Soil bacterial communities and ecosystem functioning change more strongly with season than habitat in a restored floodplain

Emanuela Samaritani^{1,2,3,*}, Edward A.D. Mitchell^{1,4}, Jeremy Rich³, Juna Shrestha⁵, Bertrand Fournier^{1,2}, Beat Frey⁵

¹ Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland

² WSL, Swiss Federal Research Institute, 1015 Lausanne, Switzerland

³ Department of Ecology and Evolutionary Biology, Brown University, 02912 Providence, RI, USA

⁴ Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

⁵ WSL, Swiss Federal Research Institute, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

Running title: Patterns of soil bacterial communities in a floodplain

Abstract

Soil bacterial community structures and associated ecosystem functions are known to vary both in space and time but spatial and temporal patterns are rarely studied simultaneously. Dynamic floodplains offer an ideal setting to study these spatio-temporal patterns and to determine the relative importance of habitat diversity and seasons in shaping soil bacterial communities.

We studied the differences between habitats and seasonal patterns of soil bacterial communities (T-RFLPs) and soil environmental conditions (temperature, moisture and time elapsed since last inundation) and ecosystem functioning proxies (basal respiration, enzymatic activity, microbial C and N) over one year in seven habitats of a restored floodplain.

Soil bacterial community structures in the two habitats closest to the river were most divergent from the others. However, although bacterial community structures differed between habitats at all sampling times, their seasonal variability was substantially higher than the habitat differences. Most of the ecosystem functioning proxies were lowest in the most dynamic habitat (gravel bar). Furthermore, while both bacterial community structure and ecosystem functions varied in relation to environmental conditions, the two were not directly correlated, and the link between communities and functions was seasonally inconsistent. The ratio of seasonal vs. habitat variability of soil bacterial communities decreased from the most dynamic to the most stable (forest) habitat. Thus in dynamic ecosystems such as floodplains also external factors (flood disturbance, seasonally changing climatic conditions) might influence ecosystem functions and bacterial communities independently - possibly because of species functional redundancy - and are keys in maintaining floodplain taxonomical and functional diversity.

Key words: Floodplain; river restoration; soil microbial diversity; T-RFLP profiling; ecosystem functioning.

Introduction

Perturbation generates spatial and temporal variability in biotic communities and associated processes (Ward et al., 2002). Riparian floodplains, at the interface between the riverbed and the surrounding upland terrestrial ecosystems (Sedell et al., 1989), are among the most diverse environments at a global scale. Flood dynamics create an important spatial and temporal heterogeneity of habitats characterized by contrasted and changing physico-chemical conditions and biotic characteristics – and a gradient of perturbation from regularly flooded gravel bars to infrequently flooded riparian forests (Stanford and Ward, 1993; Tockner et al., 1997; Brunke et al., 2003). Riparian soils contain an unusually high diversity of microfauna and microflora, but this diversity is very heterogeneously distributed among the different functional processing zones (FPZ) of floodplains (Binkley et al., 1997). Functional processing zones are patches with distinct chemical and physical properties defined by specific hydromorphic influence that determine their ecological functions, such as microbial community composition, system metabolism, productivity, organic matter dynamics, and nutrient cycling (Thorp et al., 2006). Soils of floodplain thus offer an ideal setting to investigate the effects of spatio-temporal variation on soil bacterial communities and test whether these patterns are predictable based on abiotic characteristics and vegetation composition (i.e. revealing niche specialisation) or if they are instead random (i.e. in agreement with neutral theory; Langenheder and Szekely, 2011).

Soil bacterial communities vary over space, time and in response to environmental changes. Their spatial variation occurs at fine scale (Vos et al., 2013), in relation to soil micro-structure and organic matter distribution, and at broad geographical scale (Martiny et al., 2011). Soil and vegetation types are known to influence bacterial abundance and community composition across ecological gradients and succession (Pankhurst et al., 2001; Carney and Matson, 2006; Stromberger et al., 2007; Pietri and Brookers, 2009; Yu et al., 2012). Soil bacterial communities also vary temporally, over short (e.g. rain events) to longer (e.g. seasonal, ecosystem succession) time scales (Grundmann, 2004; Prosser, 2012) and also respond to perturbations (Allison and Martiny, 2008). Temporal dynamics are likely to dominate patterns of microbial diversity in ecosystems under recurrent perturbations, such as floods in riverine floodplains

(DeLong, 2010). However high habitat heterogeneity and contrasted microhabitats should also act as ecological controls on bacterial community structure (species sorting) and thus the relative importance of temporal and habitat differences should vary across gradients of perturbation (Lindström and Langenheder, 2012).

