Spatio-temporal variability and restoration effects on below-ground biodiversity and soil ecosystem functioning at the Thur floodplain, Switzerland

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Par

Emanuela Samaritani

Acceptée sur proposition du jury:

Prof. Edward A.D. Mitchell, directeur de thèse

Dr. Beat Frey, rapporteur

Dr. Enrique Lara, rapporteur

Prof. François Gillet, rapporteur

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UNIVERSITÉ DE NEUCHÂTEL

Faculté des sciences
Secrétariat-décanat de Faculté
Rue Emile-Argand 11
2000 Neuchâtel - Suisse
Tél: + 41 (0)32 718 2100
E-mail: secretariat.sciences@unine.ch

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Madame Emanuela Samaritani

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"Spatio-temporal variability and restoration effects on below-ground biodiversity and soil ecosystem functioning at the Thur floodplain, Switzerland"

sur le rapport des membres du jury composé comme suit:

- Prof. Edward Mitchell, Université de Neuchâtel, directeur de thèse
- Dr Enrique Lara, Université de Neuchâtel
- Prof. François Gillet, Université de Franche-Comté, Besançon, France
- Dr Beat Frey, Institut fédéral de recherches WSL, Birmensdorf, Suisse

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Le Doyen, Prof. P. Kropf

Summary

Biodiversity and its link to ecosystem functioning are the main subjects of a large variety of studies nowadays, because of the tremendous rapidity at which they are altered as a consequence of direct human impact or as a delayed effect of global changes. Floodplains are acknowledged to be among the most diverse ecosystems on the planet, in terms of species diversity, but also genetic, habitat and functional diversity. Restoration projects aiming at recovering the biodiversity and associated functions and services provided by riverine floodplains are increasing and there is a pressing need for understanding their spatio-temporal complexity. In this work, I investigate the impact of floods and soil habitat diversity on the spatial and seasonal heterogeneity of soil carbon pools and fluxes, ecosystem functioning proxies, bacterial and eukariotic diversity and community structure. The study site is a restored floodplain on the Thur river in northeast Switzerland. In seven functional processes zones, over a six seasons period, soil samples were collected and soil texture and nutrient, carbon pools and fluxes, temperature, soil moisture and flooding regime, enzymatic activity, respiration and microbial biomass were measured. Soil DNA was extracted and bacterial and eukaryotic communities were analysed using respectively terminal restriction fragment length polymorphism (t-rflp) profiles and Illumina high-throughput sequencing.

Our results showed gradual changes in texture along the gradient of distance from the river, which together with water retention capacity and inundation regime, explained the spatial variability of carbon fluxes. Also increasing along this gradient was total eukaryotic microorganism diversity. Bacterial community structure was tightly linked to ecosystem functioning proxies, but what significantly influenced its variability was soil texture and nutrients. Soil diversity, nutrients and moisture also determined decomposition rates heterogeneity. Temperature and inundation regime mostly appear as indirect drivers of soil diversity and ecosystem functioning. But effects of soil conditions and climate result in patterns that vary extremely among taxa. Although the bacterial community was spatially different, temporal effect of climatic conditions dominated and resulted in strong temporal variation in the community structure. On the other hand micro eukaryotic communities were spatially much more differentiated than temporally. But different eukaryotic taxa

showed contrasting patterns. Taxa that were more abundant were also more evenly distributed across FPZ and seasons, while less abundant taxa, and especially those related to aquatic environments show higher variability.

Soil texture and nutrient content, both maintained by natural pulsing flooding regime, proved to be important determinants of bacterial community structure heterogeneity and eukaryotic diversity. Causal relationships between habitat, processes and biodiversity are highly complex and direct connections are difficult to establish, unless specific organism ecology is considered, but all biotic and abiotic factors analysed in this study showed a strong dependence on the soil structure and the natural flooding regime.

Key-words

Soil diversity, ecosystem functioning, microbial ecology, temporal and spatial heterogeneity, riparian floodplain, terminal restriction fragment polymorphism (T-RFLP), metabarcoding

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GENERAL INTRODUCTION

1. Biodiversity

Awareness of the importance of preserving natural environments is increasing. The consequences of human activities in terms of biodiversity loss, land use changes, pollution, and climate change, are thus becoming a major focus of scientific research. The concept of biological diversity and the term "biodiversity" were first created and discussed in the 1980's by Lovejoy and Wilson (Lovejoy, 1980; Wilson, 1988;1992). Initially considered as the sum of living organisms, the concept of biodiversity has since been expanded to include taxonomic and genetic diversity as well as the diversity of habitats within ecosystems and molecular diversity (Campbell 2003; CBD-Convention on Biological Diversity, 1992; IUCN-Theworld Conservation Union, 1994; McNeely *et al*., 1990 in: Reed & Frankham, 2003) . It has entered political debates since the United Nations Conference for environment and sustainable development in Rio de Janeiro in 1992. The habitat is defined as the environment and the biophysical conditions in which species occur and interact. The biotic communities and their interaction, together with the abiotic characteristics of the habitats, constitute the ecosystem.

There are innumerable services that the known biological species provide to human societies, in terms of crops, biofuels, timber production, drugs, and others. In addition, the unknown living organisms contribute most likely in a major way to societal needs (e.g. production of active molecules potentially useful for fighting diseases, wild plant species resisting to pests, or edible species able to adapt and thrive in conditions hardened by global change). In addition, biological diversity contributes to the maintenance and stability of ecosystems (Tilman, 1966; BOX 1) and helps regulate flow of water and nutrients in the soil.

In the past, conservation biology and wildlands management focused on the protection or re-introduction of selected endangered species or on the protection of specific ecosystem services such as timber production or mitigation of pollutants. In the last two decades however, the importance of maintaining the total biological diversity and the services it provides to human health has been increasingly acknowledged (Convention on Biological Diversity opened at the Earth Summit of

1992 in Rio de Janeiro, UN conference on Biodiversity in 2012). In particular, ecosystem functioning has taken an increasing importance and is now part of the main focus of restoration projects (Society for Ecological Restoration, 2004) and the (re-) introduction of species might sometimes be considered as an indirect way to achieve ecosystem functioning improvement (Seddon *et al*., 2007). The tenth meeting of the Convention on Biological Diversity held in Nagoya (Japan) in 2010 (www.cbd.int) showed that previous goals aiming at global and regionally decreasing biodiversity loss were not being achieved, and new strategies were proposed for attaining the goals in the following decade. In Switzerland following this same awareness of failure in previous goals, the Swiss parliament mandated the Federal Department of Environment, Transport, Energy and Communication to develop a strategy, in cooperation with experts from the Cantons, private sector and science researchers. This resulted in the formulation of a ten goals Swiss Biodiversity Strategy (www.sib.admin.ch) that was adopted by the Federal Council in 2012.

BOX 1

Local stability indicates the tendency of a system to return to the previous state after a small perturbation. **Global stability** is the tendency of a system to return to the previous state after a large perturbation. MacArthur (1955) suggested that the greater the complexity of a system, the greater the stability when confronted to a perturbation. MacArthur hypothesis has been since largely debated since it's publication (Elton, 1958; May, 1975; McCann, 2000; Pimm, 1979; Tilman & Bowning, 1994) and it has been suggested that diversity is only one of the factors influencing stability of an ecosystem (Ives & Carpenter, 2007).

System complexity is determined by the number of species and the number of connections between species **Resistance** is a measure of the ability of a system or organisms to not be displaced from its state by a perturbation event (Harrison, 1979; Webster & Patten, 1979). McCann (2000) defines resistance as the degree to which a variable changes after a perturbation.

Stability is the ability to return to its previous state of equilibrium after a temporary perturbation (Botton *et al*., 2006; Holling, 1973). The quicker it returns to equilibrium, the more stable the system.

Resilience is the measure of the ability of a system to absorb changes and still persist (Holling, 1973).

Since both resilience and resistance influence the ecosystem variability in response to stochastic events, the stability of the ecosystem is determined by a combination of both properties (Ives & Carpenter, 2007; Vallina & Le Quéré, 2011).

Despite this increasing popular interest in biodiversity and its conservation, its distribution and environmental drivers, especially in response to natural and human perturbations, remain to be understood. These problems can be addressed at

different spatial and temporal scales, spatial interactions being investigated by landscape ecology (Box 2). Although addressing the question of spatial and temporal dynamic in soil communities, this thesis does not focus on the landscape ecology point of view, but rather concentrates on how and why spatial differences in the soil microbial communities and their functions change over time and the impact of a natural disturbance introduced by restoration on these patterns.

BOX 2

Landscape ecology is the science studying the relationships between ecological processes in the environment and particular ecosystems within a variety of landscape scales (Wiens *et al*., 1993; Wu, 2013). Patterns are studied using GIS software and can be applied in disciplines such as land use and forest management, and regional risk assessment.

Landscapes are a spatially heterogeneous mosaic of patches that differ in size, shape, history and content (Wu, 2013).

The **scale** is the spatial or temporal measure of an object or a process (Turner & Gardner, 1991). Landscape ecology consists of four main principles: the development and dynamics of spatial heterogeneity, interactions and exchanges across heterogeneous landscapes, influences of spatial heterogeneity on biotic and abiotic processes, and the management of spatial heterogeneity. The main difference with traditional ecological studies, which frequently assume that systems are spatially homogenous, is the consideration of spatial patterns (Turner & Gardner, 1991). **Spatial heterogeneity** is usually characterized by the patch-mosaic model (Turner *et al*., 1989), in which the landscape is represented as a collection of discrete patches. The patch-mosaic model provides a simplifying framework that facilitates experimental design, analysis, and management. Landscape ecology elucidates the important links between spatial patterns and ecological processes (Turner *et al*., 2001) with the main focus on spatial heterogeneity.

Patches are the basic unit of the landscape, defined as a relatively homogeneous area that differs from its surroundings. Having definite shape and spatial configuration, they can be described by their vegetation structure using variables such as the number of trees, number of tree species, height of trees (Forman, 1995). Within patches, species might in theory follow closed system behaviours of populations regulation, but dispersal potential between patches, and other mechanisms brought in by perturbation, change and influence the communities and processes inside patches, a process called *patch dynamics* (Pickett & White, 1985). In patch-dynamic systems, classic ecological successions expect dispersal strategies and out-competition to initially favour the development and dominance of opportunistic species characterized by good dispersal, and fast-growing strategies. As nutrients availability decreases, other species with lower capacity of dispersion colonise and start to outcompete the pioneer plants. With time, the most efficient competitors increase their relative abundance outcompeting the mid-successional species. Theoretical developments in landscape ecology have emphasized the relationship between pattern and process, as well as the effect that changes in spatial scale has on the potential to extrapolate information across scales (Turner & Gardner, 1991).

2. Estimating biodiversity

Several approaches exist to assess diversity, depending on the spatial scale and the type of diversity that are being investigated. Alpha diversity corresponds to the diversity observed at the habitat scale, beta diversity the diversity between habitats or variation in the community composition, and gamma diversity is at the ecosystem scale (Whittaker, 1960). Different processes influence alpha and beta diversity (Wilson & Schmida, 1984) and the need for an independent measure of the two types of diversity is debated (Baselga, 2010). Species richness refers to the counted number of species present in the area of interest. Because species richness can be highly dependent on the size of the sample, it can be useful to draw a species accumulation curve, with the number of new species detected at each additional sample plotted against the increasing number of samples taken. The sampling is considered to cover the full species richness if it reaches a plateau.

Another way to overcome sample-size bias is to calculate diversity indexes. Diversity indexes take into account the species richness, but also the abundance and the evenness of the species in the community. Widely used examples of such an index are the Shannon diversity (entropy) index (Shannon, 1948) and the Simpson index (Simpson, 1949). The former stresses the richness component and the rare species; the latter stresses the evenness component and the dominant species. Therefore the two indexes applied on the same dataset can give contrasting results (Nagendra, 2002). A bias in the diversity index estimation can be introduced by the specific difference in the probability of being sampled (Magurran, 1988; Bunge & Fitzpatrick, 1993). Although Shannon entropy accurateness and precision has been challenged (Bent *et al*., 2007), since historically widely known and accepted in the scientific community and considered valid for communities comparison, it is still widely used (Fierer & Jackson, 2006; Marano *et al*., 2008, 2012; Yeager *et al*., 2012; Besemer *et al*., 2012; Fonseca *et al*., 2012). Because richer communities presenting more functional traits are responsible for the positive relationship between ecosystem functioning and species richness (Loreau, 2000; Tilman, 2001), functional diversity has been increasingly used (Fierer *et al*., 2012) although an important question is how to delineate functional groups (Petchey & Gaston, 2006).

Genetic diversity is the amount of variation found within a particular population, while genetic variability is a measure of the tendency of two particular individual genotypes in a population to vary from one another. Both can be calculated from genetic markers. Commonly used methods to detect genetic diversity include DNA fingerprint profiling, such as terminal restriction fragment length polymorphism (t-RFLP) modification of the amplified rDNA restriction analyses (ARDRA), introduced by Liu *et al*., 1997) or amplified fragment length polymorphism (AFLP) (Mueller & Wolfenbarger, 1999). The use of DNA fingerprint methods is controversial, because inadequate for taxonomic identification (different species can have the same restriction fragments), and species richness can not be estimated from DNA fingerprint profiles (Bent *et al*., 2007) but the ability to give an overview of the communities and reflect changes in species composition between communities has been demonstrated (Gillevet *et al*., 2009; Osborn *et al*., 2000) and the method has been widely used for microorganism community profiling (Culman *et al*., 2008; Dunbar *et al*., 2000; Frey *et al*., 2006; Green *et al*., 2004; Schütte *et al*., 2008; Yi *et al*., 2009), often in parallel to DNA cloning and sequencing (Zumsteg *et al*., 2012).

Next Generation Sequencing (NGS) techniques allowing parallel sequencing of millions of reads in a relatively short time and low-cost investment have been largely developed in the last decade. The two NGS methods (Illumina/Solexa, and Roche 454) present a different compromise between cost, precision and accuracy, length of the sequences reads, and number of reads per run of the analyses (Claesson *et al*., 2010; Zarraonaindia *et al*., 2013). These techniques are increasingly used to assess microbial diversity present in environmental samples (Fierer *et al*., 2012) and to relate it to functional diversity (Raes *et al*., 2011), although some bias persist, because of recalcitrant DNA extraction (Lombard *et al*., 2011), different susceptibility of taxa to PCR amplification (Bass *et al*., 2012), or possible creation of chimera sequences (Fonseca *et al*., 2012), typically for long reads produced by 454 (Quince *et al*., 2009) or underestimate of diversity because of lack of heterogeneity in the sequence (Krueger *et al*., 2011). The definition of species becomes controversial when comparing sequences, it is therefore generally accepted that two organism with less than 97% of DNA in common constitute a different Operational Taxonomic Unit (OTU) or phylotype.

Predictions of the global species richness vary strongly as a function of the studied group of organisms and the worldwide number of taxa is still hotly debated. For example, Erwin estimated in 1982 that there are 30 millions species of arthropods in the tropics alone (Erwin, 1982). To reach this number, he counted the number of beetle species on a tree in the tropical forest (163), multiplied it by the number of tree species. This tally was corrected to account for the percentage of beetles in arthropods (40%) and again for the proportion of arthropods on the ground compared to the trees. This procedure, was however rectified later by Ødegaard (Ødegaard, 2000), leading to an estimated arthropods richness of 5 to 10 millions species. Applying a similar approach, Grassle & Maciolek (1992) and Lambshead (1993) estimated that up to 100 millions species of invertebrates could exist in the ocean. May (2010) summarized the range of uncertainty of global species richness between 3 and 100 millions species. These estimates are based on eukaryotic organisms only. Bacterial species richness is largely unknown, because of the imprecision of techniques to assess it and difficulties related to the definition of species in morphologically similar and mainly asexual organisms. It has been tentatively estimated to tens of millions of species (Curtis *et al*., 2002; Tiedje *et al*. 1994). Mora *et al*. (2011), using the pattern of richness at different taxonomic levels, reduced estimates of eukaryotic species richness to around 10 millions.

The predicted species richness of protists is hotly debated. Historically, it has been believed that every free living microorganism was potentially everywhere. This hypothesis was based on the assumptions that, given their small size, microorganisms were extremely abundant and had high dispersion capacity (Finlay *et al*., 1996; Finlay *et al*., 1999; Finlay, 2002; de Wit & Bouvier, 2006). All microorganisms were therefore thought to be cosmopolitan, and the global diversity was thought to be approximated by local diversity and consequently be relatively low. This theory is however nowadays gradually being refuted (Foissner, 2006; Fontaneto *et al*., 2006; Mitchell & Meisterfeld, 2005; Smith *et al*., 2008; Fierer, 2008). Molecular-based assessments of microbial richness have demonstrated that classical microscopy methods tended to underestimate species diversity by not detecting hidden cryptic species (Katz *et al*., 2005; Stoupin *et al*., 2012, Kosakyan *et al*., 2012). Numerous studies have shown that geographical barrier can prevent microorganisms from colonising all places on Earth (Papke & Ward, 2004; Jenkins

et al., 2007), in a different way for different taxa (Martiny *et al*., 2006), and many cases of endemism have been recorded (Cho & Tiedje, 2000; Whitaker *et al*., 2003). Therefore microbial Eukaryotes distribution does not only depend on the heterogeneity of the environment, but it is spatially predictable (Green *et al*., 2004; Reche *et al*., 2005; Whitaker *et al*., 2003). It is currently debated if the small size of microorganisms allows for global colonisation. It was recently suggested that eukaryotic microorganisms smaller than 1mm have indeed ubiquitous dispersal and occur globally wherever habitats conditions requests are fulfilled, over 10mm organisms are much less abundant and less cosmopolitan, with a gradient of decreasing cosmopolitanism from 1 to 10mm size (Fenchel & Finlay, 2002; Fenchel & Finlay, 2004; Finlay & Fenchel, 2004). Other studies suggest threshold for cosmopolitan distribution to be much lower at 100-150 μm, at least for testate amoebae (Wilkinson, 2001) and molecular analyses corroborate the importance of size compared to abundance and ecological constraints as factors determining global distribution (Lara *et al*., 2011). Modelisation of small size particles further reduce threshold, suggesting that over 20 μm global distribution seems improbable and it becomes very unlikely over 60 μm (Wilkinson *et al*., 2011; Yang *et al*., 2010)

Appropriately applied sequencing techniques on environmental samples, combined with classical surveys of micro-organisms diversity, could significantly improve our understanding of the total diversity of living organisms and of the functional roles of this unknown diversity (Sime-Ngando *et al*., 2011).

3. Above- vs. belowground diversity

Micro-organisms are distributed in almost all places of Earth, including ecosystems characterised by the most extreme environmental conditions, such as the deep sea, the desert cryptogrammic crusts, and polar ices. But even in environments that are more familiar to us, micro-organisms are often hidden, either within other organisms or under the ground. Until relatively recently, aboveground diversity was the main focus of most biodiversity studies and the few study on soil diversity seem to indicate that soil biodiversity does not follow the same rules applied on above-ground metazoan global diversity distribution (Decaëns, 2010; Fierer & Jackson,

2006). It is now recognised that soils host an extremely important proportion of the total diversity and consequently deserve full attention for purposes of land use management and biodiversity conservation (Lavelle & Pashanasi, 1989; Suzuki & Olson, 2007).

The relationship between above ground diversity and below ground biodiversity is still debated. Global-scale surveys have demonstrated a latitudinal gradient in species richness for larger organisms, but not for protistan species (Hillebrand & Azovsky, 2001) or bacterial communities (Fierer & Jackson, 2006). Some studies suggest that aboveground plant diversity and belowground biodiversity may be inversely correlated at a global scale (Wu *et al*., 2011), or unrelated at small scale (Waldrop *et al*., 2006; Zak *et al*., 2003), or that aboveground diversity has mainly an indirect effect on microbial communities (Thoms *et al*., 2010). Others instead argue that plant diversity increases the diversity of substrate and therefore maintains a higher diversity in soil communities (Hooper *et al*., 2000). In turn, high diversity in soil organisms can contribute to increasing plant diversity (Van der Heijden *et al*., 1998). Overall, in their review on the link between aboveground-belowground diversity at different scales, De Deyn & Van der Putten (2005) conclude that habitat sizes and diversity gradients can differ significantly between aboveground and belowground organisms and between ecosystems, therefore surveys need to be adapted to the scale relevant to the organisms considered. Up to very recently, knowledge about belowground prokaryotic and eukaryotic microbial species richness and heterogeneity was very limited due to technological difficulties. In the last decade, development of new molecular approaches allowed more precise estimations of belowground biodiversity and an increasing number of studies focus on the links between richness, community composition and ecosystem functioning in soil (Peter *et al*., 2011; Urich *et al*., 2008). Thanks to these better census methods, the determinants and effects of the distribution of biodiversity can be progressively addressed.

4. Soil community structure and ecosystem functioning

Soil profiles are geologically the result of climate, parent material, topography and organisms. Interactions between soil biota and abiotic factors, under the influence of water, determine soil physical properties. In return soil texture, determined by the proportion of clay, sand and silt, and soil porosity affect soil ecology through regulation of water availability and water retention capacity. Although relatively small compared to the soil organic matter pool, microbial biomass, bacteria and fungi, thanks to its rapid turn-over and being food source for micro eukaryote grazers and source of labile nutrients for plants and other microbes constitutes an extremely important proportion of soil catabolism. Decomposition process consists in the physical and chemical transformation of dead organic matter, resulting in the turnover of nutrients. Physical litter breakdown is mostly operated by meso and microfauna, while chemical transformation is mostly lead by microbial communities (bacteria and fungi). Organic matter (OM) quality is determined by the proportion of labile substrate (sugars and starches) compared to more recalcitrant substrates (tannins and lignins) and intermediate substrates (cellulose and hemicellulose). Labile substrates are easily degraded by the bacterial community while recalcitrant substrates decomposition requires specific enzymes mostly produced by fungi. The composition of the microbial community is therefore strongly determined by OM quality. Experiments on organic litter decomposition rates with different OM quality showed that soil fauna contribution to decomposition is also dependent on OM quality (Couteaux *et al*. 1991; Gonzalez & Seastedt, 1991).

The role of soil biodiversity in maintaining the functional properties of soils has received increasing attention in relation to the impacts of land use practice, global climate change, and the remediation of degraded land (Bardgett & Cook, 1998; Brussaard, 1997; Swift *et al*., 1998; Wall *et al*., 2001). By influencing plant cover, and therefore the quality and amount of organic matter that enters the soil system, land transformation is one of the main factors associated to global change (Sala *et al*., 2000).

Soil organisms and their interactions are fundamental to processes of soil structural formation and in the transformation and transfer of materials and nutrients (Bal, 1982; Jones *et al*., 1994) (Fig. 1). Prokaryotes, thanks to their abundance, have

been estimated to contain in their cells nitrogen and phosphorus in amount comparable to that found in terrestrial plants, which illustrates the importance of these organisms for the biochemical processes of the soil (Coleman & Whitman, 2005). Larger organisms such as earthworms and termites exploit plant litter at the soil surface and substantially influence soil structure through fragmentation, mixing and transportation, and also modification of the soil water infiltration rates (Dixon & Peterson, 1971; van Eekeren *et al*., 2010; Wilkinson, 1975). They represent the so-called ecosystem engineers (Lavelle, 1996). Activities of both micro- and mesofauna contribute significantly to the formation of a soil matrix that favours efficient water movement through the soil (Morales *et al*., 2010). As a consequence, soil ability to retain moisture increases, stimulating plant root growth, and enabling plants to exploit a larger area of the soil profile for water and nutrients. Earthworms contribute to the stabilization of soil by mixing organic and mineral matter. Microorganisms also contribute to the formation and stabilization of aggregates. It is generally accepted that an increase in microbial biomass and the associated production of extracellular polysaccharides, together with fungal mycelia, increase the stability of soil aggregates (Lynch & Elliott, 1983).

The strong links between community composition and biogeochemical cycles, and the roles of particular species or assemblages of species in controlling ecosystem functions have been demonstrated, especially with respect to the role of species richness (Naeem, 1998; van der Heijden *et al*., 1998; Bell *et al*., 2005). Microbes in particular are important mediators of biogeochemical fluxes, and the presence and activity of specific groups can influence biogeochemical processes (e.g., nitrogen fixation, lignin decomposition, methane emissions)(Fig. 1). Conversely, soil physico-chemical characteristics influence soil communities dynamic and control rates of carbon processing in set patterns across the landscape (Judd *et al*., 2006). The labile pool of carbon and nitrogen in the soil, through its impact on microbial biomass and activity and on the turnover and supply of nutrients to vegetation, can alter both productivity and community structure of ecosystems (Pastor & Post, 1986).

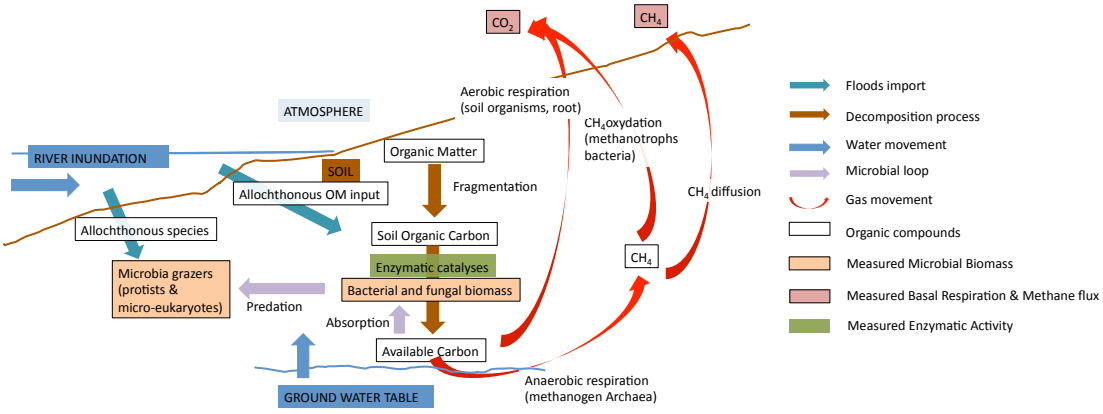

Figure 1. Schematic representation of organic matter decomposition processes and microbial communities in a floodplain. Organic matter (OM) is shred by soil fauna, then decomposed enzymatically by fungi and bacteria. CO_2 or CH_4 from microbial respiration are released depending on the oxidation state of the soil. In saturated conditions methanogens will develop, in unsaturated conditions their relative abundance will decrease. Methane diffuses through the soil and can be emitted into the atmosphere, or oxidized during diffusion by methanotrophic bacteria if oxic conditions are present. Carbon is then available for other soil organisms. Bacterial and fungi biomass depend on the organic matter input and quality and on the predation from eukaryotic microorganisms. In floodplains, OM quality and distribution depends not only on in situ productivity, but also from material deposited by floods. The same floods might also be responsible for importation of exotic species.

5. Functional importance of soil micro-Eukaryotes, the microbial loop

Soil micro-Eukaryotes and especially soil protozoa are recognized as key functional elements of the soil biota. Soil protozoa release nutrients for plant growth by preying on other microbes, stimulate plant growth through non-nutrient effects (Bonkowski, 2004), and more generally are major players in the cycling of C, N, and Si in terrestrial ecosystems (Aoki *et al*., 2007; Schröter *et al*., 2005; Wilkinson, 2008). The microbial loop describes the sequestration of organic carbon released by plants by bacteria and the following remobilisation of nutrients from protozoa consuming bacterial biomass in aquatic (Azam *et al*., 1983) and soil systems (Clarholm, 1981).

The study of soil microbial diversity was for a long time limited by the constraint of having to cultivate organisms in the lab on standard media. It is well known that only a tiny fraction of the microscopic environmental diversity can be grown into cultures (Rappé & Giovannoni, 2003). The development of culture-

independent methods such as environmental DNA surveys revealed the existence of numerous hitherto unknown taxa (Sime-Ngando *et al*., 2011), including some deep-branching clades (Epstein & López-García, 2008; Lara *et al*., 2009), although estimates of the number of novel kingdoms should be treated with caution. In a detailed screening of previously published environmental sequences, Berney *et al*. (2004) showed that the number of novel taxa revealed in those studies were overestimated because of presence of chimeric sequences, misplacing of fast-evolving sequences and incomplete sampling of described but not yet sequenced taxa, in addition to the bias introduced by different DNA amplification probability of the taxa (Berney *et al*., 2004).

Despite their acknowledged importance for soil functioning and other services relevant for land use and agronomy, the spatial distribution and temporal dynamic of soil micro-organism diversity are still poorly known. In particular, the factors that shape the communities of soil micro-organisms are mostly unknown. Soil segregation is thought to allow and maintain high local diversity (Ettema & Wardle, 2002). Horizontal species aggregation can influence diversity distribution, at different scale depending on the organisms' dispersion ability and demography, vegetation and soil physico-chemical properties and, at small scale, resource distribution (Lavelle & Spain, 2001; Jones *et al*., 1994). The activity of soil engineers might enhance local microhabitats complexity and increase local availability of resources. The scale of variation of microbial communities, as well as their evenness through time and space result in complex and nested patterns that still require to be addressed (Martiny *et al*., 2011), especially in systems that are constrained enough to limit differences due to dispersal but variable enough to present microbial communities with challenging conditions.

6. Diversity and disturbance

Biodiversity was traditionally expected to follow some relatively simple ecological rules. The Intermediate Disturbance Hypothesis (HDI) expects biodiversity to be maximal at intermediate levels of disturbance and productivity (Connell, 1978; Horn, 1975) (BOX 3). This theory is however increasingly criticized (Fox, 2013) and

studies challenging its applicability are multiplying. In their review, Mackey & Currie (2001) showed that out of 116 empirical studies investigating species richness under perturbation gradients carried out between 1985 and 1996, the expected relationship between diversity and disturbance was significant in only a limited range of experimental conditions, such as a small sample area and a low disturbance level.

BOX 3

Disturbance is a relatively discrete event that significantly alters or interrupts the course of a process, transforms the physical environment or modifies the availability of nutrients and habitat resulting in the removal of certain organisms or the modification of the community (Pickett & White, 1985; Townsend & Hildrew, 1994). **Fragmentation** is the breaking up of a habitat, ecosystem, or land-use type into smaller units (Forman, 1995). Disturbance is generally considered a natural process. Fragmentation causes land transformation, an important process in landscapes as development occurs.

Stress has been defined as a perturbation from a stressor foreign to the system, or natural but applied at excessive levels (Barrett *et al*., 1976). It is an unfavourable deflection of ecosystem performance after disturbance (Lugo, 1978; Megonigal *et al*., 1997; Odum *et al*., 1979), a perturbation that exceeds the individual or ecosystem capacity to cope with (McEwen & Wingfield, 2010). Stress cannot be entirely overcome by adaptation or species replacement and leads therefore to loss of productivity or fecundity (Hughes & Connell, 1999; Megonigal *et al*., 1997). In floodplains, the system is disturbance-dominated (disturbance being created by interacting flow, thermal and sediment pulses). Therefore the shift in timing, duration or frequency of such pulses can be seen as a stress (Tockner *et al*., 2010).

The newly emerging vision is that the disturbance level can have direct and predictable effects on diversity, but diversity response depends on the type of disturbance and the interaction between disturbance frequency and disturbance intensity, which has been shown to determine this response in experimental microbial populations (Hall *et al*. 2012).

A recent study testing the effects of competition and disturbance on experimental aquatic microcosms composed of 11 species of ciliated protists showed that the trade-off between competition and disturbance assumed by the intermediate disturbance hypothesis is not empirically justifiable and that competition can strongly influence community richness and species extinction at a broad range of disturbance level (Violle *et al*. 2010). Predation is also an extremely important factor that can dramatically influence the fate of species (Schoener *et al*., 2001). The relationship between microbial diversity and external disturbance is thus likely to be highly complex and theories developed for macro-organisms are not necessarily transposable to microbial communities.

Determining what controls the assembly of microbial communities and the consequences of species differences on the functioning of ecosystems requires a multidisciplinary study of a system subject to frequent disturbance. The bacterial and micro-eukaryotic communities and their link to environmental properties and ecosystem functioning should be characterized in heterogeneous systems, with spatial and temporal dynamic driven by natural perturbation such as riparian floodplains.

7. Floodplains are highly dynamic systems

Natural floodplains are amongst the ecosystems characterised by a high level of disturbance and spatial heterogeneity at small scale. Floodplain zones are the ecotone occupying the transition zone between a water body and the terrestrial ecosystem that is under the influence of floods. Riparian floodplains are floodplains developing along flowing waters. Rivers are the results of the drainage of all the run-off water generated in a catchment area. The topography and the local geological formations shape the river catchment and together with climate modify and influence the magnitude and type of sediments loads of river floods. River categories are defined in nested hierarchical classification going from a large-scale (km) network of stream system and hill slopes, to segments of a single streambed surrounded by floodplain (100m). Segments are composed of reaches, which are defined as lengths of streams lying between breaks in channel slope, riparian vegetation, hill-slope and can vary in size. Reaches are composed of a mosaic of habitats (10m), each habitat encompassing a system of microhabitats (Frissel *et al*., 1986, Poole 2002). This hierarchical spatial classification is subject to parallel hierarchical temporal dynamics, with transformations at the catchment scale occurring on geological times (million years) and the drying-rewetting of the reaches by the surrounding riverbed modifying the environment seasonally by disconnecting and reconnecting habitats (Labbe & Fausch, 2000). Other classifications have been developed that take into account different factors such as the level of exchanges between surface water and subsurface patches (Dent *et al*., 2001).

In 1980 Vannote introduced the River Continuum Concept (RCC) that predicted a longitudinal gradient of physical conditions controlling biotic processes

(Vannote *et al*., 1980). This gradient of physical conditions also influence the organic matter loading, transport, utilization, and storage, which are consequently expected to follow the same gradient (Thorp *et al*., 2006). The RCC has been long criticized as not applicable to real streams that are more often characterized by abrupt discontinuity than continuous gradients (Benda *et al*. 2004; Statzner & Higler 1986) and alternative concepts were proposed considering floodplains as dynamic networks. The network dynamics hypothesis (Benda *et al*., 2004) predicts the distribution of physical heterogeneity and therefore high habitat diversity, based on geomorphology and disturbance regime. Thorp *et al*. (2006) suggested that instead of a continuous longitudinal gradient of physico-chemical conditions, floodplains are characterized by a shifting mosaic of geomorphic and hydrological conditions, patches of distinct chemical and physical properties that define their ecological functions, such as community composition, system metabolism, productivity, organic matter dynamics, and nutrient cycling. These ecological functions are controlled by the physical characteristics of a specific hydromorphic patch, and are referred to as functional process zone (FPZ; Thorp *et al*., 2006).

Several sources can contribute to the inundation of floodplains, including the lateral river overflow, precipitations, groundwater and water drainage from surroundings (Tockner *et al*., 2000). Relatively moderate- and low-rate floods modify the environmental conditions and increase periodically and temporally the magnitude of ephemeral aquatic habitats, while high floods can rejuvenate, create, or eliminate water bodies and remodel the topography (Bayley, 1995; Tockner *et al*., 1997). Riparian floodplains are therefore dynamic in three spatial dimensions, longitudinally along the river flow (Vannote, 1980), laterally under the influence of the pulsing of the river discharge (Flood Pulse Concept, Junk *et al*., 1989), and vertically with the interconnection of upwelling groundwater and surface runoff. Temporal dynamic is considered as the fourth dimension of floodplain dynamics (Ward, 1989).

