Artificial groundwater recharge in forests - soil fauna and microbiology

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Dekan

Preface

“We shall not cease from exploration,

and the end of all our exploring will be to arrive where we started,

and know the place for the first time.”

(T.S. Elliot)

Water is essential to all known forms of life. About 1,460 teratonnes (Tt) of water cover 71% of the Earth's surface, mostly in oceans and other large water bodies, with 1.6% of water belowground in aquifers and 0.001% in the air as vapor, clouds, and precipitation. Saltwater oceans hold 97% of the surface water, while glaciers and polar ice caps hold 2.4%, and other land surface water such as rivers and lakes the remaining 0.6%. Water moves continually through a cycle of evaporation or transpiration, precipitation, and runoff, usually reaching the sea. Some water is trapped for varying periods in ice caps, glaciers, aquifers, or in lakes, sometimes providing fresh water for life on land. Clean, fresh water is essential to human and other life. However, in many parts of the world - especially developing countries - it is in short supply and even in central Europe we should treat water as something invaluable. Therefore, providing fresh drinking water using sustainable methods is a central issue to survival on our planet.

Soil is the naturally occurring “epidermis” of our planet presenting the major component of the terrestrial biosphere. It forms a narrow interface between the atmosphere and the lithosphere and comprises elements of both; i. e. water, a gaseous phase and mineral matter, together with a diverse range of organisms and materials of biological origin. Soils are the part of the earth’s thin surface within which organic materials are broken down to form stable humic compounds, thereby

releasing their contained nutrient elements for uptake by microorganisms and dissipating their contained energy. Major features of the soil system are habitat provision, storage of organic matter, element releases and water storage. Thus, the soil system supports, directly or indirectly, any form of life on earth.

The present thesis provides insight into the mechanisms of water purification in the area “Lange Erlen” in the city of Basel, Switzerland. The population of this city has been depending on this source providing drinking water for several decades; however, the ecological basis for the purification process was not well understood. This thesis shows that in this fascinating system, water and soil processes are directly linked to each other and work synergistically to purify water. Moreover, the results suggest that the system is not exploited to full capacity and is likely to be able to provide the local population with drinking water for many years to come. Three independent studies were carried out in this system focusing on earthworms, the structure of the microbial community, and the function of the microbial community, which are presented as individual chapters within this thesis. They can be either read separately or together – depending on the reader’s interests. As they were all carried out in the same area and the same system, they are linked to each other and accompanied by a general introduction and a general discussion.

Kirsten Schütz

Basel, March 2008

Contents

List of abbreviations

Watered sites		Non-watered sites	
GGR	Grendelgasse rechts	GM1	Gemeindematten nord
+W1	Grendelgasse rechts	-W1	Gemeindematten nord
HST	Hintere Stellimatten	GM2	Gemeindematten süd
+W2	Hintere Stellimatten	-W2	Gemeindematten süd
VW	Verbindungsweg	Br10	Brunnen 10
+W3	Verbindungsweg	-W3	Brunnen 10
		BW	Bachtelenweg

Miscellaneous

IWB	Industrial Works of Basel
AUE	Department of Environment and Energy, Basel
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
SOM	Soil organic matter
C_{mic}	Microbial biomass
qO_2	Specific respiration
PLFA	Phospholipid fatty acid
PCA	Principal component analysis
RDA	Redundancy analysis
ANOVA	Analysis of variance
ANOSIM	Analysis of similarities
SIMPER	Similarity percentages routine
nMDS	Non-parametric multidimensional scaling

General Introduction

Drinking water production

Water purification is the process of removing contaminants from a raw water source with most of this water purified for human consumption (drinking water). Water purification occurs naturally (groundwater) or artificially by various methods, such as flocculation, sedimentation, filtration (ultrafiltration, active carbon filtration, slow sand filtration, etc.), reverse osmosis and deionization or oxygen treatment.

Water supply systems get water from a variety of locations, including groundwater (aquifers), surface water (lakes and rivers), and the sea through desalination. Use of groundwater, especially for drinking water production or farming, may lower the groundwater tables. Therefore environmentally conscious planning is required to meet long term water demands whilst sustaining groundwater tables. One widely adopted approach is termed "artificial groundwater recharge" or simply "groundwater recharge". This is a practice of both, directing and simultaneously purifying water (often rain- or riverwater, but sometimes reclaimed water) into aquifers thereby raising the groundwater table and guaranteeing sufficient drinking water sources. This is the technique that has been utilized for a log time at the “Lange Erlen”, Basel (Switzerland).

The study site “Lange Erlen” – past and present

The thesis presented here investigates the drinking water production of the city of Basel (Switzerland) at the former floodplain area called “Lange Erlen”. This area is located in the northwestern part of Switzerland, northeast of Basel (Fig. 1). As a former natural floodplain area, the “Lange Erlen” extends along the river Wiese, a straightened tributary of the river Rhine that originates from the Black Forest,

Fig. 1: The study site “Lange Erlen”, Basel (Switzerland). Framed areas represent the current recharge areas (status 2008).

Germany. Before the river Wiese was canalized at the end of the 19th century, natural floodings occurred on the valley floor with alluvial soil covering the area. Up until the end of the 19th century, local groundwater wells and springs were sufficient for drinking water supply to the local population.

Together with the industrialization and growth of population, drinking water sources became insufficient and new water resources were required. In 1882, the first groundwater pumping station was built in the “Lange Erlen” and has since then continuously expanded during the 20th century. Because groundwater stores were insufficient, formerly used watering techniques for agricultural areas were adjusted to the artificial groundwater recharge technique in the “Lange Erlen” and this has been constantly improved within the last 95 years (Rüetschi 2004). Up until 1964, the water used for flooding originated from the river Wiese, but as river water quality and quantity became increasingly less dependent, the “flooding water source” was shifted to the river Rhine. Here, continuous water inflow could be guaranteed and after 1986 (Sandoz accident, Basel) a sophisticated alarm system (“Rhine-Alarm”) was built along the river to warn of industrial spills or shipping accidents. In the last 20 years, industrial discharges to the river Rhine and its tributaries were cut by 95% and levels of heavy metals including mercury, lead and copper were drastically reduced. As evidence of the improvement of water quality swimming in the Rhine has become a widespread leisure activity in Basel.

Because drinking water production in the “Lange Erlen” has always had top priority, further industrial or urban cultivation was restricted from this area. Today, the “Lange Erlen” has multiple uses: extensive agriculture, recreation and drinking water production (Industrial Works of Basel, IWB). For preserving water sources, groundwater protection zones and water conservation areas were also applied there by the “Department of Environment and Energy” (AUE, Fig. 2). The semi-natural

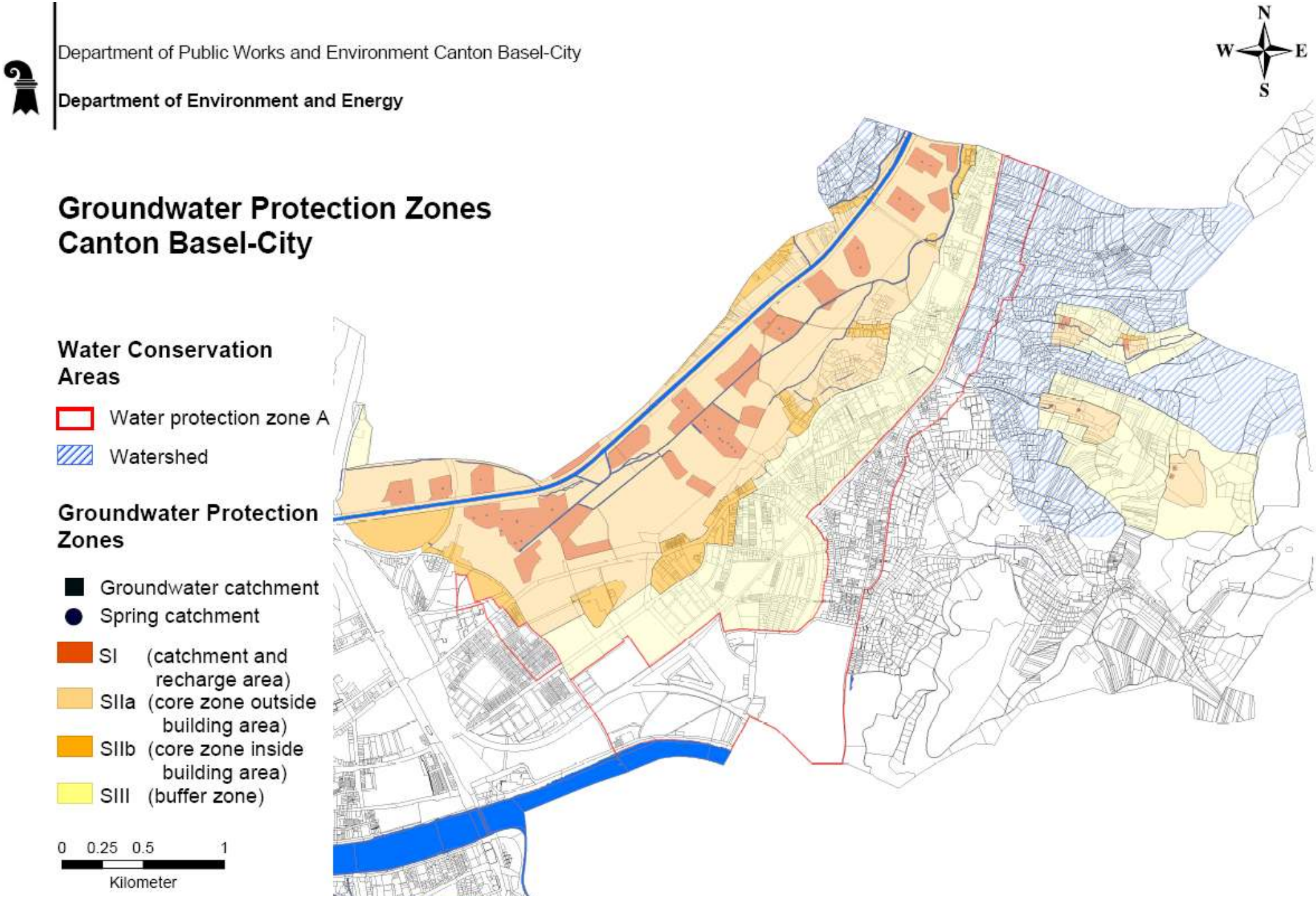

Fig. 2: Groundwater protections zones “Lange Erlen”, Basel (Switzerland). From: Department of Environment and Energy (Februar 2008).

forest sites of the “Lange Erlen” are classified as oak-hornbeam forests (Burnand & Hasspacher 1999); however the recharge areas have been partly modified by human activities, such as landfill and afforestation with poplars, ash, alders and willows.

Drinking water production in the “Lange Erlen”

In 1964 the raw water source for groundwater recharge was shifted from the river Wiese to the river Rhine. Therefore, constructions of a raw water catchment at the river Rhine, of a rapid sandfilter for water pretreatment at the “Lange Erlen” and of various water pipes were required. At present, approximately half of the drinking water for the city of Basel is obtained by the artificial groundwater recharge system in the “Lange Erlen”. The second half is provided by the “Hardwasser AG” and produced in the “Hard Forest”, west of Basel by using an artificial channel (river bank) filtration system in this forest. To the best of my knowledge, the recharge and purification system at the “Lange Erlen” (Fig. 3) is not employed elsewhere on the globe. Forested areas were chosen as recharge areas because shading the water surface reduces highly fluctuating water temperatures, pH-values, oxygen concentrations and therefore prevents algal blooms. Today, eleven embanked and subdivided forest sites (Fig. 1) of one to two hectare are periodically flooded with prefiltered water from the river Rhine to augment groundwater resources in the “Lange Erlen”, thereby transforming river water into purified drinking water (Fig. 3). In the rapid sand filter, approximately 95% of complex particulate organic and inorganic matter is eliminated prior flooding the recharge areas thereby preventing decreasing infiltration capacities due to “clogging”. To reduce maintenance work of the rapid sand filter and to prevent recharge area contaminations, the raw water catchment at the river Rhine is immediately stopped in the case of high water turbidity or river Rhine pollution (“Rhine-Alarm”).

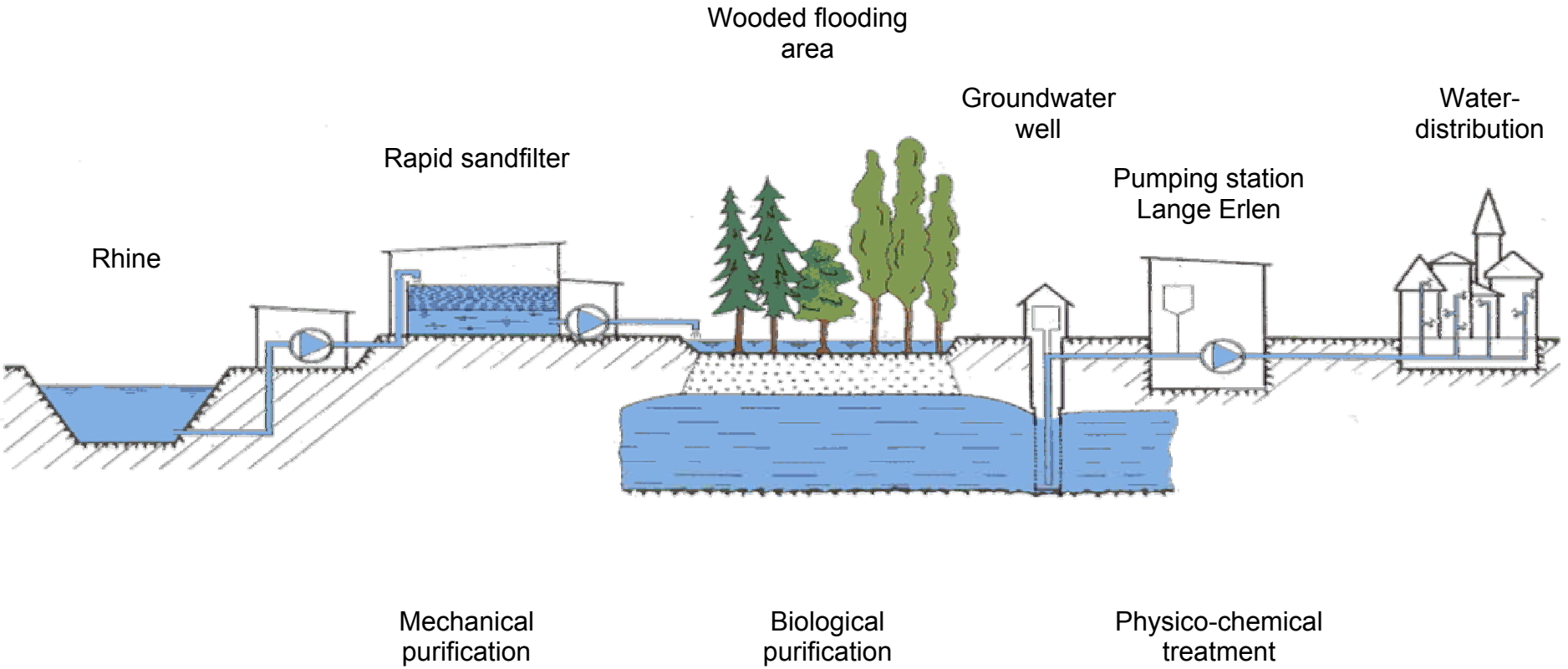

Fig. 3: The Basel System. From: Die Wasserversorgung von Basel-Stadt; IWB 2001

Flooding cycles at the recharge areas were experimentally developed in the last few decades; a balance had to be found between groundwater productivity and the ecology of that area. Too long flooding periods turned out to be ecologically harmful for the forest stands, to increase the abundance of mosquitoes and also to promote clogging of the infiltrating soil surface. Since 1974, flooding cycles usually consist of 10 days flooding and 20 days drying and regeneration representing an agreeable compromise between groundwater productivity and ecology. However, changes in these cycles occur due to maintenance work, less water demand or Rhine contaminations. Anyhow, after 10-30 days, purified water is pumped out of groundwater wells at the “Lange Erlen”, collected in the pumping station and delivered to consumers.

Generally, water purification through artificial groundwater recharge is widespread but more commonly involve unvegetated slow sand filters, dunes or channels (i.e. the “Hard Forest”) and are combined with long flooding periods (Peters et al. 1998, Duncan 1988, Weber-Shirk & Dick 1997). Slow sand filters rely on biological treatment processes for their action rather than on physical filtration. Filtration and purification depends on the development of a thin biological layer on the surface of the sand filter. However, with increasing thickness of this biofilm, infiltration capacities decrease gradually (clogging) and periodically the biofilm has to be removed and the topsoil replaced. Clogging of the infiltrating surface and the resulting reductions in infiltration rates are one major problem of artificial recharge systems (Bouwer 2002, Baveye et al. 1998). In the “Hard Forest” approximately every 10 years, the top soil of the channels has to be dredged and replaced by a new sand and gravel layer (R. Ziegler, Hardwasser AG, pers. comm.).

In the “Lange Erlen”, due to several cross-linked processes, no such biofilm is generated and therefore stripping off and renewing the soil surface is not necessary. However, because of this missing complex biological layer, purification processes can’t be limited to the top soil layer and Rüetschi (2004) concluded that deeper soil layers must be predominantly responsible for water purification. Remarkably, infiltration and purification capacities have remained constant and satisfactory since the system was first established in 1912.

Preliminary studies and target organisms

A large amount of work was carried out by Daniel Rüetschi (2004) within his PhD studies (Departement of Geography, University of Basel) and this provided the basis for further investigations in the “Lange Erlen”. Evidence for a highly linked network within the purification and infiltration system was given with different biological, physical and chemical parameters playing major roles. However, the relative importance of each parameter could not be fully addressed and some fundamental questions remained unanswered. For the purification system, the relative parts of biodegradation, adsorption and dilution with natural groundwater are being currently analyzed by Florian Storck within his PhD studies (Environmental Geosciences, University of Basel). As stated by Rüetschi (2004) the biodegradation part plays a major role in the purification system at the “Lange Erlen” and therefore, further microbial analyses are one part of this PhD thesis. Preliminary microbial studies were conducted in spring 2005 to investigate microbial biomass and activity in the “Lange Erlen”. However, since biodegradation is not only restricted to microorganisms, the investigation was extended to cover soil structure and complex organic matter decomposition promoted by the inhabiting soil fauna. Because soil fauna comprises a large complex range of different organisms including many genera and species,

preliminary studies were also conducted in spring 2005 to identify keystone species for further investigations.

Soil fauna

Soil fauna includes all animals that spend at least part of their life in the soil. They are a very diverse group, ranging from moderately large animals that excavate underground burrows to microscopic species. This includes mites (Acari), springtails (Collembola), nematodes, and microorganisms that reside in the films of water coating soil particles. The soil fauna plays an important role in decomposing and mineralizing complex organic materials, moving nutrients through soil layers and improving soil structure (Begon et al. 1998). They are divided into three groups depending on their size: (1) Macrofauna (0.5-20 cm), such as larger insects, earthworms, mice and moles; (2) Mesofauna (0.2-5 mm), such as mites and springtails and (3) Microfauna (0.002-2 mm), such as protozoa, algae, bacteria and fungi.

First studies conducted in the “Lange Erlen” showed the presence of mites (Acari), springtails (Collembola) and earthworms (Lumbricidae) in two watered sites (“Grendelgasse rechts field 1”, GGR1; “Grendelgasse rechts field 2”, GGR2) and on non-watered site (“Gemeindematten”, GM). For both mesofauna groups, mites and springtails, higher densities were found in the non-watered site (Fig. 4a), whereas earthworms reached considerably higher densities in the watered sites (Fig. 4b). The densities for springtails and mites ranged between 5000-8000 ind. m^{-2} and 10000-12000 ind. m^{-2}, respectively (Fig. 4a), and thus resembled more the usual densities of grasslands or agricultural areas than found in forests. Contrastingly, earthworms reached densities from 500-700 ind. m^{-2} in the watered sites (Fig. 4b), thereby reaching a higher density there by several orders of magnitudes. Similar to

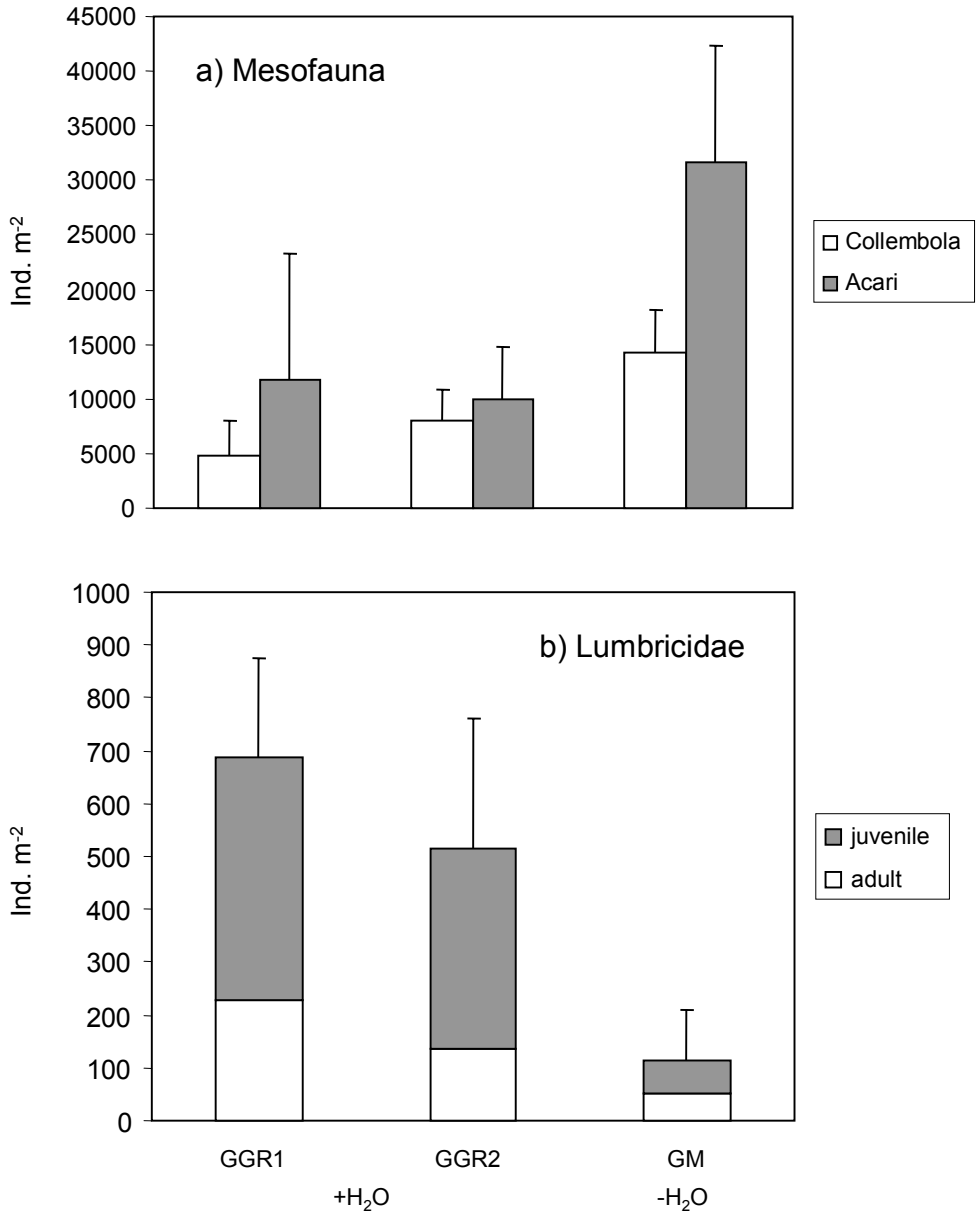

Fig. 4: Soil fauna in individuals m^{-2} ± s.d. at the watered sites GGR1 and GGR2, and the non-watered site GM. a) Mesofauna b) Lumbricidae

mites and springtails, these high earthworm densities are more applicable to grassland and agricultural areas than to forest sites, where usually earthworm densities are significantly lower. These results led to the assumption that the recharge areas in the “Lange Erlen” provide less optimal habitats for mites and springtails and in turn, even more optimal habitats for earthworms. This supports non-systematic studies on earthworms conducted by Rüetschi (2004) and Michèle

Glasstetter in the same area and consequently, suggests earthworms to be a crucial part of the soil fauna community at the recharge areas.

Earthworms

Earthworms occur in soils across the world, preferring moist habitats of moderate temperature (Lee 1985, Edwards & Bohlen 1996). The majority of earthworms in Europe belong to the taxon Lumbricidae (Annelida, Oligochaeta). In France about 180 species have been described (Bouché 1972), whereas in Switzerland and in Germany only 44 and 39 earthworm species are found, respectively (www.faunaeur.org). Earthworms are saprophagous animals and prefer feeding on dead and decaying plant residues, which vary greatly in their physical and chemical composition. Because of the limited ability of earthworms to move, they need to live close to food resources (Lee 1985). Earthworm populations are often food limited and populations increase following organic amendments (Edwards & Bohlen 1996, Scheu & Schaefer 1998). Soil characteristics are profoundly affected by earthworms: as ecosystem engineers (Lavelle et al. 1997) their burrowing activities (bioturbation), particularly that of larger deep-borrowing species (anecic) and of mineral forms (endogeic; Bouché 1977) that contribute to aeration and drainage of soils. Their ecological plasticity in disturbed environments is high and they are common even in periodically flooded sites (Klok et al. 2006). Several authors reported the number of soil macropores and soil water infiltration to correlate with earthworm numbers (Ehlers 1975, Edwards & Lofty 1982, Lee & Foster 1991). Additionally, by providing favorable habitats, earthworms indirectly affect the abundance of soil microorganisms. The walls of the burrows of primarily anecic earthworm species are enriched in nutrients due to the lining with plant debris, earthworm casts and mucus.

In comparison to the surrounding soil the abundance of soil microorganisms was shown to be increased in the burrow system of earthworms (Tiunov & Scheu 1999).

Microorganisms

The soil represents a favorable habitat for microorganisms and is inhabited by a wide range of bacteria, fungi, algae and protozoa. They are found in large numbers in soil - usually between one and ten million microorganisms are present per gram of soil - with bacteria and fungi being the most prevalent (Lavelle & Spain 2005). However, the availability of nutrients is often limiting for microbial growth in soil and most soil microorganisms may not be physiologically active in the soil at a given time. Soil microorganisms are very important since almost every chemical transformation taking place in soil involves active contributions from soil microorganisms. In particular, they play an active role in nutrient cycling, like carbon and nitrogen, which are required for plant growth. They are responsible for biodegradation of organic compounds entering the soil (e.g. plant litter) and therefore, in the recycling of nutrients in soil. Surface and subsurface microorganisms promote contaminant degradation and the maintenance of groundwater quality (Konopka & Turko 1991). Therefore microorganisms are of considerable importance acting as pollutant biodegradation processors in the vadose zone, the unsaturated zone extending from the soil surface to the groundwater table (Holden & Fierer 2005).

First soil studies of the microbial biomass and the microbial activity (down to 60 cm depth at the corresponding soil fauna sampling sites) revealed decreasing microbial biomass (C_{mic}) and respiration rates (O_2) at all sites (Fig. 5). Most of the microbial biomass and highest respiration rates were found from the top soil down to 20 cm depth. In this shallow soil layer, C_{mic} and O_2 of both watered sites exceeded the non-watered site whereas in deeper soil layers, differences were not substantial. These

missing microbial differences in deeper soil layers were intriguing because a higher microbial biomass and activity throughout the whole soil profile was expected at the watered sites due to higher substrate and nutrient input via flooding. In addition, Rüetschi (2004) presumed that the elimination of organic compounds at the recharge areas is primarily a function of the microbial biomass and mainly located in deeper soil layers. Therefore, getting more information on microbial parameters in deeper soil layers was one essential part of this thesis.

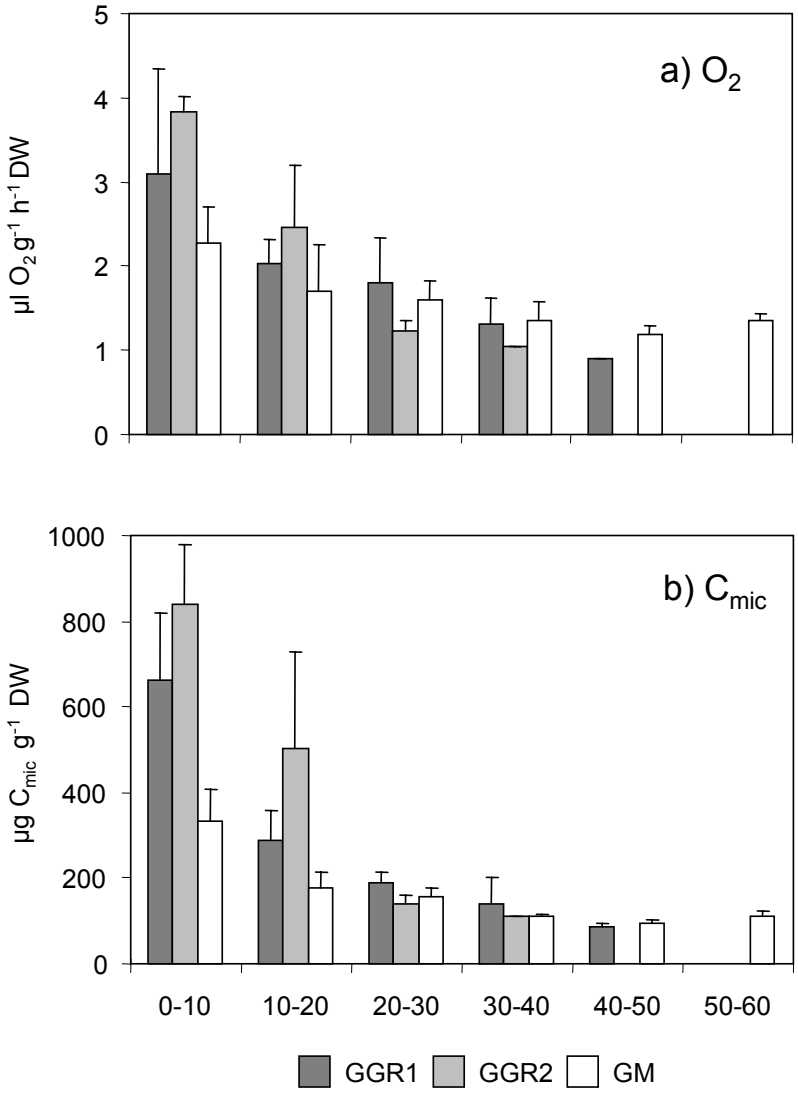

Fig. 5: Microbial community status, expressed as a) soil respiration (O_2 in µl g^{-1} h^{-1} DW ± s.d.) and b) microbial biomass (C_{mic} in µg g^{-1} DW ± s.d.), in 6 soil depths (0-10, 10-20, 20-30, 30-40, 40-50, and 50-60 cm) at the watered sites GGR1 and GGR2, and the non-watered site GM.

