[e]
	9°C	-	-	**17** (8±6)[d] - **60**(25±4)[c]	**29** (5±2)[f] - **69**(12±3)[e]	**74** (5±1)[f] - **98** (8±1)[e]

[a] 100th-300th day of operation (with atrazine from day 188)

[b] 100th-300th day of operation (with isoproturon from the start)

[c] swale 4 (Nov 11, Jul 12) – fresher substrate

[d] swale 1 (Jul 11, Nov 11)

[e] swale 5 (Nov 11, Jul 12) – fresher substrate

[f] swale 2 (Jul 11, Nov 11)

*denitrification limited by nitrate input

As in the laboratory the positive influence of HRT on relative denitrification can be observed at technical scale. At high temperatures (21°C) NO_3^- has been completely retained after less than one day. At low temperatures total denitrification was observed after 2.0 days in fresh substrate.

Higher denitrification rates in the same temperature and HRT range at technical scale have to be explained with different experimental conditions, especially in terms of availability of organic carbon. Lower average operating temperatures during the year at technical scale lead to lower consumption of the organic carbon source over time and longer lasting access to readily available species. More important, at technical scale transformed organic carbon in the saturated zone of the swale is, different from the laboratory, consistently replaced by fresh substrate from the overlaying dry/unsaturated buffer zone. Additionally, the ratio of processed NO_3^- load and original mass of organic carbon in the reactor is higher at laboratory (0.4 g-NO_3^- / g-organic matter) than at technical scale (0.25 g-NO_3^- / g-organic matter). These could be also the reasons that no use of the external carbon source was observed at technical scale.

DOC utilization

To assess the efficiency of denitrification using the organic carbon source, the utilization of the organic carbon by the major respiratory reactions was estimated. Figure 4-8 shows the use of carbon by parallel respiratory and carbon consuming processes, like O_2 depletion, denitrification, desulfurication and wash out.

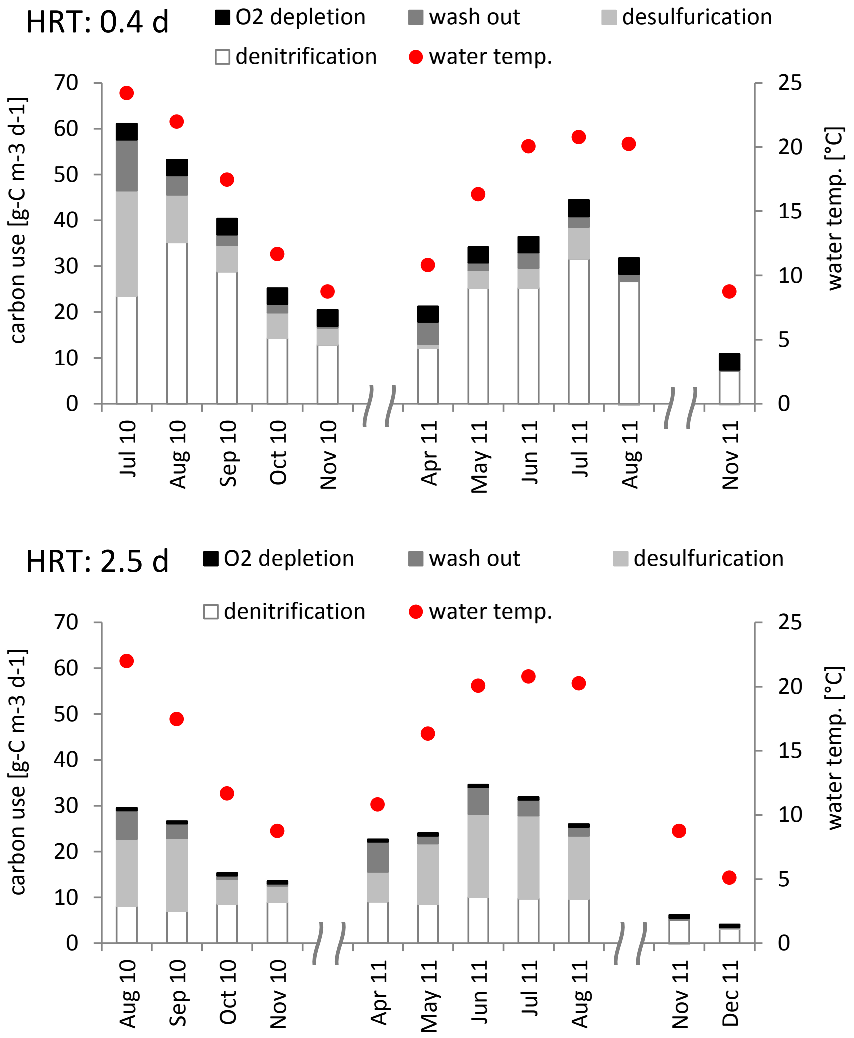


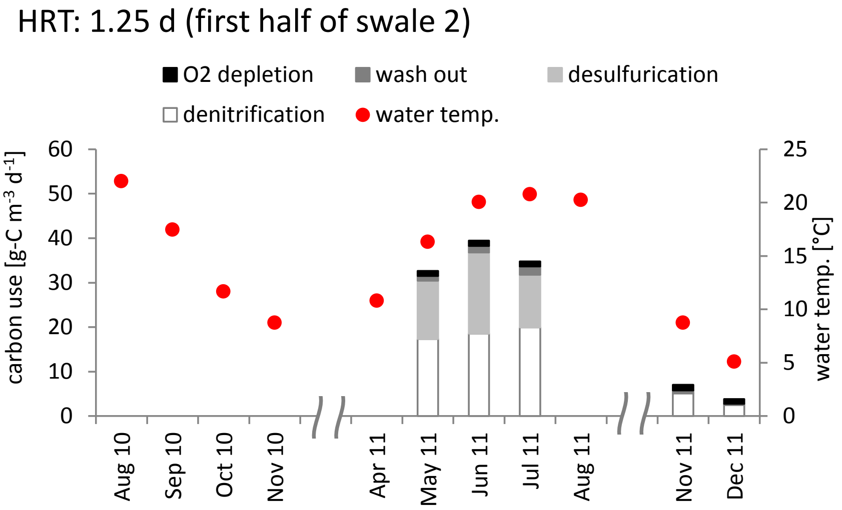
Figure 4-8: Contribution of the organic carbon source to major redox reactions and wash out in the swales 1 and 2 at HRT of 0.4 days and 2.5 days, respectively.

The swale has to be thought of as reactor and organic carbon source for different redox processes. In the first meters carbon is used for the reduction of oxygen from the inlet. When O_2 is depleted denitrification becomes the dominant process and when NO_3- is removed, desulfurication mainly uses the organic carbon source. The surplus of produced but unused DOC is washed out.

The contribution of observed redox reactions and wash out to carbon use is summarized in the following paragraph as average values for the whole period. O_2 depletion contributes to carbon consumption with 9 and 2% of the total observed DOC at HRT of 0.4 and 2.5 days, respectively. At anaerobic conditions SO_4^{3-} is used as electron acceptor by microbial processes and reduced to H_2S. Desulfurication is expected to start after NO_3^- is completely removed. Interestingly desulfurication occurs even when denitrification is not limited by NO_3- input. The transitions between the processes are gradual and the redox reactions can take place parallel in niches at micro- and macro-scale. At HRT of 0.4 days desulfurication uses only 14 % of the observed DOC and denitrification is the major DOC consumer with 70%. At HRT of 2.5 days the contribution of desulfurication to carbon use is 50%, only 34 % is used for denitrification due to limitation by NO_3- input (total removal). Total wash out is similar at both HRT with 14 % and 8 % of observed available carbon at HRT of 2.5 days and of 0.4 days, respectively. Increased wash out was observed after start and stagnation phase (at both HRT) and at high temperatures at HRT of 2.5 days, when electron acceptors in the input are nearly used up.

Considering the major respiratory reactions on basis of observed DOC use, the temperature effect on microbiological processes becomes visible in the swale 2 (HRT: 2.5 days), where denitrification is limited due to NO_3^- input (total removal).

The results of a separate contemplation on DOC consumption in swale 2 in the first and the second half with HRT of 1.25 days, respectively, are shown in Figure 4-9.



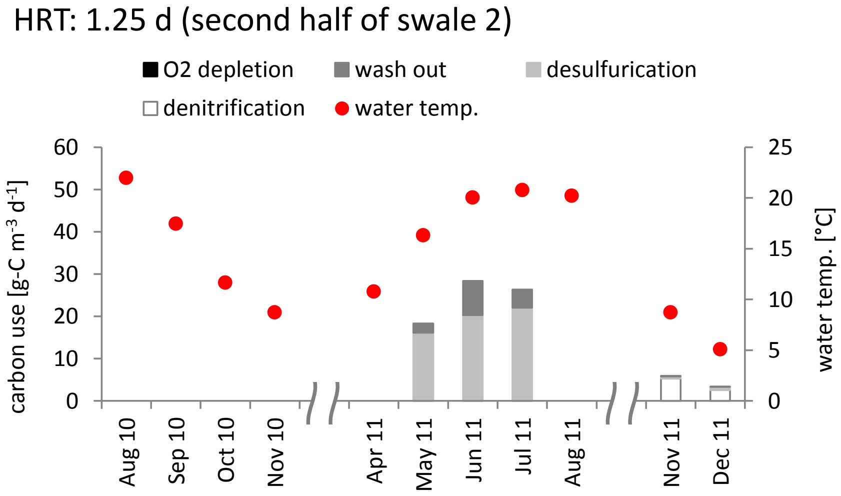


Figure 4-9: Contribution of the organic carbon source to major redox reactions and wash out in the first and the second half of swale 2.

The results (data of swale 4 and 5 not shown) indicate that the organic carbon source is consumed in dependence on the energetic yield of major respiratory processes. Total observed carbon use is higher under domination of oxidation and denitrification.

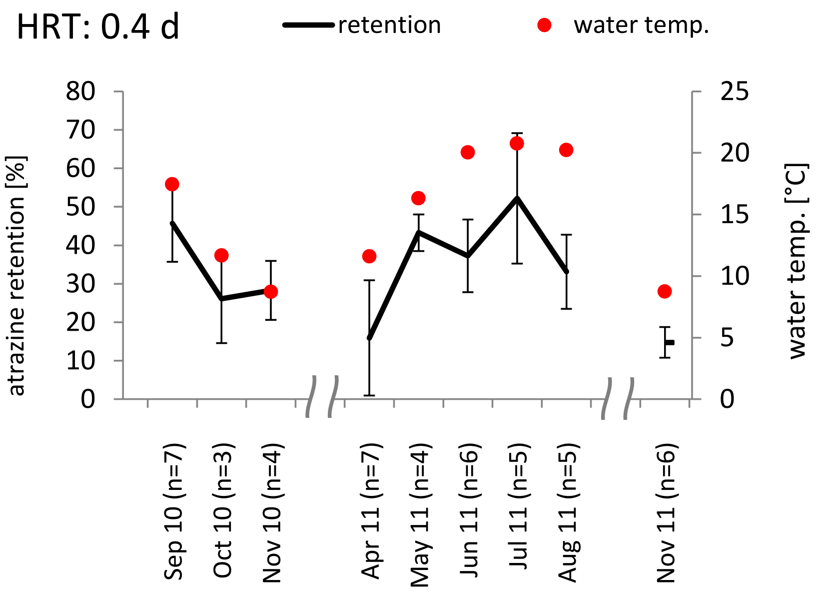
DOC seems to be produced in dependence on available electron acceptors. While in the second half of the swale even desulfurication is limited by SO_4^{2-} input at high temperatures, the DOC wash out increase only marginally and total observed carbon remains below the value of the first half. It can be expected that with lower SO43- concentrations in the input, the functionality of the substrate as organic carbon source would be ensured for even longer time as observed in the experiments.

To guarantee efficient denitrification on the basis of organic carbon source utilization, limitations by NO_3- input have to be avoided, especially when competing electron acceptors are present.

4.2.3 Mobility and persistence of atrazine and isoproturon in reactive swales

4.2.3.1 Atrazine

Figure 4-10 shows the development of atrazine retention during technical scale experiments. Relative atrazine retention (reduction of concentrations) correlates positively with temperature and flow rate with maximal values of 55% and 100% at high temperatures and minimal values of 15% and 55% at low temperatures at HRT of 0.4 and 2.5 days, respectively. In Figure 4-11the corresponding values of absolute atrazine retention are shown.



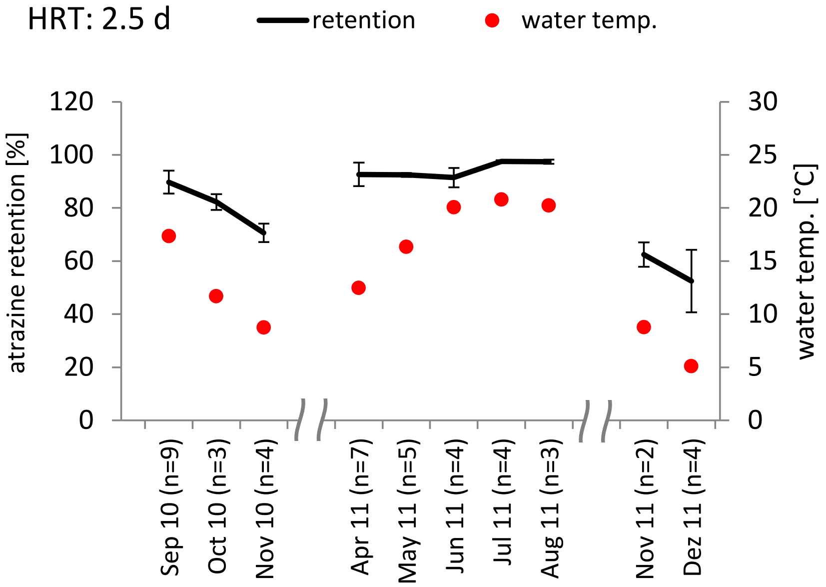
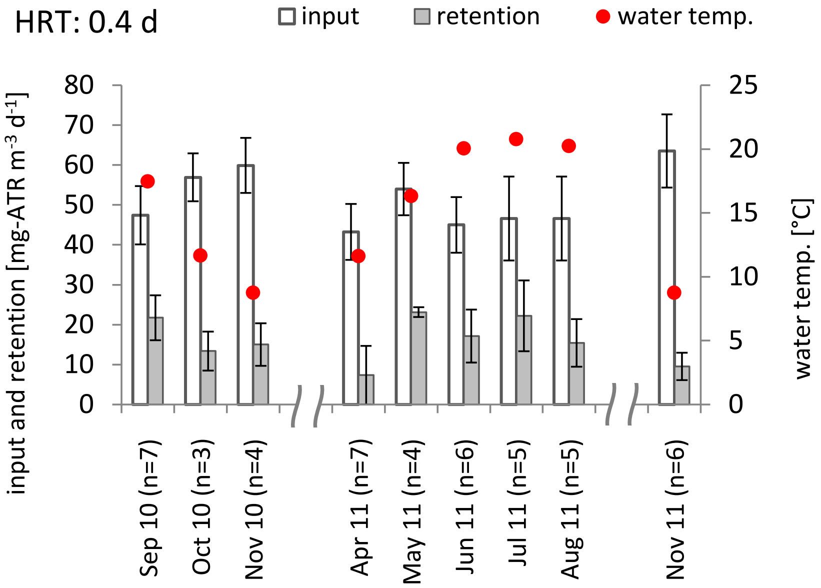


Figure 4-10: Relative atrazine retention (retention of concentrations) in the reactive swales 1 and 2 with HRT of 0.4 and 2.5 days, respectively.