Because of the more or less frequent disturbance of floods, organisms in riparian floodplains need to develop surviving strategies that allow them to thrive depending on the relative importance of the disturbance. Vegetation succession does not follow classic succession gradients, because it is the consequence of both autogenic and allogeneic processes. The flooding regime, together with the erosion

and deposition of sediments processes modulate the structure of the floodplain vegetation (Oliver & Larson, 1996). In addition, subsurface water flow influences the distribution of oxygen and nutrients in the soil and will then contribute to determine plant production and diversity (Naiman *et al*., 2005, Stromberg *et al*., 1996). Soil texture and organic matter content also influence soil water retention capacity and nutrients distribution, therefore determining plant distribution in the floodplain (Hupp & Osterkamp, 1996). In general, near the river, organisms are subjected to frequent and long inundation periods and sediment abrasion, and plants also need adaptations to develop in low nutrients and light exposed soil. Herbaceous annual species colonise the bare Gravel. Uphill further from the riverbed, young trees able to support relatively moderate floods will develop, and grow older as soon as conditions are stable enough (Fig. 2). The local drainage condition, limiting the movement of gas and liquids in the soil, determines the pore size and therefore soil moisture and nutrient cycling and in consequence also the success of the vegetation implantation (Hanley & Brady, 1997). The different vegetation succession stages host different assemblages of fauna. Usually during the aggradation phase of plant built-up and biomass accumulation, there is an increase in soil micro organisms diversity and biomass (Bardgett *et al*., 2005), but the diversity of different organisms might not be correlated (see above section on aboveground vs. belowground diversity) and contrasting response of the same taxa in response to the gradient have been reported. Overall the distribution of soil microorganisms in the spatio-temporal dynamic of floodplain successions remains poorly studied.

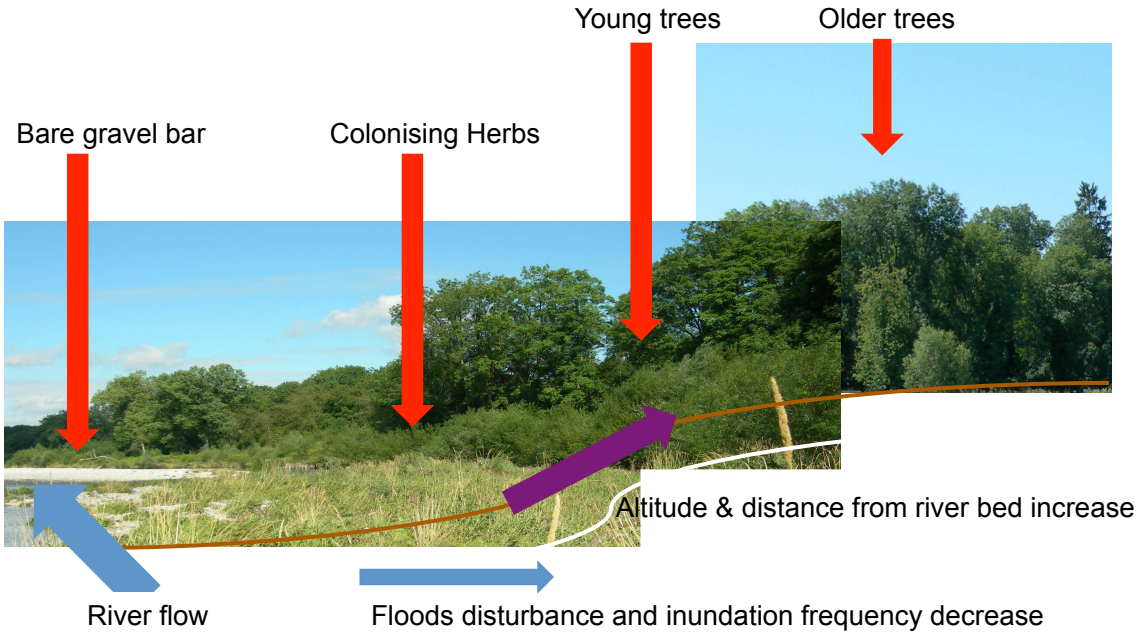

Figure 2. The succession gradient of plant colonisation and development on River Thur at Niederneunforn (Switzerland). Along the increasing distance from the river the first bare Gravel bars are colonised by herbaceous species and further replaced by young trees with strategies to resist the influence of floods and even further older mature trees in the more stable conditions.

In synthesis, the spatio-temporal variations resulting from hydrological disturbance influence the abiotic conditions along the river edge, typically by erosion-sedimentation and the removal or deposition of allochtonous organic matter, but also its biotic conditions by regularly removing the established vegetation and other organisms and by importing exotic species or propagules. Habitats that are directly adjacent to the river are consequently usually devoid of persisting vegetation and are constantly colonized by fast growing opportunist species. The average time span between floods increases with the distance from the river, allowing a higher number of large, long-lived species to develop and settle. As a result of the concomitance of various types of wetlands and small-scale heterogeneous habitats, floodplains are considered amongst the most biodiverse environments at a global scale (Brunke *et al.*, 2003; Stanford & Ward, 1993), but this diversity is very heterogeneously distributed among the different processing zones (Binkley *et al.*, 1997). While this succession of vegetation is well documented, the effects of the disturbance regimes on the response of microorganism communities and related ecosystem functioning remain to be documented.

Riparian soils being highly variable on small temporal and spatial scales constitute an excellent study system to investigate the assembly of microbial communities and its effect on ecosystem functions (Fig. 1). In particular, the heterogeneity of the microbial communities among FPZs and the effect of disturbance on community structure still need to be investigated. The effect of the microbial composition on ecosystem functions are difficult to predict as it will depend on the relative contribution of different species on the overall processes, which will interact with the sensibility of these species on external changes. These questions are however central to our understanding of riparian soils and their fate under human pressure such as degradation of the natural flooding regime.

8. River channelization and floodplain restoration

Over the millennia and especially the last ca. two centuries, most rivers have been heavily impacted by human activities and the current fluvial landscape in western Europe and many other parts of the world is the result of multiple and long-term interactions between ecosystems and societies (Cabezas & Comín, 2010; Petts *et al*., 1989). Floodplain landscapes have been transformed in order to extend the agricultural and urban areas, enable or facilitate river navigation and reduce flooding risks. More than 70% of large rivers of Europe, North America and the former Soviet Union are strongly regulated. Channelization disrupts the connectivity between the river and its floodplain, which is a major cause of habitat degradation in running waters (Allan & Flecker, 1993), leading to a loss of structural diversity, simplified flow patterns and poor retention of organic matter (Lepori *et al*., 2005; Leyer, 2005). As a direct consequence, floodplains lost their characteristic environmental heterogeneity, biodiversity and ecosystem services (Tockner & Stanford, 2002). This loss has been well characterized for above-ground biodiversity, but its effects on the diversity hidden below-ground and the associated functions are much less documented.

Nowadays, restoration of floodplain habitats and the rehabilitation of key ecosystem functions are among the major goals of environmental policy and

increasing points of interest of scientific research (Henry *et al.*, 2002; Ormerod, 2003; SER, 2004). Because of the long-lasting and diversified influence of human activity on floodplains, and given the regional differences in geology, climate, vegetation, land-use history and species distribution, it is impossible to define the pristine condition of a site to be restored. Therefore, recent river rehabilitation focuses on restoring near-natural characteristics and associated functions and values (Ramsar, 2000), such as flooding, ecological succession or species migration and nutrient exchanges as close as possible to supposed natural conditions (Dufour & Piedgay, 2009). The recreation of more natural riverine systems, by allowing more space for the rivers and through the re-establishment of natural channel-floodplain links, and their hydrological and geomorphological dynamics are some of the new strategies applied in Austria, Germany and Switzerland (Van Stokkom *et al.*, 2005; Woolsey *et al.*, 2007). The riverbed is significantly widened along a particular stretch through the removal of the embankments and the setback of the flood levees (Rohde *et al.*, 2005). In Switzerland river restoration projects are expected to multiply in the coming years following the revision on the law on water protection that became effective on January 1st, 2011 (http://www.bafu.admin.ch/umsetzungshilfe-renaturierung).

The success of restoration projects in recovering below-ground diversity, but more importantly the associated functions is unclear and difficult to assess. This question of prime importance for conservation biology requires fine scale investigation of riparian soils among rivers or sections of rivers with different degrees of perturbation.

9. Project collaborations

The work presented in this thesis was conducted as part of a multidisciplinary project called Restored Corridor Dynamics (acronym RECORD), funded by the Competence Center for Environmental Sustainability (CCES) of the ETH domain (www.cces.ethz.ch/projects/nature/Record). The main goal of the RECORD project was to acquire a mechanistic understanding of the river corridor and aquifer systems, both at the hydrological and ecological levels, in terms of ecosystem functioning and

biodiversity, and to determine changes in drinking water quality following transformations of the infiltration processes, caused by the river restoration. Three approaches were considered, monitoring of variables, *in situ* and *in lab* experimentation and modelisation of variables. The three approaches were organised around three workpackages (WP).

The first workpackage (WP1) aimed at monitoring the heterogeneity of the river subsurface and hyporheic zone exchanges, hydrochemistry, vegetation and sediment dynamic. Projects within this WP addressed questions concerning sediment distribution and infiltration processes, gas exchange in the sediments, erosion mechanisms, development of hydrogeophysical inversion methods, quantification of hyporheic exchange, river-groundwater micropollutant exchange, experimental and monitoring for quantifying vegetation-induced cohesion and Radon 220 in the groundwater. The second workpackage (WP2) investigated the heterogeneity effect of the restoration on ecosystem functioning and biodiversity. Projects within this WP focused on hydrological connectivity of biogeochemical transformation of nutrients and organic matter, on a combined approach of field work and modelling to study above ground and belowground biodiversity and understanding carbon and nitrogen processes studying plant-soil-microbe interaction in different succession stages. The third workpackage (WP3) concentrated on hydrological and ecological modelling. Projects within this WP aimed at modelling the hydrology and hydraulic, the vegetation root strengthening of otherwise cohesion less soil, the water flow and biogeochemical transformations
(www.cces.ethz.ch/projects/nature/Record/RECORD_Tasks).

The project presented in this thesis was part of WP2. The overall aim of WP2 was to understand what controls biodiversity (aquatic and terrestrial, both above- and belowground) in river systems and what it means in terms of ecosystem function. In particular, the research developed in the work package aimed at understanding the spatial and temporal effects of hydrological dynamics, associated sedimentation, and changes in habitat connectivity on biodiversity and ecosystem functions in terms of biogeochemical transformations.

This study specifically aims at investigating the spatial and temporal variability of soil diversity and associated ecosystem functions along a gradient of

distance from the river. In addition to the study presented in this thesis, parallel studies were conducted within the same WP2, which used the same sampling sites and sampling seasons. Of these, two studies are especially highly relevant to the present work, being concerned with pedological heterogeneity and organismal diversity, respectively. The study concerned with pedology and conducted in close collaboration with the present work was carried out by Juna Shrestha (PhD) under the supervision of Dr. Jörg Luster, from the Swiss Federal Institute for Forest, Snow and Landscape Research WSL Birmensdorf, and Professor Emmanuel Frossard, from the Swiss Federal Institute of Technology of Zürich. The main focus of Dr. Juna Shrestha's study was on the role of microbial transformations and plant uptake in the cycling of nitrogen in the different functional processing zones toward a better understanding of the filter function of restored river corridors and of their potential to emit greenhouse gases. The work concerned with biodiversity, closely related to the present study, was being conducted by Bertrand Fournier, under the supervision of Professor Edward A.D. Mitchell, from the University of Neuchatel, and Dr. Marco Moretti, from the Swiss Federal Institute for Forest, Snow and Landscape Research WSL Bellinzona. Bertrand Fournier's study focused on the impact of river restoration on soil fauna above ground communities, their interactions and their relations to ecosystem functioning. These collaborations resulted in the common publication of the first chapter of the present thesis (Samaritani *et al*., 2011), in a second publication entitled "Soil Nitrogen Dynamics in a River Floodplain Mosaic", which is part of Dr. Juna Shrestha thesis (Shrestha *et al*. 2012; Annexe 4) and a third publication entitled "Patterns of earthworm communities and species traits in relation to the perturbation gradient of a restored floodplain", which is part of Bertrand Fournier's thesis (Fournier *et al*. 2012; Annexe 3). These different works were highly complementary and their parallel conduct on the same samples will allow an integration of their results into synthetic works. Because my study was part of a broader project, some compromises had to be accepted between the different parts involved, and decisions about logistics or methods or nomenclature were not always under my responsibility or choice alone.

10. Aims and hypotheses

This study specifically aims at investigating the spatial (among FPZs) and temporal (seasonal) heterogeneity of soil biodiversity and associated ecosystem functions along a gradient of distance from the river. It is organised around three main topics, each topic being the main subject of one paper that is or will be published independently in an international scientific journal:

(i) soil carbon pools and fluxes as proxies of carbon dynamic

(ii) bacterial community structure dynamics and their correlation to ecosystem function proxies

(iii) eukaryotic microorganism community structure and diversity.

Biodiversity and ecosystem function proxies are studied in relation to

- soil physico-chemical properties and
- microclimatic conditions.

Communities and soil processes patterns and relationships are analysed with special focus on temporal and spatial heterogeneity.

Overall, I predict that microbial communities and ecosystem functions will vary both temporally and spatially because of environmental patch dynamics among FPZs coupled with different levels of flood-related disturbance. The relationships between biodiversity and ecosystem function proxies are also expected to vary among FPZs and seasons as the global function of bacterial communities is expected to result from a complex interplay between species identities and abundances and environmental conditions. Finally, the flooding regime is expected to be the major driver of the spatio-temporal heterogeneity of the microbial communities, since it represents the major perturbation of temporal and spatial variability in riparian environments. This effect is expected to vary among FPZs, as a function of their distance from the river, and among seasons, because of unpredicted variations in the water discharge during the year. However, given the strong and visible differences among FPZs, I hypothesize that differences among FPZs will be greater than seasonal differences.

On specific topics, an aspect of this work aims at testing the hypotheses that frequent disturbance by flood pulses in the FPZs closer to the river and stress caused

by more frequent and longer inundation affects the carbon pools and fluxes temporarily and locally and that such effects are an essential precondition to achieve a broad spectrum of conditions and processes supporting a large variety of organisms and, therefore biodiversity. Regarding bacterial diversity, I hypothesize that community structure, soil characteristics and ecosystem functions will partially covary across space and time, because these three aspects are supposed to be in part directly related to each other and in part a function of the same external factors. Despite this correlation, I hypothesize that soil characteristics, bacterial community structure and ecosystem functioning will vary in a different way in the various FPZ and seasons, because of the complex interplay between biotic and abiotic factors in natural ecosystems. Definite conclusions on the respective influence on ecosystem functioning would require manipulation of the system and are therefore out of reach of this descriptive work. Regarding eukaryotic microorganisms diversity, I expect that time (sampling date) and space (FPZs) will both have a significant effect on community composition. I expect the flooding regime to be responsible for the heterogeneity of the diversity, but that its effects will vary among taxonomic groups of Eukaryotes as a function of their life-history traits and ecological, and trophic properties. For the same reasons, I expect that relationships between eukaryotic diversity and richness, ecosystem functions proxies and microclimatic conditions will vary among taxonomic (super-) groups.

11. Structure of the thesis

The main goal of the project was to highlight the spatio-temporal heterogeneity of different aspects of the riparian system and the effect of flooding on the soil biodiversity and functioning of the study site.

Chapter 1

I first analysed the physico-chemical properties of soil sampled in multiple plots of the different FPZs. The texture and nutrients were characterized at different depths within each sampling site. The soil samples for these analyses were taken during the first field campaign in April 2008. The dynamics of carbon in the soil were then

analysed through a detailed characterization of the carbon pools and proxies for the fluxes, which were measured once a season over one year from October 2008 to August 2009 and analysed in respect to spatial (FPZ) and temporal (season) effects. The variability of these different aspects of riparian soils were analysed and discussed in relation to changing microclimatic conditions.

Chapter 2

The bacterial community was then investigated together with its links to soil properties and ecosystem functioning. Bacterial diversity was assessed with a fingerprinting technique, which was applied on the total DNA extracted from soil samples taken on the same sites in each season over one year, from July 2008 to April 2009. OTU richness, α Shannon diversity and β diversity of the bacterial community were statistically tested and discussed. Relation of β diversity to soil properties and ecosystem functioning proxies, and the interplay between these aspects of riparian ecology was discussed.

Chapter 3

The communities of soil micro eukaryotes were investigated in the same samples as bacterial diversity. Using the exact same DNA extracts, the Illumina high-throughput sequencing technique were applied to universal genetic barcodes. The organismal taxonomic unit were detected and assigned to taxonomic clades of organisms. This information was used to estimate the phylotype richness and abundance. Micro eukaryote relative abundances at two different taxonomic levels were analysed in relation to spatial and temporal heterogeneity.

Annexe 1

The soil organisms, whether bacteria (Archaea were not studied here) or eukaryotes, are directly or indirectly involved in the decomposition of organic matter. Difference in rates of organic matter decomposition throughout the floodplain were investigated with an experiment. Litterbags were installed in situ and decomposition was monitored from summer 2008 to spring 2009. Differences in rates among sites were discussed in link to microbial diversity and community structure and ecosystem functions.

Together, the parallel analysis in time and space of soil abiotic, biotic and functional characteristics of the dynamic floodplain of the Thur River allowed to shed new light on the reciprocal influences among abiotic and biotic factors, which are both constantly changing in response to climatic seasonality and floods.

Annexe 2

The metabarcoding analyses presented and discussed in chapter 3 allowed the detection of an unexpectedly large number of OTU. Those OTU were assigned at 35 different clades and their diversity, ecology and functional importance is described in this annexe.

Annexe 3

Earthworms are soil engineers, modifying its structure and composition by bioturbation, affecting water infiltration, nutrient cycling and horizon structure. Being recognized as good bioindicator of soil condition in alluvial ecosystems, their community patterns and their species traits in the different FPZ of the restored floodplain were analysed. As hypothesised, trait-based metrics reveal clearer patterns than classical approaches, strongly correlating to environmental variables and allowed detection of traits able to indicate for soil development in floodplains.

Annexe 4

Nitrogen pools and transformations were investigated in the study site and found to be influenced by soil moisture and carbon availability, both depending on inundation regime and sedimentation dynamic.

12. Study site

The Thur River originates in the limestone formation of the Mount Säntis region at 2500 m a.s.l., crosses the Swiss Plateau, and enters the Rhine River at 345 m a.s.l. Unusual for Swiss rivers of this size, there is neither lake, nor artificial reservoir along the entire course of the river that could mitigate the effects of floods. The river exhibits therefore a flashy flow regime. Flood events occur mainly during the

snowmelt period in spring, and heavy rainfall events in summer and autumn. The river flow rates range between 2 and 1'130 (mean 47) m3 s^{-1}, respectively (recording period 1904–2005: http: //www.hydrodaten.admin.ch/d/2044.htm)

The project was conducted in the river corridor at Niederneunforn (Canton Thurgau, 8º77'12''E; 47º59'10''N). It is recognized as an alluvial site of national importance, called Schäffäuli. Channeled since 1854, the Schäffäuli site has been restored in 2002; Fig. 3). The main channel was widened from 50m to 110m by removing the foreland in front of the levees. In addition, the levees were lowered in some places to reconnect the old alluvial forest with the river during high floods. Gravel bars were quickly created and floods started to inundate the alluvial forest.

Figure 3. The studied restored section of River Thur at Schäffäuli before and after restoration in 2002.

In the study site, six different functional processing zones (FPZ, Thorp *et al*., 2008) along a succession gradient of vegetation were determined based on topographic position and distance from the river (Fig. 4 & 5). A seventh FPZ was selected in the channelized section upstream of the restored section.

Close to the river the first FPZ selected was called Gravel, and it was characterized shallow coarse soil. The sparse vegetation covered, on average, 33% of the ground. The dominant species were respectively *Phalaris arundinacea*, *Barbarea vulgaris*, and *Persicaria maculosa*. In the next FPZ called Grass, vegetation was

very dense and covered almost entirely the ground. *P. arundinacea* strongly dominated the communities.

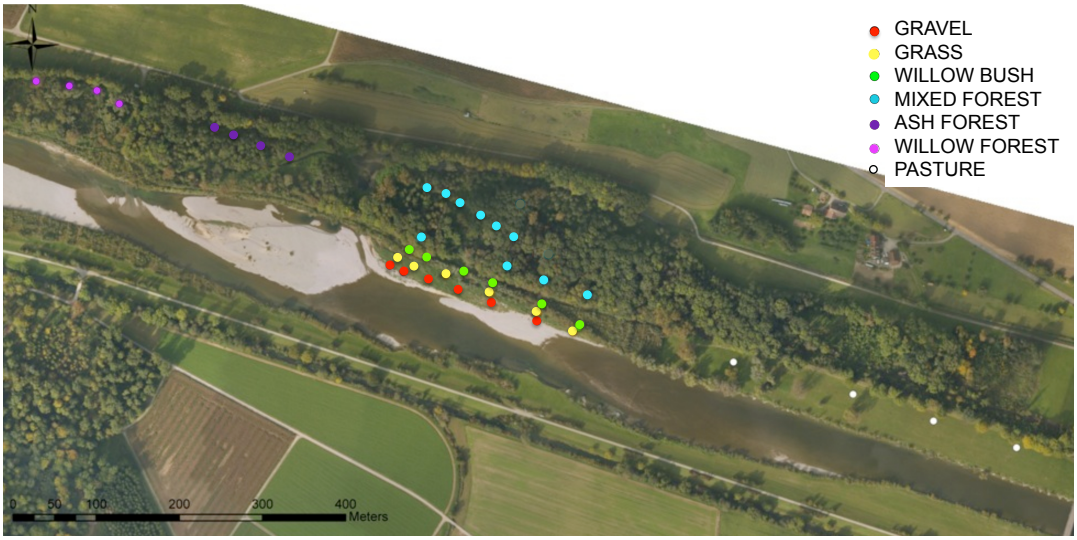

Figure 4. Seven different FPZ selected at the study site. With increasing distance and elevation we selected Gravel (GC), Grass (Ph) and Willow bush (WB), followed by Mixed forest (MF) and Pasture and then by Ash forest (AF) and Willow forest (WF) at comparable distance from the river.

The third FPZ along the gradient of increasing distance from the riverbed, Willow bush, was a strip of Willow bushes, mainly *Salix viminalis* but also *Salix purpurea* planted at the time of the restoration. Also present were *Impatiens glandulifera* and *Urtica dioica*. *P. arundinacea* could reach a height of three meters in certain places. Vegetation density was minimal in spring. The Willow bush FPZ is on a five to seven meters wide steep slope. The understory was dominated by *Rubus fruticosus* and various Grasses (e.g. *Deschampsia cespitosa, Elytrigia repens*, and *Brachypodium sylvaticum*). Uphill of the Willow bush FPZ, after crossing a pathway, lay the forest. We divided the forest in three different FPZs, depending on the dominant tree species.

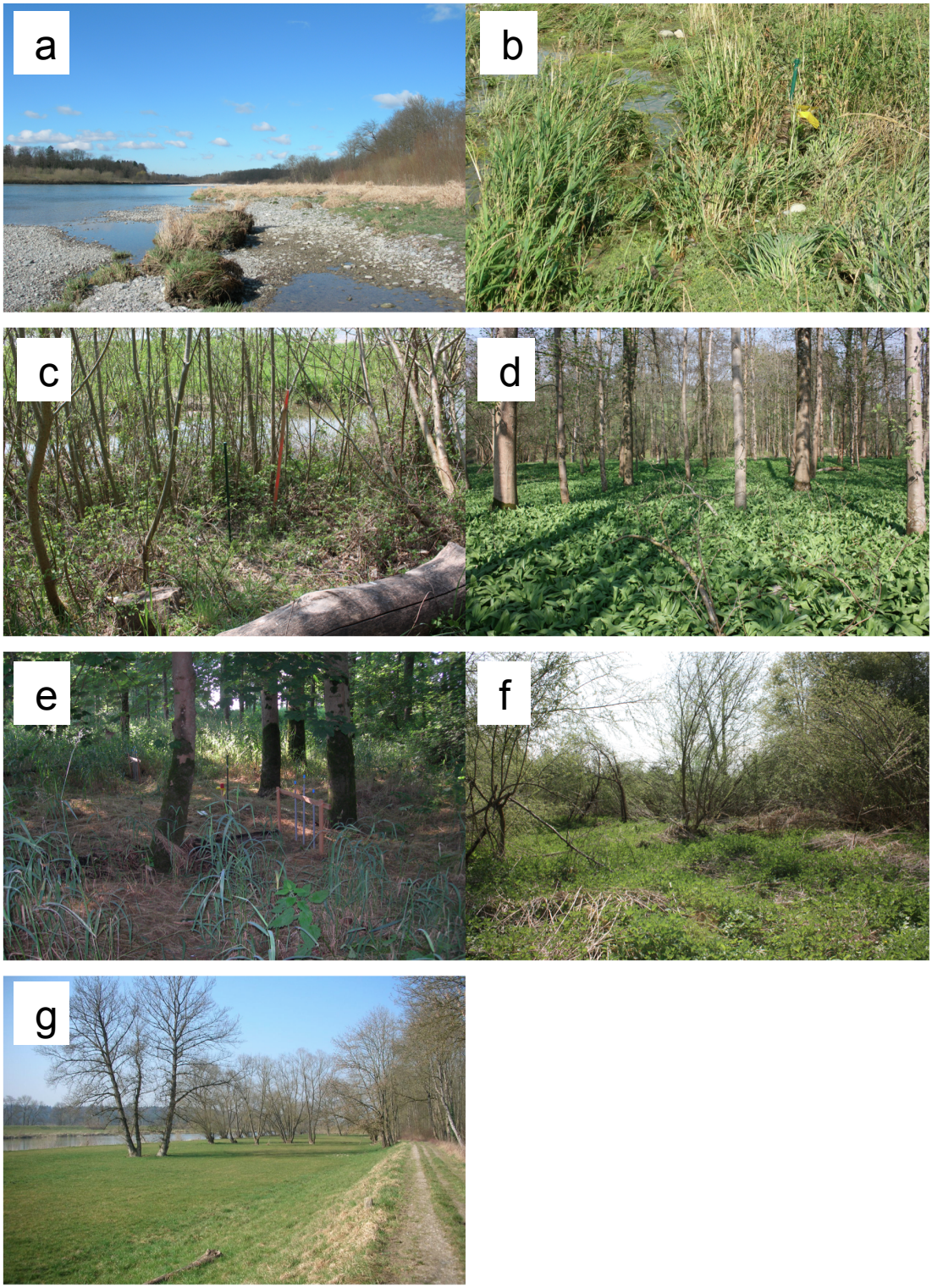

Figure 5. Pictures of the studied FPZ: a) Gravel, b) Grass, c) Willow bush, d) Mixed forest, e) Ash forest, f) Willow forest and g) Pasture.

The first forest type following the increasing distance from the riverbed was called Mixed forest. The dominant tree species were maple (*Acer pseudoplatanus*), and ash (*Fraxinus excelsior*). *Allium ursinum* and *Ranunculus ficaria* strongly

dominated the very dense understory in early spring, however later in the season the herbaceous strata decreased in cover and the vernal species were replaced by *Galium aparine*, then by *Aegopodium podagraria*, *Carex pendula*, and *Rubus fruticosus*.

Further from the riverbed and slightly downstream, another FPZ was selected and named Ash forest. This forest was dominated by ash (*Fraxinus excelsior*) with understory dominated by *P. arundinacea, R. ficaria* and *G. aparine* and *U. dioica*.

At the same distance, but further downstream, closer to the side channel, laid the Willow forest, with understory dominated by *R. ficaria* in spring, then *G. aparine* and later in the year, the understory became completely dominated by *U. dioica*. This species formed very dense, almost monospecific patches, except for a few Grasses, and its height exceeded 2.5m. Tree density was lower than in the Mixed and Ash forests.

Upstream from the restored section, the Pasture FPZ was selected as a representation of the conditions previous to the restoration. No tree or bush strata were present. The composition of the plant species community was typical of managed Grasslands, with dominance of Grasses (*Elytrigia repens*, *Dactylis glomerata*, and *Arrhenatherum elatius*) and hemicryptophytes such as *Taraxacum officinalis* and *Trifolium repens*.

In each of the three restored FPZs six plots were selected, only four plots were selected in the Ash forest, Willow forest and Pasture. In the Mixed forest we selected ten plots in order to adjust to requirements from other WP studying the groundwater flow. Each plot corresponded to an eight meters diameter circle, of which the upstream part of the half-circle was used for observation (vegetation determination and gas measurements) and downstream part of the half-circle was used for soil samples collection and other destructive samplings (sampling for earthworms; Fig. 6).

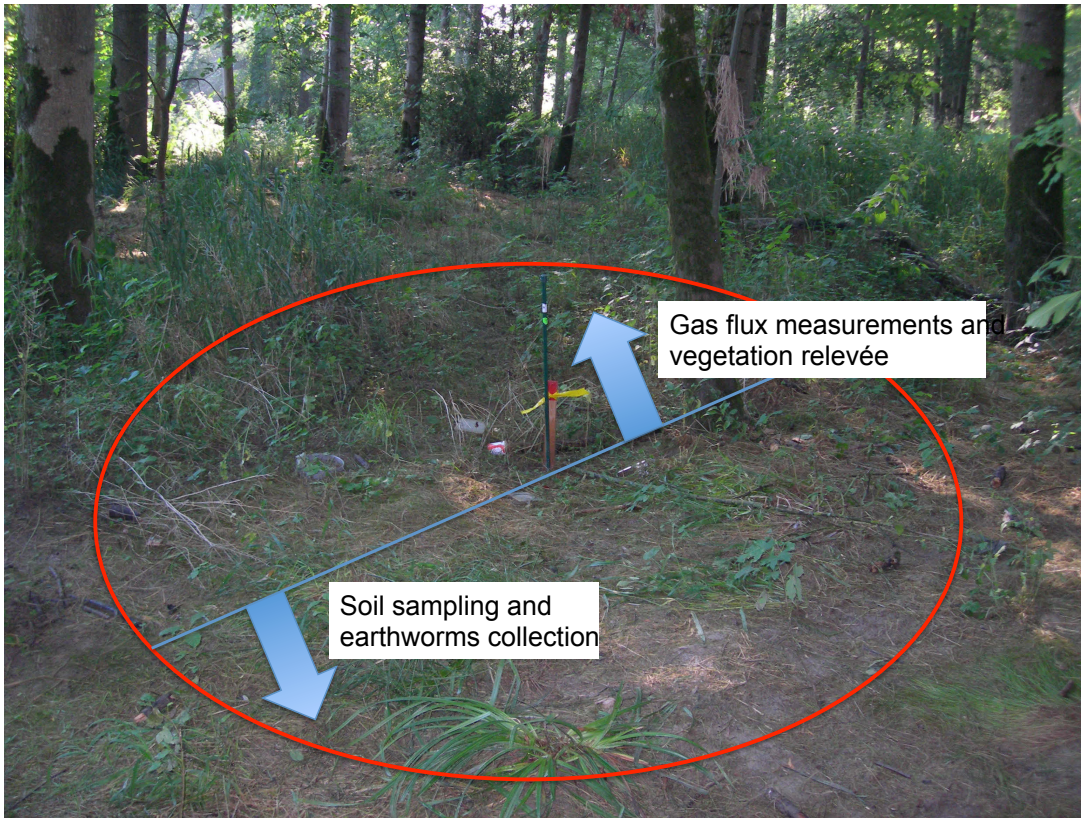

Figure 6. Schematic representation of a plot for samples collection. Sampling plots were circle of eight meters diameter, with a pole in the centre. The circle was divided for destructive sampling (downstream half-circle) and non-destructive measurements (upstream).

Chapter 1. Heterogeneity of soil carbon pools and fluxes in a channelized and a restored floodplain section (Thur River, Switzerland)

E. Samaritani[1,2,4¶], J. Shrestha[3¶], B. Fournier[1,2,4], E. Frossard[5], F. Gillet[2,6], C. Guenat[2], P.A. Niklaus[7], N. Pasquale[8], K. Tockner[9], E.A.D. Mitchell[1,2,4], J. Luster[3]

[1] Swiss Federal Institute for Forest, Snow, and Landscape Research, CH-1015 Lausanne, Switzerland

[2] Ecole Polytechnique Fédérale de Lausanne, Laboratory of Ecological Systems, CH-1015 Lausanne, Switzerland

[3] Swiss Federal Institute for Forest, Snow, and Landscape Research, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland

[4] Institute of Biology, University of Neuchatel, Rue Emile Argand 11, CH-2000 Neuchatel, Switzerland

[5] Institute of Agricultural Sciences ETH Zurich, Eschikon 33, CH-8315 Lindau, Switzerland

[6] Laboratoire Chrono-Environnement, UMR 6249 CNRS, Université de Franche-Comté, 16 route de Gray, 25030 Besançon cedex, France

[7] Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

[8] Institute of Environmental Engineering ETH Zurich, Schaffmattstrasse 6, CH-8093, Switzerland

[9] Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), and Institute of Biology, Freie Universität Berlin, Müggelseedamm 310, D-12587, Berlin, Germany

[¶]the first and second author contributed equally to this work

Personal contribution: sampling, data collection, numerical analyses, statistics, paper redaction

Placement: this work took place at the Swiss Federal Institute for Forest, Snow, and Landscape Research, both at the Lausanne (CH-1015 Lausanne, Switzerland) and Birmensdorf sections (Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland).

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Abstract

Due to their spatial complexity and dynamic nature, floodplains provide a wide range of ecosystem functions. However, because of flow regulation, many riverine floodplains have lost their characteristic heterogeneity. Restoration of floodplain habitats and the rehabilitation of key ecosystem functions, many of them linked to organic carbon (C) dynamics in riparian soils, has therefore become a major goal of environmental policy. The fundamental understanding of the factors that drive the processes involved in C cycling in heterogeneous and dynamic systems such as floodplains is however only fragmentary.

We quantified soil organic C pools (microbial C and water extractable organic C) and fluxes (soil respiration and net methane production) in functional process zones of adjacent channelized and widened sections of the Thur River, NE Switzerland, on a seasonal basis. The objective was to assess how spatial heterogeneity and temporal variability of these pools and fluxes relate to physicochemical soil properties on one hand, and to soil environmental conditions and flood disturbance on the other hand.

Overall, factors related to seasonality and flooding (temperature, water content, organic matter input) affected soil C dynamics more than soil properties did. Coarse-textured soils on Gravel bars in the restored section were characterized by low base-levels of organic C pools due to low TOC contents. However, frequent disturbance by flood pulses led to high heterogeneity with temporarily and locally increased pools and soil respiration. By contrast, in stable riparian forests, the finer texture of the soils and corresponding higher TOC contents and water retention capacity led to high base-levels of C pools. Spatial heterogeneity was low, but major floods and seasonal differences in temperature had additional impacts on both pools and fluxes. Soil properties and base levels of C pools in the dam foreland of the channelized section were similar to the Gravel bars of the restored section. By contrast, spatial heterogeneity, seasonal effects and flood disturbance were similar to the forests, except for indications of high CH_4 production that are explained by long travel times of infiltrating water favoring reducing conditions. Overall, the restored section exhibited both a larger range and a higher heterogeneity of organic C pools and fluxes as well as a higher plant biodiversity than the channelized

section. This suggests that restoration has indeed led to an increase in functional diversity.

Introduction

Embracing spatial heterogeneity is a major challenge in ecosystem ecology. The composition, spatial configuration and temporal dynamics of habitat patches determine biodiversity and ecosystem processes. Ecosystems therefore need to be considered as dynamically interacting mosaics rather than homogeneous entities (Pinay *et al*., 2002; Ward *et al*., 1999). Floodplains are an ideal model to study spatial and temporal heterogeneity.