Objectives

The goal of this thesis was to elucidate the role of the soil fauna, i.e. earthworms, and the role of microorganisms in influencing infiltration and purification capacities at the artificial groundwater recharge areas in the “Lange Erlen”. Furthermore, the effects of flooding on earthworms and microorganisms were investigated.

Because earthworms are considered to be highly relevant for the sustainability of the “Lange Erlen” system by preventing clogging, regenerating soil structures, aerating the shallow soil layer and ensuring constant infiltration rates this keystone genus was included in this thesis (Chapter 1). Periodic flooding was expected to support earthworm performance and in turn, infiltration rates at the recharge areas were expected to be positively influenced by the presence of mainly deep soil dwelling (anecic) earthworm species.

Microorganisms were considered to be mainly responsible for biodegradation and purification processes within the drinking water production at the “Lange Erlen”. Therefore, they were analyzed for their biomass (Chapter 1, Chapter 2, and Chapter 3), their community structure (Chapter 2), their physiological conditions (Chapter 2), their functional diversity (Chapter 3) and their activity (Chapter 3). Because Rüetschi (2004) stated that purification processes are located in deeper soil layers, vertical soil profiles to approximately 4 m depth were analyzed for these microbial parameters.

The water recharge sites are expected to have a different structural composition of the microbial community and to consist of functional groups adapted to the specific resource and environmental conditions resembling “purification zones” within their vertical soil profile. Similarly, the related functional activities, resulting from that different microbial structure is expected to change with soil depth and flooding. Purification capacities of the “Lange Erlen” are expected to be highly dependent on

an active microbial community and the sum of all microbial parameters analyzed within this thesis aims to provide insights into previous and actual nutrient and contaminant conditions of the recharge areas at the “Lange Erlen”.

To conclude, this thesis wants to contribute to the sustainable drinking water production system performed by the Industrial Works of Basel (IWB) by presenting more information on soil fauna and microbiology of the recharge areas in the “Lange Erlen”. Because it’s a fascinating and unique system, investigations on physical, chemical and biological soil and water parameters are necessary. Given a better understanding, the system might also be more widely adopted and used to guarantee sufficient and reliable drinking water supply to the city of Basel, and perhaps elsewhere.

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Chapter one

Structure and functioning of earthworm communities in woodland flooding systems used for drinking water production

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Abstract

Earthworms are known to influence water infiltration in soils, but most of the existing knowledge relates to grasslands and arable systems; little is known on the role of earthworms for water infiltration in forests. We studied earthworm populations and water infiltration rates in woodland flooding sites used for groundwater recharge and the production of drinking water. Intensive flooding may detrimentally affect earthworm populations and simultaneously result in clogging of the topsoil, with the latter being a common problem in groundwater recharge systems. However, clogging does not occur at our study site, the "Lange Erlen" (Basel, Switzerland) and total earthworm numbers and biomass in flooded sites exceeded those of non-flooded sites (+51% and +71%, respectively). Total earthworm numbers ($r = 0.85^{***}$), numbers of endogeic ($r = 0.64^{*}$) and epigeic ($r = 0.81^{**}$) earthworms and numbers of two species (*Lumbricus rubellus,* $r = 0.62^{*}$ and *Allolobophora chlorotica,* $r = 0.77^{**}$) significantly correlated with water infiltration rates. The results suggest that short-term flooding (max. 10 days) interrupted by longer recovery periods favor earthworm populations which likely contribute to the long-term (ca. 100 years) sustainability of the studied forest groundwater recharge system and thereby to effective and cost efficient drinking water production.

Keywords: Infiltration, Macropores, Soil invertebrates, Ecosystem engineers, Floodplain forest, Clogging, Groundwater recharge

Introduction

Earthworms occur in soils almost across the whole world, preferring moist habitats of moderate temperature (Lee 1985, Edwards & Bohlen 1996). Their ecological plasticity in disturbed environments is high and they are common even in periodically flooded sites (Ausden et al. 2001, Klok et al. 2006). Although flooding may detrimentally affect earthworm populations (Plum & Filser 2005, Ivask et al. 2007), Zorn et al. (2005) reported higher numbers and biomass of earthworms in floodplain grasslands, though directly after flooding, earthworm numbers decreased. Some species, such as the endogeic *Aporrectodea caliginosa* and *Allolobophora chlorotica*, were little affected by flooding whereas the endo-epigeic *Lumbricus rubellus* suffered from flooding but recovered quickly provided that the flooding period did not last long.

Soil characteristics are profoundly affected by earthworms: as ecosystem engineers (Lavelle et al. 1997) their burrowing activities (bioturbation), particularly that of anecic and endogeic earthworms (Bouché 1977), contribute to aeration and drainage of soils. Several authors reported the number of soil macropores and soil water infiltration to correlate with earthworm numbers (Ehlers 1975, Edwards & Lofty 1982, Lee & Foster 1991). Although infiltration via earthworm macropores is well known, most studies exclusively considered anecic earthworms, such as *Lumbricus terrestris*, and investigated open habitats, such as meadows and arable systems (Bouma et al. 1982, Edwards et al. 1992, Willoughby et al. 1997, Shipitalo & Butt 1999). For example Shipitalo et al. (2004) investigated the interactions of earthworm burrows and subsurface drainage patterns on a sandy clay field and found that infiltration rates were positively correlated to numbers and biomass of *L. terrestris.* In forests the contribution of soil invertebrates, particularly that of earthworms, to water

infiltration has not been well studied; no global figure exists for the amount of water infiltrated into soils due to the action of soil invertebrates (Lavelle et al. 2006).

In the “Lange Erlen” (Basel, Switzerland) drinking water production by artificial groundwater recharge in woodland flooding sites has been applied since 1912. This system is unique throughout the world. Embanked forest sites of one to two hectare are periodically flooded with water from the river Rhine to augment groundwater resources, thereby transforming river water into purified drinking water. Water purification through artificial groundwater recharge is widespread but common procedures usually involve unvegetated slow sand filters, dunes or channels and long flooding periods (Peters et al. 1998). In these systems a biofilm develops at the soil surface during flooding which is partly responsible for water purification. With increasing thickness of the biofilm, infiltration capacities decrease gradually (clogging) and every 1-6 months the biofilm has to be removed and the topsoil replaced. Clogging of the infiltrating surface and resulting reductions in infiltration rates are one major problem of artificial recharge systems (Bouwer 2002, Baveye et al. 1998). In the “Lange Erlen” no biofilm is generated and therefore stripping off the soil surface is not necessary. Remarkably, infiltration capacities have remained constant and satisfactory since the system has been established.

Earthworms are considered to be highly relevant for the sustainability of this system (Rüetschi 2004). Therefore, we investigated the effect of periodic flooding of the “Langen Erlen” on earthworm populations and related numbers of total earthworms, earthworm functional groups and individual earthworm species to water infiltration rates. Further, for characterizing flooding and control sites, important soil characteristics including concentrations of carbon, nitrogen, dissolved organic carbon, nitrate and microbial biomass were investigated.

Materials and Methods

Study site

The “Lange Erlen” (Fig. 1) are situated in the northwestern part of Switzerland northeast of Basel. As a former natural floodplain area the “Lange Erlen” extend along the River Wiese, a straightened tributary of the River Rhine descending from the Black Forest, Germany. Since 1912 parts of the “Lange Erlen” serve as groundwater recharge areas; today approximately half of the drinking water for the city of Basel is obtained by artificial groundwater recharge there (15×10^6 m^3 ha^{-1} y^{-1}). Before the River Wiese was canalized at the end of the 19^{th} century natural floodings occurred in the whole valley floor and alluvial soil covered the entire area (Rüetschi 2004). To date, the upper soil of the “Langen Erlen” is characterized as fluvi-eutric cambisol and the aquifer consists of 80% Rhine gravel (lower part, mostly limestone) and 20% Wiese gravel (upper part, mostly silicates and limestone, Regli et al. 2003).

Infiltration system

Rhine water, prefiltered by rapid sandfilters (80 cm quartz sand layer), is periodically seeped into 11 wooded flooding areas (total area 22 ha). Each flooding area (10,000 – 20,000 m^2) is divided into three fields (3000 – 8500 m^2) by small dams (height ca. 50 cm). Watering cycles normally consist of 10 days flooding and 20 days drying and regeneration, however, longer interruptions due to revisions occur. The water fills up to variable heights depending on soil surface structure (max. 50 cm) and seeps with a speed of 1-2 m d^{-1} through a humus and fluvial silt layer of 30-90 cm and a sand / gravel layer of 2-3 m before it reaches the groundwater table of 3-4 m depth. During flooding the groundwater table rises to approximately -2 m below floor. Subsequently, the water flows horizontally (from northeast to southwest) in the aquifer for 200-

800 m. After 10-30 days purified water is pumped out of groundwater wells, collected in the pumping station and, after a brief chemical treatment with ClO_2, delivered to consumers.

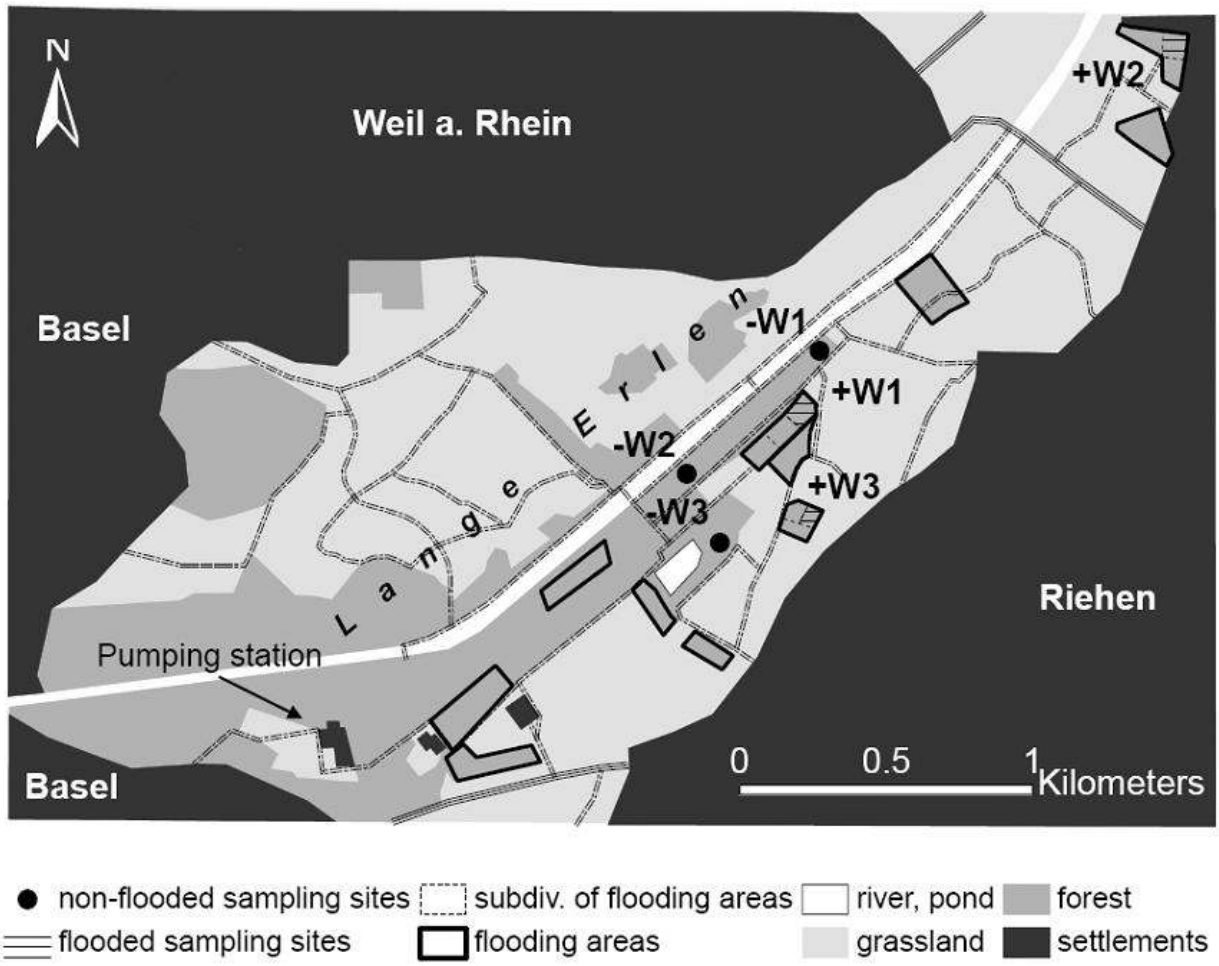

Fig. 1: The study site “Lange Erlen”, Basel, Switzerland. Flooded sites: Grendelgasse rechts (+W1), Hintere Stellimatten (+W2) and Verbindungsweg (+W3). Non-flooded sampling sites: Gemeindematten nord (-W1), Gemeindematten süd (-W2) and Brunnen 10 (-W3).

Sampling sites

Earthworm and soil sampling was conducted in three locations of non-watered and three locations of flooded sites in the “Lange Erlen”, measurement of infiltration rates took place only in flooded sites (Fig. 1). The non-flooded sites included “Gemeindematten nord” (-W1), “Gemeindematten süd” (-W2) and “Brunnen 10”

(-W3). The flooded sites included “Grendelgasse rechts” (+W1), “Hintere Stellimatten” (+W2) and “Verbindungsweg” (+W3). Non-flooded sampling sites were situated in the centre of the “Langen Erlen”, distances to the flooded sites varied between 230 m and 1700 m (Fig. 1). Non-flooded sampling sites were afforested between 1882 and 1948 as indicated by comparing the Siegfriedskarte of 1882 and the map of Riehen and Bettingen of 1948. Soils of these sites consist of alluvial materials and are rather similar as are the forests (oak - hornbeam forests; Burnand & Hasspacher 1999), whereas the flooding sites differed in various respects:

“Grendelgasse rechts” (+W1) is the oldest flooding area (1912) and is located 1900 m away from the pumping station. The site was afforested in the same time period as the non-flooded sites and on the basis of soil profiles, artificial landfill and bulldozing can be excluded (Rüetschi 2004). The soil resembles that of the non-flooded sites. The site covers 8440 m^2 of which usually 80% are submerged during flooding. The tree layer consists of *Populus canadensis, Fraxinus excelsior, Alnus nigra, Acer platanoides / pseudoplatanus, Salix* spp. and *Carpinus betulus*. The shrub layer consists of *Salix* spp., *Cornus sanguinea, Sambucus nigra, Euonymus europaea* and *Ribes rubrum*. The herb layer consists of *Rubus caesius / fruticosus, Urtica dioica, Duchesnea indica, Hedera helix, Iris preudacorus, Alliaria petiolata, Geum urbanum*, *Ranunculus ficaria* and others.

“Hintere Stellimatten” (+W2) is the youngest flooding area and is located at the border to Germany, 3700 m away from the pumping station. It began operating after afforestation in 1977; because of too high infiltration rates (11 $m^3\ m^{-2}\ d^{-1}$) artificial landfill (loess loam) and bulldozing followed in 1981. The site covers 4280 m^2 and usually the whole site is submerged during flooding. Hence, the site is mainly overgrown with an alder swamp forest (*P. canadensis, A. nigra, Salix* spp.). The herb

layer is dominated by *R. caesius* and unvegetated sites with some spots of *Poa trivialis, Potentilla reptans, Agropyron repens* and *G. urbanum*.

"Verbindungsweg" (+W3) was established after afforestation and artificial landfill (loess loam) in 1970 and is located 1800 m away from the pumping station. The site covers 3950 m^2 of which usually 70% are submerged during flooding. Currently, the forest consists of some old poplars, ash-leaved maple (*Acer negundo*) and oak trees. *Acer negundo* occurred only in this flooding site which most likely originates from the introduced soil which was used for landfill. The understory is dominated by *Lysimachia nummularia, U. dioica, Phalaris arundinacea, R. caesus / fruticosus, D. indica, I. preudacorus, Senecio aquaticus* and *Cardamine pratensis.* In the shady northern part of the site which is dominated by poplars the soil is bare of vegetation.

Infiltration

Infiltration rates were measured in the flooding sites by using the Bouwer Cylinder Infiltrometer (Bouwer 1986) with a diameter of 30 cm. This method has advantages for studying flooded sites: it is independent of water depth (double-ring infiltrometers would be drowned) and quite manageable in pathless forests. Measurement points inside the flooding sites were fixed using a grid of 20 x 20 m. Due to the different size of the sites and the degree of flooding the number of measurement points differed between sites: 13 in +W1, 11 in +W2 and 5 in +W3. Measurements were conducted during a 10 day watering period between January and March after three days of saturation. One run of measurements lasted 30-40 min; infiltration rates were calculated as described in Bouwer (1986). Upon completion of the watering period soil cores (100 cm^3) were taken at each measurement point, dried (105°C, 24 h) and weighed to determine bulk density.

Earthworms

Because of strict regulations for the “Langen Erlen” adding chemicals and large scale digging is prohibited. Therefore, the octet – method (Thielemann 1986) was applied for earthworm sampling in both flooded and non-flooded sites. The octet-method is based on electrical pulses and does not affect physical and chemical soil parameters. Earthworms were sampled in quadruplicate for each site (diameter 40 cm, depth 40-50 cm, area 0.125 m^2) early in the year within one week in March / April in between flooding periods. To ensure comparable moisture contents, the non-flooded sites were sampled two days after precipitation (24 mm; daily mean of 10 min resolution, Meteodata Station Lange Erlen, University of Basel) and the flooded sites at least four days after flooding. Earthworm sampling points in the non-flooded sites were separated at least 20 m away from paths and spaced by at least 20 m. In the flooding sites they were situated close (± 2 m) to four central water infiltration measurement points and thus separated from dams and direct water intake. Determination of earthworm species is based on Sims & Gerard (1999) and Bouché (1972) and determination of earthworm biomass (FW) was conducted on living individuals.

Soil analyses

Additional soil core samples (diameter 5 cm, depth 10 cm) were taken in September at all earthworm sampling sites two and five days after flooding in the fields +W1, +W2 and +W3. Soil samples were analysed for the concentrations of carbon (C), nitrogen (N), dissolved organic carbon (DOC), nitrate (NO_3^-), microbial biomass (C_{mic}) and the C/N-ratios. Three replicates from each site were taken, visible organic material (fine roots and leaves) and stones were removed and the soil was sieved (4 mm) and stored at -20°C until analysis. Prior to analyses soil samples were thawed in the fridge (24 h, 8°C). Carbon and nitrogen content was determined from

oven-dried (105°C, 24 h) and pulverized (swing mill, Retsch MM 200) aliquots of the bulk soil with an element analyzer (CHN 1000, Leco). DOC and nitrate was extracted from 5 g bulk soil (FW). A salt solution (0.01 M CaCl) was prepared (1:4 w/v), shaken for 1 h (200 rpm) and centrifuged for 15 min (2000 rpm). The supernatant was filtered through sterile 0.45 µm membrane filters (Millex HA) and stored in the fridge until analysis (max. 1 h). DOC was measured after acidification and air-purging (N55, O45, Carbagas, CH) with a TOC analyzer (TOC-5000 A, Shimadzu) in quintuplicate. Nitrate was measured in an ion chromatograph (IC-690, Metrohm). Microbial biomass (C_{mic}) was determined after 3 d of soil incubation at room temperature (18°C) by substrate-induced respiration (SIR) using an automated electrolytic O_2 microcompensation apparatus (Scheu 1992). For SIR fresh soil equivalent to 2 g DW was supplemented with 8 mg glucose g^{-1}. Glucose was added as an aqueous solution adjusting the water content to 80% of the water holding capacity. Respiration rates were measured at 22°C with readings every 30 min. The mean of the four lowest measurements was taken as the maximum initial respiratory response (MIRR) and microbial biomass C (µg C_{mic} g^{-1} DW) was calculated as 38 × MIRR (µl O_2 g^{-1} h^{-1}) (Beck et al. 1997).

Data analyses

Earthworm numbers and biomass were analysed in total and separated into ecological groups, species and developmental stage (juvenile, adult). Ecological groups were classified as follows: anecic earthworms (*L. terrestris, Aporrectodea longa* and *Dendrobaena platyura*), endogeic earthworms (*Al. chlorotica, Allolobophora georgii, Allolobophora rosea, Ap. caliginosa, Octolasion cyaneum* and *Octolasion tyrtaeum*) and epigeic earthworms (*Dendrobaena octaedra, Eiseniella tetraedra, Lumbricus castaneus, L. rubellus* and anecic juveniles). Earthworms were

considered adult when they were clitellate. Prior to statistical analyses data on earthworm numbers and biomass, water infiltration, bulk soil density, concentrations of soil carbon, nitrogen, DOC, nitrate, C_{mic} and C/N-ratios were checked for homogeneity of variance and log-transformed if necessary.

Numbers and biomass of total and of ecological groups of earthworms of flooded ($+H_2O$) and non-flooded ($-H_2O$) sites were analysed without transformation by a nested analysis of variance (nested ANOVA). Three fields per treatment with four replicates for earthworms and three replicates for soil characteristics were included and fields were nested in flooded and non-flooded sites. To test for differences within flooded sites we used ANOVA. Differences between means were inspected using Tukey's honestly significant difference test. Individual biomass of adult earthworm species which were sampled at least four times in each treatment were analysed with a Student's t-test. Infiltration and earthworm data in flooded sites (three sites with four replicates each) were analysed by using Pearson's correlation coefficient. All analyses were calculated with SAS 9.1. (SAS Insitute, Cary).

For visualizing differences in species composition and community structure in flooded and non-flooded sites we used ordinations. Earthworm data were log-transformed and analysed by detrended canonical correspondence analysis to determine the length of gradient. Since the gradient was rather short (3.2) data were analysed by Principal Components Analysis (Ter Braak 1995). Ordinations were calculated and visualized using CANOCO 4.5 (Ter Braak & Smilauer 2002).

Results

Infiltration rates and soil characteristics

Infiltration rates were 1960 ± 2885, 753 ± 603 and 855 ± 572 l d^{-1} m^{-2}, for +W1, +W2 and +W3, respectively. Due to high variation within sites the differences were not significant, however, data for longer periods of time and observations by staff suggest that infiltrations rates at +W1 indeed exceed those at +W2 and+W3. Bulk density differed significantly between the three flooded sites with +W2 exceeding +W1 and +W3 by 22.6% and 25.3%, respectively ($F_{2,25}$ = 9.25, P = 0.001).

Table 1: Soil characteristics of flooded (+H_2O) and non-flooded sites (-H_2O) two and five days after flooding (flooded sites). Values in parentheses represent standard errors. Means within a row without letter are not significantly different (Tukey`s HSD, P < 0.05)

		+H_2O		-H_2O	
2 days after flooding	C%	3.47 (0.86)		3.17 (0.57)	
	N%	0.31 (0.08)		0.30 (0.06)	
	C/N	11.8 (4.00)		10.6 (0.99)	
	DOC [mg g^{-1} DW]	2.09 (1.00)	**b**	6.21 (2.69)	**a**
	NO_3^- [mg g^{-1} DW]	2.02 (0.74)	**a**	1.16 (0.67)	**b**
	C_{mic} [µg g^{-1} DW]	763 (158)	**a**	618 (143)	**b**
5 days after flooding	C%	3.68 (0.82)		3.51 (0.44)	
	N%	0.30 (0.10)		0.32 (0.12)	
	C/N	12.8 (3.86)		11.7 (2.42)	
	DOC [mg g^{-1} DW]	2.22 (1.23)	**b**	4.22 (1.87)	**a**
	NO_3^- [mg g^{-1} DW]	2.44 (0.85)	**a**	1.31 (0.54)	**b**
	C_{mic} [µg g^{-1} DW]	815 (143)	**a**	609 (116)	**b**

Carbon and nitrogen concentrations and the C/N-ratios did not differ between flooded and non-flooded sites at any sampling date (Table 1). Concentrations of DOC were reduced two and five days after flooding by 66% ($F_{1,12}$ = 38.54, p < 0.001) and 47% ($F_{1,12}$ = 12.99, p < 0.01), respectively. Microbial biomass and nitrate concentrations in flooded sites exceeded that in non-flooded sites by 19% ($F_{1,12}$ = 5.62, p < 0.05) and

43% ($F_{1,12} = 10.27$, $p < 0.01$) at the first sampling date and by 25% ($F_{1,12} = 11.74$, $p < 0.01$) and 46% ($F_{1,12} = 27.15$, $p < 0.001$) at the second sampling date, respectively (Table 1).

Among the flooded sites soil nitrogen ($F_{2,6} = 14.13$, $p < 0.01$), DOC ($F_{2,6} = 4.9$, $p = 0.055$) and nitrate concentrations ($F_{2,6} = 6.54$, $p < 0.05$) measured two days after flooding differed significantly with values in +W3 being highest and in +W2 being lowest (Table 2). Similarly, five days after flooding nitrogen concentrations ($F_{2,6} = 14.13$, $p < 0.01$) and nitrate concentrations ($F_{2,6} = 5.95$, $p < 0.01$) differed significantly among the flooded sites with values being lowest at +W2. Carbon concentrations, microbial biomass and C/N-ratios did not differ significantly among flooded sites at any sampling date.

Table 2: Soil characteristics of the flooded sites (+W1, +W2, +W3) two and five days after synchronized flooding. Values in parentheses represent standard errors. Means within a row marked with the same letter or without letter are not significantly different (Tukey`s HSD, P < 0.05)

		+W1		+W2		+W3	
2 days after flooding	C%	3.77 (0.50)		2.80 (1.17)		3.84 (0.59)	
	N%	0.31 (0.04)	**a**	0.22 (0.03)	**b**	0.39 (0.03)	**a**
	C/N	12.2 (0.17)		13.3 (7.36)		9.80 (0.62)	
	DOC [mg g^{-1} DW]	1.83 (0.73)	**ab**	1.30 (0.84)	**b**	3.13 (0.31)	**a**
	NO_3^- [mg g^{-1} DW]	1.87 (0.54)	**ab**	1.40 (0.28)	**b**	2.79 (0.55)	**a**
	C_{mic} [µg g^{-1} DW]	834 (95.3)		649 (163)		807 (183)	
5 days after flooding	C%	4.14 (0.40)		2.95 (1.11)		3.96 (0.27)	
	N%	0.35 (0.07)	**a**	0.19 (0.02)	**b**	0.38 (0.06)	**a**
	C/N	12.0 (1.64)		15.8 (5.72)		10.6 (1.64)	
	DOC [mg g^{-1} DW]	1.78 (0.60)		2.27 (2.20)		2.60 (0.62)	
	NO_3^- [mg g^{-1} DW]	1.97 (0.77)	**b**	1.90 (0.26)	**b**	3.43 (0.18)	**a**
	C_{mic} [µg g^{-1} DW]	859 (60.7)		903 (137)		683 (138)	

Earthworms

Density and biomass of earthworms in flooded sites were significantly enhanced from 165 to 340 ind. m^{-2} and from 56 to 77 g m^{-2} FW, respectively (Fig. 2, Table 3). Excluding juveniles did not change this pattern (Table 3). Among ecological groups of earthworms the density was increased in endogeic (from 57 to 160 ind. m^{-2}) and anecic species (from 25 to 65 ind. m^{-2}), whereas biomass was increased in epigeic (from 11 to 29 g m^{-2} FW) and anecic earthworms (from 24 to 98 g m^{-2} FW; Fig. 2, Table 3).

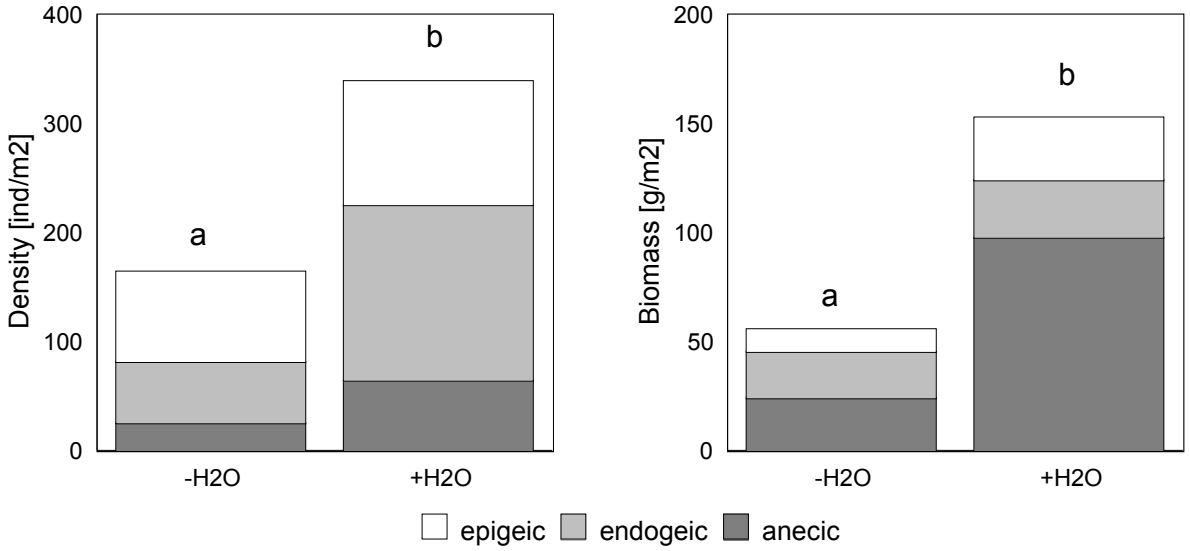

Fig. 2: Effect of flooding on density and biomass of total, epigeic, endogeic and anecic earthworms in flooded (+H_2O) and non-flooded (-H_2O) sites. Bars sharing the same letter are not significantly different (Tukey`s honestly significant difference test, $P < 0.05$).