Here, we attempted to assess the relative importance of seasonal and habitat variability as drivers of soil bacterial community structures during on a one-year discontinuous monitoring of bacterial communities and selected indicators of ecosystem functions at morphologically distinct sampling sites in the riparian zone of a river. The main factor separating them being distance from the riverbed and thus frequency and duration of flooding. Given the high habitat heterogeneity of floodplain ecosystems, we expected to find strong changes in bacterial communities among FPZs. Using terminal restriction fragment length polymorphism (T-RFLP) profiling, we analysed the habitat and seasonal variability of soil bacterial communities in relation to flood patterns, soil physico-chemical conditions and ecosystem functioning proxies such as respiration, enzymatic activity and microbial biomass in a lowland restored floodplain at Niederneunforn (river Thur) in Switzerland. These ecosystem functioning proxies can be linked to ecosystem services such as decomposition, nutrient cycling and carbon storage. Our study is part of an interdisciplinary project called RECORD (<http://www.cces.ethz.ch/projects/nature/Record>) (Linde et al., 2011; Pasquale et al., 2011; Samaritani et al., 2011; Schneider et al., 2011; Fournier et al., 2012; Shrestha et al., 2012). Along a transect in a restored floodplain several habitats corresponding to a succession gradient of plant colonisation were selected. We hypothesized (1) that bacterial community structures and ecosystem functioning proxies both would vary across habitats (from gravel bars to mature forests) and season. Given the very clear contrasts (e.g. in soil texture or vegetation structure) among the studied FPZs we hypothesized (2) that overall differences among FPZs would be greater than seasonal differences. However, given the clear gradient in inundation frequency and impact from the river to the floodplain forest, we also hypothesised (3) that the ratio of seasonal to habitat variability of soil bacterial communities would increase with the distance from the river.

Methods

Experimental design

River Thur covers a catchment area of 1700 km², from Mount Säntis, through the Swiss Plateau, and into the Rhine River. Lacking natural or artificial reservoirs, the river flow is subject to drastic and sudden changes in response to heavy rainfall events, especially in summer and autumn, and to snowmelt in spring. Long-term maximum, mean, and minimum flow rates are 1130, 50, and 2m³ s⁻¹, respectively (1904-2005: <http://www.hydrodaten.admin.ch/en/index.html>). The mean annual precipitation at the study site is 908 mm and the average monthly temperature ranges from 0.9°C in January to 19.0°C in July (<http://gate.meteoswiss.ch/idaweb>). Glacio-fluvial sandy Gravels overlaying lacustrine clays dominate the Thur aquifer.

The river was first channelized in the 1890s to protect the river valley against floods. In the last 20 years, several river sections have been widened to allow the river to braid freely, in order to decrease the strength of the floods downstream and to increase the ecological value of the floodplain. The study site is located in the river corridor at Niederneunforn (Canton Thurgau, 8°77'12" E; 47°59'10" N), where a 2 km long section was widened from 50 to 110m in 2002. The foreland in front of the levees was removed and the levees were lowered in some places to reconnect the old alluvial forest with the river during high floods. A strip of willow saplings was planted to reinforce the newly exposed banks.

Along the distance gradient from the riverbed, seven different habitats were identified based mainly on vegetation and topography and referred to as functional processing zones (FPZ) (Samaritani et al., 2011); six along the restored reach and one in an upstream adjacent channelized section. As a result of their elevation and distance from the river, these habitats are flooded at different river discharge levels and are exposed to different flooding frequencies (Table 1). Close to the river, three habitats were selected within the dynamic area. "Gravel", a mosaic of bare gravels with patches of poorly developed soil and pioneer vegetation; "Grass", characterized by a community of pioneer plants dominated by *Phalaris arundinacea*; and "Willow bush", dominated by shrubby vegetation, mainly planted *Salix viminalis*. These FPZs did not exist before the

restoration. The next three FPZs further from the river, “Mixed forest” (*Fraxinus*, *Acer*), “Ash forest” (*Fraxinus*) and “Willow forest” (*Salix alba*), which are characteristic floodplain forests differing in the dominant trees and under-storey vegetation, groundwater level and distance from the river. The FPZs were already present before the restoration but were increasingly influenced by the fluvial dynamics following the restoration. The last FPZ, “Pasture”, was considered as the pre-restoration reference condition, and was located directly upstream from the restored site in an area still protected from floods.