Floodplains are defined as low-relief areas that extend from the edge of permanent water bodies to the edge of uplands and are subject to flooding. In their natural state, the interaction between flood dynamics and geomorphic processes create a shifting mosaic of habitat patches (Naiman & Décamps, 1997; Stanford *et al*., 2005). These hydrogeomorphically distinct patches differ in age, inundation regime, and soil properties, thereby expressing a different productivity, system metabolism, organic matter dynamic, and biotic community composition. These patches can be referred to as "Functional Process Zones" (FPZs) as described by Thorp *et al*. (2008), although, in the context of the present study we apply the FPZ concept at a smaller scale to hydrogeomorphic patches within a single reach. Furthermore, we extend "functional" to ecological processes rather than to restrict the term to physical functioning of geomorphic and hydrologic forces. In dynamic floodplains, the various FPZ are arranged along distinct succession gradients (Naiman & Décamps, 1997), from recently deposited sand or Gravel to mature alluvial forests.

Due to their spatial complexity and dynamic nature, floodplains provide a wide range of ecosystem functions and related services. Because flow alteration is one of the most serious threats to ecological integrity of river-floodplain systems (Tockner *et al*., 2008), the widespread regulation of the flow regime of large rivers, in particular in Europe and North America has led to the loss of characteristic environmental heterogeneity, biodiversity and associated ecosystem services in many floodplains (Tockner and Stanford, 2002). In the last decades, restoration of

floodplain habitats and the consequent rehabilitation of key ecosystem functions has become a major goal of environmental policy, and concurrently scientific approaches to evaluate its success have been put forward (Henry *et al*., 2002; Palmer *et al*., 2005; Woolsey *et al*. 2007). Motivated to a large extent by flood protection, restoration is achieved, e.g., by widening the main river channel through the removal of embankments and by the setback of flood levees (Rohde *et al*., 2005; van Stokkom *et al*., 2005).

Ecosystem services such as provision of plant and animal resources, removal and/or degradation of pollutants, nutrient retention, and carbon (C) storage are tightly linked to organic C dynamics in riparian soils (Hill & Cardaci, 2004; Wilson *et al*., 2010). Although the need for a fundamental understanding of the factors that drive the processes involved in C cycling in heterogeneous and dynamic systems such as floodplains is recognized, knowledge is still fragmentary (Pacific *et al*., 2008; Zehetner *et al*., 2009). There have been an increasing number of publications in recent years on abundance and community structure of microorganisms in riparian soils (e.g., Rinklebe and Langer, 2006; Unger *et al*., 2009), but still little information is available on bioavailable and mobile soil organic carbon (Bishop *et al*., 1994; Hill and Cardaci, 2004). The heterogeneity of soil-atmosphere exchange of CO_2 and methane has been addressed previously (e.g. (Gulledge & Schimel, 2000; Pacific *et al*., 2008; Pulliam, 1993). However, combined studies addressing both "active" carbon pools and gas-exchange as proxies of different aspects of soil functionality have been rare.

In this study we quantify C dynamics in adjacent channelized and widened sections of the Thur River, NE Switzerland. This is the main test site of the interdisciplinary project RECORD (http://www.cces.ethz.ch/projects/nature/Record; Linde *et al*., 2011; Pasquale *et al*., 2011; Schneider *et al*., 2011). The site is composed of three different types of FPZs: (i) frequently flooded, *dynamic* patches in the restored section, (ii) mature, *stable* alluvial forests that are flooded once or twice a year in the restored section, and (iii) geomorphologically homogeneous pasture in the channelized section. The objective was to assess spatial heterogeneity (among and within FPZs) and temporal variability of selected soil organic C pools (microbial C and water extractable organic C) and fluxes (soil respiration and methane fluxes) and how they relate to physicochemical soil properties on one

hand, and to soil environmental conditions and flood disturbance on the other hand. In particular, we wanted to test the hypotheses that (i) frequent disturbance by flood pulses in the dynamic FPZs affects the C pools and fluxes temporarily and locally and (ii) such effects are an essential precondition to achieve a broad spectrum of conditions and processes supporting a large variety of organisms and, thus biodiversity. Our motivation was to better understand C dynamics in the different types of floodplain FPZs, and, as a consequence, how differences in floodplain structure, in particular between regulated and restored river sections, may affect related ecosystem services such as carbon storage and habitat provision.

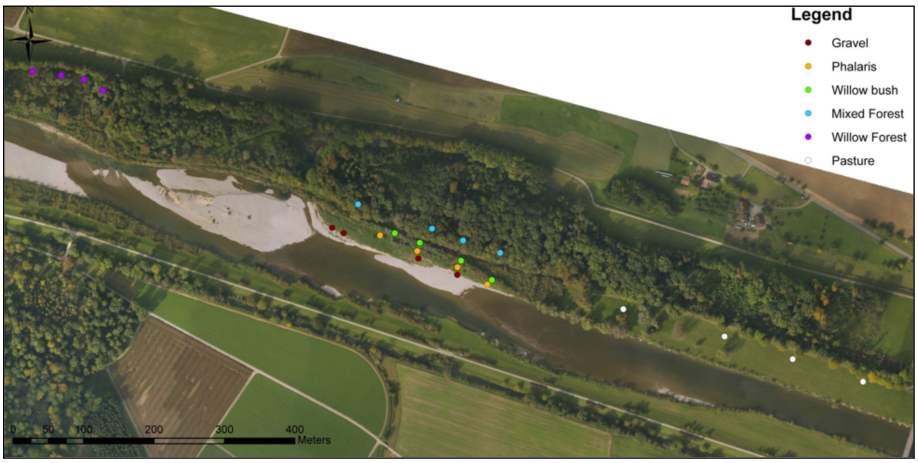

Figure 1. Aerial view of the Thur River test site near Niderneunforn (North-Eastern Switzerland) showing the different plots for each of the six Functional Process Zones.2.

Study site

The Thur River (catchment area: 1700 km^2) originates in the limestone formation of the Mount Säntis region (2500 m a.s.l.), crosses the Swiss Plateau, and enters the Rhine River at 345 m a.s.l.. The river exhibits a flashy flow regime due to the absence of reservoirs and natural lakes. Maximum, mean, and minimum flow rates are 1130, 50, and 2m^3s^{-1}, respectively (recording period 1904-2005: http://www.hydrodaten.admin.ch/d/2044.htm). Flood events occur mainly during the snowmelt period in spring, and heavy rainfall events in summer and autumn. The formerly braided river was channelized in the 1890s to protect the river valley against flooding. In the 1970s, a plan to concurrently improve the flood protection and ameliorate the ecological state of the river corridor was elaborated. Since 1993, several 1-3 km long river sections were widened to allow the formation of

alternating Gravel bars and to increase hydrological connectivity between the main channel and its riparian zone. One of these sections is the test site. Basic data on the chemical quality of the Thur River and the adjacent alluvial aquifers can be found in Hoehn and Scholtis (2011).

The test 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 restored in 2002. The main channel was widened from 50 m to 110 m by removing the foreland in front of the levees. In addition, the levees were lowered in some places to reconnect the old alluvial forest with the river during high floods. The newly exposed banks were partly enforced by tree trunks, and additionally by planting a strip of willow saplings. In the widened river channel, discharge fluctuations and sedimentation have led to the evolution of a dynamic succession of Gravel bars. At the test side, this morphodynamic has been monitor (Pasquale *et al*., 2011), and the subsurface structure of the Gravel bars was characterized with the help of geophysical methods (Linde *et al*., 2011; Schneider *et al*., 2011). The mean annual precipitation at the test site is 908 mm and the average monthly temperature ranges from 0.9°C in January to 19.0°C in July (study period; http://gate.meteoswiss.ch/idaweb).

Six FPZs were identified based on vegetation, distance to the river and topography: five in the restored section and one in an adjacent channelized section upstream (Fig. 1). As a result of their topographic position, these FPZs are flooded at different river discharge levels and are exposed to different flooding frequencies and durations (Tab. 1, Fig. 2). Starting from the riverbed, the first FPZ (Gravel) is a mosaic of bare Gravel and patchy vegetation covering on average 33% of the ground. It is frequently inundated and has very little fine soil. The second FPZ (Grass) is Gravel covered by up to 1 m of fine sediments that were trapped mainly by the dominant Grass *Phalaris arundinacea*. This plant tolerates both wet and dry conditions characteristic of soils in pulse-flooded riparian systems (Foster & Wetzel, 2005).

Table 1. Hydro-geological characteristics of the six functional process zones of the test site in the Thur River floodplain, Switzerland.

		GRAVEL	GRASS	WILLOW BUSH	MIXED FOREST	WILLOW FOREST	PASTURE
Maximum Elevation within the plots [1]	m a.s.l.	373.0	373.4	373.6	374.9	372.5	374.7
Minimum elevation within the plots [1]	m a.s.l.	371.8	372.5	372.5	373.6	371.6	374.2
Minimum river discharge for flooding lowest lying plot [2]	$m^3 s^{-1}$	75	125	150	650	400	400
Minimum river discharge for flooding highest lying plot [2]	$m^3 s^{-1}$	180	250	270	>800	400	400
Flooding frequency [3]	time/year	> 10	> 10	4 - 6	1 - 2	1 - 2 [4]	1 - 2
Flooding duration per event [3]	days	< 1 to 14	< 1 to 14	≤ 1	< 1	< 1 [4]	< 1

[1] as measured in May 2010.

[2] estimated from inundation maps produced by digital terrain modeling based on river cross section measurements

[3] approximated using the river discharge data for the years 2007 to 2009 and the minimum river discharge for flooding half of the plots within an FPZ

[4] in WILLOW FOREST more and longer inundation events can occur due to ponding of precipitation or delayed drainage

The third FPZ (Willow bush) comprises the banks composed of older sediments with shrubby vegetation dominated by planted *Salix viminalis*. Other willow species were also present, and the relatively dense understory was dominated by *Rubus* sp. and various Grass species. This strip varies in width from 5 to 10 m, and the study plots were selected in the middle of the bank slope. The last two FPZs, Mixed forest and Willow forest, are forest communities characteristic of floodplains with a deep and shallow average groundwater level, respectively (Schmider *et al*., 2003). Mixed forest is dominated by *Acer pseudoplatanus* and *Fraxinus excelsior* trees and the understory was dominated by *Allium ursinum* and *Ranunculus ficaria* in spring and *Carex pendula* and *Rubus* spp. later in the year. The North side of this FPZ is bordered by a side channel that drains the neighbouring agricultural hill slope. The Willow forest FPZ at the downstream end of the restored section is dominated by mature *Salix alba* trees. The understory was dominated by *R. ficaria* in spring, and by very dense and monospecific patches of *Urtica dioica* later in the year. The northern border of this part of the forest is formed by an old side channel that has partly silted up, but still drains the hill slope and collects back flow water from River Thur. The Pasture FPZ lies in the channelized section and is used by farmers for grazing and Grass fodder production. The plant community was typical of managed Grasslands and dominated by Grass species (mainly *Elymus repens, Dactylis glomerata*, and *Arrhenatherum elatius*) and forbs such as *Taraxacum officinale* and *Trifolium repens*.

In this study, we have considered the first three FPZs in the restored section as "dynamic" FPZs, and the two forest FPZs as "stable" FPZs. In each FPZ, four plots of eight-meter diameter were selected. The upstream half-circle was used for vegetation mapping and gas sampling while the downstream half-circle was used for destructive soil sampling.

Material & Methods

Vegetation

In each plot, all vascular plant species were recorded, and cover was estimated using Braun-Blanquet codes (Braun-Blanquet, 1964). Observations were repeated six times during the 2008 growing season and species richness was calculated from the combined data set.

Soil sampling

Topsoil sampling was carried out in April and October 2008, and in January, April and August 2009. The first sampling served to obtain basic background information on physicochemical soil properties, while the other four samplings were used for detailed measurements of C pools and fluxes. In each plot, three cores (6.5 cm diameter x 10 cm depth) were pooled. In Gravel plots, soil was collected in pits. One half of the field moist soil was sieved (2 mm) and stored at 4°C while the other half was dried (40°C, 48 h) and then sieved at 2 mm. In May 2008, two 1m long soil cores were taken with a drill corer from two plots of each FPZ except Gravel where coarse Gravel prevented the use of the equipment. Each core was split into 20 cm long segments, and the samples were dried and sieved as described before.

Basic soil properties

Soil texture of dried samples was measured using the pipette method (Gee and Brauder, 1986) after removing organic matter with hydrogen peroxide and dispersing with sodium hexametaphosphate. Grain size classes were defined as clay (< 2 μm), silt (2 – 63 μm) and sand (63 μm – 2 mm). Soil pH was measured in a 1:2 slurry of dried soil in 0.01 M calcium chloride after 30 minutes equilibration. Total N and organic and inorganic C contents of finely ground, dried soils were

determined as described by Walthert *et al*. (2010). For Olsen P as a proxy of available P, dried soil was extracted for 30 min at 25°C with 0.5 M sodium hydrogen carbonate at pH 8.5 with a soil to extractant ratio of 1:20. The extracts were filtered through Schleicher&Schuell 0790½ and the extracted phosphate measured colorimetrically using the molybdenum blue method (Kuo, 1996).

Soil environmental conditions

Soil temperature (*T*) at 5 cm depth was measured in the centre of each plot during the entire observation period (30 min resolution; TidBit v2 temperature loggers, onset, Bourne, MA, USA). The temperatures recorded at the time of the soil samplings were used in this study. Gravimetric Water Content (WC) was determined as weight loss upon drying 20 g of fresh soil at 105°C for 24 h. The elevation of the plots was measured in May 2010 by triangulation. The minimum river discharge required for flooding a plot was estimated from inundation maps for different river discharge levels as produced by a 2-D hydrodynamic model (details see Pasquale *et al*., 2011). The estimate of days after last inundation (LI) was based on the minimum discharge value for a given plot and the date at which discharge fell below this threshold.

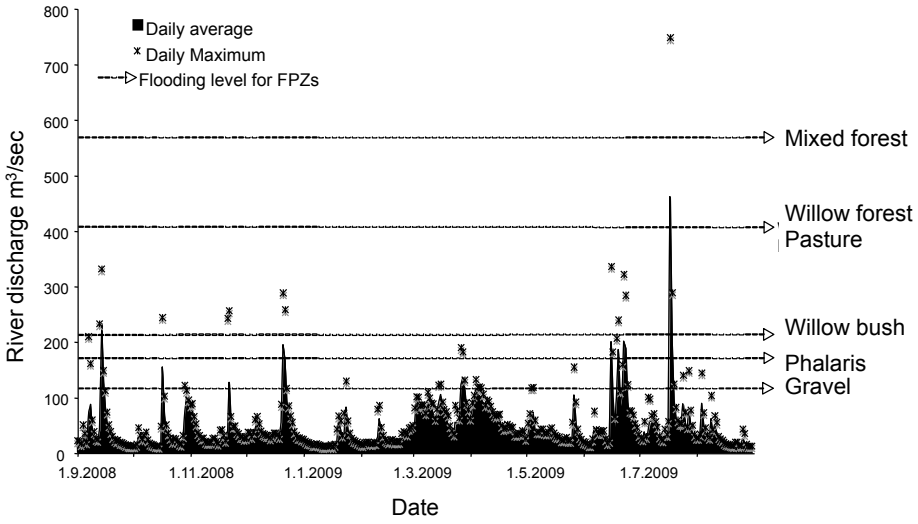

Figure 2. Daily average and maximum discharge of the Thur River at the test site. Minimum discharge required for inundation is different for each functional process zone (FPZ) and the flooding level shown here is the average elevation of the four plots for each FPZ.

Carbon pools

Water extractable organic carbon (WEOC) was extracted from dried soils with 10 mM calcium chloride at a soil:extractant ratio of 1:2 for 10 min on an end-over-end shaker (Embacher *et al*., 2007). The soil slurries were then centrifuged for 10 min at 1335 g and filtered through a 0.45 µm membrane filter. The filtered extracts were measured for non-purgeable organic C (NPOC) using a TOC analyzer (Formacs HT, Skalar Analytical, Breda, The Netherlands). Water extractable organic matter of soils, measured as WEOC, is an operationally defined proxy of dissolved organic matter in the soil solution, playing important roles as substrate of microorganisms and as transport agent (Embacher *et al*., 2007). Although WEOC also represents part of the microbial biomass when extracted from dried soils as in the present study, we consider this pool mainly as proxy of available substrate.

Microbial biomass C (MC) was determined by the chloroform fumigation extraction method (Beck *et al*., 1997). 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 for one hour with 0.5 M potassium sulphate at a 1:5 soil to extractant ratio. The extracts were filtered (0.45-µm) and frozen. The NPOC in these samples was measured using a TOC analyzer (TOC-V CPH/CPN, Shimadzu, Kyoto, Japan). Microbial C was calculated as

$$MC = \frac{(C_{fumigated} - C_{unfumigated})}{k_{EC}} \qquad (1)$$

where $C_{fumigated}$ and $C_{unfumigated}$ are the NPOC contents of chloroform-fumigated and unfumigated samples, and k_{EC}=0.45 corrects for extraction efficiency (Beck *et al*., 1997).

Carbon fluxes

For soil respiration (SR) and methane flux (MF) measurements, PVC rings with 30 cm diameter and 30 cm height (20 cm below and 10 cm above surface) were installed in each plot. Immediately before sampling, vegetation within the rings was clipped and the chamber closed with an air-tight lid. Headspace air samples were collected after 5, 25 and 45 min, injected into pre-evacuated exetainers, and analyzed for CH_4 and CO_2 using a gas chromatograph with a flame ionization

detector (Agilent 6890, Santa Clara, USA). Soil-atmosphere CH_4 and CO_2 exchange were calculated by linear regression of concentration against sampling time.

Temperature dependence of SR was modeled for each FPZ using an exponential equation (Buchmann, 2000)

$$y = a \cdot e^{(b \cdot T)} \quad (2)$$

where a and b are regression coefficients, and T is the temperature at the time of gas sampling. Q_{10} values were calculated as

$$Q_{10} = e^{(10 \cdot b)} \quad (3)$$

Soil respiration normalised to a reference temperature of 10 °C (SR_T10) was calculated according to Doering *et al*. (2011) as

$$SR\_T10 = SR \cdot e^{(b(10-T))} \quad (4)$$

Soil respiration is an indicator of the actual biological activity at the sampling site including both microbial and root respiration. Positive methane flux indicates net methane production while negative flux indicates net methane consumption in the soil.

Statistical analyses

Differences in the soil physicochemical properties among the sites were tested using one–way analysis of variance (ANOVA, SPSS 17, SPSS Inc.). Interactive effects of time and FPZ were tested by one-way repeated measures ANOVA. Post hoc tests were carried out using Tukey HSD if homogeneity of variance could be assumed or else using Games Howell (Field, 2005). Principal component analysis (PCA) was carried out for soil physicochemical properties measured in the soil profile samples. Redundancy analyses (RDA) were carried out for C pools and fluxes as multivariate response to soil properties and environmental conditions. The RDA triplot was projected using scaling method 2 (Kindt & Coe, 2005). The program R (R Team Development Core, 2010) with package vegan (Oksanen *et al*., 2010) was used for PCA and RDA.

Results

Vegetation

Mean plant species richness was lowest in Grass and Willow forest, and highest in Willow bush (Tab. 2). Spatial variability was higher in Gravel and Grass than in the other FPZs (Tab. 3). A principal component analysis of plant species composition and cover revealed that the vegetation in Pasture and in both forested FPZs was rather similar, while it exhibited completely different characteristics in the three dynamic FPZs (data not shown).

Table 2 Mean ± standard deviation of vegetation characteristics and physico-chemical soil properties in the six functional process zones (n=4) of the test site in the Thur River floodplain, Switzerland. Soil properties are for the top 10 cm of soil. Values with different superscript letters in the same row are significantly different ($P < 0.05$; Tukey post-hoc test).

FPZ	Units	Gravel	Phalaris	Willow bush	Mixed forest	Willow forest	Pasture
Vascular plants species richness		55.0 ± 13.6 ^{bc}	41.5 ± 11.2 ^{c}	79.8 ± 8.8 ^{a}	50.8 ± 4.5 ^{bc}	41.5 ± 3.1 ^{c}	67.7 ± 3.5 ^{ab}
pH		7.6 ± 0.1 ^{a}	7.4 ± 0.1 ^{a}	7.5 ± 0.0 ^{a}	7.5 ± 0.0 ^{a}	7.4 ± 0.0 ^{a}	7.5 ± 0.0 ^{a}
Sand	g kg^{-1}	806 ± 52 ^{a}	660 ± 17 ^{ab}	442± 90 ^{bc}	378 ± 57 ^{c}	245 ± 40 ^{d}	65 1± 69 ^{ab}
Clay	g kg^{-1}	53 ± 13 ^{d}	83 ± 36 ^{bcd}	117 ± 18 ^{abc}	148 ± 18 ^{ab}	177 ± 24 ^{a}	78 ± 16 ^{cd}
Inorganic C	g $CaCO_3$ kg^{-1}	355 ± 25 ^{b}	385 ± 18 ^{ab}	408 ± 5 ^{a}	390 ± 6 ^{a}	390 ± 3 ^{a}	382 ± 7 ^{ab}
Organic C	g kg^{-1}	10.1 ± 3.7 ^{c}	16.3± 5.8 ^{abc}	17.1 ± 3.2 ^{abc}	21.4 ± 3.6 ^{ab}	24.8 ± 1.5 ^{a}	12.9 ± 2.9 ^{bc}
Total N	g kg^{-1}	0.7 ± 0.2 ^{c}	1.0 ± 0.4 ^{bc}	1.1 ± 0.3 ^{abc}	1.6 ± 0.3 ^{ab}	1.8 ± 0.1^{a}	0.9 ± 0.2 ^{bc}
C:N		15.2 ± 0.5 ^{ab}	16.2 ± 1.6 ^{a}	15.2 ± 0.7 ^{ab}	13.4 ± 0.6 ^{b}	14.0 ± 0.2 ^{b}	14.0 ± 0.4 ^{b}

Basic soil properties

All soils were rich in carbonates and, accordingly, had a pH of about 7.5 (Tab. 2). In the restored section, soils became more finely textured along a gradient from Gravel to Willow forest. Total organic carbon (TOC) and total nitrogen (TN) contents increased along the same gradient, while the C/N was around 15 in all FPZs. Available P was significantly higher in Grass than in Willow bush and Mixed forest. Soil properties of Pasture were similar to Grass except for a significantly lower available P content. Spatial variability of texture and TOC content were highest in Gravel and Grass, and lowest in the two forest FPZs (Tab. 3).

Soil texture did not vary much with depth in any of the FPZs (data not shown). TOC and TN contents were also homogenously distributed within the soil profiles except for Willow forest. There, TOC and TN decreased with depth to 15 g C kg^{-1} and 1.1 g N kg^{-1} respectively. Available P decreased gradually with depth to 7 mg kg^{-1} in Willow forest, and to about 5 mg kg^{-1} in Mixed forest and Willow

bush. In Grass available P did not vary with depth and in Pasture it first decreased to less than 5 mg kg^{-1} at 20-40 cm depth and then increased to 12 mg kg^{-1} at 80-100 cm depth. The PCA (Fig. 3) showed soil texture as the main factor separating the different FPZs, and demonstrated a generally larger lateral than vertical variation of the soil properties. It also showed that Pasture soils were relatively homogeneous and overall most similar to the soil in Willow bush.

According to the world reference base for soil resources (IUSS Working Group WRB, 2006) the soils in Grass, Willow bush, Mixed forest, and Pasture can be classified as haplic Fluvisols (calcaric, humic) and those in Willow forest as haplic or gleyic Fluvisols (calcaric, humic, siltic).

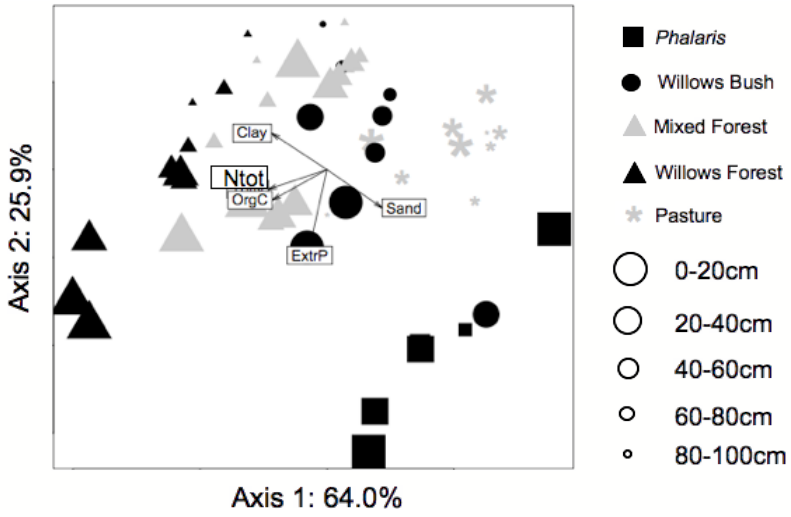

Figure 3. Five functional process zones at the test site in the Thur River floodplain, Switzerland, with two replicates each represented on the two first axes of a PCA performed on soil physico-chemical properties (total organic carbon TOC, total nitrogen TN, available phosphorus Av P, sand and clay content) measured in the soil profiles. The decreasing size of the symbols represents increasing soil depth.

Soil environmental conditions

Temperature measured in Gravel was significantly different from all other FPZs at all seasons (Tab. 4). At each sampling date, either the lowest or highest temperatures were measured there, including the extremes (-0.7 °C, and 20.2 °C). Overall, there was no significant difference in *T* among the FPZs, but in August all non-forested FPZs exhibited distinctly higher temperatures than the forested FPZs. Willow forest and Gravel represented the wettest and driest conditions, respectively. The spatial variability of soil moisture was highest in Gravel and

Grass (Tab. 3). Particularly high WCs were measured in August sampling, which was carried out two weeks after a major flood (see LI in Tab. 4), and in January when the soils were covered by snow and partially frozen.

Table 3 Coefficients of variation (CV) for species richness and soil properties (clay content Clay, total organic carbon TOC) within different functional process zones (FPZs) of the test site in the Thur River floodplain, Switzerland (n=4); mean CV for soil environmental conditions (temperature T, gravimetric water content WC), carbon pools (water extractable organic carbon WEOC, microbial carbon MC) and soil respiration (SR as measured, SR_T10 normalised to reference T of 10°C) within different FPZs (n=4) at the different sampling times (n=4).

	GRAVEL	GRASS	WILLOW BUSH	MIXED FOREST	WILLOW FOREST	PASTURE
Species richness	0.25	0.27	0.11	0.09	0.07	0.05
Clay	0.23	0.43	0.16	0.12	0.14	0.20
TOC	0.37	0.35	0.19	0.17	0.06	0.22
WC	0.36	0.20	0.11	0.09	0.08	0.08
T	0.005	0.004	0.002	0.001	0.002	0.001
WEOC	0.33	0.38	0.19	0.17	0.22	0.18
MC	0.41	0.42	0.31	0.24	0.31	0.16
SR	0.50	0.42	0.49	0.17	0.30	0.37
SR_T10	0.49	0.47	0.49	0.17	0.33	0.38

Carbon pools

On average, the WEOC contents increased from Gravel to Willow forest, and Pasture exhibited low WEOC contents (Tab. 4). WEOC was significantly higher ($P < 0.05$) in August, and lower in April, compared to other samplings. Spatial variability of WEOC was largest in Gravel and Grass (Tab. 3), with particularly high variability in Grass in April and August.

Microbial C was higher in Willow forest than in other FPZs (Tab. 4). In October, MC contents were significantly lower than at the other samplings ($P<0.05$) and highly variable. With the exception of Pasture, spatial variability of MC was large (Tab. 3).

Carbon fluxes

Soil respiration (SR and SR_T10) was lowest in Gravel and highest in Grass and Willow bush at most of the samplings, but differences were statistically not

significant (Tab. 4). Within-patch variability of SR was generally high in all dynamic FPZs (Tab. 3) with hot spots in Grass in April and August and in Willow bush in October and August. While SR values were lowest in January and highest in August, SR_T10 values varied only little with time. The Q_{10} value was highest for Gravel, while it was similar for all other FPZs (Tab. 5).

All FPZs took up methane except for August. Then uptake was observed only for Willow bush and Mixed forest, while the other FPZs emitted methane into the atmosphere. At all samplings, plots in Gravel showed very low uptake or even low emissions, while Mixed forest exhibited the highest uptake rates of all FPZs.

Table 4. Mean ± standard deviation of soil environmental conditions (days since last inundation LI, temperature *T*, gravimetric water content WC), carbon pools (water extractable organic carbon WEOC and microbial carbon MC), and fluxes (soil respiration SR as measured, SR_T10 normalised to reference *T* of 10°C, and methane flux MF) in the six FPZs (n=4) of the test site in the Thur River floodplain, Switzerland. Sampling was repeated four times from autumn 2008 to summer 2009. Also shown are results of repeated measures ANOVA over all sampling campaigns; different lower case letters in the same row indicate significant differences (P<0.05; Tukey or Games-Howell post-hoc test).

			GRAVEL	GRASS	WILLOW BUSH	MIXED FOREST	WILLOW FOREST	PASTURE
October 2008	LI	days	21	21	21	49	49	49
	T	°C	16.0 ± 1.9	14.5 ± 2.9	13.7 ± 0.4	13.4 ± 0.3	13.0 ± 0.6	13.5 ± 0.2
	WC	g kg^{-1}	171 ± 48	268 ± 37	220 ± 34	251 ± 19	302 ± 17	210 ± 11
	WEOC	mg kg^{-1}	127 ± 37	141 ±24	82 ± 15	160 ± 27	164 ± 37	80 ± 18
	MC	mg kg^{-1}	132 ± 92	168 ± 131	158 ± 119	132 ± 100	227 ± 161	73 ± 12
	SR	mmol m^{-2} day^{-1}	43 ± 22	327 ± 39	322 ± 117	194 ± 22	214 ± 47	228 ± 86
	SR_T10	mmol m^{-2} day^{-1}	17 ± 3	224 ± 24	203 ± 74	144 ± 16	150 ± 26	144 ± 55
	MF	µmol m^{-2} day^{-1}	-2 ± 1	-15 ± 3	-25 ± 9	-58 ± 11	-22 ± 9	-6 ± 6
January 2009	LI	days	14	14	14	140	140	140
	T	°C	-0.1 ± 0.4	0.5 ± 0.3	0.3 ± 0.4	0.0 ± 0.2	0.2 ± 0.3	0.5 ± 0.1
	WC	g kg^{-1}	325 ± 57	296 ± 50	255 ± 33	257 ± 26	347 ± 35	252 ± 29
	WEOC	mg kg^{-1}	85 ± 29	143 ± 22	123 ± 19	155 ± 38	147 ± 50	116 ± 11
	MC	mg kg^{-1}	208 ± 99	178 ± 71	297 ± 56	231 ± 42	471 ± 58	331 ± 49
	SR	mmol m^{-2} day^{-1}	8 ± 5	50 ± 28	58 ± 37	41 ± 9	55 ± 36	37 ± 14
	SR_T10	mmol m^{-2} day^{-1}	52 ± 34	172 ± 98	251 ± 169	128 ± 30	170 ± 111	120 ± 44
	MF	µmol m^{-2} day^{-1}	-1 ± 3	-6 ± 7	-18 ± 13	-35 ± 19	-17 ± 9	-6 ± 5
April 2009	LI	days	5	21	112	240	240	240
	T	°C	15.2 ± 2.8	11.4 ± 0.7	11.0 ± 0.7	10.3 ± 0.5	11.7 ± 0.5	10.7 ± 0.8
	WC	g kg^{-1}	169 ± 99	204 ± 43	248 ± 7	219 ± 26	276 ± 23	152 ± 13
	WEOC	mg kg^{-1}	84 ± 28	96 ± 67	117 ± 20	98 ± 16	139 ± 15	94 ± 28
	MC	mg kg^{-1}	148 ± 30	135 ± 49	210 ± 28	223 ± 29	445 ± 72	208 ± 15
	SR	mmol m^{-2} day^{-1}	91 ± 40	304 ± 143	134 ± 47	130 ± 10	178 ± 25	139 ± 43
	SR_T10	mmol m^{-2} day^{-1}	72 ± 46	303 ± 179	147 ± 49	135 ± 8	144 ± 42	118 ± 46
	MF	µmol m^{-2} day^{-1}	7 ± 23	-9 ± 2	-15 ± 5	-55 ± 5	-21 ± 5	-18 ± 3
August 2009	LI	days	2	7	14	14	14	14
	T	°C	19.3 ± 1	18.4 ± 0.8	16.6 ± 0.2	16.3 ± 0.1	16.6 ± 0.6	18.8 ± 0.2
	WC	g kg^{-1}	181 ± 70	388 ± 117	348 ± 41	365 ± 30	493 ± 44	276 ± 15
	WEOC	mg kg^{-1}	155 ± 60	324 ± 168	418 ± 103	480 ± 44	608 ± 131	297 ± 34
	MC	mg kg^{-1}	334 ± 90	351 ± 48	306 ± 46	361 ± 36	263 ± 66	190 ± 47
	SR	mmol m^{-2} day^{-1}	283 ± 127	432 ± 237	654 ± 390	260 ± 67	315 ± 48	345 ± 145
	SR_T10	mmol m^{-2} day^{-1}	50 ± 18	152 ± 90	246 ± 148	124 ± 31	147 ± 29	115 ± 46
	MF	µmol m^{-2} day^{-1}	8 ± 8	17 ± 43	-23 ± 10	-27 ± 9	52 ± 79	151 ± 236
ANOVA results	*T*		a	b	b	b	b	b
	WC		c	ab	bc	b	a	bc
	WEOC		c	bc	bc	ab	a	bc

Carbon pools and fluxes as multivariate proxy of soil C dynamics

Carbon dynamics are presented as multivariate response comprising C pools (WEOC, MC) and fluxes (SR MF), explained by soil properties and environmental

conditions (LI, *T*, WC, TOC, clay). Data were clearly distributed according to sampling date (Fig. 4). The model explained 38.0 % (adjusted R^2) of the variance of the response dataset and the two first canonical axes were significant (P=0.001, 1000 permutations). Overall, WC and *T* explained the main gradient of C pools and fluxes along axis 1, which separates samples of August from all others.

Table 5. Modeled relationship between soil respiration (y, mmol CO_2 m^{-2} day^{-1}) and soil temperature (T, °C) in different FPZs of the test site in the Thur River floodplain, Switzerland; a and b: regression coefficients; SE_a and SE_b standard errors of a and b; Q_{10}: relative increase in soil respiration upon a T increase of 10 °C; n: number of individual measurements; F, R, P: F-value, coefficient of determination, and level of significance of the regression, respectively.

FPZ	$y = a\ e^{(bT)}$	SE_a	SE_b	Q_{10}	n	F	R^2	P
GRAVEL	$y = 6.85\ e^{(0.179T)}$	0.02	2.1	6.0	13	54.7	0.83	<0.0001
GRASS	$y = 51.09\ e^{(0.128T)}$	0.02	15.1	3.6	16	28.6	0.67	<0.0001
WILLOW BUSH	$y = 43.02\ e^{(0.148T)}$	0.02	9.86	4.4	16	55.8	0.80	<0.0001
MIXED FOREST	$y = 40.84e^{(0.117T)}$	0.01	3.53	3.2	16	235.7	0.94	<0.0001
WILLOW FOREST	$y = 44.83e^{(0.116T)}$	0.02	8.46	3.2	16	56.1	0.80	<0.0001
PASTURE	$y = 33.96\ e^{(0.124T)}$	0.01	6.18	3.4	16	77.9	0.85	<0.0001

Soil respiration was positively correlated with *T* and negatively correlated with the number of days since the last inundation. WEOC correlated mainly with WC, whereas MC was strongly linked with clay and TOC content.

