Zürcher Hochschule
für Angewandte Wissenschaften

Life Sciences und
Facility Management

ZURICH UNIVERSITY OF APPLIED SCIENCES
SCHOOL OF LIFE SCIENCES AND FACILITY MANAGEMENT
INSTITUTE OF NATURAL RESOURCE SCIENCES

The impact of hydropower on microbial diversity and community structure in floodplains

Master thesis by
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29 September 2017

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Antenen, N. (2017). The impact of hydropower on microbial diversity and community structure in floodplains

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Keywords**:** Habitat heterogeneity, T-RFLP, floodplain patterns, residual flow, hydropeaking

Title: Sense floodplain © water.wwf.ch

Abstract

Within freshwater ecosystems, floodplains are among the most diverse ecosystems on earth but the increasing human demand for water, energy and food resulted in a decrease of biodiversity. Even though hydropower is often seen as “green energy”, it creates several negative impacts on riverine systems. In order to produce energy, river water is abstracted and released, leading to hydropeaking and residual flow sections, both representing a major disturbance on the natural functions and dynamics of a river. The ecological impact of hydropower production on riverine systems has been extensively investigated, however, mostly focusing on structural aspects. The present study focused on the microbial diversity and community structure in three hydrologically different floodplain sections, two impacted by hydropower and one in a natural reference system.

Abiotic characteristics of habitats and genetic fingerprinting (T-RFLP) of the microbial communities were used to assess differences and similarities between and within these regimes. Within the latters we investigated sediments of seven different floodplain habitat types over a sampling period of one and a half year (summer 2015 until autumn 2016) consisting of six sampling seasons. Six abiotic parameters (temperature, water content, total organic matter, total nitrogen, total carbon and grain size distribution) were used to analyse patterns amongst habitats, regimes and seasons. Organic matter, water content, total nitrogen and total carbon content showed significant differences between the habitats whereas of the regimes the hydropeaking showed relevant influences on abiotic factors. Furthermore, we could illustrate several relationships between abiotic factors and microbial diversity, mainly within the same habitats and regimes as the distinct abiotic properties were highlighted. While the microbial communities clearly differ between aquatic and terrestrial habitats as well as between seasons, there are little differences among the regimes.

Linking abiotic characteristics, shaped by the hydrological regimes, to microbial diversity and community structure allows the consideration of the latter as possible indicators of disturbances in river systems. The use of the genetic fingerprinting technique (T-RFLP) proved to be an appropriate method to gain first insights into patterns of microbial diversity and communities. In this context, the results from this study are a first contribution towards assessments of disturbances by microbial diversity and community analysis and possibly to a future development of a functional indicator for disturbance.

Content

1 Introduction

1.1 Floodplain ecology

Within freshwater ecosystems, floodplains are among the most diverse ecosystems on earth (Junk et al., 1989; Tockner and Stanford, 2002). They are shaped by changing water levels, complex groundwater-surfacewater exchange processes, and repeated erosion and deposition of sediments by recurrently floods (Amoros and Bornette, 2002; Tockner et al., 2008). They form various aquatic and terrestrial habitats, which are hydrologically connected. These habitats are strongly influenced by the exchange of organic matter and biota and shape diverse species communities (Ward et al. 2002; Naiman et al. 2010). Due to this habitat heterogeneity and its effects on ecosystem functioning and biodiversity, floodplains are ideal model ecosystems to study multiple stressor effects such as water pollution, flow modification, destruction or degradation of habitat (Tockner et al., 2010). These disturbances caused by the increasing “human demand for water, food and energy”, result in declining biodiversity of freshwaters greater than in most affected terrestrial ecosystems (Bunn, 2016; Dudgeon et al., 2006; Vörösmarty et al., 2010). Therefore, the knowledge on responses (resistance and resilience) of such complex ecosystems to stressors or disturbances is important for a development of solution-oriented recommendations on how to resolve the trade-offs when optimizing simultaneously different ecosystem services (Pahl-Wostl et al., 2013).

1.2 Microbial diversity as an ecological indicator for disturbance

For the assessment of disturbance and restoration success of aquatic and terrestrial ecosystems, ecological indication is widely used. However, it has traditionally focused more on structural measures such as species richness and density, water quality, algae, macrophytes, fish and river morphology but not on functional measures (Dziock et al., 2006; Friberg et al., 2011; Pander and Geist, 2013). Functional indicators are directly linked to ecosystem processes and involve the transfer of energy and materials such as primary productivity, respiration, litter decomposition or nutrient cycling (Wallace, 2007). They are considered to be important measures for ecosystem health and react rapidly to environmental changes, and therefore find a lot of attention in current river and stream ecology (Young et al.2008). In combination with structural indicators they can improve understanding of complex ecosystem relationships and predictions of ecosystem processes (Feio et al., 2010; Graham et al., 2016). Heterotrophic bacteria are the key players in ecosystem processes in both terrestrial and aquatic ecosystems. Mainly unexplored the diversity of microbes can help to understand complex interactions in nutrient cycling of soil and sediments (Torsvik et al., 1996). Nevertheless, past studies on functional indicators often focused on metabolism measurements as leaf breakdown, respiration rates and enzyme activities rather than on microbial diversity or microbial community structures (Bodmer et al., 2016; Gessner et al., 1999).

Due to the recent replacement of culture-based methods with PCR-based culture-independent molecular community analysis, it is possible to obtain a deeper insight into environmental microbial community structure, their diversity and their relationship to environmental parameters (Simon and Daniel, 2009; Tringe et al., 2005). For example microbial diversity in terrestrial soil habitats is strongly influenced by pH, typically showing higher diversity with a neutral pH rather than a high or low one (Lauber et al., 2009). Higher community respiration rates were shown to relate with increased microbial diversity (Bell et al., 2005). In

addition, other environmental factors such as soil temperature, plant diversity, moisture and nutrient availability also affected bacterial communities and their diversity (Carney and Matson 2005; Torsvik et al. 1996). Similar tendencies were found in aquatic ecosystems, with distinct bacterial communities, and strong relationship between physico-chemical properties, microbial structure and functioning (Freimann, 2012).

1.3 Ecological impact of hydropower

On a global scale, hydropower is boosted by the efforts to increase renewable energy supply, but it is also one of the main reasons of disturbances of riverine ecosystems. The obvious advantages for the global CO_2 balance have caused a trend of huge dam constructions all over the world with at least 3,700 major dams planned or already under construction, primarily in countries with emerging economies (Zarfl et al., 2015).

In Switzerland, with its rich water resources, and advantageous topography, the use of hydropower has a long history. In the 1970s, before the first nuclear power plant was build, 90% of the country's energy came from hydropower. Currently, Switzerland is still among the largest hydroelectricity producers in the European Alps with 604 hydropower plants over 300 kW and an average production of approximately 36 TWh/a corresponding to approximately 56% of the country's total electricity supply (Bundesamt für Energie (SFOE), 2015).

Even though hydropower is often seen as "green energy" production, it creates several negative impacts on riverine systems. In order to produce energy, river water is abstracted and released. This leads to hydropeaking and residual flow sections, both representing a major impairment on the natural functions and dynamics of a river.

Hydropeaking causes frequent discharge fluctuations, creating daily flood-like conditions. The subsequent amounts of water cause regular movement and transport of solid materials in the river channel bed and can, therefore, build harsh conditions for any harbouring organisms (Bruno et al., 2009). The rapidly increasing and decreasing discharge can lead to drift and casting away of organisms, such as fish and macroinvertebrates. Furthermore the intensity and frequency of the hydropeaking events can lead to destruction of natural habitats (Robinson, 2012; Schweizer et al., 2015). On the contrary, the residual flow is characterised by a constant minimal discharge, whereas the missing discharge dynamics lead to sedimentation and reduction of aquatic habitats (Dewson et al. 2007).

The intense use of hydropower together with land reclamation, flood protection, channelization, obstructions, etc. led to a massive degradation of floodplain ecosystems leaving only 20% with natural floodplain dynamics (BUWAL, 1993). In need to protect and restore degraded river and floodplain ecosystems the revised Swiss Water Protection Act, a federal law on water protection from January 24, 1991 (Swiss Water Protection Act, WPA; SR 814.20) will support the revitalisation (morphological improvement) of flowing waters (Art. 38a WPA) in the next 80 years (Göggel, 2012). Moreover, to reduce the adverse effects of hydropower on riverine ecosystems, hydropower plant owners must take appropriate mitigation measures by 2030 (Art. 83a WPA). Several actions will be implemented to reduce hydropeaking impacts (Art 39a WPA), bedload deficit (Art. 43a WPA), and to improve fish migration (Art.9 & 10 Federal law on fisheries). In addition, the existing law on appropriate residual flow conditions (Art. 80 WPA) will further improve river and floodplains located below hydropower infrastructures. These actions together with the large global

investments in hydropower, emphasize that the complex and important riverine ecosystems are in focus of multiple socio-economic and ecological interests.

In this context this study aims to answer the following question:

How does hydropower influence microbial diversity and community structure in floodplains?
We hypothesise that
i) the hydrological regime (hydropeaking, residual flow, natural floodplain) shapes the ecosystem properties of the soil and sediment in the different floodplain habitats
ii) subsequently the hydrological regime shapes the harbouring microbial diversity and community structure

To answer these questions, we investigated two different hydropower-impacted floodplain sections in the River Sarine, one residual flow and one hydropeaking section, and one natural reference system; the floodplain of the River Sense. In these river sections we assessed the structural properties of soil and sediments (total carbon and total nitrogen content, grain size distribution, water content) in different aquatic, semi aquatic and terrestrial habitats (main channel, side channel, island, riparian forest, open and vegetated gravel bar). In the same local areas, the spatial and temporal changes of microbial diversity and community structures were assessed by the use of the genetic fingerprinting technique Terminal Restriction fragment length polymorphism (T-RFLP). A wide array of studies showed the use of genetic fingerprinting methods such as T-RFLP is appropriate for assessing microbial ecology in different habitats (Blaud et al. 2015; Osborn et al. 2000). Nevertheless T-RFLP has rarely been used for riverine environments and there are no existing studies which combine the use of T-RFLP based microbial analyses for the assessment of ecosystem disturbances.

This master thesis is part of the project Hydroecology ans Floodplain Sustainability in Application (HyApp), which was founded over the Swiss National Science Foundation (SNF). The project is a collaboration between the Research Group Ecohydrology of the Zurich University of applied Sciences (ZHAW), the University of Zurich, the aquatic research institute of ETH (Eawag) and the Ecole polytechnique fédérale de Lausanne (EPFL).

2 Material and methods

2.1 Research Area

This study was conducted from August 2015 to November 2016 on the rivers Sarine and Sense in the canton of Fribourg, Switzerland (Figure 1).

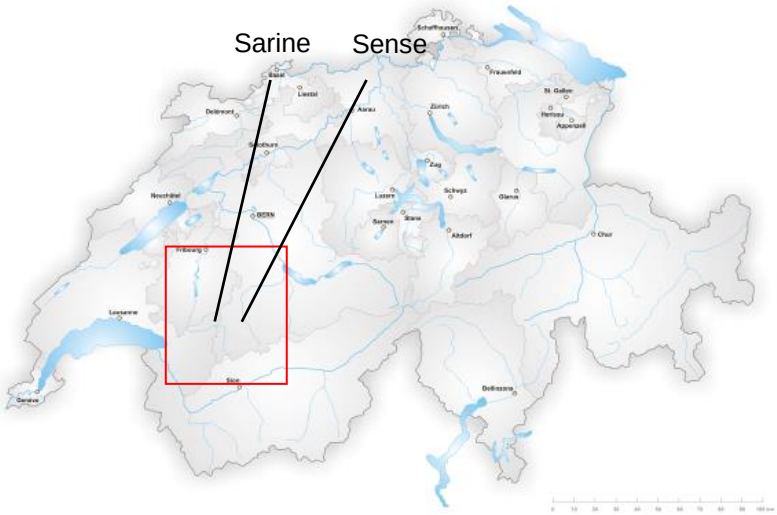

Figure 1: Location of the study area with the two rivers Sarine (N 46° 46.515 E 7° 7.284) and Sense (46°43'45.0"N 7°17'44.7"E).

The river Sarine is a hydropower-influenced river originating in the Bernese alps near Gstaad at 2344 m a.s.l. and flows into the Aare river after 126 km. Two sections of the Sarine were used as the main study area; i) the residual flow section between the Rossens dam (672 m a.s.l.), which is located between the Lac de Gruyère, and the hydropower plant in Hauterive (572 m a.s.l.) and ii) the hydropeaking section between the hydropower plant in Hauterive and Pont de Perolle (560 m a.s.l.,City of Fribourg). Within the two sections, three reaches have been chosen where the following floodplain habitats were present: riparian forests, islands, vegetated gravel bars (only in the residual flow section), open gravel bars, main channels and side channels (Figure 2).

The river Sense is one of the few rivers in Switzerland without any hydropower-influenced alteration in the flow regime and has been declared as one of the most natural rivers in the Northern alps (Hettrich and Ruff, 2011). Due to its neighbouring location to the Sarine as well as its similar settings, the Sense with its natural nivo-pluvial hydrological regime is an ideal reference floodplain for comparative studies. The Sense originates in the Gantrisch area, canton of Fribourg, and is officially called Sense after the confluence of the cold and the warm Sense at Zollhaus (871 m a.s.l.). Exactly 35 km later, the Sense flows into the Sarine at the village of Laupen (486 m a.s.l.). The study site at the River Sense, includes the following floodplain habitats: riparian forests, islands, areas with large woody debris, open gravel bars, the main channel, and side channels (Figure 3). The river Sarine and the river Sense both contain floodplain areas of national interest (BAFU, 2007) without any bigger morphological changes. Hence, the main difference between the three river sections compared in this study is their hydrological regime and consequently their discharge fluctuations (Figure 4, 5, 6).

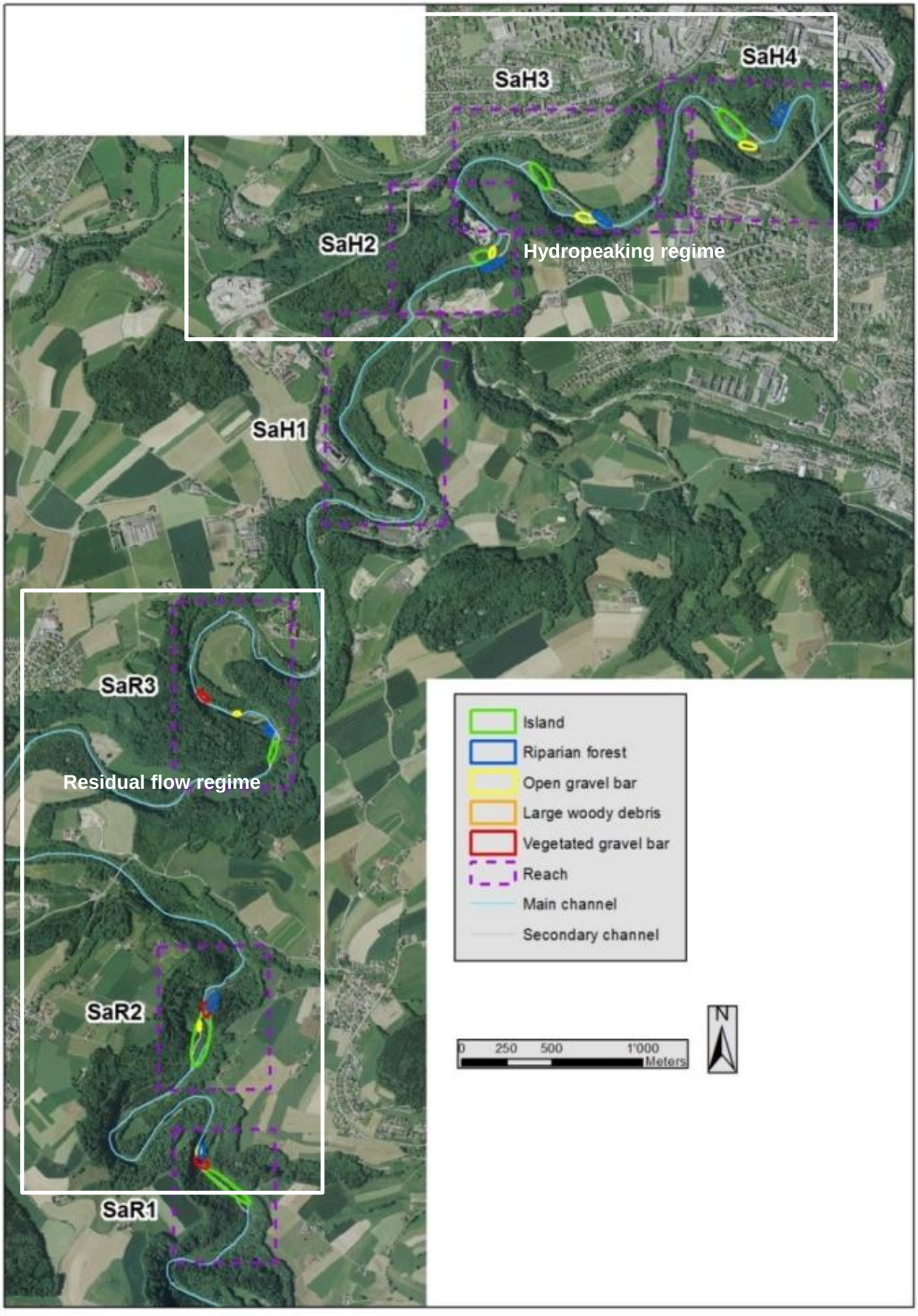

Figure 2: River Sarine with the hydropeaking regime and the three river reaches (SaH2-SaH4) on the upper part and the residual flow section with the three reaches (SaR1-SaR3) on the lower part of the figure. Marked in colors are the locations of the floodplain habitats. SaH1 was not part of this study.