In each FPZ, four to six plots were selected; six in Gravel, Grass, and Willow bush, four in the Mixed forest, Ash Forest, Willow forest and Pasture along a slight topographic and vegetation gradient (Samaritani et al., 2011). Each plot was constituted of an eight-meter diameter circle. The upstream half-circle was used for vegetation mapping and gas sampling (Samaritani et al., 2011) while the downstream half-circle was used for destructive soil sampling.

Soil sampling and analyses

Four sampling campaigns over one year (July, October, January and April) were carried out. In each plot, three soil cores (6.5 cm diameter x 10 cm depth) were collected and pooled. In Gravel plots, soil was collected in pits (10 cm x 10 cm x 10 cm) because it was impossible to take cores owing to the high proportion of large pebbles. Soil samples were sieved at 2 mm on the collection day, then stored at 4°C for microbial analyses. Soil subsamples were put in extraction buffer for DNA extraction the same day and stored at -20°C.

Soil properties, and environmental conditions

Soil texture of dried samples was measured using the pipette method (Gee and Bauder, 1986) after removing organic matter with hydrogen peroxide and dispersing with sodium hexametaphosphate. Soil pH was measured in a 1:2 slurry of dried soil in 0.01M calcium chloride after 30 min equilibration. The percentage of total organic carbon (TOC) of dried, homogenized soils was measured in duplicate using a TOC analyzer (Shimadzu, Tokyo, Japan) after HCl (10%) acid digestion to remove carbohydrates. Total carbon and

nitrogen contents were measured in duplicates using an automatic element analyzer (Shimadzu, Tokyo, Japan). Microclimate variables (soil temperature, soil moisture and inundation regime) were monitored for this and other related studies conducted at the same site (Samaritani et al., 2011). Soil temperature (T) at 5 cm depth was continuously measured during this study in the centre of each plot at 30 minutes resolution with TidBit v2 temperature loggers (Bourne, MA, USA). Soil Moisture (SM) was estimated at each sampling time by measuring the weight loss upon drying 20 g of fresh soil at 105°C for 24 h.

We estimated the minimum river discharge required for flooding a plot from inundation maps for different river discharge levels as produced by a 2-D hydrodynamic model (details in Pasquale et al., (2011). The number of days after the last inundation (LI) was based on the minimum discharge value required for flooding a plot and the date at which this discharge level was reached, compared to the sampling date.

Basal respiration, microbial biomass and enzymatic activity

Basal respiration (BR) was measured in a closed soil-chamber system connected to a Li-8100 infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA). Soil samples at field moisture level, previously stored at 4°C, were left at room temperature for at least 3 hours before placing in the soil containers. Soil samples (40 g per container) were incubated for 2 days at 20°C. The soil containers were then connected to the CO₂ analyzer. CO₂-free air flowed at a rate of about 0.16 L min⁻¹ through the containers, and entrained the CO₂ just released from the soil to the infrared gas analyzer. After reaching a steady state situation, the gas flow and the CO₂ concentration were recorded and the BR was calculated according to Rieder and Frey (2013). Fluxes are reported as $\mu\text{mol CO}_2 \text{ h}^{-1} \text{ g}^{-1}$ soil dry weight.

Microbial biomass Carbon (MC) and Nitrogen (MN) were determined by chloroform fumigation-extraction (Vance et al., 1987; Frey et al., 2006; Samaritani et al., 2011). Fresh soil samples corresponding to 10 g dry mass were placed in a desiccator containing chloroform. The desiccator was evacuated and left in the dark for 24 h. These fumigated soil samples and another set of fresh soil samples were extracted with 0.5 M potassium sulphate and filtered. The extracts were measured with a Total Organic

Carbon/Nitrogen Analyzer (TOC-VC/TN, Shimadzu, Kyoto, Japan). Microbial C and N were calculated as the difference in extractable organic carbon and nitrogen content between the fumigated and control subsamples using a conversion factor of 0.45 for C (Beck et al., 1987) and 0.54 for N (Brookes et al., 1985). MC and MN data were expressed in mg kg⁻¹ soil dry weight.