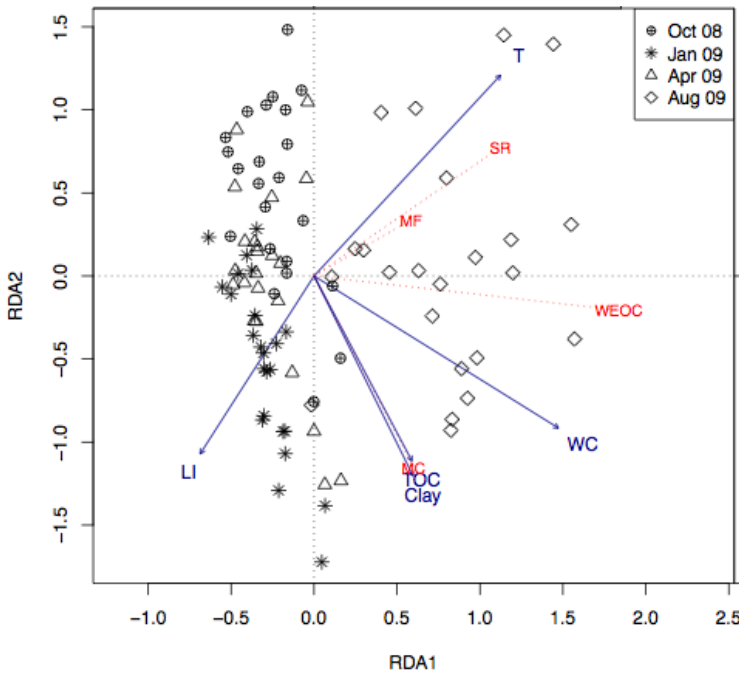

Figure 4. RDA triplot for carbon pools and fluxes in the six functional process zones at the test site in the Thur River floodplain, Switzerland (water extractable organic carbon WEOC, microbial carbon MC, soil respiration SR, methane flux MF) as multivariate response variables (red font), constrained by soil properties and environmental conditions (days since last inundation LI, soil temperature T, gravimetric water content WC, clay content Clay and total organic carbon TOC) as explanatory variables (blue font). Four soil sampling dates are represented with different symbols. Axis 1 explains 35.4% and axis 2 11.3% of the variance. Data were projected using scaling method 2.

Discussion

Our data allow us (i) to relate differences between soil C dynamics in different functional process zones (FPZs) of the Thur River floodplain to differences in physicochemical soil properties on one hand and to effects of flood disturbance as driving force of a geomorphically dynamic system on the other hand, and (ii) to evaluate the relative magnitude of temporal variability as well as among and within-FPZ spatial heterogeneity of C dynamics. Based on this, conclusions about the effects of river restoration on C dynamics can be drawn.

Soil properties, environmental conditions and degree of disturbance in different FPZs

With their high carbonate content the soils at our test site are representative of young, weakly developed alluvial soils (Guenat *et al.* , 1999). The mostly homogeneous distribution of soil properties with depth and the strong correlation

between TOC and TN contents and soil texture indicates soil formation by fluvi (Cabezas & Comín, 2010). Further homogenization can be attributed to bioturbation by earthworms. This was especially clear in the forested FPZs where earthworm biomass was highest (Fournier *et al*., unpublished data), most likely because of the lower frequency of inundation and fluvial dynamics (Guenat *et al*., 1999). On the other hand, the depth gradient of TOC and TN in Willow forest, representing an advanced stable FPZ, indicates *in-situ* pedogenesis. Soil texture, the main factor differentiating the FPZs according to the PCA (Fig. 3), reflects the average sedimentation conditions with texture becoming finer with decreasing stream energy (Nanson & Croke, 1992). The TOC contents are within the range found in floodplain sediments of other large rivers in Europe (Graf *et al*., 2007; Pies *et al*., 2007). The low C/N ratios and high available P contents (Morel *et al*. , 1992) indicate high nutrient availability in all FPZs, which is characteristic of many river floodplains (Tockner and Stanford, 2002). The particularly high P availability in Grass can be explained by high sedimentation rates (Steiger and Gurnell, 2003). Low C/N values also indicate favourable conditions for organic matter degradation, which is confirmed by the observation of fast mineralisation of leaf litter in most FPZs and by the humus morphology (carbonate-rich Mull; data not shown).

The high variability and extreme values of *T* measured in Gravel are consistent with the general finding of bare Gravel bars as extreme environments. The absence of stable vegetation cover to buffer temperature variations accompanied by high thermal conductivity due to low water contents explain this harshness (Tonolla *et al*., 2010). The differences in WC among FPZs can be explained mainly by a combined effect of precipitation, inundation frequency and water retention capacity of the soils. In particular, the maximum WC in Willow forest can be attributed largely to the finely textured soil that retains water efficiently. The additional effects of shading and water uptake by the plants on WC via evapotranspiration are not obvious from the data.

Considering the days after the last inundation (LI), the samplings carried out over the course of this study represent conditions that are typical for this site (Fig. 2), i.e. inundation of the low-lying dynamic FPZs on a regular basis, and flooding of the entire floodplain once or twice a year. The high spatial heterogeneity of physicochemical soil properties, environmental conditions, and plant species

richness in Gravel and Grass (Tab. 3) reflects the patchy and dynamic geomorphology due to the frequent disturbance by flooding, while the low variability in the forested FPZs can be related to stable conditions leading to homogenization of properties, conditions and communities. The low variability in Pasture can in addition be explained by its particularly homogeneous geomorphology strongly reducing erosion and sediment deposition. In agreement with the hypothesis of maximum biodiversity at intermediate levels of disturbance or connectivity (J. V Ward *et al*., 1999), the pattern of plant species richness in the restored section FPZs reflects the degree of disturbance. This hypothesis predicts highest species richness in habitats characterised by intermediate inundation frequency (i.e. Willow bush), and lower diversity under high or low degrees of disturbance (i.e., Grass and the two forested FPZs, respectively) where ruderal or competitive species dominate, respectively (in particular *Phalaris arundinacea* as flood tolerant species in Grass (Foster & Wetzel, 2005)). Considering the low inundation frequency, the relatively high species richness observed in Pasture can be explained mainly by the regular harvesting, which reduces the effect of competition.

Carbon pools and fluxes as related to soil properties, environmental conditions and disturbance in different FPZs

The strong correlation between MC and TOC suggests C-limitation of microbes, which is especially common in nutrient rich soils (Wardle, 1992). The pattern of WEOC suggests an influence of both TOC and WC. The correlation with TOC indicates similar solubility of soil organic matter across FPZs. The influence of WC is mainly a flooding effect as demonstrated by the highest WEOC contents in August after the major flood. On one hand, this pattern suggests temporally increased soluble C pools due to input of non-structured fine soil and fresh litter along with the decreased aeration in the waterlogged soils. During soil saturation, dissolved organic matter production is expected to increase (Kalbitz *et al*., 2000). On the other hand, flooding has also been shown to increase the rates of enzymatic soil organic matter degradation (Wilson *et al*., 2010). The flood-related increase in available C is also reflected by increased MC, except in Willow forest where the almost completely saturated soil suggests longer unfavourable conditions for microbial growth (Rinklebe & Langer, 2006; Unger *et al*., 2009).

The measured range of SR, which includes root and microbial respiration, was similar to results from other floodplains (Pulliam, 1993; Gulledge & Schimel, 2000; Pacific *et al*., 2008; Doering *et al*., 2011). The strong correlation of SR with *T* has been commonly observed (Buchmann, 2000; Lloyd & Taylor, 1994) and explains the differences between the samplings to a large extent. The temperature dependence in terms of Q_{10} values in most FPZs is similar to riparian and uphill forests (Buchmann, 2000; Doering *et al*., 2011), while the Q_{10} value of Gravel is much higher than in similar systems (Doering *et al*., 2011). According to Pacific *et al*. (2008) soil CO_2 efflux is determined both by CO_2 production and diffusive transport in the entire soil, and soil moisture levels observed in our study would support high respiration in all FPZs most of the time. Considering this, the often highest CO_2 efflux in Grass and Willow bush can be explained on one hand by the coarse soil texture allowing optimal gas diffusion, on the other hand by the frequent and large input of available organic C during flooding. In addition, the sediment translocations during high floods may increase the content of available organic C also at greater depths. Together with the high spatial variability in sedimentation, this can explain the hot spots of SR observed in these two FPZs. The low SR in Gravel is likely due to the low fine soil content.

Consumption of atmospheric methane is largely determined by CH_4 diffusion in the soil (Dörr *et al*., 1993), and CH_4 produced in water saturated soil layers can be consumed in upper aerated soil layers (Boon & Lee, 1997). Net CH_4 production can therefore be considered as an indicator of the balance between overall soil aeration and underlying CH_4 production. The observed decrease of net CH_4 production along the elevation gradient from Gravel to Mixed forest is in line with the aeration increasing with the average thickness of unsaturated soil, and confirms earlier studies showing a strong influence of landscape position on CH_4 consumption (Burke *et al*.,1999; Gulledge & Schimel, 2000). This interpretation is supported by an increase in earthworm diversity (Fournier *et al*., unpublished data). In Willow forest, net CH_4 production was higher than expected at that elevation, which suggests a relatively weak aeration, confirmed by hydromorphic features in upper soil layers (data not shown) and/or high CH_4 production.soil texture found in this FPZ m(Dörr *et al*., 1993). The high net CH_4 production in the relatively high laying Pasture, characterized by a sandy soil texture, suggests generally high CH_4

production in the water-saturated layers of this FPZ. This can be explained by the relatively long travel time of infiltrating water in the channelized section of the river (Vogt *et al.*, 2010), favouring reducing conditions in deeper soil layers.

In summary, microbial and available C pools are determined mainly by physicochemical soil properties with some additional effects of flooding via WC. By contrast, C fluxes are strongly influenced by flood disturbance, and either *T* (SR) or geomorphology (net CH_4 production).

Temporal variability and within-FPZ heterogeneity of soil C pools and fluxes

Carbon pools and fluxes as multivariate proxy of soil C dynamics differed more among sampling dates than among FPZs. This indicates that overall factors related to seasonality and flooding (*T*, WC, and organic matter input) influence soil C dynamics more than differences in soil physicochemical properties in the test site.

The high spatial heterogeneity of all C pools and fluxes within Gravel and Grass can be related to the variability in both soil properties and environmental conditions caused by frequent flooding disturbance. The high variability of MC in all FPZs of the restored section cannot be explained exclusively by the large-scale variability between replicate plots but might in addition be due to small-scale variability at the soil aggregate level as well as to additional heterogeneity brought by the rooting pattern and related exudation of plants. Similarly, it can be speculated that hot spots of CO_2 and CH_4 emissions in otherwise homogeneous FPZs (Willow bush, Pasture) are due to small-scale heterogeneities in substrate availability and water saturation in the subsoil (Ramakrishnan *et al.*, 2000; Sey *et al.*, 2008).

Conclusions

This study of organic C dynamics in the Thur River floodplain revealed that in the dynamic FPZs of the restored section characterised by low TOC contents and coarse-textured soils, frequent disturbance by flood pulses temporarily and locally increased SR and the otherwise low base-levels of organic C pools. By contrast, in the stable forested FPZs, the finer texture of the soils was responsible for higher TOC contents and water retention capacity both leading to high base-levels of C pools. Spatial heterogeneity was smaller than the effects of major floods and

seasonal T differences on C pools and fluxes. The Pasture FPZ stood out by i) low C pools due to coarse-textured soils low in TOC, as in the dynamic FPZs, ii) spatial heterogeneity, seasonal effects and flood disturbance, similar to the forest FPZs, and iii) high CH_4 production that can be explained by slow travel times favouring reducing conditions.

Irrespective of the FPZ, the input of non-structured allochthonous soil material and possibly the destruction of local aggregates during flood pulses appear to be the driver for a temporary and, in dynamic FPZs, local increase of microbial activity. The related variability in available carbon or soil respiration cannot be explained by the spatial and temporal heterogeneity of bulk soil properties or the variability of environmental conditions. Our results thus confirm our first hypothesis that spatial and temporal C variability are affected mainly by flood disturbance. However, they also show that the temporal effects are not restricted to dynamic FPZs. The strong increase in plant biodiversity brought about by the recurrent rejuvenation of the habitats seems to support our second hypothesis, that frequent disturbance - defined as temporary and strong changes in environmental conditions and substrate availability- creates a large functional diversity. Our results therefore support recent findings that short-term inundations are important drivers of microbial habitat structure and function in floodplains (Wilson *et al*., 2010). Further comprehensive studies in similar as well as contrasted sites are required for generalisation of the results. In particular, since soil organic matter turnover differs between acidic and carbonate-containing soils (Walse *et al*., 1998), studies in sites with carbonate-free fluvial source material would be of great interest.

Based on our results, we recommend that river restoration, in order to achieve maximum recovery of ecosystem functions, should aim at creating near-natural floodplains comprising both dynamic Gravel bars and stable alluvial systems. On one hand, this ensures the provision of a large diversity of habitats. On the other hand, the complex interplay of organic matter input and hot spots of both mineralisation and incomplete degradation strongly affects the potential of floodplains to store carbon, an ecosystem service of great current interest (Cierjacks *et al*., 2010). River widening combined with hydrological reconnection with former floodplains (from the time before channelization) as in the example presented here, is likely to be a successful recipe to achieve this goal, at least for a river

characterised by pulse flooding. The Thur River example also shows that doing so on a rather small scale is sufficient to achieve a high heterogeneity of carbon pools and habitats. In cases where, in contrast to the Thur, the river is dammed upstream, this may have to be combined with controlled outflow mimicking the natural discharge regime including a few larger floods.

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Chapter 2. Seasonal variability of soil bacterial diversity, community structure and associated ecosystem functions dwarf spatial patterns in a restored floodplain

Emanuela Samaritani[1,2,3,*], Edward A.D. Mitchell[1], Jeremy Rich[3], Juna Shrestha[4], Beat Frey[4]

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

[2] WSL, Swiss Federal Institute for Forest, Snow, and Landscape Research, 1015 Lausanne, Switzerland

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

[4] WSL, Swiss Federal Institute for Forest, Snow, and Landscape Research, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

Personal contribution: sampling, data collection, numerical analyses, statistics, paper redaction

Placement: data collection presented in this work took place at the Swiss Federal Institute for Forest, Snow, and Landscape Research, both at the Lausanne (CH-1015 Lausanne, Switzerland) and Birmensdorf sections (Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland). Data analyses and paper redaction were carried out at the university of Neuchâtel (2009 Neuchâtel, Switzerland) and at Brown University (02906 Providence, RI, USA).

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Abstract

The patterns of soil bacterial diversity and associated ecosystem functions are known to vary in space and time but the spatial and temporal variability are rarely studied simultaneously. Dynamic natural ecosystems such as floodplains offer an ideal setting to study these spatio-temporal patterns and to determine the relative importance of habitat diversity, seasons and external perturbation in shaping soil bacterial communities and associated processes.

We studied the spatial and seasonal patterns of soil bacterial communities (T-RFLPs), soil environmental conditions (soil temperature, soil moisture and time elapsed since last inundation), and ecosystem functioning proxies (basal respiration, enzymatic activity, microbial carbon and nitrogen) over a full year in seven habitats of a revitalised floodplain.

Bacterial community structure and ecosystem functioning proxies were significantly correlated to soil environmental conditions and physico-chemical properties. Physico-chemical conditions were predictably more extreme and variable and bacterial community structure was most divergent in the two habitats closest to the river. However, although bacterial communities differed among habitats at all sampling times, their temporal variability and associated ecosystem functions were substantially higher than the spatial variability. Indeed, in a redundancy ordination analysis, the spatial pattern was nested within the temporal effect. However, while both bacterial community structure and ecosystem functions varied in relation to environmental conditions, the two were not directly correlated.

Although microbial communities, environmental conditions and ecosystem functions were correlated, the link between communities and functions was seasonally inconsistent. Thus in dynamic ecosystems such as floodplains, external factors (flood disturbance, seasonally changing climatic conditions) influence ecosystem functions and microbial communities independently, 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 is a major process generating spatial and temporal variability in biotic communities and associated processes (Ward *et al*., 2002). The relative importance of spatial versus temporal variability differs among ecosystems depending on regular seasonal changes or different types of perturbations (e.g. extreme climatic events, floods, landslides, fire). Our focus here is on soil microbial communities and processes in floodplains.

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 and represent an ideal setting to investigate the effects of spatio-temporal variations on soil bacterial communities. 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 (Brunke *et al*., 2003; Stanford & Ward, 1993; Tockner *et al*., 1997). 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 community composition, system metabolism, productivity, organic matter dynamics, and nutrient cycling (Thorp *et al*., 2006). Thus floodplain soils offer an ideal setting to study the spatio-temporal patterns in structure and function of soil organisms and assess if these patterns are predictable based on abiotic characteristics and vegetation patterns (i.e. revealing niche specialisation) or if they are instead random (i.e. in agreement with neutral theory).

Soil bacterial communities vary over space, time and in response to environmental changes. Spatially they vary at fine scale, 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 (Aciego Pietri & Brookes, 2009; Carney & Matson, 2006; Pankhurst *et al*., 2001; Stromberger *et al*.,

2007; Yu *et al*., 2011). Soil bacterial communities vary temporally, over short (e.g. rain events) to longer (e.g. seasonal, ecosystem succession) time scales (Grundmann, 2004; Prosser, 2012). They also respond to perturbations, and this often affects microbial processes (Allison & 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 spatial heterogeneity and contrasted microhabitats should also act as ecological filters controlling bacterial community structure (species sorting) and thus the relative importance of temporal and spatial differences should vary across gradients of perturbation (Lindström & Langenheder, 2012).

Linkages between physico-chemical characteristics, microbial community composition and ecosystem functions (e.g., nitrogen fixation, lignin decomposition) have been largely demonstrated (Coleman *et al*., 2004; Judd *et al*., 2006; Naeem, 1998; Schimel, 1995; Schimel & Schaeffer, 2012; van der Heijden *et al*., 1998). The close ties among soil condition, ecosystem function and microbial diversity should theoretically be observable independently of their spatial and temporal patterns across contrasted habitats of dynamic ecosystems such as floodplains. These relationships should also be detectable in human-impacted and restored ecosystems. Indeed, restoration ecology has been qualified as an ideal field to test ecological theories (Young *et al*., 2005).

Analyses of DNA extracted from the environment have revealed the huge extent of bacterial diversity in soil (Curtis *et al*., 2006; Hugenholtz *et al*., 1998; Torsvik *et al*., 1990) and made it possible to process high numbers of samples to assess community patterns, taxonomic and functional diversity of soil bacteria, and the influence of ecosystem perturbations such as floods in floodplain ecosystems (e.g. Rinklebe and Langer 2006; Song *et al*. 2008; Unger *et al*. 2009). Using terminal restriction fragment length polymorphism (T-RFLP) profiling, we analysed the spatial and temporal variability of soil bacterial communities in relation to flood patterns, soil physico-chemical conditions and soil ecosystem functioning proxies in a restored floodplain from Switzerland. 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; Schneider *et al*., 2011; Shrestha *et al*., 2011; Samaritani *et al*., 2011; Fournier *et al*., 2012).

We expected to find both spatial (gravel bars to mature forest) and temporal (seasonal) patterns, both in microbial community structure and ecosystem functioning proxies. We hypothesized that 1) bacterial community structure, soil characteristics and ecosystem functions would be correlated across space and time. Given the very clear contrasts among FPZs we also hypothesized that 2) differences among FPZs would be greater than seasonal differences. Finally, we hypothesized that 3) soil characteristics and the bacterial community structure variability would determine the diverse response of the ecosystem functioning proxies.

Material & Methods

River Thur covers a catchment area of 1700 km^2, from Mount Säntis, through the Swiss Plateau, and into the River Rhine. Lacking natural or artificial reservoirs, the river flow regime 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 2m^3s^{-1}, respectively (1904-2005: http://www.hydrodaten.admin.ch/d/2044.htm). 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. We considered as dynamic the three FPZ closer to the river: "Gravel", a mosaic of bare Gravel and patchy vegetation, "Grass", characterized by a community of pioneer plants dominated by *Phalaris arundinacea*, and "Willow bush", dominated by shrubby vegetation, mainly planted *Salix viminalis*. Those FPZ did not exist before the restoration. We considered as stable 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, elevation and distance from the river. The stable FPZ were already present before the restoration. The last FPZ, "Pasture", was considered as the pre-restoration reference condition, and is located upstream from the study section, in the channelized section.

In each FPZ, four to ten plots were selected; six in the dynamic FPZs (Gravel, Grass, and Willow bush), four in the Ash Forest, Willow forest, and Pasture and ten in the Mixed forest 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 (July and October 2008; January and April 2009) were carried out for this project. Samples collected in the last three samplings, together with an additional in August 2009, were used for a study of carbon cycle and ecosystem functioning in relation to soil physico-chemical conditions (Samaritani *et al*., 2012). 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 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. Soil subsamples were put in buffer for soil extraction the same day. Three sets of samples (October 2008, January and April 2009), together with another set of samples collected in August 2009, were analysed for carbon pools and fluxes and their spatio-temporal variability under the influence of floods (Samaritani *et al*. 2011).

Soil properties, and environmental conditions

Soil properties (pH, sand and clay percentage, inorganic carbon, organic carbon and total nitrogen content, C:N ratio and available P) and vascular plant species richness for each FPZ were presented and discussed elsewhere (Samaritani *et al*., 2012). These data are compared to patterns of bacterial communities.

Soil pH, texture and nutrients (inorganic C, organic C, total N, C:N, available P) were determined from samples taken in April 2008, using standard protocols (see Samaritani *et al*. 2011 for details). 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 measured in the centre of each plot from April 2008 to April 2009 at 30 minutes resolution with TidBit v2 temperature loggers (Bourne, MA, USA). Soil Moisture (SM) was estimated 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 estimate of days after 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 estimated using an Infrared Gas Analyser (Licor 8100). Soil samples at field moisture level, previously stored at 4°C, were left at room temperature for at least 3 hours before measuring by placing 40 g of the soil in Licor 8100-102 survey chambers and CO_2 emissions were monitored for 9 minutes. All measured CO_2 fluxes were highly stable (R^2>98%). Fluxes are reported as μmol CO_2 $s^{-1}g^{-1}$ soil dry weight.

Microbial biomass Carbon (MC) and Nitrogen (MN) were determined by chloroform fumigation-extraction (Beck *et al*. 1997; Vance *et al*. 1987; Brookes *et al*. 1985) as previously described in Samaritani *et al*. (2011). MC and MN data were expressed in μg 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 (Medzon and Brady, 1969; Schnurer and Rosswall, 1982; Söderström, 1977). Two replicates per sample, 0.5g of fresh soil each, were treated with 200μl of FDA solution (10^{-3} M in acetone) and 10ml of sterilized phosphate buffer. One blank per sample was prepared adding 200μl of pure acetone in sterilized phosphate buffer. After 45min of gentle mixing, the reaction was stopped abruptly by adding 10ml of pure acetone. One reference sample was analysed after adding 200μl of FDA solution to 10ml pure acetone. Samples were centrifuged at 4000rpm during 5min and the fluorescence measured at 490nm by spectrophotometer. 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 490nm and A_{st}=Absorbance of reference sample. Results were converted in μg of degraded FDA $h^{-1}g^{-1}$ soil dry weight.

Soil bacterial community structure

Total DNA was extracted as follows: a 0.5g subsample of fresh soil and 0.75g glass beads (0.1 mm diameter) were suspended in 1ml extraction buffer (0.2M Na_3PO_4 [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 5.5m s^{-1} for 40s followed by centrifugation for 5min at 13,000rpm (Frey *et al*., 2006). DNA was purified by chloroform extraction with 2ml chloroform, vortexed and then centrifuged 5min at 13,000rpm. 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 15min at 15,000rpm. The pellets were washed with 1.5ml of 70% EtOH and centrifuged for 2min at 10,000rpm. The supernatant was removed and the samples were air dried for 20min, and re-suspended in AE buffer (10mM TrisCl, 0.5mM EDTA, pH9) at 1ml AE per g of extracted soil (dry weight equivalent). Extracted DNA were examined by electrophoresis on agarose gels (1% w/v in TBE) 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. 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'; Heuer *et al*., 1997). In a total volume of 25μl, we added 5μl of pre-treated and 1:10 diluted DNA, 10x PCR buffer, 0.5mM $MgCl_2$, 0.2μM of each primer, 400μM of each dNTP (Promega), 0.3mg ml^{-1} of BSA, and 0.05U μl^{-1} of Hotstar *Taq*-polymerase (Qiagen). PCR amplification was performed with a PTC-100 thermocycler (MJ Research, Waltham, MA, USA). PCR conditions applied an initial activating step for HotStarTaq® polymerase (15min at 95°C), followed by 35 cycles with denaturation at 94°C for 45s, annealing at 48°C for 45s, and extension at 72°C for 2min. The PCR amplification was followed by an additional extension step at 72°C for 5min. 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 20min. 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). 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. 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-RF, from both the *Msp*I and *Hae*III restriction enzymes.

Numerical analyses

Differences in the soil physico-chemical properties among sites were tested using one–way analyses of variance (ANOVA, SPSS 17, SPSS Inc.; Field, 2005). Effect of sampling dates (hereafter referred to as Sampling) and FPZ on ecosystem functioning

proxies were analysed by multivariate analyses of variance on the distance matrices (software R, function adonis, *vegan* library).

α diversity was estimated both in terms of bacterial richness, defined by the number of T-RF detected in each plot for the two enzymes, and by Shannon diversity index. ß diversity was estimated both in terms of the ratio of γ/α-1 (Whittaker, 1972) and also as the mean of pairwise Bray-Curtis dissimilarities (Hill *et al*., 2004; Langenheder *et al*., 2012; Pereira *et al*., 2011). Sampling and FPZ effects were tested with TukeyHDS tests. F-test analyses of variance on Bray-Curtis distance matrices were performed with the package *adonis*. The bacterial communities were plotted on a non-metric multidimensional ordination (NMDS) based on Bray-Curtis distance matrices. Ecosystem functioning proxies, soil and climatic conditions were independently fitted were also projected. The software R (R Team Development Core, 2010) was used for all statistic analyses.

Results

Soil properties, vascular plant species richness and ecosystem functioning

Significant differences among FPZs were recorded for all soil variables except pH (details given in Samaritani *et al*., 2011). Ecosystem functioning (Basal Respiration BR, Enzymatic Activity EA, Microbial Carbon MC and Microbial Nitrogen MN) varied significantly among FPZs (ANOVA F test, R^2=15.8%, p=0.001) and sampling date (R^2=20.4%, p=0.001) and cross-effect (R^2=16.9%, p=0.002)(Fig. 1).

Basal Respiration (BR) was significantly higher in fall than in winter, spring and summer ($p<0.001$). Enzymatic activity (EA) was higher in spring than winter and summer ($p<0.05$). Microbial carbon (MC) was lowest in fall, intermediate in winter and spring and highest in summer ($p<0.05$). Microbial nitrogen (MN) was higher in fall compared to winter and spring ($p<0.05$).

With respect to spatial variability, basal respiration tended to be higher in the three forested FPZs (Ash, Mixed and Willow forests), but no significant difference was found. EA was significantly higher in Grass FPZ than Pasture ($p<0.05$). The lowest values were found in Gravel and Pasture. MC was lowest in Gravel, and intermediate in the Pasture. Grass, Willow bush and the forests had comparably high

MC, but only Grass and Willow forest were 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.

Basal Respiration was correlated negatively to Microbial Carbon and to the time elapsed since last inundation (LI) and positively to Microbial Nitrogen (MN) (Tab. 1). Enzymatic Activity was positively correlated to, soil moisture (SM), MN and LI. MC was positively correlated to MN and SM. Soil moisture was negatively correlated to soil temperature (T).

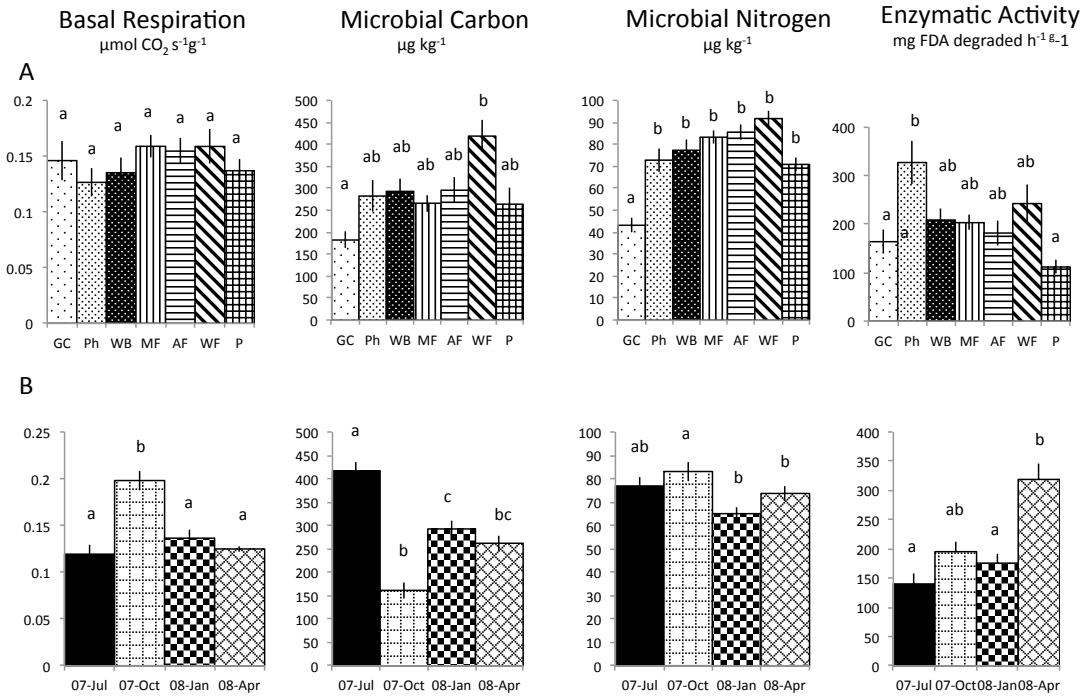

Figure 1. Mean values (n=4 to 6) and standard error of the ecosystem functioning proxies (Basal Respiration, Enzymatic Activity, Microbial Carbon and Microbial Nitrogen) for each functional process zone (A) and sampling date (July and October 2008 and January and April 2009)(B). GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, AF=Ash forest, WF=Willow forest and P=Pasture). Means for each FPZ individually at each Sampling are shown in Annexe1 of this chapter.

Table 1. Correlation matrix between ecosystem functioning proxies (basal respiration BR, enzymatic activity EA, microbial carbon MC and microbial nitrogen MN) and environmental conditions (soil moisture SM, soil temperature T and days after last inundation LI). For units see Fig. 1. Stars indicate statistical significance: *=p-value<0.05, **=p-value<0.01, ***=p-value<0.001.

	EA	MC	MN	SM	temp	LI
BR	NS	-0.18*	0.21**	NS	NS	-0.19*
EA	-	NS	0.35***	0.37***	NS	0.32***
MC	-	-	0.38***	0.43***	NS	NS
MN	-	-	-	0.32***	0.17*	NS
SM	-	-	-	-	-0.36***	NS
temp	-	-	-	-	-	NS
LI	-	-	-	-	-	-

Bacterial community, richness and diversity

Phylotypes (OTU) accumulation curve on the bacterial terminal restriction fragment profiles shows that the number of phylotypes detected saturates after 20 sites (Fig. 2). This indicates that the sampling effort was sufficient to detect the overall richness.

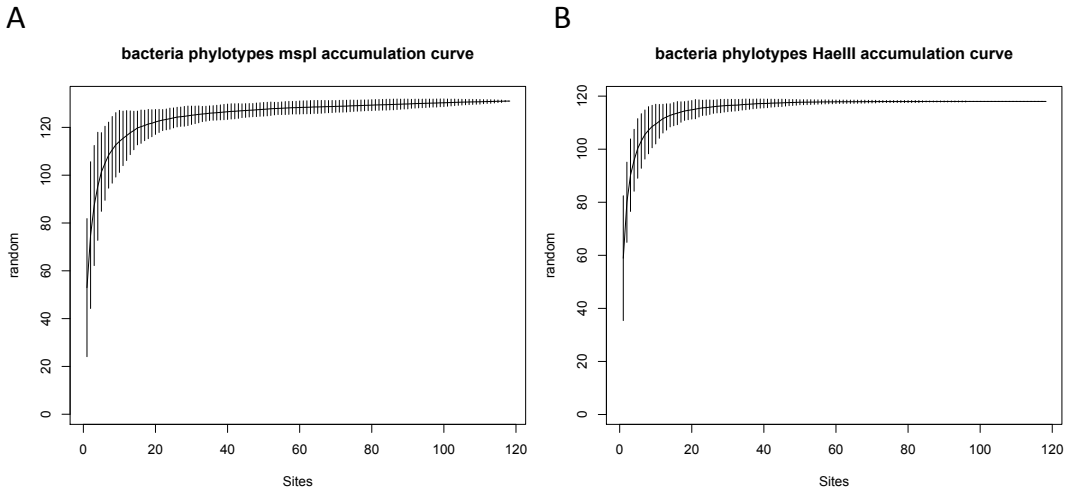

Figure 2. Bacterial phylotype richness accumulation curve, determined by number of terminal restriction fragments detected with two restriction enzymes *Msp*I (A) and *Hae*III (B).

Bacterial richness estimated on T-RFLP profiles were not comparable among the two restriction enzymes, with the exception of the total richness (ε), which was maximal in April in both cases, with 110 *Msp*I OTU and 94 *Hae*III OTU (Tab. 2). *Msp*I α diversity was very low in October, ranging from 25 to 36 OTU, compared to

46 to 60 in July, 54 to 56 in January and 68 to 74 in April (Tab. 2). *Msp*I ε diversity was intermediate in summer and winter (79 and 76 OTU, respectively), maximal in spring (110 OTU) and minimal in autumn (51 OTU). *Msp*I β diversity was highest in October (ranging from 0.32 to 0.60), intermediate in April (ranging from 0.25 to 0.40), and lower in July and January (ranging from 0.17 to 0.37 and 0.14 to 0.35, respectively).

*Hae*III α diversity ranged from 46 to 61 OTU in July, compared to 61 to 68 in October, 56 to 64 in January and 49 to 61 in April. *Hae*III ε diversity was lowest in July, with 77 OTU, intermediate in October and January, with 85 and 88 OTU respectively, and highest in April (94 OTU). *Hae*III β diversity was highest in April (ranging from 0.29 to 0.55), lowest in October, and July (ranging from 0.10 to 0.27 and 0.08 to 0.33, respectively), and intermediate in January (ranging from 0.20 to 0.38) (Fig. 3).

Table 2. α, β, γ, and ε bacterial biodiversity *sensu* Whittaker (1972) measured on terminal restriction fragments richness estimated independently on two restriction enzymes, *Msp*I and *Hae*III, for the seven FPZ (Gravel, Grass, Willow bush, Mixed forest, Ash forest, Willow forest and Pasture) in the four sampling seasons (July 2008, October 2008, January 2009 and April 2009).