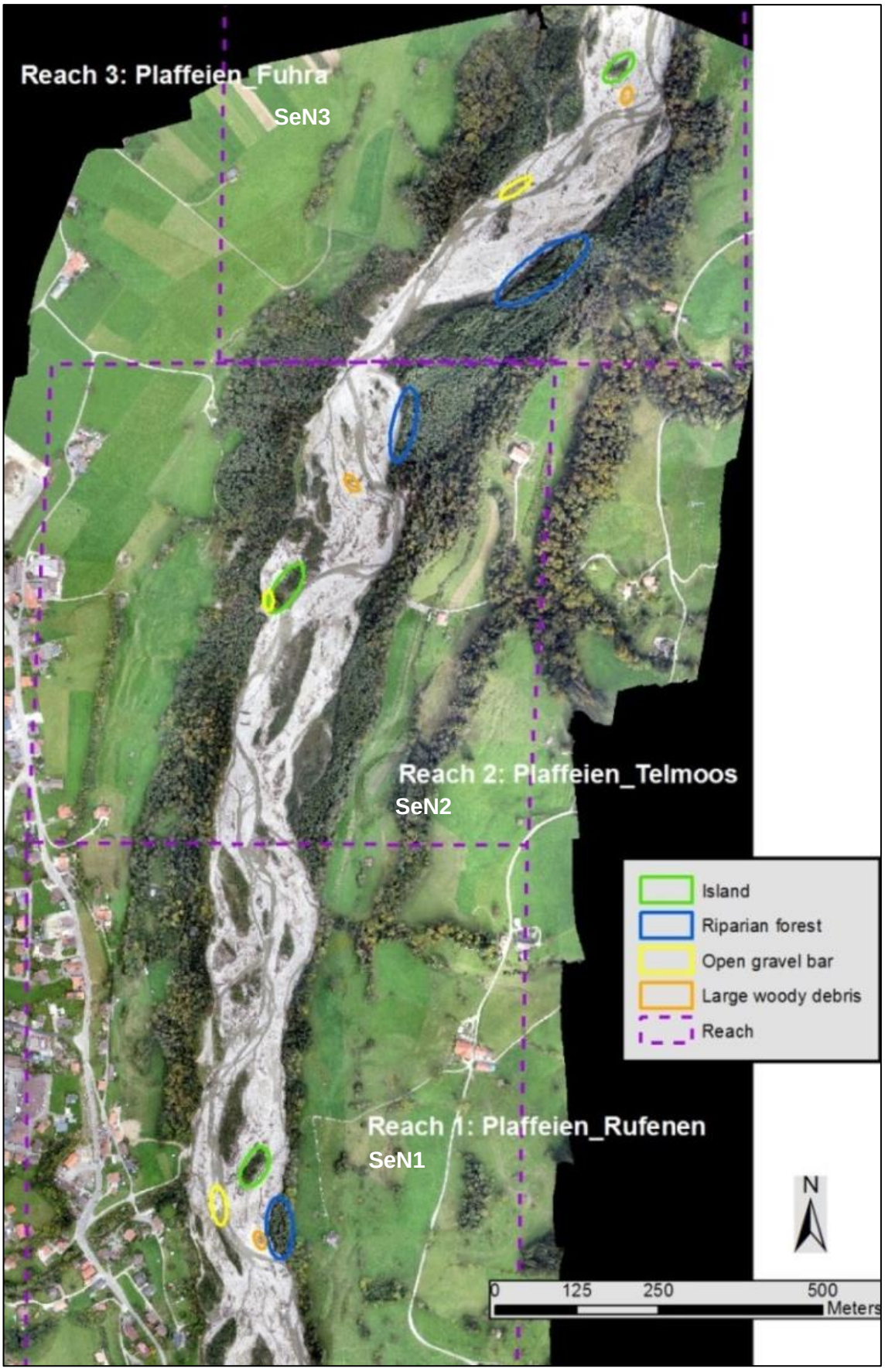

Figure 3: Floodplain of the river Sense as natural reference system showing an overview of the three river reaches (SeN1- SeN3) and the location of the different floodplain habitats.

2.2 Hydrology

The discharge in the residual flow section of the River Sarine consists of the controlled lake outflow from the Rossens dam, which accounts for a constant amount of 2.5m^3/s from 1 October - 19 May and 3.5m^3/s from 19 May - 30 September and occasional overflows from the dam (Figure 4, data from (Groupe-e, 2016). During the sampling years 2015 and 2016 the residual flow section of the river Sarine showed an annual mean discharge (MQ) of 3.82 m^3/s, a mean annual flood discharge (MHQ) of 95.3 m^3/s and a mean annual low water discharge (Q_{347}) of 2.5 m^3/s (calculated after Pfaundler et al. 2011).

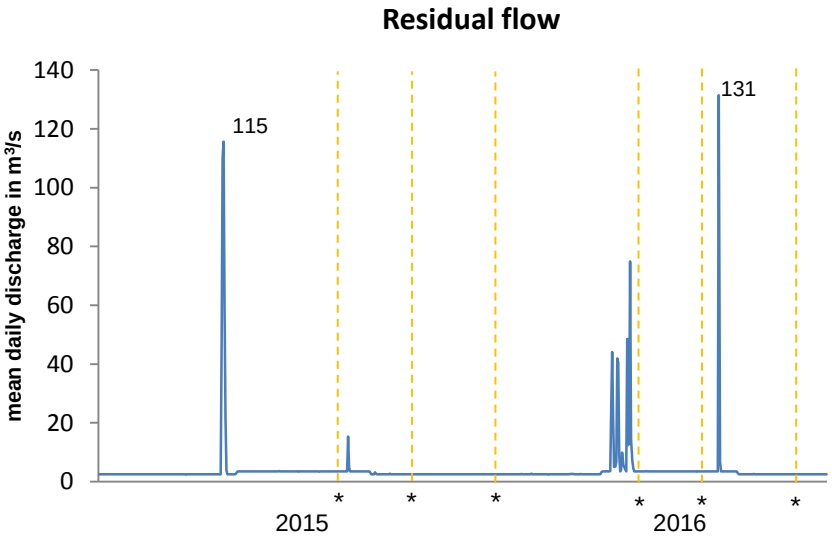

Figure 4: Mean daily discharge in m^3/s in the residual flow section of the river Sarine for the years 2015 and 2016. Numbers in the graph show the discharge of the largest flooding events during the sampling periods in m_3/s and stars mark the approximate sampling periods.

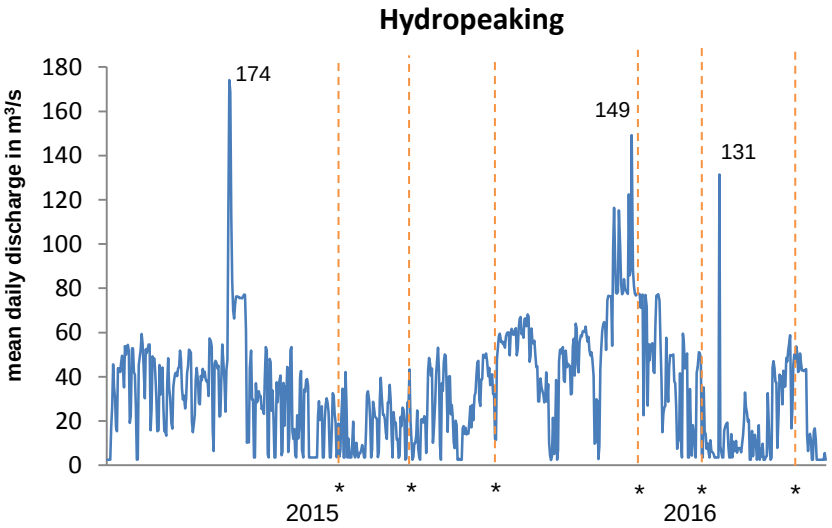

Figure 5: Mean daily discharge in m^3/s in the hydropeaking section of the river Sarine for the years 2015 and 2016. Numbers in the graph show the discharge of the largest flooding events during the sampling periods in m_3/s and stars mark the approximate sampling periods.

In the hydropeaking section of the river Sarine the discharge is controlled by the hydroelectric power plant in Hauterive where the water, abstracted at Rossens dam, is released to the river producing electricity and a daily fluctuation of discharge levels up to 70 m_3/s (Groupe-e, 2016). On average there were peaking periods

twice a day, in the morning and in the evening (Figure 5). The discharge flow for the hydropeaking section consists of the daily input from the hydroelectric plant added to the residual flow coming from up the stream. During the sampling years 2015 and 2016 the hydropeaking section of the river Sarine had an annual mean discharge (MQ) of 33.9 m^3/s, a mean annual flood discharge (MHQ) of 161.7 m^3/s and a mean annual low water discharge (Q_{347}) of 3.2 m^3/s (calculated after Pfaundler, 2011). The two influent rivers La Glâne und La Gérine were not taken into account.

The natural floodplain regime from the river Sense is mainly influenced by snowmelt and rain (nivo-pluvial) with often high discharge levels in spring. For mean discharge measures at the Sense an estimation was made based on a hydrological model (E-dric.ch, 2017). The annual mean discharge (MQ) is 4.3m^3/s and the mean annual flood discharge (MHQ) is 36.5 m^3/s where the annual low water discharge (Q_{347}) is 0.7m^3/s (Figure 6).

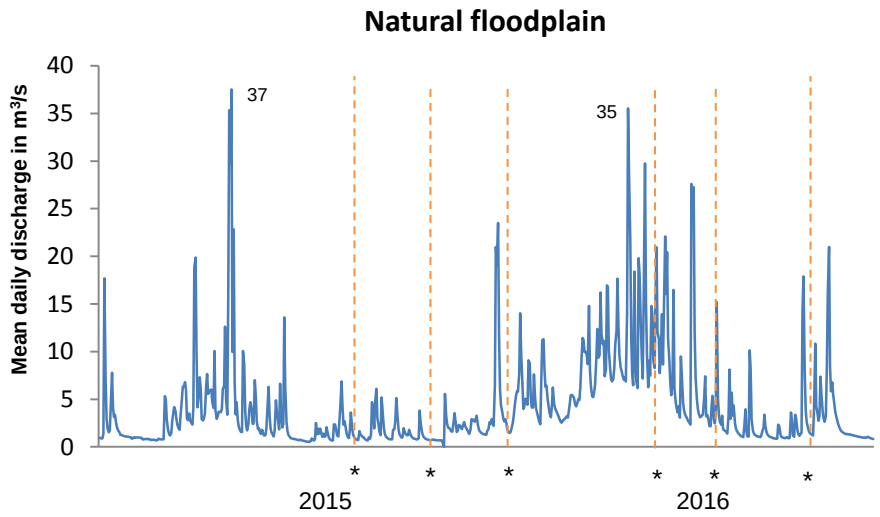

Figure 6: Mean daily discharge in m^3/s in the natural floodplain of the river Sense for the years 2015 and 2016. Numbers in the graph show the discharge of the largest flooding events during the sampling periods in m^3/s and stars mark the approximate sampling periods.

2.3 Sampling

A seasonal sampling was conducted from Summer 2015 through Autumn 2016 (see Table 1 or for more detailed sampling design see Appendix 1). Within each section, three reaches containing five to six different habitats were sampled with a total number of 52 samples collected during each sampling period. The according habitat characteristic can be seen in (Table 2).

Table 1: Seasonal sampling with the number of samples collected per season and section.

Season	Summer 2015	Autumn 2015	Winter 2016	Spring 2016	Summer 2016	Autumn 2016
Date of sampling	26 - 31 Aug 15	30 Oct - 7 Nov 15	14 - 30 Jan 16	22 June - 3 July 16	3 - 12 Aug 16	4 - 12 Nov 16
Sarine Residual Flow Section (SaR)	n=18	n=18	n=18	n=18	n=18	n=18
Sarine Hydropeaking Section (SaH)	n=15	n=15	n=15	n=15	n=15	n=15
Sense Natural Floodplain (SeN)	n=18	n=18	n=18	n=18	n=18	n=18

Table 2: Characterisation of terrestrial and aquatic habitat sites examined in this study adapted from Doering et al., 2011a.

Habitat type	Characteristics
Riparian Forest	Predominantly forested terrestrial* habitat type, fringing the active tract of the floodplain and characterised by developed soil
Island	Predominantly terrestrial* habitat type characterised by sandy substrata and developed soil
Vegetated gravel bar**	Predominantly terrestrial* areas characterised by vegetated gravel deposits
Open gravel bar	Predominantly terrestrial* areas characterised by bare or sparsely vegetated gravel deposits
Channels	Permanent lotic primary and secondary channels composed of coarse permeable gravel sediments and typically fringed by gravel bars
Large Woody Debris***	Predominantly terrestrial* accumulations of large wood trapping mainly fine sediments and organic matter

*These habitats possibly become aquatic habitats during floods.

** Vegetated gravel bar only present in the residual flow section.

*** Large woody debris only present in the natural floodplain.

In each habitat, three soil or sediment samples were taken. In the aquatic habitats, the sampling sites were selected as much towards the middle of the stream as possible, however stream sediment sampling was limited by flow velocity. At each sampling site, the upper layer of 10 cm was removed before collecting between 500-600 ml of sediment with a small garden hand shovel. The three sediment samples were pooled, mixed and sieved through a <8mm sieve. One sample was separated for DNA extraction (<2mm sieved, 50 ml falcon tube) and one for nutrient analysis (<2mm sieved, 50 ml falcon tube). The residue (~ 1000g) was filled in a plastic bag (1500 ml, clear polyethylene) for analysis of total organic matter contents by combustion, water content and grain size distribution analyses. Samples for DNA extraction were stored at -20°C, other samples at -4°C or -20°C until proceeding.

The temperature at each sampling site was measured with a temperature needle probe (Multi-Thermometer DT-300, VOLTCRAFT, Switzerland) and averaged over each habitat.

2.4 Sample processing

2.4.1 Abiotic and chemical parameters

Within twelve hours from sampling the water content (percentage water of dry soil or sediment) was determined by weighing, drying at 105°C for 24 h and reweighing. To determine the organic matter content, between 500 and 800 g of soil or sediment was dried at 105°C for 24 h, weighed, ashed at 500°C for 3 h and expressed as g ash free dry mass (AFDM) kg^{-1} dry weight. Ashed sediments were used to asses grain size distribution using sieves (Retsch GmbH, Germany) with mesh sizes 0.063, 2, 4, 8 mm. Total nitrogen and total carbon content of the sediments were measured and analysed by combustion using a Carbon-Hydrogen-Nitrogen-Analyzer (TrueSpec CHN Makro Analyser, Leco, USA).

2.4.2 Microbial diversity and community analysis using T-RFLP

Microbial diversity and communities were analysed using terminal restriction fragment length polymorphism (T-RFLP). This is a DNA fingerprinting method, allowing detecting of microbial community diversity and richness by laser detection of fluorescently end-labelled endonuclease-digested PCR products.

DNA of the sediment samples was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following the suppliers protocol which involved three main steps: i) homogenization of the environmental sample (0.25 g) by bead beating involving cell lysis by mechanical and chemical interaction, ii) capturing DNA on a silica membrane in a spin column, and iii) ethanol based washing and eluting the DNA from the membrane. For bead beating the MP FastPrep®-24 (MP Biomediacals, Solon, OH, USA) was used with following set up: 4m/s rotation speed, MP:2x24 for 45 seconds.

From the extracted DNA the partial 16S rRNA gene was amplified by PCR using two fluorescently labelled primers: forward primer 8F_Red (5'-AGA GTT TGA TCC TGG CTC AG-3') with fluorophore AT565 and reverse primer 534R_Green (5'-ATT ACC GCG GCT GCT GGC-3') with fluorophore AT532 (Microsynth AG, Balgach, CH) and (2U/µl) Thermo Scientific™ DyNAzyme™ II DNA Polymerase within a suitable mastermix created according to instructions from the supplier (Table 3). PCR amplifications were carried out in a T100 Thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) running the cycling program as described in Table 4.