Enzymatic activity (EA) was estimated by fluorescein diacetate analysis. Fluorescein diacetate (FDA) is hydrolysed by proteases, lipases and esterases and can therefore be used to determine the amount of active microbes in the soil (Söderström, 1977; Schnürer and Rosswall, 1982; Adam and Duncan, 2001). The formation of a yellow color was assessed at 490 nm by spectrophotometer. The intensity of the resulting yellow color is indicative of the amount of enzymatic cleavage of the FDA molecule (colorless) and the overall enzymatic activity in the sample. Two replicates per sample, 0.5 g of fresh soil each, was used for enzymatic assays. Enzymatic activity was calculated as follows:

$$EA = \frac{400 \frac{A}{A_{st}}}{0.75h \times 0.5g \times \% \text{ of dry weight}}$$

where A=Absorbance measured at 490 nm and A_{st}=Absorbance of reference sample. Results were converted in µg of degraded FDA h⁻¹g⁻¹ soil dry weight.

Soil bacterial community structure

Total DNA was extracted as follows: a 0.5 g subsample of fresh soil and 0.75 g glass beads (0.1 mm diameter) were suspended in 1ml extraction buffer (0.2M Na₃PO₄ [pH 8], 0.1 M NaCl, 50 mM EDTA, 0.2% CTAB) and treated three times with 1 ml extraction buffer and a bead beating procedure using a FastPrep bead beater (FP120, Savant Instruments) at 5.5 m s⁻¹ for 40 s followed by centrifugation for 5 min at 13,000 rpm (Frey et al., 2006). DNA was purified by chloroform extraction with 2 ml chloroform, vortexed and then centrifuged 5min at 13000 rpm. DNA was precipitated by the addition of 3 ml of precipitation solution (20 % PEG 6000, 2.5M NaCl), gently mixed, incubated at 37°C for 1 h and then centrifuged for 15 min at 15000 rpm. The pellets were washed

with 1.5 ml of 70% EtOH and centrifuged for 2 min at 10000 rpm. The supernatant was removed and the samples were air dried for 20 min, and re-suspended in AE buffer (10mM TrisCl, 0.5mM EDTA, pH9; Qiagen, Hilden, Germany) at 1ml AE per g of extracted soil (dry weight equivalent). Extracted DNA was examined by electrophoresis on agarose gels (1% w/v in Tris-Borate-EDTA buffer), quantified using PicoGreen and stored at -20°C.

DNA samples were pre-treated with bovine serum albumine (BSA, molecular biology grade, Fluka, Buchs, Switzerland) by heating for 5min at 95°C (Frey et al., 2006). DNA were amplified by PCR using fluorescently labelled primers 27F (FAM-labelled forward primer, corresponding to positions 8 to 27 of the 16S rRNA from *Escherichia coli*, corresponding to GenBank entry J01695; 5'-AGAGTTTGATCMTGGCTCAG-3') and 1378R (unlabelled reverse primer, positions 1378 to 1401; 5'-CGGTGTGTACAAGGCCCGGGAACG-3') (Bent et al., 2007). In a total volume of 25 µl, we added 5 µl of pre-treated and 1:10 diluted DNA, 10x PCR buffer, 0.5mM MgCl₂, 0.2 µM of each primer, 400 µM of each dNTP (Promega), 0.3 mg ml⁻¹ of BSA, and 0.05 U µl⁻¹ of Hotstar *Taq*-polymerase (Qiagen, Hilden, Germany). PCR amplification was performed with a PTC-100 thermocycler (MJ Research, Waltham, MA, USA). PCR conditions applied an initial activating step for HotStarTaq® polymerase (15 min at 95°C), followed by 35 cycles with denaturation at 94°C for 45 s, annealing at 48°C for 45s, and extension at 72°C for 2 min. The PCR amplification was followed by an additional extension at 72°C for 5 min. Amplified products were then digested 3 hours at 37°C with two restriction enzymes (*Msp*I and *Hae*III). The enzymes were then inactivated by incubation at 65°C for 20 min. The terminal restriction fragments (hereafter called T-RF) were then separated with a genetic analyser (ABI310, Applied Biosystems Inc., Foster City, USA) equipped with a 36 cm capillary and POP 4 polymer (Applied Biosystems), with internal size standard ROX500 (Applied Biosystems) and fragment size converted to numeric data using Genotyper 3.6 NT software (Applied Biosystems) (Frey et al., 2009). The percentage abundance of each T-RF between 50 and 500 bp of length was calculated as the integration of the surface under the peak of fluorescence given by the Genotyper analyses, divided by the total integration for all peaks (Frey et al., 2006; Rieder et al., 2013; Hodel et al., 2014). Only