Table 3: Nested ANOVA table of F-values and degrees of freedom (df) on the effect of flooding (Treat) and field (Field nested in Treat) on density and biomass of earthworms (total, adult, epigeic, endogeic, anecic).

		Density					Biomass				
	df	Total	Adult	Epigeic	Endogeic	Anecic	Total	Adult	Epigeic	Endogeic	Anecic
Treat	1	25.1***	44.5***	1.3	49.2***	21.0***	29.0***	19.8***	9.6**	0.7	26.1***
Field (Treat)	4	11.2***	4.38*	5.1**	25.4***	2.2	2.42(*)	0.74	2.4(*)	7.6***	0.66

***P < 0.001; ** P < 0.01; *P < 0.05; (*), P < 0.1

In addition to the general differences between flooded and non-flooded sites, total earthworm density and ecological group density among the flooded sites varied considerably. With 562 ind. m^{-2} the density of earthworms at +W1 exceeded that of the other flooded sites considerably (224 and 234 ind. m^{-2} for +W2 and +W3, respectively; $F_{2,9} = 27.83$, $P = 0.0001$). Especially endogeic earthworms were responsible for these differences reaching 296 ind. m^{-2} in +W1 but only 124 and 60 ind. m^{-2} at +W2 and +W3, respectively ($F_{2,9} = 44.57$, $P < 0.0001$).

Table 4: Pearson correlation coefficients for the correlation between earthworm groups and species and water infiltration rate and bulk density in periodically flooded sites

	Infiltration rate	Bulk density
Total earthworm number	0.85 ***	-0.42
Anecic	0.55 (*)	-0.45
Endogeic	0.64 *	-0.31
Epigeic	0.81 **	-0.34
Allolobophora chlorotica (Savigny, 1826)	0.77 **	-0.45
Allolobophora georgii Michaelsen, 1890	0.30	-0.48
Allolobophora icterica (Savigny, 1826)	0.39	-0.39
Allolobophora rosea (Savigny, 1826)	-0.35	0.19
Aporrectodea caliginosa (Savigny, 1826)	0.50 (*)	-0.42
Aporrectodea longa (Ude, 1885)	0.28	-0.30
Dendrobaena octaedra (Savigny, 1826)	0.11	-0.23
Dendrobaena platyura (Fitzinger, l833)	-0.01	-0.29
Eiseniella tetraedra (Savigny, 1826)	0.51 (*)	-0.49
Lumbricus castaneus (Savigny, 1826)	0.57 (*)	-0.30
Lumbricus rubellus Hoffmeister, 1843	0.62 *	-0.17
Lumbricus terrestris Linnaeus, 1758	0.38	0.06
Octolasion tyrtaeum (Savigny, 1826)	0.19	0.10

***P < 0.001; ** P < 0.01; *P < 0.05; (*), P < 0.1

Overall, 14 earthworm species were recorded in this study. Thirteen species were found in the flooded sites (Table 4) and 8 species in the non-flooded sites (*Al. chlorotica, Al. rosea, Ap. caliginosa, Ap longa, L. castaneus, L. rubellus,*

L. terrestris, O. cyaneum and *O. tyrtaeum*). Species diversity as measured by the Shannon-Index was at a maximum at +W1 (2.21, 11 species), followed by -W1 (1.81, 8 species), +W3 (1.77, 10 species), +W2 (1.71, 8 species), -W2 (1.27, 5 species) and –W3 (0.99, 6 species).

PCA clearly separated the flooded sites +W1 and +W3 from the non-flooded sites, however, in different directions; +W2 was placed close to the non-flooded sites (Fig. 3). Three species (*Al. chlorotica, Al. georgii, E. tetraedra*) only occurred in the flooded sites +W1 and +W3 but not in +W2. *Lumbricus terrestris* was absent in +W3, whereas *D. platyura* only occurred at this site. The first two axes explained 57% of the variance in the species data with the two axes being of similar importance, representing 30% and 27% of the variation, respectively.

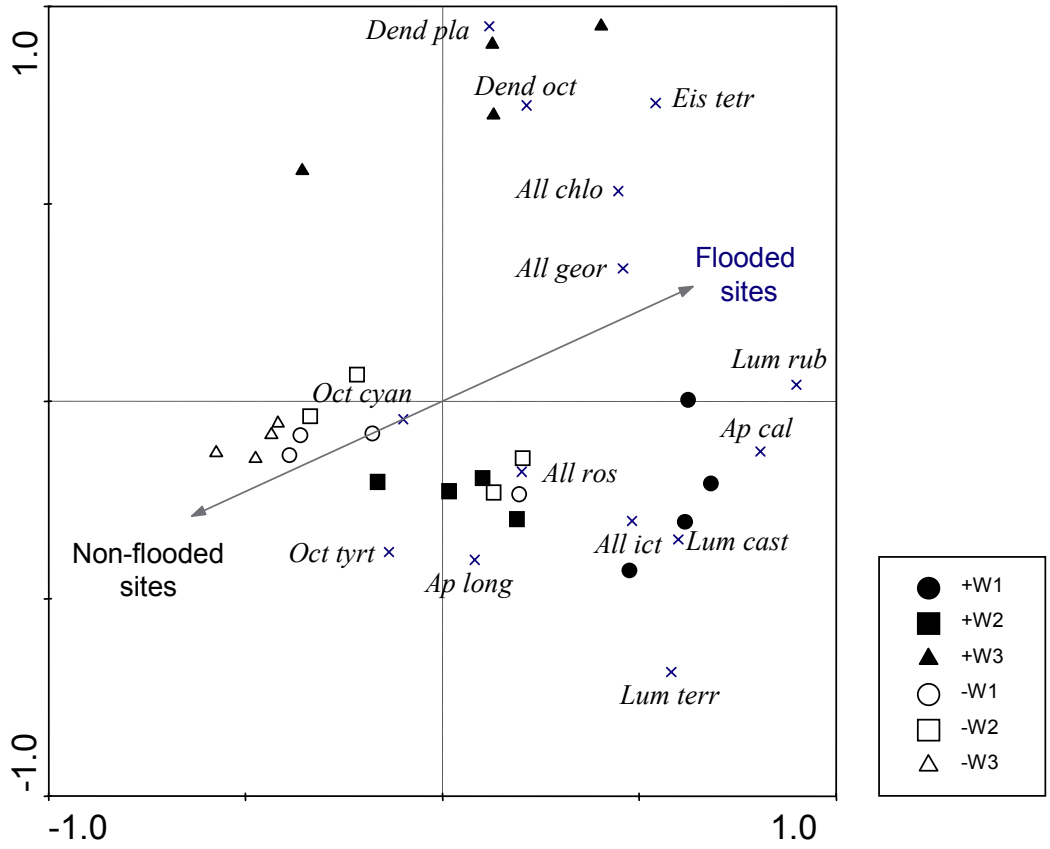

Fig. 3: PCA diagram of earthworm species in three flooded (+W1, +W2, +W3) and three non-flooded (-W1, -W2, -W3) sites (four replicates each). The first axis represents 30% and the second axis 27% of the variation in species data.

Individual biomass of adult earthworms which were sampled at least four times in each treatment was compared between flooded and non-flooded sites Two endogeic earthworm species, *Al. rosea* and *O. tyrtaeum*, reached lower biomass in flooded sites, 49% and 50% of that in non-flooded sites, respectively. In flooded sites only the body mass of the endo-epigeic species *L. rubellus* exceeded that in non-flooded sites by 43% (Table 5).

Table 5: T-test results and mean weights of adult earthworm species which occurred at least four times in flooded (+H_2O) and non-flooded sites (-H_2O).

	+H_2O			-H_2O				
Species	Mean	SD	n	Mean	SD	n	t-value	
Allolobophora rosea	0.18	0.06	18	0.35	0.14	6	$2.84_{unequal}$	*
Lumbricus castaneus	0.20	0.05	16	0.19	0.05	14	-0.18_{equal}	
Lumbricus rubellus	0.73	0.23	32	0.51	0.31	6	-2.07_{equal}	*
Octolasion tyrtaeum	0.54	0.28	8	1.08	0.41	8	3.08_{equal}	**
Aporrectodea caliginosa	0.29	0.33	33	0.97	0.71	4	$1.90_{unequal}$	
Aporrectodea longa	1.74	0.49	9	2.58	1.16	4	$1.39_{unequal}$	

** P < 0.01; *P < 0.05

Infiltration rates and earthworms

Infiltration rates significantly correlated with the density of total earthworms, endogeic and epigeic earthworms, and two earthworm species (*Al. chlorotica* and *L. rubellus*). For these groups and species higher earthworm numbers correlated positively with higher infiltration rates (Table 4, Fig. 4). The density of anecic earthworms and three other species, *Ap. caliginosa, E. tetraedra* and *L. castaneus,* also correlated with infiltration rates, however, not significantly (Table 4). Bulk density was generally not correlated with earthworm density (Table 4).

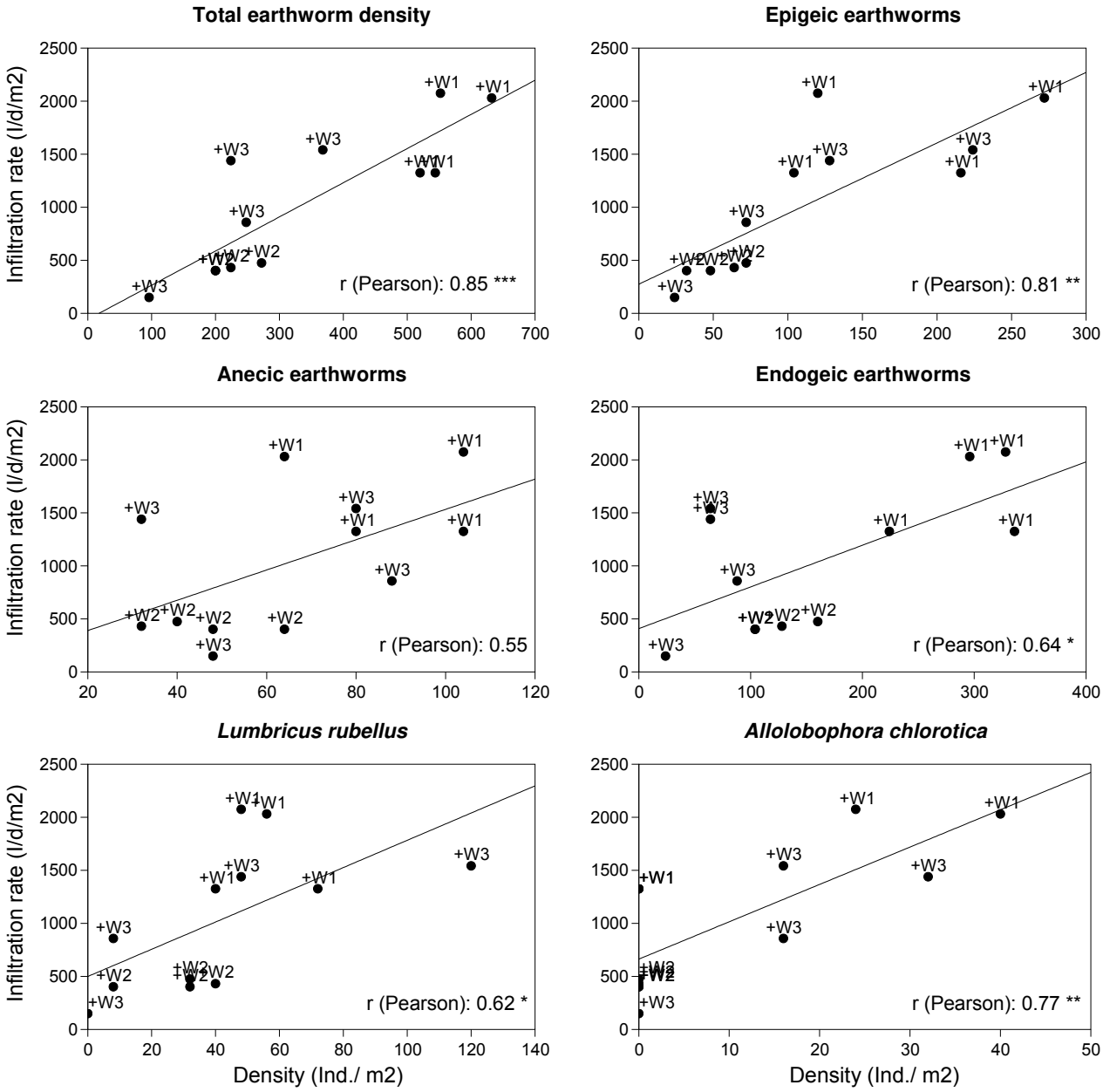

Fig. 4: Scatterplot of infiltration rates and earthworm density (total, epigeic, anecic, endogeic, *L. rubellus, Al. chlorotica*) in three flooded sites (+W1, +W2, +W3) with four replicates each. r, Pearsons correlation coefficient; *, $P < 0.05$; **; $P < 0.01$; ***, $P < 0.001$.

Discussion

Soil characteristics

Before the River Wiese was canalized in the end of the 19th century natural floodings occurred in the whole valley floor and alluvial soil covered the entire area (Rüetschi 2004). Since 1912 parts of the “Langen Erlen” serve as groundwater recharge areas and thus artificial flooding only occurs there. To date, all other parts are either forest or extensively managed grassland (Fig. 1) and the whole area is used multiply for drinking water production, recreation and extensive agriculture. Flooded (+H_2O) and non-flooded sampling sites (-H_2O) were exclusively located in forests and, although artificial landfill (loess loam) was conducted in +W2 and +W3, soil carbon and nitrogen concentrations and C/N-ratios were similar in each of the sites (Table 1). In contrast, nitrogen supply and microbial biomass was enhanced in the top soil layer of the flooded sites presumably due to additional input of organic matter by the flooding water. Interestingly, DOC was reduced in flooded sites suggesting that increased microbial populations effectively degraded and assimilated DOC. Organic carbon is known to be removed during groundwater recharge due to a combination of physical, chemical and biological processes (Thurman 1985), and Rauch & Drewes (2005) reported that microbial biodegradation of DOC mainly occurs in top soil layers.

Soil carbon concentrations and C/N ratios did not differ among the flooded sites at both sampling dates. However, nitrogen, DOC and nitrate concentrations varied significantly among the flooded sites and were lowest in +W2 and highest in +W3 two (and in part five) days after flooding. These differences presumably relate to the different histories of the sites; e.g. groundwater recharge at +W2 was established considerably later than at +W1 and +W3. Interestingly, however, the differences between the flooded sites did not reflect the land fill *vs.* natural construction of the

sites, suggesting that the construction history is of minor importance for the functioning of the groundwater recharge systems.

General earthworm performance

In general, earthworm densities in the studied forest were in a similar range as in other oak, beech or mixed woodlands (Edwards & Bohlen 1996). However, in flooded grasslands, Zorn et al. (2005) reported higher density and biomass of earthworms than at our sites, although flooding may detrimentally affect earthworm populations (Ausden et al. 2001, Zorn et al. 2005, Plum & Filser 2005, Ivask et al. 2007). Investigations of earthworm populations in periodically flooded forests are scarce; hence it is difficult to draw comparisons because grassland and forest earthworm communities differ in species composition.

Investigating earthworm populations in flooded and non-flooded deciduous forests with similar carbon and nitrogen concentrations we found earthworm density, biomass and species richness in flooded sites to significantly exceed that in non-flooded sites. Increased moisture and nutrient input by flooding with Rhine water likely promoted earthworm reproduction and lead to continuously enhanced earthworm performance. It is well known that earthworms can use a variety of organic materials for food, and even at adverse conditions they can extract sufficient nourishment from organic matter and microorganisms to survive (Edwards & Bohlen 1996). Regarding density, endogeic and anecic earthworms were responsible for these differences; biomass differed for epigeic and anecic earthworms. Earthworm populations actually benefited from periodic flooding and this was most pronounced in the oldest flooding site (+W1). In contrast to the other flooding sites this site was not affected by artificial landfill and bulldozing and together with its age this may explain the high earthworm density.

Earthworms can cope well with submergence during short-term floods; Roots (1956) even reported survival of earthworms (*Al. chlorotica, Ap. longa, D. subrubicunda, L. rubellus* and *L. terrestris*) for 31-50 weeks in submerged soil. A number of life-history adaptations of earthworms to floods have been documented, e.g. flood-tolerant cocoons and accelerated maturation at lower weight (Lytle & Poff 2004, Klok et al. 2006): Survival and fostering of earthworm populations suggest that the periodical floods did not result in long lasting anaerobic conditions. Further, the intermittent dry periods were long enough to allow maturation and reproduction of earthworms (Klok et al. 2006). Roots (1956) observed that adult earthworms submerged for one year did not reproduce, although cocoons of *Al. chlorotica* hatched under water and young worms fed and grew when totally immersed. In the "Lange Erlen" watering cycles usually consist of 10 days flooding followed by 20 days drying and regeneration. However, non-flooding and regeneration periods may be longer during phases of low drinking water consumption, maintenance work or pollution of the river Rhine. The watering protocols of the last 10 years reflect a more or less annual rhythm of low flooding intensity in spring, regular flooding in summer (due to higher drinking water consumption) and intermediate flooding intensity in autumn / winter (Industrielle Werke Basel, W. Moser, pers. comm.).

Higher earthworm numbers at the flooded sites might also have been due to more efficient earthworm extraction with the octet method in the moister flooded soils. As the octet method is the least invasive sampling method for earthworms (Schmidt 2001), which is important at the highly sensitive sites used for the production of drinking water, we tried to minimize effects due to differences in soil moisture by careful timing of the sampling events. Earthworms were sampled in early spring before plant growth started, i.e. when soil moisture is high due to precipitation during the winter months. Further, samples at the non-flooded sites were taken two days

after rainfall (24 mm; Meteodata Station Lange Erlen, University of Basel) to ensure that the litter and upper topsoil is moist and comparable to that of the flooded sites. At the flooded sites samples were taken at least four days after flooding to allow drainage and equilibration to field capacity.

Earthworm community

Different earthworm communities developed over decades in the investigated flooded and non-flooded sites of the “Langen Erlen”. As indicated by PCA, especially the earthworm communities from the two oldest studied recharge areas, +W1 and +W3, separated both sampling sites from all the others and also from each other. The genus *Dendrobaena* only occurred in +W3 which presumably results from the artificially introduced soil prior to initial operation of this recharge site. The sole occurrence of *A. negundo* in +W3 supports this conclusion. Surprisingly, *L. terrestris* was absent at this site and in the PCA plot *D. platyura* and *L. terrestris* were farthest away from each other. Possibly, both anecic species exclude each other by occupying similar spatial and trophic niches. In the PCA plot three riparian species, *Al. chlorotica, Al. georgii* and *E. tetraedra* were situated between +W1 and +W3 and *Ap. caliginosa, Al. icterica* and the genus *Lumbricus* was associated with +W1. All these species or genera are known to be well adapted to floods, some of them even have been classified as riparian species (Roots 1956, Sims & Gerard 1999). The earthworm communities from all non-flooded sites and the youngest flooded site, +W2, grouped together and correlated with non-flooded sampling sites. Only two species, *O. cyaneum* which only occurred in the non-flooded sites and *O. tyrtaeum* characterized the non-flooded sites suggesting that these species are more sensitive to flooding.

Earthworm body size

Biomass of individual adult earthworm species which occurred at least four times in flooded and non-flooded sites was analysed. In flooded sites individual biomass of L. *rubellus* exceeded that in non-flooded sites whereas the opposite was true for *Al. rosea* and *O. tyrtaeum*. Flood dependent decrease in individual biomass of earthworms has been documented by Zorn et al. (2005) and Ausden et al. (2001). However, Ausden`s studies only showed a significant reduction after 120 days of flooding. Higher individual biomass of *L. rubellus* in flooded sites contradict results of Klok et al (2006) who found significant lower individual biomass of *L. rubellus* in flooded grasslands. Food availability, soil characteristics and pollutants did not provide convincing explanations for their results and they assumed the lower biomass to result from adaptation to the local inundation regime. At our study site regular flooding with Rhine water for decades increased microbial biomass and the availability of nutrients (i.e. nitrate), thus presumably promoting the growth of plants and thereby litter input. Obviously, *L. rubellus*, as an endo-epigeic hygrophilous r-strategist, and the riparian earthworm species can cope well with the watering regime in the “Lange Erlen”.

Changes in individual biomass of specific earthworm species compensated significant overall differences of epigeic and endogeic earthworms in flooded and non-flooded sites: although the density of endogeic earthworms in flooded sites exceeded that in non-flooded sites, overall biomass of endogeic earthworms did not differ significantly between these sites. In contrast, overall biomass of epigeic species in flooded sites exceeded that in non-flooded sites whereas their density did not differ significantly. Higher individual weight of *L. rubellus* (epigeic) and the lower individual weights of *Al. rosea* and *O. tyrtaeum* (endogeic) in flooded sites were responsible for these effects.

Earthworms and water infiltration

A number of studies documented that earthworms increase water infiltration rates (Ehlers 1975, Lee & Forster 1991, Weiler & Naef 2003). Our correlations between total earthworm density and water infiltration rates support these findings. Both, earthworm density and water infiltration rates, were at a maximum in +W1 exceeding those at the other two flooding sites by a factor of about 2.5. However, the factors responsible for these correlations could not be detected in our study.

By separating earthworms into ecological groups, significant and positive correlations between earthworm density and water infiltration rates were detected for epigeic and endogeic earthworms, but not for anecic earthworms. Most earthworm – macropore – infiltration studies deal with the anecic species *L. terrestris* since this species constructs large vertical burrows extending deep into the soil thereby strongly influencing infiltration rates (Edwards et al. 1992, Willoughby el al. 1997, Shipitalo et al. 2004). Surprisingly, in the “Lange Erlen” density of anecic earthworms correlated less with infiltration rates than the density of endogeic and epigeic earthworms. Similar results were obtained with biomass data (data not shown). At the “Langen Erlen” numbers of epigeic and endogeic earthworms exceeded those of anecic earthworms; potentially, density of anecic earthworms was too low for significantly correlating with infiltration rates. *Lumbricus terrestris*, as the main representative of anecic earthworms in European temperate forests, may generally be less common in river banks (Sims & Gerard 1999), however, this assumption is mainly based on data from grassland sites and knowledge on forest river banks is scarce.

The species closely correlated with infiltration rates were *L. rubellus* and *Al. chlorotica*. *Lumbricus rubellus* was found in all flooded sites, whereas *Al. chlorotica* only occurred in +W1 and +W3 but not in all samples. Density and biomass of earthworm species suggests that *L. rubellus* benefited most from the

periodical flooding in the “Lange Erlen”. This was unexpected, since burrow construction of *L. rubellus* is restricted to the uppermost soil layers (Edwards & Bohlen 1996). Potentially, mixing of upper soil layers is more important for infiltration rates than vertical macropores or soil density. In fact, bulk density of the soil did not correlate significantly with the density of total earthworm numbers or of individual species. However, as indicated by results of other studies (Edwards & Shipitalo 1998, Shipitalo & Le Bayon 2004) this might be specific to the studied forest ecosystem. Presumably, by mixing litter materials with mineral soil *L. rubellus* contributes in preventing the development of clogging thereby ensuring long-term functionality of the studied filtration system for drinking water production. However, since the role of *L. rubellus* for long-term infiltration of water in periodically inundated forests has not been studied before, this assumption needs further testing.

Conclusions

The main factors that contribute to the efficient, artificial recharge and purification system in the “Lange Erlen” include watering cycles with longer regeneration periods in combination with highly active decomposer species, increased microbial biomass and woodland sites. Particularly earthworms turn over the upper soil and litter layers, thereby functioning as ecosystem engineers. We showed that earthworms can cope well with the applied watering regime and most likely, species specific life history adaptations are responsible for their differential performance. Presumably, the different functional groups of earthworms, in particular epi- and endogeic species, interact in regenerating soil structure during regeneration periods, thereby stimulating microbial biomass, preventing clogging and enhancing infiltration rates. At the species level *L. rubellus* appeared to be most important and anecic earthworm species were subordinated. Overall, the data suggest that earthworms contribute to

the functioning of the “Lange Erlen” filtration system keeping it working effectively without larger maintenance for almost 100 years. With few signs of degeneration, the system may well work for another century or longer. In addition to providing economic drinking water production in a semi-natural floodplain forest ecosystem, it provides refuge for a large number of plant and animal species (Luka 2004, Rüetschi 2004). Due to its simplicity and functionality the system may be widely adopted for drinking water production in future.

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Chapter two

Flooding forested groundwater recharge areas modifies microbial communities along a vertical soil profile down to the groundwater table

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Abstract

Most studies in microbial communities of belowground systems are limited to the top soil layer and disregard deeper horizons. However, subsurface microorganisms are crucial for contaminant degradation and maintenance of groundwater quality. We assessed the microbial community composition (by phospholipid fatty acids, PLFAs), and physical and chemical soil characteristics at woodland flooding sites of an artificial groundwater recharge system used for drinking water production. Vertical soil profiles to approximately 4 m at two watered and one non-watered site were analyzed. Considerable fractions of the microbial biomass (25-42%) were located in 40-340 cm depth. The microbial community structure differed significantly between watered and non-watered sites, predominantly below 100 cm depth. Proportions of the bacterial PLFAs 16:1ω5, 16:1ω7, cy17:0 and 18:1ω9t, and the long chained PLFAs 22:1ω9 and 24:1ω9 were more prominent at the watered sites, whereas branched, saturated PLFAs (iso/anteiso) dominated at the non-watered site. PLFA community indices indicated stress response (trans/cis ratio), higher nutrient availability (unsaturation index) and less thermal adjustment of membrane fluidity (iso/anteiso ratio) due to flooding. In conclusion, water recharge processes resulted in a microbial community adapted to resource and environmental conditions, which was located in a biologically active layer between 100-220 cm soil depth.

Keywords: Flooding, Groundwater recharge, Fatty acids, Microorganisms, Vadose zone, PLFA indices

Introduction

Water purification through artificial groundwater recharge is widespread, and common routines comprise unvegetated slow sand filters, such as dunes or channels, in combination with long flooding periods (Peters et al. 1998). In these systems a biofilm matrix, comprising microorganisms, microfauna and a range of aquatic insect larvae, develops at the surface of the sand filter. This complex biological layer is predominantly responsible for water purification, whereas the underlying sand acts as support medium (Duncan 1988, Weber-Shirk & Dick 1997). The present study investigates the drinking water production of the city of Basel (Switzerland) at the former floodplain area called “Langen Erlen”. This system is unique throughout the world. Embanked forest sites with either artificial landfill (loess loam) or fluvi-eutric cambisol are periodically flooded with water from the Rhine river to augment groundwater resources. Thereby river water is transformed into purified drinking water, but in contrast to slow sand filter systems no biofilm is generated. Nevertheless infiltration and purification capacities remain constant and satisfying since the system has been established in 1912 (Rüetschi 2004).

Generally, surface and subsurface microorganisms play an important role in soil biogeochemistry, contaminant degradation, and maintenance of groundwater quality (Konopka & Turko 1991). Thereby processes of pollutant biodegradation in the vadose zone, the unsaturated zone extending from the soil surface to the groundwater table, are of considerable importance (Holden & Fierer 2005). The vadose zone is a three-phase system comprising solid, aqueous and gaseous components and the soil water content can change dramatically from saturated conditions to desiccation over short time intervals (Kieft & Brockman 2001). Chemicals, such as pesticides, hydrocarbons, pharmaceuticals and metals, applied at the soil surface are transported through the vadose zone and a capable biological

activity is essential for their biotransformation. Konopka & Turko (1991) reported that sorption, as a function of clay content, was more appropriate for atrazine and metachlor, whereas phenol and aniline were metabolized by microorganisms. Comparably, mineralization of benzene and toluene was located rather in sandy than in clayey soil samples (Aelion 1996). Microbial degradation in contaminated aquifers was also shown for hydrocarbons such as jet fuel, toluene and petroleum derivates (PHC) (Aelion 1996, Pelz et al. 2001). Moreover, antibiotics such as streptomycin, bambermycins, tylosin and penicillin were mineralized in agriculture soil within 30 days (Gavalchin et al. 1994).

Due to the function of the vadose zone as a natural mechanism for pollutant attenuation, there is need to better understand the microbial ecology of this unsaturated subsurface. However, as the density of microorganisms is highest in the top soil layer (0-20 cm), most studies have predominantly investigated abundance, community structure and physiological status there (Konopka & Turco 1991, Hinojosa et al. 2005, Williams & Rice 2007). Microbial biomass declines rapidly within the first meter of soil profiles but then stabilizes (Federle et al. 1998, van Gestel 1992). Nevertheless, the summed biomass of a soil profile down to 2 m depth can comprise approximately 35% of the surface layer (Fierer et al. 2003) and thus should not be ignored in biodegradation processes. These microbial communities residing at depth are not simply diluted analogs of the surface populations; their composition and structure changes significantly with soil depth and resource availability (Blume et al. 2002, Fierer et al. 2003).

Few attempts have been made to understand how microorganisms are distributed with depth through soil profiles, and hardly any studies exist on microbial communities in deep soil profiles under periodical flooding events. Impacts of flooding or water stress in the top soil (0-20 cm) were found to result in a decline of

fungi, increased abundance of Gram-positive bacteria, decreased monoenoic fatty acids (used as aerobic indicator) and increased methane production under submerged conditions (Bossio and Scow 1998, Williams 2007). Williams and Rice (2007) documented a reduction in the physiological stress of the Gram-negative microbial community in continuous moisture treatments. In the upper layer of floodplain soils Rinkelrebe & Langer (2006) observed the highest microbial biomass and proportion of monoenoic fatty acids in short-time flooded sites (234 days within 1.5 years), whereas long-time flooded sites (330 days within 1.5 years) displayed a converse pattern.