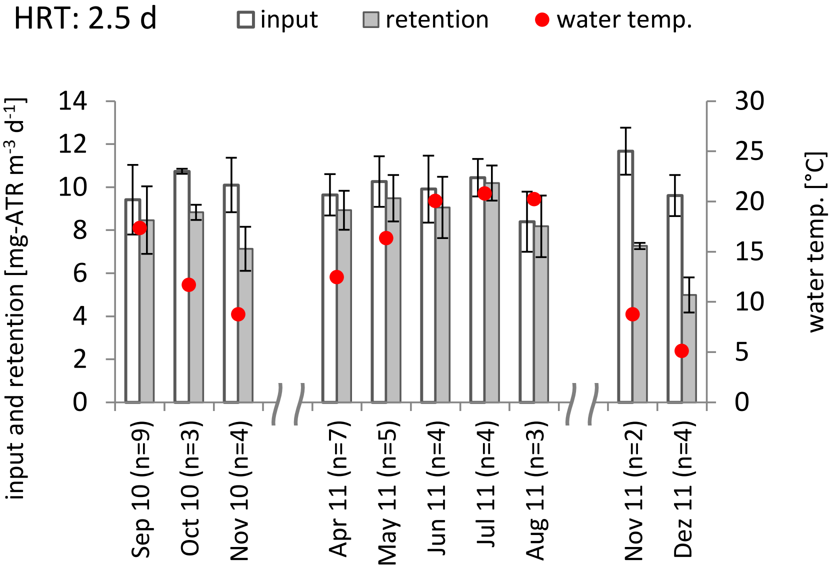
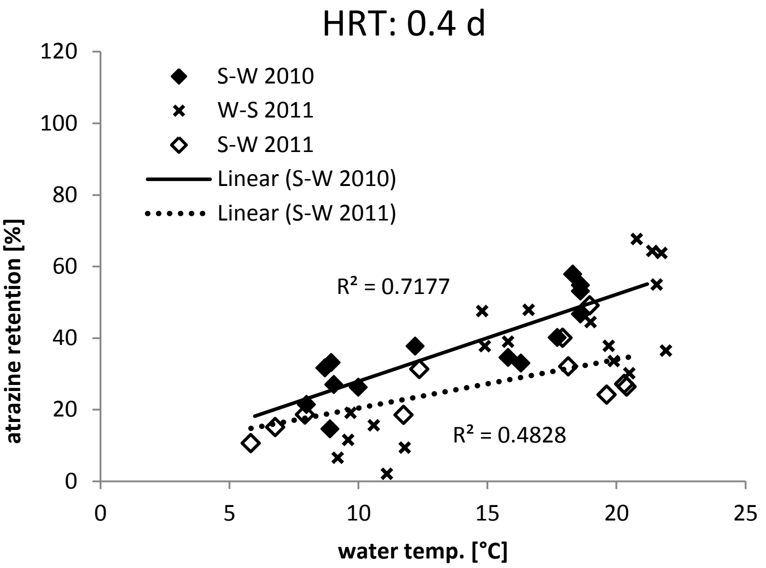


Figure 4-11: Absolute atrazine retention (retention of loads) in the reactive swales 1 and 2 with HRT of 0.4 and 2.5 days, respectively.

Atrazine retention shows similar dependence on temperature as denitrification but is less limited by input load, only at HRT of 2.5 days at high temperatures. The ability of the substrate to retain atrazine seems to be less influenced by substrate aging. Figure 4-12 shows, that there is no clear shift of atrazine retention at same temperatures and different times of operation.



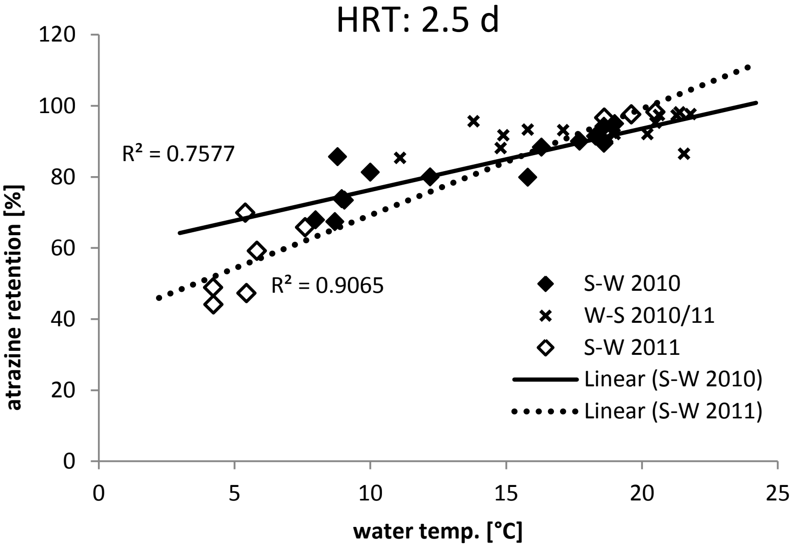


Figure 4-12: Correlation of relative atrazine retention and water temperature and effect of substrate aging on relative atrazine retention at HRT of 0.5 and 2.0 days.

Processes responsible for NO_3^- and atrazine retention are of different origin. While NO_3^- is sustainably reduced by microbial metabolism, atrazine seems to be predominantly retained by binding to the matrix of the organic substrate. Literature studies (Gendrault 2004) indicate rather formation of bound residues instead of degradation as the dominant dissipation path. There is proof for the formation of the metabolite HA, but only of about 13 % and 20 % of the retained atrazine load at HRT of 2.5 days and of 0.4 days, respectively (Figure 4-13). The high relative HA production at HRT of 2.0 days as observed in laboratory and with that a substantial contribution of degradation to atrazine retention could not be confirmed. Release of DEA and DIA, metabolites relevant for drinking water production, was marginal. Concentrations did not exceed 0.2 µg L^{-1}.

The results are in accordance with other studies, which also show positive correlation of atrazine adsorption and temperature. Schreiber et al. (2007) confirms that atrazine adsorption to activated carbon increases with increasing temperature (atrazine conc.: 2 mg L^{-1}; temp.: 5°C, 20°C, 35°C). Ovez and Höll (2008) found in batch experiments the same dependence also for low concentrations (atrazine conc.: 10µg L^{-1}; temp.: 25°C, 35°C, 45°C). De Andrea et al. (1997) observed in batch experiments with Brazilian soil (atrazine conc.: 13 mg L^{-1}; matrix solution ratio: 3:1) that formation of bound residues and simultaneous chemical degradation (hydrolysis) to HA were directly related to temperature (10°C, 20°C, 30°C, 40°C). The bound residues were predominantly hydroxyatrazine (90%). The decrease of atrazine retention over time is probably a result of the advancing saturation and the system immanent successive decomposition of available binding sites.

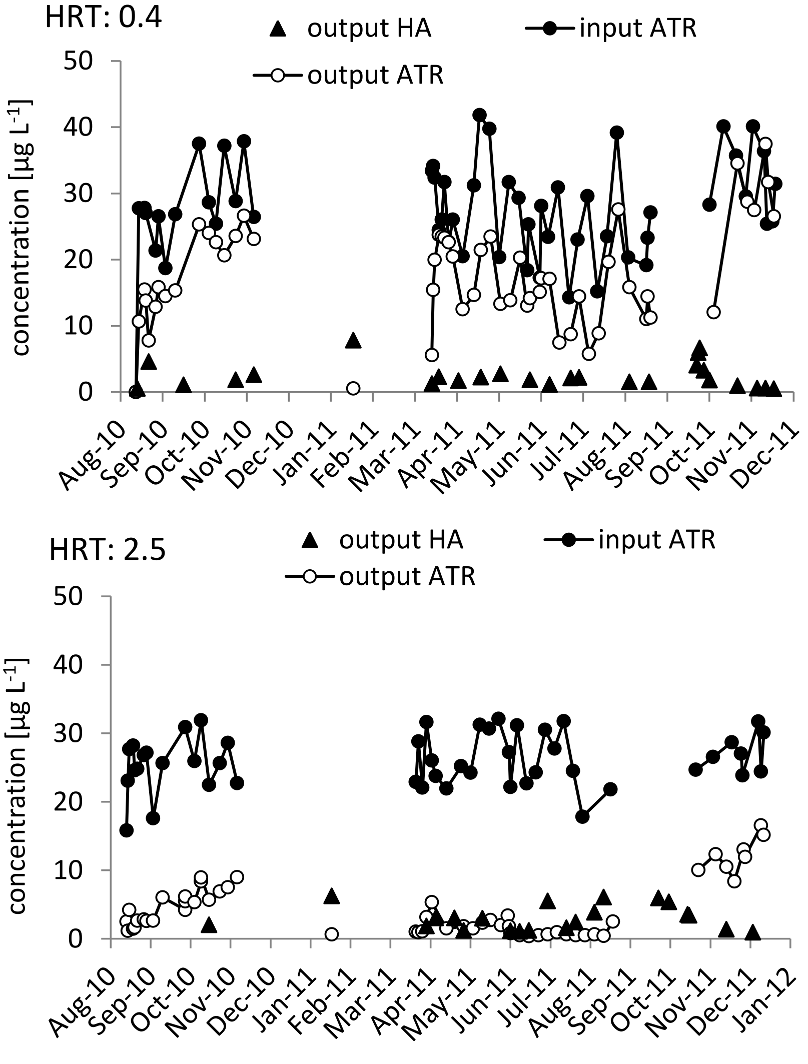


Figure 4-13: Development of atrazine (ATR) input and output concentrations as well as hydroxy-atrazine (HA) output concentrations in the swales 1 and 2.

In Table 4-4, the atrazine retention in the swales and in the laboratory column at 21 °C is compared. As for NO_3^- increased HRT affects positively the relative ATR removal (reduction of concentrations). At HRT of 2.5 days the evaluation of relative and absolute atrazine retention potential is hindered, because atrazine input limitations led at high temperatures to total removal of atrazine. The investigation of atrazine retention in the first half of the swale at HRT of 2.5 days reveals, that atrazine has already retained after one day (data not shown). The overlay of possible input effect and HRT effect as well as the influence of different scales prohibit sure assessment of single effects on absolute ATR retention. Whether higher ATR retention due to higher input loads at lower HRT could have counterbalanced higher ATR retention due to higher contact times at higher HRT remains subject for further studies.

Table 4-4: Atrazine retention performance at different HRT and different scales at temperatures at 9 and 21°C.

HRT		0.2 days (lab)	0.4 days (lab)	0.4 days (tec)	1.25 days (tec)*	2.5 days (tec)
Atrazine retention [% of input (mg-ATR m^{-3} d^{-1})] at	21°C	**22** (15±3)[a]	**44** (15±3)[b]	**52** (22±9)[c]	**94** (20±2)[d]	**98** (10±1)[d]
	9°C	**n.d.**	**n.d.**	**15** (10±3)[c]	**49** (11±7)[d]	**62** (7±1)[d]

[a] 350th – 500th day of operation
[b] 700th – 850th day of operation
[c] swale 1 (Jul 11, Nov 11)
[d] swale 2 (Jul 11, Nov 11)
*first half of swale 2

As in the laboratory experiments, the influence of drying of the swales on atrazine retention was investigated. Unsaturated and wet conditions that were established during the drying phase, were expected to be more favourable for microbial degradation of bound atrazine than saturated conditions.

Likewise, it was found reduction of extractable bound atrazine during the drying phase (data not shown), but the extractable atrazine was again only a small part of the atrazine which has dissipated during the experiment (1 to 2%). It seems that the extraction method was not adequate for aged bound residues of atrazine.

The observations are insufficient to approve that the drying phase improves atrazine retention. Increased metabolite concentrations during flushing with un-spiked water after the drying phase (short peak of HA in the range of 25% of atrazine input concentrations) present only a marginal fraction of the amount of atrazine that was retained during the whole experiment (Figure 4-13) and could also be caused by desorption of bound HA from the matrix due to decomposition of the organic substrate. Equally negligible short duration peaks of atrazine in the range of input concentrations, which were observed along with the HA peaks, support the suspicion of washed out residues.

Since the amount and the composition of the bound residues are not clear and not all possible metabolites have been analysed in solution, the effect of the drying phase as well as the faith of atrazine in the straw-bark mulch bioreactor cannot be stated conclusively.

Literature indicates high rates of metabolized atrazine (HA) and stable bindings. Capriel et al. (1985) detected 9 years after atrazine application still 50% of bound residues in humic matter, mainly HA and HA metabolites, but also around 13 % of initial atrazine and confirmed the stability of bound residues.

Proof for low risk of the availability of atrazine residues under saturated conditions is shown by leaching tests with un-spiked process water at the end of the experiment. After 1.5 years of experiment duration, the tests resulted in marginal wash out of atrazine with progressively decreasing concentrations (from 6 to 1 µg L^{-1} within 6 weeks in the swale at HRT of 0.4 days and from 9 to 0.5 µg L^{-1} within 3 weeks in the swale at HRT of 2.5 days).