T-RF peaks higher than 0.1 % and T-RF present in at least 5% of the samples were retained, in order to avoid extremely rare fragments. This filtering resulted in 119 samples, with a total of 254 T-RFs, from both the *MspI* and *HaeIII* restriction enzymes. It should be noted that not every terminal restriction fragment identified by the T-RFLP analysis necessarily represents a single species, since it is possible that different species have restriction fragments with exactly the same number of base pairs. Thus, we define the individual restriction fragments as operational taxonomic units (OTUs), which can be considered a conservative estimate of the number of bacterial species present in our system. However, the use of two restriction enzymes allowed us to assess the relative differences in bacterial community structures in different FPZs and seasons. We estimated Shannon diversity index based on the T-RFLP profiles (alpha diversity), while beta diversity was estimated on Bray-Curtis distance matrices calculated based on normalized and square root transformed OTU abundances (Rime et al., 2016).

Numerical analyses

Differences in the ecosystem functioning proxies and alpha diversity (Shannon diversity index) among the habitats and season were tested using two-way analysis of variance (ANOVA) with FPZ, season and their interactions (FPZ x season). Post-hoc tests (Tukey's honest significance test) were further performed to study the effects of habitat and season on ecosystem functioning and alpha diversity. In order to compare habitat and seasonal effects on the bacterial community structures, we performed permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis distance matrices (Frey et al., 2016). Bacterial communities were plotted on a non-metric multidimensional ordination (NMDS) based on Bray-Curtis distance matrices. Ecosystem functioning proxies and climatic conditions were independently fitted and also plotted. To assess the relation between bacterial community structures, ecosystem functioning proxies and the environmental conditions we calculated Pearson correlation coefficients between the dissimilarity matrices of the T-RFLP profiles, the different ecosystem functioning proxies (BR, EA, MC and MN) and the environmental conditions (SM, Temp and LI) and tested the significance of correlations for the different sampling times by Mantel tests, as implemented in the *vegan* library of software R (R Core Team 2013). All statistic

analyses were performed with the program R.

Results

Soil properties and ecosystem functioning

Significant differences ($p < 0.001$) among FPZs were recorded for all soil variables except for pH (Samaritani et al., 2011). Ecosystem functioning proxies (Enzymatic Activity EA, Microbial Carbon MC and Microbial Nitrogen MN) varied significantly among FPZs and sampling dates (two-way ANOVA, Table 2). In contrast, basal respiration (BR) only differed significantly ($p < 0.001$) between seasons but not ($p = 0.23$) between FPZs. Cross-effects were only significant ($p = 0.002$) in EA (Table 2).

With respect to habitat variability (Fig. 1A), basal respiration tended to be higher in the three forested FPZs (Ash, Mixed and Willow forests), but the difference was not significant. MC was lowest in Gravel, and intermediate in the Pasture. Grass, Willow bush and the forests had comparably high MC, but only Willow forest was significantly different ($p < 0.05$). MN increased along the gradient from Gravel, to Grass, Willow bush, Mixed forest, Ash Forest and Willow forest, but only Gravel was significantly different ($p < 0.001$). Pasture MN values were comparable to Grass and Willow bush. EA was significantly higher in Grass FPZ than Pasture ($p < 0.05$). The lowest values were found in Gravel and Pasture.

With respect to seasonal variability (Fig. 1B, Supplementary Fig. 1), basal respiration (BR) was significantly higher in autumn than in winter, spring and summer ($p < 0.001$). Microbial carbon (MC) was lowest in autumn, intermediate in winter and spring and highest in summer ($p < 0.05$). Microbial nitrogen (MN) was higher in autumn compared to winter and spring ($p < 0.05$). Enzymatic activity (EA) was higher in spring than in winter and summer ($p < 0.05$).

Bacterial community structure

Alpha diversity estimated using the Shannon diversity index based on T-RFLP profiles differed significantly ($p = 0.003$) between seasons but not ($p = 0.18$) between FPZs with no significant cross-effect (Table 2). Shannon diversity index was lowest in Gravel and highest in Pasture (Fig. 2A) but these differences were not significant. Shannon diversity

index in spring was significantly ($p < 0.05$) higher than in summer and autumn but not in winter (Fig. 2B).