Table 3: Mastermix (50 µl) used for PCR Reaction based on DyNAzyme™ II DNA Polymerase 2U/µl

Components	Volume per reaction [µl]	Final concentration
Forward Primer 8 F Red 10 µM	2.5	0.5 µM
Reverse Primer 534 Green 10 µM	2.5	0.5 µM
Buffer $MgCl_2$ free 10x	5	1x
BSA 100x	2.5	5x
$MgCl_2$ (50mM)	2.5	2.5 mM
dNTP's (2mM)	5	0.2 mM
DyNAzyme II DNA Polymerase (2U/µl)	0.5	0.2U/ µl
ddH_2O	25.5	-
Total mastermix	**46**	**-**
+ Template	4	

Table 4: RAD Thermocycler cycling protocol, including steps with corresponding temperatures [C°], time durations [min] and number of cycles.

	Step	Temperature[C°]	Time [min]	Cycles
	Initial Denaturation	94	2	
Cycles	Denaturation	94	0.3	35 x
	Annealing	55	0.3	
	Extension	72	0.5	
	Final extension	72	5	

Successful PCR amplification was verified by gel electrophoresis on 1.25 % agar. The PCR product was end-treated to correct for the effect of overhanging ends, by pipetting 2.34 µl Klenow-Mix (Table 5) to each amplified PCR product, incubating for 2 h at room temperature and inactivating for 10 min at 75°C in the thermocycler. PCR products were cleaned by filtering through a Millipore MultiScreen PCRµ96 filter plate (Merck KGaA, Darmstadt, Germany) using a vacuum pump, washed with ddH_2O (2x40 µl) and resuspended in 25 µl ddH_2O.

Table 5: Klenow-Mix for the end-treatment of the PCR product, based on Klenow Fragment (Thermofisher Scientific™, MA, USA)

Components	Volume per reaction [µl]	Final concentration
10x Polymerase-Buffer	2.2	9.4x
dNTP's (10µM)	0.1	0.427µM
Klenow Fragment 2U/ µl	0.04	0.03U/ µl
Total Klenow-Mix	**2.34**	

For each sample the purified PCR amplicon was digested by a single restriction enzyme (RE). Four different restriction enzymes (*Msp*I, *Rsa*I, *Hae*III, *Alu*I) with a recognition sequence of 4 base pairs were selected by *in silico* digestion of 16S rRNA gene sequences from microorganisms expected to be present in the environmental soil or sediment samples. Evaluating the pre-runs on the ABI Sequencer, *Alu*I and *Hae*III showed better results than *Msp*I and *Rsa*I in terms of fragment numbers, equalized signal strengths and amplitude and were retained for subsequent analyses. A total volume of 4 µl of PCR product was digested with 0.5 µl (see digestion mix in Table 6) of each single RE at 37°C in the thermocycler for 2 h and 30 min and the REs were inactivated at 80°C for 20 min. Of each digestion product 1 µl was mixed with 18.65 µl formamide and 0.35 µl GS LIZ 600 Size Standard (Thermofisher Scientific™, MA, USA) denatured for 10 min. at 95°C, directly incubated on ice for 5 min and analysed on an ABI 3500 capillary sequencer (Applied Biosystems) using a 50-cm capillary array filled with POP7 Polymer.

Table 6: Digestion Mix for the restriction enzymes *Hae*III and *Alu*I

Components	Volume per reaction [µl]	Final concentration
Tango Buffer/Buffer R 10x	1	1.67x
*Hae*III/*Alu*I 10U/µl	0.5	0.83U/ µl
H_2O	4.5	
Total Digestion Mix	**6**	
+ cleaned PCR Product	4	

2.5 Data analysis

2.5.1 Dataprocessing T-RFLP

The T-RFLP profiles (Figure 7) were analysed using GeneMapper® Software 5 (Applied Biosystems). Terminal restriction fragments (TRFs) between 40 and 500 bp were included in the analysis and exported as raw data from GeneMapper.

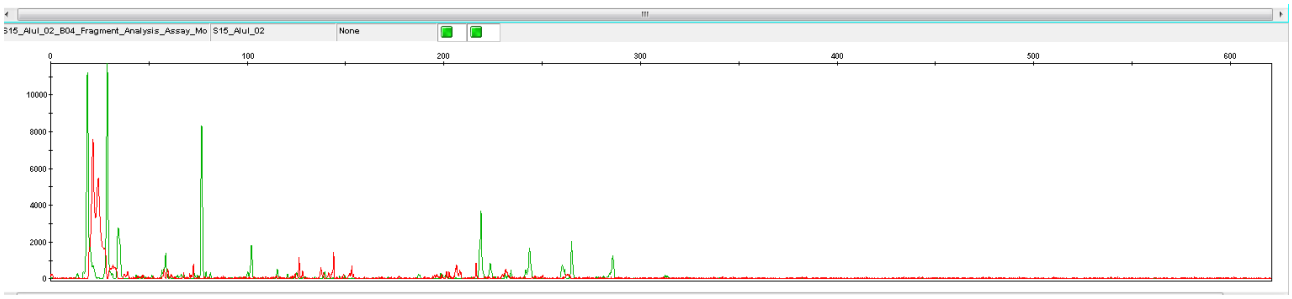

Figure 7: Example of a T-RFLP profile from ABI 3500 Sequencer (Applied Biosystems) processed by GeneMapper® Software 5 (Applied Biosystems). Fragment size in base pairs is shown at the top, while peak heights are shown as fluorescent units detected on y-axis. Red peaks show fragment lengths from the forward primer and green peaks show fragment lengths from the reverse primer.

Further data processing was carried out using the software T-REX: Software for the Processing and Analysis of T-RFLP data (Culman et al., 2009). The software filters noise over true peaks (Abdo et al., 2006). This approach identifies true peaks with an area which is higher than the standard deviation (assuming zero mean) over all peaks and multiplies them by factor 1. This procedure continues until no new true peaks are found.

Peak alignment was also performed in T-REX using the approach of the T-Align software program, where the smallest peak over all samples is identified and peaks within the specified clustering threshold are grouped into one terminal restriction fragment (TRF) (Smith et al., 2005). This procedure continues until all peaks are aligned in TRFs.

The two main data-outputs that were created in T-REX after aligning and filtering were i) TRF abundance data matrices based on the relativized peak areas of the present TRFs per sample, ii) total TRF numbers per sample. The abundance matrices were used to calculate the Shannon index (Shannon, 1948), to create distance matrices (Beals, 1984) and furthermore cluster analyses based on similarity (or dissimilarity). The number of total TRFs is expressed in total richness of the T-RFLP profiles and served as a measure of diversity. Comparisons of presence/absence matrices to abundance matrices showed abundance to be more adequate for similarity and cluster analyses because presence/absence matrices weighs rare species equally to common ones, whereas the abundance matrix includes information on peak heights or area (Culman et al., 2008)

The use of different REs is suggested in several studies (Blaud et al., 2015). Therefore, for this study we decided to use two different REs to classify the results.

2.5.2 Statistical analysis

All abiotic and chemical parameters were tested for normal distribution by Shapiro-Wilk test and for homogeneity of variance or homoscedacity with a Bartlett test (Shapiro et al. 1968). Results indicated non-

normal distributed data. Homoscedacity was not given for most of the parameters. For this reason, non-parametric tests were used. Kruskal-Wallis test was used to test on significant differences between the factors regime (hydropower influence), habitat and season and paired Wilcoxon rank sum test was applied to evaluate, which factors differed the most (Kruskal and Wallis, 1952; Wilcoxon, 1945).

Principal component analysis (PCA) was conducted on abiotic parameters to test the main components influencing the variance between regimes and habitats.

The number of terminal restriction fragments (TRF) per sample from T-RFLP analysis is equal to the number of taxa. Shannon-index was calculated on the relative abundance of TRFs (calculated on peak area) and was used as microbial diversity measure together with taxa richness (Hill et al., 2003). Spearman rank correlation was used to analyse the relationship between diversity (Taxa richness) and abiotic parameters.

Data from the T-RFLP abundance matrices were square root transformed and non-metric multidimensional scaling (NMDS) was created by the rank order of Bray-Curtis dissimilarity matrices with the function metaMDS from R package vegan (Oksanen et al., 2013). 2D Stress values have been calculated for each of the created and displayed NMDS plots which show the mismatch between the rank similarity matrices and the NMDS 2D representation. A 2D stress smaller 0.05 provides an excellent representation in reduced dimensions, a stress smaller 0.1, a great representation, smaller 0.2 a sufficient representation and values above 0.2 show only a poor representation.

All data analysis was performed using R Studio version 3.1.2 (R Core Team, 2014).

3 Results

3.1 Abiotic characteristics in hydropower influenced floodplain habitats

Sediment characteristics were analysed on six abiotic parameters which are summarized as mean and standard deviations (mean±SD) (Table 7). All parameters were pooled per habitat within the three different hydrological regimes and tested for significane with a Kruskal-Wallis test for the overall comparison (Table 8) and with a Wilcoxon rank sum test for the pairwise comparison of the habitats (Table in Appendix B).

Mean sediment temperature ranged from 9.8 °C to 13.0 °C being highest in the open gravel bar sediments of the hydropeaking section and lowest in the island sediments of the natural floodplain. The differences were not significant between the habitats or the hydrological regimes.

The highest mean organic matter contents were found in the terrestrial habitats riparian forest and islands, ranging from 70.24 g AFDM kg^{-1} in the hydropeaking regime to 41.91 g AFDM kg^{-1} in the natural floodplain regime. Lowest contents were measured in the open gravel bar habitat and the main channel ranging between 21.29 AFDM kg^{-1} in the residual flow regime and 12.9 AFDM kg^{-1} in the hydropeaking and the natural floodplain regime. All differences were highly significant (p-values <0.01).

Mean gravimetric water contents of the sediments ranged between 24.9 % and 6 % dry weight with significantly lower values in the open gravel bar habitats of all the three regimes (p-values <0.01). High water contents were measured in the terrestrial habitats riparian forest and island followed by the main and side channels.

Mean total carbon values were in a similar range between the habitats in the residual flow regime and in the natural floodplain. They ranged only slightly between 5.8 and 5 % dry weight, without being significantly different. In the hydropeaking regime total carbon values ranged between 5.02 % and 3.75 % dry weight, decreasing from the highest values in the terrestrial sediments riparian forest and island towards the lowest values in the sediments of the open gravel bar and the main channel and side channel. High and low values were significantly different (p-value <0.01). Total nitrogen values showed the same pattern, they were only significantly different between the habitats of the hydropeaking regime (p-value <0.05) and ranged between 0.31 and 0.17 % dry weight, both decreasing from the highest values in the terrestrial sediments of the riparian forest and island habitat, towards the lowest values in the aquatic sediments of the main channel, side channel and the sediments at the open gravel bar, which is influeced from daily flooding (see Figure 5) .

The analysis of grain size distribution showed the presence of bigger grain sizes (2-4 mm, 4-8 mm) in the sediments of the aquatic habitats main channel and side channel, whereas the smaller grain sizes (<0.063, 0.063-2mm) were more present in the sediments of the terrestrial habitats. An exception hereby was the residual flow regime, where the highest shares of the bigger grain sizes were found in the two gravel bar habitats, open gravel bar and vegetated gravel bar.

Within the single grain size distribution categories, significant differences were found between some of the habitats mainly between the terrestrial habitats island and riparian forest and the aquatic channel habitats (Appendix C).

Table 7: Mean and SD of abiotic parameters in floodplain habitats separated by regime (hydropeaking, residual flow and natural floodplain) calculated from pooled data over all six sampling seasons (August 2015 – November 2016) (n=248). Colour gradient: dark colours = high values, light colours=low values, no colour= minimal values.

*Values calculated for permanent aquatic sediments.

								Grain size distribution:			
			Temperature	**Organic Matter**	**Water Content**	**Total Carbon**	**Total Nitrogen**	**<0.063 mm**	**0.063 mm - 2mm**	**2 - 4 mm**	**4-8 mm**
	Habitat		[°C]	[g AFDM kg-1]	[%] d wt	[%] d wt	[%] d wt	[%] d wt	[%] d wt	[%] d wt	[%] d wt
Hydropeaking	Riparian Forest	Mean	10.7	70.24	23.58	5.02	0.31	2.0	68.5	8.1	21.4
		SD ±	6.0	28.87	9.71	1.21	0.13	2.1	29.6	7.9	24.9
	Island	Mean	10.5	54.12	18.66	4.41	0.24	2.1	64.4	8.5	24.8
		SD ±	6.0	44.71	9.27	0.52	0.09	2.0	46.2	6.9	24.3
	Open Gravel Bar	Mean	13.1	12.90	7.39	4.00	0.20	2.9	63.5	12.0	21.6
		SD ±	9.0	8.79	3.23	0.52	0.11	2.7	26.3	10.9	19.7
	Main Channel	Mean	12.0	19.44	15.49*	4.26	0.19	1.1	46.2	20.8	31.9
		SD ±	5.1	24.29	3.66	0.66	0.10	1.4	27.6	21.3	22.4
	Side Channel	Mean	11.6	13.87	16.27*	3.75	0.17	2.4	61.2	11.0	25.4
		SD ±	5.1	12.07	5.10	0.48	0.07	1.9	28.7	9.7	21.1
ResidualFlow	Riparian Forest	Mean	10.4	62.49	20.67	5.80	0.29	3.0	68.3	8.4	20.3
		SD ±	5.4	59.53	8.69	1.30	0.16	2.6	23.8	7.1	19.0
	Island	Mean	10.7	36.88	15.24	5.49	0.26	2.1	72.6	8.1	17.8
		SD ±	1.6	4.92	1.78	0.29	0.02	1.7	21.4	6.3	16.0
	Open Gravel Bar	Mean	11.4	21.29	6.08	5.26	0.21	1.2	52.5	13.3	32.9
		SD ±	6.0	41.08	3.26	1.36	0.08	1.4	23.4	7.2	18.5
	Vegetated Gravel Bar	Mean	12.1	32.33	14.00	5.21	0.24	1.3	52.5	12.1	34.1
		SD ±	6.2	31.25	9.56	1.39	0.09	1.7	26.9	8.1	22.9
	MainChannel	Mean	10.5	32.11	18.84*	5.03	0.20	3.0	65.4	8.8	22.7
		SD ±	4.0	46.67	6.85	1.06	0.13	3.0	25.6	6.4	21.8
	Side Channel	Mean	10.8	30.43	17.02*	5.33	0.21	2.1	64.4	10.7	22.8
		SD ±	3.3	36.45	8.03	1.25	0.09	2.1	26.0	9.5	21.8
Natural Floodplain	Riparian Forest	Mean	11.3	41.91	24.97	5.36	0.23	3.0	62.7	10.5	23.7
		SD ±	4.0	24.43	7.61	0.89	0.09	3.4	27.1	7.8	23.2
	Island	Mean	9.8	40.86	12.93	5.39	0.19	2.1	72.6	8.1	17.8
		SD ±	5.5	40.31	5.97	0.50	0.11	1.7	21.4	6.3	16.0
	Open Gravel Bar	Mean	11.1	40.30	9.57	5.24	0.21	7.5	65.6	9.1	17.8
		SD ±	8.4	57.34	5.79	0.68	0.09	23.8	25.4	7.5	14.9
	Large Woody Debris	Mean	12.4	18.64	17.33	5.17	0.19	2.1	61.1	12.2	24.7
		SD ±	6.6	6.49	4.47	0.85	0.07	2.6	29.5	10.5	22.7
	Main Channel	Mean	10.1	12.95	17.53*	5.21	0.20	2.9	46.9	14.7	35.5
		SD ±	5.7	9.75	1.98	0.64	0.12	6.8	21.1	8.0	18.7
	Side Channel	Mean	12.8	32.02	18.87*	5.41	0.18	2.3	59.9	12.6	25.3
		SD ±	5.2	41.29	3.17	0.48	0.07	2.4	24.6	7.1	20.2

Table 8: Summary on Kruskal-Wallis test for abiotic parameters with p-value and degree of freedom (df). Overall comparison between the regimes, between habitats within each individual regime and between seasons.