To date, the water purification processes in the artificial groundwater recharge system “Langen Erlen” are not understood in detail. Presumably they consist of a combination of groundwater dilution, particle sorption and biodegradation. Therefore, it is of particular interest to study the presence and structure of subsurface microbial communities that can intercept or immobilize pollutants. In this study, we analyzed soil depth transects (approx. 4 m deep) comprising surface soil, vadose zone, and capillary fringe for their microbial community structure as well as physical and chemical characteristics in two watered and one non-watered site. Microorganisms were assessed using phospholipid fatty acid (PLFA) pattern to determine biomass, community structure, physiological conditions (e.g. stress response), and presence of functional groups (i.e. gram-positive and gram-negative bacteria, fungi). Our goal is to deepen the understanding in microbial ecology of the unsaturated subsurface, predominantly the vadose zone. Firstly, microbes residing there live under conditions of relatively low water availability, and the periodic flooding is expected to alter microbial community structure, activity and physiological status. Secondly, the vadose zone is usually treated as a relatively static entity, with strong gradients from top to bottom. We hypothesize that the water recharge sites have a different spatial

organization, with biologically active layers below the rooted zone. Thirdly, the microbial communities located at these “purification sites” are assumed to be distinct from the communities above and below, and to consist of functional groups adapted to the specific resource and environmental conditions.

Materials and Methods

Study site

The study site “Lange Erlen” (size ~3 km^2) is situated northeast of the city of Basel, Switzerland. This former natural floodplain area extends along the river Wiese, a straightened tributary of the river Rhine, descending from the Black Forest, Germany. Since 1912 part of the “Lange Erlen” serves as groundwater recharge area, and today approximately half of the drinking water for the city of Basel originates there (15×10^6 m^3 ha^{-1} y^{-1}).

The semi-natural forest sites of the “Lange Erlen” are classified as Galio - Carpinetum oak - hornbeam forests (Burnand & Hasspacher 1999). The flooded sites have been modified by human activities, such as landfill and afforestation with poplars (*Populus canadensis*), ash (*Fraxinus excelsior*), alder (*Alnus nigra*) and willows (*Salix* spp.). The upper soil is characterized as fluvi-eutric cambisol, and the aquifer consists of 80% Rhine gravel (lower part, mostly limestone) and 20% Wiese gravel (upper part, mostly silicates and limestone).

The first sampling site “Hintere Stellimatten” (HST) is the most recent flooding area and started operating after afforestation in 1977. Too high infiltration rates (11 m^3 m^{-2} d^{-1}) led to artificial landfill (loess loam) and bulldozing in 1981. The soil profile down to approximately 1 m consists of landfill loess loam, followed by sand and gravel with interspersed silt and clay lenses and partial FeMn deposition. Average pH across the soil depth profile is 7.3.–The water content (% DW soil)

increases from 81 to 94%, whereas the C/N-ratio declines from 14.6 to 8.2 with depth. The site is mainly overgrown with an alder swamp forest (*P. canadensis, A. nigra, Salix* spp.*)* and the herb layer is dominated by *Rubus caesius and Urtica dioica.* Unvegetated sites comprise some spots of *Poa trivialis, Potentilla reptans, Agropyron repens* and *Geum urbanum*.

The second site, “Verbindungsweg” (VW), was established as flooding area after afforestation and artificial landfill (loess loam) in 1970. Landfill loess loam is approximately 0.5 m deep, and is followed by silt, sand and gravel with interspersed clay lenses and partial FeMn deposition. pH-values and water content (% DW soil) increase with depth from 7.2 to 7.5 and 79 to 92%, respectively. C/N-ratio decreases with soil depth from 9.3 to 7.6. The forest consists of some old poplars, ash-leaved maple (*Acer negundo*) and oak trees. The understory is dominated by *Lysimachia nummularia, Urtica dioica, Phalaris arundinacea, R. caesus / fruticosus, Duchesnea indica, Iris preudacorus, Seneco aquaticus* and *Cardamine pratensis.* In the shady northern part of the site, dominated by poplars, the soil is bare of vegetation.

The non-watered site “Bachtelenweg” (BW) is situated between these two flooding areas and is agriculturally used with extensive biological grassland management. The soil profile is similar to VW, pH values range between 7.5 in the top soil and 5.9 in the deepest soil layer. The C/N-ratio declines from 9.7 to 7.3, and water content (% DW soil) increases from 86 to 94% with depth.

Infiltration system

Rhine water, prefiltered by rapid sandfilters (80 cm quartz sand layer), is periodically seeped into 11 wooded flooding areas (total area 22 ha). Each flooding area (10,000 – 20,000 m^2) is divided into three fields (3000 – 8500 m^2) by small dams (height ca. 50 cm). Watering cycles usually consist of 10 days flooding and 20 days drying and

regeneration, however, longer interruptions due to revisions occur. The water fills up to variable heights depending on soil surface structure (max. 50 cm) and seeps with a speed of 1-2 m d^{-1} through a mull humus and fluvial silt layer of 30-90 cm and a sand / gravel layer of 2-3 m before it reaches the groundwater table at 3-4 m depth. Subsequently, the water flows horizontally (from northeast to southwest) in the aquifer for 200-800 m. After 10-30 days purified water is pumped out of groundwater wells, collected in the pumping station and, after a brief chemical treatment with ClO_2, delivered to consumers.

Soil Sampling

Soil sampling was conducted in triplicate at the two watered sites (HST, VW) and the non-watered site (BW). The groundwater level at the watered sites was lowered by a stop of the flooding regime four weeks before sampling. Three vertical core drillings (diameter 25 cm) from the soil surface down to a maximum of 4.5 m depth, or alternatively groundwater level, were conducted in HST and VW by the construction firm GLANZMANN (Basel) in November 2005. Soil cores were excavated in 0.5 m intervals and placed carefully into soil core boxes, representing the full soil profile along the depth transect. Subsequently, soil samples (100 g) were taken in 30 cm distances with a shovel from the inner part of the cores, transported to the laboratory and frozen (-20 °C) until analysis. Overall, 45 soil samples were taken in each, HST and VW.

Three horizontal soil samplings through the face of a trench down to 3.5 m depth were performed in the non-watered site BW in connection with drain installations for flood protection in December 2005. This was the unique chance for deep soil sampling outside the flooding areas in the same time period. Three depth transects were randomly chosen, and, respective to the soil layers in HST and VW, soil

samples were directly taken from the inner trench wall. A total of 34 soil samples were taken and frozen (-20 °C) until analysis.

Prior to all analyses the required soil substrate was thawed in the fridge (24 h, 8 °C). Visible organic material (fine roots and leaves) and stones were removed, and soil was sieved (5 mm).

Soil analysis

Chemical and physical soil characteristics

The soil samples were analyzed for a variety of physical and chemical characteristics to determine the gradients in pH, carbon (C), nitrogen (N), dissolved organic carbon (DOC), nitrate (NO_3^-), sulfate (SO_4^{2-}) and moisture content. Total carbon and nitrogen content were determined from oven-dried (65 °C, 3 days) and pulverized (swing mill, Retsch MM 200) aliquots of the bulk soil with an element analyzer (CHN 1000, Leco, USA). Results are expressed in % per soil dry weight. Soil pH was measured in soil salt (0.01 M $CaCl_2$) solution (1:4 w/v) after stirring and incubating at room temperature for 1 h. Soil moisture content was determined by the mass difference before and after drying at 105 °C for 24 h.

DOC, nitrate and sulfate was extracted from 5 g soil (fresh weight) for the 0-200 cm depths and from 10 g for the 200-450 cm depths. A soil salt (0.01 M $CaCl_2$) solution was prepared (1:4 w/v), shaken for 1 h (200 rpm) and centrifuged for 15 min (2000 rpm). The supernatant of each soil solution was filtered through sterile 0.45 µm membrane filters (Millex HA) and stored in the fridge until analysis (max 1 h). To avoid contaminations, particularly in low concentrated sample solutions of deeper soil samples, only glass ware was used exclusively in the whole procedure. The sample solutions were analyzed for DOC after acidification and air-purging (N55, O45, Carbagas, CH) with a TOC analyzer (TOC-5000 A, Shimadzu) in quintuplicate.

Nitrate and sulfate were measured in an ion chromatograph (IC-690, Metrohm). Results are expressed in mg g^{-1} DW soil.

Phospholipid fatty acid (PLFA) analysis

Lipid extraction and methylation was conducted on soil samples thawed for 24 h in a fridge (8 °C) using previously described procedures (Frostegård et al. 1993). PLFAs were extracted and quantified from 3 g soil (fresh weight) for the 0-40 cm depths, from 6 g for the 40-100 cm depths, and from 10 g for the 100-400 cm depths in order to compensate for the decrease in microbial biomass with depth. PLFAs were identified and quantified by using gas chromatography interfaced to a electron ionization mass spectrometer (GC/EI-MS). Analyses were performed with a 3400/Saturn 4D iontrap GC/MS system (Varian, Darmstadt, Germany). The split/splitless injector was operated in splitless mode for 2 min and kept at 225 °C. The carrier gas (helium, purity 5.0) was used at a constant pressure of 22.5 psi. A HP 5 crosslinked 5% phenyl 95% methyl polysiloxane column (50 m, 0.32 mm i.d., 0.17 µm d_f; Varian Chrompack, Middelburg, The Netherlands) was installed in the GC oven. The GC oven temperature was programmed as follows: 50 °C (2 min hold time), then at 5 °C/min to 180 °C (2 min), and at 5 °C/min to 300 °C (50 min). The transfer line was heated to 300 °C. The manifold was set to 200 °C, and the multiplier voltage was set at 2400 V. At a filament emission current of 15 µA, the mass range was scanned from *m/z* 50 to *m/z* 600 twice a second after a solvent delay of 5 minutes. 1 µL of sample extract was injected each run.

PLFAs were determined with a combination of retention time and fragment ions: *m/z* 74 and 87 were used for saturated PLFAs, *m/z* 81 and *m/z* 87 for monoenoic and cyclic PLFAs and *m/z* 79 and *m/z* 81 for polyenoic PLFAs as described in Thurnhofer and Vetter (2005). A standard mixture composed of 37 different FAMEs that ranged

from C11 to C24 (Sigma-Aldrich, St Louis, USA) was used to verify retention time. For the quantification of PLFAs, an internal standard (19:0) with known concentration and the best visible fragment ion of the specific PLFA was included in the calculation. The abundance of individual PLFAs was expressed in nmol g^{-1} DW soil and in mol%. The ω notation was used to classify fatty acids, where unsaturated acid are ascribed according to the number of carbon atoms from the terminal methyl group (ω end) to the nearest double bond, i.e. ω9, ω6, ω3 (IUPAC-IUB, 1978). Four PLFA-indicator ratios were calculated as supplementary variables. The fungal to bacterial ratio (F/B) comprised 18:2ω6, 18:3ω6 as representatives of fungal and i15:0, a15:0, i16:0, i17:0, cy17:0, cy19:0 and 16:1ω7 of bacterial PLFAs (according to Frostegård et al., 1993; Frostegård & Bååth, 1996; Zelles, 1999). The PLFA 16:1ω5 as biomarker for mycorrhizal fungi was excluded, because this exclusively fits for the rooted zone. Furthermore, the bacterial gram-positive/gram-negative ratio (i.e. iso + anteiso/cyclo PLFAs) and the iso/anteiso ratio of the PLFAs 15:0 and 17:0 as temperature (Kaneda 1991, Peterson & Klug 1994) and aerob/anaerob indicators (Parkes & Taylor 1983, Mauclaire 2007) were estimated. The *trans*/*cis*-ratio for 18:1ω9 was used as stress indicator (Heipieper et al. 1992). As a measure of membrane fluidity the unsaturation index (UI) was calculated as UI = ((C:1 * 1 + (C:2 * 2) + (C:3 *3) + (C:4 * 4) + (C:5 *5))/100, where C:1, C:2, C:3, C:4 and C:5 represent the proportion (%) of FAs with 1, 2, 3, 4 and 5 double bonds, respectively.

Statistical analyses

Overall, sampling was conducted in triplicate for each field site and summarized in 7 depths: The surface soil (rooted zone) with 0-40 and 40-100 cm, the upper vadose zone with 100-160 and 160-220 cm and the lower vadose zone with 220-280, 280-340 and 340-400 cm. Calculations were conducted on LOG transformed

environmental data, which also included the sum of PLFA, mol% PLFA and the different PLFA ratios. Environmental variables, PLFA amounts and -ratios were analyzed for field- and depth-differences using repeated ANOVA procedures. As the 340-400 cm depth was only sampled at the watered sites HST and VW, this layer was excluded from analysis. Pair wise comparison of means was performed using Tukey`s honestly significance difference test (HSD). Pearson correlation coefficients for depth gradients were calculated for single PLFAs including all depth layers. Correlations and repeated measure ANOVAs were calculated using SAS (SAS Institute, Cary, NC).

Multivariate data analysis of PLFA data was carried out by analysis of similarities (ANOSIM), the similarity percentages procedure (SIMPER) and non-parametric multi-dimensional scaling (nMDS) to determine whether the microbial assemblages differed between sites and/or depths. Prior to analyses, the Bray-Curtis similarity index was applied to the data set. Two-way ANOSIM was calculated on the mol % data of the overall PLFA pattern (two-way nested ANOSIM with the factors “depth” nested in “field”). One-way ANOSIM with “field” as factor was applied to the bulked functional layers: surface soil, upper vadose zone and lower vadose zone (without the 340-400 cm layer). On this tripartite soil profile, nMDS was applied for visualizing the differences between fields and depths on the basis of mol% PLFA data. The nature of community groupings identified by ANOSIM was further explored by applying SIMPER procedure to determine the contribution of individual PLFAs to the average dissimilarity between sites in single soil layers. Multivariate data analyses were calculated with Primer 6.1.5 (Clarke & Warwick 2001).

To visualize differences in PLFA composition in relation to environmental variables biplot ordinations of redundancy analysis (RDA) and the Monte Carlo permutation test (CANOCO 4.5, Ter Braak & Smilauer 2002) was used. First, data were analyzed

by detrended canonical correspondence analyses (DCCA) to determine the length of gradient. Since this gradient was rather short (1.36), data were analyzed and visualized by linear RDA.

Results

Soil parameters

Concentrations of dissolved organic carbon (DOC), total carbon, total nitrogen, and the C/N-ratio in the soil significantly decreased with soil depth at all sites (Tab. 1). DOC ranged from 2.2 (HST) to 42.1 mg g^{-1} DW (BW) in the top soil layer, and from 0.2 (HST) to 6.0 mg g^{-1} DW (BW) in 280-340 cm depth. This corresponds to reduction rates between 85 and 87% in all fields along the depth transect (Fig. 1).

Table 1: Repeated measure ANOVA table of *F*-values and degrees of freedom (*df*) on the effect of field (watered sites: HST, VW; non-watered site: BW) and soil depth (0-40, 40-100, 100-160, 160-220, 220-280, and 280-340 cm) for soil parameters and phospholipid fatty acid ratios (PLFA-ratios).

	Within subjects effects		Between subjects effects
	depth (*df* 5)	depth x field (*df* 10)	field (*df* 2)
Soil parameters			
pH	1.68	3.40**	3.11
%C	24.66***	0.75	2.61
%N	20.86***	1.46	0.05
C/N	5.66***	1.15	11.1**
DOC [mg g^{-1} DW]	11.35***	1.03	163.31***
NO_3^- [mg g^{-1} DW]	41.2***	6.91***	8.68*
SO_4^{2-} [mg g^{-1} DW]	15.4***	0.73	36.53***
PLFA [nmol g^{-1} DW]	64.58***	1.94	10.46*
PLFA - ratios			
[1]Fungi/[2]Bacteria	0.35	1.01	0.47
[3]Gram+/Gram-	2.14	5.73***	11.85**
Unsaturation Index	2.42	3.19**	38.31***
i/a 15:0	1.22	3.36**	10.18*
i/a 17:0	12.96***	2.72*	14.31**
[4]Trans/cis	1.66	4.26**	31.18***

[1] 18:3ω6, 18:2ω6
[2] i15:0, a15:0, 15:0, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, cy19:0
[3] (i15:0 + a15:0 + i17:0 + a17:0) / (cy17:0 + cy19:0)
[4] 18:1ω9t / 18:1ω9c

The concentrations for DOC were significantly higher in the non-watered site BW at all depths (average increase 15 fold), compared to the watered sites, HST and VW, which contained comparable amounts.

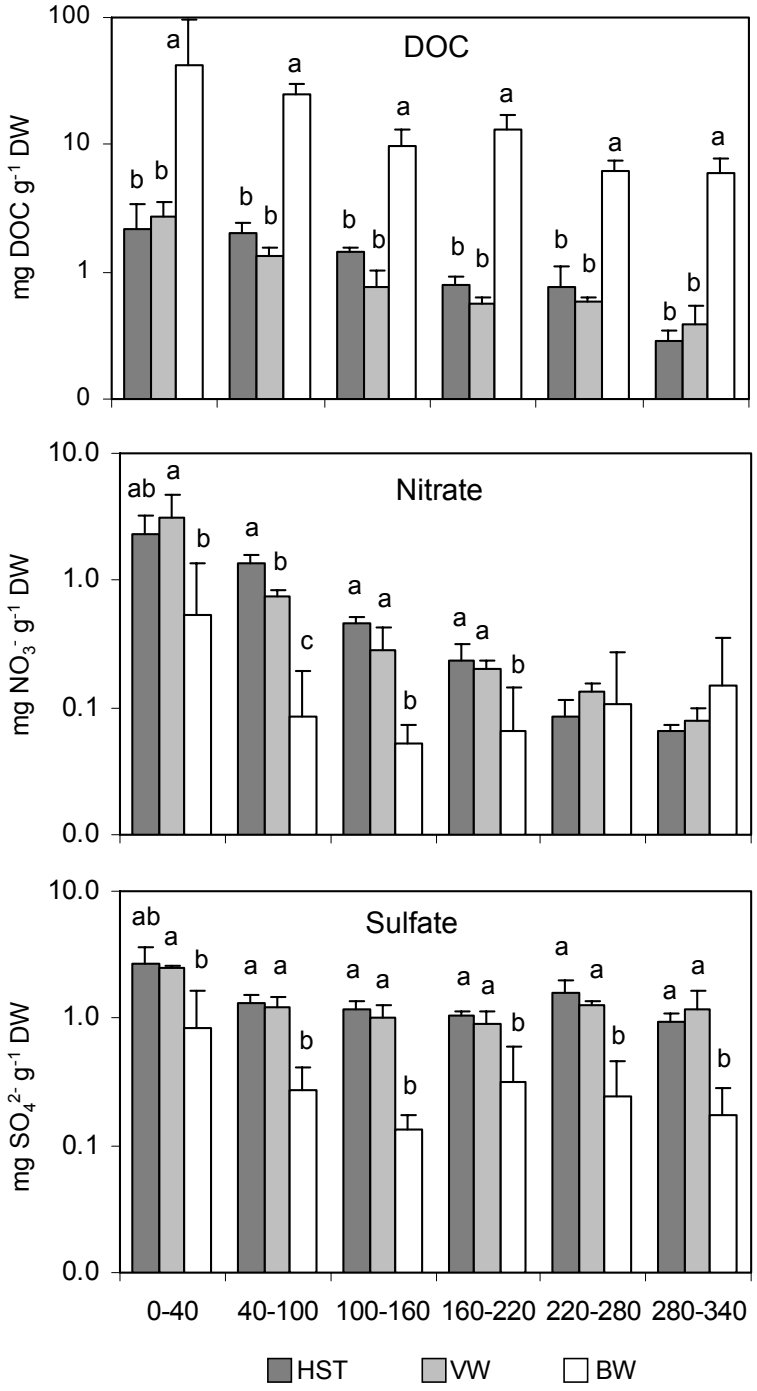


Fig. 1: Nutrient status, expressed as dissolved organic carbon (DOC in mg g^{-1} DW ± s.d.), nitrate (NO_3^- in mg g^{-1} DW ± s.d.) and sulfate (SO_4^{2-} in mg g^{-1} DW ± s.d.) in six soil depths (0-40, 40-100, 100-160, 160-220, 220-280, and 280-340 cm) at the watered sites HST and VW, and the non-watered site BW. Bars of the same depth sharing the same or no letter are not significantly different (Tukey`s HSD, $P < 0.05$)

Total soil carbon content (% DW soil) was reduced within the soil profile to about 88% in the watered sites and 82% in the non-watered site, i.e. from 2.05 to 0.25, 2.14 to 0.26, and 1.74 to 0.32% for HST, VW and BW, respectively (data not shown). Soil nitrogen content (% DW soil) decreased with depth by about 80% with 0.14 to 0.01, 0.23 to 0.03, and 0.18 to 0.04% for HST, VW, and BW, respectively (data not shown). Correspondingly, the C/N-ratio declined slightly with soil depth from 14.6 to 8.2 in HST, 9.3 to 7.6 in VW, and 9.7 to 7.3 in BW. Carbon and nitrogen content were significantly affected by depth, whereas the C/N ratio was significantly influenced by both, soil depth and field site, without interaction (Tab. 1).

Nitrate (NO_3^-) concentrations in the top soil layer ranged between 2.29 (HST) and 3.12 (VW) mg g^{-1} DW of the watered sites, and 0.53 mg g^{-1} DW in the non-watered site BW (Fig. 1). Sulfate (SO_4^{2-}) concentrations in the top soil layer ranged between 2.71, 2.45, and 0.84 mg g^{-1} DW in HST, VW, and BW, respectively. At all sites NO_3^- and SO_4^{2-} concentrations significantly decreased with soil depth (Tab. 1, Fig. 1). Initial nitrate concentrations in the top soil layer were reduced by about 97% in the lower vadose zone in both watered sites, whereas in the non-watered site the decrease was less (71%). Sulfate concentrations declined by about 34, 47, and 21% in deeper horizons of HST, VW, and BW, respectively. Both watered sites had significantly higher nutrient concentrations in -40 cm to -220 cm depth for nitrate, and -40 cm to -340 cm depth for sulfate, compared to the non-watered site BW (Fig. 1).

pH-values in VW increased slightly with soil depth from 7.18 to 7.53, whereas in HST they remained about constant at pH 7.3. The pH values in BW decreased with depth from 7.1 to 6.6 (data not presented). These contrasting depth gradients for VW and BW, with lowest pH-values in the lower vadose zone at BW and highest at VW, are reflected in a significant depth x field interaction (Tab. 1).

Phospholipid fatty acids (PLFAs) and soil depth

The total amount of PLFAs, a measure for the microbial biomass, uniformly decreased from 53.2±28.1 to 1.8±1.1 nmol $g^{-}1$ DW soil with depth across sites (Tab. 1, Tab. 2). Highest amounts of PLFAs were observed in the top soil layer (0-40 cm). However, an average of 42%, 26% and 25% of the total microbial biomass was located in -40 to -340 cm depth in HST, VW and BW, respectively. Differences between sites occurred in the 40-100 and the 100-160 cm layer where the sum of PLFAs in HST surpassed VW and BW (Tab. 2).

In total 25 predominant PLFAs were determined in the soils of the investigated sites (Tab. 3, Fig. 2). The concentrations of individual PLFAs decreased significantly with soil depth, and only minor fractions were located in the lower vadose zone (data not shown). On the other hand, proportions (%) of individual PLFAs within the profile of single soil layers distinctly varied with depth (Tab. 3). A uniform decrease in mol% occurred for the PLFAs 16:1ω5, i17:0, cy19:0 and 24:0, and an increase for 18:3ω6 at all sites. In contrast, the proportions of the gram-positive bacterial PLFAs a15:0 and a17:0 were negatively correlated with depth at the watered sites HST and VW, and positively correlated at the non-watered site BW. Decreasing proportional amounts in 18:1ω9t, 20:4ω6 and 20:5ω3 were detected in BW soils only. Contrasting distributions were observed for the bacterial PLFAs i15:0 and i16:0, where proportions declined with depth in the watered sites, but remained constant in the non-watered site. Exclusively in VW the PLFAs 16:1ω7, cy17:0 and 22:0 reached higher proportions with depth.

Table 2: Total amount of PLFA (nmol/gDW) and different PLFA-ratios in 6 soil depths (0-40 cm, 40-100 cm, 100-160 cm, 160-220 cm, 220-280 cm, 280-340 cm) at the watered sites HST and VW, and the non-watered site BW. Values in parentheses are the standard deviation. Means within a row with the same or no letter are not significantly different (Tukey`s HSD, $P < 0.05$)

	Depth (cm)	HST		VW		BW	
Total amount	0-40	53.77 (49.0)		54.86 (23.79)		50.95 (13.5)	
(nmol/gDW)	40-100	18.10 (2.27)	**a**	7.03 (0.63)	**b**	6.82 (1.61)	**b**
	100-160	13.33 (1.20)	**a**	3.89 (1.57)	**b**	3.22 (0.86)	**b**
	160-220	4.17 (2.13)		3.29 (0.43)		3.08 (1.32)	
	220-280	2.14 (0.58)		2.60 (1.14)		1.48 (1.31)	
	280-340	1.76 (0.78)		1.58 (0.11)		1.95 (1.96)	
[1]Fung/[2]Bact	0-40	0.06 (0.00)		0.07 (0.03)		0.08 (0.04)	
	40-100	0.05 (0.01)		0.07 (0.03)		0.12 (0.10)	
	100-160	0.06 (0.08)		0.05 (0.03)		0.09 (0.04)	
	160-220	0.07 (0.03)		0.05 (0.06)		0.05 (0.05)	
	220-280	0.07 (0.06)		0.07 (0.09)		0.07 (0.05)	
	280-340	0.04 (0.04)	**b**	0.14 (0.04)	**a**	0.07 (0.04)	**ab**
[3]Gram+/Gram-	0-40	2.71 (0.58)		2.67 (0.67)		2.79 (0.74)	
	40-100	2.79 (0.76)		2.37 (0.39)		4.07 (2.38)	
	100-160	1.90 (0.42)	**c**	3.00 (0.34)	**b**	5.28 (0.50)	**a**
	160-220	1.88 (1.43)	**b**	2.55 (1.45)	**b**	8.25 (1.23)	**a**
	220-280	2.05 (0.58)	**b**	1.68 (0.17)	**b**	9.50 (4.98)	**a**
	280-340	1.73 (1.23)	**b**	1.06 (0.37)	**b**	10.52 (5.04)	**a**
[4]UI	0-40	0.50 (0.06)		0.52 (0.04)		0.50 (0.06)	
	40-100	0.39 (0.04)		0.47 (0.05)		0.47 (0.11)	
	100-160	0.66 (0.04)	**a**	0.56 (0.03)	**a**	0.37 (0.07)	**b**
	160-220	0.55 (0.04)	**a**	0.64 (0.03)	**a**	0.28 (0.09)	**b**
	220-280	0.55 (0.09)		0.60 (0.06)		0.43 (0.17)	
	280-340	0.61 (0.16)		0.69 (0.06)		0.43 (0.10)	
i/a 15:0	0-40	1.25 (0.03)		1.28 (0.19)		1.22 (0.44)	
	40-100	1.15 (0.07)		1.18 (0.06)		1.07 (0.29)	
	100-160	1.26 (0.06)	**a**	1.23 (0.05)	**a**	0.67 (0.26)	**b**
	160-220	1.41 (0.03)	**a**	1.27 (0.07)	**a**	0.46 (0.20)	**b**
	220-280	1.30 (0.38)		1.19 (0.08)		0.76 (0.36)	
	280-340	1.54 (0.31)	**a**	1.38 (0.27)	**ab**	0.53 (0.46)	**b**
i/a 17:0	0-40	1.00 (0.08)		0.94 (0.16)		0.98 (0.24)	
	40-100	0.98 (0.05)		0.80 (0.03)		0.85 (0.21)	
	100-160	0.90 (0.04)	**a**	0.72 (0.04)	**b**	0.57 (0.06)	**c**
	160-220	0.84 (0.13)	**a**	0.70 (0.06)	**ab**	0.49 (0.09)	**b**
	220-280	0.70 (0.19)	**a**	0.68 (0.04)	**ab**	0.36 (0.14)	**b**
	280-340	1.00 (0.09)	**a**	0.61 (0.26)	**ab**	0.39 (0.09)	**b**
[4]*trans*/*cis*	0-40	1.10 (0.10)		1.09 (0.22)		0.98 (0.18)	
	40-100	0.84 (0.21)		1.04 (0.11)		0.94 (0.19)	
	100-160	1.26 (0.08)	**a**	1.13 (0.17)	**a**	0.45 (0.22)	**b**
	160-220	1.32 (0.09)	**a**	1.04 (0.04)	**a**	0.54 (0.27)	**b**
	220-280	1.33 (0.22)	**a**	1.16 (0.36)	**a**	0.24 (0.06)	**b**
	280-340	1.00 (0.41)	**a**	1.00 (0.06)	**a**	0.39 (0.20)	**b**

[1] 18:3ω6, 18:2ω6
[2] i15:0, a15:0, 15:0, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, cy19:0
[3] gram-positive: i15:0, a15:0, i17:0, a17:0; gram-negative: cy17:0, cy19:0
[4] UI: unsaturation index
[5] 18:1ω9t / 18:1ω9c