Beta diversity estimated on distance matrices differed significantly (PERMANOVA, $p = 0.001$) among FPZs and sampling periods, with cross-effects (Table 2). We observed a habitat pattern of decreasing beta diversity along the increasing distance from the river (Fig. 3A) with highest beta diversity in July and April (Fig. 3B). In October and January, beta diversity was more homogeneous among FPZs, except for a peak in Ash forest in October.

The non-metric multidimensional scaling (NMDS) grouped T-RFLP profiles primarily according to the season (Fig. 4). Soil environmental conditions and ecosystem functioning proxies were independently fitted on the ordination. Among climate variables, LI and T strongly correlated with the bacterial community structure, while the correlation of SM was lower on the first two axes. Among ecosystem functioning proxies, BR correlated better with October communities, MC with July communities and MN with both of them. The correlation of EA was lower on the first two axes.

Mantel tests between distance matrices showed weak correlation (significant only in winter) between bacterial communities and ecosystem functions (Table 3). By contrast, correlations were stronger and always significant with environmental conditions. A positive significant correlation was found between ecosystem functioning proxies and soil environmental conditions at all time except spring (Table 3).

Discussion

Here, we attempted to assess the effects of seasonal and habitat variability as drivers of soil bacterial community structures during on a one-year discontinuous monitoring of bacterial communities and selected indicators of ecosystem functions at morphologically distinct sampling sites in the riparian zone of a river. The main factor separating them being distance from the riverbed and thus frequency and duration of flooding. Given the high habitat heterogeneity of floodplain ecosystems, we expected to find strong changes in bacterial communities among FPZs. Microbial communities have been shown to be organised in several nested levels, even within the centimetre- to meter-scale (Franklin and Mills, 2003). The main finding was that the bacterial community

structure varied both in time and in space, but it was the seasonal variation which has a stronger impact. Habitat variability was also detected, especially for July and April communities that were characterised by decreasing beta diversity with increasing distance from the river.

In ecosystems driven by seasonally pulsed inputs of resources (e.g. water in semi-arid ecosystems), such pulses frequently emerge as major determinants of seasonal variation of soil microbial biomass (Wardle, 1998). A corollary of this is that the species-environment correlations weaken when tested over long periods of time, because different environmental factors may influence the microbial community in different seasons (e.g. limiting factors may shift over time) (Schütz et al., 2009). Soil microbial community composition (Zogg et al., 1997; Schimel and Gulledge, 1999; Unger et al., 2009) and biomass (Pesaro et al., 2003; Pesaro et al., 2004) are expected to be influenced by soil temperature, soil moisture, flooding events and carbon supply (Briar et al., 2011). Although the bacterial Shannon diversity index might not change after a perturbation episode, the bacterial community structure is modified, because some bacterial groups are strongly affected by changing conditions, while others benefit and may outcompete sensitive ones or become more active (Chowdhury et al., 2011). Resilience after disturbance is therefore a function of the community structure (e.g. the relative contribution of sensitive and resistant species) as pointed out by Allen-Morley and Coleman (1989).

Ecosystem functioning proxies were also spatially heterogeneous and temporally (except for basal respiration) dynamic like bacterial community structures. Time had a statistically stronger effect than habitat. The duration, frequency, and timing of floods in riparian floodplain play a crucial role in the ecosystem functioning (Langhans and Tockner, 2006; Shrestha et al., 2012). In the same floodplain, the different habitats might present different sensitivity to factors influencing ecosystem functioning (Doering et al., 2011; Samaritani et al., 2011). Major determinants of the ecosystem functioning proxies were the soil conditions (texture, organic carbon, total nitrogen and phosphorus) and microclimatic conditions (Samaritani et al., 2011). The soil conditions were in turn very strongly influenced by floods indicating that floods influence directly or indirectly all the abiotic conditions of riparian soils, which themselves determine at least partially the

response of biotic components. However, the inter-related effects of environmental and biotic factors on the ecosystem functioning remained poorly understood because of a lack of studies encompassing multiple aspects of ecosystems.