*p-value <0.1, ** p-value <0.05, ***p-value <0.01

							Grain size distribution			
Factor		**Sediment temperature**	**Organic Matter**	**Water Content**	**Total Carbon**	**Total Nitrogen**	**<0.063 mm**	**0.063 mm - 2mm**	**2 - 4 mm**	**4-8 mm**
Regime (n=248)	p-Value	0.8616	0.653	0.6458	9.958e-15***	0.12	0.7116	0.9223	0.8545	0.7088
	df	2	2	2	2	2	2	2	2	2
Regime HP * Habitat (n=76)	p-Value	0.8965	2.932e-08***	1.468e-06***	0.002966***	0.01131**	0.1357	0.1655	0.03026**	0.5491
	df	4	4	4	4	4	4	4	4	4
Regime RF * Habitat (n=85)	p-Value	0.9678	0.000518***	0.0003376***	0.8842	0.3269	0.05*	0.1102	0.2161	0.2022
	df	5	5	5	5	5	5	5	5	5
Regime NF * Habitat (n=76)	p-Value	0.676	0.001552***	2.551e-06***	0.837	0.5453	0.03225**	0.02222**	0.1191	0.01898**
	df	5	5	5	5	5	5	5	5	5
Season (n=248)	p-Value	< 2.2e-16***	9.41e-06***	0.01929**	0.6439	2.303e-16***	0.1647	0.01322**	0.8015	0.01477**
	df	5	5	5	5	5	5	5	5	5

Seasonal influence on abiotic parameters was high. Sediment temperature (Kruskal-Wallis p-value <0.01), organic matter content (Kruskal-Wallis p-value <0.01), water content (Kruskal-Wallis p-value <0.05) and total nitrogen content (Kruskal-Wallis p-value: <0.01) differed significantly between the seasons (Table 8).

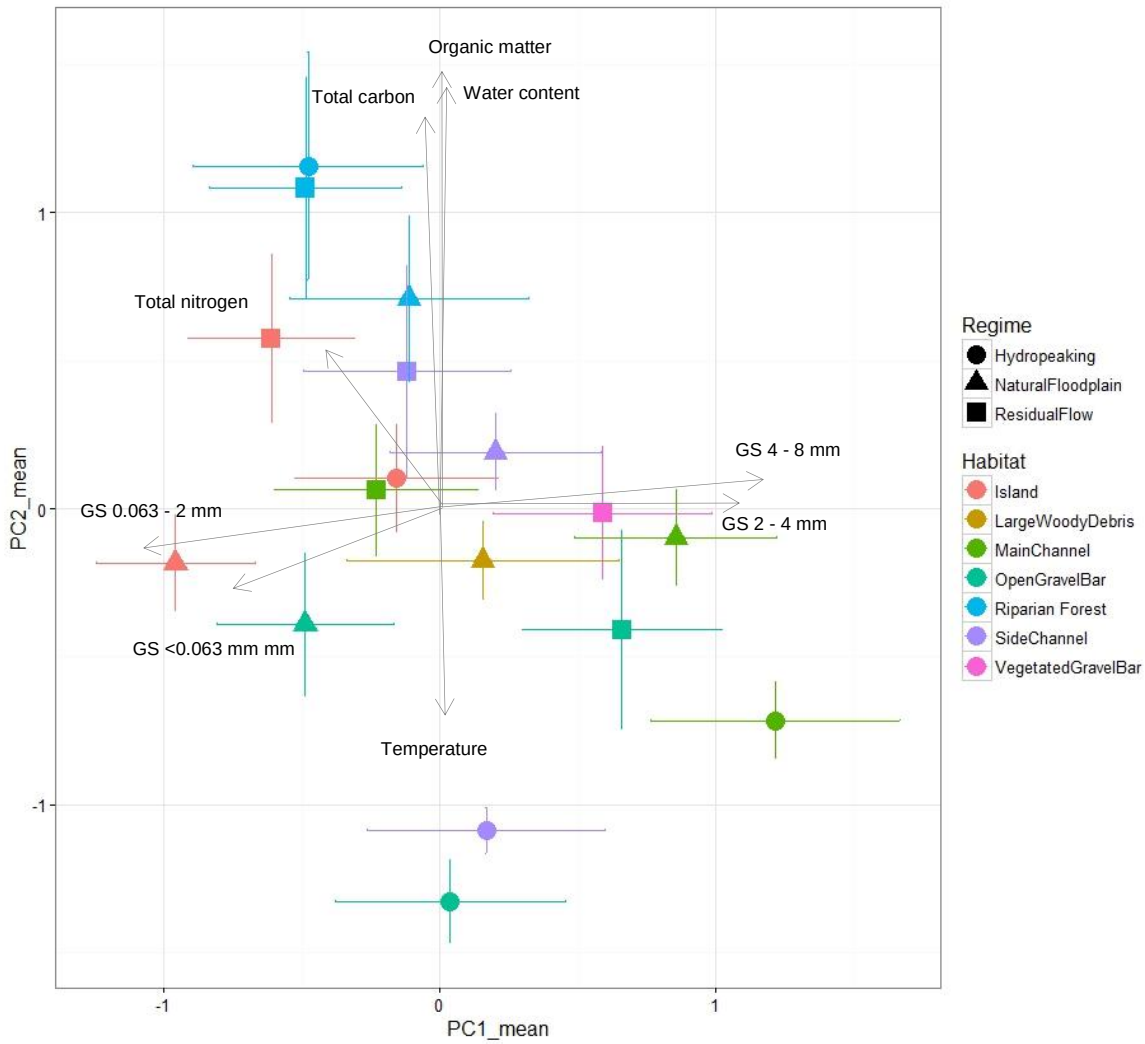

Figure 8: Biplot of principle component analysis (PCA) with abiotic parameters temperature [°C], water content [% dry weight], total organic matter [g AFDM kg-1], total carbon [% dry weight], total nitrogen [% dry weight], grain size distribution [GS, % dry weight]. Data from all season was used and grouped per habitats within the individual regimes (mean and standard error). PC1 explains 27.5 % of data variation and PC2 axis explains 23.3% of data variation, both axis explain 50.8% of data variance.

Ordination with a principle component analysis (PCA) confirmed the different abiotic characteristics of the selected sampling sites and showed a separation between the habitats, as expected mainly between the aquatic and the terrestrial habitats (Figure 8). The separation on the level of the regime was less clear but the aquatic habitats of the hydropeaking regime were separated on the lower side of the graph. PCA was conducted with all abiotic parameters. Samples were grouped by regime and habitat and calculated as mean and standard deviations. A PCA calculates the contribution of each parameter to the spatial separation of the samples. Loading of the parameters show the grain size (GS) being the parameter explaining horizontal variation for axis PC1 (27%) and total nitrogen, total carbon, organic matter, water content, temperature explaining vertical variation for axis PC2 (23.3%).

3.2 Microbial diversity

All analyses on the T-RFLP data (community and diversity analyses) were applied on the results from both the two restriction enzymes (RE) *Alu*I and *Hae*III. Though for reasons of simplification, values and graphs are only showed for the RE *Alu*I, this step is justified by a strong correlation (r_s = 0.752) between the number of T-RFs of the two enzymes *Alu*I and *Hae*III (Figure 9).

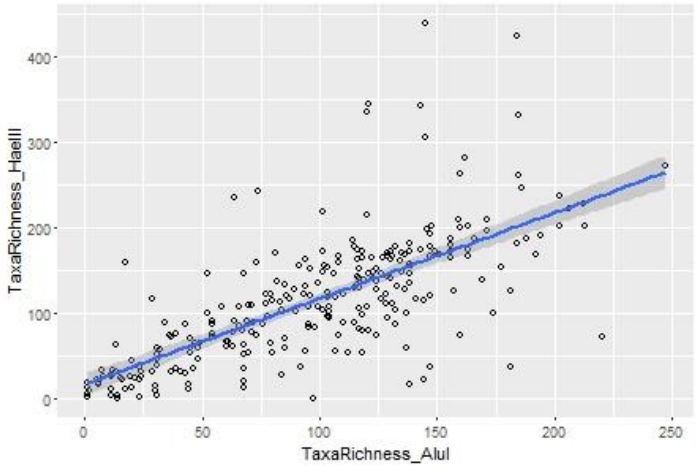

Figure 9: Scatterplot of the correlation between the taxa richness from the two applied restriction enzymes *Alu*I and *Hae*III (spearman rank coefficient, r_s = 0.752, p-value< 0.01).

Microbial diversity was measured by taxa richness, based on the numbers of terminal restriction fragments (TRF) and furthermore Shannon-Index was calculated on the relative abundance of TRF Peaks from the TRFLP profiles (Appendix D). Shannon-Index and taxa richness showed a strong correlation (r_s=0.703) and therefore boxplots are only shown for taxa richness assuming the same trends for the Shannon-Index.

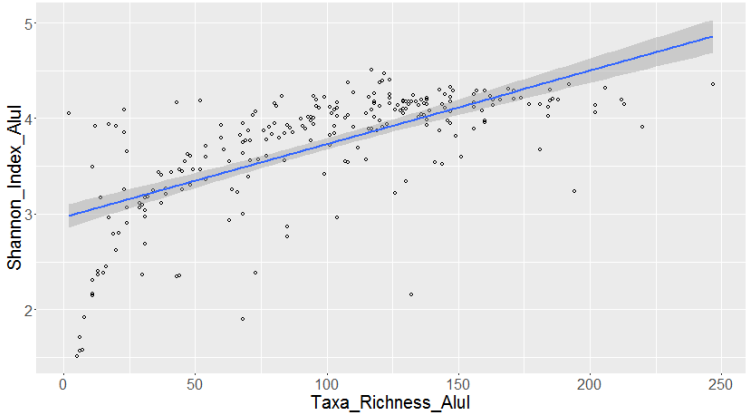

Figure 10: Scatterplot of the correlation between the taxa richness and the Shannon-Index over all data (spearman rank coefficient, r_s = 0.703, p-value< 0.01).

Overall taxa richness ranged between 63±40 and 118±57. Comparing the taxa richness between the floodplain habitats within each regime showed the lowest taxa numbers in the habitat open gravel bar for both of the hydropower systems (Table 9) but only in the hydropeaking system differences were significant (Kruskal-Wallis test, p-value 0.028) (Figure 11). Higher numbers of taxa were found in the sediments of the aquatic habitats main and side channel and in the two terrestrial habitats island and riparian forest (Table 9). On the contrary the natural floodplain regime showed a different pattern with lower taxa richness in the aquatic habitats and higher taxa richness in the terrestrial habitats (Table 9).

Table 9: Microbial diversity measures for each habitat expressed in mean ± SD taxa richness (number of TRFs), mean ± SD Shannon index (calculated on the relative abundance of TRF peaks area by RE AluI), separated by regime.

		All habitats	Main Channel	Side Channel	Open Gravel Bar	Vegetated Gravel Bar	Large Woody Debris	Island	Riparian Forest
Hydro-peaking n=76	Taxa richness	95±51	111±42	85±52	63±40	-	-	108 ± 58	108±50
	Shannon Index	3.70±0.65	3.88±0.3	3.56±0.84	3.4±0.56	-	-	3.89±0.51	3.74±0.84
Residual flow n=85	Taxa richness	106±48	118±57	115±40	91±35	109±59	-	98±50	104±45
	Shannon Index	3.8±0.5	3.77±0.54	3.9±0.47	3.71±0.5	3.87±0.4	-	3.75±0.58	3.77±0.53
Natural floodplain n=87	Taxa richness	94±56	71±53	87±41	85±62	-	98±71	110±52	118±45
	Shannon Index	3.7±0.64	3.49±0.59	3.81±0.43	3.61±0.84	-	3.43±0.82	3.92±0.47	3.83±0.46

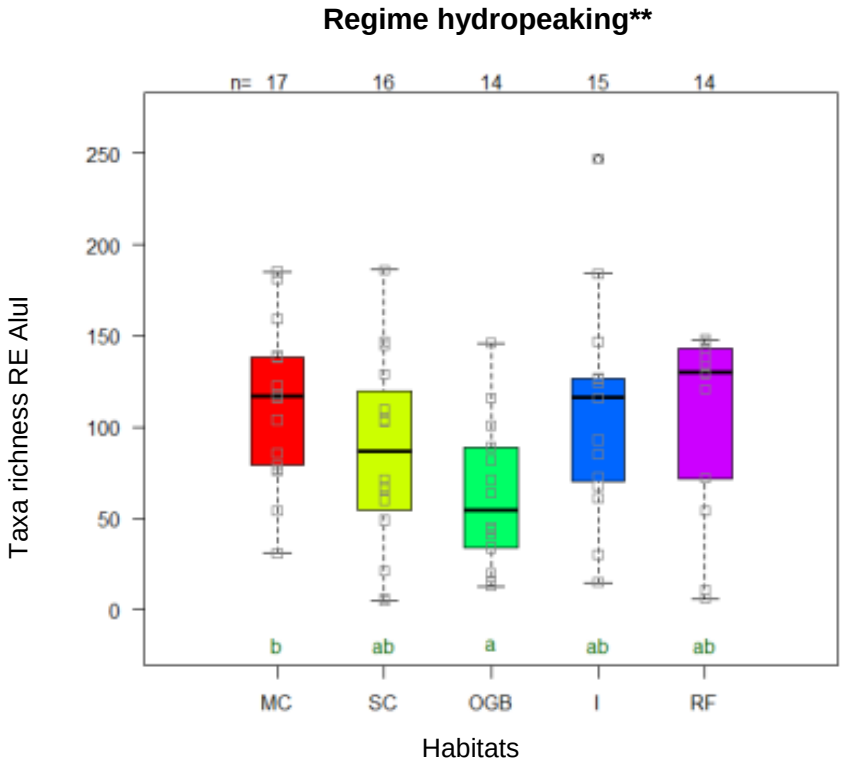

Figure 11: Boxplots on differences in taxa richness RE *Alu*I between habitats (MC=main channel, SC =side channel, OGB=open gravel bar, VGB= vegetated gravel bar, LWD=large woody debris, I=island, RF=riparian forest) within the regime hydropeaking (Kruskal-Wallis, p-value 0.028**, B). Letters show significant differences based on wilcoxon rank sum test (p-value <0.05).

The comparison between the hydrological regimes within one single habitat type showed significant differences in the main channel habitat between the hydropeaking regime and the natural floodplain (Figure 12). The other habitat differences were not significant, neither they were significant between the regimes (Appendix D).

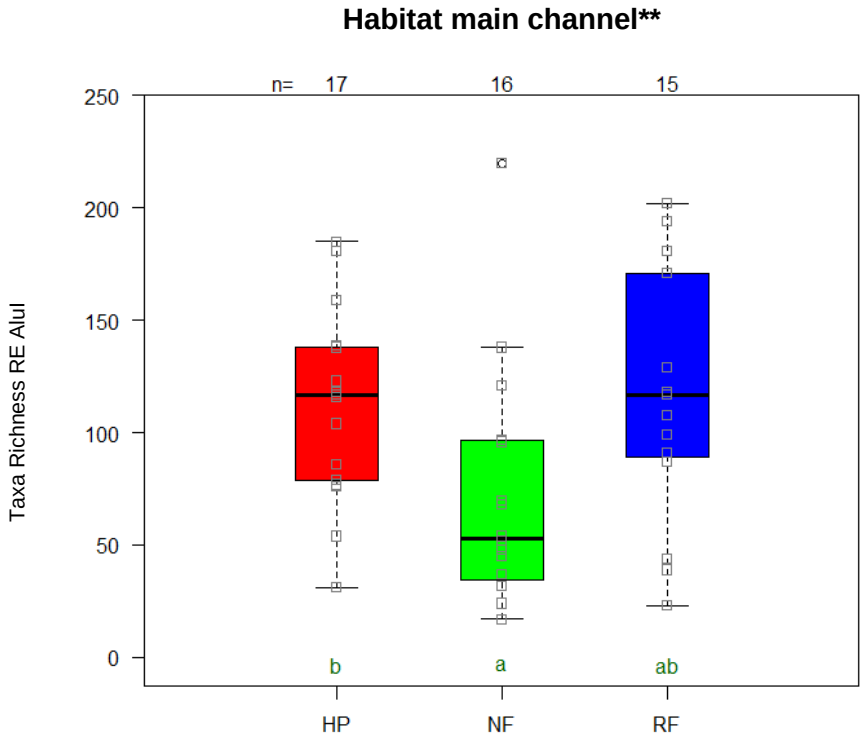

Figure 12: Boxplots on differences in taxa richness RE *Alu*I between regimes (HP=Hydropeaking, NF= Natural floodplain, RF=Residual flow) within the individual habitat type main channel (Kruskal-Wallis test, p-value <0.019**). Letters show significant differences based on wilcoxon rank sum test (p-value <0.05).

Seasonal influence on diversity was analysed separately. Boxplots show significant seasonal differences in all the regimes (Kruskal-Wallis p-values <0.01, Figure 13 A) B) C). In the hydropeaking regime pairwise tests located the significant differences between the summer and autumn 2015 samplings (Figure 13 A) and in the residual flow regime between autumn 2016, spring 2016 and the other seasons (pairwise Wilcoxon rank sum test p-values < 0.01). In the Natural Floodplain taxa richness was significantly lower in autumn 2016 than in all the other seasons (Figure 13 C).