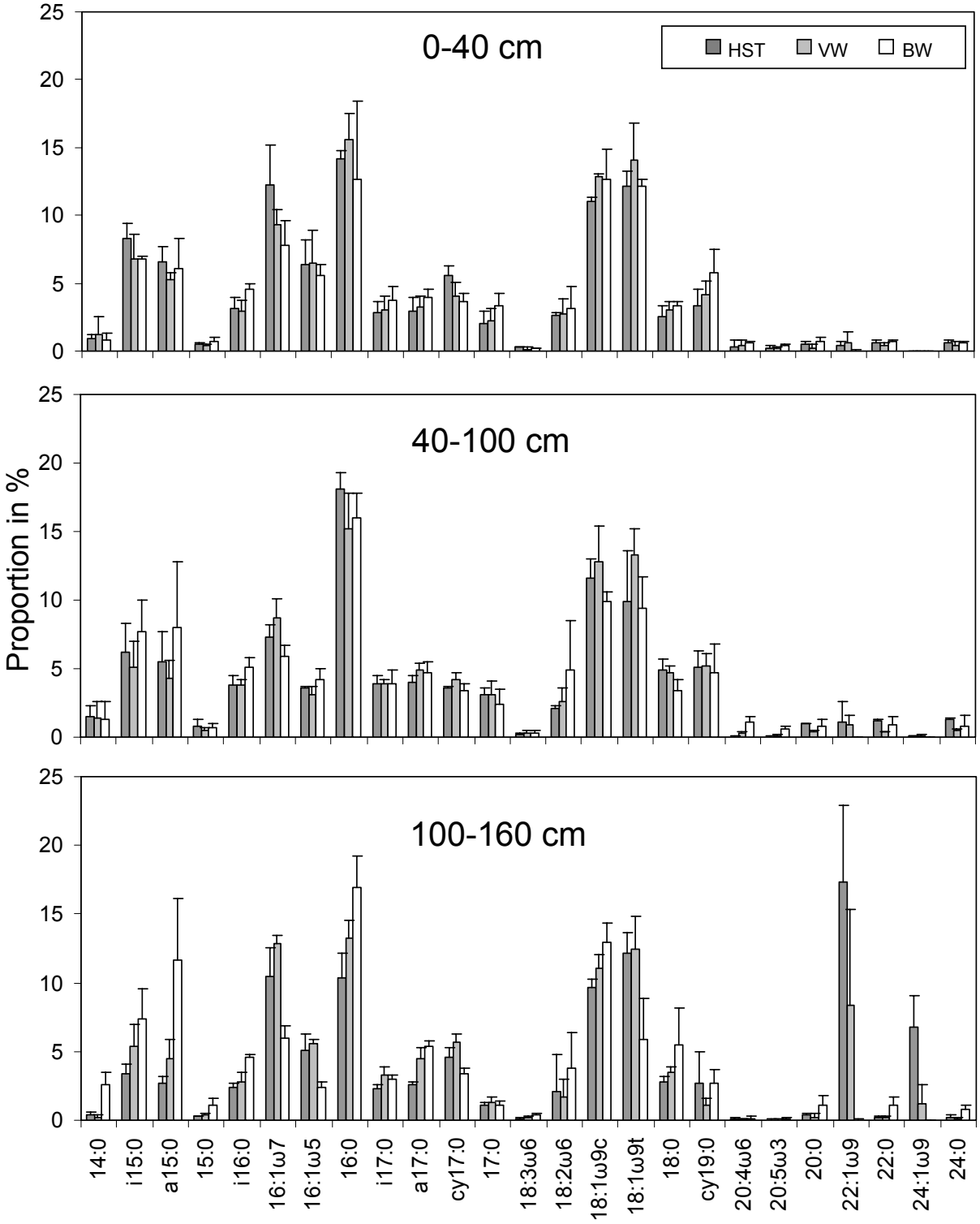
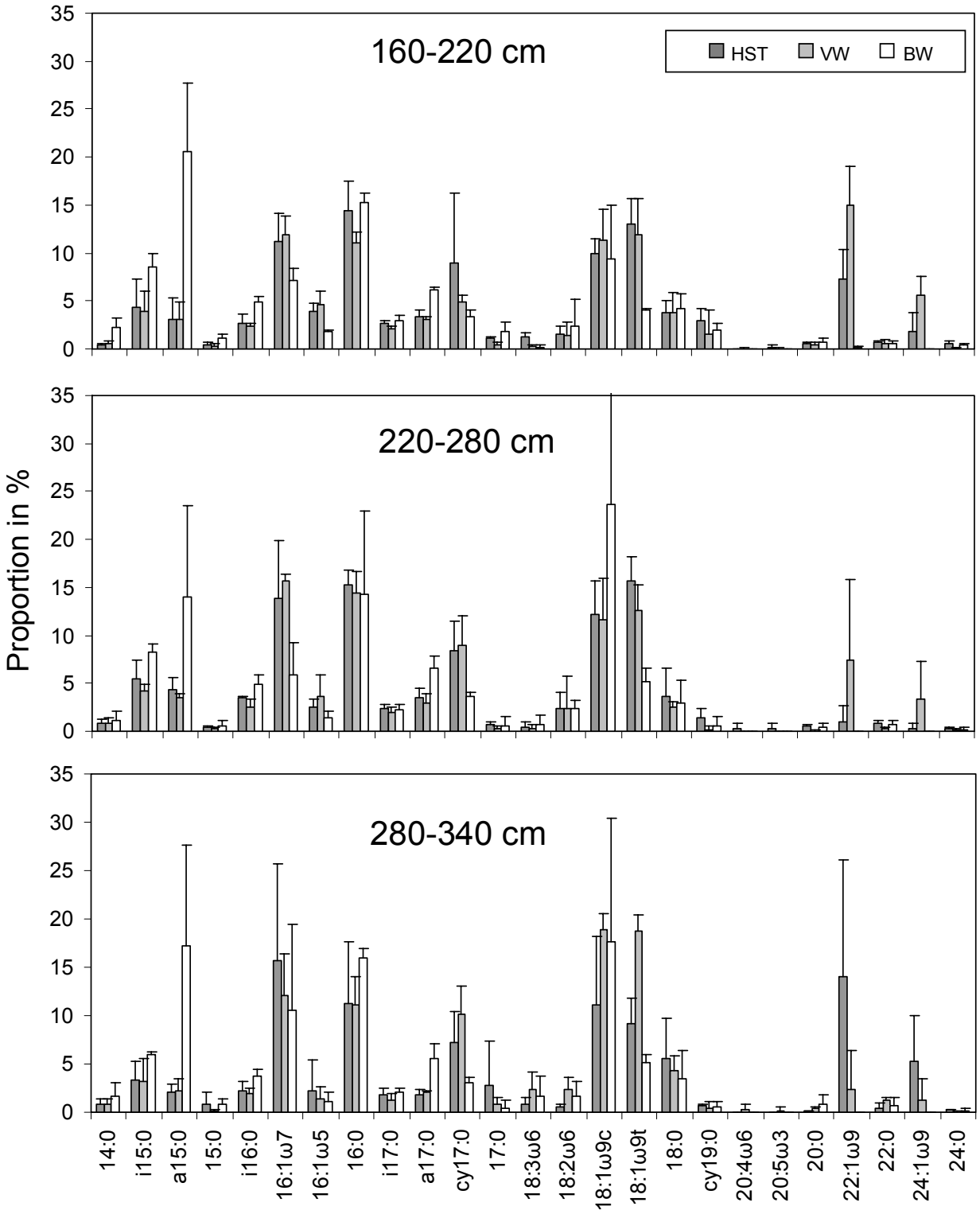


Fig. 2: Phospholipid fatty acid pattern (PLFA in % ± s.d., proportion > 1%) in six different depths (0-40, 40-100, 100-160, 160-22, 220-280, and 280-340 cm) at the watered sites HST and VW, and the non-watered site BW.



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PLFAs and soil parameters

Redundancy analysis (RDA) and Monte Carlo permutation test of PLFA proportions (%) and LOG transformed environmental variables separated the watered sites from the non-watered site, with HST and VW being closer related to each other compared to BW (Fig. 3a,b). The first axis ($F = 14.01$, $P = 0.001$) explained 56.1% and the second axis 14.5% of the variation in the PLFA and soil parameter data set. Gram-positive bacterial PLFAs (iso/anteiso) were more prominent in the non-watered site, whereas monoenoic PLFAs and cy17:0, with the latter as marker for Gram-negative or anaerob bacteria, occurred predominantly in the watered sites. Higher DOC concentrations ($F = 12.04$, $P = 0.001$) in BW, and higher pH-values ($F = 3.13$, $P = 0.009$) and SO_4^{2-} concentrations ($F = 2.7$, $P = 0.017$) in HST and VW, significantly contributed to the separation of watered and non-watered sites (Fig. 3b). Higher microbial biomass, expressed as sum of PLFAs ($F = 2.35$, $P = 0.038$), as well as higher carbon concentrations ($F = 1.9$, $P = 0.087$) separated the top soil (0-100 cm depth) from deeper soil layers, which is supported by the contrasting vector of depth ($F = 3.66$, $P = 0.005$).

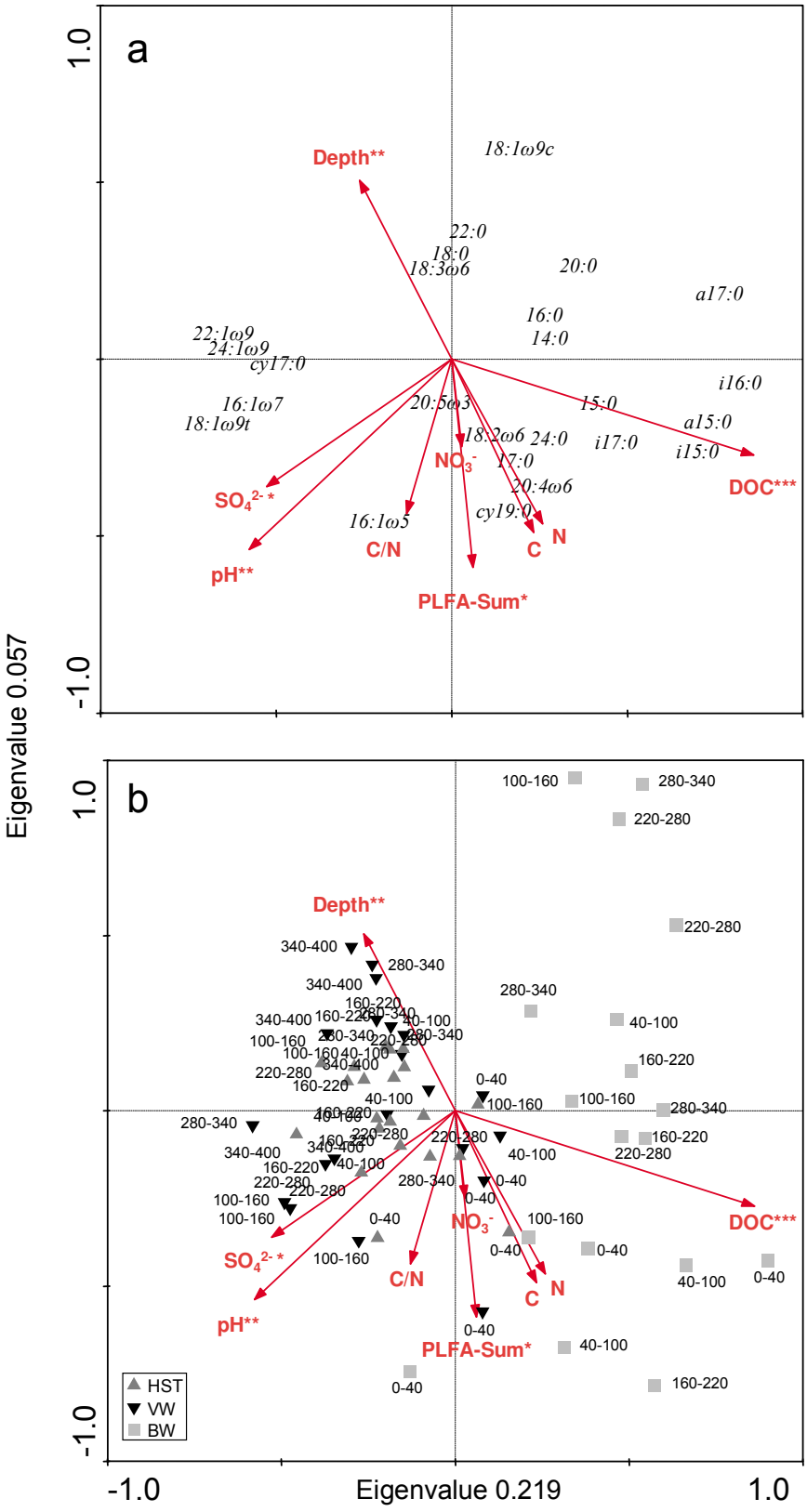

Fig. 3: Redundancy analysis (RDA) ordination biplots of phospholipid fatty acid pattern (%) and LOG transformed soil parameter data. a) species score plot with individual PLFAs, b) sample score plot. *, **, *** significant contribution to separation with $P < 0.05, 0.01, 0.001$, respectively.

PLFA pattern

Two-way nested ANOSIM ("depth" nested in "field") revealed different PLFA patterns between all fields (Global $R = 0.608$, $P = 0.001$; HST-VW: $R = 0.445$, $P = 0.002$; HST-BW: $R = 0.667$, $P = 0.002$; VW-BW: $R = 0.751$, $P = 0.001$) and at all depths (Global $R = 0.41$, $P = 0.001$). Grouping PLFAs into the depth compartments surface soil (0-100 cm), upper vadose zone (100-220 cm) and lower vadose zone (220-340 cm) indicated distinct differences due to water recharge practice by one-way ANOSIM and nMDS (Fig. 4a, b, c). In the surface soil all fields displayed comparable PLFA profiles, i.e. microbial community patterns (Global $R = 0.115$, n.s.; Fig. 4a). In the upper vadose zone the model was significant (Global $R = 0.63$, $P = 0.001$) and separated the watered sites from the non watered site (HST-VW: $R = -0.085$, n.s.; HST-BW: $R = 0.939$, $P = 0.002$; VW-BW: $R = 0.974$, $P = 0.002$; Fig. 4b). This corresponds to the differences in microbial community pattern of the lower vadose zone (Global $R = 0.41$, $P = 0.001$; HST-VW: $R = -0.074$, n.s.; HST-BW: $R = 0.504$, $P = 0.002$; VW-BW: $R = 0.807$, $P = 0.001$; Fig. 4c).

SIMPER analysis of the PLFA pattern of individual soil layers supported these findings with a more pronounced increase in average dissimilarity with depth for HST/BW and VW/BW, compared to HST/VW (Tab. 4). In general, PLFAs with a medium chain length accounted for the dissimilarity in the surface soil, whereas in the upper and lower vadose zone the contribution of long-chain PLFAs increased. Mainly responsible for the differences between watered and non-watered sites was the gram-positive bacterial PLFA a15:0 with higher proportions in BW from -40 to -340 cm (Tab. 4, Fig. 2). The dissimilarity contribution was at maximum in 160-220 cm depth with 23.76 and 20.34 for HST and VW, respectively, accounting for about half of the overall dissimilarity in this layer. Further, higher proportions of the bacterial PLFA 16:1ω7 contributed to the separation between all fields in the top soil

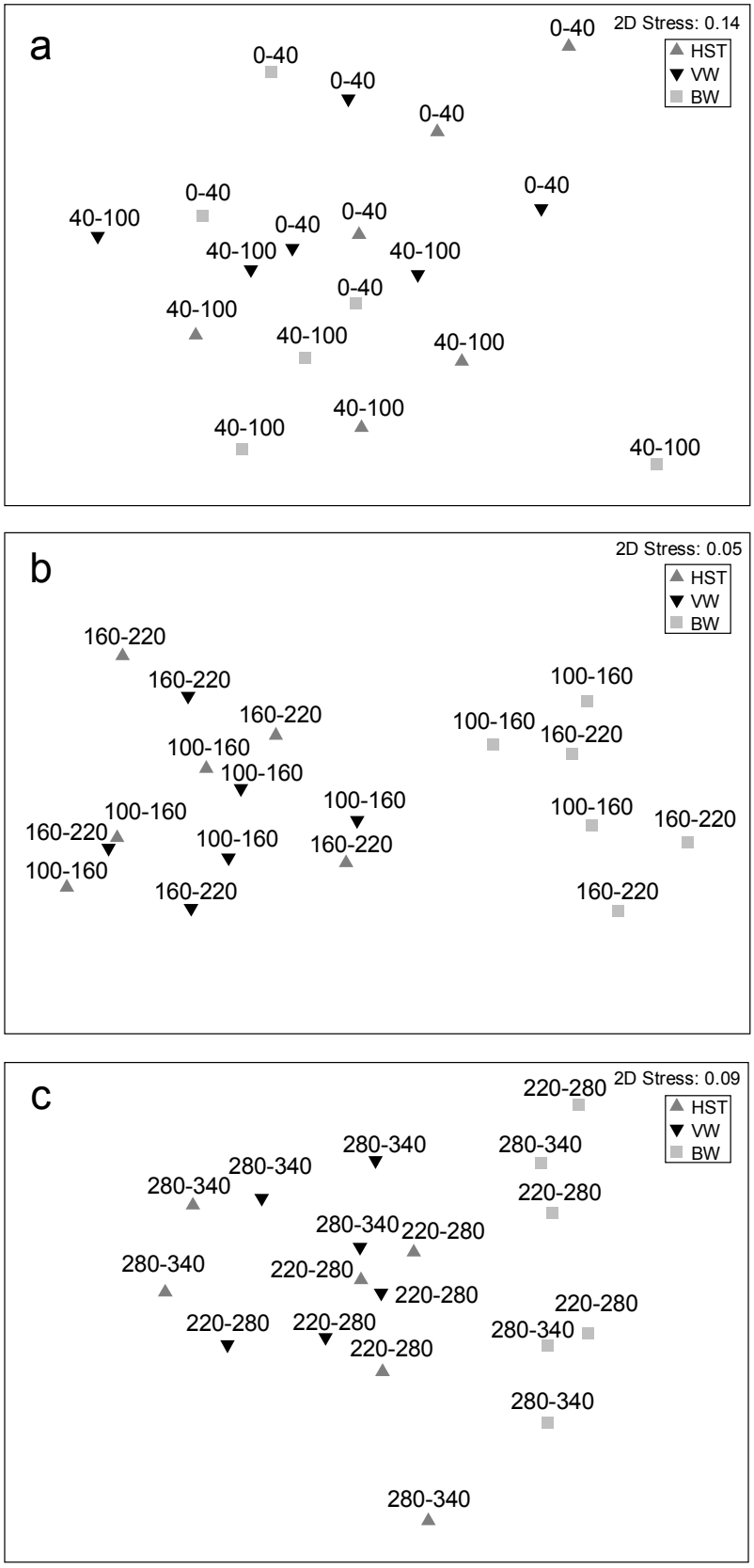

Fig. 4: Nonmetric multi-dimensional scaling (nMDS) of the watered sites HST and VW, and the non-watered site BW in 3 depths based on the proportion (%) of individual phospholipid fatty acids. a) shallow soil layer: 0-100 cm. b) upper vadose zone: 100-220 cm. c) lower vadose zone: 220-340 cm. Similarity index: Bray curtis.

layer (0-40 cm) and in the lower vadose zone. The stress attributed *cis* to *trans* conversion of the PLFA 18:1ω9 in bacteria occurred to a higher proportion in the upper and lower vadose zone of the watered sites. Higher proportions of the either plant or fungal derived PLFA 22:1ω9 were responsible for the separation between fields along the entire vadose zone. The impact of other fungal PLFAs was minor and only apparent in the surface soil layer, with highest proportions of the VA mycorrhizal biomarker 16:1ω5 in VW, and highest portions of the common fungal biomarker (saprotrophs, ectomycorrhiza) 18:2ω6 in BW. Interestingly, the predominantly eukaryotic nervonic acid (24:1ω9) contributed to the separation between fields in the upper vadose zone (Tab. 4).

PLFA indicator ratios

Generally, all PLFA based indices applied showed no differences between sites in the top soil layer from 0-100 cm (Tab. 2). Moreover, we found no differences in the fungal to bacterial PLFA ratio with depth or field (Tab. 1). On the other hand, the ratio of gram-postive to gram-negative bacterial PLFAs increased strongly with soil depth in BW, whereas it slightly decreased in HST and VW. The unsaturation index (UI), a measure of membrane fluidity, showed a reversed pattern in the upper vadose zone (100-220 cm depth) with significantly lower values in the non-watered site BW. The iso to anteiso ratios of the PLFAs 15:0 and 17:0, which denote changes in temperature and oxygen conditions, strongly decreased in BW, but not in the watered sites. Differences were most pronounced in the upper and lower vadose zone for i/a 15:0 with higher values in watered compared to non-watered sites. Generally, the i/a17:0 ratio had highest values in HST, followed by VW and the non-watered site BW. The stress indicating *trans*/*cis*-ratio of 18:1ω9 decreased with depth in BW, but

remained constant in HST and VW, resulting in significantly higher ratios in the watered sites in the upper and lower vadose zone (Tab. 2, Fig. 4).

Table 4: Average dissimilarity and contribution of single pospholipid fatty acids (PLFAs in%) to dissimilarities between fields (watered sites: HST, VW; non-watered site BW) in 6 depths (0-40 40-100, 100-160, 160-220, 220-280, and 280-340 cm). The four dominant PLFAs in each depth are presented. The field site with the greater proportion of the individual PLFA is indicated by the respective name. One-way SIMPER analysis. Similarity index: Bray curtis.

Depth (cm)	HST-VW			HST-BW			VW-BW		
0-40	average dissimillarity		12.86	average dissimillarity		15.56	average dissimillarity		14.87
	i15:0	7.77	HST	16:1ω7	14.85	HST	16:1ω7	6.9	VW
	16:1ω7	12.56	HST	16:0	15.77	HST	16:1ω5	6.38	VW
	16:1ω5	7.81	VW	cy17:0	6.28	HST	16:0	18.16	VW
	18:1ω9t	10.34	VW	cy19:0	7.69	BW	18:1ω9t	8.98	VW
40-100	average dissimillarity		13.22	average dissimillarity		16.08	average dissimillarity		17.83
	i15:0	7.83	HST	i15:0	7.54	BW	i15:0	8.37	BW
	16:0	11.31	HST	a15:0	11.65	BW	a15:0	11.26	BW
	18:1ω9c	8.03	VW	18:2ω6	9.74	BW	18:2ω6	8.32	BW
	18:1ω9t	15.28	VW	18:1ω9t	9.80	HST	18:1ω9t	10.91	VW
100-160	average dissimillarity		19.94	average dissimillarity		40.42	average dissimillarity		29.75
	16:1ω7	6.26	VW	a15:0	11.15	BW	a15:0	12.17	BW
	16:0	7.38	VW	16:0	8.11	BW	16:1ω7	11.48	VW
	22:1ω9	24.14	HST	22:1ω9	21.37	HST	18:1ω9t	11.14	VW
	24:1ω9	13.76	HST	24:1ω9	8.32	HST	22:1ω9	14.03	VW
160-220	average dissimillarity		22.16	average dissimillarity		36.94	average dissimillarity		43.00
	16:0	8.15	HST	a15:0	23.76	BW	a15:0	20.34	BW
	cy17:0	10.36	HST	cy17:0	7.52	HST	18:1ω9t	9.09	VW
	22:1ω9	17.58	VW	18:1ω9t	12.18	HST	22:1ω9	17.21	VW
	24:1ω9	8.50	VW	22.1ω9	9.61	HST	24:1ω9	6.56	VW
220-280	average dissimillarity		22.04	average dissimillarity		36.17	average dissimillarity		42.69
	16:1ω7	11.53	VW	a15:0	13.35	BW	a15:0	12.25	BW
	18:1ω9c	8.92	HST	16:1ω7	11.31	HST	16:1ω7	11.51	VW
	18:1ω9t	7.95	HST	18:1ω9c	17.05	BW	18:1ω9c	15.21	BW
	22:1ω9	15.97	VW	18:1ω9t	14.55	HST	18:1ω9t	8.81	VW
280-340	average dissimillarity		36.27	average dissimillarity		46.99	average dissimillarity		41.49
	16:1ω7	10.38	HST	a15:0	16.06	BW	a15:0	18.12	BW
	18:1ω9c	11.26	VW	16:1ω7	10.3	HST	16:1ω7	8.62	VW
	18:1ω9t	13.22	VW	18:1ω9c	11.03	BW	18:1ω9c	12.46	VW
	22:1ω9	18.18	HST	22:1ω9	14.85	HST	18:1ω9t	16.44	VW

Discussion

Effects of depth and flooding on soil parameters and microbial biomass

The physical and chemical soil characteristics at all sites showed a strong depth dependency, which was particularly evident for DOC, C, N, NO_3^- and SO_4^{2-}. Clearly, such plant derived solution-phase resources are readily mineralized in the surface soil. DOC, as a measure for groundwater quality, was reduced to about 86% in all fields, likely due to biodegradation and sorption processes (Thurman 1985). However, DOC concentrations of the non-watered site BW significantly exceeded both watered sites. This is presumably caused by the artificial groundwater recharge procedure, where higher water turnover rates may function as flush, diluting river- and litter-borne DOC in the interstitial water of flooded fields.

In contrast to DOC, regular flooding with river water enhanced the SO_4^{2-} and NO_3^- concentrations in watered compared to non-watered sites. That also applies to the groundwater received from the area in relation to river water (-71% for DOC, but +19% and +60% for SO_4^{2-} and NO_3^-, respectively; Rüetschi 2004). This indicates that the nutrient input due to water recharge is too high for their entire degradation from surface soil to groundwater table. Nevertheless DOC, NO_3^- and SO_4^{2-} concentrations of the groundwater in the “Langen Erlen”, which is a mixture of natural and artificial recharge sources, have remained low since decades (Waterworks Basel, Groundwater monitoring) and fulfill the tolerance values of the Swiss “Foreign Substance and Ingredient Order” (FIV). On average pH-values in both watered sites tended to be higher as in the non-watered site, most pronounced in deeper soil layers. Apparently, this is caused by the regular flooding with Rhine water at a relatively constant pH value of 8.2 (Waterworks Basel, Rhine water monitoring).

Generally, high recharge-vadose zones are characterized by high numbers of microorganisms due to the amendment with metabolizable organic substrates (Kieft

& Brockman 2001). However, microbial biomass, determined by the total amount of PLFAs, declined with soil depth in all fields, but did not differ due to water recharge treatment. This is likely related to the stopping of the flooding regime four weeks before sampling, i.e. comparable soil moisture contents and relatively low organic input at that date. Seasonal studies of the surface soil (0-10 cm) at the "Langen Erlen" (Schütz et al. 2008) revealed higher microbial biomass in the watered sites throughout the year, indicating that differences may be pronounced under flooding conditions. The significantly higher microbial biomass at HST in 40-160 cm depth was likely due to the deeper humus and fluvial silt horizon at that site.

In sum the nutrient supply was increased by regular flooding with river water, which was also apparent by the occurrence of nitrogen indicator plants such as *Urtica dioica, Rubus caesius/fruticosus,* and *Geum urbanum* at the watered sites. The improved nutritional status led to a rapid degradation of DOC by microorganisms using the additional nutrients as co-substrates. This is in line with Aelion & Bradley (1991), who found biodegradation of hydrocarbons to be best in the presence of nitrate. However, nutrient amendment via flooding did not result in an increased microbial biomass along the soil depth transect as reported by other studies (Balkwill et al. 1998, Ludvigsen et al. 1999, Kieft & Brockman 2001). This indicates that at comparable biomass to the non-watered field, the microorganisms at the watered fields effectively degraded supplementary substrate inputs, suggesting a more active microbial community at these sites.

Structure and physiological status of the microbial community

Considerable fractions of the total microbial biomass (42%, 26% and 25% in HST, VW and BW, respectively) were located in 40-340 cm soil depth, which contradicts the widespread opinion that subsurface microbial biomass is negligible. Fierer et al.

(2003) estimated a comparable distribution of microorganisms in a two meter deep soil profile with approximately 35% of the total biomass below 25 cm depth. This sizable biomass and the apparent high activity, as revealed by the capability for biodegradation, indicates that the contribution of the vadose zone microbial community cannot be ignored.

The microbial community structure, determined by PLFA patterns, differed between fields, predominantly between watered and non-watered sites. PLFAs are essential components of microbial membranes, and in soil they are in close contact with the environment. Changes in PLFA composition therefore directly reflect changes in the active microbial community to altered soil conditions (Frostegård et al. 1993, Zelles 1999). In the shallow soil layer, the microbial community of all fields showed comparable PLFA patterns, whereas in the vadose zone differences became obvious due to flooding. Either physical transport of microorganisms due to flooding or river water ingredients may have determined distinct microbial populations in watered sites. In common slow sand filter plants biodegradation processes are related to a biofilm matrix located on the top soil layer, which develops on a supporting sand corpus (Duncan 1988, Weber-Shirk & Weber-Shirk & Dick 1997). However, at our watered sites neither sand layer nor biofilm matrix are present. The shallow soil layer consists of loess loam, with litterfall and root exudates enhancing DOC concentrations (Rüetschi 2004). Thus, in the “Langen Erlen” the microbial community living below this uppermost soil compartment likely is responsible for biodegradation and water purification. Rüetschi (2004) showed highest purification rates in the vadose zone assessed by breakthrough curves of DOC and SAK254 (spectral adsorption coefficient at 254 nm). He suspected a combination of DOC adsorption during the 10-day flooding period, and DOC biodegradation in the following regeneration period as driving forces. The importance of vadose zone

microorganisms in purification processes is strongly supported by our study, where the differences in community composition between watered and non-watered sites were most pronounced in the upper vadose zone, but still distinct in the lower vadose zone.

With increasing depth, the proportion of the bacterial PLFAs 16:1ω5, 16:1ω7, cy17:0 and 18:1ω9t, and the long chained PLFAs 22:1ω9 and 24:1ω9 became more prominent in the flooded sites, whereas the proportions of the branched, saturated PLFAs i15:0, a15:0, i16:0 and a17:0, as representatives for gram-positive bacteria, became more dominant in the non-watered site. Bossio & Scow (1998) reported PLFA patterns of a rice field soil to respond to flooding and organic matter input; but contradicting to our results they observed reduced monoenoic PLFAs and increased branched PLFAs under flooding. However, the observations of Bossio & Scow (1998) are limited to the top soil layer, and therefore not directly comparable to our findings in the vadose zone. On the other hand, water stress, as investigated by Williams (2007) in irrigated and drought-prone prairie soils (0-10 cm depth), led to an increase in 16:1ω5 and 18:1ω9t, and a decrease in 15:0 and a17:0, which is comparable to our results. The two long chained PLFAs 22:1ω9 and 24:1ω9, which increased at depth, are usually not found in bacteria. Moreover, they are both scarcely described and their occurrence at depth remains obscure.