To learn more about the binding behavior of atrazine and its metabolites to the organic substrate further studies are required.

4.2.3.2 Isoproturon

As in the laboratory experiments, isoproturon shows different behaviour in the reactive swale compared to atrazine. In contrast to atrazine, relative isoproturon retention decreases with increasing HRT, which indicates that adsorption is a less dominant removal process (Figure 4-14).

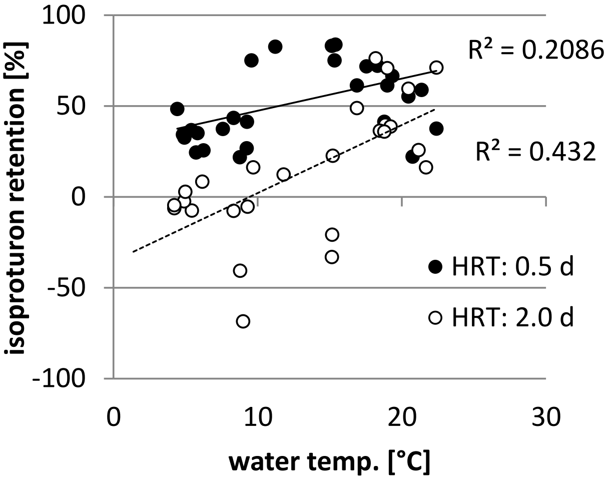
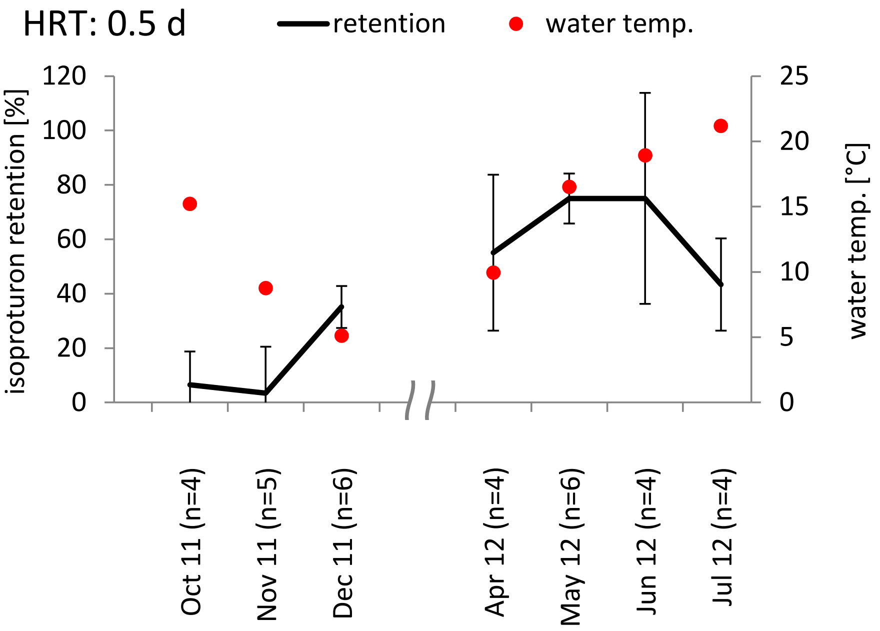


Figure 4-14: Correlation between relative isoproturon retention and temperature - Dec 11 to Jul 12.

A first hint for different dissipation pathways of isoproturon and atrazine is the observation of lower persistence of isoproturon under aerobic conditions. Hydraulic residence times of isoproturon in the mixing cell were 3 h and 14 h in the swale with HRT of 0.5 and 2.0 days and resulted in isoproturon reduction by an average of 35 and 65%, respectively. This is in contrast to atrazine concentrations of the analogue swales that decreased by only 4%. In the swales, isoproturon was further reduced.

In Figure 4-15 it can be seen that at HRT of 0.5 days during the starting phase isoproturon retention is inhibited. At this time NO_3^- is completely removed due to the high amount of readily available DOC; and redox conditions are anaerobic (Figure 7-10 in appendix 8). Beginning in December 2011, after the reservoir of readily available DOC is depleted and denitrification becomes dependent on DOC formation, conditions turn suboxic and absolute isoproturon retention rates increase with increasing temperature and increasing input loads. That anaerobic conditions really seem to disturb the dissipation process of isoproturon can be observed in summer 2012. Absolute isoproturon retention stagnates at water temperatures between 10 and 20°C and relative isoproturon retention decreases at water temperatures > 15°C (Figure 4-15) maybe due to high denitrification, which provokes oscilation between suboxic and anaerobic conditions (Figure 7-10 in appendix 8). Strong desulfurication in the same time range (May to July 2012) gives indication for at least anaerobic zones in the swale (Figure 7-11 in appendix 9).



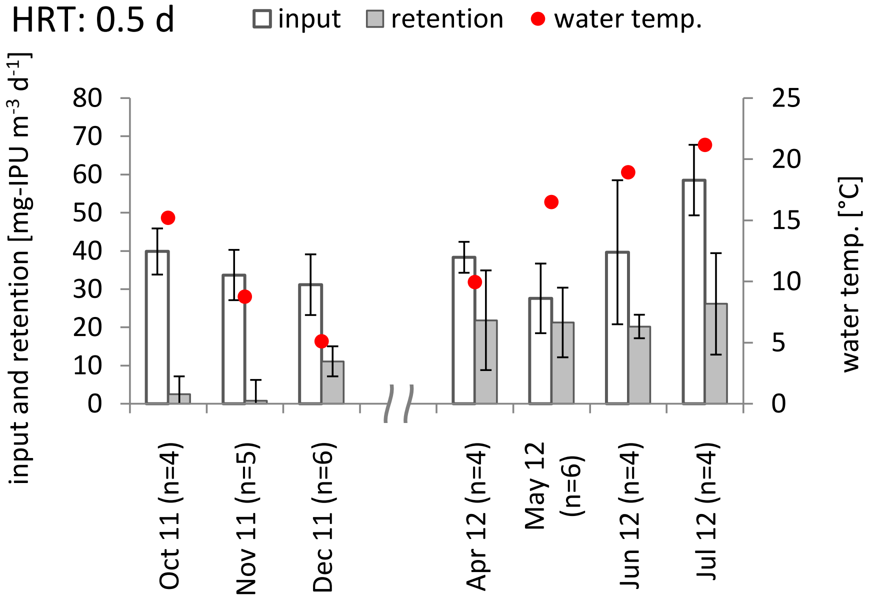


Figure 4-15: Relative and absolute isoproturon retention in swale 4 at HRT of 0.5 days during the experiment.

Relative retention (retention of concentrations) is low at low temperatures (35% at 5°C), has an optimum at medium temperatures (75% at 17°C) and decreases again at higher temperatures (40% at 21 °C), supposedly due to disturbance of dissipation processes as result of anaerobic conditions (see above).

Figure 4-16 presents the development of isoproturon retention over time at high HRT of 2.0 days. The release of isoproturon in October and November of 2011 seems to be the result of decreasing input concentrations, due to increasing reduction in the mixing cell, and retardation of isoproturon in the swale. The swale acts as a buffer and delays the effect of reduction processes in the mixing cell on output concentrations (Figure 7-12 in appendix 9). This observation indicates a lower affinity of isoproturon to form irreversible connections to the matrix of the organic substrates than atrazine. At low temperatures (5 to 10°C) no retention of isoproturon is observed. At temperatures around 21°C, relative isoproturon retention increase to 40%.

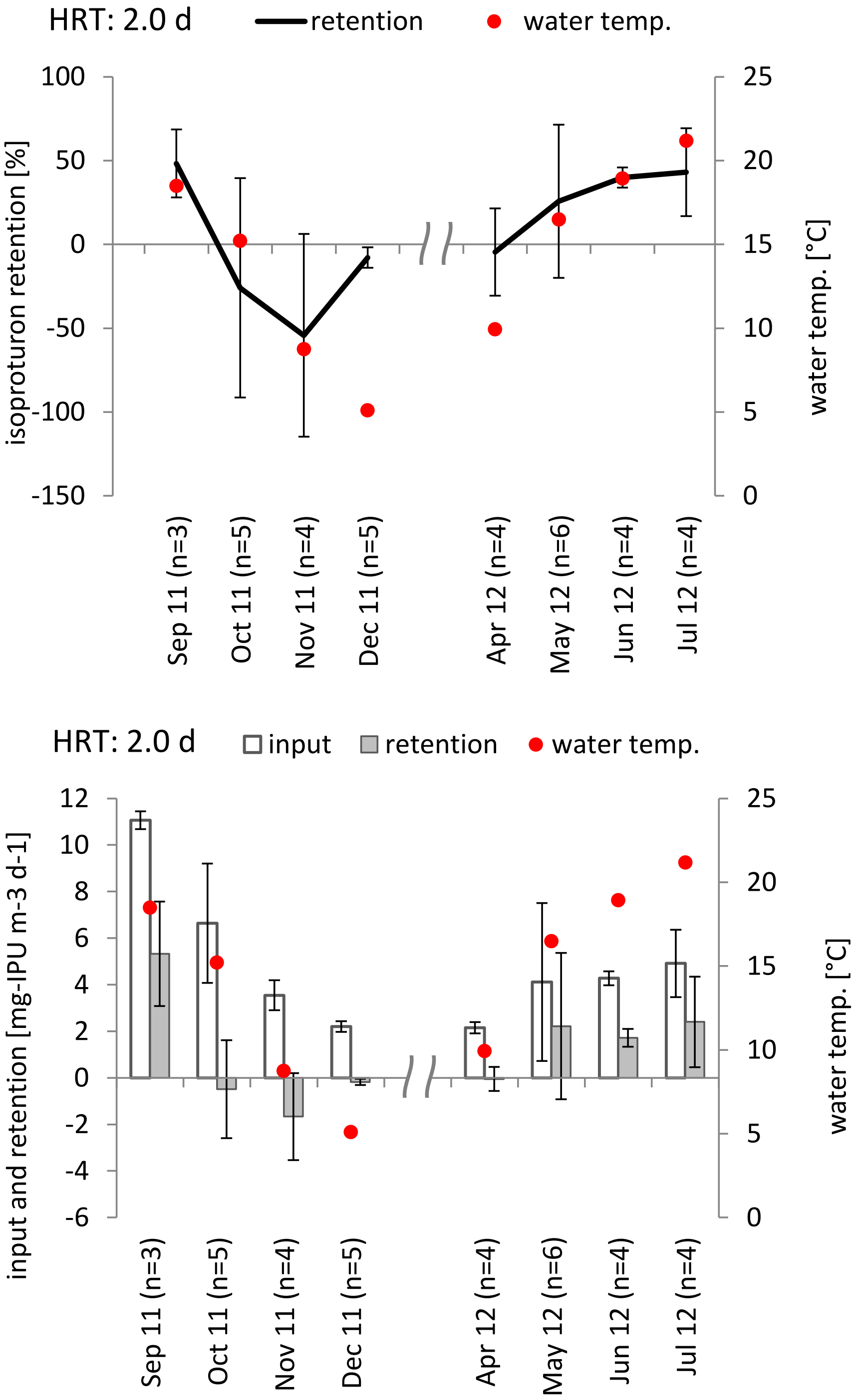


Figure 4-16: Relative and absolute isoproturon retention in swale 5 at HRT of 2.0 days during the experiment.

The theory of redox conditions as limiting factor for isoproturon retention becomes more apparent, comparing different HRTs. Temporary anaerobic conditions at HRT of 0.5 days at temperatures around 21°C result in relative isoproturon retention, which is similar to values at HRT of 2.0 days, where anaerobic conditions prevail throughout the experiment (Figure 7-10 in appendix 8).

Moreover, a closer look at swale 5 with HRT of 2.0 days reveals, that under anaerobic (sulphate reducing) conditions relative retention of isoproturon is mostly suppressed. In Table 4-5 the development of the redox potential and isoproturon retention along swale 5 is presented. At summerly temperatures the highest contribution to isoproturon reduction is found in the mixing cell and in the first part of the swale at aerobic to suboxic conditions, while in the second half of the swale at anaerobic conditions isoproturon retention is marginal. Contribution of the mixing cell to isoproturon retention is lower than at the start of the experiment, because HRT in the mixing cell was lowered by constructional measures.

Table 4-5: Redox potential and corresponding isoproturon retention in swale 5 at HRT of 2.0 days at a single sampling date in July 2012 (after adjustment of inflow structure, which resulted in reduced residence time in the mixing cell)

Sampling points	mixing cell	outflow first half	outflow second half
Redox potential [mV]	+ 320	+ 120	- 145
isoproturon retention [% of input]	20	60	0

Leaching tests with un-spiked process water before the winter break resulted in wash out of isoproturon with progressively decreasing concentrations (10 to 1 µg/L within 2 weeks in swale 4), which is marginal when compared to the retained fraction of isoproturon.

As the technical scale experiments have shown, isoproturon retention depends at first on the redox potential. Under suboxic conditions isoproturon retention seems to correlate positively with temperature. However, the influence of temperature cannot be completely isolated from the influence of input (varying inflow concentrations due to removal in mixing cell) and therefore it remains an uncertainty about the effect of temperature. Anaerobic conditions interfere with isoproturon retention. In the swales isoproturon retention is optimal at high temperatures as long as redox conditions remain suboxic.

In Table 4-6 comparable results of the laboratory and the technical scale study, regarding temperature, are presented. It can be confirmed that under suboxic conditions higher retention is possible than in an environment with at least anaerobic phases or zones. According to observations (Table 4-5) isoproturon retention at HRT of 2.0 days is expected to occur in the first half of the swale, where conditions are still suboxic.

Table 4-6: isoproturon retention in dependence on redox potential at different HRT and in the temperature range around 21°C.

HRT	0.4 days (lab)	0.5 days (tec)	2.0 days (tec)
retention [% of input (mg-IPU m^{-3} d^{-1})]	20 (7) to 90 (30)*	40 (25)	40 (2)
redox potential** [mV]	+260	-100 to +250	-200

* supposedly adaptation of microorganisms (Figure 3-9); ** at the outlet

Temporary higher isoproturon retention at similar HRT in laboratory than at technical scale could be the result of more favourable conditions for dissipation processes due to constantly high temperatures and higher redox potential.