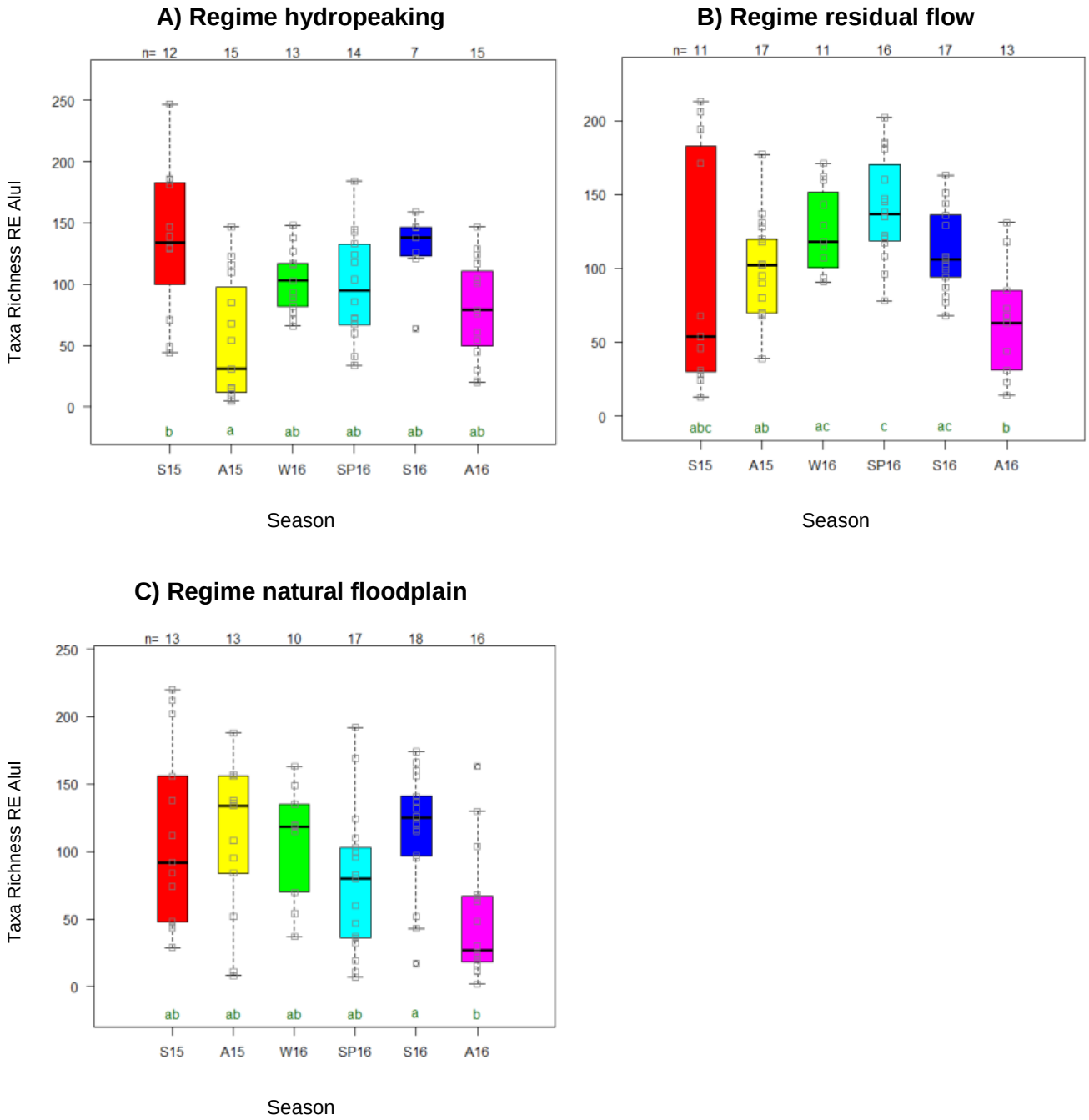

Figure 13: Boxplots for taxa richness RE AluI grouped by season (S15=summer 2015, A15=autumn 2015, W16=winter 2016, SP16=spring 2016, A16= autumn 2016). A) pooled data from the Hydropeaking regime (Kruskal-Wallis Test, p-value < 0.01), B) pooled data from the residual flow regime (Kruskal-Wallis Test, p-value < 0.01), C) pooled data from the Natural Floodplain Regime (Kruskal-Wallis Test, p-value < 0.01). Letters show significant differences based on wilcoxon rank sum test (p-value <0.05).

Taxa richness did not correlate significantly with abiotic factors on the overall pooled data, neither when the data was pooled per regime (Table 10). Looking at the correlations between taxa richness and abiotic factors within the habitats in the individual regimes there were a few significant correlations. Within the habitat open gravel bar of the Hydropeaking regime taxa richness correlated positively with the total organic matter content and two of the grain size categories (2-4 mm and 4-8 mm), whereas negative correlation was found for the other two grain size categories (<0.063 mm and 0.063-2mm). In the habitat main channel, taxa richness correlated positive with temperature, and in the side channel negative with the total carbon content. In the habitats of the residual flow regime, none of the abiotic parameters showed any significant correlations with taxa richness and in the natural floodplain regime, there were only two parameters correlating significantly: total carbon in the habitat open gravel bar and grain size category <0.063 mm in the habitat side channel.

Table 10: Spearman rank correlations (rs values) of taxa richness and all abiotic factors for each regime and its individual habitats from pooled data over all six sampling seasons (August 2015 – November 2016), *=p-value < 0.05.

Regime	Habitat	Temperature [°C]	Total Organic Matter [g AFDM kg-1]	Water Content [%] dry wt	Total Carbon [%] dry wt	Total Nitrogen [%] dry wt	Grain Size Distribution <0.063 mm [%] dry wt	0.063-2 mm [%] dry wt	2 - 4 mm [%] dry wt	4 - 8 mm [%] dry wt
Hydropeaking		0.27	0.2	0.14	0.033	0.15	-0.25	-0.19	0.3	0.18
Residual Flow		0.07	-0.092	0.19	-0.22	-0.25	0.13	0.062	0.092	0.063
Natural Floodplain		0.019	-0.053	0.0041	0.11	0.1	0.065	0.25	-0.16	-0.18
All Data		0.11	0.043	0.092	0.015	0.0021	-0.0061	0.057	-0.01	-0.029
Hydropeaking	Riparian forest	0.17	0.39	0.073	0.18	0.11	-0.13	0.062	0.092	0.063
	Island	-0.37	0.11	-0.2	-0.46	0.063	0.0088	0.5	-0.23	-0.45
	Open gravel bar	0.09	0.71*	0.21	-0.46	0.026	-0.65*	-0.61*	0.71*	0.51*
	Main channel	0.66*	-0.28	-0.39	0.065	-0.053	-0.0066	0.2	0.23	0.17
	Side channel	0.36	0.36	0	-0.55*	0.15	-0.1	-0.42	0.48	0.48
Residual Flow	Riparian forest	0.12	-0.22	0.36	-0.22	-0.14	0	0.39	-0.25	-0.33
	Island	-0.12	0.23	0.35	-0.062	-0.48	0.26	0.0059	-0.097	-0.14
	Open gravel bar	-0.26	0.26	0.42	-0.51	-0.21	-0.064	0.19	0.13	0.037
	Vegetated gravel bar	0.23	-0.28	0.015	-0.35	0.11	-0.3	-0.1	0.21	0.12
	Main channel	0.28	-0.47	-0.016	-0.079	-0.38	0.2	0.14	-0.29	-0.093
	Side channel	0.24	-0.042	-0.12	-0.23	-0.21	0.4	0.049	0.047	-0.2
Natural Floodplain	Riparian forest	0.36	-0.16	0.18	-0.34	-0.13	0.12	-0.018	-0.018	0.064
	Island	0.12	0.11	-0.31	0.14	0.23	0.16	0.081	-0.0033	-0.069
	Open gravel bar	-0.16	-0.057	-0.075	0.56*	0.072	-0.084	0.41	-0.075	-0.17
	Large woody debris	0.24	0.26	0.071	-0.45	0.15	0.16	0.081	-0.0033	-0.069
	Main channel	0.31	-0.4	0.26	0.33	0.42	0.2	0.3	-0.09	-0.26
	Side channel	-0.38	-0.46	-0.34	0.42	0.13	-0.59*	0.16	0.0022	-0.13

3.3 Microbial community structure

The microbial community structure was analysed based on the differences between each sample in the abundance matrix of the relative peak areas from the T-RFLP profiles per sample. The similarities were calculated using the Bray Curtis distance and a non-metric multidimensional scaling (NMDS) was used to visualize the distances between the samples.

Summarizing the means and standard errors (mean±SE) of the nMDS calculations showed stronger clustering by habitats than by regime (Figure 14). Within the regimes there were more dissimilarities between the samples in the hydropeaking and the natural floodplain regime compared to the residual flow, which can be seen on the wider scattering of the samples compared to the clustering of the residual flow samples in the middle of the plot. The dissimilarities on the level of the habitats showed a clear separation between the aquatic (upper left side) and the terrestrial habitats (lower right side). The habitat open gravel bar clustered more with the aquatic habitats than with the terrestrial.

Summarizing (mean±SE) the samples by season and regime, showed a much clearer separation by season than by regime (Figure 15). A vertical separation is visible especially between the seasons summer 2015 (S15), autumn 2015 (A15) and the seasons summer 2016 and autumn 2016. On the horizontal axis summer seasons were clustering towards the right side.

The summary (mean±SE) of seasons and habitats showed a similar pattern with a stronger separation between the seasons than between the habitats (Figure 16).

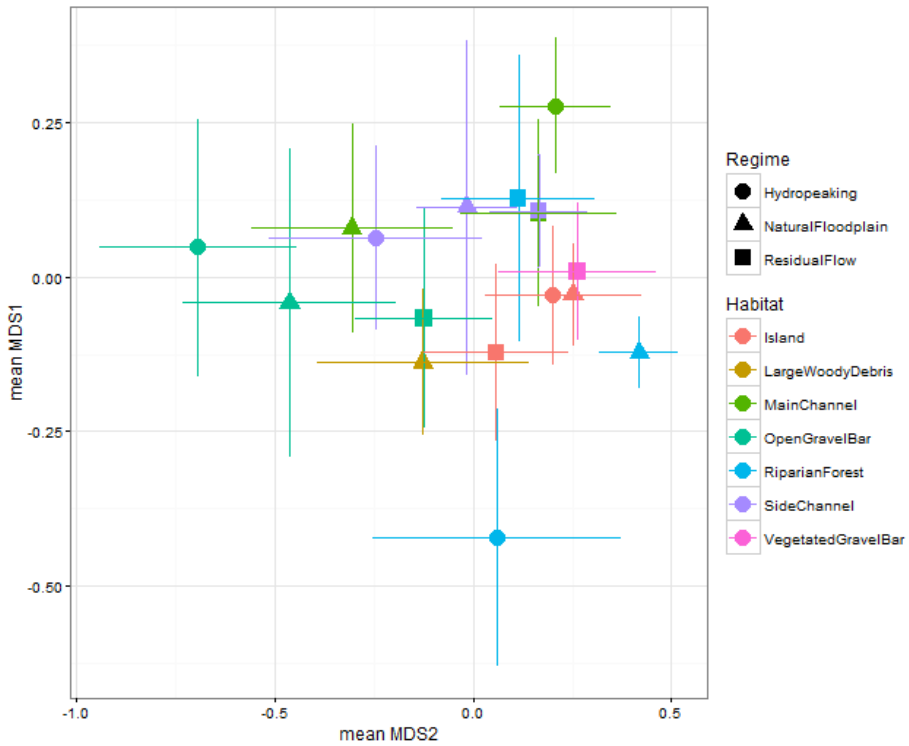

Figure 14: nMDS plot showing seasons based on bray-curtis similarities of the bacterial community composition generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzyme *Alu*I. Mean and standard error is shown for the nMDS statistics and summarized for habitats and regimes. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

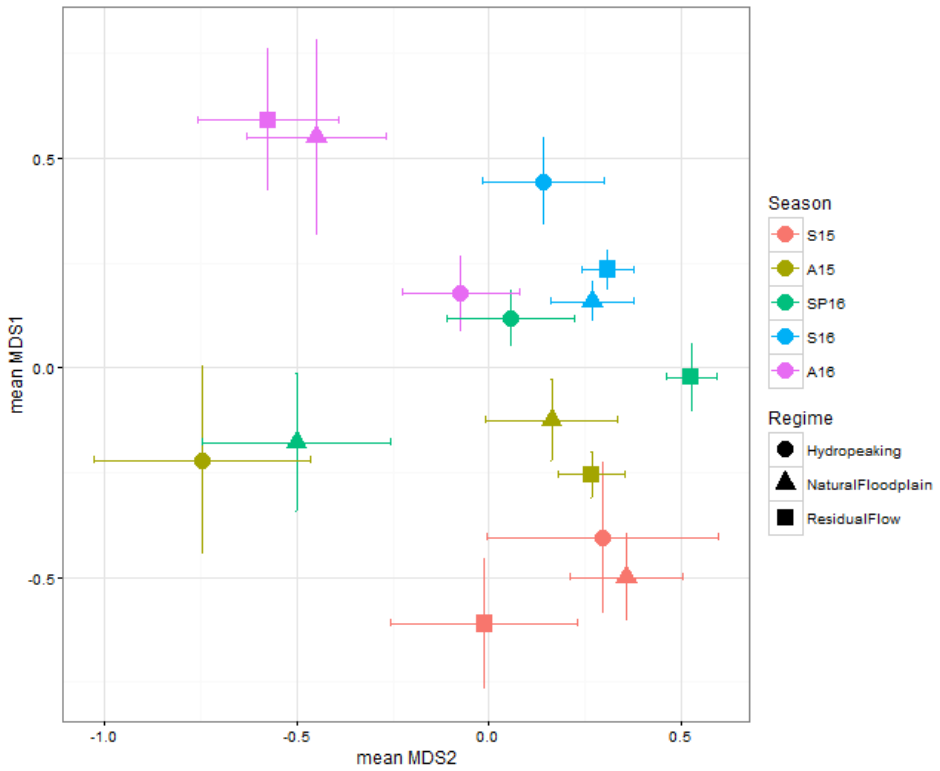

Figure 15: nMDS plot showing seasons based on bray-curtis similarities of the bacterial community composition generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzyme *Alu*I. Mean and standard error is shown for the nMDS statistics and summarized for seasons and regimes. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

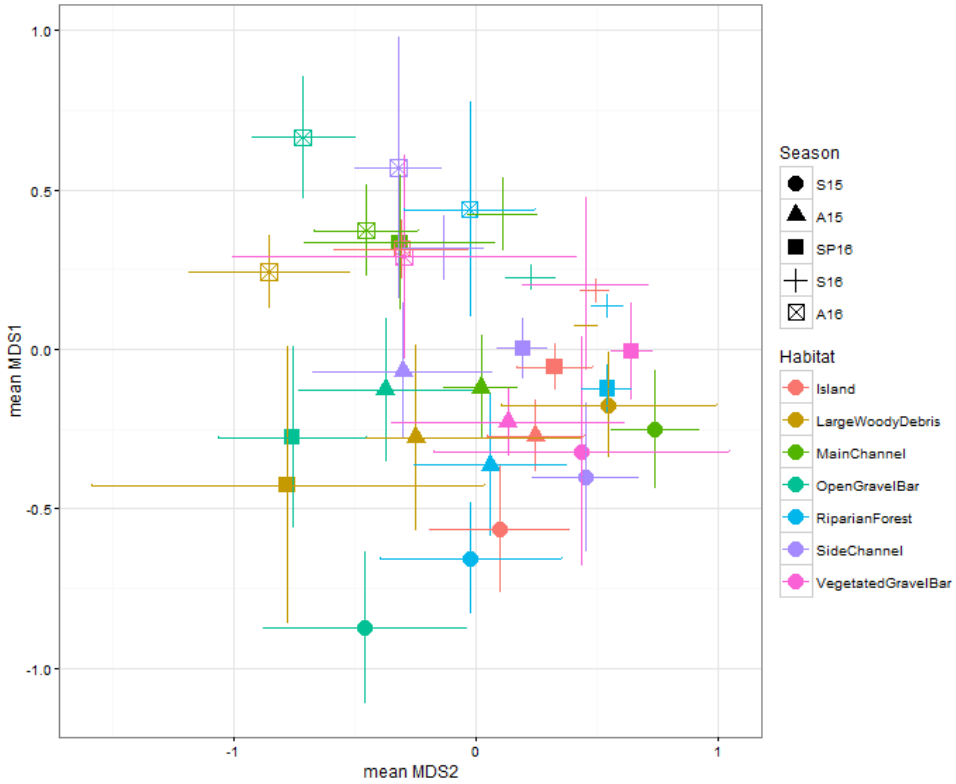

Figure 16: nMDS plot showing seasons based on bray-curtis similarities of the bacterial community composition generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzyme *Alu*I. Mean and standard error is shown for the nMDS statistics and summarized for seasons and regimes. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

4 Discussion

The present study focused on the microbial diversity and community structure in three hydrologically different floodplain sections. Two of the systems are impacted by hydropower and one served as a natural reference system. Abiotic characteristics of the floodplain habitats and genetic fingerprinting (T-RFLP) of the microbial communities were used to assess differences and linkages between and within the three floodplain sections and the present floodplain habitats.