Bacterial community structures and ecosystem functioning were not directly correlated. Causal relationships between habitat, processes and community structure are highly complex and direct connections are difficult to establish (Stark et al., 2007; Bardgett and Van der Putten, 2014). Different bacterial taxa are not expected to contribute equally to ecosystem functions, and particular groups can be more sensitive to changes in soil parameters or nutrients (Rasche et al., 2011) or functionally redundant (Pereira e Silva et al., 2012, Allison and Martiny, 2008). The resulting link of the community structure on the ecosystem functions can therefore be poorly predicted on the basis of T-RFLP profiling. An increased understanding of the physiological traits present in the community, which are related to the ecosystem functioning, might improve predictions of the response of specific communities to disturbance (Allison and Martiny, 2008).

The habitat heterogeneity resulting from the presence of different plant communities and soil conditions in each FPZ explains the significant habitat variability in the bacterial community structure (Grayston et al., 2001; Thoms et al., 2010). For example, some plant species select for soil biota that facilitate the decomposition of their own residues (Cookson et al., 1998), at least at small spatio-temporal scales (Dennis et al., 2010), and the successional patterns of microbial community will follow the vegetation succession along the gradients of distance from the river (Duineveld et al., 1998), and litter quality (Bardgett et al., 1999; Judd et al., 2006). However, the differences in bacterial community structures among FPZs are relatively small compared to the continuous and dynamic shifts in the community structures caused by the seasonally changing conditions due to either internal or external factors (flooding, soil moisture, anaerobic conditions, soil temperature). Over the seasons, also differences in plant community composition (stage of plant development, litter quality) drive bacterial community structure through nutrient availability (Schütter et al., 2001; Bardgett et al., 2005) and carbon availabilities of the litter (Liu et al., 2016)

Here, the T-RFLP method clearly demonstrated the importance of the season and

habitat as drivers for soil bacterial community structures. This approach is valuable for exploratory evaluations of microbial community data from environmental settings (Ramette, 2007; Kaurin et al., 2015) and remains useful to compare community structures in soils (Zhang et al., 2008; Orcutt et al., 2009). Ordination of the environmental data obtained on the same samples can be overlaid on the ordination of T-RFs to determine which, if any, of the environmental parameters are related to the observed statistical groupings of samples (Osborne et al., 2006; Ollivier et al., 2014). However, a phylogenetic description of the main responders is not possible with T-RFLP profiling. Sequencing approaches of 16S rRNA gene amplicons might be of use to confirm the observed response pattern by T-RFLP profiling and amplicon sequencing although comparative results of high-throughput sequencing data were in agreement with profiling methods (Pilloni et al., 2012; Hartmann et al., 2015).

Conclusions

Our results show that ecosystem functioning proxies and bacterial community structure differ significantly among seasons and FPZs, in agreement with our expectations. The bacterial community covariability with soil conditions and ecosystem functioning changed over the seasons. In the general perspective of floodplain ecology and management, this study reveals the role of seasonal flooding and resulting changes in micro-environmental conditions for structuring soil bacterial communities and maintaining heterogeneity of ecosystem functions. A surprising result was that bacterial community composition varied less among the strongly contrasted floodplain habitats than seasonally.

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Figure legends

Figure 1. Mean values (n=4 to 6) and standard error of the ecosystem functioning proxies (Basal Respiration, Enzymatic Activity, Microbial Carbon and Microbial Nitrogen) for each functional process zone (A) and sampling date (July and October 2008 and January and April 2009) (B). GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, AF=Ash forest, WF=Willow forest and P=Pasture). Basal respiration are reported as: $\mu\text{mol CO}_2 \text{ h}^{-1} \text{ g}^{-1}$ soil dry wt.; microbial carbon and nitrogen as mg kg^{-1} soil dry wt.; enzymatic activity as μg of degraded FDA $\text{h}^{-1} \text{ g}^{-1}$ soil dry wt. Means for each FPZ individually at each sampling are shown in Supplementary Figure 1. Differences in the ecosystem functioning proxies among habitat (FPZs) and season (sampling date) were tested using two-way analysis of variance (ANOVA) with FPZ, season and their interactions (FPZ x season, see Table 2). Post-hoc tests (Tukey's honest significance test) were further performed to study the effects of habitat and season on ecosystem functioning. Statistical differences between FPZ and season were given in small letters.

Figure 2. Habitat (A) and seasonal (B) variability in Shannon alpha diversity index calculated on T-RFLP data using two restriction enzymes, *MspI* and *HaeIII*. Values are separated by functional process zone: GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, AF=Ash forest, WF=Willow forest, P=Pasture; and by sampling date: July 2008, October 2008, January 2009 and April 2009. The box upper and lower lines represent 25th (Q25) and 75th (Q75) percentiles, the bold line represents the median and the upper whiskers the last point included in the 1.5 times the interquartile range + Q75 and the lower whiskers the last point included in the Q25- 1.5 times the interquartile range.