Generally, relative to gram-negative bacteria, gram-positive bacteria become more abundant with depth (Blume et al. 2002, Fierer et al. 2003). Balkwill & Ghiorse (1985) determined by transmission electron microscopy that 85-90% of cells from vadose zone samples were gram-positive, *Arthrobacter*-like bacteria, and Colwell (1989) reported a majority (84%) of gram-positive bacteria among heterotrophic plate count isolates from deep vadose zone samples. These results are in line with the composition of the microbial community at the non watered site BW. However,

periodic flooding resulted in a relatively constant gram-positive / -negative ratio with depth. Firstly, gram-negative bacteria show a positive correlation with moisture (Kieft & Brockman 2001). Secondly, they are considered to be fast growing, to utilize a variety of C sources, and to adapt quickly to changing environments (Pondor and Tadros 2002, Hinojosa et al. 2005). Environmental conditions, e.g. nutrients and moisture, regularly alternate at the watered sites, which likely favored the presence of gram-negative bacteria. Ludvigsen et al. (1999) found a two fold increase of both, gram-positive and gram-negative bacteria close to a landfill leachate contaminated aquifer (DOC > 25 mg l^{-1}), and an increase in the proportions of saturated PLFAs at the uncontaminated site. If we assume higher nutrient availability in the watered sites these findings only partly support our results and solely concern gram-negative bacteria and saturated PLFAs. Obviously, periodic flooding and concomitant factors altered the microbial community at the watered sites, which is most pronounced in the upper vadose zone. Presumably, this is the main water purification site, and the predominant responsible functional group are gram-negative bacteria.

Generally, increasing *trans/cis* or cyclo/precursor ratios of unsaturated PLFAs are used as indicators for starvation or stress in microorganisms (Guckert et al. 1986, Heipieper et al. 1992, Kieft et al. 1994, White et al. 1996), thereby the term “stress” is used in a broad sense and comprises different environmental conditions like the exposure to toxins, starvation or desiccation. At our watered study sites the *trans/cis* ratio of 18:1ω9 exceeded the non-watered site from 100 cm downwards with 1-1.33 to 0.24-0.54, respectively. Firstly, bacteria transported downwards by water recharge, are exposed to altered environmental conditions and to enhanced competition with established species, resulting in the higher stress values observed (Balkwill 1998). Secondly, the residing microbial community at depth may be affected by alterations in temperature or oxygen conditions. In contrast, at the non-watered site bacterial

movement through macropores is limited and environmental conditions likely less variable. In line with Bossio & Scow (1998) flooding with river water did not affect stress indicators (*trans/cis* and cyclo/precursor ratios) in the shallow soil layer. Williams & Rice (2007) even found a reduction in the physiological stress (cyclo/precursor ratio) of the microbial community in soils of irrigation plots (0-10 cm). Thus, a total or selective transport of microbes through the soil profile and the changes in environmental conditions in the vadose zone appear to be the main factors for increasing or constantly higher stress there.

This impact of flooding on physiological conditions of the microbial community residing at the "purification sites" was apparent in further metabolic indicators. Like for the *trans/cis* ratio, similar changes with soil depth and differences between watered sites and BW were observed for the *iso/anteiso* ratio of 15:0 and 17:0. These PLFAs predominantly derive from gram-positive bacteria (Haack et al. 1994), and changing ratios can be related to different environmental conditions for this functional group. Increasing *iso/anteiso* ratios were reported at higher temperatures, due to maintenance of membrane fluidity (Kaneda 1991, Peterson & Klug 1994). In the watered sites, soil temperature is periodically determined by the temperature of the infiltrating Rhine water. Compared to air temperature flooding leads to enhanced (+8 °C in November), and more homogenous temperatures within the soil profile throughout a year (Rüetschi 2004). The increase in *anteiso* series at BW likely is a metabolic respond to maintain membrane fluidity at the lower soil temperatures there. However, it should be noted that some gram-negative, anaerobe sulfate reducing bacteria (e.g. *Desulfovibrio, Desulfobacter*, *Desulfobulbus*) also contain *iso* and *anteiso* PLFAs (Parkes & Taylor 1983, Taylor & Parkes 1983, Kaneda 1991) and produce, relative to oxygen availability, higher proportions of the *anteiso* branching. Consequently, higher ratios in the vadose zone of the watered sites can either be

caused by relatively constant, respectively higher soil temperatures in wintertime and/or periodically anaerobic conditions during flooding. Anaerobic conditions are also indicated by higher amounts of cyclopropyl PLFAs, whereas aerobic conditions can be linked to the occurrence of monoenoic PLFAs (Guckert at al. 1985; Vestal & White 1989). At the watered sites we found higher amounts of cy17:0 as well as monoenoic PLFAs compared to the non-watered site, thus both conditions, aerob and anaerob, may occur.

The unsaturation index (UI), another measure for membrane fluidity, showed significantly higher ratios in the upper vadose zone of the watered sites. Moreover, it is used as a marker for C limitation, or more generally, nutrient limiting conditions in soil (Zelles et al. 1992, Kieft et al. 1997, Bossio & Scow 1998), with higher ratios (UI) in soils with high organic input and lower ratios in soil under nutrient deprivation. The enhanced unsaturation index (UI) in the vadose zone fits to our assumption of higher nutrient input and turnover in this biologically active layer of the watered sites.

Conclusions

Groundwater recharge performed in the “Langen Erlen” clearly affected community structure, physiological status and metabolic activity of the soil inhabiting microorganisms. These changes likely derive from enhanced resource availability and altered environmental conditions due to flooding with river Rhine water. Differences between watered sites and the non-watered were most pronounced in the upper vadose zone (100-220 cm), but still apparent in the lower vadose zone (220-340 cm). Thus, a biologically active layer has developed below the rooted zone, which is mainly responsible for water purification. Besides nutrient input, flooding may cause passive transport of microbial cells as well as changes in temperature and aerobic/anaerobic conditions. Under these circumstances, the competitive ability

of each microbe determines the microbial community. This has led to the establishment of functional groups well adapted to the specific resources and environment.

Microbial biomass was not significantly enhanced throughout the soil profile at the watered sites, indicating the community at the “purifications sites” in the upper vadose zone to be highly active. General terrestrial nutrient limitations (e.g. nitrogen) are reversed at the watered sites and thereby led to a capable microbial community, which effectively degrades organic compounds. The slightly increased nitrate and sulfate concentrations in the groundwater of the recharge site may be a hint that the inhabiting microbial community is working under full capacity. However, with few signs of degeneration, the system may well function for another century or longer.

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Chapter Three

Soil structure and nutrient availability determines enzyme activity in a vertical transect from top soil to groundwater table

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Abstract

Subsurface microorganisms are essential constituents of the purification processes associated with groundwater quality. Thereby, soil enzyme activity determines biodegradation of natural and xenobiotic organic compounds passing through the soil profile. Depth transects to approximately 4 m from two groundwater recharge sites and one control site were investigated for chemical and physical soil characteristics. The microbial communities were analyzed for their biomass (C_{mic}) and activity (CO_2 and specific respiration, qCO_2) via substrate induced respiration (SIR) and their functional biodiversity via eight hydrolytic enzymes. Microbial activity was highest at the watered sites, whereas biomass did not differ between sites. Acid phosphatase was the dominant active enzyme, followed by L-leucine aminopeptidase and β-glucosidase, across sites and depth. No differences in *absolute* and *specific* enzyme activities between watered sites and the non-watered site indicated complex organic matter input at recharge sites to be impeded by flooding water pretreatment. The *relative* enzyme activity pattern significantly separated all sites, presumably due to differences in soil structures and not to flooding regime. Overall this implies, that adding labile C (i.e. DOC by flooding) to a soil where additional nutrients are limiting microbial growth (i.e. P as indicated by acid phosphatase) increases microbial activity but not biomass, and results in waste respiration by overflow metabolism.

Keywords: Exoenzymes, Flooding, Groundwater recharge, Microbial activity, Vadose zone, Filtration

Introduction

Subsurface microbial, chemical and physical soil processes directly influence groundwater chemistry by biodegradation, adsorption and dilution of organic and inorganic matters. These mechanisms are essential for purification processes associated with drinking water production. The latter is performed by artificial groundwater recharge near Basel (Switzerland) in a former floodplain area called “Lange Erlen” since 1912. Embanked forest sites are periodically (max. 10 days) flooded with water from the river Rhine to augment groundwater resources. This contrasts the commonly used unvegetated slow sand filter systems with long flooding periods and a biologically active layer at the soil surface predominantly responsible for water purification (Duncan 1988, Weber-Shirk & Dick 1997, Peters et al. 1998). The recharge areas in the “Lange Erlen” comprise no such biofilm, but show constant and satisfactory purification capacities since the system has been established (Rüetschi 2004). There is need to assess the metabolic activity and function of subsoil microorganisms in order to understand the transformation and degradation of both, natural and xenobiotic compounds passing through the soil profile.

Kandeler et al. (1996) hypothesized, that the microbial composition of a soil determines it’s potential for substrate catalysis since most of the processes occurring in soil are microbe-mediated and carried out by enzymes. Soil enzyme activities therefore are a measure of the metabolic requirements and available nutrients for soil microbial communities. Extracellular enzymes are produced by microorganisms to decompose complex insoluble polymers (e.g. cellulose, chitin, protein) to dissolved soil organic matter (SOM). This exoenzyme-driven breakdown predicts a flow of low molecular weight substrates (DOC, DON) suitable for microbial uptake (Schimel & Weintraub 2003). Generally, free enzymes are short-lived as they can be rapidly denatured, degraded or inhibited (Sarkar et al. 1989). However, a considerable

proportion may be bound to clay and humic colloids in soil, presenting an immobilized or accumulated enzyme fraction, which persists even in harsh conditions, where microbial activity is inhibited (Burns 1981, Sarkar et al. 1989, Nannipieri et al. 2002, Marx et al. 2005).

Exoenzyme production in microorganisms can either be induced by the occurrence of specific substrates (particulate organic matter) or by the lack of nutrients, whereas the addition of superior catabolites (i.e. glucose) can result in repression (Chrost 1991, Beck & Beck 2000, Shackle et al. 2000, Nannipieri et al. 2002). Speir & Ross (2002) reported that intra- and extracellular soil enzyme activities increased in proportion to microbial biomass and soil organic carbon content. Slightly different results were shown in contamination field studies, where at moderate levels of oil pollution, some soil enzyme activities declined and some increased, whereas most microbial populations increased (Kiss et al. 1998). Moreover, carbon supply studies revealed that the addition of complex carbon sources, such as cellulose or sewage can lead to enhanced bGLC activities (Shackle et al. 2000).

Flooding in groundwater recharge areas and the load of organic material herewith, affects mineralization and degradation performed by microorganisms. Mentzer et al. (2006) studied wet prairie macrocosms and showed increased *specific* enzyme activities (*absolute* enzyme activity divided by microbial biomass) at constant flood treatments (up to 3 months), whereas *absolute* enzyme activity was only little affected. They suggested an increase of either microbial activity or mobility of extant soil enzymes being responsible for the higher enzyme activities. Burns & Ryder (2001) found strong temporal peaks of *absolute* enzyme activities in floodplain sediments at the beginning of flooding (1 to 7 days), followed by a general decline on day 7 to 21, likely caused by substrate limitation or inhibition by end products of hydrolysis. Moreover, they showed a strong positive correlation between dissolved

organic carbon (DOC) concentration and glucosidase activity, providing evidence that DOC is a potential food resource in semi terrestric or aquatic systems. Hendel et al. (2001) investigated extracellular enzymes of an artificial groundwater recharge plant and observed decreasing enzyme activities in water samples along the water purification passage. Based on this they concluded that DOC was effectively degraded and the recharge plant fulfilled its function.

Transport, adsorption and mobilization of organic and inorganic matter through the soil profile are also influenced by microbial metabolic activity. However, studies on exoenzymes at soil depth are scarce. Taylor et al. (2002) present data from 0 to 4.2 m, and Venkatesan & Senthurpandian (2006) from 0 to 2 m depth. Both studies report significant decreases in enzyme activity with increasing depth, and relate this to the corresponding decrease in soil organic matter content and microbial biomass. To the best of our knowledge, no investigations combining the impact of flooding and soil depth on extracellular soil enzyme activities exist. However, knowledge on the functional microbial ecology in the unsaturated subsurface, predominantly the vadose zone, is essential in terms of the capable biological activity necessary for biodegradation of organic matter in water purification processes.

The present study investigates the microbial biomass, activity and functional diversity of subsurface communities in soil depth transects (approx. 4 m deep) from two watered groundwater recharge sites and one non-watered site. Additionally, physical and chemical soil parameters were analyzed. Microbial parameters were investigated using the substrate induced respiration method (SIR) and functional diversity was assessed by a range of hydrolytic enzymes, involved in C, N, and P processes, using a fluorimetric microplate assay. Periodic flooding is expected to enhance nutrient supply and alter microbial community structure. The aim of the present study was to assess the related functional activities resulting from that potential. Enzyme activities

can increase due to added suitable substrates that prompt production, and/or a corresponding stimulated growth of the microbial population. Physicochemical changes in the soil environment by flooding events likely superimpose on these processes and initiate activation of extracellular enzymes immobilized in soil. Overall, these effects are expected to result in distinct exoenzmye activities along soil depth transects and with flooding regime.

Materials and Methods

Study site

The study site “Lange Erlen” serves as groundwater recharge area for the city of Basel (Switzerland) since 1912. Today approximately half of the drinking water for the city is gained here (15 x 10^6 m^3 ha^{-1} y^{-1}). The former natural floodplain area (size ~3 km^2) extends along the river Wiese, a straightened tributary of the river Rhine, descending from the Black Forest, Germany. The semi-natural forest sites are classified as Galio - Carpinetum oak - hornbeam forests (Burnand & Hasspacher 1999). Along with water recharge processes the recharge areas have been modified by human activities, predominantly with landfill and afforestation (*Populus canadensis*, *Fraxinus excelsior*, *Alnus nigra*, *Salix* spp.) The soil texture, pH-values, carbon and nitrogen concentrations, the C/N-ratios and the soil moisture contents of the study sites are summarized in Table 1.

Table 1: Soil parameters in 6 soil depths (0-40, 40-100, 100-160, 160-220, 220-280, and 280-340 cm) at the watered sites HST and VW, and the non-watered site BW. Values in parentheses are the standard deviation. Means within a row with the same or no letter are not significantly different (Tukey`s HSD, $P < 0.05$)

	Depth	HST	VW	BW
Texture	0-40	landfill loam	landfill loam	landfill loam, gravel
	40-100	landfill loam	gravel, sand	silt, sand, gravel
	100-160	landfill loam, gravel, sand	gravel, sand	gravel, sand
	160-220	gravel, sand, silt, clay	gravel, sand, silt	gravel, sand, silt, clay
	220-280	gravel, sand, silt, clay	gravel, sand, silt	gravel, sand, clay
	280-340	gravel, sand	gravel, sand, silt, (clay)	gravel, sand, clay
pH	0-40	7.39 (0.13)	7.18 (0.07)	7.08 (0.79)
	40-100	6.96 (0.23)	7.37 (0.11)	6.87 (0.68)
	100-160	7.38 (0.13)	7.42 (0.07)	6.73 (0.70)
	160-220	7.31 (0.19)	7.47 (0.05)	6.68 (0.50)
	220-280	7.30 (0.10) **ab**	7.43 (0.18) **a**	6.62 (0.49) **b**
	280-340	7.28 (0.13) **ab**	7.45 (0.07) **a**	6.56 (0.66) **b**
%C	0-40	2.05 (0.80)	2.14 (1.16)	1.74 (0.63)
	40-100	1.11 (0.60)	0.50 (0.13)	0.56 (0.21)
	100-160	0.60 (0.21)	0.34 (0.07)	0.27 (0.09)
	160-220	0.43 (0.07) **ab**	0.27 (0.04) **a**	0.64 (0.23) **b**
	220-280	0.41 (0.17)	0.25 (0.01)	0.31 (0.25)
	280-340	0.25 (0.01)	0.26 (0.02)	0.32 (0.22)
%N	0-40	0.14 (0.02)	0.23 (0.11)	0.18 (0.07)
	40-100	0.11 (0.05)	0.06 (0.01)	0.06 (0.03)
	100-160	0.06 (0.02) **a**	0.05 (0.01) **a**	0.03 (0.01) **b**
	160-220	0.05 (0.01) **ab**	0.04 (0.01) **a**	0.07 (0.02) **b**
	220-280	0.05 (0.02)	0.03 (0.00)	0.04 (0.03)
	280-340	0.03 (0.01)	0.03 (0.00)	0.04 (0.03)
C/N	0-40	14.15 (3.64)	8.97 (1.12)	9.76 (0.55)
	40-100	9.22 (2.06)	8.73 (0.89)	8.85 (0.95)
	100-160	10.27 (0.95) **a**	7.39 (0.16) **b**	8.09 (1.41) **ab**
	160-220	8.83 (0.24)	7.50 (0.38)	8.88 (1.06)
	220-280	8.23 (1.16)	7.82 (0.24)	6.80 (2.38)
	280-340	8.42 (1.74)	7.48 (0.58)	7.01 (0.78)
% dry weight	0-40	80.69 (3.64) **ab**	79.05 (1.20) **a**	86.10 (2.62) **b**
	40-100	83.42 (0.66) **b**	90.89 (0.18) **a**	90.29 (3.30) **a**
	100-160	89.99 (0.67) **b**	92.96 (0.53) **a**	94.83 (1.07) **a**
	160-220	92.38 (0.85)	91.92 (1.02)	89.39 (3.32)
	220-280	91.99 (0.74)	92.34 (0.17)	93.64 (3.23)
	280-340	93.60 (0.95)	91.98 (0.22)	93.73 (1.53)

The first sampling site “Hintere Stellimatten” (HST) is the youngest flooding area and started operating after afforestation in 1977. Due to excess infiltration rates (11 m^3 m^{-2} d^{-1}) an artificial landfill (loess loam) and bulldozing was performed in 1981. The site is mainly overgrown with an alder swamp forest (*P. canadensis, A. nigra, Salix* spp.*)* and the herb layer is dominated by *Rubus caesius and Urtica dioica.* Unvegetated sites comprise some spots of *Poa trivialis, Potentilla reptans, Agropyron repens* and *Geum urbanum*. The second site, “Verbindungsweg” (VW), was established as flooding area after afforestation and artificial landfill (loess loam) in 1970. The forest consists of some old poplars, ash-leaved maple (*Acer negundo*) and oak trees. The understory is dominated by *Lysimachia nummularia, Urtica dioica, Phalaris arundinacea, R. caesus / fruticosus, Duchesnea indica, Iris preudacorus, Seneco aquaticus* and *Cardamine pratensis.* The shady northern part is dominated by poplars and the soil bare of vegetation. The non-watered site “Bachtelenweg” (BW) is situated between these two flooding areas and is agriculturally used with extensive biological grassland management.

Infiltration system

Wooded flooding areas (11, total area 22 he) are periodically flooded with prefiltered water from the river Rhine. Prefiltration is performed by a rapid sandfilter (80 cm quartz sand layer), where approximately 95% of complex particulate matter is eliminated (Rüetschi 2004). Each wooded flooding area (10,000 – 20,000 m^2) is divided into three fields (3000 – 8500 m^2) by small dams (height ca. 50 cm). Watering cycles usually consist of 10 days flooding and 20 days drying and regeneration, however, longer interruptions due to revisions occur. Depending on soil surface structure the water fills up to variable heights (max. 50 cm) and drains with a speed of 1-2 m d^{-1} through the soil surface (Tab. 1). After 2-3 days flooding, the

groundwater table rises from -4-5 m to -2-3 m depth. Subsequently, the water flows horizontally (from northeast to southwest) in the aquifer for 200-800 m. After 10-30 days purified water is pumped out of groundwater wells, collected and, after a brief chemical treatment with ClO_2, delivered to consumers.

Soil Sampling

Three vertical core drillings (diameter 25 cm) from the soil surface down to a maximum of 4.5 m depth, or alternatively groundwater level, were conducted at each watered site (HST, VW) in November 2005. Four weeks before drilling, the groundwater level was lowered by a stop of the flooding regime. Soil cores were excavated in 0.5 m intervals and placed carefully into soil core boxes, representing the full soil profile along the depth transect. Soil samples (100 g) were taken in 30 cm distances with a shovel from the inner part of the cores, transported to the laboratory and frozen (-20°C) until analysis. Overall, 45 soil samples were taken in each, HST and VW. During drain installations in the non-watered site BW, three horizontal soil samplings through the face of the trench down to 3.5 m depth could be performed in the same time period. Three depth transects were randomly chosen and 34 soil samples were directly taken from the inner trench wall and frozen (-20°C) until analysis. Prior to all analyses the required soil substrate was thawed in the fridge (24 h, 8°C). Visible organic material (fine roots and leaves) and stones were removed, and soil was sieved (5 mm).

Soil analysis

Chemical and physical soil characteristics

Soil samples were analyzed for pH, soil moisture, carbon (C), nitrogen (N), dissolved organic carbon (DOC), nitrate (NO_3^-), and sulfate (SO_4^{2-}) content. Soil pH was

measured in soil salt (0.01 M CaCl) solution (1:4 w/v) after stirring and incubating at room temperature for 1 h. Soil moisture content was determined after soil sieving by the mass difference before and after drying at 105°C for 24 h. Total carbon and nitrogen content were determined from oven-dried and pulverized (swing mill, Retsch MM 200) aliquots of the bulk soil with an element analyzer (CHN 1000, Leco, USA). Results are expressed in % per soil dry weight. DOC, nitrate and sulfate was extracted from 5 g soil (fresh weight) for the 0-200 cm depths and from 10 g for the 200-450 cm depths. A soil salt (0.01 M CaCl) solution was prepared (1:4 w/v), shaken for 1 h (200 rpm) and centrifuged for 15 min (2000 rpm). The supernatant of each soil solution was filtered through sterile 0.45 µm membrane filters (Millex HA) and stored in the fridge until analysis (max 1 h). The sample solutions were analyzed for DOC after acidification and air-purging (N55, O45, Carbagas, CH) with a TOC analyzer (TOC-5000 A, Shimadzu) in quintuplicate. Nitrate and sulfate was measured in an ion chromatograph (IC-690, Metrohm). All results are expressed in mg g^{-1} DW soil.

Microbial biomass (C_{mic}), soil respiration (O_2) and specific respiration (qO_2)

Microbial biomass (C_{mic}), soil respiration (O_2) and specific respiration (qO_2) were determined after 3 d of soil incubation at room temperature (18°C) by substrate-induced respiration (SIR) using an automated electrolytic O_2 microcompensation apparatus (Scheu 1992). The initial weight of soil samples depended on their depth: for the 0-40 cm depths 2 g and for all other depths 4 g soil (DW) was taken in order to compensate for the decrease in microbial biomass with depth. Soil respiration (O_2) was measured at 22°C with readings every 30 min and determined from the average O_2 consumption (µl O_2 g^{-1} h^{-1}) after equilibration in the hours 10-20.

For the determination of microbial biomass, soil samples were supplemented with 8 mg glucose g^{-1} DW. Glucose was added as an aqueous solution adjusting the water content to 80% of the water holding capacity. The mean of the four lowest measurements was taken as the maximum initial respiratory response (MIRR) and microbial biomass C (µg C_{mic} g^{-1} DW) was calculated as 38 × MIRR (µl O_2 g^{-1} h^{-1}) (Beck et al. 1997). From the data on microbial biomass and soil respiration the specific respiration (qO_2) was calculated as µl O_2 mg^{-1} h^{-1} C_{mic} DW.

Enzyme analyses

A range of hydrolytic enzymes, involved in C, N, and P processes, were investigated using a fluorimetric microplate assay (Marx et al. 2001). The activities of α-glucosidase (EC 3.2.1.20), β-glucosidase, (EC 3.2.1.21), N-acetyl-β-glucosaminidase (EC 3.2.1.30), β-xylosidase (EC 3.2.2.37), β-cellobiohydrolase (EC 3.2.1.91), acid phosphatase (EC 3.1.3.2), leucine aminopeptidase (EC 3.4.11.1) and tyrosine aminopeptidase (EC 3.4.11) were measured using 4-methylumbelliferyl (MUB)-α-1,4-glucoside, MUB-β-1,4-glucoside, MUB-N-acetyl-β-1,4-glucosaminide, MUB-β-xylopyranoside, MUB-β-cellobioside, MUB-phosphate, L-leucine-7-amido-4-methyl-coumarin and L-tyrosine-7-amido-4-methyl-coumarin as substrates, respectively. Enzyme abbreviations and enzyme functions are listed in Table 2. Enzyme activities were determined as the rate of release of 4-methylumbelliferone (MUB) and 7-amino-methylcoumarin (AMC) from the MUB- or AMC- labeled substrates, respectively. Before measuring enzyme activity an aqueous soil suspension (freshweight soil: water = 1:50 for the 0-40 cm depth and 1:25 for the deeper soil layers) was prepared from each soil sample. Of each 50 µl was transferred in triplicate into the vials of a 96-well microplate, 50 µl of buffer (100 mM MES acid buffer, pH 6.1 for carbohydrases and phosphatase or 50 mM TRIZMA buffer, pH 7.8 for

aminopeptidase) and 100 µl substrate was added, giving a final substrate concentration of 500 µM. Microplates were incubated at 30°C in an incubator, and the release of MUB and AMC was measured after 0, 30, 60, 120 and 180 min using an automated luminescence spectrophotometer (Biolumine^{TM}, 960 Molecular Dynamics; emission 446 nm, excitation 377 nm). The fluorescence produced was converted into an amount of MUB/AMC according to a soil-specific standard calibration. This consisted of increasing amounts of substrates (0, 10, 20, 50, 80 and 120µl) and constant amounts of soil solution (50µl), and corrects for possible quenching effects on the fluorescence intensity of MUB/AMC. Absolute enzyme activities were reported in nmol MUB/AMC g^{-1} h^{-1} DW. The specific enzyme activity was calculated by dividing absolute enzyme activity by the microbial biomass and reported as (nmol MUB/AMC g^{-1} $h^{-1}C_{mic}$ DW).

Further, enzyme activity ratios were determined from β-glucosidase / acid phosphatase and from acid phosphatase / N-acetyl-β-glucosaminidase to get insight into the microbial community response to changing nutrient resources and the relative importance of different nutrients (Caldwell 2005).

Table 2: Enzymes analyzed with abbreviations and respective general function

Enzyme	Abbreviation	Cycle	Function
α-D-Glucosidase	aGLUC	C	Starch (maltose) degradation
β-D-Glucosidase	bGLUC	C	Cellulose degradation
Cellobiohydrolase	CELL	C	Cellulose degradation
β-Xylosidase	XYLO	C	Hemicellulose (xylan) degradation
N-Acety-β-Glucosaminidase	GLAM	C, N	Chitin and glycoprotein (murein) degradation
L-Leucine	LEU	N	Peptide degradation
L-Tyrosine	TYR	N	Peptide degradation
Phosphatase	PHOS	P	Poly- / ester-phosphate degradation

Statistical analyses

Samplings were conducted in triplicate for each field site and summarized in 7 depths: The surface soil (rooted zone) with 0-40 and 40-100 cm, the upper vadose zone with 100-160 and 160-220 cm and the lower vadose zone with 220-280, 280-340 and 340-400 cm. Statistical analysis were performed on enzyme ratios, LOG transformed soil parameters, microbial parameters and enzyme (*absolute* and *specific* activity) data and on *relative* enzyme activities (%) in the different soil depths. Soil parameters, microbial parameters, *absolute* and *specific* enzyme activities and enzyme ratios were analyzed for field- and depth- differences using repeated ANOVA procedures. As the 340-400 cm depth only exists for the watered sites HST and VW, this layer was excluded from analysis. Pair wise comparison of means was performed using Tukey`s honestly significance difference test (HSD). Repeated measure ANOVA and Tukey`s HSD were calculated using SAS (SAS Institute, Cary, NC).

Multivariate data analysis of the *relative* enzyme activity pattern was carried out with proportional amounts (%) of individual enzymes in distinct depths by analysis of similarities (ANOSIM), to determine weather the *relative* enzyme activity pattern differed between sites and/or depths. Prior to analyses the Bray-Curtis similarity index was applied to the data set. Two-way ANOSIM was calculated with the factors “depth” nested in “field”. Multivariate data analyses were calculated with Primer 6.1.5 (Clarke & Warwick 2006).

To visualize study site differences in the *absolute* enzyme activity pattern in relation to soil and microbial parameters, triplot ordinations of redundancy analysis (RDA) and the Monte Carlo permutation test (MCPT) was used. Additionally, the relation between structural and functional diversity at each field site was analyzed by RDA and MCPT on the basis of the respective PLFA pattern (Schütz et al. 2008b) and the

respective *specific* enzyme activity pattern. The use of the linear method RDA was justified by the length of gradients being shorter than 3.0 in a detrended canonical correspondence analysis (DCCA) (CANOCO 4.5, Lepš & Šmilauer 2003).

Results

Soil parameters

The investigated field sites differed in soil texture predominantly in the upper soil layers (Tab. 1). Landfill loess loam expanded to about 160 cm depth at HST, whereas it constituted only the top soil layer (0-40 cm) at VW and BW. Subsequently, sand and gravel followed at all sites down to 340 cm depth. Interspersed silt and clay lenses were equally present at HST in 160-280 cm depth, but not detected in 280-340 cm depth. At VW, silt was predominantly found below 160 cm and only scattered clay parts were present in 280-340 cm depth. At BW clay lenses were primarily observed below 160 cm depth. Partial Mn depositions and rust spots were detected in deeper soil layers at all sites, indicating periodical waterlogging and anoxic conditions.

pH-values at the watered sites VW and HST were generally higher compared to the non-watered site BW (Tab. 1). At VW soil pH increased slightly with soil depth, whereas at HST it remained constant at about pH 7.3, whereas at BW the soil pH decreased from 7.1 to 6.6 with depth. These contrasting depth gradients for VW and BW resulted in significantly lower pH values at BW in the lower vadose zone (Tab. 1, Tab. 3) and were reflected in a significant depth x field interaction (Tab. 3). Average pH-value of the river Rhine water used for flooding at VW and HST was 8.2.

Total soil carbon content (% DW soil) was significantly reduced with depth to about 88% in the watered, and 82% in the non-watered sites, with the latter exceeding significantly the watered sites in 160-220 cm depth (Tab. 1, Tab. 3). Soil nitrogen content (% DW soil) decreased significantly with depth by about 80% at all fields. In the upper vadose zone (100-220 cm) the watered sites HST and VW had higher nitrogen contents than the non-watered site BW. However, repeated measure ANOVA results revealed no differences between field sites and no interactions between depth and field (Tab. 3). C/N-ratios declined significantly with soil depth by about 40, 17 and 28% for HST, VW and BW, respectively. In the 100-160 cm depth HST significantly exceeded VW and repeated measure ANOVA showed a significant field separation (Tab. 1, 3).