Due to the experimental conditions no distinct correlation between HRT and retention can be observed. The redox potential, which is amongst others controlled by the HRT seems to have the decisive influence on isoproturon retention.

Since isoproturon sorption on the matrix seems to be unstable and its retention is influenced by factors which drive microbial processes, indications of laboratory scale that isoproturon retention is a result of degradation and therefore sustainable can be confirmed. To learn more about metabolites, adaptation of possible microorganisms and potential mineralization rates under the experimental conditions and to prove the theory of microbial degradation, further studies have to be conducted.

4.3 Summary of technical scale results

Technical scale experiments confirm that the mixture of bark mulch and straw provides high hydraulic permeability on a long term basis.

At HRT of 0.4 days relative denitrification (retention of concentrations) correlated positively with water temperature. At HRT of 2.5 days total denitrification was observed (limited by NO3 input), even at low temperatures in first winter. However, denitrification rates became dependent on temperature in second winter.

NO_3^- concentrations were reduced by 17-82% at HRT of 0.4 days and 74-99% at HRT of 2.5 days over a period of 1.5 years (9-21°C).

Denitrification performance decreased with increasing substrate age. In fact, substrate age (availability of organic carbon) had higher influence on denitrification than temperature. With fresher, less processed substrate higher denitrification was observed even at lower temperatures (45-81% at HRT of 0.5 days at 5°C, 98-99% at HRT of 2.0 days at 21°C)

Retained loads at HRT of 0.4 days yielded between 8 g-N m^{-3} d^{-1} (9°C) and 30 g-N m^{-3} d^{-1} (21°C) and at HRT of 2.5 days between 5 g-N m^{-3} d^{-1} (9°C) and 8 g-N m^{-3} d^{-1}(21°C).

At HRT of 0.4 days efficient use of the organic substrate and high rates in comparison with comparable studies were observed. About 70% of the observed DOC was metabolized by denitrification

At HRT of 2.5 days denitrification was more effective (total denitrification) but less efficient in the scope of DOC use. Since denitrification was limited by NO_3^- input the carbon source was substantially used for desulfurication. Only about 30% of observed DOC was used for denitrification at high (21°C) and only about 50% at low temperatures (9°C).

It is indicated that under the experimental conditions denitrification rate correlates positively with the DOC production rate, which depends on temperature, HRT, presence of electron acceptors, energetical yield of redox reactions and resistance of organic carbon species.

Low wash out of DOC during operation, even when electron acceptors like O_2, NO_3^- or SO_4^{2-} in input are limited, indicate that the organic carbon source has to be located in the reactor for efficient use of DOC in denitrification. Microorganisms dissolve organic carbon for respiration, when DOC in solution is not sufficient in relation to present electron acceptors. Also, in absence of electron acceptors longevity of the reactor seems to be prolonged, because the organic carbon source is spared.

Atrazine concentrations were reduced by 15-52% at HRT of 0.4 days and by 62-98% at HRT of 2.5 days over a period of 1.5 years. Corresponding absolute atrazine retention yielded 10-22 mg-ATR m^{-3} d^{-1} at HRT of 0.4 days and 7-10 mg-ATR m^{-3} d^{-1} at HRT of 2.5 days in the range of 9 to 21°C.

Technical scale experiments substantiate assumptions on the base of laboratory experiments that atrazine retention is predominantly driven by adsorption, only a minor part of the retained atrazine could be observed as dissolved metabolite (HA). The

metabolites of DEA and DIA are of no concern. Atrazine seems to create stable bound residues at the substrate matrix, partially in form of HA as literature indicates.
The risk for desorption and salting out is low, given the observations that atrazine and HA is permanently retained during the 1.5 year experiment and only marginally washed out in the end. Unfortunately the composition of bound residues is uncertain and the left bound atrazine can be set free after decomposition of the binding sites.
In conclusion, there is the risk for set free of a fraction of atrazine, on the other side there is a good chance for long-lasting retention and partial transformation to a high extend.

Atrazine retention and formation of bound residues correlate positively with temperature. Under the experimental conditions substrate aging has low negative influence on atrazine retention.

For isoproturon the technical scale experiments reinforced the indications of laboratory experiments that degradation is a more influencing process than adsorption. Degradation of isoproturon is linked to the energetic efficiency of respiratory processes, indicated by the redox potential. The decrease of the redox potential into the anaerobic zone, which is the result of complete denitrification, interferes with isoproturon retention. Isoproturon removal seems to correlate positively with temperature.

Isoproturon concentrations were reduced by 35-75% at HRT of 0.5 days in the range of 5 and 17 °C and by 0-40% at HRT of 2.0 days in the range of 5 and 21 °C over a period of one year. Corresponding absolute isoproturon retention yielded 11-27 mg-IPU m^{-3} d^{-1} at HRT of 0.5 days and 0-2 mg-IPU m^{-3} d^{-1} at HRT of 2.0 days.

Risks

The implementation of a bioreactor, which supply organic carbon to microbiological processes is also accompanied with a serie of risks for the receiving waters. An obvious drawback of the fragile carbon source like straw, which is responsible for the enhanced denitrification, is the risk to turn into a source of DOC after start and stagnation phases. The decrease of DOC wash out over time is a result of increased resistance of the residual organic substrate in the bioreactor. Also wash out of acidity (minimum pH of 4.5) during start phase was observed, which is originated from bark mulch. PH increased to 6 within one month at HRT of 2 to 2.5 days and to 7 within 1.5 months at HRT of 0.4 days (data not shown).

At low temperatures during first winter and following spring, increased concentrations of nitrite (NO_2^-), a fish toxin, ranging from 3 up to 9 mg L-1, suggest that the reduction process from NO_3^- to N_2 is disturbed at low HRT (0.4 days) and release of further intermediate products like N_2O, which acts as glasshouse gas, is indicated. Interestingly, release of NO_2^- decreases in the second winter to at max 0.4 mg L^{-1}, maybe due to adaptation of microorganisms (data not shown).

At HRT of 2.0 to 2.5 days total denitrification was observed and concentrations of NO_2^- in the outlet did not exceed 0.5 mg L^{-1}. On the other hand complete reduction of NO_3^- in combination with a fragile carbon source improve conditions for methane (CH_4) formation and release of another potent glasshouse gas, although CH_4 is less effective than N_2O.

But with respect to the total nitrogen balance over a watershed, Moorman et al. (2010) noted that if NO_3^- is not efficiently treated in a bioreactor, more N_2O may be released downstream under less efficient conditions.

When SO_4^{2-} is present in the inlet, in the bioreactor hydrogen sulfide (H_2S) is formed. H_2S is a fish toxin. H_2S has also negative effect on denitrification, because it disturbs the last two transformation steps from NO_3^- to elementary nitrogen (Soerensen et al. 1980) and decrease the ratio of N_2/N_2O. Desulfurication also enables mercury methylation (Christianson 2011). According to Clifford and Liu (1993) biological denitrification bring along oxygen demand, turbidity and additional bacteria in the receiving waters.

However, due to Schipper et al. (2010) the adverse effect potential of washed out contaminations depends on the ratio of drainage and receiving water discharge and on the nature and quality of receiving waters.

Chapter 5

Effects of experimental design on the removal of nitrate and selected herbicides

- NO_3^- retention

  laboratory scale experiment

  - straw is a readily available organic carbon source and supports high denitrification performance (10 to 45 g-N m^{-3} d^{-1}) at low HRT (0.2 days) and at temperatures around 21°C
  - Relative denitrification (retention of concentrations) increases with increasing HRT at high temperatures (21°) from 42% at HRT of 0.2 days to 67% at HRT of 0.4 days to complete denitrification at HRT of 2.0 days
  - Organic substrates, especially straw, emit high concentrations of DOC for a short period of few weeks after start and after stagnation phases, which lead to complete or increased denitrification and increased DOC wash out (at HRT of 0.2 to 0.5 d: 250 mg L^{-1} after start, 100 mg L^{-1} after days, 10 mg L^{-1} after weeks).
  - bark mulch is a more resistant organic carbon source with moderate denitrification performance at low HRT (3 to 15 g-N m^{-3} d^{-1}) and at temperatures around 21°C
  - efficiency of bark mulch as carbon source can be enhanced by addition of straw (co-metabolic decomposition)
  - realistic concentrations of bentazone, atrazine and isoproturon in drainage waters have no negative influence on denitrification in straw and bark mulch

  technical scale experiment

  - under realistic temperate climate conditions the mixture of straw and bark mulch supports efficient denitrification (5 to 38 g-N m^{-3} d^{-1}) at HRT from 0.4 to 2.5 days and at temperatures between 5 and 21°C
  - Relative denitrification (retention of concentrations) increases with increasing HRT at high temperatures (21°) from 82% at HRT of 0.4 days to 99% at HRT of 2.5 days and at low temperatures (9°C) from 17% at HRT of 0.4 days to 74% at HRT of 2.5 days
  - denitrification correlates positively with temperature and negatively with substrate age (availability of organic carbon)
  - realistic concentrations of atrazine and isoproturon in drainage waters have no negative influence on denitrification in straw and bark mulch

- Herbicide retention

  laboratory and technical scale experiment:

  - retardation of atrazine and isoproturon (buffer of peaks) due to high capacity of available sorption sites in bark mulch can be observed
  - substantial retention of atrazine (7 to 22 mg-ATR m^{-3} d^{-1}), potentially by formation of bound residues at the substrate and successive chemical degradation to HA , predominantly at bark mulch, under suboxic to anaerobic conditions (HRT between 0.2 and 2.5 days, temperature between 5 and 21°C)
  - relative atrazine retention correlates positively with temperature and HRT (15% at 9°C to 52% at 21°C at HRT of 0.4 days and 62% at 9°C and 98% at 21°C at HRT of 2.5 days)
  - At HRT between 0.5 and 2.0 days and temperature between 5 and 21°C retention of isoproturon (0-27 mg-IPU m^{-3} d^{-1}) seems to be predominantly caused by microbial degradation under suboxic conditions; under anaerobic conditions retention seems to be inhibited
  - isoproturon retention correlates positively with temperature, when conditions are suboxic (35% at 5°C and 75% at 17°C at HRT of 0.5 days)
  - at HRT of 2.0 days and at high temperatures at HRT of 0.5 anaerobic zones decrease isoproturon retention (0% at 5°C and 40% at 21°C)
  - it can be assumed that herbicides, which belong to the same chemical groups as atrazine (triazines e.g. simazine, therbutylazine) and isoproturon (phenylurea herbicides e.g. diuron, chlortoluron, linuron) and have similar chemical properties would be similarly retained in the reactor of organic substrate
  - the experimental design is not suitable for herbicides, which are persistent in the absence of oxygen and more mobile than atrazine and isoproturon as shown for bentazone. The same is expected for related (thiadiazines) and equally mobile herbicides

Chapter 6

Recommendations for efficient design of mitigation systems

Efficient designs of mitigation zones should include considerations that enhance denitrification and the removal of herbicides. NO_3^- is the main relevant contaminant in agriculture. The use of herbicides is increasing due to e.g. development of resistances in targeted herbs. The faith of herbicides in natural compartments is often incompletely understood and the contamination of raw water for drinking water production is of growing concern. The positive influence of organic carbon addition on NO_3^- removal is well known and comprehensively studied for low-flow conditions (subsurface flow/ground water). If steady low-flow discharge of drainage systems is expected, a more resistant organic carbon source like bark mulch, wood chips, saw dust etc. is suitable for long lasting NO_3^- retention. Unfortunately, periodic high flow events can reduce the overall efficiency of mitigation systems drastically, because contaminant loads and the risk of bypassing untreated water are increasing and DOC production from resistant organic carbon sources is limited by low contact times. For situations like that, the use of more readily available organic carbon sources is necessary like straw, weed, sugar cane, corncobs etc. The experiments show that the mixture of less resistant organic substrates with readily accessible organic carbon (e.g. straw) and more resistant organic substrates with stabile structure and high effective porosity (e.g. bark mulch) is capable of retaining high fractions of NO_3^- at low HRT and has the potential to keep high effective permeability for a long time. The mixture of bark mulch and straw also seems to provide a supportive environment for effective attenuation of atrazine and isoproturon, two preferentially aerobically degraded herbicides with high leaching potential, by adsorption and degradation under suboxic conditions. It can be assumed that herbicides, which belong to the same chemical groups as atrazine (triazines e.g. simazine, therbutylazine) and isoproturon (phenylurea herbicides e.g. diuron, chlortoluron, linuron) and have similar chemical properties would be similarly retained in the reactor of organic substrate.

The usage of organic substrates as organic carbon sources in denitrification involves the risk of disadvantageous side effects on the quality of downstream waters, e.g. oxygen demand, turbidity, odour and contamination with by-products e.g. excess of organic carbon (dissolved and particular), microorganisms, nitrite and hydrogen sulfide. Hydrogen sulphide has the potential to hinder the formation of N_2 by disturbing the denitrification process on the level of nitrogen oxides, which are powerful greenhouse gases.

If the disadvantages can be properly managed and minimized, the addition of straw and bark mulch seems to be an optimal, cost- and performance-efficient solution to improve conventional mitigation zones. Reactive swales or basins promise to be the most efficient designs for successful denitrification and herbicide removal in mitigation zones as literature study and experiments have shown.

According to the experimental conditions (input: 100 mg-NO_3^- L^{-1}, 25-30 µg-ATR L^{-1}, 20-25 µg-IPU L^{-1}, water saturated reactor, continuous flow) and results, a HRT of 0.5 days would describe an effective ratio between reactor volume and inflow to ensure efficient denitrification (17-82 %, 9-21°C) and atrazine (15-52%, 9-21°C)and isoproturon (35-75%, 5-17°C) removal at high flow. Around 70% of the observed DOC, which was metabolized for respiratory means of the microorganisms or washed out, was utilised in the denitrification process. If O_2 and SO_4^{2-} concentrations in the inlet would be lower as in the experiment, the efficiency of DOC use could be even higher (up to 90 %).