4.1 Abiotic characteristics in hydropower influenced floodplains

The investigated study sites differed in abiotic characteristics in spatial (habitat), hydrological (regime) and temporal (season) dimensions.

The floodplain habitats mainly differed in contents of organic matter and water which allowed a separation between terrestrial habitats with higher values and the aquatic influenced habitats with lower values. The sediments of the habitat open gravel bar stood out with the minimum overall values for all the abiotic measurments, except temperature. The habitat open gravel bar is stated as "predominantly terrestrial habitat, which becomes possibly an aquatic habitat during floods" (Doering et al., 2011). In the hydropeaking regime with daily discharge fluctuations of 70 m^3/s and in the natural floodplain regime with its nivo-pluvial influence the open gravel bar can therefore count as mainly aquatic habitat. The hydrological influence in combination with the high representation of the bigger grain sizes could possibly foster the flushing out of the organic matter and nutrients and would therefore explain the low values for these parameters. In general open gravel bar is known for its harsh environment characterised by extreme temperature variation, high water stress and low productivity (Doering et al., 2011). Eventhough we didn't measure the same parameters our results suggest a similar characterisation of this habitat.

In contrast, the sediments of the terrestrial habitats island and riparian forest showed high organic matter, high water, and high nutrient contents. Riparian forests and islands are known to provide large organic carbon amounts to a floodplain system due to leaf-decomposition (Langhans et al., 2008). This input together with a lower hydrological impact is an explanation for the higher values and therefore might be an explanation for the clear separation between terrestrial habitats and the aquatic influenced habitats.

In natural floodplains the interaction between the terrestrial and aquatic habitats, is an important driver of ecosystem properties (Langhans et al., 2006). In our study, the separation between terrestrial and mainly aquatic habitat properties can lead to the conclusion that there was no regularly hydrological exchange between the terrestrial habitats and the aquatic habitats in all of the investigated regimes. However, it remains unknown in which extent a hydrological exchange within the terrestrial and aquatic floodplain habitats happened.

The most relevant differences between aquatic and terrestrial habitats were observed in the hydropeaking regime. Several studies showed an alteration of abiotic characteristics caused by repeated hydropeaking events and floodings (Robinson, 2012). In this context the strong influence of the daily discharge fluctuations in the hydropeaking section might be a possible explanation for the minmal organic matter, nutrient and water contents.

Previous studies showed a strong seasonal effect on abiotic properties, mainly driven by temperature (Mulholland and Hill, 1997; Tang et al., 2006). The significant seasonal differences in sediment temperature in this study supports this assumptions. However, it seems obvious, that temperature has a strong effect on all the other abiotic parameters. But in this study it remains unclear if temperature is solely responsible for the seasonal effect or if other seasonal influences as for example naturally flooding events due to rain or snow melt could be an important influence as well. The discharge measurements of the three investigated systems show several flooding events during the sampling period (of which one was an artificial triggered flood) which also could possibly explain some of the seasonal variations. In our study we analysed data from six different sampling seasons. As just illustrated there were effects of seasonality visible. The question remains, how far the seasonal influence concerns the other two dimensions (spatial, hydrological) and if there is a trade-off in effects on abiotic characteristics.

4.2 Abiotic characteristics influence microbial diversity and community structure

Microbes are the main drivers of nutrient cycling and organic matter turnover and therefore it is not surprising that our results show a linkage between some of the illustrated abiotic habitat characteristics and the microbial diversity and community structures.

The lowest taxa richness was found in the sediments of the open gravel bar in the hydropeaking regime. This corresponds with the previous observations that the habitat open gravel bar represents harsh conditions for biotic processes. Various studies show connections between soil and sediment characteristics and microbes but mainly measured the microbial activity by respiration or gross primary production rates (Buchmann, 2000; Doering et al., 2011b; Uehlinger and Naegeli, 1998). One study which had a closer look at the bacterial community structure in an aquatic environment, showed strong relationships between physico-chemical parameters and microbial community structures and therefore confirm the assumed linkage (Freimann, 2012).

The microbial diversity within the floodplain habitats showed unexpected differences between the two hydropower impacted systems and the natural floodplain. Surprisingly the taxa richness was highest in the sediments of the main channels of both the two hydropower impacted systems. Taxa numbers were decreasing from the habitat main channel towards the gravel bar with the lowest value and showed increasing values in the terrestrial habitats. Due to a lack of studies which investigate the influence of hydropower on microbiology, the comparison to other biotic indicators is a possibility. Studies on the influence of hydropeaking and residual flow on macroinvertebrates for example are numerous but results are not equivocal. The reduced discharge in the residual flow section of a hydropower influenced river or floodplain system is usually leading to a reduction of taxa numbers for macroinvertebrates induced by a loss of habitat diversity due to low heterogenity in flow velocities and a reduction of the wet area (Cazaubon and Giudicelli, 1999; Dewson et al., 2007). On the contrary a study on macroinvertebrates within this project showed highest taxa numbers of macroinvertebrates in the residual flow corresponding with the high number of microbial taxa richness in this study (Kipfer and Schneeberger, 2016, unpublished). One reason for higher taxa in the residual flow section could be that the river Sarine, even though influenced by a reduced flow regime, is still a floodplain with the presence of the typical floodplain habitats, compared to other studies on more morphologically impacted rivers.

The high number of taxa in the main channel of the hydropeaking regime is contradictory to most of the literature on hydropeaking effects and also to the results of macroinvertebrate studies in this project (Englund and Malmqvist, 1996; Kipfer and Schneeberger, 2016 unpublished; Schülting et al., 2016). A possible explanation hereby is that microbes are more resistant or resiliant to disturbances than macroinvertebrates. A study about the effects of experimental floods showed that after three years of regularly experimental floods a regime shift took place and the taxa decrease from macroinvertebrates was reduced by 30% suggesting a shift to a more adapted community (Robinson, 2012). The transfer of these results to microbial analysis would suggest the conclusion, that the community in the main channel of the hydropeaking section is more resiliant to flood disturbance. A faster colonization rate of some micobial species or the much smaller sizes of bacteria compared to macroinvertebrates might be another possible explanation, but further research is needed to support these assumptions. However, when the data of the habitats was pooled and only differences between the regimes were observed, there was no significant difference in taxa numbers between the three hydrological regimes, therefore it is difficult to insist on these linkages.

The lower taxa richness in the aquatic habitats of the natural floodplain can be explained due to different overall nutrient availability in the river Sense which can be seen especially in the very low taxa levels in the channels. The river Sarine shows much higher nitrogen and phosphorus concentration which could possibly be due to the much longer distance between source and sampling sites and the subsequent multiple agricultural influences (Kipfer and Schneeberger, 2016, unpublished).

Our results showed only a few statistically significant correlations between abiotic parameters and taxa richness. Nevertheless these correlations corresponded well with the already presented results. Significant correlations were found in the main channel habitat of the hydropeaking section. Taxa richness strongly correlated with temperature which points again towards the importance of the temporal aspect because sediment temperature highly corresponded with the sampling season. In the habitat open gravel bar of the hydropeaking regime, there was a positive correlation between taxa richness and organic matter content. This relationship makes sense because both taxa richness and organic matter content was low in the open gravel habitat. Another positive correlation was found between the presence of bigger grain sizes (2-4 mm and 4-8 mm) and taxa richness which stays in contrast to the assumption that fine grain sizes have a higher surface-to-volume ratio and therefore a higher colonisable area (Hargrave, 1972). However, there were similar patterns observed when analysing the microbial community structure by dissimilarity analyses. Cluster analyses showed a visible separation between the aquatic and terrestrial habitats but little evidence was found for distinct communities between the three hydrological regimes. The individual seasons showed clearly separated communities.

4.3 Methodological Considerations

This study showed trends and patterns of distinct microbial communities and relationships between abiotic factors and microbial diversity but just partly with clear evidence. The differences in microbial diversity and community structure would have been expected to be stronger. This could be interpreted in various directions. One explanation is that bacterial diversity and community structure was not affected as much as expected or there are several trade-offs between the different influences of hydropower, habitat structure or seasonality on microbial communities. Another possibility is that the T-RFLP method and the further data

processing and statistics have limited possibilities for the assessment of such complex ecosystems. Currently the next generation sequencing techniques (NGS) are emerging the assessment of microbial diversity and community structures. Nevertheless, studies with the T-RFLP method are still being frequently used in the research due to lower costs and less intensive data processing. T-RFLP allows making statements on microbial diversity and community structure on the level of distinguishable communities and taxa numbers, allowing the link to other structural or functional patterns. However, the results of this study demonstrate that in the broader scale of assessing whole ecosystems, T-RFLP seems an appropriate time and money saving method for a first overview. For a more detailed analysis on microbial species and abundances a first overview by T-RFLP can help to conduct more targeted NGS analyses.

4.4 Conclusion and outlook

To our knowledge, this study is the first study assessing microbial diversity and community structures in hydropower impacted floodplains and it can serve as a first overview on the effects of disturbances on riverine systems caused by hydropower plants. Interesting properties of floodplain habitats, the hydrological regimes and physical and chemical characteristics could be highlighted as for example a correlation between organic matter and taxa richness at some of the study sites or clearly different community structures between the sampling seasons.

The first hypothesis, that the hydrological regime shapes the ecosystem properties of the soil and sediment in the different floodplain habitats was partly supported by the study. The main differences in ecosystem properties, were observed between aquatic and terrestrial habitats, within each of the three hydrologically different regimes (hydropeaking, residual flow, natural floodplain). Besides this linkage, this study also revealed distinct abiotic properties in the hydropeaking regime, especially in the highly impacted habitat open gravel bar, which allows a partial confirmation of the first hypothesis. Nevertheless, the strong influence of seasonality could have caused trade-off effects especially in the less disturbed residual flow and natural floodplain regimes.

With this study, we tried to illustrate several relationships between abiotic factors and microbial diversity within the same habitats and regimes and highlight the distinct abiotic properties. The microbial community structure analysis did not show totally distinct communities but it could illustrate first insights that the communities clearly differ between aquatic and terrestrial habitats. The difference was weak between different regimes but strong between different seasons. With this, we can partly confirm the second hypothesis stating, that, as a consequence of the first hypothesis, the hydrological regime shapes indirectly the harbouring microbial diversity and community structure.

Furthermore, the results cannot confirm the suitability of microbial diversity and community structure analyses for the assessment of disturbances but they show a trend that suggests and justifies a further research. An interesting further research approach would be to include quantifying measures such as qPCR or analysing the activities of the bacteria by RNA based analyses. Both techniques would allow a deeper insight into the linkages between microbes and the important ecosystem processes and support the knowledge on the effects of human induced disturbances on ecosystems.

Acknowledgement

I would like to thank Michael Döring for initiating this thesis and his help and support during the whole project. For the support especially during the field work I would like to thank Diego Tonolla. I also want to thank the other field team members, especially Christa Gufler and Cristian Gees for the support during the sampling periods. Furthermore I want to thank Fabio Rezzonico and his team for the great support in the lab and for the reading and evaluating of this thesis as Co-referee, Theo Smits for his general support and his great inputs on the topic and Rahel Wanner and Nicola for the support with the nutrient analyses. Additionally I want to thank my team colleagues Linda Tschirren, Dominik Refardt, Zala Schmautz, Ranka Junge, Constanze Pietsch and Sophia Egloff for their support with the statistics software r and the efforts in proofreading my thesis.

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Appendix

Appendix A: Sampling Design

Appendix B: Wilcoxon-pairwise test results

Appendix C: Additional boxplots

Appendix E: Additional NMDS Plots

Appendix A: Detailed sampling Desing

Sarine - Residual flow			**Sarine - Hydropeaking**		
River Reach	**Sampling site**	**Sample Code**	**River Reach**	**Sampling site**	**Sample Code**
1	Vegetated gravel bar 1	SaR1_VGB1	2	Island 1	SaH2_I1
	Island 1	SaR1_I1		Riparian forest 1	SaH2_RF1
	Riparian forest 1	SaR1_RF1		Open gravel bar 1	SaH2_OGB1
	Main channel 1	SaR1_MC1		Main channel 1	SaH2_MC1
	Secondary channel 1	SaR1_SC1		Secondary channel 1	SaH2_SC1
	Open gravel bar 1	SaR1_OGB1			
2	Vegetated gravel bar 2	SaR2_VGB2	3	Island 2	SaH3_I2
	Island 2	SaR2_I2		Riparian forest 2	SaH3_RF2
	Riparian forest 2	SaR2_RF2		Open gravel bar 2	SaH3_OGB2
	Main channel 2	SaR2_MC2		Main channel 2	SaH3_MC2
	Secondary channel 2	SaR2_SC2		Secondary channel 2	SaH3_SC2
	Open gravel bar 2	SaR2_OGB2			
3	Vegetated gravel bar 3	SaR3_VGB3	4	Island 3	SaH4_I3
	Island 3	SaR3_I3		Riparian forest 3	SaH4_RF3
	Riparian forest 3	SaR3_RF3		Open gravel bar 3	SaH4_OGB3
	Main channel 3	SaR3_MC3		Main channel 3	SaH4_MC3
	Secondary channel 3	SaR3_SC3		Secondary channel 3	SaH4_SCD3
	Open gravel bar 3	SaR3_OGB3			
	Total samples	**18**		**Total samples**	**15**

Sense - Natural floodplain		
River Reach	**Sampling site**	**Sample Code**
1	Island 1	SeN1_I1
	Riparian forest 1	SeN1_RF1
	Open gravel bar 1	SeN1_OGB1
	Main channel 1	SeN1_MC1
	Secondary channel 1	SeN1_SC1
	Large woody debris 1	SeN1_LWD1
2	Island 2	SeN2_I2
	Riparian forest 2	SeN2_RF2
	Open gravel bar 2	SeN2_OGB2
	Main channel 2	SeN2_MC2
	Secondary channel 2	SeN2_SC2
	Large woody debris 2	SeN2_LWD2
3	Island 3	SeN3_I3
	Riparian forest 3	SeN3_RF3
	Open gravel bar 3	SeN3_OGB3
	Main channel 3	SeN3_MC3
	Secondary channel 3	SeN3_SC3
	Large woody debris 3	SeN3_LWD3
	Total samples	**18**

Total	**51**

Appendix B: Results on Wilcoxon rank sum tests for significant abiotic parameters

Regimes and all habitats

TC	Hydropeaking	NaturalFloodplain
NaturalFloodplain	6.10E-10	-
ResidualFlow	3.40E-14	0.44

Habitats in hydropeaking section

OM	main channel	side channel	open gravel bar	island
side channel	1	-	-	-
open gravel bar	1	1	-	-
island	0.00476	0.00012	2.00E-05	-
riparian forest	0.0002	5.50E-07	9.00E-07	0.055

WC	main channel	side channel	open gravel bar	island
side channel	0.68716	-	-	-
open gravel bar	6.20E-05	0.00037	-	-
island	0.68716	0.68716	0.00098	-
riparian forest	0.01175	0.03958	3.50E-06	0.68716

TC	main channel	side channel	open gravel bar	island
side channel	0.195	-	-	-
open gravel bar	0.605	0.605	-	-
island	0.737	0.019	0.345	-
riparian forest	0.459	0.039	0.195	0.605

TN	main channel	side channel	open gravel bar	island
side channel	1	-	-	-
open gravel bar	1	1	-	-
island	0.492	0.351	0.717	-
riparian forest	0.093	0.029	0.182	0.717

GS 2-4 mm	main channel	side channel	open gravel bar	island
side channel	0.586	-	-	-
open gravel bar	0.522	1	-	-
island	0.032	1	1	-
riparian forest	0.036	1	1	1

Habitats in residual flow section

OM	main channel	side channel	open gravel bar	island	riparian forest
side channel	0.8305	-	-	-	-
open gravel bar	0.7544	0.8305	-	-	-
island	0.2361	0.3829	0.0025	-	-
riparian forest	0.2141	0.2412	0.0058	0.8305	-
vegetated gravel bar	0.8305	0.6571	0.0173	0.8305	0.6133

WC	main channel	side channel	open gravel bar	island	riparian forest
side channel	1	-	-	-	-
open gravel bar	0.00031	0.00633	-	-	-
island	0.84527	1	0.00564	-	-
riparian forest	1	1	1.20E-05	0.84527	-
vegetated gravel bar	1	1	0.44758	1	0.84527

GS1	main channel	side channel	open gravel bar	island	riparian forest
side channel	1	-	-	-	-
open gravel bar	0.73	1	-	-	-
island	1	1	0.73	-	-
riparian forest	1	1	0.25	1	-
vegetated gravel bar	0.99	1	1	0.73	0.11

Habitats in natural floodplain

OM	main channel	side channel	open gravel bar	island	riparian forest
side channel	1	-	-	-	-
open gravel bar	0.1436	1	-	-	-
island	0.0046	0.7014	1	-	-
riparian forest	0.0027	0.762	1	1	-
large woody debris	0.1068	1	1	1	0.1928