Table 3: Repeated measure ANOVA table of *F*-values and degrees of freedom (*df*) on the effect of field (watered sites: HST, VW; non-watered site: BW) and soil depth (0-40, 40-100, 100-160, 160-220, 220-280, and 280-340 cm) for soil parameters.

	Within subjects effects		Between subjects effects
	depth (df 5)	depth x field (df 10)	field (df 2)
pH	1.96	3.52 **	1.97
%C	24.66 ***	0.75	2.61
%N	20.86 ***	1.46	0.05
C/N	5.66 ***	1.15	11.1 **
DOC [mg g^{-1} DW]	11.35 ***	1.03	163.31 ***
NO_3^- [mg g^{-1} DW]	41.2 ***	6.91 ***	8.68 *
SO_4^{2-} [mg g^{-1} DW]	15.4 ***	0.73	36.53 ***
C_{mic} [µg g^{-1} DW]	34.24 ***	1.82	2.65
O_2 [µl g^{-1} h^{-1} DW]	16.66 ***	1.17	7.73 *
qO_2 [µl O_2 g^{-1} h^{-1} C_{mic}]	8.7 ***	1.88	8.46 *

Moisture content ranged between 86 to 95% of DW soil and increased with depth at all sites by approaching the groundwater level (Tab. 1). Significant differences between sites were apparent in the layers from 0 to160 cm, particularly evident for HST. However, this may not reflect the original conditions. Generally, the soil was

sieved prior to further processing, and to achieve this, the moist loamy soil samples (i.e. predominantly from HST) had to be briefly pre-dried at room temperature. Therfore initially HST likely had the highest water content among sites.

Soil nutrient status

Concentrations of dissolved organic carbon (DOC), nitrate (NO_3^-) and sulfate (SO_4^{2-}) significantly decreased with soil depth at all sites (Tab. 3). Moreover, significant field effects were detected. Details on soil nutrient conditions are given in Schütz et al. (2008b). In brief, the following significant differences were observed: (1) DOC concentrations at the non-watered site BW were highest throughout the whole soil profile, (2) nitrate concentrations were elevated at both watered sites in 40 cm to 220 cm depth, and (3) sulfate concentrations at both watered sites exceeded the non-watered site in 40 to 340 cm depth.

Biomass and activity of soil microorganisms

The microbial biomass ranged from 359 to 52, 538 to 58, and 320 to 70 µg g^{-1} DW at HST, VW and BW, respectively (Fig. 1). Biomass significantly declined with soil depth by 85, 89 and 78% for HST, VW and BW, respectively (Tab. 3). Highest microbial density was in the upmost soil layer (0-40 cm) with an average of 42 to 58% of the total biomass. However, a considerable proportion of microorganisms with 42 to 58% was located in 40 to 340 cm depth. Differences between sites were negligible and only detected in the 100-160 cm layer where the microbial biomass of HST surpassed VW and BW.

Microbial respiration, measured as O_2 consumption rate per hour and gram DW soil, ranged between 3.2 to 1.4, 4.1 to 1.1, and 1.6 to 0.6 µl g^{-1} h^{-1} DW at HST, VW and BW, respectively (Fig.1). Respiration significantly declined with soil depth at all sites

(Tab. 3). O_2-concentrations were strongest reduced with depth in VW (-74 %), followed by BW (-65 %) and HST (-55 %). Generally, O_2-concentrations of watered sites were higher compared to the non-watered site. This was most pronounced in the top soil layer and the upper vadose zone, but effects were still apparent in the deepest soil layer (280-340 cm; Fig. 1).

Specific respiration (qO_2), an indicator for higher metabolic activity and/or stress among the microbial community, increased distinctly in watered sites within the soil profile (+68 and +58% for HST and VW, respectively; Fig. 1). The increase was particularly evident below 100 cm depth. In contrast, the qO_2 in the non-watered site remained more or less stable throughout the soil transect, despite slightly elevated amounts in 160 to 280 cm depth. In the deepest soil layer the qO_2 of both watered sites exceeded significantly the non-watered site. Moreover, HST in 0-40 cm and VW in 100-160 cm depth had higher specific respiration than BW.

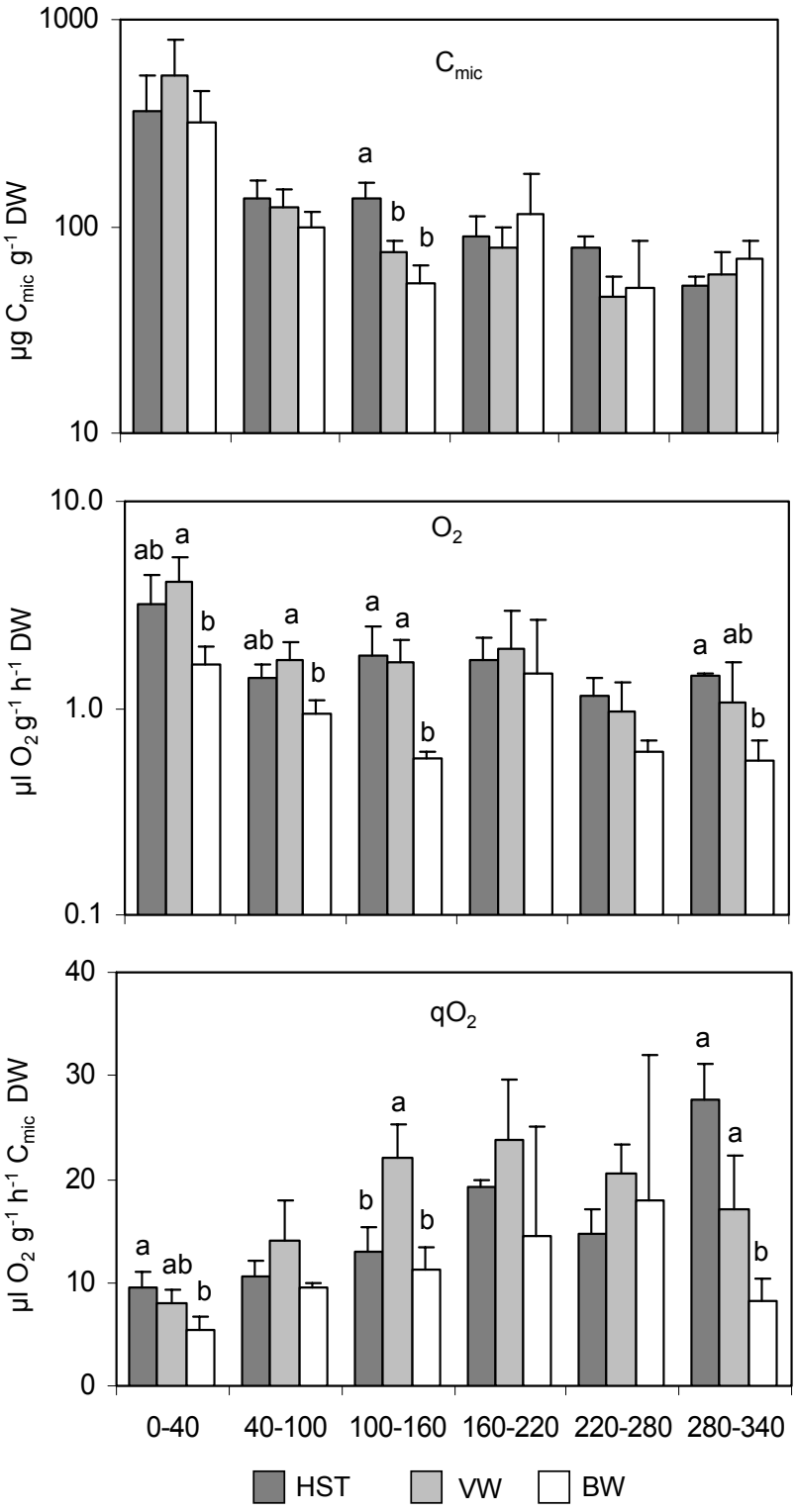

Fig. 1: Microbial community status, expressed as microbial biomass (C_{mic} in µg g^{-1} DW ± s.d.), soil respiration (O_2 in µl g^{-1} h^{-1} DW), and specific respiration (qO_2 in µl CO_2 mg^{-1} h^{-1} C_{mic} DW) in 6 soil depths (0-40, 40-100, 100-160, 160-220, 220-280, and 280-340 cm) at the watered sites HST and VW, and the non-watered site BW. Bars of the same depth sharing the same or no letter are not significantly different (Tukey`s HSD, $P < 0.05$)

Absolute and *specific* soil enzyme activities

Activities of the following eight enzymes of the soil C, N and P cycle were analyzed: α-D-glucosidase (aGLUC), β-D-glucosidase (bGLUC), cellobiohydrolase (CELL), β-xylosidase (XYLO), n-acety-β-glucosaminidase (GLAM), L-leucine (LEU), L-tyrosine (TYR) and phosphatase (PHOS). For the respective enzyme function see Table 2. We measured *absolute* and calculated *specific* soil enzyme activities, with the latter as *absolute* enzyme activity divided by the total microbial biomass of the respective sample. By this, enzyme activities across differences in microbial biomass, e.g. due to depth gradients, are normalized (Waldrop et al. 2000, Mentzer et al. 2006). *Absolute* (nmol g^{-1} h^{-1} DW soil) and *specific* (nmol g^{-1} h^{-1} C_{mic} DW soil) enzyme activities showed comparable amounts, therefore only *absolute* enzyme activity data are given in Table 4. Highest *absolute* activities of all exoenzymes were found in 0-40 cm depth and only minor or even no activity could be measured in 280-340 cm depth. This corresponds to reduction rates of 98 to nearly 100% from the top soil to ground water table, and is reflected in significant depth effects for *absolute* and *specific* enzyme activities (Tab. 5). Significant overall field differences were detected for CELL, GLAM, and PHOS, for both *specific* and *absolute* enzyme activity, and for bGLUC in *absolute* activity only (Tab. 5). Furthermore significant depth x field interactions occurred for bGLUC, CELL, GLAM, XYLO and TYR, which were less pronounced in *specific* compared to *absolute* enzyme activity.

Tab. 4: Absolute enzyme activities in 6 soil depths (0-40, 40-100, 100-160, 160-220 , 220-280 , and 280-340 cm) at the watered sites HST and VW, and the non-watered site BW. Values in parentheses are the standard deviation. Values in bold are the three most active enzymes within the respective soil layer across all sites. Means within a row with the same or no letter are not significantly different (Tukey`s HSD, $P < 0.05$)

Enzyme activity [nmol g^{-1} h^{-1} DW]	Depth	HST			VW			BW		
α-D-Glucisidase	0-40	9.84	(7.63)		7.96	(5.53)		4.70	(1.89)	
	40-100	2.81	(0.45)	**a**	0.70	(0.66)	**b**	0.64	(0.31)	**b**
	100-160	0.90	(0.76)		0.02	(0.02)		0.00	(0.00)	
	160-220	0.21	(0.35)		0.00	(0.00)		2.82	(2.27)	
	220-280	0.35	(0.61)		0.24	(0.29)		0.94	(1.63)	
	280-340	0.03	(0.04)		0.05	(0.08)		0.94	(1.62)	
β-D-Glucosidase	0-40	**289**	**(166)**		237	**(164)**		197	**(132)**	
	40-100	**116**	**(17.3)**	**a**	10.2	**(7.34)**	**b**	20.3	**(9.71)**	**b**
	100-160	**20.8**	**(5.79)**	**a**	1.40	(1.55)	**b**	1.57	(1.36)	**b**
	160-220	**10.9**	**(12.1)**	**a**	0.38	(0.65)	**b**	10.7	**(1.12)**	**a**
	220-280	0.96	(0.67)		0.74	(0.18)		7.90	**(13.7)**	
	280-340	0.53	(0.68)		1.27	(0.95)		3.38	**(5.32)**	
Cellobiose	0-40	42.3	(23.6)		56.4	(61.1)		39.4	(29.3)	
	40-100	20.7	(1.62)	**a**	0.79	(0.58)	**b**	1.90	(1.65)	**b**
	100-160	2.94	(1.01)	**a**	0.07	(0.12)	**b**	0.00	(0.00)	**b**
	160-220	0.85	(1.11)		0.00	(0.00)		1.01	(0.78)	
	220-280	0.13	(0.22)		0.17	(0.15)		0.16	(0.28)	
	280-340	0.00	(0.00)		0.00	(0.00)		0.40	(0.68)	
N-Acety-β-Glucosaminidase	0-40	101	(53.0)		96.4	(68.2)		79.0	(54.2)	
	40-100	55.7	(12.1)	**a**	6.01	(5.41)	**b**	8.55	(3.69)	**b**
	100-160	10.6	(3.35)	**a**	0.87	(1.04)	**b**	0.05	(0.07)	**b**
	160-220	7.34	(9.88)		0.16	(0.28)		2.25	(1.69)	
	220-280	0.52	(0.21)		0.47	(0.34)		0.47	(0.81)	
	280-340	0.13	(0.22)		0.19	(0.26)		0.65	(1.12)	
β-Xylosidase	0-40	37.3	(14.2)		44.6	(35.2)		41.4	(28.5)	
	40-100	21.5	(2.96)	**a**	3.25	(2.78)	**b**	4.15	(2.84)	**b**
	100-160	4.40	(2.19)	**a**	0.23	(0.34)	**b**	0.06	(0.11)	**b**
	160-220	1.29	(1.54)		0.01	(0.02)		1.40	(1.12)	
	220-280	0.19	(0.33)		0.16	(0.18)		0.35	(0.60)	
	280-340	0.00	(0.00)		0.08	(0.12)		0.58	(1.00)	
Phosphatase	0-40	**260**	**(40.5)**		323	**(202)**		299	**(221)**	
	40-100	**181**	**(12.1)**	**a**	44.5	**(23.2)**	**b**	46.1	**(33.7)**	**b**
	100-160	**48.8**	**(21.0)**	**a**	6.91	**(6.60)**	**b**	2.67	**(1.38)**	**b**
	160-220	**16.1**	**(7.22)**	**ab**	1.83	**(2.85)**	**a**	47.5	**(34.8)**	**b**
	220-280	**8.89**	**(2.99)**		3.95	**(1.65)**		14.7	**(25.5)**	
	280-340	**3.97**	**(0.17)**		5.88	**(0.98)**		13.0	**(20.4)**	
L-Leucine-aminopeptidase	0-40	**280**	**(193)**		206	**(96.6)**		271	**(222)**	
	40-100	**71.6**	**(23.1)**	**a**	23.4	**(5.58)**	**b**	28.8	**(10.2)**	**b**
	100-160	**17.3**	**(4.39)**	**a**	5.76	**(3.53)**	**b**	5.19	**(1.69)**	**b**
	160-220	**7.93**	**(4.14)**		4.13	**(2.48)**		11.32	**(5.10)**	
	220-280	**3.30**	**(1.64)**		2.71	**(1.51)**		3.95	**(5.67)**	
	280-340	**2.37**	**(0.47)**		3.47	**(0.20)**		5.44	**(4.84)**	
L-Tyrosine-aminopeptidase	0-40	81.2	(48.2)		91.7	(65.5)		174	(129)	
	40-100	39.4	(13.7)	**a**	7.96	(2.33)	**b**	15.3	(6.05)	**b**
	100-160	7.93	(0.97)	**a**	2.73	(0.91)	**ab**	2.49	(1.68)	**b**
	160-220	4.48	(2.12)		2.09	(0.75)		4.15	(2.39)	
	220-280	**2.53**	**(1.79)**		1.54	**(0.28)**		1.36	(1.83)	
	280-340	**1.10**	**(0.26)**		1.63	**(0.17)**		1.55	(1.96)	

Generally, PHOS had highest *absolute* activities at all field sites throughout the soil depth transect (except HST 0-40 cm, VW 160-220 cm, BW 100-160 cm; Tab. 4). On average, LEU was the second and bGLUC the third most active soil enzyme throughout the whole soil depth transect and across all field sites. In 40-100 cm depth all eight measured *absolute* soil enzyme activities of the watered site HST exceeded the watered site VW and the non-watered site BW. With the exception of aGLUC, the same pattern was continued in the 100-160 cm depth. At 160-220 cm depth the *absolute* enzyme activity of bGLUC and PHOS was lowest at VW, compared to HST and BW.

Table 5: Repeated measure ANOVA table of *F*-values and degrees of freedom (*df*) on the effect of field (watered sites: HST, VW; non-watered site: BW) and soil depth (0-40, 40-100, 100-160, 160-220, 220-280, and 280-340 cm) for absolute and specific enzyme activities and soil enzyme ratios. Enzyme ratios are calculated based on *absolute* activities. For abbreviations see Table 2.

	Within subjects effects		Between subjects effects
	depth (df 5)	depth x field (df 10)	field (df 2)
Absolute enzyme activity [nmol h^{-1} g^{1-} DW]			
aGLUC	18.02 ***	1.88	1.74
bGLUC	50.3 ***	3.33 ***	11.78 ***
CELL	76.76 ***	4.98 ***	5.2 *
GLAM	86.78 ***	4.43 ***	9.07 *
XYLO	86.96 ***	4.02 ***	4.13
PHOS	27.25 ***	2.71 *	7.35 *
LEU	63.47 ***	1.24	4.12
TYR	68.02 ***	1.83	3.86
Specific enzyme activity [nmol h^{-1} g^{1-} C_{mic}]			
aGLUC	6.8 ***	1.88	2.41
bGLUC	14.52 ***	3.2 *	2.98
CELL	32.5 ***	4.2 **	6.07 *
GLAM	32.23 ***	3.54 **	5.53 *
XYLO	35.52 ***	3.48 **	2.64
PHOS	7.87 ***	2.37 *	5.4 *
LEU	16.77 ***	0.84	1.59
TYR	35.44 ***	2.52 *	1.37
Enzyme ratios			
bGLUC / PHOS	7.48 ***	1.39	1.86
PHOS / GLAM	5.77 ***	1.96	0.45

Microbial community indicator ratios

Enzyme ratios of bGLUC to PHOS and PHOS to GLAM were used as indicators for microbial community response to altered nutrient availability. Enzyme ratios were calculated based on absolute activities. The average ratio over all field sites for bGLUC / PHOS decreased with soil depth from 0.86 ± 0.34 in 0-40 cm to 0.23 ± 0.15 in 280-340 cm (data not presented). The average ratio over all field sites for PHOS / GLAM increased with soil depth from 3.4 ± 0.9 in 0-40 cm to 25.7 ± 16.7 in 280-340 cm (data not presented). Ratios for bGLUC / PHOS and PHOS / GLAM were significantly affected by depth, but did not differ between field sites (Tab. 4).

***Relative* soil enzyme activities**

The proportional, i.e. *relative*, enzyme activity differed significantly across sites and entire soil depth transect: two way nested ANOSIM ("depth" nested in "field") revealed different *relative* enzyme patterns between all fields (Global $R = 394$, $P = 0.003$; HST-VW: $R = 0.362$, $P = 0.026$; HST-BW: $R = 0.417$, $P = 0.011$; VW-BW: $R = 0.433$, $P = 0.001$) and at all depths (Global $R = 0.654$, $P = 0.001$).

Highest proportional activities for all soil enzymes occurred in the top soil at 0-40 cm depth at all fields (> 50%; Fig. 2). However, at the watered site HST, high *relative* enzyme activities were still detected in 40-100 cm (22-35%) and 100-160 cm depth (5-10%; Fig. 2a). At the watered site VW, between 82% (LEU) and 98% (CELL) of the *relative* enzyme activities were located in the 0-40 cm depth, but strongly declined with depth in the subsequent soil layers (Fig. 2b). At the non-watered site BW, the pattern for *relative* enzyme activities showed the following pattern (Fig. 2c): (1) Both amino-peptidases (LEU and TYR) were comparable in depth distribution to VW. (2) Both glucosidases showed remarkable high *relative* enzyme activities in 160-220 cm depth (26 and 6% for aGLUC and bGLUC, respectively) and in 220-280 cm

depth (14 and 8% for aGLUC and bGLUC, respectively). (3) PHOS activity reached high amounts even below 160 cm depth.

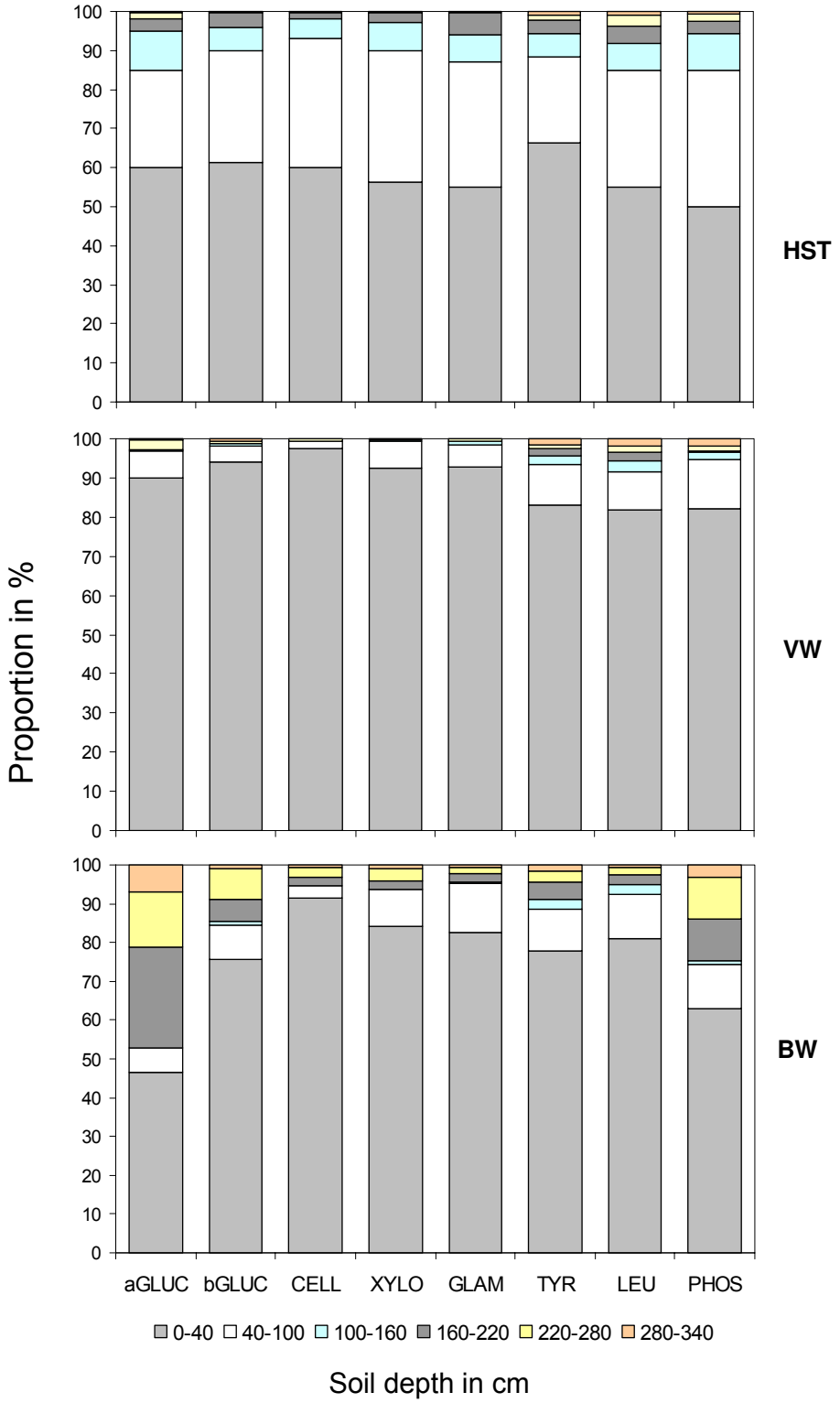

Fig. 2: Relative enzyme activity (absolute enzyme activity in % per soil layer) in 6 soil depths (0-40, 40-100, 100-160, 160-22, 220-280, and 280-340 cm) at the watered sites HST and VW, and the non-watered site BW. For abbreviations see Table 2.

***Absolute* soil enzyme activities, soil parameters and soil nutrient status**

Redundancy analysis (RDA) and Monte Carlo permutation test of *absolute* soil enzyme activities, soil parameters and soil nutrient status slightly separated the watered sites from the non-watered site in the top soil layer with HST and VW being closer related to each other than to BW (Fig. 3). Deeper soil layers showed no clear separation between sites. The first axis ($F = 299$, $P = 0.002$) explained 98.7% and the second axis 0.7% of the variation in the data set.

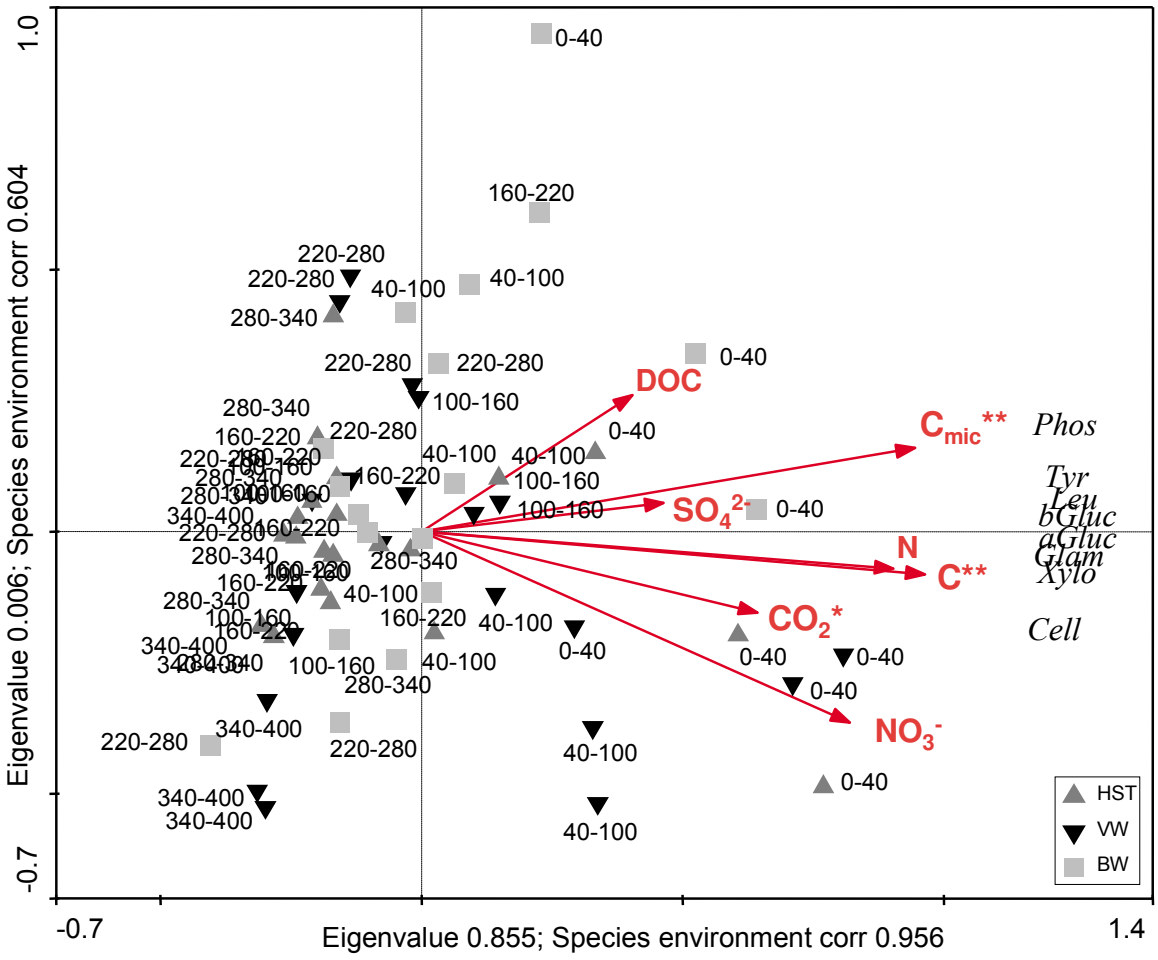

Fig. 3: Redundancy analysis (RDA) ordi-nation triplot of LOG transformed *absolute* enzyme activities and LOG transformed soil parameter data. *, **, *** significant contribution to sepa-ration with $P < 0.05$, 0.01, 0.001, respect-tively.

Across all fields, separation of the top soil from deeper soil layers mainly occurred on the first axes with %C ($F = 225.27$, $P = 0.001$), C_{mic} ($F = 14.08$, $P = 0.001$) and CO_2 ($F = 3.66$, $P = 0.035$) being significantly involved. Moreover, higher CO_2 concentrations in the watered sites contributed to their separation from non-watered sites in the top soil layer. Increased %N concentrations ($F = 2.71$, $P = 0.069$) also tended to contribute to the separation of the top soil layers to the deeper soil layers, whereas SO_4^{2-} ($F = 0.87$, n.s.) did not correlate with the *absolute* soil enzyme activity pattern.

Relation between structural and functional diversity

Generally, enzyme activity represents the functional diversity of microorganisms in soil, whereas phospholipid fatty acids (PLFAs) are a measure for structural diversity of the microbial community (Kandeler et al. 2000, Waldrop 2000, Mentzer 2006). The RDA analysis above revealed strong relations between the *absolute* enzyme activity pattern and microbial biomass (C_{mic}). Further, Waldrop et al. (2000) stated that the *specific* activity of extracellular soil enzymes was more closely related to community composition than the *absolute* activity. Therefore, in this study the PLFA pattern (data are presented in Schütz et al. 2008b) was related to the *specific* enzyme activities in the respective soil samples. Here we analyze these two community parameters by application of a Monte Carlo permutation test (MCPT) for the separated field sites (Tab. 6).

Table 6: Monte Carlo permutation test results of explained variability (%) and *F*-values on the basis of the PLFA pattern (% nmol) and *specific* enzyme activities (LOG) in the watered sites HST and VW, and the non-watered site BW. *, **, *** with $P < 0.05, 0.01, 0.001$, respectively. For abbreviations see Table 2.