Figure 3. Beta diversity ($\pm$ SE) estimated on Bray-Curtis distance matrices averaged per FPZ (A) and Sampling (B). GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, AF=Ash forest, WF=Willow forest, P=Pasture.

Figure 4. Nonmetric multi-dimensional ordination of microbial terminal restriction fragment profiles measured on two restriction enzymes, *MspI* and *HaeIII*. The

multidimensional scaling is based on Bray-Curtis distance matrices, with arrows indicating independently fitted ecosystem functioning proxies (BR=basal respiration, EA=enzymatic activity, MC=microbial carbon and MN=microbial nitrogen), and climatic conditions (T=soil temperature, SM=soil moisture & LI=inundation). Different symbols represent different FPZ and different colours represent different sampling dates (see legend in the picture).

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691 Table 1. Physicochemical soil properties, hydrological and vegetation characteristics in the seven functional process zones of the study site in the Thur River
692 floodplain, Switzerland. Soil properties are for the top 10 cm of soil.

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Variable	Unit	Gravel	Grass	Willow bush	Mixed forest	Ash forest	Willow forest	Pasture
Flooding frequency	times year ⁻¹	>10	>10	4 -6	1-2	1-2	<1	<1
Flooding duration per event	days	>10	~10	~2	1	1	<1	<1
Gravimetric water content	%	21 ± 7	24 ± 8	26 ± 3	23 ± 4	29 ± 6	32 ± 4	21 ± 4
Texture (Sand/Silt/Clay)	%	81/14/5	66/26/8	44/44/12	38/47/15	33/50/17	25/58//18	65/27/8
pH (CaCl ₂)		7.6 ± 0.1	7.4 ± 0.1	7.5 ± 0.0	7.5 ± 0.1	7.5 ± 0.0	7.4 ± 0.0	7.5 ± 0.0
Organic C	g kg ⁻¹	10.1 ± 3.7	16.3 ± 5.8	17.1 ± 3.2	21.4 ± 3.6	22.7 ± 2.1	24.8 ± 1.5	12.9 ± 2.9
Total N	g kg ⁻¹	0.7 ± 0.2	1.0 ± 0.4	1.1 ± 0.3	1.6 ± 0.3	1.6 ± 0.2	1.8 ± 0.1	0.9 ± 0.2
Dominant vegetation		Various seasonal herbs, patchy	Phalaris arundinacea	Salix viminalis	Acer pseudoplatanu s & Fraxinus excelsior	Fraxinus excelsior	Salix alba	Managed grassland dominated by Trifolium sp.
FPZ type		dynamic	dynamic	dynamic	stable	stable	stable	channelized
Distance from the river	m	3 - 6	5 - 15	10 - 25	50 - 70	70 - 80	70 - 80	40 - 50

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Table 2. Main effects of habitat and season on the ecosystem functioning proxies (BR=basal respiration, EA=enzymatic activity, MC=microbial carbon and MN=microbial nitrogen), alpha-diversity (Shannon Diversity index) and beta-diversity (Bray-curtis distance matrices) and their interactions.

	Habitat	Season	Habitat x Season
BR ⁺	0.23	< 0.001	0.2
EA	0.01	0.029	0.002
MC	0.001	< 0.001	0.07
MN	< 0.001	0.004	0.072
Alpha-Diversity	0.18	0.003	0.81
Beta-Diversity*	0.0001	0.0001	0.0001

⁺ Ecosystem functioning proxies and Alpha-Diversity were analysed by two-way ANOVA

* Beta-Diversity was analysed by permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis distance matrices

Table 3. Mantel tests between distance matrices of bacterial communities (bact), ecosystem functioning BR, EA, MC and MN (ecofun) and soil environmental conditions SM, temp and LI (envcond) for the different sampling times. R and p-value are given (n.s.=not significant, *=p<0.05, **=p<0.01, ***=p<0.001).

	Global	July08	Oct08	Jan09	Apr09
bact vs. ecofun	n.s.	n.s.	n.s.	0.296***	n.s.
bact vs. envcond	0.219***	0.073**	0.430***	0.766***	0.376*
ecofun vs. envcond	0.047*	0.249**	0.205*	0.318***	n.s.