WC	main channel	side channel	open gravel bar	island	riparian forest
side channel	1	-	-	-	-
open gravel bar	0.00585	0.00248	-	-	-
island	0.21862	0.04838	0.23889	-	-
riparian forest	0.04697	0.08987	0.00026	0.00303	-
large woody debris	1	1	0.00388	0.19828	0.04838

GS 1	main channel	side channel	open gravel bar	island	riparian forest
side channel	0.611	-	-	-	-
open gravel bar	0.611		-	-	-
island	0.064	0.661	0.661	-	-
riparian forest	0.611	1	1	1	-
large woody debris	1	1	1	0.693	1

GS 2	main channel	side channel	open gravel bar	island	riparian forest
side channel	1	-	-	-	-
open gravel bar	0.3531		-	-	-
island	0.0052	0.3072	1	-	-
riparian forest	1	1	1	0.699	-
large woody debris	1	1	1	1	1

GS 4	main channel	side channel	open gravel bar	island	riparian forest
side channel	1	-	-	-	-
open gravel bar	0.1595		-	-	-
island	0.0049	0.4034	1	-	-
riparian forest	1	1	1	1	-
large woody debris	1	1	1	1	1

Habitats all data

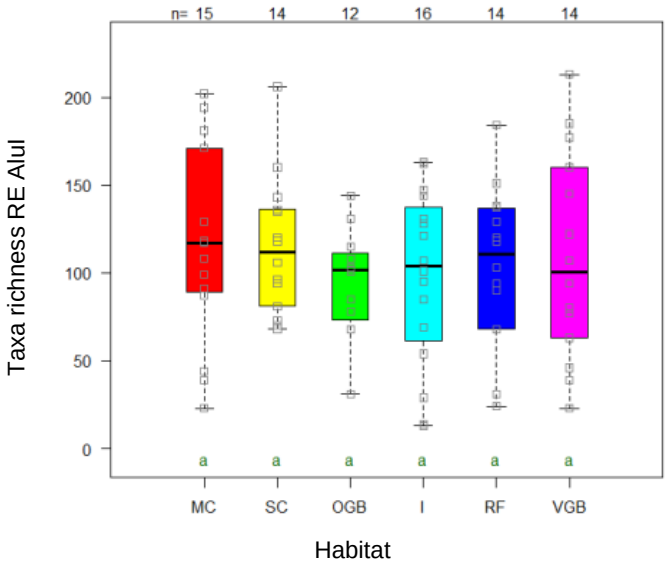
OM	main channel	side channel	open gravel bar	vegetated gravel bar	large woody debris	island
side channel	1	-	-	-	-	-
open gravel bar	1	1	-	-	-	-
vegetated gravel bar	0.04537	0.09615	0.0664	-	-	-
large woody debris	0.30485	0.47191	0.44697	0.47191	-	-
island	1.80E-06	4.40E-05	4.00E-06	0.47191	0.00406	-
riparian forest	9.80E-08	6.80E-06	8.00E-07	0.04473	0.00014	0.13149

TN	main channel	side channel	open gravel bar	vegetated gravel bar	large woody debris	island
side channel	1	-	-	-	-	-
open gravel bar	1	1	-	-	-	-
vegetated gravel bar	1	1	1	-	-	-
large woody debris	1	1	1	1	-	-
island	0.666	0.542	1	1	1	-
riparian forest	0.042	0.016	0.229	1	0.339	1

WC	main channel	side channel	open gravel bar	vegetated gravel bar	large woody debris	island
side channel	1	-	-	-	-	-
open gravel bar	4.30E-10	2.50E-08	-	-	-	-
vegetated gravel bar	1	1	0.45639	-	-	-
large woody debris	1	1	1.90E-05	1	-	-
island	1	1	5.40E-06	1	1	-
riparian forest	0.00625	0.02731	5.50E-11	0.06441	0.17477	0.00068

Appendix C: Additional boxplots

C.1) Residual flow



C.2) Natural floodplain

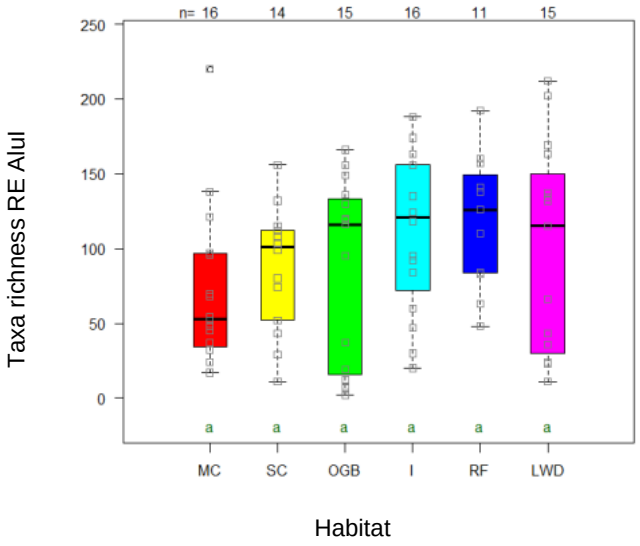
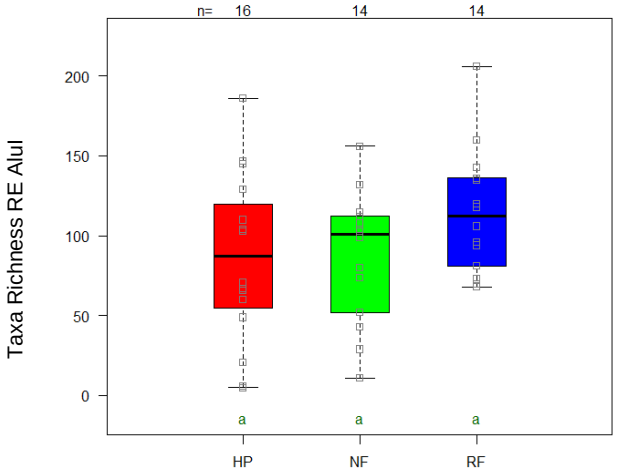
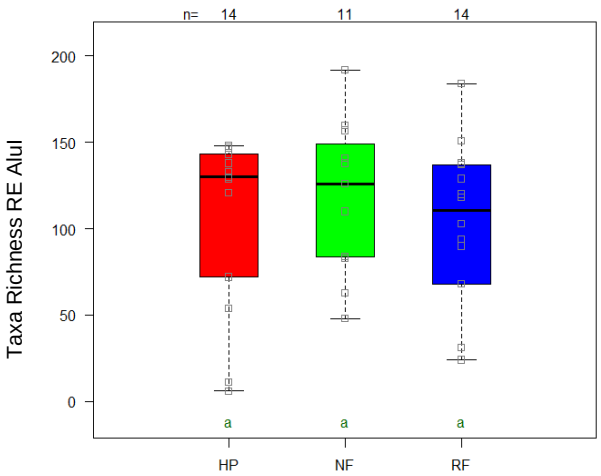


Figure C.1), C.2): Boxplots on differences in taxa richness RE *Alu*I between habitat types (MC=main channel, SC =side channel, OGB=open gravel bar, VGB= vegetated gravel bar, LWD=large woody debris, I=island, RF=riparian forest) within the individual hydrological regimes C.1) Residual flow (Kruskal-Wallis, p-value 0.817, C.2) Natural floodplain (Kruskal-Wallis, p-value 0.191. Letters show significant differences based on Wilcoxon rank sum test (p-value <0.05).

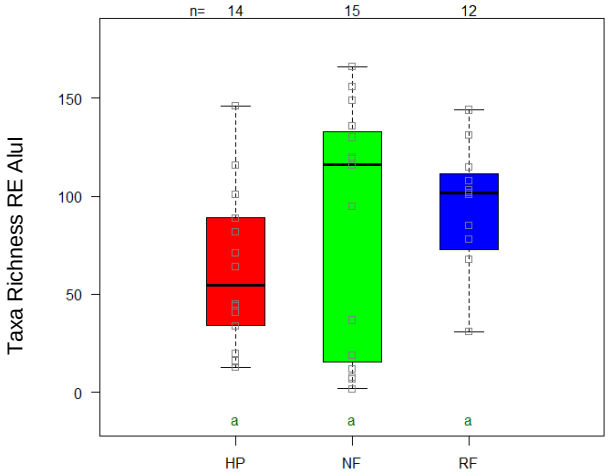
C.3) Side channel



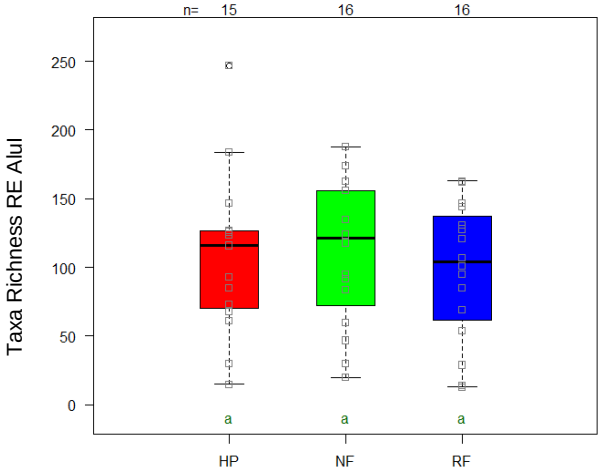
C.4) Riparian Forest



C.5) Open Gravel Bar



C.6) Island



C.7) All habitats together

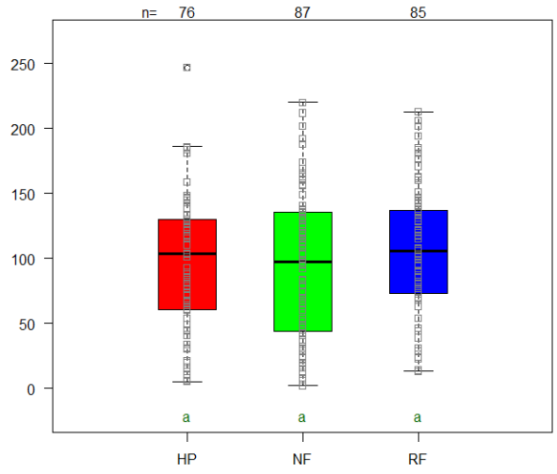
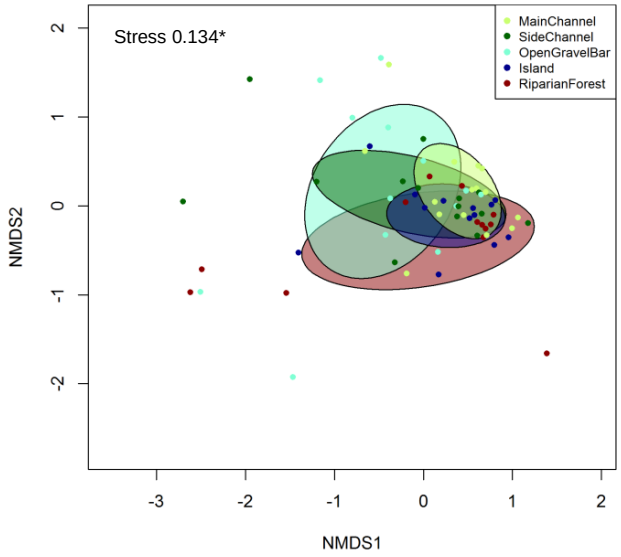


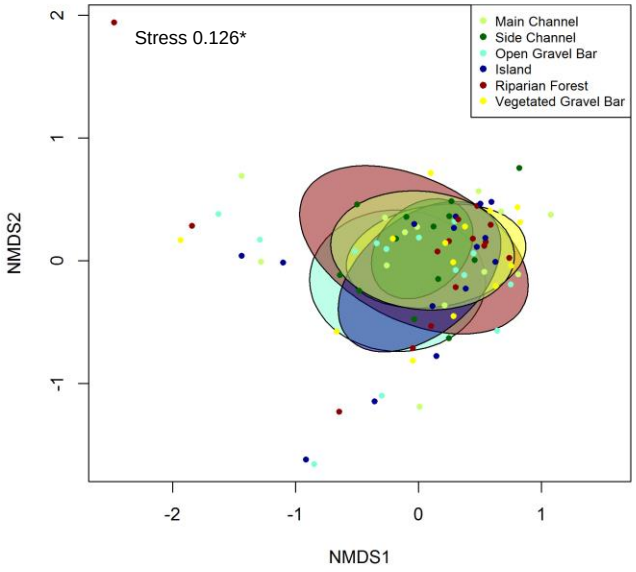
Figure C.3-C.7: Boxplots on differences in taxa richness RE *Alu*I between hydrological regimes (HP=Hydropeaking, NF= Natural floodplain, RF=Residual flow) within the individual habitat types C.3) Side channel (Kruskal-Wallis p-value 0.198, C.4) Open gravel bar (Kruskal-Wallis Test, p-value 0.337), C.5) Island (Kruskal-Wallis Test, p-value < 0.817), C.6) Riparian forest (Kruskal-Wallis p-value 0.647), C.7) All habitats types together (Kruskal-Wallis p-value 0.3249). Letters show significant differences based on Wilcoxon rank sum test (p-value <0.05).

Appendix D: Additional nMDS plots

D.1) Hydropeaking (n=95)



D.2) Residual flow (n=93)



D.3) Natural floodplain (n=101)

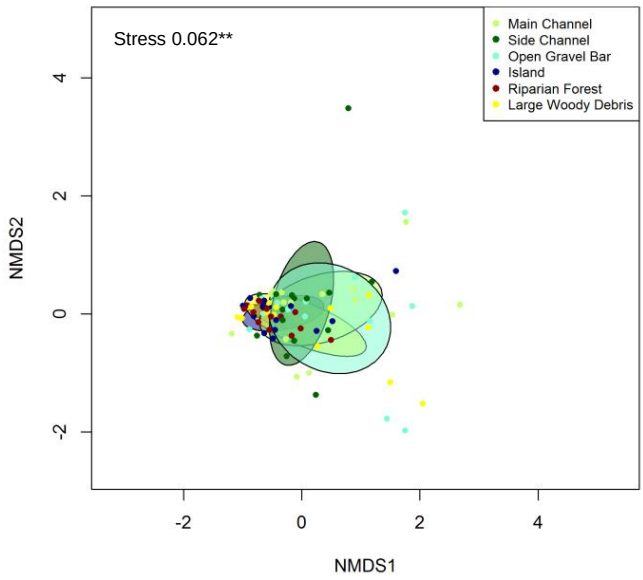
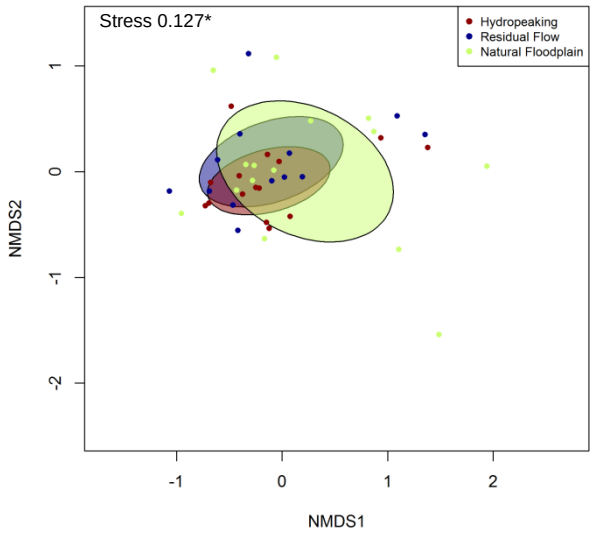
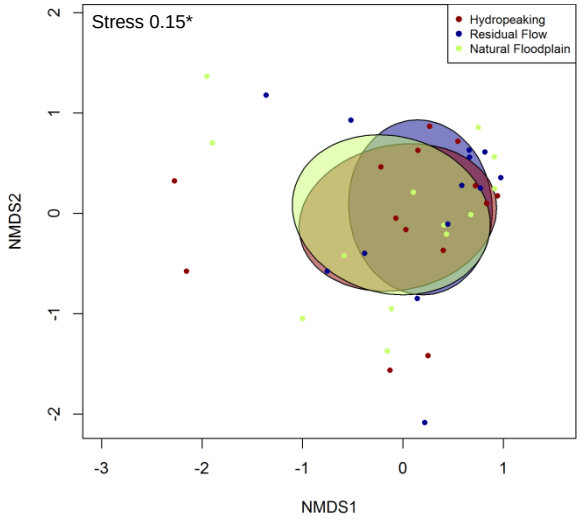


Figure D.1-D.3: Single nMDS plot on the individual regimes, grouped by habitat type, based on bray-curtis similarities of the bacterial community composition generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzyme *Alu*I. D.1) Hydropeaking D.2) Residual flow D.3) Natural floodplain. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