	HST		VW		BW	
	Explained variability (%)	*F*	Explained variability (%)	*F*	Explained variability (%)	*F*
aGLUC	2	0.47	5	1.25	11	3.74 *
bGLUC	8	2.07	2	0.51	9	3.29 *
CELL	6	1.33	3	0.48	1	0.26
XYLO	2	0.6	5	1.22	21	5.79 **
GLAM	8	1.88	12	2.99 *	0	0.41
LEU	3	0.65	3	0.69	8	3.92 *
TYR	14	3.03 *	3	0.85	6	2.84 *
PHOS	2	0.39	15	3.35 *	25	4.51 **
Σ Explained variability (%)	45		48		81	

MCPT revealed connectivity between functional and structural diversity in HST only for TYR, and in VW for GLAM and PHOS. In contrast, several significant relations in BW were detected: the *specific* activity of aGLUC, bGLUC, XYLO, LEU, TYR and PHOS showed significant relations to the structural diversity, i.e. the PLFA pattern. In sum, the explained variability by the individual enzymes reached 81% for the non-watered site BW, whereas only 45 and 48% were explained in the watered sites HST and VW, respectively.

Discussion

Effects of flooding, soil parameters and depth on the microbial community

Pore size, nutrient limitations, availability of electron acceptors, and large surface areas for attachment, all have major effects on microbial abundance and activity in vadose zone and aquifer material (Ghiorse & Wilson 1988). These environmental

parameters likely contributed to the observed strong decline in microbial biomass from top soil to groundwater table at the investigated sites. Water recharge was expected to foster microorganisms by the additional input of dissolved organic carbon (DOC) from flooding sources. However, microbial biomass did not differ between watered and non-watered sites. This is in contrast to other studies which observed high numbers of microorganisms due to the amendment with metabolizable organic substrates by water recharge practice (Balkwill et al. 1998, Kieft & Brockman 2001). Presumably, stopping the flooding regime four weeks before sampling, and hence the relatively low organic input at that date, resulted in comparable microbial biomass between sites. Additional studies at the “Lange Erlen” (0-10 cm soil depth) under flooding conditions revealed higher microbial biomass in watered compared to non-watered sites (Schütz et al. 2008b). This indicates different soil conditions under water cover, and further suggests that the surface soil transect (0-40 cm) in the present study likely was too coarse to obtain differences. The latter is supported by soil infiltration studies from Rauch-Williams & Drewes (2006), who detected differences in microbial biomass from zero to approximately 10-30 cm depth.

Overall, considerable fractions of the total microbial biomass (58%, 42% and 55% in HST, VW and BW, respectively) were located in 40-340 cm soil depth. At HST microbial biomass was elevated in 100-160 cm depth, likely due to the deeper humus and fluvial silt horizon at that site. These findings contradict the widespread opinion that subsurface microbial biomass is unimportant. Of the few studies available for subsoil microorganisms Fierer et al (2003) reported approximately 35% of the total biomass below 25 cm in a two meter deep soil profile. Regarding this and the results of the present study with about half of the biomass located below 40 cm depth, the corresponding capability of the vadose zone microorganisms for biodegradation in deeper soil layers cannot be ignored.

Microbial respiration (CO_2 – emission rate) decreased with soil depth at all field sites, and was generally higher in the watered sites throughout the soil profile. Specific respiration (qCO_2 = CO_2 production per unit C_{mic}) increased with soil depth in the watered sites, but remained more or less constant at the non-watered site. Both microbial parameters can either be linked to higher activity or to stress (Anderson & Domsch 1993, Wardle & Ghani 1995). In the vadose zone of watered sites the frequently altered habitat characteristics due to regular flooding may produce stress, thereby leading to enhanced CO_2 and qCO_2 respiration in microbial communities. Particularly oxygen availability, temperature, organic solvents and soil compression may be responsible for this (Anderson & Domsch 1990, Kandeler et al. 1993, Fliessbach et al. 1994). Previous studies at the watered site VW revealed oxygen saturation during a 10-day watering period to be reduced from 90% to 65% in summer, and from 100% to 85% in winter, for filtrate- and groundwater, respectively (Rüetschi 2004). Moreover, the groundwater table at the watered sites rises from ca. 4-5 m to 2-3 m depth after 3-4 days of flooding, thereby leading to fluctuating aerobic and anaerobic conditions.

On the other hand, flooding introduces nutrients, such as DOC and DON, which may lead to excess-C and -N conditions at watered sites. This further changes the ratio of carbon / nitrogen / phosphorus in soil, and consequently other nutrients than C or N may become limiting factors for the growth of the microbial biomass. This is supported by preliminary fertilizer studies with C, N and P (data not shown) where at all sites fast growing microorganisms were predominantly C and N limited, as indicated by a distinct increase in SIR within the first 15 h. However, exclusively slow growing microorganisms from the watered sites reacted to the addition of CNP with an increased respiration after 2 days. Thus, enhanced CO_2 and qCO_2 values at the watered sites likely occur due to nutrient limitation and a subsequent overflow

metabolism in microorganisms (Postma et al. 1989, Russell & Cook 1995, Teixeira de Mattos & Neijssel 1997, Dauner et al. 2001). Microorganisms excessively emit CO_2 into the surrounding environment at the watered sites, which may also cause the observed higher pH-values in deeper soil layers there. Similar effects are documented due to ongoing biotic CO_2 production and low rates of belowground diffusion by Davidson and Trumbore (1995), and Richter and Markewitz (1995).

The enhanced nutrient input via flooding over decades was evident by the nitrogen indicator plants growing at the recharge sites. Presumably the microorganisms at the watered sites effectively absorbed supplementary substrate input. However, apparent nutrient shortage prevented microbial biomass to increase and induced an overflow metabolism. Most likely phosphorus was limited as indicated by the enzyme activities discussed in the next paragraph.

Effects of flooding, soil parameters and depth on enzyme activities

As expected, all measured *absolute* and *specific* enzyme activities significantly decreased with soil depth at all field sites. This corresponds to results from Taylor et al. (2002) and Venkatesan & Senthurpandian (2006), who found decreasing *absolute* enzyme activities of arylsulphatase, dehydrogenase, bGLUC, acid and alkaline PHOS, urease and protease with soil depth. Freeman et al. (1995) also investigated *absolute* enzyme activities of bGLUC, PHOS and sulphatase in a finer divided depth transect from the surface soil down to -40 cm and identified the top soil layer (0-10 cm) as the enzymatically most active horizon.

Flooding did not result in different exoenzyme activities throughout the soil depth transect, although increasing activities in top soils due to flooding were reported previously (Freeman et al. 1995, Mentzer et. al. 2006). Freeman et al. (1995) found an increase in *absolute* enzyme activities in bGLUC and PHOS at riparian wetland

sites from soil surface down to 3 cm. Increased *specific* enzyme activities of bGLUC, XYLO, CELL, GLAM, aGLUC and alkaline PHOS in silty loam top soils have been reported under constant flood (3 months) treatments (Mentzer et. al. 2006). However, in short-timed flood treatments (up to 2 weeks) *specific* activities did not differ, and *absolute* enzyme activities showed only little differences. This corresponds to our study sites with cycles of 10 day flooding, and subsequent 20 days drying and regeneration. Moreover, the investigated top soil layers with 0-40 cm may be too large to reveal differences as observed in the thin layers analyzed by Freeman et al. (1995). Overall, enzyme activity did not respond to flooding, which is in line with the stable microbial biomass, and presumably caused by nutrient and/or substrate limitation as discussed above.

Nevertheless, we detected differences in *absolute, specific* and *relative* enzyme activities of single enzymes between field sites. Additionally, the *relative* enzyme activity pattern significantly separated all sites from each other. *Absolute* and *specific* enzyme activities at HST from 40 to 160 cm depth exceeded the watered site VW and the non-watered site BW by several orders of magnitude, most likely caused by differences in soil structure. Because of too high infiltration rates, artificial landfill was carried out at HST in 1981 and approximately 1 m of loess loam was applied to the soil surface. Therefore loess loam (i.e. predominantly silt) at HST reaches considerably greater depths than at the two other sites. Extracellular soil enzyme activity is strongly affected by soil particle size fractions and in particular silt and clay size micro-aggregates provide attractive locations (Kandeler et al. 1999, Nannipieri 2002). Corresponding to our results, Taylor et al. (2002) reported negative correlations of *absolute* soil enzyme activities (arylsulphatase, dehydrogenase, bGLC, phosphomonoesterase, urease) with sand, and positive with silt and partly

clay content in primarily sandy soils. Marx et al. (2005) found highest *relative* enzyme activities (CELL, GLAM, bGLUC, XYLO, LEU, PHOS) in the silt and clay size fractions of a loamy soil. Hence, the expansion of silt in greater depths at HST had a major positive impact on enzyme activity there.

In contrast to watered sites, the *relative* enzyme activities at the non-watered site BW were remarkable high in the lower half of the soil profile (from 160 cm downwards). Effects were predominantly apparent for bGLUC, aGLUC and PHOS. As discussed above, this may be caused by more frequent clay lenses there (Marx et al. 2005). This suggests that the enzyme activity pattern along the depth transects reflects the silt dominated upper part of the profile (0-160 cm) at the watered site HST, and the clay dominated lower half of the profile (160-340 cm) in the non-watered site BW. Moreover, a large proportion of total soil P is bound to clay and humic colloids in soil (Syers 1969), resulting in high proportions of PHOS in those particle size fractions (Marx et al. 2005). Similarly, predominant sorption of organic matter in clay size fractions is well known (Guggenberger & Kaiser 2003). Thus, considering the low water turnover rates and diluting processes in the non-watered site BW compared to watered sites, higher *relative* a- and bGLUC activities in clay size fractions of deeper soil layers are reasonable.

Generally, PHOS was the most important exoenzyme at all field sites and mostly exceeded carbohydrases and aminopeptidases at all depths. Exoenzyme are produced by microorganisms for the degradation of complex insoluble polymers (such as proteins, nucleic acid or chitin), which are too large to pass directly through microbial membranes (Beck & Beck 2000). They therefore reflect nutrient requirements, and an inverse relationship between phosphatase activity and inorganic phosphate availability is reported (Shackle et al., 2000). The high PHOS activity therefore strongly suggests that P is limiting. In water and sediment samples;

this trend (PHOS > carbohydrases) has been observed in previous studies (Miettinen et al. 1996, Hendel et al. 2001, Kuhbier et al. 2002). This is in line with the relatively low total phosphate and ortho-phosphate concentrations (0.05 mg l^{-1} and 0.02 mg l^{-1}, respectively; Department of Environment and Energy (AUE) 2006) in the river Rhine water used for flooding. However, as PHOS activity was elevated in watered as well as in non-watered sites, P supply seems to be below demand in all fields.

Functional indicator ratios

Ratios of C-, N- and P-processing enzymes can provide insight into the microbial community response to changing nutrient resources and the relative importance of different nutrients (Caldwell 2005). We determined enzyme ratios of bGLUC/PHOS and PHOS/GLAM and found significant depth effects (-374% and +760% for bGLUC/PHOS and PHOS/GLAM, respectively). Kuhbier et al. (2002) studied the impact of a wastewater discharge site for the river Horloff (Germany) and found significantly higher bGLUC/PHOS ratios downstream of discharge due to enhanced bGLUC activity. However, in our study the ratio of bGLUC/PHOS declined at all sites, suggesting that the relative importance of polyphosphates increases, and of complex organic compounds decreases, with soil depth. Data from a 4-million-year chronosequence in Hawaii were analyzed and showed ratios of PHOS/GLAM in organic and mineral horizons to increase with soil age (Olander & Vitousek, 2000; Caldwell, 2005). This relates to increasing importance of organic phosphorus relative to organic nitrogen with soil age (development). Hence, the PHOS/GLAM at soil profiles of the “Langen Erlen” indicates relatively young top soil layers (i.e. artificial landfill) and older deeper soil layers.

Effects of soil parameters and nutrients on the functional microbial pattern

Combining *absolute* enzyme activities with soil parameters in one analysis (RDA) slightly separated the surface soil layers from watered sites and the non-watered site. However, the separation mainly occurred on the y axes and only CO_2 concentrations contributed significantly. On the other hand, the distinct separation of the top soil layer from deeper soil layers mainly occurred on the x axes based on %C, C_{mic}, and CO_2. Remarkably, all measured nutrients (DOC, NO_3^- and SO_4^{2-}) did not significantly correlate with the *absolute* exoenzyme activity pattern. Such dissolvable micronutrients are liberated by exoenzymes from complex soil organic matter (SOM), i.e. exoenzymes are produced by microorganisms for this purpose, which was apparently not the case at the investigated sites. This suggests that flooding with river water did not introduce additional SOM to the system, and indeed SOM content is similar at all sites, as indicated by comparable total %C and %N contents. This is due to the effectively working rapid sandfilter technique, which assures that complex organic compounds are not excessively introduced by flooding the recharge areas. On the other hand, flooding increased the input of DOC and DON, i.e. readily available substrate, but this did not alter enzyme activity. This is in line with studies in arable, forest or wetland soils where amendment with complex carbon sources, such as cellulose or sewage led to enhanced bGLUC activities, but addition of glucose did not (Dilly & Nannipieri 2001; Shackle et al. 2000). In contrast, Burns & Ryder (2001) found correlations between glucosidases activity and DOC in floodplain sediments. However they assigned DOC as particles up to 0.7 µm, whereas in our study DOC particles were smaller than 0.45 µm as defined by Thurman (1985). In sum the lack in response of exoenzymes indicates that DOC reduction is rather a function of bacterial degradation (absorption) than of enzyme activity at our sites.

Relations between structural and functional microbial community composition

The PLFA pattern and the *specific* enzyme activity pattern in the soil were compared to reveal relationships between structural and functional microbial community composition. Generally, the *specific* activity, which normalizes enzyme activity to microbial biomass, was more closely related to community composition than the *absolute* activity (data not shown). This is in line with Waldrop et al. (2000) who found this measure of functional diversity to be closely connected to structural diversity assessed by PLFA pattern. Generally, enzymes (except CELL and GLAM) of the non-watered site BW were significantly associated with the structural community composition, whereas at the watered sites only two enzymes showed significant relations. This pattern reflects the lack in external nutrient input at the non-watered site, where a considerable amount of nutrients has to be gained by decomposition of SOM. In contrast, a comparable high microbial biomass can be maintained at watered sites without the need for degradation of complex organic substrates, i.e production of exoenzymes, due to the enhanced nutrient supply by river water (Fig. 4). Therefore, the functional diversity pattern of the watered sites is not related to the structure of the microbial community.

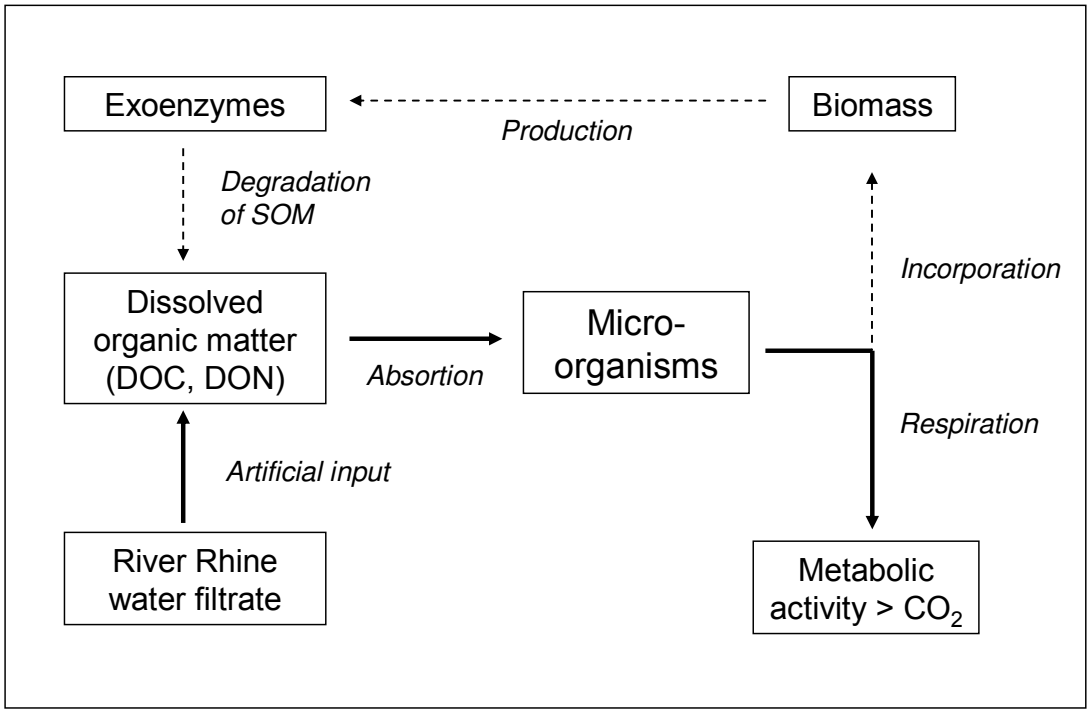

Fig. 4: Carbon flux model for the watered sites HST and VW. Solid lines indicate flows of carbon as indicated by the data, dashed lines assign potential decomposition processes, which were not supported by the measurements of microbial community parameters.

Conclusions

Previous investigations at the water recharge area “Lange Erlen” revealed distinct microbial community structures, most likely due to altered environmental conditions caused by flooding (Schütz et al. 2008a). However, in the present study, enzyme activities showed no differences between watered and non-watered site, whereas functional diversity based on the *relative* exoenzyme pattern separated all sites from each other. The latter was rather caused by differences in soil structure, i.e. silt and clay depositions where exoenzymes can be stabilized by their interactions with the soil matrix, than by flooding regime.

Structural and functional microbial diversity were linked to each other at the non-watered site. Amendment with readily available substrates (DOC, DON) via flooding uncoupled this relation and resulted in an enhanced microbial respiration, indicating a highly active microbial community at the recharge sites (Fig. 4). Yet this did not result in a corresponding increase in microbial biomass, but in an overflow metabolism due to nutrient limitation. Enzyme activity suggested evidence for P limiting conditions, likely introduced by high C and N availability along with the Rhine water. On the other hand, excessive input of complex organic matter was inhibited by the rapid sand filter technique, which is confirmed by comparable soil enzyme activities irrespective of flooding. Thus, larger contaminations at the recharge areas (past and present) can be excluded. However, as decomposition processes are carried out by a large number of microorganisms a decline of one group or species may have little effect, since other organisms can fulfill the function. This resilience is a feature of many soil ecosystems and most likely holds for the forested recharge areas, indicating that the system of drinking water production in the “Lange Erlen” can be widely preceded in future.

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General Discussion and Conclusion

Most studies on soil fauna and microbial communities of belowground systems are limited to the top soil layer and disregard processes occurring in deeper soil horizons. However, in this thesis, I took a comprehensive look at soil fauna and microorganisms from top soil to the groundwater table (approx. -4 m) to ask why the water purification of the city of Basel in the area “Lange Erlen” is so effective. I show that soil fauna and microorganisms play a decisive role in subsurface water processes such as soil regeneration, water infiltration, contaminant degradation and maintenance of groundwater quality at the “Lange Erlen”. Clearly, the effects were bilateral; while water processes were affected by soil inhabitants, soil inhabitants were also affected by water processes. In the beginning of this study, intensive flooding was thought to affect soil structure, earthworm populations, and to change structure and function of microbial communities. Vice versa, earthworms were thought to affect soil structure and water infiltration rates. In addition, structure and function of the microbial community was thought to influence water purification processes. These ideas are largely tenable based on my results where I show, that watered and non-watered sites at the “Lange Erlen“ perform different in abundance, biomass and community composition of earthworms and microorganisms.

I found that intensive flooding affected several biological soil parameters, i.e. earthworm density, biomass, species richness and the microbial biomass of the top soil layers (0-10 cm). These parameters were significantly increased in the watered versus the non-watered sites. It became obvious that earthworms at the “Lange Erlen” can cope well with the applied watering regime, which consists of 10 days flooding followed by 20 days drying and regeneration cycles, but also includes some larger interruptions due to less water demand, deficient Rhine water quality, or

maintenance work. Most likely, the increased moisture and nutrient input by flooding with Rhine water are factors that promote earthworm reproduction and lead to continuously enhanced earthworm performance in the recharge areas. Furthermore, infiltration rates were positively correlated with earthworm densities. Presumably, the different functional groups of earthworms, in particular epi- (litter dwellers) and endogeic (mineral forms) species, interact to regenerate soil structure during regeneration periods, thereby stimulating microbial biomass of the top layer, preventing clogging, and enhancing infiltration rates. At the species level, *L. rubellus* appeared to be the most important and anecic (deep-borrowing earthworm species), which were thought to have the greatest impact, seemed to play a less important role.

When I looked at the deeper soil layers, I found that flooding resulted in an altered structure of the microbial community in the vadose zone. Furthermore, the flooding uncoupled the functional microbial cycle of organic matter degradation, nutrient absorption, microbial growth, and exoenzyme production which resulted in a higher metabolic activity (CO_2 – emission) at the recharge areas. Moreover, I identified a biologically distinct layer in the upper vadose zone (-100 to -220 cm depth) which was partly continued in the lower vadose zone (-220 to -340 cm depth). This layer was characterized by a completely different microbial community, special dominant functional groups (gram-negative and in part anaerobe bacteria) as well as higher nutrient, temperature, and stress indices. This clearly supports the assumption of Rüetschi (2004) that purification processes are mainly located in deeper soil layers. The measurements of several “stress indices” showed, that the microbial community residing at larger depths in the recharge areas suffered from the periodic flooding and the corresponding alternated environmental conditions. Furthermore, the microbial community was not able to increase its biomass at depths below 20 cm.

This happened despite that fact that basic nutrient supply (carbon and nitrogen) seemed to be sufficient or even more than enough. Perhaps other nutrients (i.e. phosphate) were limiting microbial growth at these sites and resulted in overflow or waste metabolism and nutrient leaching into the groundwater. Nevertheless, groundwater recharge processes resulted in a microbial community adapted to the resources and environmental conditions. During the water purification process, DOC (dissolved organic carbon), a measure for water quality, is reduced by about 71%. This indicates that most introduced organic carbon must be degraded within the soil, i.e. respired effectively by the inhabiting microorganisms. Because extracellular enzymes, which are also produced during higher organic substrate availability, can be stabilized over long time periods in the soil matrix, they can be regarded as an enzymatic memory of the catalytic history of a soil. In our soil profiles no evidence was found for higher organic loadings at the watered sites, indicating that larger contaminations did not happen within the last decades.

Overall, my data strongly support the idea that earthworms and microorganisms synergistically contribute to the functioning of the “Lange Erlen” filtration system. This synergism and balance is likely what kept this unique system working efficiently and without larger maintenance for almost 100 years. The main factors that contribute to the efficient artificial recharge and purification system in the “Lange Erlen” include a prefilter treatment of the raw water as well as watering cycles with longer regeneration periods in combination with highly active and adapted decomposer species, microorganisms, and woodland sites. Possible interferences by chemical contamination or physical disturbances might be absorbed by the “Lange Erlen” system because the purification process is carried out by a large number of soil- and microorganisms. A small reduction in any group of species is expected to have little

effect on overall soil processes probably because other organisms could compensate and fulfill these functions. This spare capacity or resilience is a general feature of most soil ecosystems, and here I show convincingly that it is also a feature of the “Lange Erlen” system. In addition to providing economic and sustainable drinking water production in a semi-natural floodplain forest ecosystem, it provides refuge for a large number of plant and animal species. Due to its simplicity and functionality the groundwater recharge and purification system in the “Lange Erlen” could potentially be widely adopted for drinking water production in future.

Every experience is a paradox in that it means to be absolute,
and yet is relative; in that it somehow always goes beyond itself
and yet never escapes itself.
(T.S. Elliot)

Summary

At present, approximately half of the drinking water for the city of Basel (Switzerland) is obtained by an artificial groundwater recharge system in a former floodplain area called “Lange Erlen”. Generally, the use of groundwater for drinking water production may lower groundwater tables. Artificial groundwater recharge is a practice of directing and simultaneously purifying water into aquifers, thereby raising the groundwater table and guaranteeing sufficient drinking water sources. Water purification through artificial groundwater recharge is widespread. However, it more commonly involves areas without vegetation, i.e. slow sand filters, dunes or channels and is combined with long flooding periods. In contrast, at the “Lange Erlen”, forested areas are periodically flooded (max. 10 days) with water from the river Rhine. This routine is interrupted by longer regeneration periods. To date, water infiltration and purification processes have remained constant and satisfactory since the system has been established almost 100 years ago. However, detailed knowledge on the belowground processes that have been sustaining the water purification capacity of “Lange Erlen” is scarce.

Intensive flooding may detrimentally affect earthworm populations and result in clogging of the topsoil, which is a common problem in groundwater recharge systems. Earthworms are known to influence water infiltration and aeration of soils, but most of the existing knowledge relates to grasslands and little is known about the role of earthworms for water infiltration in forests. To better understand the “Lange Erlen” system in the shallow soil layer, earthworm populations, microbial parameters (substrate induced respiration, SIR) and water infiltration rates were studied at the recharge areas. The findings suggest that earthworms are an important factor of the long-term sustainability of this system (for the past ~100 years). The total earthworm

numbers and biomass in watered sites exceeded those of non-watered sites (+51% and +71%, respectively). Total earthworm numbers, numbers of endogeic (mineral forms) and epigeic (litter dwellers) earthworms, and numbers of two species (*Lumbricus rubellus* and *Allolobophora chlorotica*) significantly and positively correlated with water infiltration rates. Microbial biomass and activity was significantly enhanced in the top soil layer of the watered sites. The results imply that the flooding regime at the “Lange Erlen” favors earthworm populations which in turn prevent soil clogging, aerate the top soil layer, and stimulate microbial growth.

Groundwater quality is directly influenced by subsurface microbial, chemical and physical soil processes. However, most studies on microbial communities have been limited to the top soil layer. These studies disregarded deeper soil horizons although subsurface microorganisms are crucial for the degradation of natural organic compounds or contaminants and the maintenance of groundwater quality. Therefore, vertical soil profiles down to approximately 4 m of depth from two watered sites and one non-watered site were investigated for the structural (phospholipid fatty acids, PLFAs) and the functional (extracellular hydrolytic enzymes) microbial community composition. Furthermore, additional microbial (by SIR), physical and chemical soil parameters were obtained from the same soil samples.

The microbial biomass did not differ between watered sites and the non-watered site, however considerable fractions of the microbial biomass (25-42% by PLFA and 42-58% by SIR) were located in 40-340 cm depth at all sites. The microbial activity (CO_2 emission) and the specific respiration (qCO_2) were highest at the watered sites. The microbial community structure differed significantly between watered and non-watered sites (predominantly below 100 cm depth), whereas the functional structure (based on the *relative* enzyme pattern) differed significantly between all sites. The latter finding could probably be explained by different soil structures in each soil

profile rather than by flooding. Proportions of the bacterial PLFAs 16:1ω5, 16:1ω7, cy17:0 and 18:1ω9t, and the long chained PLFAs 22:1ω9 and 24:1ω9 were more prominent at the watered sites, whereas branched, saturated PLFAs (iso/anteiso) dominated at the non-watered site. The PLFA community indices indicated stress response and higher nutrient availability due to flooding. The analysis of extracellular soil enzymes revealed that acid phosphatase showed highest *absolute* activities at all field sites throughout the soil depth transect and was followed by L-leucine aminopeptidase and β-glucosidase. Combining the structural and the functional diversity of the microbial community in one analysis revealed significant correlations between the PLFA pattern and *specific* enzymes activities in the non-watered site. However, at the watered sites these relationships were not detected and the same factors appeared uncoupled from each other.

Overall, this implies that adding labile nutrients (i.e. DOC or DON by flooding) to a soil where other nutrients are limiting microbial growth (i.e. P as indicated by acid phosphatase) increases microbial activity but not biomass. This in turn results in waste respiration by overflow metabolism. Additionally, slight nutrient leaching (e.g. nitrate) into the groundwater is observed due to P-limiting conditions.

No differences in *absolute* and *specific* enzyme activities between watered sites and the non-watered site indicated complex organic matter input at the recharge sites to be impeded by flooding water pretreatment. In conclusion, water recharge processes resulted in a microbial community adapted to resource and environmental conditions, which was predominantly located in the upper (100-220 cm depth) and partly in the lower vadose zone (220-280 cm depth). Given a better understanding, the system may be more widely adopted and used to provide sufficient and reliable drinking water to the city of Basel.

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Education

2005 - 2008	PhD thesis “Artificial groundwater recharge in forests – soil fauna and microbiology“.
	Department of Environmental Sciences, University of Basel. Supervisor: Prof. P. Nagel, Co-referee: Prof. S. Scheu
1994 - Aug 1998 Oct 1999 - 2002	Diploma in Biology (Zoology, Ecology, Animal Physiology), Technical University of Darmstadt.
	Diploma thesis: “Effects of Collembola and fertilizers on plant performance (*Triticum aestivum*) and aphid reproduction (*Rhopalosiphum padi*)”. Institute of Zoology, TU Darmstadt. Supervisor: Prof. S. Scheu
1986 - 1993	Grammer school (Abitur), Darmstadt
1984 - 1986	Transition stage, Roβdorf
1980 - 1984	Primary school, Reinheim / Zeilhard

Activities

2002 - 2005	Periodic employment as graduate assistant at the Institute of Zoology, University of Darmstadt
Since 2001	Instructor of the federal youth leader training team (German Alpine Club, JDAV)
Sept 1998 - Aug 1999	Gap year taken to do voluntary work in the environmental sector (FÖJ). OUTWARD BOUND Baad, Kleinwalsertal, Austria

Publications

Schütz K, Nagel P, Vetter W, Kandeler E, Ruess L (200?) Flooding forested groundwater recharge areas modifies microbial communities from top soil to groundwater table, accepted for publication in FEMS Microbiology Ecology

Schütz K, Nagel P, Dill A, Scheu S (2008) Structure and functioning of earthworm communities in woodland flooding systems used for drinking water production. Applied Soil Ecology, 39 (3): 342-351

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Ruess L, Schütz K, Haubert D, Häggblom MM, Kandeler E, Scheu S (2005) Application of lipid analysis to understand trophic interactions in soil. Ecology, 86 (8): 2075-2082