In Table 6-1 to Table 6-3 the expected mitigation zone dimensions and the contaminant attenuation performance of mitigation zones on basis of the technical scale results at HRT of 0.5 days, taking into account concentrations of 100 mg-NO_3^- L^{-1}, 27 µg-ATR L^{-1} and 20 to 25 µg-IPU L^{-1} in the drainage water, are estimated for a 10 ha field (drainage coefficient: 12 mm).

Table 6-1: Expected dimensions and performances of denitrification on the basis of different temperatures and treated percentages of flow at HRT of 0.5 days (area: 10 ha, drainage coefficient: 12 mm[a], effective porosity in organic substrate: 80 %, input concentration: 100 mg-nitrate L^{-1}).

Percentage of treated flow [%]	Volume of filter [m³]	Fraction of field area for filter, when depth is 1 m [%]	Summer (water: 21°C)		Winter (water: 9°C)	
			Denitrification [kg-nitrate-N d^{-1}] [b]	Denitrification [%]	Denitrification [kg-nitrate-N d^{-1}] [b]	Denitrification [%]
100	750	0.8	23	**82**	6-19[c]	**17-60[c]**
50	375	0.4	11	41	3-10[c]	8-30[c]
25	188	0.2	6	20	1-5[c]	4-15[c]

[a] drainage coefficient: depth of water, a drainage system should remove from a field in 24 hour period; in humid climate zones without surface inlet common values are between 6 and 18 mm for mineral and organic soils, respectively.

[b] under the premise of a discharge of 12 mm per day

[c] higher value results from addition of fresh substrate short before drop of temperatures

Table 6-2: Expected dimensions and performances of atrazine retention on the basis of different temperatures and treated percentages of flow at HRT of 0.5 days (area: 10 ha, drainage coefficient: 12 mm[a], effective porosity in organic substrate: 80 %, input concentration: 27 *µ*g-atrazine L^{-1}).

Percentage of treated flow [%]	Volume of filter [m³]	Fraction of field area for filter, when depth is 1 m [%]	Summer (water: 21°C)		Winter (water: 9°C)	
			Retention [g-atrazine d^{-1}] [b]	Retention [%]	Retention [g-atrazine d^{-1}] [b]	Retention [%]
100	750	0.8	17	**52**	8	**15**
50	375	0.4	8	26	4	8
25	188	0.2	4	13	2	4

[a] drainage coefficient: depth of water, a drainage system should remove from a field in 24 hour period; in humid climate zones without surface inlet common values are between 6 and 18 mm for mineral and organic soils, respectively.

[b] under the premise of a discharge of 12 mm per day

Table 6-3: Expected dimensions and performances of isoproturon retention on the basis of different temperatures and treated percentages of flow at HRT of 0.5 days (area: 10 ha, drainage coefficient: 12 mm[a], effective porosity in organic substrate: 80 %, input concentration: 20 μg-isoproturon L^{-1}(winter); 25 μg-isoproturon L^{-1}(summer)).

Percentage of treated flow [%]	Volume of filter [m^3]	Fraction of field area for filter, when depth is 1 m [%]	Summer (water: 17°C)		Winter (water: 5 °C)	
			Retention [g-isoproturon d^{-1}] [b]	Retention [%]	Retention [g-isoproturon d^{-1}] [b]	Retention [%]
100	750	0.8	16	**75**	8	**35**
50	375	0.4	8	37	4	17
25	188	0.2	4	18	2	9

[a] drainage coefficient: depth of water, a drainage system should remove from a field in 24 hour period; in humid climate zones without surface inlet common values are between 6 and 18 mm for mineral and organic soils, respectively.

[b] under the premise of a discharge of 12 mm per day

Lower HRT than 0.5 days is critical for contaminant removal at low temperatures (5°C). With HRT higher than one day the volume of the reactor or the bypass of untreated water and the risk for anaerobic conditions and accompanying negative side effects as described above and in detail in the summary of technical scale experiments is increasing. Due to limitations by NO_3^- input at HRT of 2.0 days only 33% of the observed DOC, which was metabolized for respiratory means of the microorganisms or washed out, was utilized for denitrification.

Next to the standard design of the swales (mixure of bark mulch and straw, water saturated flow) variations are possible according to site conditions.

If there is no area for implementation of a bioreactor on site, an instream reactor (Robertson and Merkley, 2009) could be a solution. If land is available between drainage outflow and surface water a bio reactor in the bank or in surface water inflow, maybe an additional storage basin is recommended. Dependent on slope and water head between drainage outflow and receiving water vertical flow reactor (higher depth, less area) or horizontal flow reactor have to be considered. If the flow is constant and low preferable substrates would be bark mulch or comparable.In case low and high flow events are alternating, the reactive swale could be built with a bottom layer of a resistant organic carbon source like bark mulch, saw dust or wood chips, for treatment of the base flow and an upper layer consisting of a mixture of a mixture of fragile (straw, sugar cane, reed, corn cobs) and resistant organic carbon sources (see above) for high flow events. Due to relatively rapid depletion of the fragile substrate under unsaturated and wet conditions, the upper layer would need to be exchanged more frequently. The exchange interval depends on the load of NO_3^- and competing electron acceptors and climate conditions on site, which determine the degradation rate of the organic substrate under aerobic to suboxic conditions. Since the homogeneous mixture of the fragile and resistant organic carbon sources could be a technical problem, the substrates could be added in horizontal layers.

The addition of the readily accessible organic substrate should be accomplished shortly before the drop of temperature and/or start of the rainy season. The short process age allows high denitrification at low temperatures, and under high flow conditions, reduction of concentrations of washed out DOC by dilution is higher.

Combining surface wetlands and reactive swales is worth to be considered as a possible solution in general. The efficiency of wetlands can be increased by implementing a reactive swale downstream, which results in much lower need of surface area or reactor volume for the wetland.

For example, taking into account wetland studies at average HRT of 10 days and a NO_3^- removal of 185 mg NO_3-N m^{-2} d^{-1} compared to reactive swale studies at HRT of 0.4 days, an effective porosity of 50%, a reactor height of 0.4 m and a minimal NO_3^- removal of 6 g NO_3-N m^{-3} d^{-1}, as observed at low temperatures, which translates to 2400 mg NO_3-N m^{-2} d^{-1}, the surface area of the mitigation zone can be hypothetically reduced by a factor of 10 and denitrification performance would be still about the same.

Utilizing the wetland to increase storage volume for the swale would prevent bypass of untreated water, furthermore the swale could be kept saturated to reduce decomposition of the organic carbon sources. Taking the example of 100% treated flow in Table 6-1 as a basis and into account that (i) the annual groundwater recharge rate is 200 mm (Berlin, Germany), (ii) the average annual denitrification performance is 50 % (HRT: 0.5 d) and (iii) 10 g straw are needed to denitrify 1 g nitrate-N (this study, Soares and Abeliovich 1998) straw had to be refilled after 10 years. Enhanced sulphate reduction in times of NO_3^- input limitation, e.g. in phases of stagnation would shorten exchange intervals. Under unsaturated conditions the decomposition of straw would be further accelerated because of higher oxygen input. According to results of this study it is recommended to add fresh substrate to the bioreactor every one or two years, shortly before drop of temperatures and/or rainy season. Decontamination performance would benefit from adapted microorganisms in the established, remaining substrate and from fresh readily available organic carbon under critical conditions like low temperatures and/or high discharge. Under high flow conditions DOC concentrations would be reduced by dilution. The surface wetland could be used for herbicide degradation by aerobic and photolytic or hydrolytic processes and for sedimentation of particles to prevent clogging of the swale. A surface wetland downstream of the swale could improve quality of outflow (DOC reduction and oxygen enrichment).

As always, a successful design has to be adapted to site-specific conditions.

According to the results of the experiments at UBA, the amendment of mitigation zones with organic substrate for efficient and parallel retention of NO_3^- and certain herbicides at short HRTs is strongly recommended. It has to be underlined that conditions in the experiment are worst case considerations, since concentrations of the contaminants are above average and continuously added, so that in real systems the longevity of the organic substrate as efficient organic carbon source and adsorbent is expected to be higher. The studied design has the potential to be a valuable and effective tool in the set of best management practices.

Bibliography

Abdelhafid, R., Houot, S., Barriuso, E., 2000. Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils. Soil Biology & Biochemistry. 32, 389-401.

Armstrong, D. E., Chester, C., Harris, R. F. 1967. Atrazine Hydrolysis in Soil. Soil Sci.Soc.Amer.Proc. 31, 61-66.

Aslan, A. Türkman, A., 2005. Combined biological removal of nitrate and pesticides using wheat straw as substrates. Process Biochemistry. 40, 935–943.

Boivin, A., Cherrier, R., Schiavon, M., 2005. A comparison of five pesticides adsorption and desorption processes in thirteen contrasting field soils. Chemosphere. 61, 668-676.

Bouwer, W.W.M., Boesten, J.J.T.I., Siegers, W.G., 1990. Adsorption of transformation products of atrazine by soil. Weed research. 30,123-128.

Capriel, P., Haisch, A., Khan, S. U., 1985. Distribution and Nature of Bound (Nonextractable) Residues of Atrazine in a Mineral Soil Nine Years after the Herbicide Application. J. Agric. Food Chem. 33, 567-569.

Chung, K.H., Ro, K.S., Roy, D., 1996. Fate and enhancement of Atrazine biotransformation in anaerobic wetland sediment. Wat Res. 30(2), 341-346.

Clifford, D., Liu, X., 1993. Ion exchange for nitrate removal. Journal AWWA, 85 (4), 135–143.

Crawford, J.J., Sims, G.K., Mulvaney, R.L., Radosevich, M., 1998. Biodegradation of Atrazine under denitrifying conditions. Appl Microbiol Biotechnol. 49, 618-623.

Christianson, L. I., 2011. Design and performance of denitrification bioreactors for agricultural drainage. Dissertation. Iowa State University. Available at URL: http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1348&context=etd

De Andrea, M.M., Matallo, M.B., Tomita, R.Y., Luchini, L.C., 1997. Effect of temperature on dissipation of [^{14}C]-atrazine in a brazilian soil. Pesq. Agropec. Bras. 32(1), 95-100.

De Haan, J., Van Der Schoot, J.R., Verstegen, H., Clevering, O., 2010. Removal of nitrogen leaching from vegetable crops in constructed wetlands. Acta Horticulturae. 852**,** 139-144.

De Wilde, T., Spanoghe, P., Ryckeboer, J., Jaeken, P., Springael, D., 2009. Sorption characteristics of pesticides on matrix substrates used in biopurification systems. Chemosphere. 75, 100–108.

Gendrault, S., 2004. Etude d'un traitement combiné bio-physico-chimique pour la décontamination des eaux polluées en atrazine. Dissertation. National institute of applied sciences in Lyon. Available at URL: http://theses.insa-lyon.fr/publication/2004ISAL0045/these.pdf [In French]

Gibert, O., Pomierny, S., Rowe, I., Kalin, R.M., 2008. Selection of organic substrates as potential reactive materials for use in a denitrification permeable reactive barrier (PRB). Bioresour. Technol. 99, 7587–7596.

Greenan, C.M., Moorman, T.B., Kaspar, T.C., Parkin, T.B., Jaynes, D.B., 2006. Comparing carbon substrates for denitrification of subsurface drainage water. J. Environ. Qual. 35, 824–829.

Herzberg, M., Dosoretz, C.G., Tarre, S., Beliavski, M., Green M. 2004. Biological granulated activated carbon fluidized bed reactor for atrazine remediation.
Water Science and Technology. 49, 215-222.

Ilhan, Z.E., 2010. Fate of agrochemicals in wood chip denitrifying reactors and their impacts on wood chip microbial ecology. Graduate Theses and Dissertations. Paper 11340. Available from URL: http://lib.dr.iastate.edu/etd/11340

Ilhan, Z.E., Ong, S.K., Moorman,T.B., 2011. Dissipation of Atrazine, Enrofloxacin, and Sulfamethazine in Wod Chip Bioreactors and Impact on Denitrification. J. Environ. Qual. 40, 1816-1823.

Jablonowski, N.D., Modler, J., Schaeffer, A., Burauel, P., 2008. Bioaccessibility of environmentally aged 14C-atrazine residues in an agriculturally used soil and its particle-size aggregates. Environ Sci Technol. 42(16), 5904-5910.

Jaynes, D.B., Kaspar, T.C., Moorman, T.B., Parkin, T.B., 2008. In situ bioreactors and deep drain-pipe installation to reduce nitrate losses in artificially drained fields. Journal of Environmental Quality. 37, 429-436.

Jørgensen, P.R., Urup, J., Helstrup, T., Jensen, M.B., Eiland, F., Vinther, F.P., 2004. Transport and reduction of nitrate in clayey till underneath forest and arable land. Journal of Contaminant Hydrology. 73, 207-226

Kögel-Knabner, I., 2010. Organische Bodensubstanz, in: Blume, H.-P., Brümmer, G.W., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., Wilke, B.-

M., (Eds.), Scheffer/Schachtschabel -Lehrbuch der Bodenkunde. 16th ed. Heidelberg, Berlin (GER): Spektrum Akademischer Verlag. pp. 51-81. [In German]

Krause, B., Heise, S., Litz, N., 2010. Properties of Atrazine and Bentazone - Project acronym: Aquisafe 2. Berlin (GER). Available from URL: http://www.kompetenz-wasser.de/index.php?id=482&type=0&jumpurl=..%2Ffileadmin%2Fuser_upload%2Fpdf%2Fforschung%2FAquisafe%2FD5_1_report_pesticides_final.pdf

Lerch, R.N., Blanchard, P. E., Thurma,n E. M., 1998. Contribution of Hydroxylated Atrazine Degradation Products to the Total Atrazine Load in Midwestern Streams. Environ. Sci. Technol. 32, 40-48.

Lesan, H.M., Bhandari, A., 2004. Contact-time-dependent atrazine residue formation in surface soils. Water Res. 38(20), 4435-45.

Loiseau, L., Barriuso E., Zegouagh Y., Largeau C., Mariotti A.; 2000. Release of the atrazine non-extractable (bound) residues of two soils using degradative techniques. Agronomie. 20(5), 513-524.

Ma, L., Selim, H.M., 1996. Atrazine retention and transport in soils. Rev Environ Contam Toxicol. 145, 129-73.