	Jul-08			Oct-08			Jan-09			Apr-09		
*Msp*I	**α**	**β**	**γ**	**α**	**β**	**γ**	**α**	**β**	**γ**	**α**	**β**	**γ**
Gravel	59	0.19	70	31	0.35	42	56	0.32	74	73	0.25	91
Grass	57	0.33	76	32	0.53	49	56	0.20	67	73	0.27	93
Willow Bush	51	0.37	70	38	0.29	49	56	0.14	64	70	0.27	89
Mixed Forest	53	0.30	69	36	0.33	48	55	0.35	74	72	0.40	101
Ash Forest	60	0.17	70	25	0.60	40	55	0.29	71	73	0.30	95
Willow Forest	NA	NA	NA	30	0.30	39	54	0.33	72	74	0.00	74
Pasture	46	0.00	46	NA	NA	NA	54	0.20	65	68	0.28	87
Total (ε)	79			51			76			110		

	Jul-08			Oct-08			Jan-09			Apr-09		
*Hae*III	**α**	**β**	**γ**	**α**	**β**	**γ**	**α**	**β**	**γ**	**α**	**β**	**γ**
Gravel	61	0.25	76	62	0.27	79	58	0.26	73	49	0.55	76
Grass	46	0.33	61	61	0.20	73	56	0.23	69	62	0.37	85
Willow Bush	60	0.12	67	65	0.14	74	59	0.34	79	51	0.53	78
Mixed Forest	60	0.12	67	64	0.23	79	61	0.38	84	58	0.53	89
Ash Forest	59	0.08	64	68	0.10	75	64	0.20	77	57	0.30	74
Willow Forest	NA	NA	NA	63	0.22	77	60	0.27	76	59	0.00	59
Pasture	61	0.00	61	NA	NA	NA	63	0.30	82	62	0.29	80
Total (ε)	77			85			88			94		

α = mean richness in the FPZ
γ = total richness/FPZ
β = γ/α-1
ε = total richness/Sampling

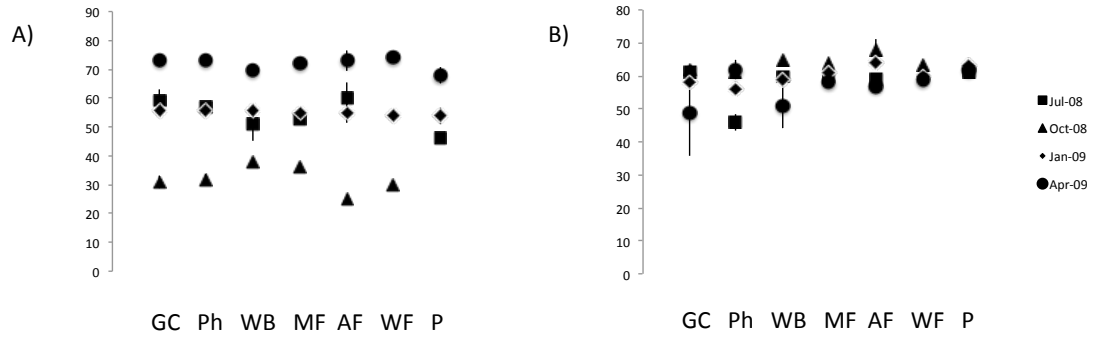

Figure 3. α diversity (±se) determined as terminal restriction fragments OTU richness estimated independently on two restriction enzymes, *Msp*I (A) and *Hae*III (B), averaged for each of seven FPZ (n=2-10; GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, AF=Ash forest, WF=Willow forest and P=Pasture) in the four sampling seasons (July 2008, October 2008, January 2009 and April 2009).

α diversity estimated using the Shannon diversity index (Fig. 4) was significantly lower in Gravel than in the other FPZs except for Grass, which was not different from any other FPZ. Shannon diversity was significantly higher in January and April than in July and October ($p<0.05$).

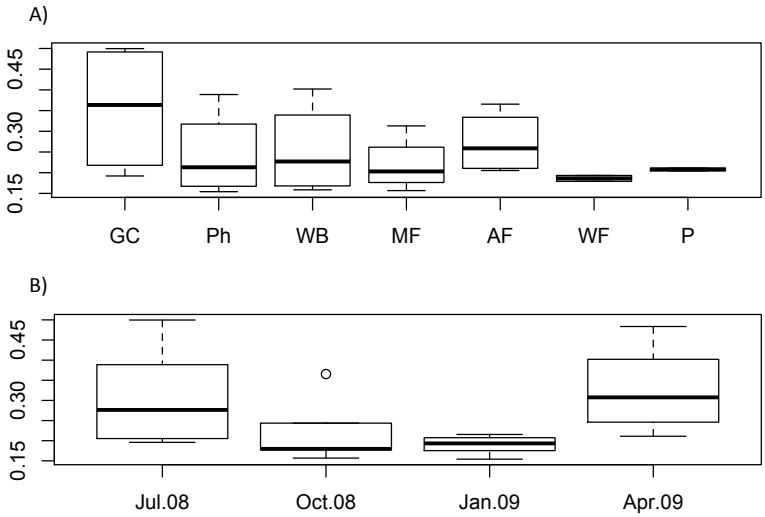

Figure 4. Spatial (A) and temporal (B) variability in Shannon α diversity index calculated on t-RFLP data using two restriction enzymes, *Msp*I and *Hae*III. Sampling per functional process zone: GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, AF=Ash forest, WF=Willow forest, P=Pasture; per sampling date: July 2008, October 2008, January 2009 and April 2009.

β diversity estimated on distance matrices differed significantly among FPZs and sampling periods (R^2=0.12, $p<0.001$ and R^2= 0.57, $p<0.001$ respectively, cross-effect R^2= 0.08, $p<0.001$). In July and April, β diversity reached its highest level in

Gravel, Grass (Fig. 5B), leading to a spatial pattern of decreasing diversity along the increasing distance from the river (Fig. 5A). In October and January, β diversity was more homogeneous among FPZs, except for a peak in Ash forest in October.

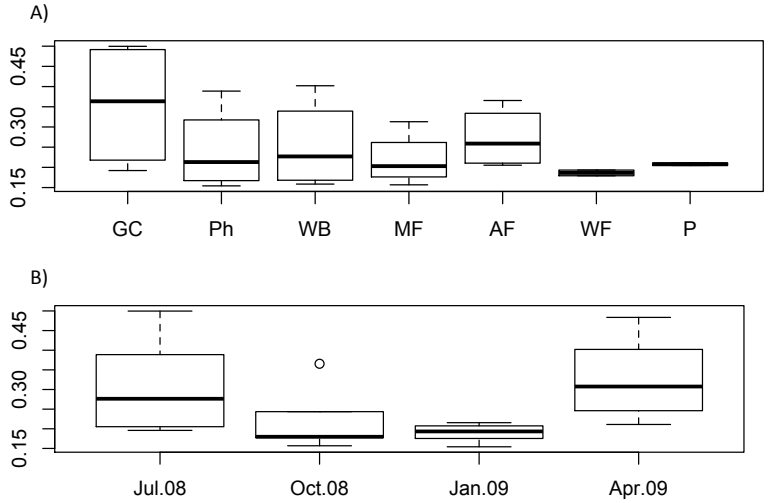

Figure 5. β diversity (± se) estimated on Bray-Curtis distance matrices averaged per FPZ (A) and Sampling (B). GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, AF=Ash forest, WF=Willow forest, P=Pasture.

The non-metric multidimensional scaling (NMDS, Fig. 6) grouped T-RFLP community data primarily according to the season. Climatic conditions, ecosystem functioning proxies and soil conditions 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. The three soil conditions fitted in the ordination correlated similarly, and mostly with January (OrgC) and July (Sand and avP) communities while, 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.

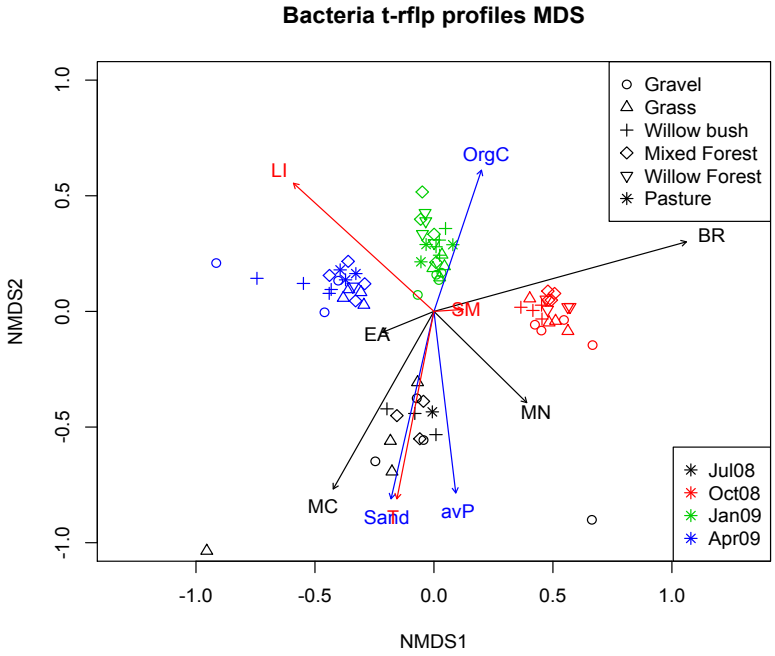

Figure 6. Nonmetric multi-dimensional ordination of microbial terminal restriction fragment profiles measured on two restriction enzymes, *Msp*I and *Hae*III. The multidimensional scaling is based on Bray-Curtis distance matrices, with arrows indicating independently fitted ecosystem functioning proxies (BR=basal respiration, EA=enzymatic activity, MC=microbial carbon & MN=microbial nitrogen), soil conditions (Sand, avP=available Phosphorus & OrgC=organic carbon) and climatic conditions (T=soil temperature, SM=soil moisture & LI=inundation). Different symbols represent different FPZ and different colours represent different Sampling.

Discussion

Terminal restriction fragment polymorphism (T-RFLP) rDNA community profiling fingerprinting enable the processing of many samples and comparative analyses of community structure. Although inadequate for taxonomic identification and accurate estimations of species richness (Bent *et al*., 2007; Dunbar *et al*., 2000, Navaro *et al*., 2007), this method was shown to give reliable results at relatively small diversity, although rare taxa might be unaccounted for (Moyer *et al*., 1996; Orcutt *et al*., 2009), and is useful to compare community structures (Orcutt *et al*., 2009; Zhang *et al*., 2008). The two restriction enzymes we used yielded different and uncorrelated OTU richness results, which is not uncommon (Zhang *et al*., 2008). Engebretson & Moyer (2003) tested 18 restriction enzymes and concluded that *Msp*I detected 70% of the

diversity in a population composed of 50 OTUs and 60% in a 100 OTU community, while *Hae*III detected about 5% fewer OTUs in both communities.

OTU richness estimated on *Hae*III restriction enzyme in this study showed no significant difference among seasons, while *Msp*I richness was significantly higher April and lower in October, which can be explained by a combination of flooding effects and seasonal shifts in the decomposer community (Rinklebe *et al*., 2009; Unger *et al*., 2009), although consequences of redox state changes following flooding are expected to be more drastic on bacterial community structure than bacterial richness (Song *et al*., 2008).

Temporal vs. spatial variation

Microbial communities have been shown to be organised in several nested levels, even within the centimetre- to meter-scale (Franklin and Mills, 2003). Therefore, and given the high spatial heterogeneity of floodplain ecosystems, we expected to find a strong turnover of bacterial communities among FPZs. In our study temporal variability was however much more important (Fig. 6). Spatial variability was also detected, especially July and April communities that were characterised by high but homogeneous richness (Fig. 3) and decreasing beta diversity (Fig. 5B) with increasing distance from the river. While October and January communities showed low richness, especially January, and rather homogeneous beta diversity, except for Willow forest. 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).

Are changes in ecosystem functioning and bacterial community structure predictable?

Soil microbial community composition (Zogg *et al*., 1997; Schimel *et al*., 1999; Unger *et al*., 2009) and biomass (Pesaro *et al*. 2003, 2004) are expected to be influenced by soil temperature, soil moisture, flooding events and carbon content (Briar *et al*., 2011). We found that microbial carbon and nitrogen were both positively correlated with soil moisture and with soil carbon content (Tab. 1). Although total

bacterial diversity might not change after a perturbation episode, the 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). Thus resilience after disturbance is mostly a species-specific characteristic rather than a function of the community structure itself (Allen-Morley & Coleman, 1989).

The spatial heterogeneity resulting from the presence of different plant communities and soil conditions in each FPZ explains the significant spatial 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 scale (Dennis *et al*., 2010), and the successional patterns of microbial community will follow the vegetation succession along the gradient of distance from the river (Duineveld *et al*., 1998), and the litter quality (Bardgett, *et al*., 1999; Judd *et al*., 2006). Over the seasons, these differences in plant community composition also drive bacterial community structure through nutrient availability (Schütter *et al*., 2001). It follows that the significant differences among FPZs in the community composition are relatively small compared to the continuous and dynamic shifts in the community structure caused by the seasonally changing conditions due to either internal or external factors (flooding, temperature, soil moisture), which indirectly also influence the shift in biological conditions (stage of plant development, litter composition) (Bardgett *et al*. 2005).

Although both microbial community structure and ecosystem functioning covaried with soil conditions (Mantel test, data not shown), we did not find a direct relation between the variability of the community structure and the soil conditions. In this study soil conditions were measured only once at the beginning of the study therefore their effect on the seasonal variation in the bacterial community cannot be interpreted directly. Soil properties and environmental conditions act in concert to alter the ecosystem functioning and microbial community in ways that are difficult to predict. The bacterial community composition (e.g. identity of species) is expected to influence ecosystem functions along a gradient of soil moisture (Jiang & Krumins, 2006; Peralta *et al*., 2010), and their activity may transform their microenvironment (Morales *et al*., 2010). But changes in bacterial community structure might have a

different effect on soil function depending on the initial soil conditions (Schimel & Schaeffer 2012). It follows that soil function rates may not necessarily vary in relation to microbial community structure (Griffiths *et al*., 2001).

Conclusions

T-RFLP profiles method for studying bacterial community structure proved useful to highlight temporal and spatial turnover. However, the two restriction enzymes used for T-RFLP analyses gave different results in terms of α, β, γ, and ε bacterial biodiversity among Samplings and FPZs. Therefore, T-RFLP remains a very useful and robust approach to estimate patterns of microbial communities, e.g. to identify sites of interest to be targeted by alternative methods that allow a more exhaustive and accurate identification of the phylotypes present but not for OTU richness.

Our results showed that ecosystem functioning proxies and bacterial community structure differed significantly among seasons and FPZs, in agreement with our expectations. The bacterial community covariability with soil conditions, ecosystem functioning and soil properties changed over the seasons.

In the general perspective of floodplain ecology and management, this study reveals the importance of seasonal flooding and resulting changes in micro-environmental conditions for maintaining high microbial diversity and heterogeneity of ecosystem functions.

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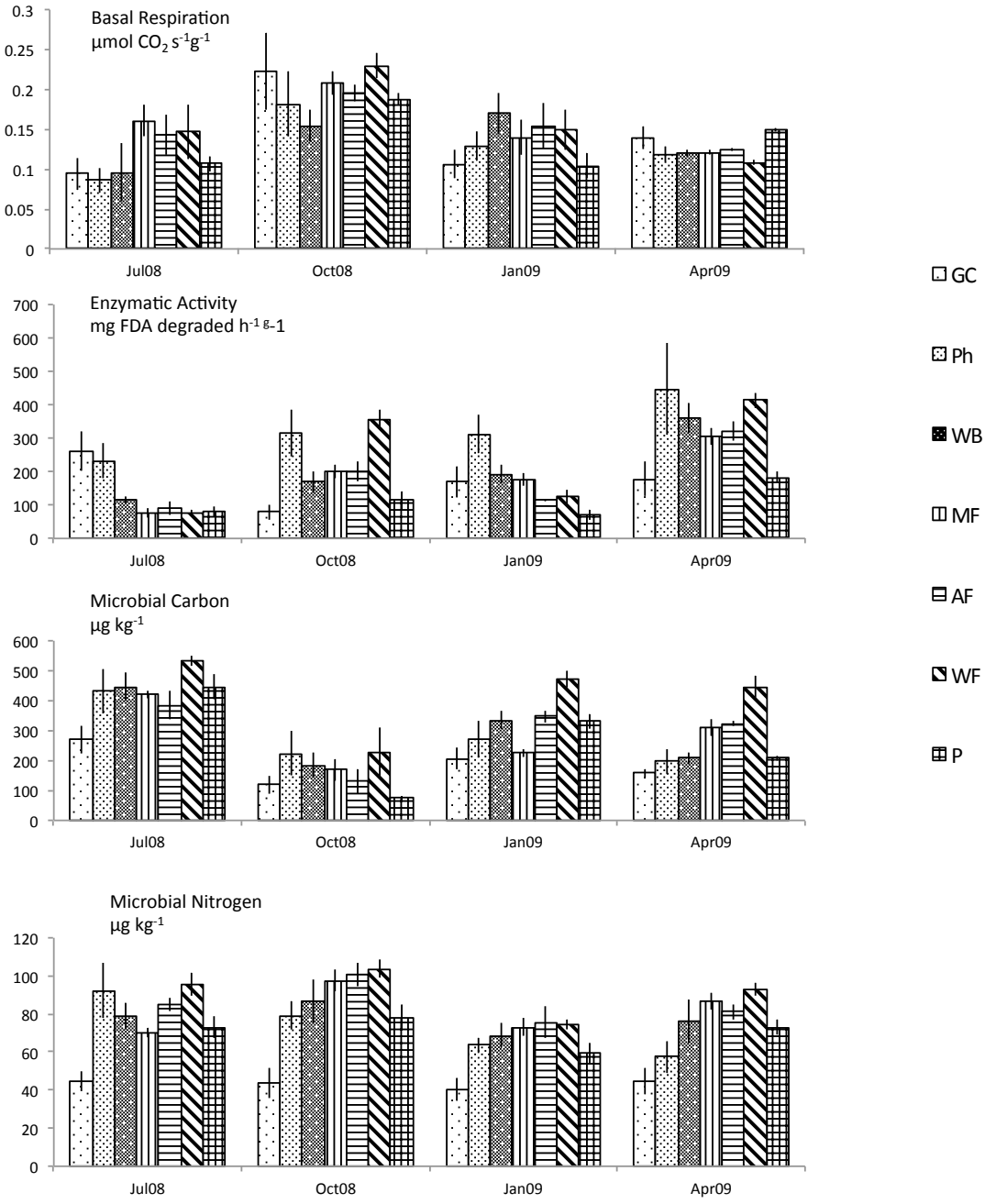
ANNEXE 1

Mean and standard error of ecosystem functioning proxies at each FPZ and Sampling individually.



Chapter 3. Spatio-temporal patterns of eukaryotic microbial diversity in a restored floodplain assessed by high-throughput sequencing

Emanuela Samaritani [1,2], Enrique Lara [1], Jeremy J. Rich [2], Beat Frey [3], Lassaâd Belbahri [1] and Edward A.D.Mitchell [1]

[1] Institute of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland

[2] Department of Ecology and Evolutionary Biology, Brown University, Providence, RI, USA

[3] Swiss Federal Institute for Forest, Snow, and Landscape Research, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

Personal contribution: sampling, data collection, numerical analyses, statistics, paper redaction

Placement: This work was performed at Brown University (02912 Providence, RI, USA).

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Abstract

Biodiversity in floodplain has been largely studied but mainly focusing on macroscopic organism or bacteria. Although specific groups of Eukaryotes have been investigated, the total diversity of micro Eukaryotes has rarely been addressed in dynamic ecosystems such as floodplain and it rarely considered both the spatial and temporal heterogeneity of communities along the gradient of perturbation. We tagged amplified the eukaryotic SSU hypervariable V9 region and metabarcoded 96 DNA environmental samples extracted from six different FPZ along a gradient of distance from the riverbed of a restored floodplain at four different seasons. Of the 202'240 unique reads that were taxonomically assigned to Eukaryotes, 59% were Opisthokonta, 12% Stramenopiles, 11% Archaeplastida and 9% Rhizaria. Micro Eukaryotes communities were significantly different among FPZs and among seasons, but spatial effect was greater. OTU richness, defined as number of unique reads, and abundance of eight supergroups and 14 subgroups show extremely different temporal and spatial patterns. Each habitat contributed to the overall diversity, highlighting the importance of spatial heterogeneity and seasonal variability in increasing diversity in natural floodplains.

Key words: floodplain, ecosystem functioning, SSU rRNA, soil micro Eukaryotes diversity

Introduction

Wetlands restoration and biodiversity conservation project are increasing worldwide. After centuries of floods control by channelization of rivers, societies adopted in the last decades another approach towards floodplains managements, aiming at balancing floods control with biodiversity conservation and ecosystem functioning (SER, 2004). The spatial complexity of juxtaposition of microhabitats characterized by different environmental conditions and the dynamic characterizing floodplains naturally, periodically rejuvenated by floods, make them difficult to understand, in an ecosystem functioning point of view. This understanding is crucial for a sustainable management of restoration of floodplain and it is therefore essential to conduct studies covering measurements of ecosystem functioning and its link to soil biodiversity, especially taking into account the spatial and temporal variability (Ward *et al*., 2001).

Numerous studies in restored or non-restored floodplains have investigated the role played by floods on biological communities, but focus has often been on macro-flora and fauna (Fournier *et al*., 2012; Paetzold *et al*., 2007; Renöfält *et al*., 2006) and on prokaryote microbes (chapter 6; Unger *et al*., 2009). Soil microorganisms play many important roles in maintaining soil fertility and productivity, and in the cycling of nutrients. The application of metabarcoding on environmental DNA has produced an impressive amount of new data on microbial communities in the soil (Bartram *et* al., 2011; Damon *et al*., 2012; Karmono *et al*., 2013)

Increasing attention has been given to eukaryotic microorganisms, but most studies concentrate on marine environments (Amaral-Zettler *et al*., 2009; Pawlowski *et al*., 2011) and forest soil (Damon *et al*., 2012). To our knowledge, no previous study investigated the spatio-temporal distribution of eukaryotic microbial communities in the soil of floodplains, using high-throughput sequencing. With this study we aimed at elucidating the dynamic of microbial Eukaryotes community structure and diversity along the gradient of distance from the riverbed and determine the influence of environmental conditions and the link to ecosystem functioning proxies.

We expected to find significant effect of time and space on diversity and community structure heterogeneity. We expected microorganism community to be

correlated to ecosystem functioning and soil structure and the relative importance of the community driver to vary spatially and temporally.

Material & Methods

Study site

The study site is a restored floodplain along the Thur river in the northeast of Switzerland. The previously channelized river runs in the Swiss plateau, originating at the Mount Säntis, and ending into River Rhein. The river does not have any artificial or natural reservoir; the water regime is therefore strongly influenced by heavy rains, especially in summer and fall, and the snow melting in early spring. The restoration project took place in 2002 and consisted in removing the foreland in front of the levees along 2 km long river stretches, lower the levees to allow flooding of the alluvial forest and reinforce the newly exposed river banks by planting a strip of Willow saplings. The study site is located in the river corridor at Niederneunforn (Canton Thurgau, 8°77'12" E; 47°59'10" N). The river has been widened from the former 50 up to 110m.

Along the gradient of distance from the river six different habitats were selected. We defined them as Functional Processing Zone (FPZ; Samaritani *et al*. 2011). Starting from the riverbed we defined first two dynamic FPZ: Gravel (GC; mostly bare Gravel with some sediment accumulation and sparse patches of colonising plants) and Grass (Ph; highly dominated by the Grass *Phalaris arundinacea*). Further along the gradient on the levee Willow bush FPZ (WB). Then Mixed (MF) and Willow forest (WF) and finally as an indication of the situation previous to restoration, located upstream of the other FPZ, the Pasture (P). The six FPZs differ in vegetation cover, soil texture, soil nutrient pools and fluxes, flooding regime and bacterial and earthworms communities (Samaritani *et al*. 2011; Shrestha *et al*. 2012; Fournier *et al*. 2012). In each FPZ four plots were selected and in each plot three soil-cores (4cm diameter and 10cm depth) were sampled, homogenised, 2mm sieved and stored at 4°C. Soil sampling was repeated four times during one year (July and October 2008 and January and April 2009) resulting in a 96 samples set. From the same plot were measure as proxies of ecosystem functioning: basal respiration (BR), enzymatic activity (EA), microbial carbon (MC), and microbial

nitrogen (MN), and environmental factors: soil moisture (SM), soil temperature (T) and time elapsed between the last inundation of the FPZ and the sampling date (LI).

Basal respiration was measured on 20g of soil, by accumulation under a chamber connected to a Li-COR. Enzymatic activity was estimated by fluorescine diacetate method. Microbial nitrogen and carbon were measured by fumigation-extraction method. Soil moisture was estimated by weighting 10g of dry equivalent of soil samples before and after drying for 24hours at 105 °C. Soil temperature was measured by inserting a HOBO probe into the soil at 5cm depth and measuring every 30sec over the whole sampling year. Last inundation indicates the number of days elapsed between the last inundation and the sampling (more details on the methods are found in chapter 1 and 2 of this thesis).

DNA extraction

Total DNA was extracted from 0.5g subsample of fresh soil. Soil samples were initially suspended with 0.75g glass beads (0.1 mm diameter) in 1ml of extraction buffer (0.2M Na3PO4 [pH 8], 0.1 M NaCl, 50 mM EDTA, 0.2% CTAB). Later they were treated by bead beating procedure using a FastPrep bead beater (FP120, Savant Instruments) at 5.5 m s^{-1} for 40s with 1 ml extraction buffer, three times. Followed centrifugation during 5min at 13,000rpm (Frey *et al*., 2006). DNA was then purified by 2ml chloroform extraction, then vortexed and centrifuged during 5min at 13,000rpm. DNA precipitated was obtained by first adding 3 ml of precipitation solution (20% PEG 6000, 2.5M NaCl), then gently mixing, incubating 1 h at 37°C and finally centrifuged at 15,000rpm for 15min. 1.5ml of 70% EtOH was used to wash the pellets. After centrifugation at 10,000rpm for 2min, the supernatant was removed. The samples were let air-drying for 20min, and then re-suspended in AE buffer (10mM TrisCl, 0.5mM EDTA, pH9) at a volume of 1ml AE per each g of extracted soil (dry weight equivalent). Extracted DNA were examined by electrophoresis in agarose gels (1% w/v in TBE) and stored at -20°C. Pre-treated with bovine serum albumine (BSA, molecular biology grade, Fluka, Buchs, Switzerland) by heating for 5min at 95°C was applied.

DNA Library preparation

DNA samples were amplified in the V9 SSU rRNA hypervariable region using the eukaryotic specific forward and reverse primers combination 1380f/1510r (Tab. 1). The forward primers were tagged with 96 different 9 nucleotides long keys.

Table 1. Forward and reverse primers combination for V9 hypervariable region amplification. X indicate the barcode keys

Name	Sequence
1380f	XXXXXXXXXCCCTGCCHTTTGTACACAC
1510r	CCTTCYGCAGGTTCACCTAC

In a total volume of 30μl we added 1ng of DNA, 6μl of 10xPCR buffer, 0.6μl of each primer, 0.6μl of each dNTP 400μM (Promega), and 0.2μl of 0.05U μl-1 Hotstar Taq-polymerase (Qiagen). PCR amplification was performed with a PTC-100 thermocycler (MJ Research, Waltham, MA, USA). Each PCR reaction was repeated in triplicates and a negative control for each primer pair was run. Amplification conditions followed Amaral-Zettler *et al*. (2009) (modified from Sogin *et al*. (2006)) as follows: 3 minute denaturation at 94°C, followed by 30 cycles of 30s at 94°C, 60s at 57°C, and 90s at 72°C. Final extension at 72°C for 10 minutes.

The three PCR products/sample were pooled together, purified through Zymo columns and DNA concentration measured with Qubit quantification kit. The 96-tagged samples were pooled together at 4ng of DNA each (total DNA 960ng). The resulting sample was precipitated to 84μl, and resulting DNA concentration re-quantified (total DNA 663ng) and DNA library prepared for Illumina sequencing following New England Biolabs's kit NEBNext DNA Sample Prep Master Mix Set 1 (Illumina compatible) (http://www.neb.com/nebecomm/ManualFiles/manualE6040.pdf). Briefly it consists in the step of fragmented DNA end repair, followed by dA-tailing, adaptor ligation, and a final step of PCR enrichment. The 100bp paired-end sequencing run was performed by the HiSeq2000 sequencer at the Genomics Core Facility of Brown University (http://www.brown.edu/Research/CGP/core/). To avoid underestimation of clustering due to low diversity in the first bases of the sequences, we spiked-in 25% of PhiX library in the DNA library. PhiX library was not indexed, therefore those sequences were not retained downstream in the sequences dataset.

Sequencing resulted in 370k/mm2 clusters and provided 221'625'392 barcoded reads in total. Reads 1 (forward) were in average 71bp long and reads 2 (reverse) 80bp. Reads 1 and 2 were merged if they had length higher than 75 bp and overlapped by a minimum of 5bp, using Phyton script. The trimming process resulted in 148'595'988 reads, with an average of 760'723 per sample (min 202'870, max 3'029'180). Merged reads were then scanned for quality and all sequences with quality below 28 were discarded (quality of 20 indicates base call accuracy at 99%, quality of 30 indicates base call accuracy at 99.9%).

The sequences were then compared each against all the others in order to detect unique reads and their abundance in each sample. Singletons were discarded. The remaining 498'000 unique reads were blasted against a reference database (SSURef_108_tax_silva.fasta) from SILVA database (www.arb-silva.de). Taxonomy, percentage of identity and percentage of cover were recovered. Since a difference of even only a nucleotide in a different position of the sequence can indicate or not a different OTU, we decided to keep all unique reads individually in the database for statistical analysis, even when the blasting resulted in the assignment of the same OTU. Blast with cover below 98% and identity below 97% were discarded.

Numerical analysis

Relative abundance of OTU richness and abundance at two different taxonomic levels were compared on pie charts and temporal and spatial patterns were discussed.

Non-metric multidimensional scaling analysis was applied on the community dataset, temporal and spatial effect tested by non-parametric permutation MANOVA. Sequences reads trimming and pairing was performed with Python and the pipeline script with Perl. Downstream statistical analyses were done in Software R.

Results

With clustering at cover higher than 70% and identity higher than 97% the reference database blasting allowed the detection of 202'240 unique reads attributed to Eukaryotes, with cover higher than 98% and identity higher than 97%, we were able to retrieve 95'779. The latter dataset was used in this study. The phylotype accumulation curve of unique reads for all micro Eukaryotes detected with cover

higher than 98% and identity higher than 97% over the 96 samples showed that saturation was not reached (Fig. 1).

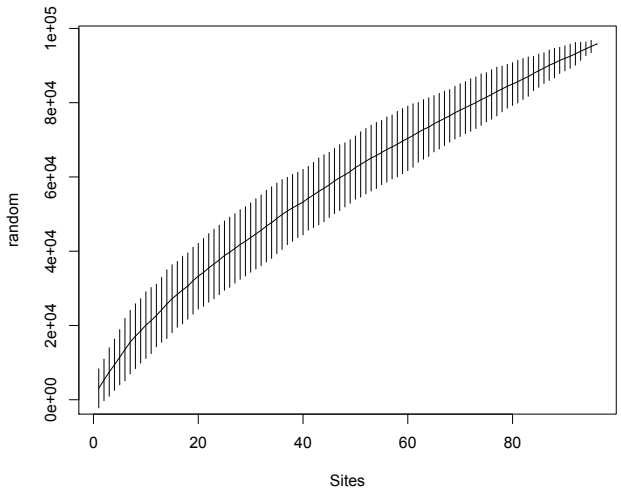

Figure 1. Phylotype accumulation curve of unique reads for micro Eukaryotes over the 96 soil samples.

Eukaryotes phylotypes were assigned to eight supergroups: Opisthokonta, Amoeboza, Heterokonta, Alveolata, Rhizaria, Hacrobia (Okamoto *et al*., 2009), Archaeplastida, Excavata, and others. Number of unique reads (hereafter OTU richness) and their abundance for all Eukaryotes and micro Eukaryotes only (Eukaryotes excluding animals and land plants) shows similar pattern (Fig. 2). (Hacrobia being relatively poorly represented it dos not appear in the pie charts). MicroEukaryotes relative OTU richness was largely dominated by Opisthokonta (44%), Stramenopiles (25%), Rhizaria (13%), and Alveolata (7%). Each supergroup was subsequently divided in the following hierarchical taxonomic groups, resulting in 32 taxa: Opisthokonta includes Chanoflagellida, Fungi, Nucleariidae, Ichthyosporea. Amoebozoa includes Tubulinea, Flabellinea, Archamoeba and Variosea. Stramenopiles includes Bacillariophyceae, Oomycetes, Labirynthulomycetes, Bicosoecida, Chrysophyceae, and Eustigmatales. Alveolata includes Ciliophora, Dinoflagellata and Apicomplexa. Rhizaria includes Cercozoa, Proleptomonas and Foraminifera. Hacrobia includes Cryptophyta, and Centrohelida. Archaeplastida includes Streptophyta, Chlorophyta, Rhodophyta. Excavata includes Kinetoplastida, Euglenida, Heterolobosea, Trimastix, Parabasalia and Jakobida. Unclassified includes Apusozoa. Other groups were detected in the initial unique reads assignment with 70% cover threshold: Reticulamoebae, Micronuclearia, Katablepharidophyta, Glaucocystophyta, Eccrinales, Corallochytrium, Breviata and Ancyromonadidae.

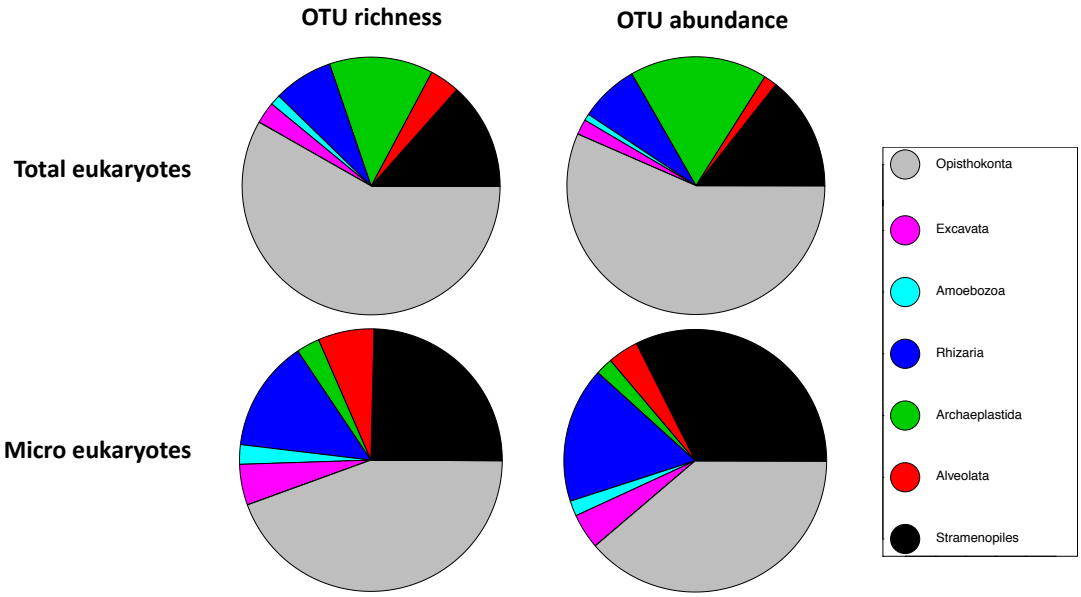

Figure 2. Relative OTU richness and abundance of the eight major supergroups, all Eukaryotes and micro Eukaryotes (Eukaryotes excluding land plants and animals) for OTUs with identity assignment higher than 97%. Richness was calculated as the number of unique reads in all samples pooled together, abundance was the sum of all samples abundances for all phylotypes in the supergroups.