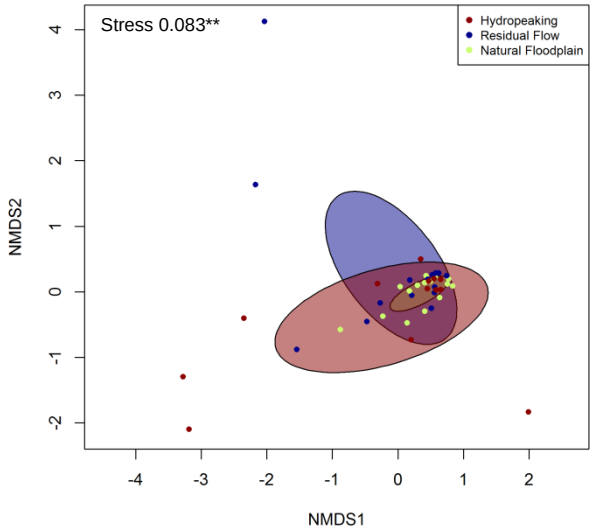
D.4) Main Channel (n=43)



D.5) Open Gravel Bar (n=43)



D.6) Riparian Forest (n=43)



D.7) All Data (n=254)

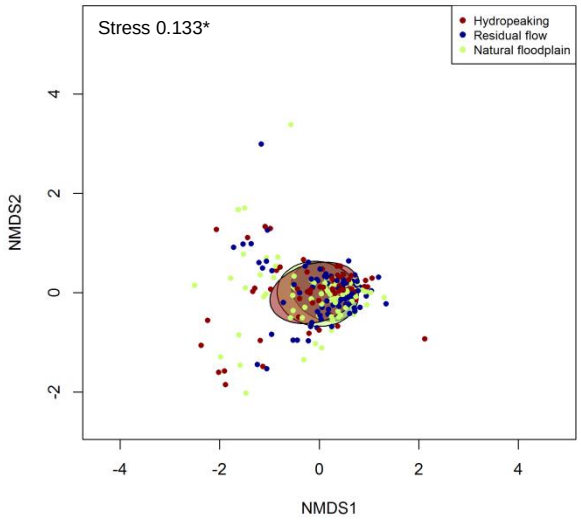


Figure D.4-D.7: Single nMDS plots on the individual "habitats", grouped by "regime" , based on bray-curtis similarities of the bacterial community composition generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzymes *Alu*I. A) Main channel with B) open gravel bar C) Riparian forest D) all data. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

D.8 All habitats together (n=254)

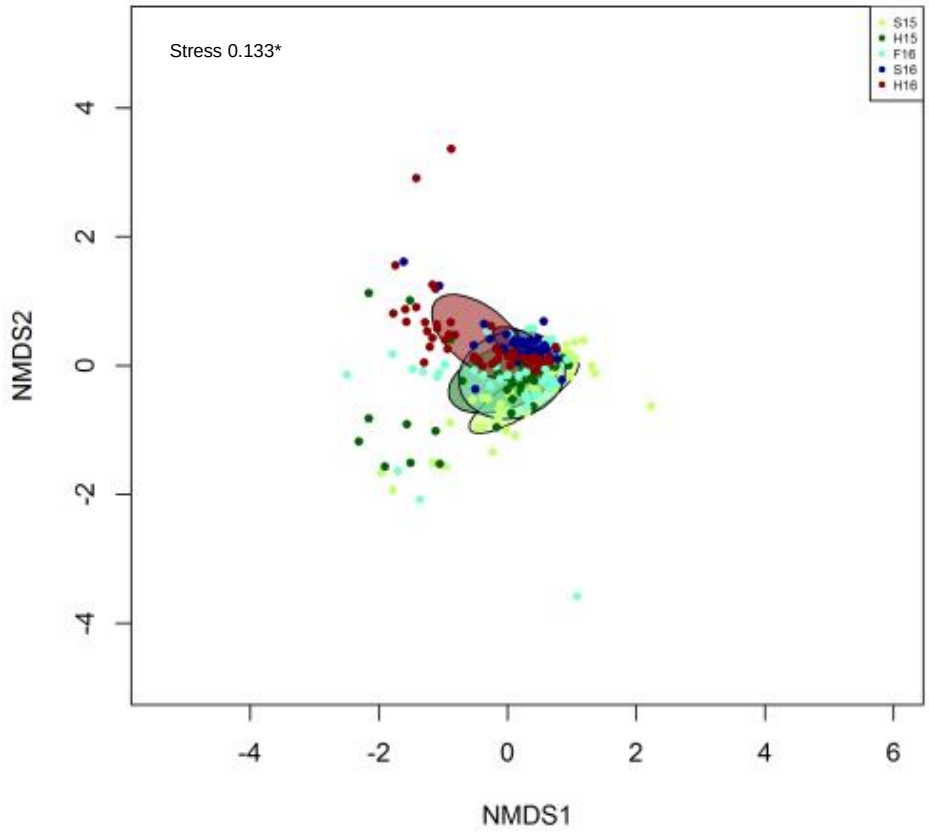
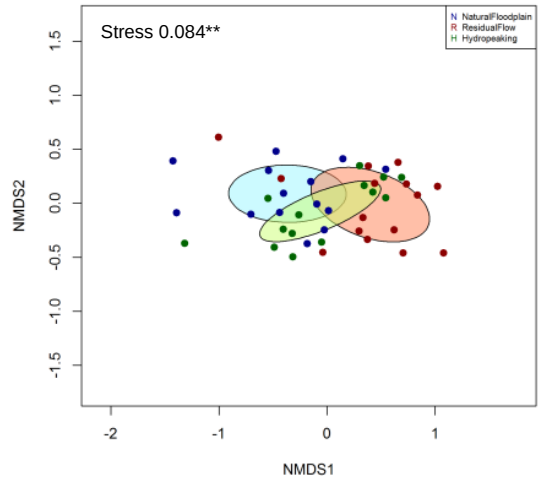


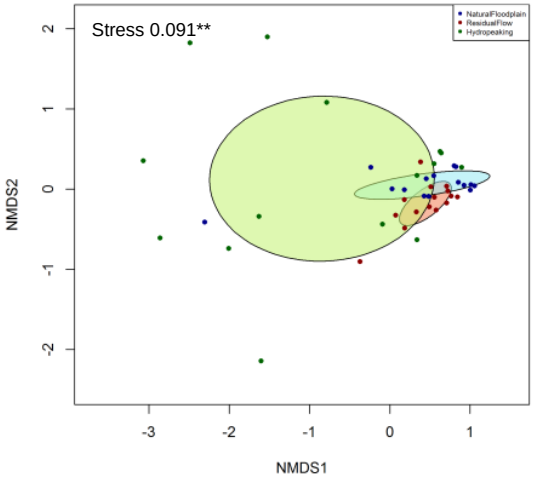
Figure D.8: Single nMDS plot, showing sampling seasons based on bray-curtis similarities of the bacterial community composition (Summer 2015 until Autumn 2016) generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzyme *Alu*I. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

Figure D.9-12: nMDS plots showing the three different hydrological regimes (blue=Natural Floodplain, red=Residual Flow, green=hydropeaking) based on bray-curtis similarities of the bacterial community composition along a seasonal gradient (Summer 2015 until Autumn 2016) generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzyme *Alu*I. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

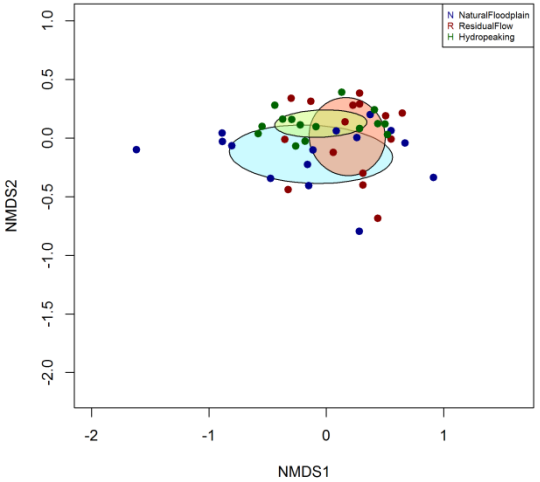
D.9) Season Summer 2015



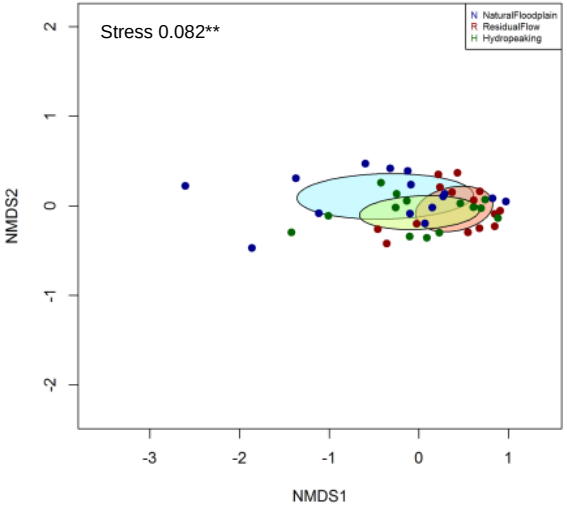
D.10) Season Autumn 2015



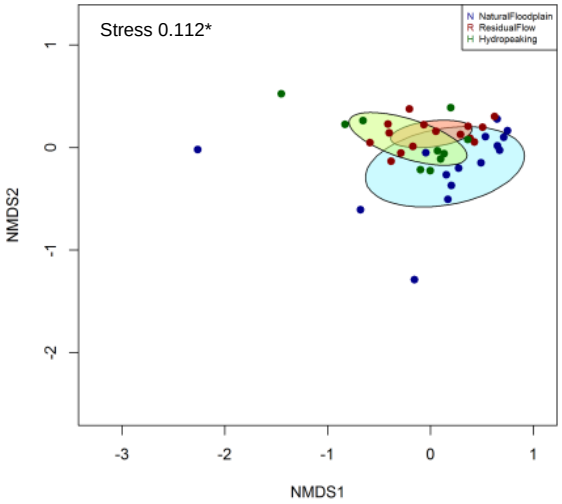
D.11) Season Winter 2016



D.12) Season Spring 2016



D.13) Season Summer 2016



D.14) Season Autumn 2016

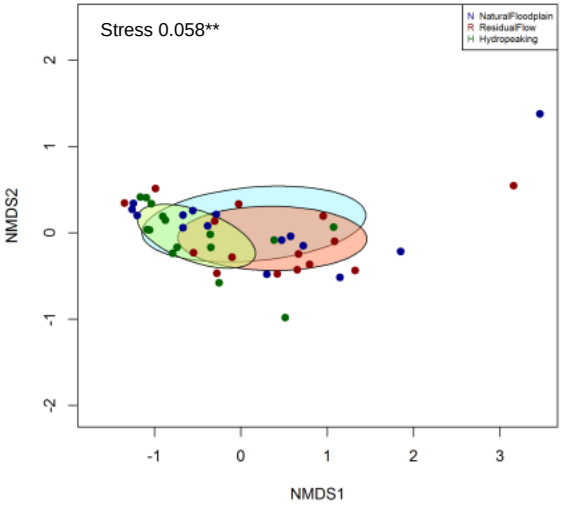
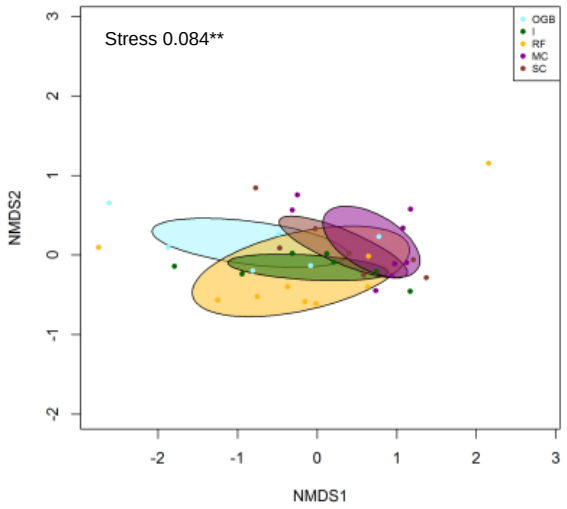
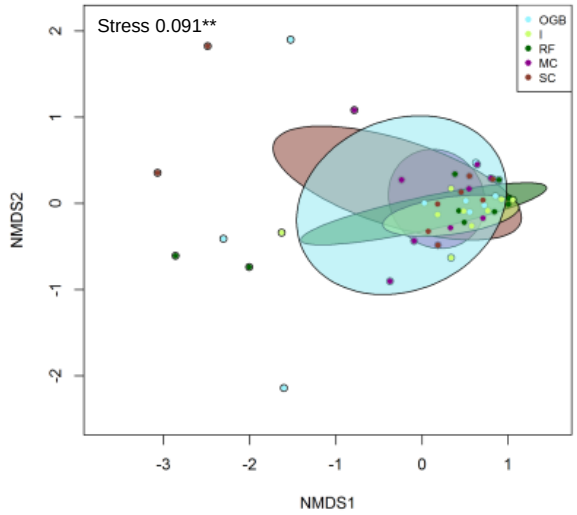


Figure D.15-D.20: Single nMDS plots showing five different habitat types (lightblue=OGB, darkgreen=I, yellow=RF, purple=MC, magenta=SC) for each sampling season (Summer 2015 until Autumn 2016), based on bray-curtis similarities of the bacterial community composition generated from the relative abundance matrix of TRFLP-Data digested with the restriction enzyme *Alu*I. The 2D stress is given for each nMDS plot (stress < 0.05 provides an excellent representation in reduced dimensions***, < 0.1 is great**, < 0.2 is good/ok*, and stress < 0.3 provides a poor representation).

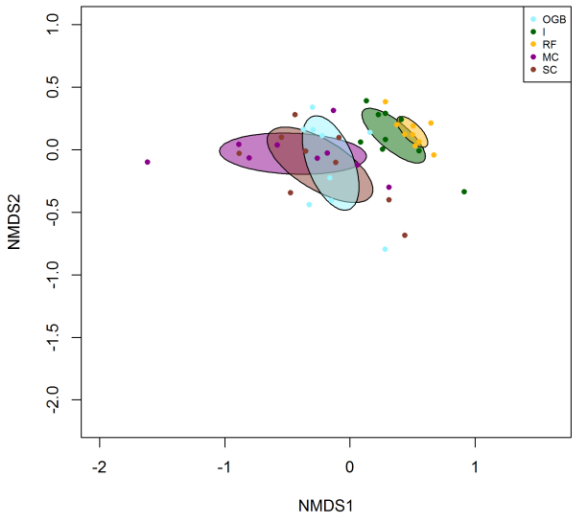
D.15) Season Summer 2015



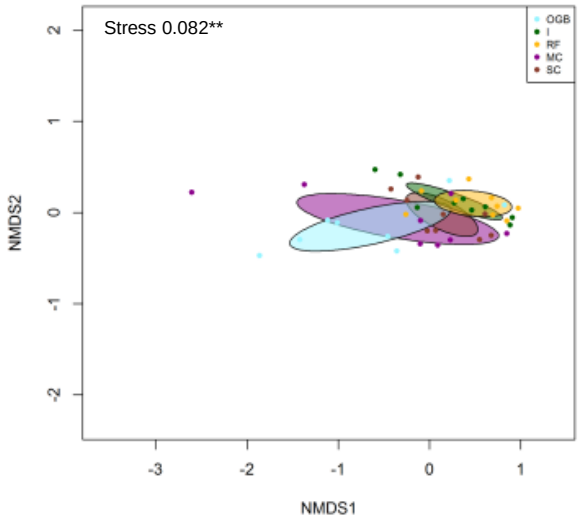
D.16) Season Autumn 2015



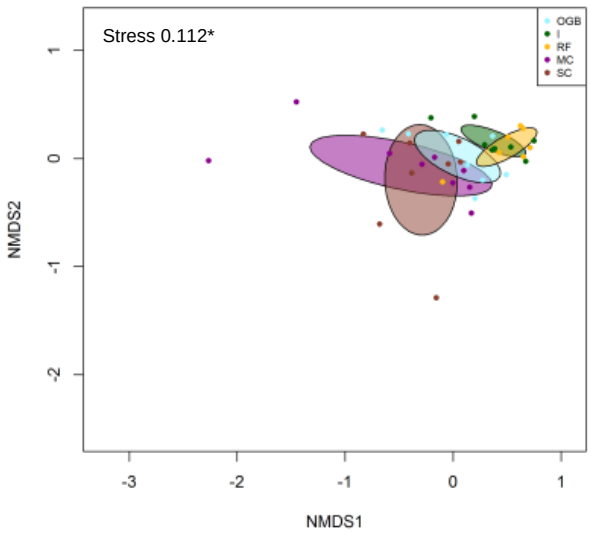
D.17) Season Winter 2016



D.18) Season Spring 2016



D.19) Season Summer 2016



D.20) Season Autumn 2016