Munier-Lamy, C., Feuvrie,r M.P., Choné, T., 2002. Degradation of 14C-atrazine bound residues in brown soil and rendzina fractions. J Environ Qual. 31(1), 241-247.

OECD 106, 2000. Guideline for the testing of chemicals. Adsorption - Desorption Using a Batch Equilibrium Method. Paris (FRA):The organisation. Available from URL: http://www.oecd-ilibrary.org/environment/test-no-106-adsorption-desorption-using-a-batch-equilibrium-method_9789264069602-en.

Ovez, B., Höll, H.H., 2008. Adsorption of Atrazine and Simazine from Aqueous Solutions onto Poly(ε-Caprolactone). CLEAN – Soil, Air, Water. 36(10-11), 900–904.

Park, J.-H., Feng, Y., Ji, P., Voice, T.C., Boyd, S.A., 2003. Assessment of Bioavailability of Soil-Sorbed Atrazine. Appl. Environ. Microbiol. 69(6), 3288-3298.

Périllon, C., Matzinger, A., 2010. Identification of existing mitigation systems that can attenuate nitrates during high flow events from drained, agricultural fields. Report of the Aquisafe 2 project. KompetenzZentrum Wasser Berlin. Available from URL: http://www.kompetenz-wasser.de/index.php?id=482&type=0&jumpurl=fileadmin%2Fuser_upload%2Fpdf%2Fforschung%2FAquisafe%2FD1_1_Existing_mitigation_systems_final.pdf

Robertson, W.D., Merkley, L.C., 2009. In-stream bioreactor for agricultural nitrate treatment. Journal of Environmental Quality. 38,230-237.

Rolf, F., 2002. Weitergehende Abwasserreinigung in kleinen Kläranlagen. Dissertation. Brandenburgisch Technische Universität Cottbus. Available from URL: http://www-docs.tu-cottbus.de/wassertechnik/public/Publikationen/Schriftenreihe/Heft_7.pdf [In German]

Schipper, A.L., Barkle, G.F., Vojvodic-Vukovic, M., 2005. Maximum rates of nitrate removal in a denitrification wall. Journal of Environmental Quality. 34**,** 1270-1276.

Schipper, L.A., Robertson, W.D., Gold, A.J., Jaynes, D.B., Cameron, S.C., 2010. Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. Ecological Engineering. 36, 1532–1543.

Schreiber, B., Schmalz, V., Brinkmann, T., Worch, E., 2007. The effect of water temperature on the adsorption equilibrium of dissolved organic matter and atrazine on granular activated carbon. Environ Sci Technol. 41(18), 6448-6453.

Soares, M., I., M., Abeliovich, A., 1998. Straw as substrate for water denitrification. Wat. Res. 32 (12), 3790-3794.

Sørensen, J., Tiedje, J. M., Firestone, R. B., 1980. Inhibition by sulfide of nitric and nitrous oxide reduction by denitrifying Pseudomonas fluorescens. Applied and environmental microbiology, 39(1),105-108.

Sørensen, S.R., Bending, G.D., Jacobsen, C.S., Walke,r A., Aamand, J., 2003. Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields.FEMS Microbiology Ecology. 45(1), 1–11.

Kretzschmar, R., 2010. Chemische Eigenschaften und Prozesse, in: Blume, H.-P., Brümmer, G.W., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., Wilke, B.-M., (Eds.), Scheffer/Schachtschabel -Lehrbuch der Bodenkunde. 16th ed. Heidelberg, Berlin (GER): Spektrum Akademischer Verlag. pp. 121-170. [In German]

Spongberg, A.L., Ganglian, L., 2000. Adsorption of atrazine and metolachlor in three soils from Blue Creek wetlands, Waterville, Ohio. Science of soils. 5(1),1-9.

Tedesco, L., Hall, B. E., Barr, R. C., Matzinger, A., Morel-Fatio, A., 2009: Diffuse trace contaminants with relevance for drinking water production in rural and semi-rural environments. Project report. Kompetenzzentrum Wasser Berlin. http://www.kompetenz-wasser.de/index.php?id=383&type=0&jumpurl=fileadmin%2Fuser_upload%2Fpdf%2Fforschung%2FAquisafe%2FKWB_Aquisafe_D_1_1_Aug_2009.pdf

Trigo, C., Koskinen, W.C., Celis, R., Sadowsky, M.J., Hermosín, M.C., Cornejo, J., 2010. Bioavailability of organoclay formulations of atrazine in soil. J Agric Food Chem. 58(22), 11857-11863.

Van Driel, P.W., Robertson, W.D., Merkley, L.C., 2006a. Upflow reactors for riparian zone denitrification. J. Environ. Qual. 35, 412-420.

Van Driel, P.W., Robertson, W.D., Merkley, L.C., 2006b. Denitrification of agricultural drainage using wood-based reactors. Transactions of the ASABE. 49**,** 565-573.

Wang, X.M., Wang, J.L., 2012. Denitrification of nitrate-contaminated groundwater using biodegradable snack ware as carbon source under low-temperature condition. Int. J. Environ. Sci. Technol. 9, 113–118.

Widenfalk, A., Svensson, J.M., Goedkoop, W., 2004. Effects of the pesticides captan, deltamethrin, isoproturon, and pirimicarb on the microbial community of a freshwater sediment.Environ Toxicol Chem. 23(8), 1920-1927.

Witz, U., 2005. Festbettnitrifikation und –denitrifikation mit festen Kohlenstoffquellen – Biologische Stickstoffelimination in der Nachsorge von Deponien. Dissertation. Bergische Universität Wuppertal. Available from URL: http://elpub.bib.uni-wuppertal.de/edocs/dokumente/fbd/bauingenieurwesen/diss2005/witz/dd0502.pdf [In German]

Chapter 7

Appendices

Appendix 1 – Literature

Table 7-1: Nitrate attenuation and hydraulic residence time (HRT) in field experiments in systems to mitigate agricultural pollution (Périllon and Matzinger, 2010)

Type of system	NO3- retention	Average HRT	Average temperature	References
	g-N $m^{-3}_{\text{of reactor}}$ d^{-1}	days		
Surface flow wetland (without carbon addition)	0.04	9.5	n.a.	Review of 25 studies by Périllon and Matzinger (2010)
Horizontal subsurface flow system with straw	1.98	5-23	n.a.	De Haan et al. (2010)
Wood chip denitrification wall	2.3	n.a.	7-9	van Driel et al. (2006a)
Wood chip denitrification wall	0.62	2-32	10	Jaynes et al. (2008)
Wood chip upflow reactors	1.2-5	1-5.6	9-13	van Driel et al. (2006b)
Wood chip bioreactor in stream	0.33-6.66	0.8	3-14	Robertson and Merkley (2009)

Appendix 2 – Column experiment (lab)

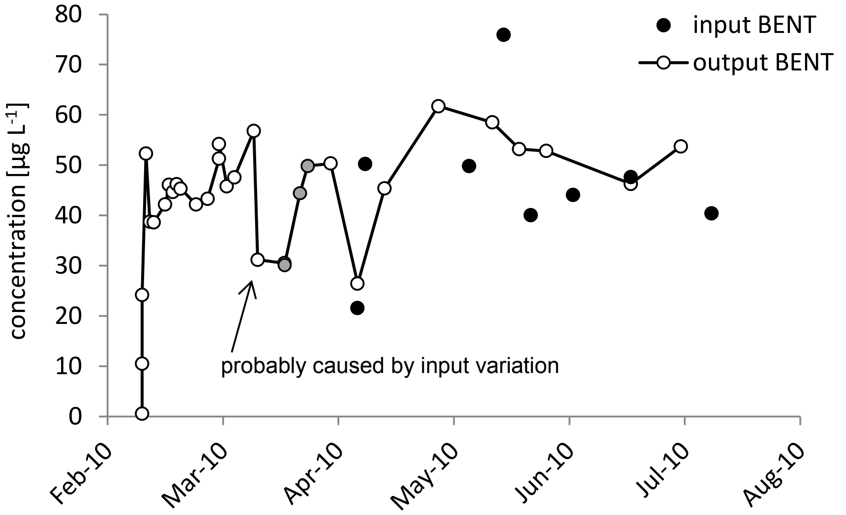


Figure 7-1: Concentrations of bentazone (BENT) in the in- and outflow of the straw-bark mulch column at HRT of 0.2 days. The grey symbols represent the phase of increased HRT (0.4 days)

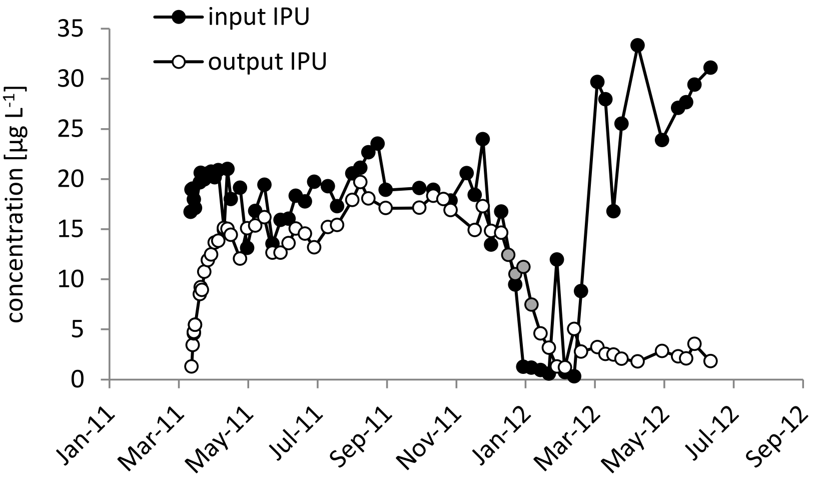
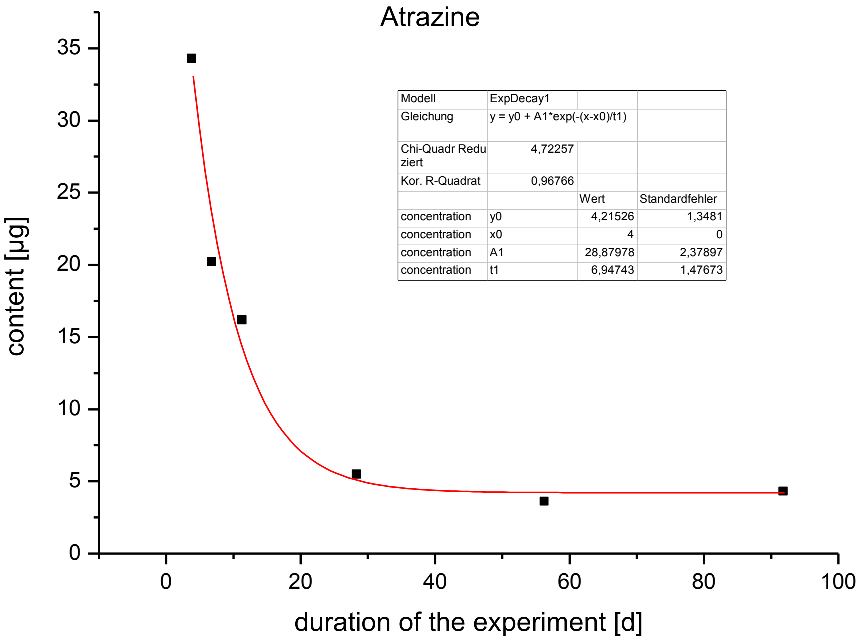
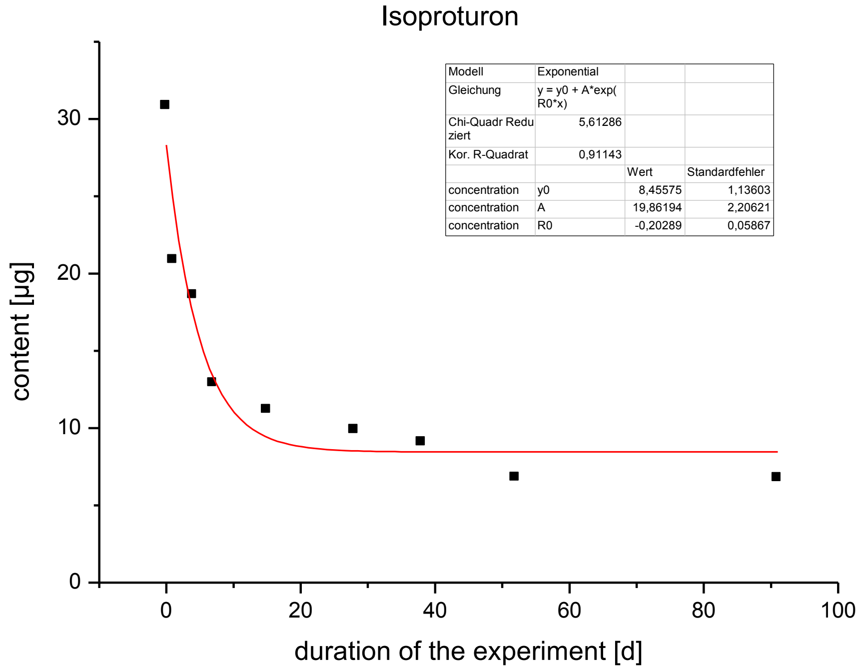


Figure 7-2: Concentrations of isoproturon (IPU) in the in- and outflow of the straw–bark mulch column at HRT of 0.4 days. The grey symbols represent the phase of increased HRT (2.0 days)

Appendix 3 – Dissipation experiment (lab)



Modell	ExpDecay1		
Gleichung	y = y0 + A1*exp(-(x-x0)/t1)		
Chi-Quadr Reduziert	4,72257		
Kor. R-Quadrat	0,96766		
		Wert	Standardfehler
concentration	y0	4,21526	1,3481
concentration	x0	4	0
concentration	A1	28,87978	2,37897
concentration	t1	6,94743	1,47673



Modell	Exponential		
Gleichung	y = y0 + A*exp(R0*x)		
Chi-Quadr Reduziert	5,61286		
Kor. R-Quadrat	0,91143		
		Wert	Standardfehler
concentration	y0	8,45575	1,13603
concentration	A	19,86194	2,20621
concentration	R0	-0,20289	0,05867

Figure 7-3: Development of total extractable herbicide content in solution and substrate over time under anaerobic conditions in the dissipation experiment. Retention of atrazine starts after lag-phase of 4 days.