Micro Eukaryotes abundance, was dominated by Opisthokonta at 38% (42'498 phylotypes with abundance 2'070'808), Stramenopiles at 32% (23'587 phylotypes with abundance1'732'530) and Rhizaria at 17% (13'129 phylotypes with abundance 892'841; Fig. 2). OTU richness and abundance for each of the 32 groups detected are shown in Annexe 1 of this chapter.

Opisthokonta abundances are represented at 98% of fungi. Stramenopiles are represented at 86% by Diatoms (unicellular algae characterized by their silica shells), 8% Chrysophyceae (yellow-green flagellate algae), 6% Oomycetes (fungus-like filamentous organisms, some of them are pathogens of plants and crops). Rhizaria are mainly represented by Cercozoa (99.8%). Alveolata are represented at 98% of Ciliates, and 1.5% Dinoflagellates (common in freshwater, many are photosynthetic). The rest of protists is represented by at 4.4% by Excavata (67% Kinetoplastida and 32% Heterolobosea), 1.9% by Amebozoa (63% Tubulinea, 37% Flabellinea), 2.1% of Archaeplastida (99.4% Chlorophyta) and <1% Hacrobia (69% Cryptophyta, and 31% Centrohelida).

Supergroup relative abundances shows different pattern both temporally and spatially (Fig. 3). Gravel and Grass are strongly dominated by Stramenopiles. The

other FPZs are dominated by Opisthokonta, with Stramenopiles dominance decreasing along the increasing distance from the river. They are poorly represented in Pasture. In all FPZ there is a temporal increase of relative abundance of Rhizaria from July to April and a spatial increase with distance from the river and are very strongly represented in Pasture.

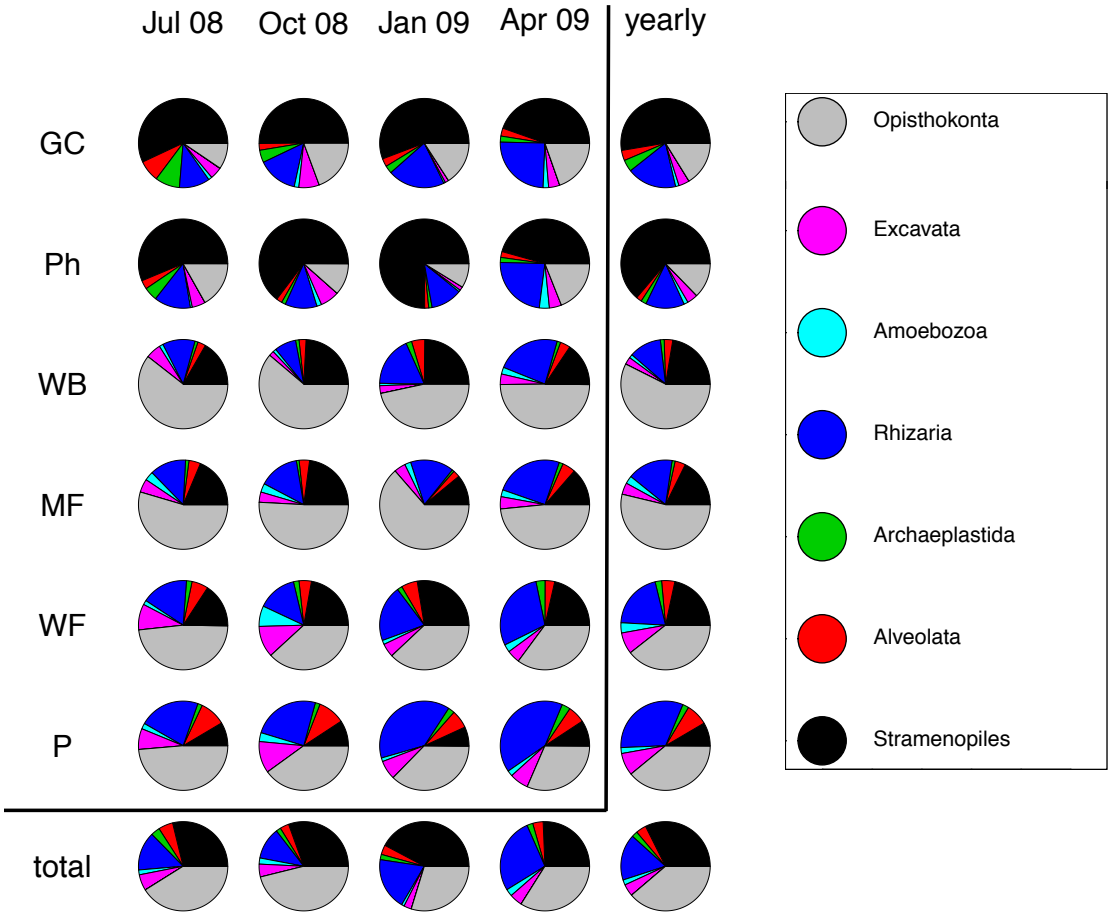

Figure 3. Relative OTU abundance of 8 supergroups of microEukaryotes averaged per Habitat (GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, WF=Willow forest, P=Pasture) in each Sampling July and October 2008 and January and April 2009)(n=4). The bottom row (total) shows the relative OTU abundance in all FPZ together per each Sampling, the right column (yearly) show the relative OTU abundance in all Sampling together per each FPZ. Bottom right pie shows abundance proportions in all samples together and corresponds to the bottom right pie chart in Fig. 2.

Alveolata are seasonally homogeneously represented in all FPZ except Gravel and Grass where they are much more important in July. The highest proportions of Alveolata are found in Pasture. Archaeplastida in Gravel and Grass are fairly represented in July and as Alveolata, they decrease in importance along the seasons. They also present in Willow forest and Pasture. Hacrobia are very poorly represented

all over the seasons and FPZ, therefore they are not visible in the pie charts. Amoeboza are proportionally more important in Mixed forest and Willow forest and Pasture all over the seasons and in April in Gravel, Grass and Willow bush. Excavata generally increase in importance with increasing distance from the river, except in October.

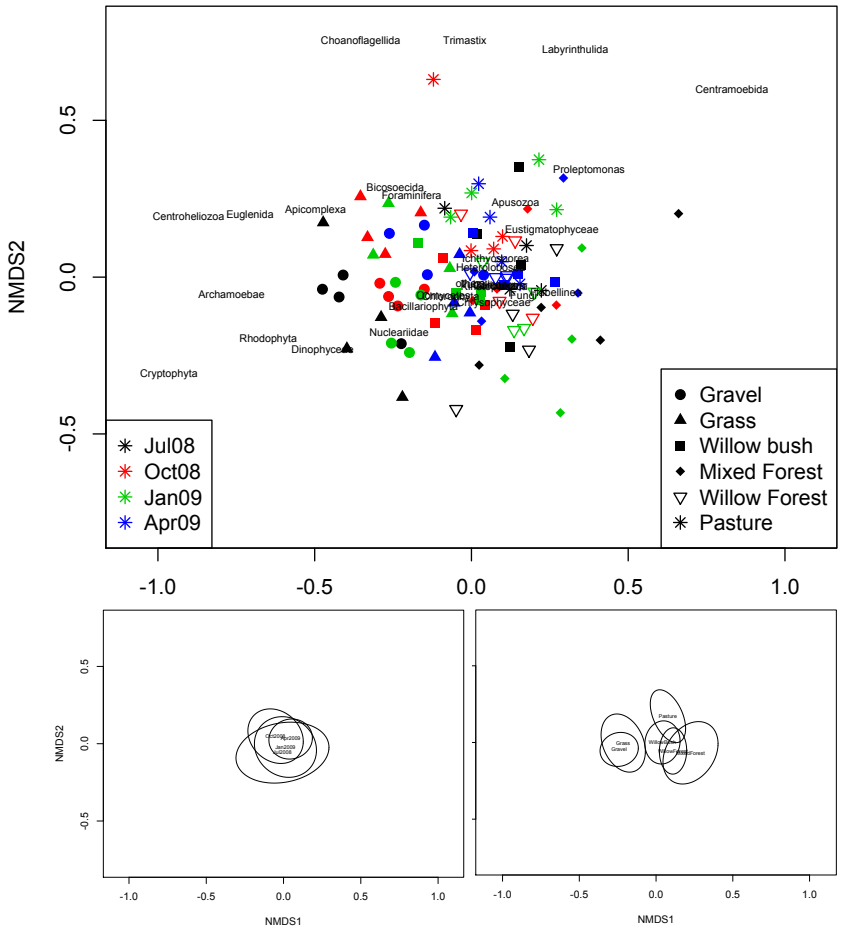

Figure 4. Non-metric multidimensional scaling (NMDS) representation of the spatio-temporal community distribution based on sum of phylotypes abundances of 42 groups of micro Eukaryotes. Samplings are indicated with colours (black=July 2008, red=October 2008, green=January 2009 and blue=April 2009) and FPZ with symbols (dot=Gravel, triangle=Grass, square=Willow bush, diamond=Mixed forest, upside-down triangle=Willow forest and star=Pasture). The bottom ellipse projections show the Sampling (left) and FPZ (right) grouping.

Subgroups taxonomic assignment detected 32 groups (OTU richness and abundance are listed in Annexe 1 of this chapter). Non-metric multidimensional scaling analyses on the phylotype abundances show spatial community turnover (Fig. 4). Both FPZ and Sampling have significant effect ($R^2=0.22$, $p<0.001$ and $R^2=0.07$, $p<0.001$, respectively). The analyses shows that Gravel and Grass are very different

from the other FPZ, especially important in the spatial differentiation are the presence and abundance of Apicomplexa, Euglenida, Centrohelida, Bicosoecida, Foraminifera, Archamoebae, Rhodophyta, Dinoflagellata (=Dinophyceae), Cryptophyta, Nucleariidae and Bacillaryophyta. Pasture is differentiated by presence and abundance of Choanoflagellida, Labyrinthulomycetes (=Labyrinthulida), Centramoebida, Trimastix and Prolptomonas. Mixed forest is more differentiated by some Flabellinea, Eustigmatophyceae and Proleptomonas. Spatial and temporal relative abundance of these groups are studied more in detail (Fig. 5). We excluded from the visual analyses three groups with abundance too high to allow visualisation of other groups (Bacillaryophyceae, Nucleariids and Flabellinea) and one group with very low abundance and one OTU (Archamoeba).

Relative OTU abundance in the taxonomic zoom-in (Fig. 5) shows that Apicomplexa are mainly represented in Gravel in January and April, Grass at all seasons and Willow bush in October and January. Centrohelida are poorly represented and only in Gravel and Grass, very abundant in Gravel in April. Choanoflagellida are poorly represented in Gravel, Grass and Pasture. Chryptophyta are only present in Gravel, especially in July. Dinoflagellata, as Bicosoecida, are present at all FPZ at different Samplings, always very abundant and sometimes dominating. Euglenida are relatively abundant in Grass and Willow bush, but only in October. Eustigmatophyceae are also present at different FPZ at different Sampling, extremely abundant in Mixed forest in April. Foraminifera are very abundant in all Samplings in Grass, Willow bush and Mixed forest, although in MF in April their abundance is relatively low compared to Eustigmatophyceae. Labirynthulida are extremely abundant in Mixed forest in April and present in Pasture in October and April. Proleptomonas are present and particularly abundant in Willow forest in October and Pasture in January. Rhodophyta are only present in Gravel, Grass and Willow bush. Trimastix is only found in Pasture, at all seasons and Variosea are very abundant in Mixed forest in January.

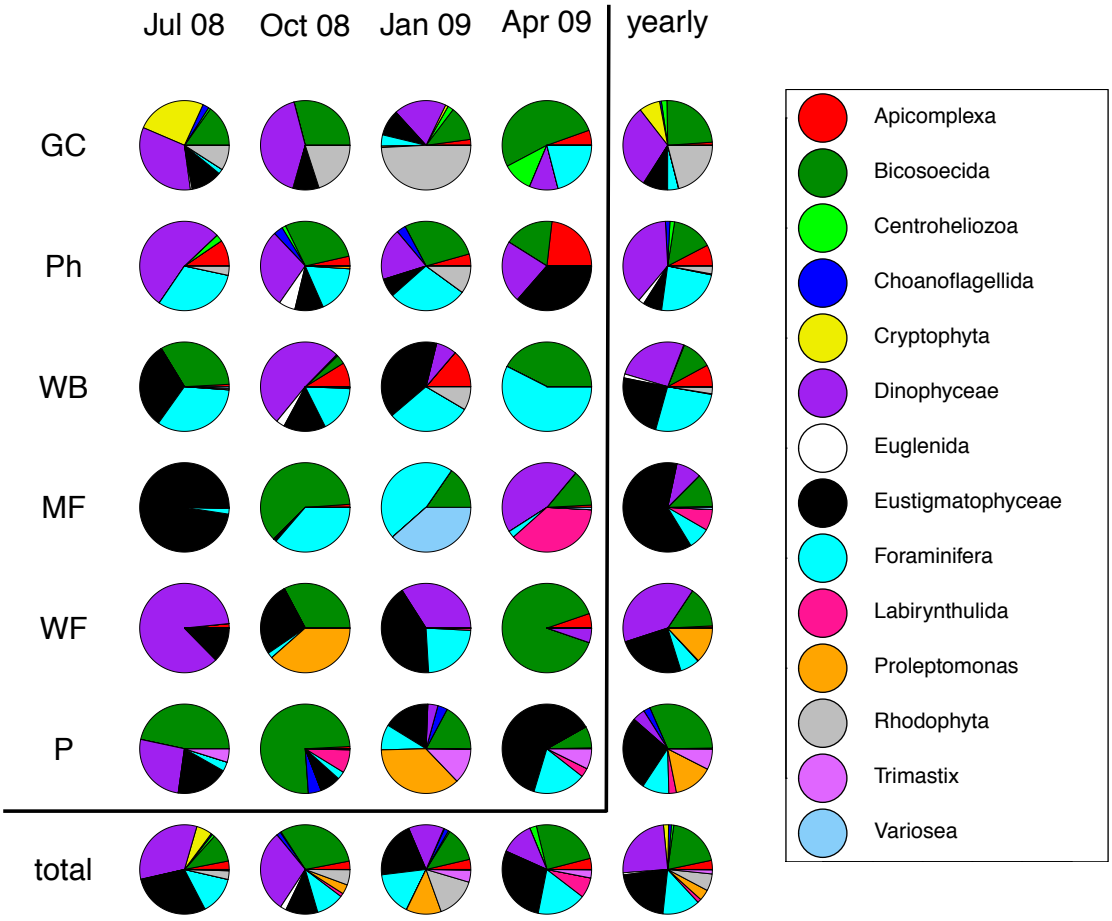

Figure 5. Relative OTU abundance of 14 groups of microEukaryotes averaged per Habitat (GC=Gravel, Ph=Grass, WB=Willow bush, MF=Mixed forest, WF=Willow forest, P=Pasture) in each Sampling July and October 2008 and January and April 2009)(n=4). The bottom row (total) shows the relative OTU abundance in all FPZ together per each Sampling, the right column (yearly) show the relative OTU abundance in all Sampling together per each FPZ. Bottom right pie shows abundance proportions in all samples together.

The yearly average showed decreasing relative abundance of Bicosoecida and Dinophyceae along increasing distance from the river and Pasture and increasing Eustigmatophyceae. Rhodophyta are very abundant in Gravel and decrease in Grass and Willow bush while Apicomplexa increase along these three same FPZ. FPZ average shows alternate dominance of Dinophyceae, Eustigmatophyceae, Foraminifera and Bicosoecida.

Discussion

Advantages and limits of high-throughput sequencing

It has been suggested that high-throughput sequencing of SSU rRNA clone libraries was not able to sample the whole environmental diversity (Edgcomb *et al*., 2002). This was confirmed by our study as, despite sequencing depths that exceed by far those achieved with the first metabarcoding methods, the number of OTUs detected did not saturate with the number of samples added (Fig. 1). This is mainly due to the enormous number of different sequences that were detected in our soil samples. In the absence of knowledge about the genetic differentiation between species of micro Eukarotyes, we considered each unique sequence as a different OTU. It is however possible that some sequences that diverged by a small number of positions belonged to the same species or even the same individuals. This problem is not easily solved in the absence of independent identification of species, for example based on morphological character. However, it was possible to tackle this issue by working with abundance per higher-level taxonomic groups. This completely removed the need to identify species and enabled comparative analyses independently of the problem of species concepts.

Although the use of the V4 hypervariable region of the SSU-rDNA has been shown to correlate better with results generated by long rDNA fragments (Dunthorn *et al*., 2012) it has also proved to be more subject to errors than the V9 region (Behnke *et al*., 2011). The use of the V9 region for detection of diversity in environmental samples has been shown to be a good compromise between precision of taxonomic identification and diversity detected (Amaral-Zettler *et al*., 2009; Pawlowski *et al*., 2011; Stoeck *et al*., 2009). This method might not be suitable to discuss the species absolute abundances because a positive correlation between genome size and rRNA copy number has been proved (Birnstiel, 1971; Prokopowich *et al*., 2003). Moreover, there is large variation in gene number between species (Kobayashi, 2011), but also among individuals of polymorphic populations and in plant species presenting different levels of ploidy (Long & Dawid, 1980). Considering that and the fact that our sampling effort did not saturate OTU diversity (Fig. 1) it is not surprising that richness and abundance in our study were strongly correlated (R^2 0.97, $p<0.001$). More than 60% of the unique reads detected belong to

animals (Metazoa), fungi, and plants (Land plants). Considering the relatively small amount of soil samples (1g), DNA of meso- and macroscopic organisms is mostly extracted from residues. Only micro Eukaryotes were considered in the following analyses. Despite the fact that we worked on a very small proportion of the total soil microorganisms and we chose to set cover threshold in sequences blasts very high, to limit wrong taxonomic assignments, we detected an enormous number of different sequences. Since taxa are likely to have been missed by our sequencing efforts, as shown by our OTU accumulation curve (Fig. 1), and the use of a non-specific eukaryote primer (Bråte *et al*., 2010; Howe *et al*., 2009), the total number of unique sequences in our soils is probably higher. Therefore, high throughput sequencing reveals the incredible diversity present in environmental samples and hidden behind the lack of explicit morphological characters to differentiate some groups and difficulties to cultivate microorganisms in laboratories.

Micro Eukarotyes in space and time

The relative abundance of the eight supergroups for each FPZ and at each sampling (Fig. 3) generally reflected the overall relative abundances (Fig. 2), with a strong dominance of Opisthokonta, Stramenopiles and Rhizaria. This indicates that these three groups tend to dominate each site at each period. However, interesting quantitative variation was observed both among FPZ and among sampling dates, particularly concerning habitats closest to the river, Gravel and Grass. The strong dominance of Stramenopiles in these FPZ is not surprising, since two of the three dominant Stramenopiles subgroups, Diatoms and Chrysophyceae, are common algae and the third, Oomycetes, thrive better in moist environments. Gravel and Grass are both tightly connected with river fluctuations, allowing aquatic organism to establish, at all seasons. However, Stramenopiles were also very abundant in the other FPZ, including the Pasture, which shows how strongly all the FPZ are linked to the aquatic environment.

Rhodophyceae were until recently described as mostly multicellular macroscopic marine algae, but studies applying pyro-sequencing techniques allowed their detection in environmental soil samples and floodplains (Acosta-Martinez *et al*., 2008; Yuan *et al*., 2012; Bradford *et al*., 2013), and our study confirm their findings. Rhizaria, the third most abundant supergroup in all FPZs and samplings, was largely

represented by Cercozoa. However Foraminifera, representing only 3 % of Rhizaria and only recently being attributed to soil communities (Lejzerowicz *et al*., 2010), was found determinant in the differentiation of communities. The frequent occurrence of a taxonomic group in an environment from which it was unknown based on classical surveys based on direct observations shows both how our knowledge of micro Eukaryotes is limited and the power of high-throughput sequencing to shed new lights on the microbial diversity.

Unlike Stramenopiles, Opisthokonta showed an extreme dominance in the FPZ that were further from the river. Long term inundation and anoxia in soils closer to the river are disadvantageous for fungi (Langer & Rinklebe, 2009) but aquatic fungi might have contrasted trends and their diversity and temporal variability can be considerable (Gessner & Van Ryckegem, 2003; Nikolcheva & Bärlocher, 2004). Closer analyses of fungal species composition and diversity in our site and correlations with environmental factors would improve understanding of our results, and contribute to the still debated fungi phylogeny (Blackwell, 2011; Hibbett *et al*., 2011). Amoebozoa and Excavata as well increased with distance from the river, but further spatial and temporal diversity analyses of Flabellinea, Tubulinea, Heterolobosea and Kinetoplastida are necessary to interpret this trend.

Supergroups and subgroups relative abundance both showed extreme spatial and temporal variation (Fig. 3 and 5), which confirms the importance of multiple temporal and spatial samplings to capture the entire soil micro Eukaryotes diversity in a dynamic floodplain, as already suggested by Nolte *et al*. (2010). In addition, it shows that the heterogeneity of floodplains, mainly due to different flooding frequency among FPZs and across the year, create a differential representation of phylogenetic groups. The differences were mainly observed among FPZs, suggesting that the physico-chemical environment of each FPZ acts as a major filter on the type of micro Eukaryotes that can establish. However, some temporal variation still affected each FPZ, showing that temporal factors, linked to seasonal variation as well as floods, act on top on site-specific conditions to determine the groups that will be encountered in a specific point in space of time in a floodplain.

Further analyses should be done aiming at linking micro Eukaryotes richness and abundance to ecosystem functioning and soil conditions. Indeed, different taxonomic groups might be expected to differ in their functional characteristics,

although variation might be in some case present within specific clades. In addition, incorporating the ecology of subgroups and their functional traits in statistical models would improve our understanding of the system and the specific influence of flooding regime (Raes *et al*., 2011).

Conclusions

Absolute abundance or diversity data are not directly retrievable from our data, because the number of DNA copy per individual and the number of unique sequences per species are not known (Weber & Pawlowski, 2013). However, the number of reads for each taxon is proportional to both the real abundance of the taxon and the total number of reads. Our results thus reliably show the huge diversity present in river floodplain soils and the added value of Gravel and Grass habitats in terms of biodiversity to the whole ecosystem. These two habitats, which were not present before the restoration, differ both from each other and from the other, more stable habitats. It is therefore imperative to study soil eukaryotic microorganisms community and diversity for assessing total biodiversity and its link to ecosystem functioning, especially in the context of ecosystem restoration.

Acknowledgments

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Annexe 1

Supergroups and subgroups OTU detected in the study site with sequence assignment cover higher than 98% and identity higher than 97%, richness and OTU abundance.

Supergroup	group	OTU richness	OTU abundance
Alveolata	Apicomplexa	27	366
	Ciliophora	6565	198569
	Dinoflagellata	88	2734
Amoebozoa	Archamoeba	1	3
	Flabellinea	1097	35118
	Tubulinea	1114	60049
	Variosea	7	15
Archaeplastida	Chlorophyta	2881	112601
	Rhodophyta	22	681
Excavata	Euglendia	4	82
	Heterolobosea	1397	75108
	Jakobida	2	2
	Kinetoplastida	3514	156983
	Parabasalia	23	414
	Trimastix	3	163
Hacrobia	Centroheliozoa	7	93
	Cryptophyta	7	207
Opisthokonta	Choanoflagellida	13	112
	Fungi	43596	2025521
	Ichthyosporea	673	32834
	Nucleariidae	241	12341
Rhizaria	Cercozoa	13847	890896
	Foraminifera	64	1503
	Proleptomonas	16	440
Stramenopiles	Bacillariophyceae	18006	1472937
	Bicosoecida	75	2161
	Chrysophyceae	2902	143207
	Eustigmatales	102	2369
	Labirynthulomycetes	4	155
	Oomycetes	2630	98213
Others	Apusozoa	80	1990

GENERAL DISCUSSION

The duration, frequency, and timing of floods in riparian floodplain play a crucial role in the transport of sediments and their redistribution (Hassan *et al*., 2006; Bertoldi *et al*., 2009), the evolution of the morphology of the river bed (Bertoldi *et al*., 2009b), the maintenance of hydrologic connectivity (Ward *et al*., 2002), the structure of floodplain communities (Tiegs *et al*., 2005; Fournier *et al*., 2012), and the ecosystem functioning (Fierer & Schimel, 2002; Langhans & 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) and habitats can be highly dynamic even over short-time scale (Van der Nat *et al*., 2003). 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.

The main aim of this study was to investigate soil community diversity and heterogeneity of bacteria and micro-Eukaryotes and to relate the community patterns to ecosystem functioning, carbon dynamics and microclimatic conditions of the soil. In particular, I investigated the spatial and temporal dynamics of those relationships in a restored floodplain, under the influence of a recently restored natural flooding regime. Floodplains characterised by episodic and pulsed inundations are spatially very heterogeneous because the duration, frequency, velocity and therefore sedimentation processes of floods have a different impact depending on the distance from the riverbed and topography.

The Thur River is the only river in Switzerland that is not characterised by any reservoir or natural or artificial lakes. Its flooding regime is therefore flashy and directly dependent upon precipitation events and snowmelt in spring in the catchment area. A restoration project started at the end of last century aimed at recreating near-natural conditions of natural floodplains in several 2 km-long reaches of the previously channelized river, lowering the levees and thus allowing the floodplain to be inundated. In order to be able to analyse and discuss the direct consequences of the introduction of a near-natural flooding regime on the soil communities, analyses of the latter should have been done both before and after the restoration took place. Although there have been some previous studies in the site (Acuña *et al*., 2008;

Borsuk *et al*., 2008; Bratrich 2004; Schager & Peter, 2005) none included all the variety of FPZ I studied and more importantly, none included analyses of bacterial and micro-eukaryotic communities. My project started after the restoration phase, so that a before/after comparison was impossible. Another way to quantify the impact of the restoration project would have been to compare communities studied in the same way in a similar ecosystem presenting similar conditions, except for the flooding regime. Unfortunately, defining the properties that really matter and consequently make systems similar enough to be compared is impossible without a perfect understanding of the ecosystem functioning. Identifying multiple sites representing similar enough conditions would consequently have been very challenging. In addition, the large amount of data collected in the present study, embracing different domains such as soil pedology, carbon dynamics, vegetation relevees, bacteria community profiling and micro-eukaryotic environmental DNA massive sequencing, covering multiple sites and multiple time points, was challenging enough without considering other study site.

In order to circumvent the absence of data for a reference stage before the restoration, some plots were studied along the channelized section of the river, slightly uphill of the restored section. These were considered as a proxy for the soil conditions and communities in the absence of restoration. Those plots cannot be used statistically as a control, but they give nevertheless an indication of what might have been found in the site, if it was still a channel instead of a dynamic floodplain. The strength of the present study resides in the multidisciplinary approach. As part of a broader project, the data produced here will be combined and analysed with information originating from other research areas in order to create a massive database and address questions from a broader point of view. Unfortunately, because of individual schedules, challenges, issues, and limitations, the attempt to coordinate the spatial and temporal choice of data collection has not been successful. It was consequently not possible to include many other data in the type of analyses performed in the present study, except for some of the ones which were part of the same work package. The first chapter of the present study is indeed the result of a good coordination and successful collaboration of the three different PhD projects in WP 2.2 and shows that it is possible to achieve fruitful collaboration and common publications among PhD students.

1. Spatial heterogeneity

In order to investigate the spatial heterogeneity of soil bacterial and micro-eukaryotic biodiversity and their link to ecosystem functions, the restored reach of River Thur at the Schaffäuli site was selected as it represents a mosaic of patches ranging from Gravel bars to alluvial forest periodically inundated (Turner *et al*., 1989). Seven habitats were defined as functional processes zones (FPZ; Thorp *et al*., 2006) based on a visual description of their vegetation, distance from the river and topography.

The spatial difference among FPZs in terms of vegetation, which was assumed a priori based on a visual inspection, was confirmed statistically a posteriori based on vegetation relevé analyses (Braun-Blanquet, 1964; Chapter 1). The significant heterogeneity of the site was also supported by soil texture and nutrient contents typical of riparian system under the influence of natural flooding regime. Close to the riverbed the soil texture was coarse and total organic carbon and nitrogen contents were low. With increasing distance from the river in the restored section soil texture gradually changed, with a decrease of the relative fraction of sand and an increase of clay content. Total organic carbon and nitrogen increased with the distance from the riverbed. Higher spatial variability was generally observed within the FPZ closer to the river, indicating finer scale heterogeneity in the soil conditions, and microclimate. This confirms that the external disturbance caused by fluctuation in the river flow and the resulting more frequent flooding of the FPZs closest to the riverbed lead to a high diversity of micro-environments (Doering *et al*., 2011; Langhans *et al*., 2008; Naiman *et al*., 2005; Tockner *et al*., 2010). The forested FPZ and especially Pasture were spatially more homogeneous, with respect to the carbon pools and fluxes, the ecosystem functioning proxies and the bacterial communities. The spatial differences in the soil texture and nutrient content reflected differences in vegetation, suggesting that visual determination of different FPZs based on vascular plants structure is a valuable method for selecting habitat for such a study on spatial variability.

2. Multifaceted description of riparian soils

The analyses of soil texture showed a gradient of decreasing sand percentage along the gradient of increasing distance from the river (chapter 1). Texture and therefore water retention capacity, together with the flooding frequency, have been found to be the main factors influencing total organic carbon content of the soil, carbon fluxes proxies (e.g. soil respiration and methane emissions) and nitrogen processes in this study. Gravel bars, which developed considerably following the restoration and are characterized by both frequent inundation and high sediment deposition rates showed high nitrogen turnover rates. High rates of nitrogen turnover were also measured in the fine-textured alluvial forest where anaerobic microsites facilitated coupled nitrification–denitrification processes (Shrestha *et al*., 2012, Annexe 4). Gravel bars were also characterised by very high ratios of epigeic versus anecic earthworms. Because of the coarser texture and/or soil saturation anecic earthworms were rare in the more dynamic sites, but also in the more waterlogged but stable soils of Willow forest. The ratio of the relative abundance of epigeic and anecic species, and the differences in species composition within anecic and endogeic ecological types of earthworms were identified as indicators of soil development in floodplains (Fournier *et al*., 2012, Annexe 3). The spatial variability observed at the level of the macro-flora was consequently mirrored by variability at the smaller scales, including the soil texture and micro-fauna. This strongly supports a beneficial effect of the restoration, by increasing the diversity observed at multiple levels.

Although texture leads to and maintains significant spatial heterogeneity among FPZs, time had a statistically stronger effect on differentiating carbon pools and fluxes. This was explained by the comparatively much stronger impact of flooding on the carbon balance. Flooding frequency was also the main driver of seasonal heterogeneity of microbial community structure, microclimatic conditions and ecosystem functioning proxies (Chapter 2). Although microbial community and ecosystem functioning were strongly correlated (as indicated by Mantel tests; data not shown), the major determinants of the ecosystem functioning proxies were the microclimatic conditions and the soil conditions (texture, organic carbon, total nitrogen and phosphorus). The soil conditions were in turn very strongly influenced by floods. This means that floods influence directly or indirectly all the abiotic

conditions of riparian soils, which themselves determine at least partially the response of biotic components.

Even though microbial community structure does change significantly spatially and temporally as a consequence of the flooding regime, those changes have no direct influence on the ecosystem functions. The spatial heterogeneity provided by the floodplain, itself modulated by floods, maintains a great diversity in the microbial community, but it is ultimately the direct impact of flooding regime that determines the ecosystem functioning. The eukaryotic microorganism community, even though mainly driven by the same flooding disturbance, was more heterogeneous spatially than temporally (chapter 3), at the highest taxonomic level. This suggests that differences among FPZs in the type of soils and the microclimatic conditions such as temperature and water content influence the type of microorganisms that can develop as well as their relative abundance. It is especially apparent when looking deeper in the taxonomic groups. Depending on the ecology of individual groups, their communities present contrasting distributions. This indicates that environmental conditions varying on very small scales affect differentially each taxonomic group. Since the conditions leading an increased biodiversity are clade specific, a high overall level of biological diversity can be achieved only through an optimisation of the general environmental diversity.

Soil structure and nutrient content, both maintained by naturally pulsing flooding regimes, have proven to be essential to host bacterial heterogeneous community structures and eukaryotic diversity in this study. Ecosystem functioning and decomposition rates were both spatially heterogeneous and temporally dynamic but were not directly correlated to belowground biodiversity. Causal relationships between habitat, processes and biodiversity are highly complex and direct connections are difficult to establish, unless specific organism ecology is considered, but all biotic and abiotic factors analysed in this work showed a strong dependence on the natural flooding regime. In conclusion, the restoration of the flooding regime appears as a relatively easy and very efficient method to increase diversity, through the induction of stochastic variation spread spatially and temporally, which over time generate many different combinations of factors across a small area.

3. Effect of inundation regime on soil microbial biomass and decomposition

During the period of this project, the studied site was subject to environmental conditions typical of the region. River discharge was most of the time under 50m^3/sec, but frequently increased over 110 m^3/sec, the minimum river discharge required to inundate Gravel FPZ and only once reached over 400 m^3/sec, the minimum river discharge required to inundate the forests and pasture FPZ (Figure 2, chapter 1). The variable to quantify the inundation regime used in this project was the number of days elapsed between the sampling date and the last time floods inundated the considered FPZ (Last Inundation; LI). LI varied between a minimum of 2 days measured in Gravel and 240 days measured in the Mixed forest, Willow forest, Ash Forest and Pasture. Inundation regime is predicted to have a significant influence on the microbial decomposer community. Initial soil flooding should result in a flush of carbon, nitrogen and phosphorus, because of leaching from leaf litter (Baldwin *et al*., 1999). Increasing flooding time can alter the bacteria:fungi ratio (Unger *et al*., 2009) and decrease fungal colonisation capacity by reduced sporulation (Aziz, 1995).

In this study, I did not find a significant increase in microbial biomass carbon along the increasing distance from the river. The flooding effect on the microbial biomass might be obfuscated by the high variability of carbon pools and fluxes observed within each FPZ. Indeed, decreasing variability along the increasing distance from the river was observed for total organic carbon, water extractable organic carbon and microbial carbon (Tab. 3, chapter 1). Different flooding frequency/intensity and drought period can trigger a different response of the community biomass and activity (Artigas *et al*., 2009). It would be of high interest to estimate those relationships in a longer period, to be able to compare the effect of specific events, such as a very long summer drought to a very regularly flooded summer.

4. Effect of soil temperature on soil microbes and decomposition

Soil temperature measured during the period covered by this study reflected seasonal variations typical of the region. Daily average ranged between 29˚C and -2˚C, both extremes being measured in Gravel FPZ. When Gravel is not taken into account, differences among FPZs in the daily average were less than 5˚C. Gravel experienced extremely high temperatures, with up to 7˚C more than the next hotter FPZ in summer daily average. During the growing season, between April 2008 and April 2009, there was a pattern of decreasing temperature from Gravel to Grass, Willow bush and Mixed forest, while the three forests were not distinguishable. The cover of high vegetation decreases solar radiations reaching the soil and traps cooler, more humid air, preventing a strong increase of soil temperature in the middle of the day. The temperature in Pasture was similar to that in Willow bush. Between October and March, temperatures were not significantly different among FPZs, but in April 2009 the rise of temperature was again stronger in the FPZ adjacent to the river. The effect of temperature on microbial community composition, abundance and activity is well documented (Zogg *et al*., 1997; Pietikäinen *et al*., 2005). Allison & Martiny (2008) in their review analysed the response of microbial community composition to perturbations, such as temperature, in a variety of studies and found temperature to have a significant impact on microbial species composition.