Table 7-2: Comparison of dissipation parameters from batch and column experiments with atrazine and isoproturon

Parameter	Unit	Flow through reactor (F)			Batch reactor (B)
		First 10 weeks	Intermediate 10 weeks	Last 10 weeks	
λ_{ATR}	d^{-1}	~5.8	~1.98	~1.42	~0.15
$DT_{50, ATR}$ [a]	d	~0.12	~0.35	~0.49	~7.9 [b]
λ_{IPU}	d^{-1}	~0.63	-	~22.5	~0.2
$DT_{50, IPU}$ [a]	d	~1.1	-	~0.03	~3.2
λ_{ATR}	d^{-1}				1.4 (20°C, Gendrault 2004)

[a] DT_{50} means time for dissipation of 50% of input by degradation and permanent adsorption

[b] including lag-phase of 4 days

Appendix 4 – Sorption experiment (lab)

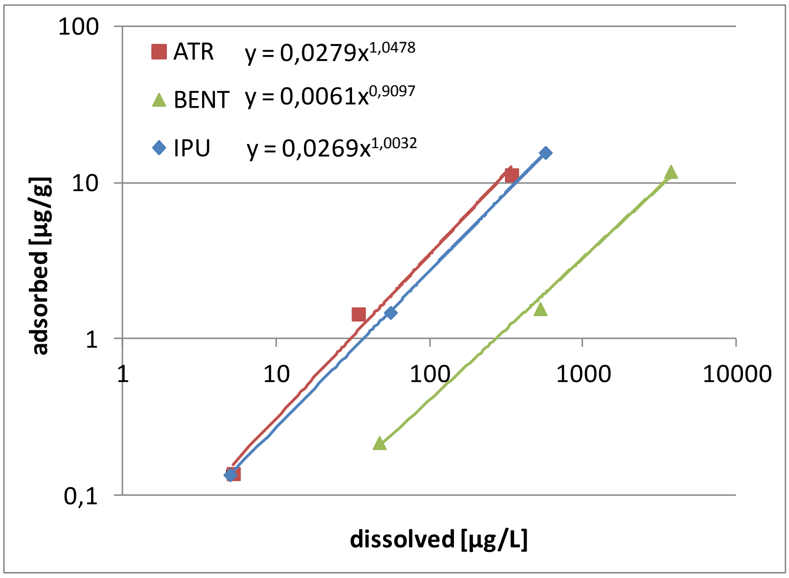


Figure 7-4: Distribution of herbicides in liquid and solid compartments after adsorption experiment with FREUNDLICH-coefficient and FREUNDLICH-exponent

Table 7-3: Adsorbed herbicide partitions after adsorption and desorption experiment

	adsorbed after 24 h adsorption step [%]	adsorbed after two 24 h desorption steps [%]
Atrazine	82-88	65-73
Isoproturon	82-83	52-60
Bentazone	35-65	9-37

Table 7-4: Adsorption potential of the straw-bark mulch mixture in comparison with other organic substrates and soils

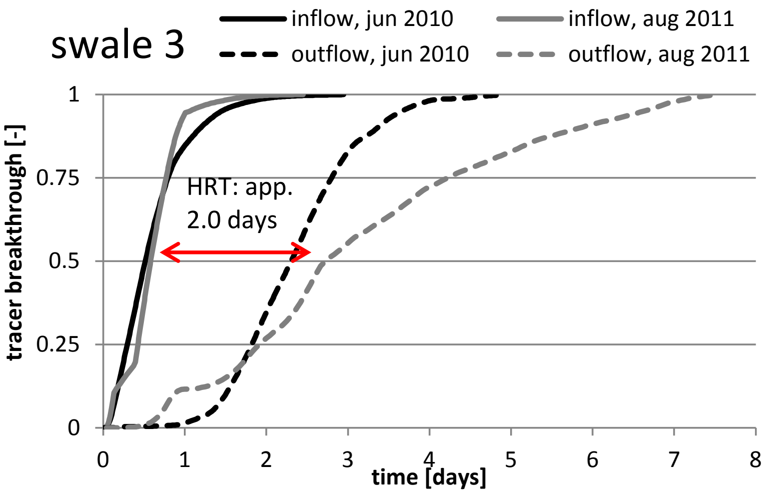
Substance	Concentration range	Substrate (C_{org})	K_F	Source
	mg L^{-1}	%	L kg^{-1}	
Atrazine	1-20	Bark (40)	155 [a]	Gendrault 2004
	0.5-8	Wood (46.5)	66 [a]	Ilhan 2010
	0.03-2.3	Straw-bark mulch mixture (46-51)	33 [b]	This study
	0.003-0.8	Bark (40)	23 [a]	Gendrault 2004
	N/A	Straw (N/A)	15	Abdelhafid et al. 2000
	0.25-10	Soil (0.62-3.5)	1.3-6.3	Boivin 2005
	0.2-3	Soil (0.18-1.63)	1.7-2.6	Sponberg and Ganglian 2000
Isoproturon	0.024-2.6	Straw-bark mulch mixture (46-51)	27	This study
Bentazone	0.085-5.8	Straw-bark mulch mixture (46-51)	3	This study
	1-5000	Straw (-)	2.45	De Wilde 2009
	0.25-10	Soil (0.62-3.5)	1.2-1.9	Boivin 2005

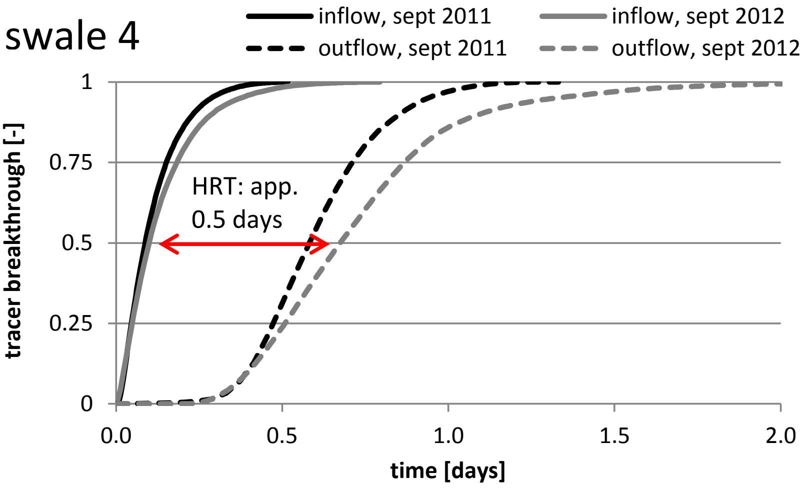
[a] liquid to solid ratio: 10
[b] liquid to solid ratio: 5

Appendix 5 – Construction (technical scale)

Figure 7-5: Pictures from the construction site of the swales

Appendix 6 – Hydraulic residence times (technical scale)





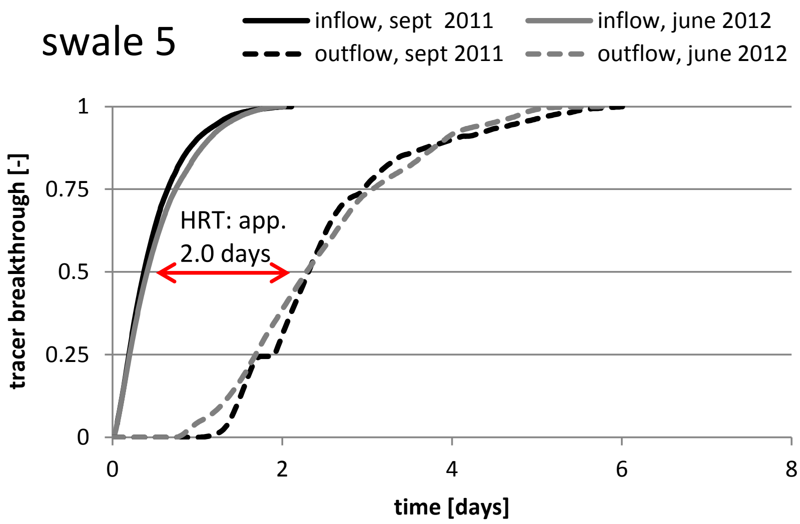


Figure 7-6: Tracer breakthrough curves at the inlet and outlet of the swales 3 to 5 at the start and at the end of the experiment.

Appendix 7 – Nitrate (technical scale)

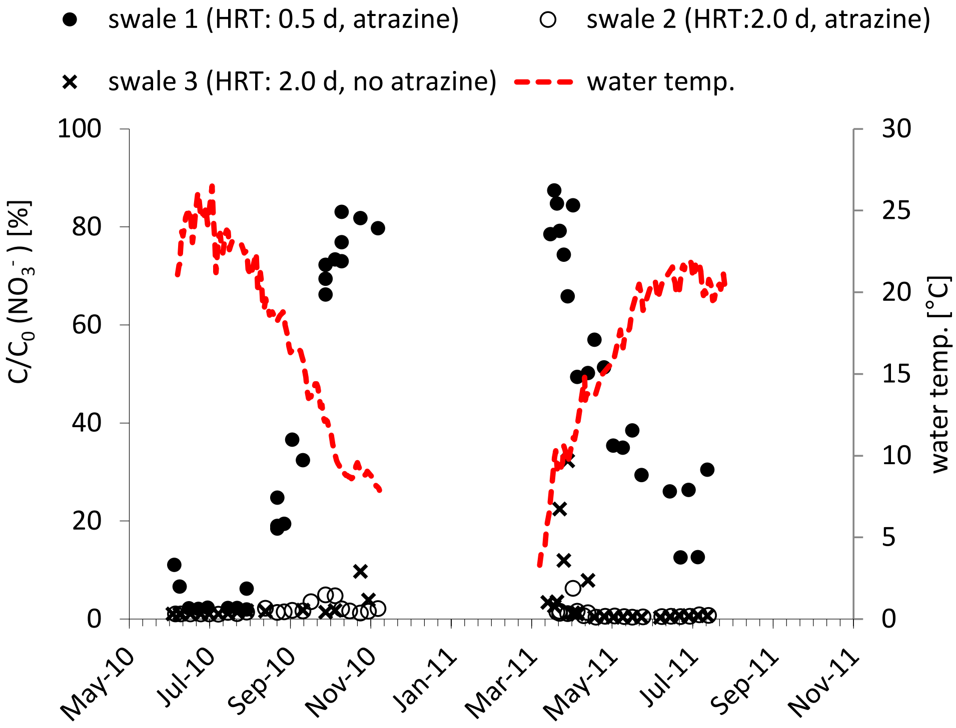
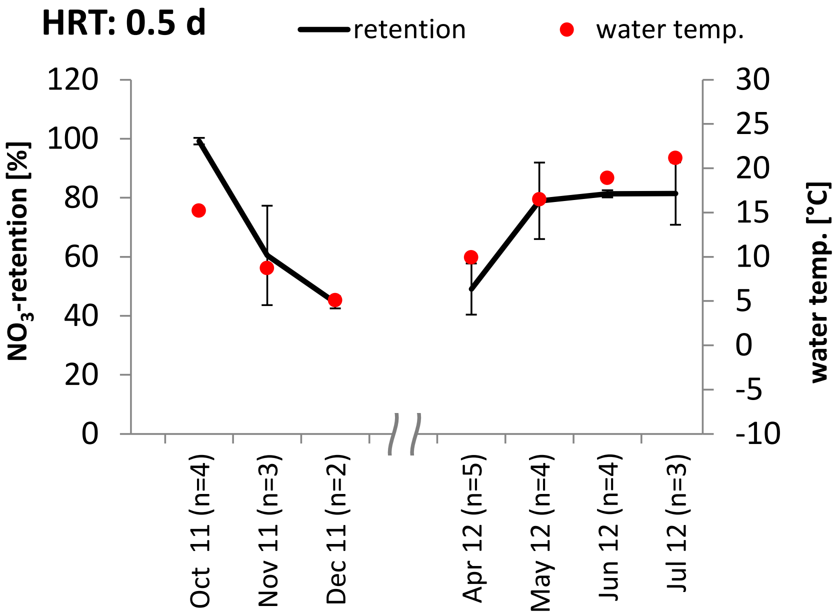


Figure 7-7: Breakthrough of nitrate in the swales 1 and 2 with atrazine and in swale 3 without atrazine (control). HRT stands for hydraulic residence time.



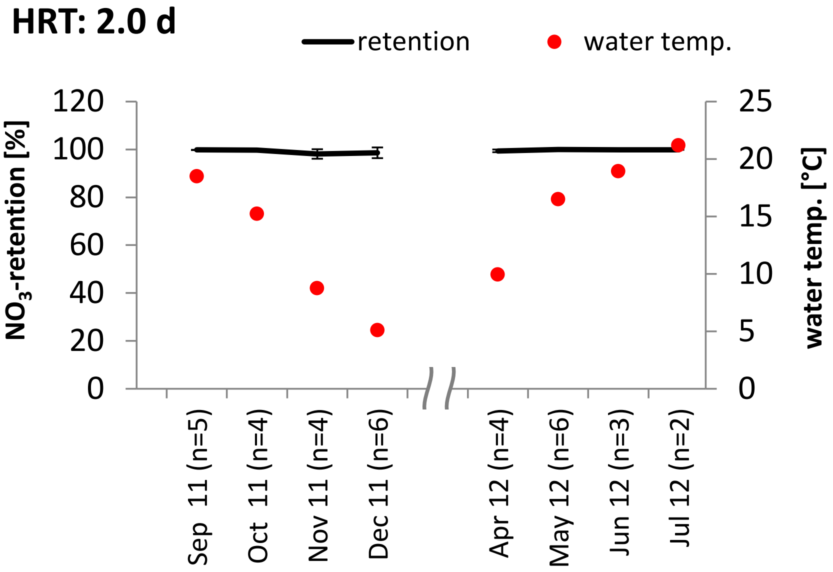


Figure 7-8: Relative denitrification (retention of concentrations) in the reactive swales 4 and 5 with HRT of 0.5 and 2.0 days, respectively.

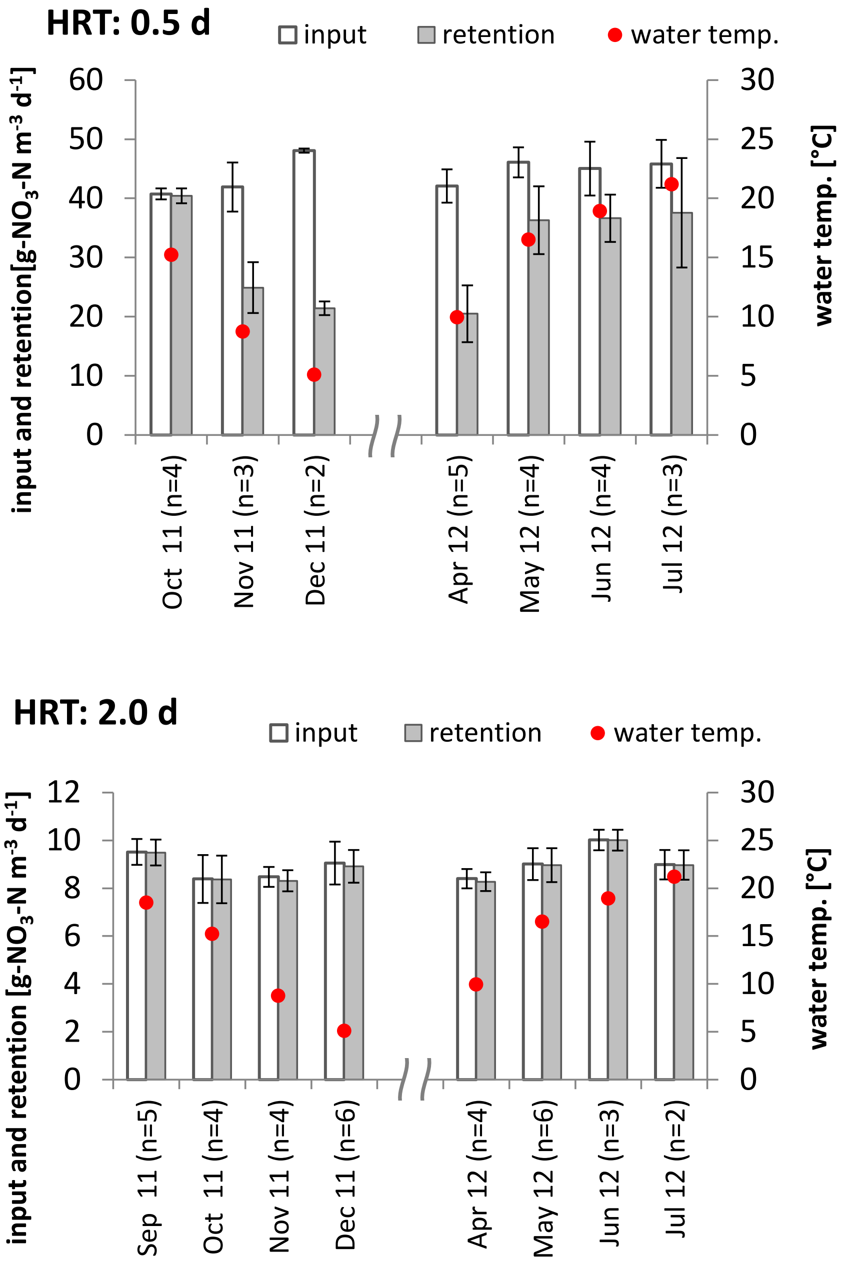


Figure 7-9: Absolute denitrification (retention of loads) in the reactive swales 4 and 5 with HRT of 0.5 and 2.0 days, respectively.

Appendix 8 – Redox potential (technical scale)

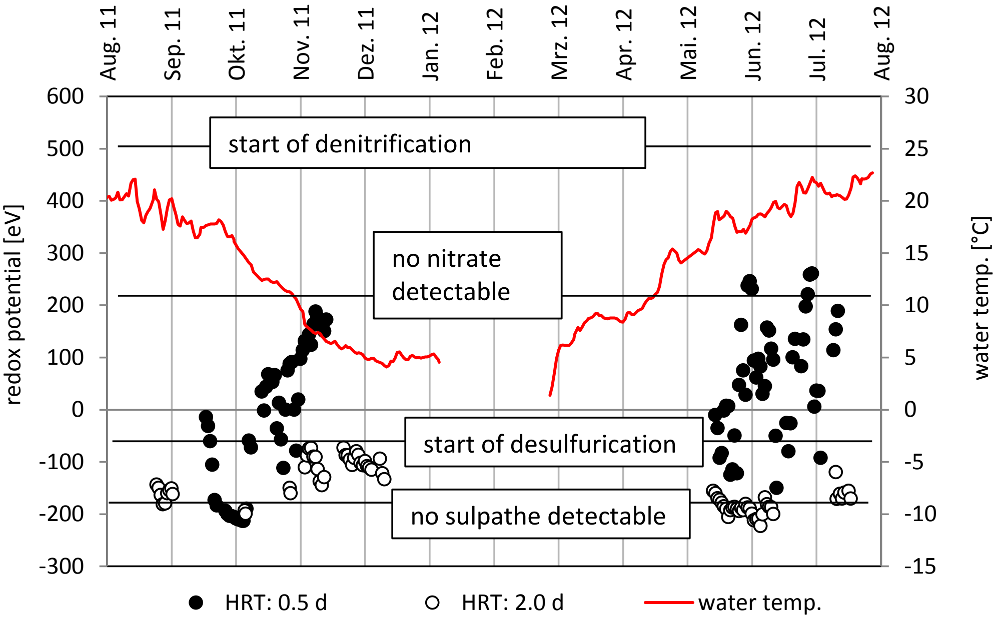
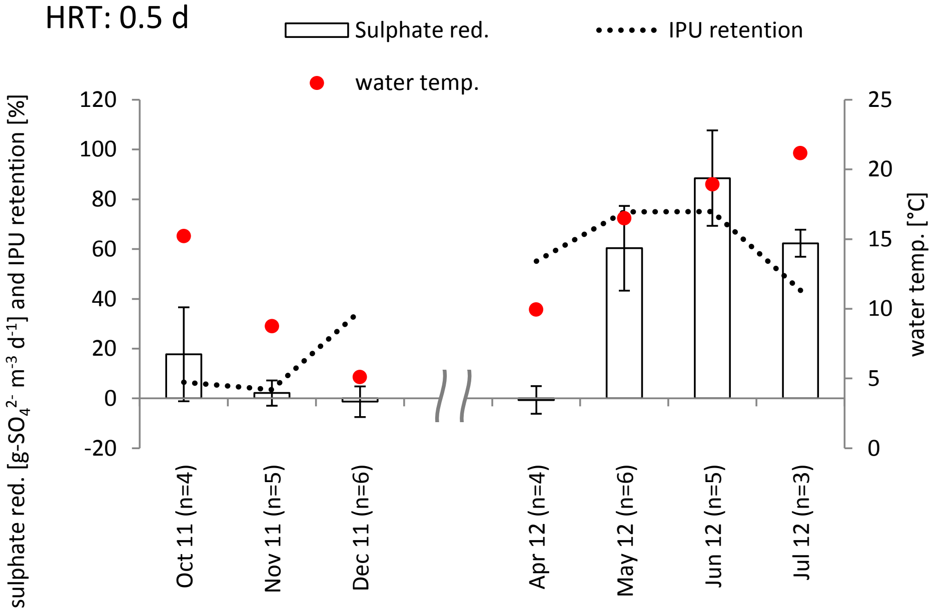


Figure 7-10: The redox potential at the outlet of the swales 4 and 5 as indicator for nitrate retention (experimental limits for different redox reactions by Kretzschmar, 2010)

Appendix 9 – Isoproturon (technical scale)



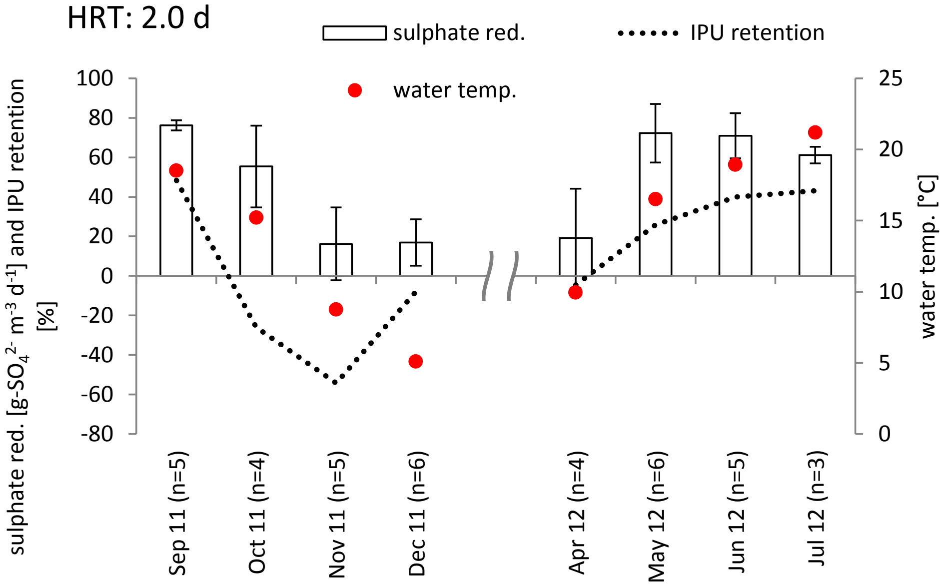
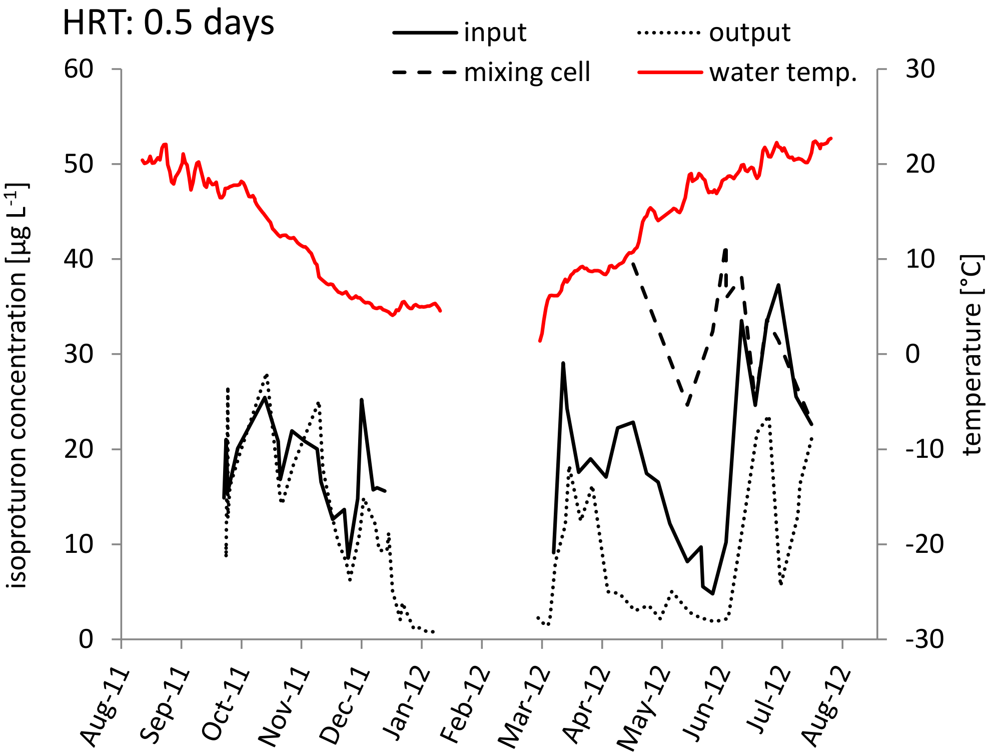


Figure 7-11: Relation of isoproturon retention, water temperature and redox condition illustrated by SO_4^{2-} reduction (no to low SO_4^{2-} reduction = suboxic conditions; high SO_4^{2-} reduction = anaerobic conditions).



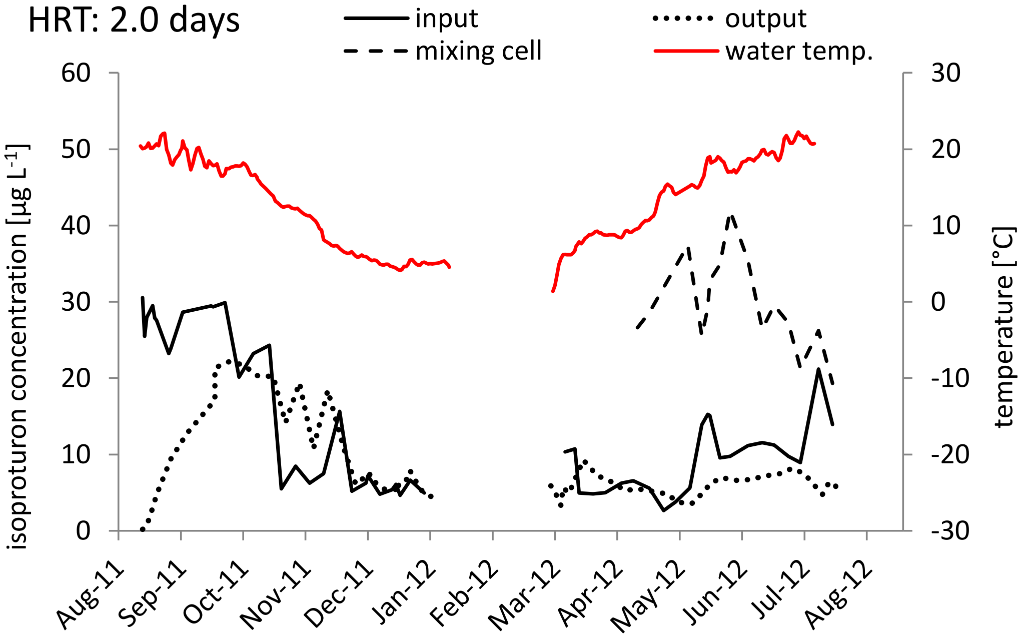


Figure 7-12: Input and output concentrations of isoproturon at HRT of 0.5 and 2.0 days in swale 4 and 5, respectively.