Microbial activity changes as an effect of the shift of different groups (Balser & Firestone, 2005), but a global study in decomposition rates showed that temperature by itself cannot explain the variability in decomposition rates (Giardina & Ryan, 2000). With temperature, microbial abundance and activity are expected to increase and microbial community to vary (Blume *et al*., 2002), although rain events might also have a major effect (Castro *et al*., 2010). Indeed Bachar *et al*. (2010) along a gradient of precipitation observed that soil bacteria abundance decreased with precipitation, but diversity varied independently. At our site, precipitation events are not different among FPZs, although the open (Gravel, Grass and Pasture) versus forested structure of the vegetation might interact with the effect of precipitations. Differences among FPZs in terms of microenvironmental conditions are likely mainly the consequences of differences in temperature, which adds on differences due to soil texture.

5. Effect of soil moisture on soil communities and functions

Soil moisture is expected to significantly influence microbial biomass and microbial and microfaunal community structure (Frey *et al*., 1999; Simmons & Coleman 2008; Wagener & Schimel, 1998). Bacterial diversity was shown in some cases to be less influenced than bacterial abundance by changes in the oxygen content associated with water content fluctuation (Song *et al*., 2008). The effect on microbial activity might depend on the water potential level (Griffin, 1981). Fluctuation of the redox state in the soil is expected to shift the bacterial community composition and function, for instance by favouring the development of methanogens (Blume *et al*., 2002). Net soil methane production is an indication of the balance between soil production in the saturated layers and methane oxidation in the unsaturated layers (Boon & Lee, 1997; see Figure 1 in the General Introduction section of this thesis). Therefore with increasing distance from the river, methane production is expected to decrease, while methane oxidation is expected to increase, resulting in lowered soil emissions or increased uptake. In this site, methane fluxes showed an increase in soil uptake of atmospheric CH_4 along the increasing distance from the river up to the Mixed forest, which is explained by a combination of decreasing soil texture and opposite increasing water retention capacity and therefore gas diffusion velocity and oxidation potential in the oxygenated layer (Conrad & Rothfuss, 1991; Boeckx *et al*., 1997).

Fungal species are also expected to differ in tolerance to soil moisture (Anderson *et al*., 1984; Miller, 2000; Miller & Bever, 1999; Rickerl *et al*., 1994). Tremolières *et al*. (1998) found higher EM (ectomycorrhiza) fungal richness in unflooded stands of the Rhine River when compared to stands that still receive overbank flooding; however, Jacobson (2004) found higher EM colonization on *Populus* at sites that were frequently flooded and higher AM (arbuscular mycorrhiza) fungi colonization at sites that were infrequently flooded. Both studies attribute these differences to variation in soil nutrients, texture or moisture between the flooded and unflooded reaches. The effect of soil moisture on fungi was not directly tested in the course of this thesis. This could be done by performing detailed analyses of sequences corresponding to different groups of fungi extracted from the Eukaryotes diversity screening study (Chapter 3; see further discussion in Perspectives below). Such a

project would be a first step toward the elucidation of clade specific properties that together determine large-scale patterns of functional and organismal diversity.

6. Diversity of microorganisms

The bacterial community composition in the soil is expected to be mainly driven by soil type and seasonality (Björk *et al*. 2008, Bossio *et al*., 1998). The response of the community to abiotic factors is expected to change over time (Langenheder *et al*., 2012), but soil structure and topography can explain that variation in microbial communities are more pronounced spatially than temporally (Bach *et al*., 2010; Banning & Murphy, 2008). The community composition of some microbial groups can be sensitive to environmental conditions and not resilient to disturbance, so that changes in the environment due to disturbance can directly affect the ecosystem functioning (Allison & Martiny, 2008). However, a lack of direct relationship between bacterial community structure heterogeneity and ecosystem functioning has been previously recorded (Stark *et al*., 2007). Different strains of bacteria are not expected to contribute equally to ecosystem functions, and particular groups can be more sensitive to soil climatic changes or nutrients (Rasche *et al* 2011) or functionally redundant (Pereira e Silva *et al* 2012, Allison & Martiny, 2008). The resulting link of the community structure on the ecosystem functions can therefore be poorly predicted on the basis of fingerprint 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 & Martiny, 2008). The probable shift of the decomposers community from being bacterial dominated to fungal dominated along the vegetation (and therefore litter quality) succession (Wardle, 2002) is not retrievable from my data. This might be because fine changes in the abundance of functionally important taxa are masked by large changes in functionally similar species. Gaining insights into the question of the relationship between changes in the microbial communities and ecosystem functions would require detailed analyses of specific organismal taxonomic units or functional groups. The data produced here provide the framework in which to conduct such analyses, since a detailed description of the ecosystem properties is accompanied by

detailed census of the microbial communities. The next step will require a detailed characterization of specific taxa. The problem is that each fraction of soils can contain hundreds of thousands of organisms, so that identifying the key players can be an impossible endeavour. As similar data accumulates for other sites, meta-analyses could identify species whose abundance is consistently associated with specific changes in the ecosystem function, while decreasing the rate of false positives due to changes in the abundance of species that do not impact the function. Such an approach is used to identify genes responsible for key traits, with the comparison of a large number of samples and the identifying of markers from high-throughput sequencing consistently associated with a given trait of interest (Bräutigam *et al*., 2011).

The diversity of micro-Eukaryotes increases along the gradient of distance from the river at all seasons, except in spring, when diversity is comparable among habitats, indicating homogenisation at this season (chapter 3). This observation does not support the Intermediate Disturbance Hypothesis (Horn, 1975; see introduction ch.6), confirming Fox (2013) and Mackey & Currie (2001) findings. It is possible that our gradient of FPZ does not cover the whole spectrum of possible disturbance levels. Even the more stable FPZ from our study site are subject to episodic disturbance and might therefore represent and level out of all the possible disturbance regimes. This hypothesis might be tested by comparing the diversity of microorganisms found in our sites to that measured in similar environments that are never subject to flooding. Such a comparison is not currently possible as studies conducted on other ecosystems are not statistically comparable. At lower taxonomic levels, the links that the taxonomic groups might have with ecosystem functioning, environmental factors and soil conditions are more contrasted. This shows that the determinants of diversity are clade specific, urging caution when using models that do not take into account the taxonomic diversity to predict the distribution of biodiversity.

Further analyses of causal relationships between individual eukaryotic groups and bacterial community, soil conditions and ecosystem functioning in response to microclimatic dynamic conditions will bring major insights into microorganism responses to flooding regime and spatial and temporal heterogeneity. However, the present study already contributed to a significant improvement of our understanding of the interplay between external disturbance, and soil environmental and microbial

diversity. Working at an intermediate level between large-scale models and specific characterization of selected organisms, this study can benefit both fields. On the one hand, my findings that disturbance affects differentially each group of organisms and that the overall changes in microbial community are not connected to changes in ecosystem function should be incorporated to improve models of the effect of climate change on diversity and ecosystem functioning worldwide. On the other hand, the knowledge gained about the composition of microbial communities and the variation in ecosystem functioning can constitute the basis for investigation of the ecological significance of different clades of microbes.

7. Characterization of individual Functional Processing Zones (FPZ)

The FPZ were identified *a priori*, based on the visual characteristics of different regions of the riparian system, mainly linked to the topography and vascular vegetation. These were confirmed *a posteriori* with a description of the vegetation occupying these FPZ. The work presented in this thesis accumulated data on the soil characteristics and communities independently for several plots within each FPZ, allowing a detailed description of these different types of habitats geographically clustered in the studied riparian floodplain.

Gravel

The Gravel FPZ was initially described as being at lower altitude, closer to the river, subject to more frequent and longer inundation events compared to the other FPZ, and covered by sparse herbaceous vegetation. The results of the different studies presented in this thesis allowed us to determine that this FPZ is also subject to significantly more extreme temperature ranges. It is composed of coarser sandy soil, and is poor in total organic and inorganic carbon and nitrogen. Spatial variability within the FPZ was the highest measured in the gradient concerning soil water content, temperature, total organic carbon, soil respiration and soil respiration sensitivity to temperature increase. This latter finding contrasted with studies predicting that microbial community regularly subject to higher temperature or redox fluctuations would be better adapted to temperature or redox fluctuations effect

(Pettersson & Bååth, 2003; Pett-Ridge & Firestone, 2005). The microbial and micro eukaryote community structures in Gravel were both very different from the other FPZ (chapter 2 Fig. 2 and chapter 3 Fig. 3). The added value to the system in terms of diversity of the Gravel FPZ was particularly striking when considering the Stramenopiles, a supergoup that was more abundant in the Gravel FPZ. This supergroup of organisms is composed at 63% by diatoms, 16% oomycetes and 14% yellow algae, all organisms characteristic of aquatic or moist freshwater environment (see Annexe 2 for detailed description). The presence of these groups shows the importance of allochtonous introduction of organic matter and therefore carbon biomass in this FPZ that might explain local increases of microbial activity (Chapter 1).

Grass

The Grass FPZ is situated slightly uphill compared to Gravel and vegetation patterns are completely different, with *Phalaris* largely dominating the exclusively herbaceous cover, although a few willow saplings are present. These willows could indicate that soil conditions are shifting toward a more stable soil allowing the development of young trees. Major flooding events did sometimes temporally reduce *Phalaris* populations closer to the river, but recolonisation afterwards was fast, which might be mediated by rapid non-vegetative growth using resources stored in the parts of the plants that were not destroyed. Compared to Gravel, soil temperature was lower in the growing season, and higher in winter, while water content was usually higher. Carbon pools and content were less variable within the FPZ, indicating more homogeneous conditions, and usually quantitatively higher than in Gravel, indicating very active microorganisms community. These results are supported by the measures of decomposition rates, which were significantly higher in Grass than the other FPZ (Annexe 1). Available phosphorus (P) content was especially high in this FPZ, further indicating good decomposition conditions (Enriquez *et al.*, 1993), because organic acids commonly released during decomposition prevent phosphorus fixation on clay minerals and therefore improve its availability for plants and soil organisms. Baldwin & Mitchell (2000) reviewed soil nutrient cycling in floodplains and concluded that when sediments dry, they increase affinity for P and promote nitrification-denitrification processes, therefore limiting availability of nitrogen and P. With

increased desiccation, this affinity decreases, resulting in P and N being released by dying bacteria and being made available for absorption by colonising plants. The ecosystem functioning proxies in Grass FPZ were comparable to the Willow bush FPZ. On the other hand, the micro-eukaryotic community was more similar to Gravel FPZ, with the relative abundances dominated by Stramenopiles. This might indicate that Gravel FPZ, having similar community, has the same potential of ecosystem function ability as the most active of the studied FPZ (i.e. Grass), but that a limiting factor, probably a combination of high temperature, low soil moisture, and short pulses of nutrients, prevents the community to be as active as in Grass FPZ.

Willow bush

The Willow bush FPZ was considered *a priori* the intermediate FPZ along the gradient of distance from the river and therefore the impact of the flooding regime was expected to be intermediate too. Soil texture and nutrient content were confirmed to represent an intermediate stage along the gradient of distance from the river, but soil respiration showed particularly high values and variability in the Willow bush FPZ, especially in summer and fall. Methane uptake from soil was observed all year. This might be the consequence of a combination of relatively coarse soil texture, thanks to accumulated sedimentation process, allowing good diffusion of gas, and oxidation of methane because of poor retention capacity and pulse of allochtonous organic matter introduced by floods.

Ash and Mixed forest

Overall, Ash forest and Mixed forest did not significantly differ from each other in soil conditions, microclimatic conditions or ecosystem functioning proxies, but they did differ regarding the composition of the vascular plants community and decomposition rates, which were higher in Ash forest than in Mixed forest. Given the proximity of the FPZ and the similar influence of inundation regime and microclimatic conditions, microbial populations developed in a similar way. Micro-Eukaryotes or micro-fauna differences might explain the difference in the decomposition rates between those FPZ. Unfortunately, those data were not collected for Ash forest because of a lack of resources. Mixed forest has the highest elevation of the study site and was inundated only once during the project. Vascular plant

richness was lower than in Willow forest, Willow bush and Pasture. Mixed forest also had the lowest C:N ratio, and high available P, indicating good conditions for decomposition, although differences with other FPZ were not always significant throughout the year (Chapter 1). Although Mixed forest covers an larger area than the other FPZ, and a higher number of plots were consequently selected within this FPZ, very low spatial variability was observed in the soil texture, nutrient contents and carbon pools and fluxes. The micro-Eukaryotes community structure in Mixed forest was characterised by a high abundance of Amoebozoa (mostly Tubulinea and Flabellinea) and Euglenozoa (mostly Kinetoblastida), and overall a very high Shannon entropy (Chapter 3).

Willow forest

Willow forest was different from the other forested FPZ. Positioned at lower altitude compared to Gravel, but closer to the side channel, it presented a vascular plant richness comparable to that measured in the Grass FPZ. Texture showed very low sand content and high clay content. Together with the highest concentration of organic carbon and total nitrogen, and very high available Phosphorus, those soil properties putt this FPZ at the edge of the gradient of texture and nutrients measured in the site. Soil moisture (Water Content WC, Chapter 1) was significantly higher than in the other FPZ, indicating a very good water retention capacity, which leads to less methane uptake compared to the Mixed forest and actual methane emissions in August 2009 after the important flood event that covered the whole floodplain. The highest values for Microbial carbon and Water extractable organic carbon were also measured in this FPZ, on average, at each season, suggesting accumulation of organic carbon, because decomposition is retarded by soil saturation (Sjögersten *et al*., 2006). The finer texture and higher water content of the soil might increase micro-pores density and connectivity, therefore allowing development of a major number of bacteria and good diffusion of nutrients, thus increasing microbial biomass.

Pasture

Pasture FPZ showed contrasting soil and biological community structure. Soil texture and nutrients were very similar to those of the Grass FPZ, with the exception of the

C:N ratio and available P, which were closer to Willow bush values. With the exception of the Enzymatic Activity, which was very low and comparable to Gravel FPZ, ecosystem functioning proxies in Pasture were intermediate and comparable to the Grass FPZ. Decomposition rates were however more similar to those observed in Willow bush (those observed in Grass were very high), and microbial and micro-Eukaryotes community structures were significantly different from the other FPZ. This FPZ presented similarities with different FPZ from the restored riparian floodplain. However, the properties observed in the Pasture FPZ for the different dimensions studied in this thesis represented only a small fraction of those measured in the restored section of the floodplain and the distribution of all variables within the restored section overlapped with that of the Pasture FPZ, indicating that restored riparian floodplains exhibit a larger palette of conditions, a small subsample of which are also present in the Pasture FPZ. Samples collected and studied in Pasture were fewer than the rest of the study site together, however the constant homogeneity within FPZ, compared to the other very heterogeneous FPZ could suggest that even increasing number of samples would not significantly increase heterogeneity in this FPZ.

	Gravel	Grass	Willow bush	Mixed forest	Willow forest	Pasture
Altitude	+	++	+++	>	++++	++++
Distance from river	+	++	+++	++++	>	++++
Texture (Sand %)	>	++++	+++	++	+	+++
Flooding frequency	>>	>>	>	+	+	+
Temperature sensitivity	>	+++	++++	+	+	++
Organic carbon	+	+++	+++	++++	++++	+
Inorganic carbon	+	++	+++	++	++	++
Total nitrogen	+	++	+++	+++	++++	++
Available phosphorus	+++	++++	++	++	+++	+
Basal respiration	+	+	+	+	+	+
Enzymatic activity	>>	++	++++	++++	>	+
Microbial carbon	++	+	++	++	+++	++
Microbial nitrogen	+	++	+++	++++	>>	++
Decomposition rates	+	+++	++	++	+++	+
Micro eukaryotes Supergroups richness	>	++++	>>	+++	+	++
Micro eukaryotes Supergroups abundance	++++	+++	>	+++	++	+
Bacteria Shannon α diversity	+	+	++	++	+	++
Bacteria β diversity	+++	++	++	++	+	+

\+ = increasing value
\> = very high value
>> = extremely high value

Figure 1. Soil physico-chemical conditions, ecosystem functioning and microorganism richness and diversity trends. Pasture in the figure is slightly separated because unlike the other FPZ it does not follow the gradient of increasing distance from the river.

8. Effects of restoration

The studied section of the river was recently restored. Unfortunately, the soil microorganism communities before restoration were not investigated for the exact same sites and the effect of the restoration can consequently not be directly tested. However, the research presented in this thesis has shown that the study site, although small in size, is spatially very diverse and temporally extremely dynamic. This diversity concerns the abiotic conditions but also the biotic conditions including aboveground vegetation, earthworm communities, belowground bacteria and belowground micro-Eukaryotes. In addition, ecosystem functioning, which can be linked to ecosystem services, such as decomposition, nutrient cycling and carbon storage are also diverse within the study site. I have shown that this high diversity is due in large part to the frequency of floods, which are the major source of disturbance. The spatial heterogeneity is also due partially to differences in the soil

texture, which is in itself under the direct influence of floods. The diversity of ecosystem functions is mainly due to the variety of environmental conditions among FPZ. Going backward, the variety of environmental conditions can be related to differences in soil texture and aboveground vegetation and these differences are directly or indirectly the consequence of floods. Therefore, the heterogeneity observed among FPZs in all aspects of the ecosystems is the direct or indirect consequence of recurrent floodings, but also differential floodings along the elevation gradient.

In a channelized river inundations are rare, as only exceptionally high river discharges inundate sites above the levees, such as the Pasture. In addition, the presence of levees leads to a more homogeneous elevation above the river level, so that inundations will affect homogenously the whole area adjacent to a channelized river. The diversity of disturbance regimes among the different sites within the studied restored floodplain are therefore the direct consequences of the restoration project. The overall variety of soils, ecosystem functions and microbial communities described in this thesis is thus likely the consequence of the restoration, which should be considered a success from a soil biology point of view.

9. Concluding remarks

Soil bacterial and micro-eukaryotic communities, ecosystem functioning, nutrients dynamic, decomposition rates and microclimatic conditions are all the subjects of a large and complex literature that involves different methods of analyses, groups of organisms of interest, consequences explored (e.g. global change impact, invasive species impact on communities, soil degradation and restoration) and system analysed. The originality and challenge of this project was to analyse all of the above-mentioned variables, and detect patterns of spatial and temporal dynamic in riparian ecosystems, considered as hotspots of biodiversity but also extremely modified and threatened environments. I was expecting to find a very significant spatial difference in the soil microorganisms distribution and diversity and in the ecosystem functioning proxies, reflecting the aboveground vegetation structure and topography. Although the communities and processes were significantly different among FPZs, I found the

temporal effect of the inundation regime to be often of greater influence than the spatial heterogeneity. That was not the case for micro-Eukaryotes diversity. The spatial pattern of increasing diversity with distance from the river, and the gradual decrease of relative importance of the dominant Supergroup of aquatic organisms (Stramenopiles) to another group of organisms, seems to be relatively stable along the year. In both bacteria and micro-Eukaryotes communities, the inundation regime was the main driver of the community structure variation in the soil and related functions. The dramatic effects, apparent after only 10 years from the re-introduction of the flooding regime, strongly point at natural flooding regime being an essential asset for riparian floodplain restoration. One acknowledged issue of this project that should be mentioned, was the absence of measurement performed before the restoration, which might have been used for comparative purposes. Although Pasture FPZ was sometimes used as a reference for the conditions present before restoration in the dynamic section (first three FPZ along the distance from the river) it cannot (and was not) considered as a control. That prevents me from definitely concluding that my results are a consequence of the restoration project and let me only speculate on the benefits the restoration project brought in terms of increased spatial and temporal heterogeneity of biodiversity, ecosystem functioning and soil diversity.

10. Perspectives

The description of the ecosystem functions aimed to assess the heterogeneity of functions provided by the floodplains and to link them to differences in organismal diversity. One important ecosystem service includes the processes linked to nutrient cycling, derived from litter originated in the site, or deposited by the river during inundation events. In order to evaluate the rate of degradation in the different zones of the floodplain, I left bags filled with different types of organic matters and monitored the degradation of this matter over a relatively long period of time (Annexe 1). Unfortunately, several litterbags were taken away by floods and could not be included in the experiment. In addition, the degradation of some of the fillings used in this experiment was too rapid for the selected period to precisely assess the degradation rate. This important experiment should consequently be re-conducted over

consecutive short periods of time (e.g. a couple of weeks) with a regular monitoring of the state of the organic matter, alongside long term monitoring, to investigate the dynamic of spatial difference between FPZ at different time scales. The litterbags experiment included in this thesis showed some spatial difference in the decomposition rates between FPZs, but the amount data was not sufficient to significantly detect the effects. This first result should be taken forward with a better experimental approach, built on my own experience. It would be very interesting to repeat the same experiment measuring the C:N:P ratio in the litter before and after deposition in the soil, to monitor independently the decomposition rate of the different components present in organic matter. In addition, using more bags and filling them with a greater variety of organic matter, with different concentration of recalcitrant compounds would allow establishing the decomposition rate shifts at different stages of decay.

The investigations of the prokaryotic and eukaryotic microorganism communities initiated in this thesis has resulted in an impressive amount of data, especially concerning the soil eukaryotic community, for which I capitalized on recent advances in sequencing technologies (Chapter 3). The focus of this thesis was on the spatial and temporal heterogeneity of the community at the largest (super groups) and directly following taxonomic scale, but innumerable other questions can be answered by investigating the different super groups at small taxonomic levels. The number of different OTUs retrieved in the sequencing of the DNA contained in a relatively small amount of soil exceeded by far the number of species described in riparian soil. This strongly suggests that our approach sampled a high number of species that were unknown to science, or at least whose presence in riparian soils was unexpected. The strength of this dataset lies in its association with environmental and ecosystem functioning datasets sampled in parallel. This vast amount of data could be further investigated to gain important insights into specific taxonomic groups. For example, the detection of OTUs that are not expected in such environments as floodplains might be investigated in more details, to identify the organism and find explanations for its presence. Such a research program would strongly increase our specific knowledge of riparian soil microorganism biology and might highlight unexpected connections and similarities with other environments with regular exchanges of organisms. Similarly, the spatial and temporal distribution of organisms

typical of aquatic habitats across the different zones of the floodplain might lead to new insights about the species colonization and survival capacity between different ecosystems. Indeed, the temporal and spatial prevalence of specific species can allow a distinction between taxa that are consistently present and those that colonize riparian soils episodically from surrounding environments, as a function of the presence of ephemeral conditions.

The work conducted in this study aimed at investigating the dynamics characterizing a real ecosystem. As with any experiment conducted in the field, the high number of interacting uncontrolled factors makes causality difficult to prove with confidence. This shortcoming could be tackled by following the descriptive work presented in this thesis with more experimental works, where the different confounding factors can be controlled. For example, an innovative experiment would consist in collecting soil samples from the different FPZ and randomly redistribute them in other FPZ and analyse the soil nutrients and the prokaryotic and eukaryotic communities before and after the spatial shift to assess migration and colonization capacities of the microbial species. Floods could be mimicked by watering some portions of the riparian soil. Manipulating a single aspect of the environment would improve our capacity to find causal relationships. Combined with the descriptive works presented in this study, they would strengthen our understanding on the dynamics acting on riparian soil microbial communities and their effects on the functioning of the whole ecosystem.

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Annexe 1. Spatial heterogeneity and flood impact on decomposition rates in a restored floodplain in northeast Switzerland.

Abstract

Organic matter (OM) decomposition rate depends on the quality of the OM, the soil physico-chemical conditions, the microclimate and the decomposer community. In a dynamic floodplain, OM distribution is strongly influenced by the flooding regime, but the determinants of decomposition rates remain partially understood.

In a restored floodplain in northeast Switzerland, we selected seven different functional processes zones (FPZ) differing in their distance from the river, topography and vascular plant community. In each FPZ, we estimated the decomposition rates of *Phalaris* litter and cotton strips in litterbags of two different mesh sizes over two periods of time of eight and 12 months.

The decay coefficient was significantly different among FPZs for *Phalaris* litter in the first period. Decomposition was faster in the more dynamic FPZ, but not in the site closest to the river. In the forests, decomposition rates measured over one year were higher than over eight months, indicating an acceleration of decay in spring.

No correlation was found between *Phalaris* and cotton decomposition in any of the mesh size or harvest time. Small and large mesh size decomposition rates were significantly correlated in the first period, indicating a spatially stable proportion of small versus large decomposer community. This stability was lost in spring, indicating higher spatial variability in the decomposer community in this season.

Flooding regime and soil temperature influenced only indirectly decomposition rates, while enzymatic activity, soil moisture and soil diversity and phosphorus content were directly linked to both the small and large decomposer community. Based on these results, we discuss the potential factors affecting the decomposition rates in an ecosystem subject to natural pulsing floods.

Introduction

Floodplains are composed of a complex mosaic of aquatic and terrestrial habitats, and are considered to be amongst the most productive ecosystems on Earth (Naiman *et al*., 2005; Naiman & Décamps, 1997). Organic matter (OM) enters floodplain ecosystems both from autochtonous production and from allochtonous material, which is introduced by floods. The quantity and quality of autochtonous litter entering floodplains is temporally variable, depending on litter phenology and therefore from the regional climatic conditions (Gonzalez, 2012). In addition, flooding duration and frequency can also significantly influence the system productivity (Megonigal *et al*., 1997). OM and energy movements are consequently under the influence of floods (Malard *et al*., 2000; Molles *et al*., 1995), typically during short pulses (Tockner *et al* 1999), and can be distributed very heterogeneously across space (Langhans *et al*., 2008).

Decomposition is a very important process driving nutrient cycling in the soil (Cleveland *et al*., 2004). The decomposition rate of organic matter depends on the physico-chemical composition of the OM, the physico-chemical environmental conditions and the functional diversity of the decomposer community (Coulson & Butterfield, 1978; Elliott *et al*., 1993; Langhans *et al*., 2008).

Organic matter breakdown is carried out mostly by microorganisms communities, including bacteria and fungi. The proportion of bacteria and fungi depends on the organic matter quality, with fungi decomposing the more recalcitrant fractions such as lignin and cellulose. The oxidation state of the soil can also have an indirect effect, through the control on the types of microorganisms present. The proportion of fungi and bacteria can also vary vertically, with fungi contributing more to the decomposition of the surface litter and bacteria more to the decomposition of the buried litter (Beare *et al*., 1992). Finally, inundation regime, timing, duration and frequency significantly influence decomposition rates in riparian, in particular decreasing inundation duration will affect leaf decomposition rates, while a reduction in flow variation will decrease leaf decomposition heterogeneity (Langhans & Tockner, 2006). The decomposition rates are therefore expected to be determined by complex interactions between biotic and abiotic factors. However, the relative contribution of these factors might vary among ecosystems.

In this study we studied the decomposition rate of OM and its spatial variation in relation to soil conditions (texture and nutrients), microbial activity and biomass, and microclimatic conditions (soil temperature and moisture and inundation) in the soil of different Functional Processes Zone (FPZ) of a regenerating floodplain. Because of the differences in biological community composition, soil texture and nutrient content, characteristic of dynamic floodplains, decomposition rates between the different FPZs are expected to be very heterogeneous. Analysing these differences in light of the biotic and abiotic conditions of the FPZs defined in other chapters will shed new light on the determinants of decomposition rates.

Material & Methods

In a restored floodplain of the river Thur, northeast Switzerland, seven different functional processes zones (FPZ) were selected based on topography, distance from the river and vascular plant community. With increasing distance from the river the FPZ were named Gravel, Grass, Willow bush, Mixed forest, Ash forest, and Willow forest. The seventh FPZ was selected upstream of the restored section, in the channelized section covered with pasture. Pasture distance from the river is comparable to Grass and its altitude similar to that of the forests. In each FPZ, four to ten plots were randomly selected. Four plots were selected in the more stable FPZs (Ash forest, Willow forest and Pasture), while six plots were defined in the more dynamic FPZs (Gravel, Grass and Willow bush) and in the Mixed forest.

Using polyester nets of two different mesh sizes (5mm and 0.5mm), we cut and sew a total of 288 litterbags. Seventy-two bags of each size were filled with 4g of air-dried *Phalaris* litter from the previous year collected *in situ*. The remaining bags were filled with 4x2cm (~280mg) unbleached commercial organic cotton strips (96% cellulose). Two replicates of each combination mesh size-OM were installed in each of the 36 plots in July 2008. One replicate was left on the site for the whole duration of summer, fall and winter (240 days), while the second replicate remained for the whole of summer, fall, winter and spring (360 days). In order to avoid weight bias from sediment accumulation in the litterbags, the litter was extracted from the bags after collection, dried at 105ºC, weighted, and then weighted again after calcinations

at 550ºC. Organic litter mass was estimated as the weight loss by calcination. Organic litter decomposition follows an exponential decay curve:

$$X_t = X_0 e^{-kt} \quad \text{Eq. 1}$$

where X_0 is the initial mass of organic matter in the litter bags, X_t is the organic matter mass at the time of removal from the field, and t is the time elapsed in days.

Soil properties, environmental conditions and ecosystem functioning proxies

Soil texture and nutrients (organic C, total N, available P) were determined from samples taken in April 2008 using standard protocols (See Samaritani *et al*., 2011).

As microclimatic variables, we measured soil temperature (T) at 5 cm depth in the centre of each plot, soil moisture (SM) estimated by measuring the weight loss upon drying 20 g of fresh soil at 105°C for 24 h, and the time elapsed since last inundation (LI) 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 (BR) was estimated using an Infrared Gas Analyser (Licor 8100). Fluxes are reported as μmol CO_2 $s^{-1}g^{-1}$ soil dry weight. Microbial biomass carbon (MC) and nitrogen (MN) were determined by chloroform fumigation-extraction (Beck *et al*. 1997; Vance *et al*. 1987; Brookes *et al*. 1985). Data are expressed in mg kg^{-1} soil dry weight. Enzymatic activity (EA) was estimated by fluorescein diacetate analysis. Activity is indicated as mg of degraded FDA $h^{-1}g^{-1}$ soil dry weight.

Bacteria Shannon diversity index was calculated on the terminal restriction fragment length polymorphism profile based on two restriction enzyme digestion (*Msp*I and *Hae*III) of the 16S rRNA. All of these techniques are described in more details in the previous chapters.

Results & discussion

Due to unexpected difficulties with the experiment, more than 50% of the litterbags could not be integrated in the dataset used for statistical analyses. A few were flushed away by the floods and were never recovered. Many were filled with sediments, causing the seam to break and a few (especially cotton strips) were so degraded that it was not possible to recover them. At t_2 no litterbag was recovered from Gravel, Grass

or Pasture, and no small mesh size-cotton strips were recovered from Willow forest. In total 102 litterbags were collected at t_1=240 days and 46 at t_2=360 days. Because of the incomplete dataset in the cotton litterbags, only *Phalaris* litterbags were used for analyses. Mass loss percentage of *Phalaris* in litterbags with small mesh size (0.5mm) and large mesh size (5mm) are shown in figures 1.

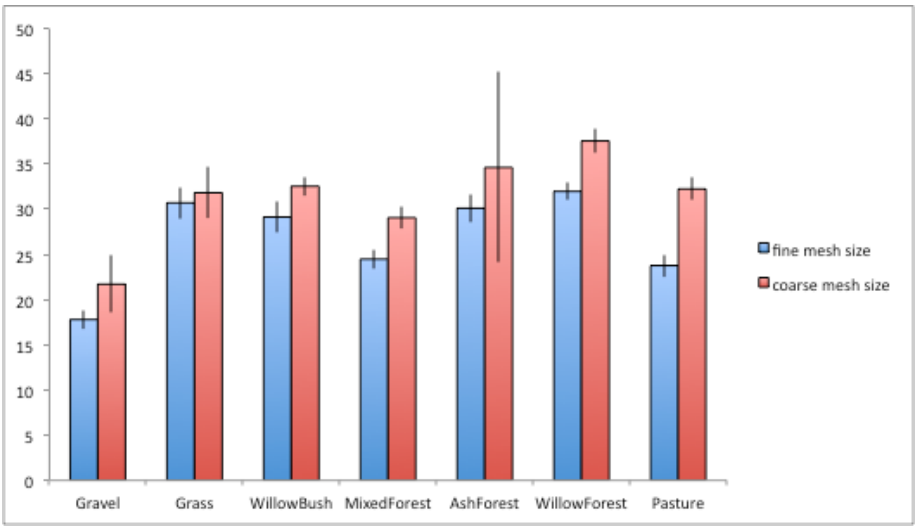

Figure 1. Mean (± SE) mass loss percentage estimated for *Phalaris* litter, with fine (blue bars) and coarse (red bars) mesh size, after 240 days in the field. Decay rates were calculated as indicated above (Equ. 1).

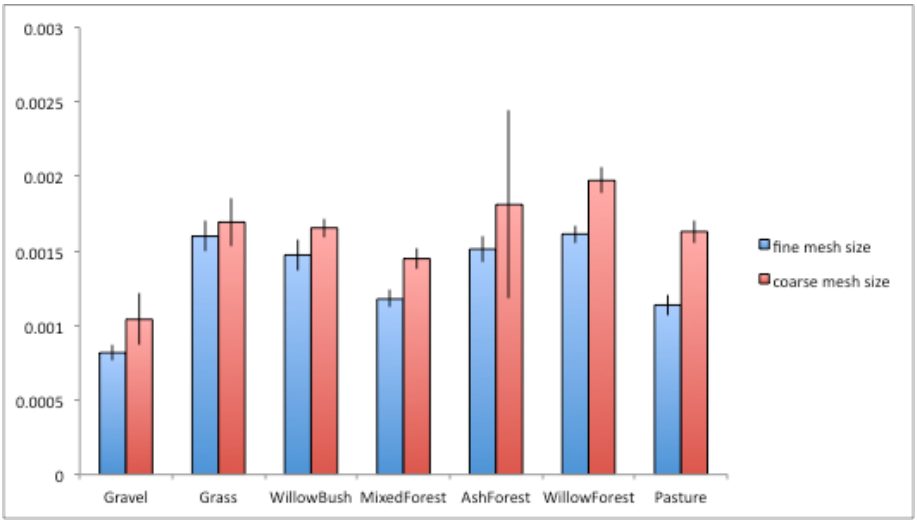

Figure 2. Mean (± SE) exponential decay rates (k) estimated for *Phalaris* litter, with fine (blue bars) and coarse (red bars) mesh size, after 240 days in the field.

Mass loss and decay coefficients difference among FPZ were tested by non-parametric Kruskal-Wallis test and found to be non-significant, TukeyHSD test showed also no significant difference between pairwise FPZ.

Linear model showed that in small mesh size litter bags, 42% of mass loss variance is explained by a combination of soil texture (sand and silt), nutrients (organic carbon and available Phosphorus), soil moisture and enzymatic activity ($R^2_{adj}=0.58$, $p<0.001$). Bacterial diversity did not significantly correlate to decomposition rates.

In riverine floodplains, the inundation regime and frequency influence directly the decomposition processes (Langhans & Tockner, 2006). In aquatic systems, decomposition is generally faster because of more active microbes and leaching and especially fast decomposition rate is measured after water retreats (Molles *et al*., 1998; Tiegs *et al*., 2007). The decay rates we found were comparable to those found by Tiegs *et al*. (2007) in the Tagliamento river (Italy) and twenty streams in Switzerland, as well as by Schädler & Brandl (2005) near Marbirg, Germany. Mass loss and decay rates were higher in the coarse mesh size litterbags, except for the Ash Forest, where fine mesh and coarse mesh values overlapped. The difference in decomposition rates measured with the two sizes might be due to the contribution to the decomposition process of mesofauna too that are too large to penetrate the small size litterbags. However, the actual contribution cannot be precisely quantified, because of the contribution of fragmentation, erosion and leaching (Boulton & Boon, 1991). In the future, the use of a modified version of decomposition and consumption tablets (DECOTAB) could improve the standardisation among habitats (Kampfraath *et al*., 2012). We measured the highest decomposition rates in Grass and Willow forest. Those two FPZ were also characterised by very high enzymatic activity, especially in October, and in general high soil moisture. In Grass FPZ this is explained by the proximity of the riverbed, coupled with the very dense Grass coverage that buffers the effect of high temperature and drought. Litterbags in the Grass FPZ were systematically covered by sediments after each flooding episode, which can contribute to higher decomposition rates (Harner *et al*., 2009). In Willow forest, the high decomposition rates could be explained by the proximity of the side channel coupled with finely textured soil that enhance water retention capacity. Inundation regime is expected to directly influence the decomposition rates (Irons *et al*., 1994). I did not find a significant correlation between decomposition rates and flood frequency, but other studies have shown that flooding duration might be the main driver of decomposition rates, soil texture and carbon in the soil (Baker *et al*., 2001; De Jager *et al*., 2012; Langhans & Tockner, 2006).

In this study, decomposition rates were explained by a combination of soil texture, soil nutrients, soil moisture and enzymatic activity. Surprisingly, temperature did not correlate with decomposition, but the effect of temperature is expected to change with seasons, because of annual variation in the sensitivity of enzymes to temperature (Conant *et al*., 2011). Therefore, short-term experiments on litterbag decomposition should be carried out in order to avoid seasonal effects of the temperature sensitivity (see perspectives in General Discussion section of this thesis). Combined effects of soil fauna and temperature can also indirectly contribute to decomposition rates (Briones *et al*., 2010). Available phosphorus concentration appeared to be a good indicator of decomposition process, as expected (Enriquez *et al*., 1993). Soil texture strongly influence the decomposition rates through soil aggregates and pore sizes (Krull *et al*., 2001; Strong *et al*., 2004), physical protection of carbon from decomposition where it cannot be reached by decomposers, influence on water retention and oxygen availability, and therefore on the portion of decomposers that will thrive (Franzluebbers, 1999; Juarez *et al*., 2013; Kilbertus, 1980).

Microbial communities are major contributors to decomposition process. Therefore, microbial abundance and community structure and diversity are expected to influence the decomposition rates. I found microbial biomass and bacterial enzymatic activity to be positively correlated to decomposition rates and to increase along the gradient of distance from the river. However, microbial diversity was not correlated with decomposition rates. Experimental studies have shown that the effect of reducing microbial diversity on ecosystem functioning can result in complex patterns, notably by accelerating degradation of some compounds and slowing down others (Degens, 1998; Griffiths *et al*., 2001). Chemical analyses of litter before and after the experiment would improve our understanding of the different rates found in the site and uncover heterogeneity in the decomposer activity. Litter from different origins can have non-additive effect on decomposition process and decomposer communities (Schädler & Brandl, 2005; Swan & Kominoski, 2012). Litterbags experiment with litter from different origins and different proportions should consequently also be included. Direct correlation between microbial diversity and decomposition rates is especially difficult to predict in complex and dynamic environment such as floodplains. The diversity of functional traits might consequently

be a better proxy than species diversity to assess the influence of microbial community on rates of decomposition in the spatio-temporally heterogeneous riparian floodplains (Kominoski *et al*., 2011).

Conclusions

This experiment, which aimed at assessing the decomposition rates heterogeneity, underwent several unanticipated practical problems. The results are consequently limited in scope, hampering definitive conclusions about the interplay between biotic and abiotic factors on the decomposition rates, one of the most important services provided by ecosystems. Despite these limits, our results suggest that the activity of decomposers is spatially heterogeneous and varies among FPZs. In addition, the difference can be tentatively explained by a combination of abiotic and biotic factors. These encouraging preliminary results should motivate future studies, with an improved design to avoid the same problems as experienced in my own work.

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Annexe 2. Short description of the 35 eukaryotic clades detected more than five times in at least four samples, when taxonomic assignment with cover higher than 70% and identity higher than 97% was accepted.

Ancyromonas (=*Planomonas* (Cavalier-Smith *et al*., 2008; Heiss *et al*., 2010))(<10μm) widespread and common, from marine to terrestrial ecosystems. Have 2 flagella. Probably consume bacteria. Previously classified as Infusoria. There are 13 described species. Morphological description and molecular analyses suggest that *Ancyromonas* is a plausible candidate for the closest relative to the common ancestor of metazoans, fungi, and choanoflagellates (the Opisthokonta), but more studies are needed to confirm this (Atkins et *et al*., 2000). Ancyromonas might group with Apusozoa (Cavallier-Smith *et al*., 2008).

Apicomplexa (former Sporozoa**)**(4-9μm long 1-3 μm wide) firstly described by Levine (1970). They are obligate parasites of Metazona, characterized by the apical complex, a group of secretory organelles used during infection stage (Lee *et al*., 2000; Levine, 1973). More than 5000 species have been described, seven of which are human parasites (e.g. *Plasmodium*, the organism responsible for malaria; *Toxoplasma gondii*, *Cryptosporidium parvum*), but some estimate between 1.2 to 10 million species exist (Adl *et al*., 2007), probably at least one for each vertebrate species (Slapeta, 2011). Many of them are insect and earthworm parasites (Gregarines). Grouped with Ciliates and Dinoflagellates into the Alveolata.

Apusozoa (5-20μm) gliding, generally biflagellated, ubiquitous in marine, freshwater, and soil environments (Adl *et al*., 2012; Paps *et al*., 2013). Are characterized by an organic shell under the dorsal surface of the cell. Feed on bacteria. The group contain three orders: Apusomonadida, Planomonadida and Mantamonadida (Glücksman, 2011).

Bacillariophyceae (=*Diatomea*; Adl *et al*., 2012)(<140 μm). Yellow-brown unicellular algae, most live singly, but some form colonies. The shape is round or bilaterally symmetrical (pennate Diatoms). There are ca. 10'000 species, separated in three groups: Coscinodiscophyceae, Mediophyceae, and Bacillariophyceae (Medlin & Kaczmarska, 2004). Characterized by cell wall of silica called frustule, composed by two valves. Common in marine and freshwater, soil and on moist surfaces of plants. They live free or attached to a surface (e.g. rocks or aquatic plants). They are a major component of plankton and are estimated to carry out 20-25% up to 40% of all carbon fixation.

Bicosoecida (2-5μm) group of small unicellular biflagellates, most often planktonic and sometimes sessile (genus *Bicosoeca*). They can be found in marine, freshwater and soil environments. One flagellum anchors them to a surface, while the other brings food particles to an ingestion area. Solitary or colonial, they are bacterivores. Can have a lorica (genus *Bicosoeca)*. Unlike many other Stramenopiles, they lack photosynthesizing plastids and are therefore colourless. Phylogeny is still under revision (Karpov, 2000); Park *et al*., 2006).

Breviatea (previously Mastigamoebae) (8-12μm) are long oval or nearly cylindrical shaped, anaerobic or micro-aerophilic free-living amoeboid flagellate. Considered part of Amoebozoa (Minge *et al*. 2009), even though no morphological affinity has been found, they might be closer to the *Apusozoa* (Walker *et al*., 2006). They present unique combination of morphological, behavioural (e.g. locomotion) and ultrastructural characteristics: They have a single flagellum, like a unikont, but two basal bodies attached to that flagellum, like a bikont, their position in the eukaryotic tree is thus currently debated (Adl *et al*., 2012; Heiss *et al*., 2013).

Centramoebida (15-35μm)(=Acanthopodida) Small amoebozoans frequent in soil and freshwater. Mostly free-living bacterivores, some species opportunistic pathogens. Possess a centrosome, organelle responsible for controlling the activity of microtubules. Were placed in the Amoebozoa, but the phylogeny of amoeboid protists still need to be untangled (Fahrni *et al*., 2003; Pawlowski *et al*., 2009; Smirnov & Burki, 2011) and the taxon Centramoebida is not always recognized (Smirnov & Brown, 2004). Members of the group Discosea (Cavalier-Smith, 2004)

Centrohelida (30-80 μm) unicellular round body with radiating axopods supported by arrays of microtubules, include both mobile and sessile forms. Common in freshwater and marine environments, sometimes also in soils (Li *et al*., 2010). Most have an organic or siliceous coat and feed on other protozoa. This group may be polyphyletic with yet unclear phylogeny (Burki *et al*., 2009; Pawlowski & Burki, 2009; Sakaguchi *et al*., 2005).

Cercozoa (5-50 μm) heterotrophic, they are the most numerous eukaryotes found in soil, but are also present in marine and freshwater environments. Typically unicellular, some are parasite, but most are free-living predators feeding by means of filose pseudopods on bacteria, fungi, other protists or microscopic animals. Have diversified in many distinct body lineages, there are probably tens of thousands of species (Bass & Cavalier-Smith, 2004) and numerous taxa are added after molecular analyses (Chantangsi & Leander, 2010). Separated

in two main groups, Filosa and Endomyxa (Cavalier-Smith & Chao, 2003). They are closely related to Foraminifera (Longet *et al*., 2003) and together with Retaria form the supergroup Rhizaria.

Chlorophyceae (10-24 μm) green algae. Live in fresh water and terrestrial habitats, some marine (Graham & Wilcox, 2000). Can be unicellular, filamentous or form colonies and present different types of thallus. Can have flagella, temporarily or not. Most are free-living, but some (*Chlorella* ssp.) can be endosymbionts of animals or protists. There are approximately 2650 species. Together with Prasinophyceae, Ulvophyceae and Trebouxiophyceae form the group Chlorophyta (Lewis & McCourt, 2004). Green algae are widely used models for research on the evolution of modern land plant from the common ancestor Streptophyta algae (Wodniok *et al*., 2011).

Choanoflagellata (3-10 μm) small free-living or colonial, mobile or sessile, unicellular, round or ovoid, bacterivorous organisms found both in fresh and marine water. Characterised by a collar of microvilli surrounding the base of their flagella that creates current to propulse them and to trap bacteria and detritus. As they are the sister group of Metazoa, they are used as models to study the evolution of multicellularity in animals. There are more than 125 described species (King *et al*., 2008). Some form colonies, some have a lorica.

Chrysophyceae (4-45 μm) golden algae (contain fucoxanthin pigment) found mostly in freshwater, but also moist soil and mosses. Usually unicellular, but some multicellular, with 2 flagella, some are sessile, can form colonies and can form a lorica. Secondarily heterotrophic (soil species) or mixotrophic. Produce cysts; loricate species encyst within the shell. There are around 1000 species described, possibly many more..

Ciliophora (ciliates; 10-500 μm, some as big as 2mm) unicellular common in all aquatic environments, soils and sediments, symbiotic and (ecto or endo)parasitic. They are primarily covered with flagella (cilia), most are heterotrophic, feeding on bacteria, algae and detritus, other protists and even other ciliates; a few are mixotrophic. Characterised by the presence of two different types of nuclei (macro-nucleus and micro-nucleus). There are around 8000 described species.

Cryptophyta (2-20 μm) small algae, typical phytoplankton common in freshwater (greater abundance) and marine water (greater diversity), but also found in sandy beaches. Dorsoventrally flattened, they have 2 flagella. Characterised by ejectosomes, extrusomes able

to propel them away of disturbance. Most are photosynthetic, may exhibit mixotrophy. Some form cysts to survive unfavourable conditions, some form sessile stages. Can be important food source for small plankton, including Ciliates and Dinoflagellates. Having acquired photosynthesis by secondary endosymbiosis (Gould *et al*., 2008), they are largely studied to understand endosymbiosis and evolution of plastids.

Dinoflagellata (10-100 μm) unicellular, form marine plankton, but also common in freshwater, some species are found in snow and wet sand. They have usually two dissimilar ventral flagella. Can have many different form, their cell is covered by a complex cover called amphiesma composed of alveoli that support cellulose plates to form an armour, the theca, when it is present. Can be mixotrophes, photosynthetic (some with chloroplasts, others host a phototrophic symbiont) or prey on bacteria, cyanobacteria, diatoms, ciliates and other dinoflagellates. Some are endosymbiont of marine invertebrates (e.g. corals) or protists (e.g. Foraminifera; Pochon & Pawlowski, 2006), some are parasitic. Their nucleus, called dinokaryon, host chromosomes that are constantly in a condensed state and their genome is exceptionally big and varied, rich in non-coding and repetitive sequences (Lin, 2011). Can form cysts during environmental stress. There are almost 2300 species described. The phylogeny and diversity of the group is a work in progress (Daugbjerg *et al*., 2000; Murray *et al*., 2012; Percopo *et al*., 2013).

Euglenida (<400 μm) free-living, aquatic flagellates, a few are endosymbiotic. Has been linked to high organic material richness. Unicellular (mostly free-living but sometimes colonial). Many are photosynthetic, others feed on bacteria or smaller flagellates by phagocytosis or absorb organic particles (osmotrophy). Characterised by a pellicle, strips of proteins along the length of the cell that gives them a distinctive striation. There are approximately 1400 species described, with very high diversity of morphology, behaviour and locomotion. Together with Kinetoplastida, Fornicata, Diplomids, Parabasalia Oxymonads, and others they form the supergroup Excavata, but the phylogeny and diversity of the group is still under investigation (Breglia *et al*., 2007; Chan *et al*., 2013; Simpson *et al*., 2006; Yamaguchi *et al*., 2012).

Eustigmatophyceae (2-4 μm) marine, freshwater and soil living, non-motile unicellular algae (Graham & Wilcox, 2000), characterised by a large orange-red eyespot, called eustigma. Very small group with less than 20 described species, however the genetic diversity suggests higher diversity (Prior *et al*., 2009). They produce lipids and hydrocarbon that might be exploited as biofuel (Hu *et al*., 2008). Belong to the supergroup Stramenopiles.

Flabellinea (15-35 μm) broad and fan shaped naked amoeba with flattened subpseudopodia with no flagella and well developed glycocalix, a protective layer of proteins and polysaccharides. Live on bacteria biofilms and feed on bacteria. Members of the new taxon Discosea (Cavalier-Smith *et al*., 2004), their diversity is still unresolved (Kudryavtsev *et al*. 2005; Pawlowski *et al*., 2009; Smirnov, 2011).

Foraminifera (50 μm <1mm, but some as big as 18cm recorded) mostly marine but also freshwater and soil habitats (Lejzerowicz *et al*., 2006). Most species produce a test of calcium carbonate or agglutination of sediments, but some are naked. They are sensitive to environmental conditions such as temperature and salinity and are therefore used as bioindicators of past and present climatic and environmental conditions (.e.g isotope ratio in foraminiferal carbonate are proxies of ocean pH). Many have photosynthetic algae as endosymbionts, or capture plastids from their prey (kleptoplastidy) others feed on dissolved organic particles, bacteria diatoms, and small copepods. Characterised by net of thin reticulopodia that can emerge from shell for locomotion or predation. Some carry out complete denitrification (Risgaard-Petersen *et al*., 2006). Estimated diversity: 4000 species.

Glaucophyceae (=Glaucocystophyta)(<10 μm) freshwater algae, sometimes found in soil, whose plastids are surrounded by a peptidoglycan layer, which is thought to be a relic of endosymbiotic cyanobacteria. As they are the basal-most archaeplastids, they are studied to understand the evolution of chloroplasts (Keeling, 2004). Form colonies, may be motile, non-motile, or both by stages. Estimated 13 species, not common, therefore poorly studied ecologically. Together with green plants and red algae they for the supergroup Archaeplastida, but the phylogeny of the group is still debated (Nozaki *et al*., 2009, Stiller & Harrel, 2005).

Haptophyta (=Prymnesiophyta) (2-3 μm) mostly marine, a few found in freshwater, unicellular algae with 2 slightly different flagella and 1 haptonema (flagella-like structure that can anchor them to substrate). They are typically covered with external organic scales. The group Coccolithophores is one of the most abundant organisms in nanoplankton, they are very important primary producers. Most are photosynthetic, but some can be phagotrophic or mixotrophic. About 300 described species, but the diversity is likely much higher (Bittner *et al*., 2013).

Heterolobosea (<65 μm) mostly unicellular, found in soil or fresh water, sometimes marine, amoeboid that do not form true pseudopods but instead advance by eruptive waves. Many can alternate between stages of amoeboid form, flagellate form, and cyst in response to environmental stress. A few are facultative endosymbiont of vertebrates and invertebrates. Most feed on bacteria, some on diatoms and other microeukaryotes. The group of Acrasids can form aggregative cellular slime moulds. About 140 species described morphologically, but many more estimated molecularly (De Jonckheere *et al*., 2011a;b). Intragroup phylogeny still needs elucidation (Pánek & Čepička, 2012). Together with Euglenozoa, Jakobida, Parabasalia, Fornicata and Preaxostyla form the supergroup Excavata.

Ichtyosporea (=Mesomycetozoa)(100-360 μm?) small group, mostly parasites of fish and crustaceans, but also symbionts and saprotrophs and some free-living stages in marine, fresh water and soil environment (Mendoza *et al*., 2002; Glockling *et al*., 2013). Most produce flagellate spores, amoeboid stages, and can form cysts. Some present septated hyphae. Phylogenetically near the divergence of fungi and animals (Ragan *et al*., 1996). Around 15 species described, but more are being added to the group (Lohr *et al*., 2010; del Campo & Ruiz-Trillo, 2013).

Kathablepharids (10 μm) colourless heterotrophic flagellates found both in freshwater and marine environments. Have an ejectisome as the Cryptophyta, which are their closest relatives. 5-6 species described, more are increasingly added to the group (Nishimura *et al*., 2012; Okamoto *et al*., 2009).

Kinetoplastida (10-100 μm long, <20 μm large) unicellular flagellate mostly parasitic. Some are found in soil and freshwater and feed mostly on bacteria (Boenigk & Arndt, 2000). Characterised by presence of a kinetoplast, a granule containing DNA in the single large mitochondrion, closely associated with the basal body of the flagellum. A well studied group because it contains the subgroup Trypanosomatida contains parasites that cause major human diseases ((e.g. *Leishmania*, *Trypanosoma*), and also *Ichtyobodo necator*, an economically relevant fish parasite. Together with Euglenida they form the group Euglenozoa, a group that diverged very early from other Eukaryotes (Dacks & Dolittle, 2001).

Labyrinthulomycetes (2-20 μm) single-celled, they are mainly marine decomposers, or parasite of algae, seagrass and molluscs, or with mutualistic and commensalistic relationships. Terrestrial species have also been described as parasites of grass (Bigelow *et al*. 2005). They are very abundant where they occur and their biomass can be comparable or

even occasionally exceed that of bacteria in some environments (Raghukumar & Damare, 2011). Substrata seem to be species specific (Leander *et al*., 2004). Some have a specialised organelle called bothrosome that produces an external cytoplasmic network of filaments, that absorbs nutrients, fix them to its substrate and to glide on. Produce zoospores. Because of their high accumulation of secondary metabolite, they are of industrial interest as biofuel They are members of the Stramenopiles group, 48 species described but many are added by phylogenetic molecular affiliation (Gomaa *et al*., 2013).

Micronuclearia (5μ) spherical naked filose amoebae, non flagellate, with a single pseudopod found in freshwater. Feed on bacteria. Member of group Apusozoa (Cavalier-Smith *et al*., 2008).

Nuclearia (<50 μm) naked amoebae with elongated filopodia, changing shape, may form a cyst. Typically colourless. They are the sister group of all Fungi. Found usually in freshwater or soil. Widespread but not common. Feed on algae and bacteria by phagocytosis. 9 species described.

Oomycota (hyphae diameter 10-50 µm, oogonia 50-130 µm) filamentous heterotrophic osmotrophic protists, saprophytic or parasites. Mostly found in moist to freshwater environment, some are pathogens of major crops, trees and animals (e.g. fish). Some (*Rozellopsis, Olpidiopsis*) are unicellular but most are filamentous. More than 800 species described. Most produce motile zoospores. Because of their fungal-like appearance they were historically considered as fungi, but phylogenetic studies showed they are more related to Brown Algae, Bacillaryophyta and Xanthophyta. Phylogenetic relationships within the group are still to be clarified (Carpenter *et al*., 2010; Cooke *et al*., 2000; Lara & Belbahri, 2011; Schröder *et al*., 2011).

Parabasalia (10-200 μm) anaerobic, single-celled flagellate, parasite, including human pathogens, or symbionts in animals especially termites and cockroaches, but also free-living. Lack mitochondria. Members of supergroup Excavata. Phylogeny in progress (Gile *et al*., 2013; Malik *et al*., 2011).

Proleptomonas (5-10 μm) unicellular, with elongated ellipsoid body, widespread, non–phagotrophic, found in nitrogen-rich soil or dung. Only 1 species described *Proleptomonas faecicola*. Supergroup Rhizaria, Cercozoa (Vickerman *et al*., 2002).

Reticulamoeba (3-60 μm) amoeba-flagellate found in marine and freshwater environments and soil, with rounded body shape, irregular outline and long and branched reticulopodia. Feed on diatoms, among others. Supergroup Rhizaria, Cercozoa. Recently described clade, new taxa are added (Bass *et al*., 2012; Grell, 1994). But phylogenetic affiliations still debated (Pawlowski & Burki, 2009).

Rhodophyceae (<15cm) usually multicellular, macroscopic red algae, but some are unicellular or colonial forms. Mostly marine but also found in freshwater (<10% of species) with no flagella. Estimates around 4-5000 species. Economically important as food and for production of compounds such as agar and food additives. Significant primary producers, they also provide shelter for other marine organisms. Some are parasites. Some secrete a calcium carbonate shell and contribute to coral reef formations. Members of supergroup Archaeplastida, together with Glaucophyta, Chlorophyta and Viridiplantae. The large morphological diversity and cryptic species within the group makes species richness estimation difficult and increasing diversity is currently uncovered (Ciniglia *et al*., 2004; Schneider *et al*., 2011).

Trimastix (10-25 μm) free-living heterotrophic flagellates (4 flagella), anaerobes or microaerophiles, they lack mitochondria. Phagocyte small suspended particles and bacteria. Studied in relation to the evolution of mitochondria (Hampl *et al*., 2011). Member of supergroup Excavata. Phylogenetic assignments still ongoing (Hampl *et al*., 2009; O'Kelley *et al*., 1999).

Tubulinea (<0.5mm) cylindrical or sub-cylindrical lobose amoeba including naked (e.g. *Amoeba*) and testate amoebae (e.g. *Arcella*, *Difflugia*). Have no flagella, most use tubuline proteins to form lobopods. Members of supergroup Amoebozoa. Phylogeny still ongoing (Brown *et al*., 2011; Cavalier-Smith *et al*., 2004; Lahr *et al*., 2013; Smirnov *et al*., 2005).

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Patterns of earthworm communities and species traits in relation to the perturbation gradient of a restored floodplain

Bertrand Fournier[a,b,c,*], Emanuela Samaritani[a,b], Juna Shrestha[d], Edward A.D. Mitchell[a,b,c], Renée-Claire Le Bayon[e]

[a] Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland
[b] Swiss Federal Research Institute WSL, Site Lausanne, Station 2, CH-1015 Lausanne, Switzerland
[c] Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Ecological Systems, Station 2, CH-1015 Lausanne, Switzerland
[d] Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland
[e] Laboratory of Soil and Vegetation, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland

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ABSTRACT

Little is known about the diversity and ecology of earthworms in floodplains, as well as their response to natural and anthropic perturbations (*e.g.* floods, river channelisation, floodplain restoration). We characterised the patterns of earthworm communities and species traits in the different habitats of a lowland restored floodplain in Switzerland. In addition to classical species-based metrics, such as species richness and Shannon diversity, species traits were used to calculate the community weighted means (CWMs) of traits and functional dispersion (FDis). We hypothesised that trait-based metrics would reveal clearer patterns than classical approaches. The distribution of earthworm traits varied among habitats in relation to changes in flooding frequency: poorly developed gravel bar soils most exposed to flooding were characterised by high abundance of small epigeic species and low abundance of large anecic species. Differences in anecic and endogeic earthworm community structure matched flood frequency. In agreement with our hypothesis, CWMs were more strongly correlated to environmental variables than species composition, diversity, or functional diversity. Based on these results, the ratio of the relative abundances of epigeic and anecic species, and the differences in species composition within anecic and endogeic ecological types of earthworms were identified as indicators of soil development in floodplains.

1. Introduction

Floodplains are among the most threatened ecosystems worldwide (Malmqvist and Rundle, 2002; Tockner and Stanford, 2002). In the last decades, a paradigm shift has taken place in river management, the dominant view shifting from controlling rivers to restoring their natural states and functions. This has led to major changes in policy, such as the water framework directive (WFD; 2000/60/EC) in the EU. As a result of these policy changes, an increasing number of river restoration projects are being conducted in Switzerland and worldwide (Nakamura et al., 2006; Palmer and Bernhardt, 2006; Palmer et al., 2005; Wohl et al., 2005). These projects generally aim to improve the flood protection and biodiversity reservoir functions of floodplains. However, their impact on the terrestrial ecosystems of floodplains remains poorly understood, especially with respect to the soil fauna. Soil organisms include many potential indicators of river restoration success but this potential has not yet been studied much (Bullinger-Weber et al., 2007; Fournier et al., 2012; Guenat et al., 1999). Among the candidates, earthworms are recognised as good bioindicators of soil conditions in alluvial ecosystems (Bullinger-Weber et al., 2012; Salomé et al., 2011) and could therefore provide useful information for monitoring of restoration projects.

Earthworms are present in most terrestrial ecosystems of the world. Their abundance in soils is principally affected by soil properties (*i.e.* texture, organic matter, pH, depth, and water content), land management (*e.g.* land use, agricultural practices), climate, and other biotic factors (Edwards, 2004; Edwards and Bohlen, 1996). Earthworms modify soils mainly through bioturbation (Meysman et al., 2006) thus participating actively to soil pedogenesis. Their activity affects water infiltration (*e.g.* Shipitalo et al., 2004), nutrient cycling (Butenschoen et al., 2009; Sheehan et al., 2006), organic matter cycling (Koutika et al., 2001), soil structure (Shipitalo and Le Bayon, 2004) and horizon texture (Lavelle, 1997; Lavelle et al., 1997). Their potential as bioindicators of landscape structure, land use and soil pollution has been well studied in many ecosystems

* Corresponding author at: Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland. Tel.: +41 32 718 31 08; fax: +41 32 718 30 01.
E-mail address: bertrand.fournier@unine.ch (B. Fournier).

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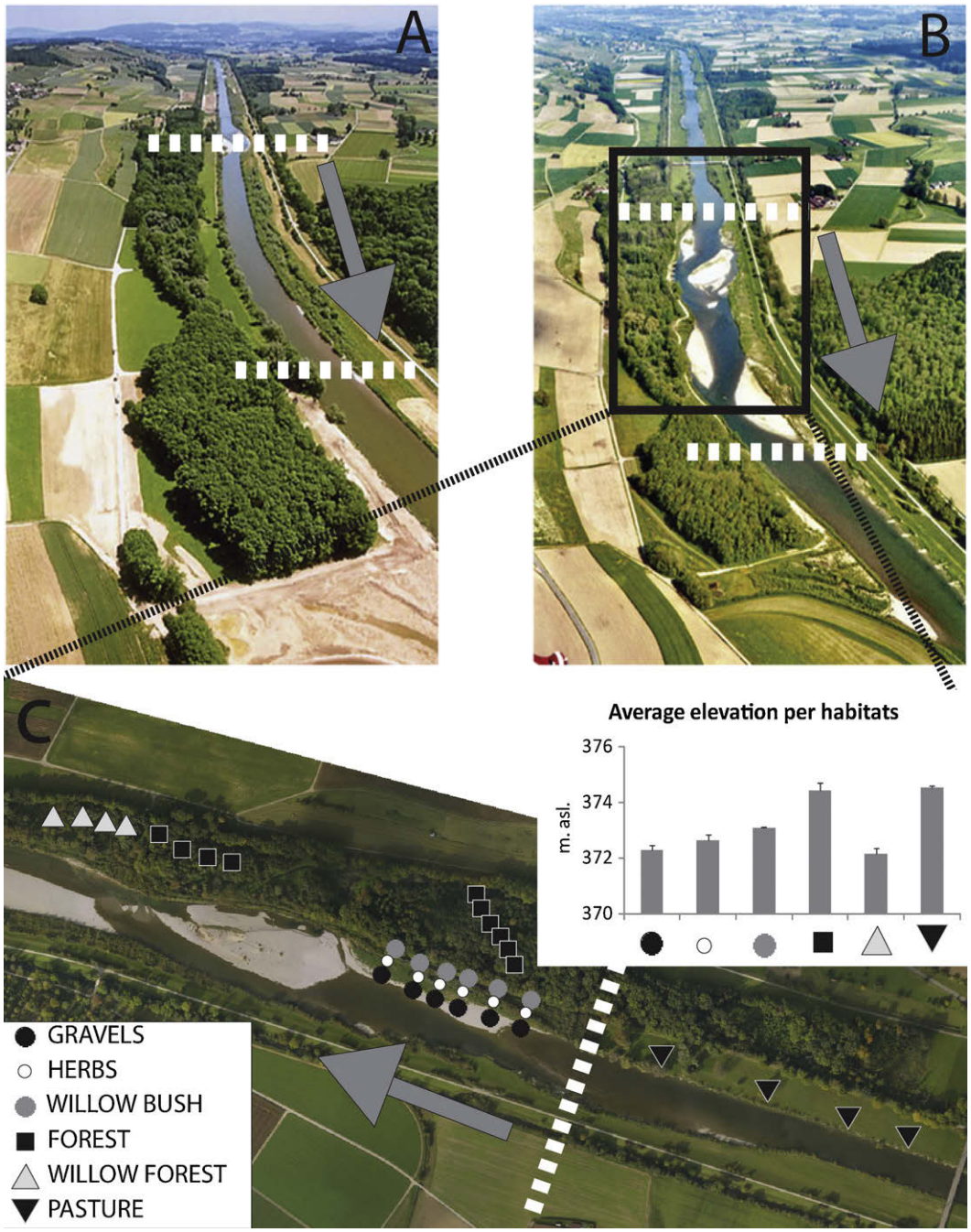

Fig. 1. Thur River (A) before (June 2001), and (B) after the 2002 restoration (May 2004); (C) aerial view of the study site in 2008 showing the plots, and the average elevation of each habitat. Error bars are standard errors. (Pictures A and B: C. Herrmann, BHAteam, Frauenfeld; picture C: CCES RECORD project.) The dashed white lines delimit the restored area; grey arrows give the direction of the river flow.

(Krivolutsky et al., 1982; Paoletti, 1999; Paoletti et al., 1998; Suthar et al., 2008). However, there are comparatively few data on the ecology of earthworms in floodplains (Kamitani and Kaneko, 2007; Zorn et al., 2005).

In flood prone areas, the water holding capacity as well as the organic matter content of the soil are key factors controlling earthworm abundance (Plum and Filser, 2005). Flooding generally has a negative impact on earthworms (Ausden et al., 2001; Ivask et al., 2007; Plum and Filser, 2005), but this effect is species-specific. For example, flooding reduced the total biomass of *Lumbricus terrestris* and *L. rubellus* whereas it had no or little effect on that of *Allolobophora chlorotica* and *Aporrectodea caliginosa* (Zorn et al., 2005, 2008). In subalpine floodplains, epigeic species are considered as bioindicators of recent flood events because of their relation to topsoil texture and organic matter quality (Bullinger-Weber et al., 2012). River restoration was shown to affect negatively *L. rubellus* biomass through a reduction of suitable habitats and an enhanced exposure to contaminants (Thonon and Klok, 2007). Inundations were reported to increase earthworm abundance and biomass in a human transformed ecosystem used for drinking water production by artificial groundwater recharge (Schütz et al., 2008). These observations, and more generally the central role of earthworms in ecosystem development and functioning (Lavelle et al., 1997), lead us to hypothesise that earthworms could be useful bioindicators for monitoring floodplain restoration.

Research in ecology has shown that the analysis of species traits is a useful and powerful approach for understanding ecosystem functioning (Diaz and Cabido, 2001; Díaz et al., 2007; Loreau et al., 2001). Indeed, species traits are often more closely associated to environmental conditions than the actual species (Grime, 1998; Hooper et al., 2005; Tilman et al., 1997). The dominant idea behind this approach is that environmental conditions filter species through their traits. As a result, a species with a given set of characteristics can only survive in a range of conditions that together constitute its ecological niche. These ground concepts in ecology have led to the development of theories such as the habitat templet theory (Southwood, 1977) and are increasingly studied and challenged by ecologists. The trait approach offers an alternative to species abundance or biomass for bioindication that present interesting advantages. Relating species traits to environmental characteristics allows more intuitive understanding of ecosystem functioning as compared to individual species abundance. The trait approach is not hampered by taxonomic difficulties (at least for morphological traits) and not biased by species biogeography. A bioindicator trait can be used across all biomes where the target taxonomic group is present. Earthworm traits have received little attention in ecological studies except for ecological categories as defined by Bouché (1977). However, given the functional importance of earthworms and their sensitivity to waterlogging, we hypothesised that earthworm traits could be used to develop bioindicator tool for environmental management.

In this context, this paper aims at (1) characterising the patterns of earthworm community structure, species composition and species traits in the different habitats (gravel bars to floodplain forests) of a lowland floodplain in Switzerland, (2) assessing the relationships between these patterns and environmental variables, and (3) discussing the potential use of earthworms as bioindicators of restoration.

2. Material and methods

2.1. Study site

The study site is a floodplain located along the Thur River, a tributary of the Rhine, in north-eastern Switzerland (8°77′12″E; 47°59′10″N). It is situated at 365 m a.s.l. and has a temperate climate (annual precipitation ca. 1000 mm year^{-1}, average annual temperature 7.9 °C; http://gate.meteoswiss.ch/idaweb). The average annual flow (1904–2005) of the river is 47 m^3 s^{-1} with peaks above 1000 m^3 s^{-1} (http://www.hydrodaten.admin.ch/d/2044.htm). The site was channelised and levies built until 2002 (Fig. 1A) when it was restored through widening of the riverbed from 50 m to 150 m and bank stabilisation by plantation of willows (Fig. 1B). See Hostmann et al. (2005) for more technical details on the study site restoration.

The Thur River site constitutes an ideal lowland river restoration study case to assess in detail the impact of changed inundation regime on the soil fauna. The study site is divided into a restored section and a non-restored section (Fig. 1). Six different habitats were selected based on elevation and distance to the river, soil type, vegetation structure, and impact of restoration (Samaritani et al., 2011). The French soil classification (Baize and Girard, 2009) was preferred over the FAO World Reference Base for Soil Resources (IUSS Working Group WRB, 2006) because the latter does not discriminate different types of Fluvisols (the dominant soil taxon within the study site). Close to the river, three habitats were selected within the dynamic area. Bare gravels with patches of poorly developed soil – FLUVIOSOLS BRUTS – and pioneer vegetation constituted the first habitat (GRAVELS). The second habitat (HERBS) was an area with more developed soils – FLUVIOSOLS JUVENILES – but showing high spatial and temporal heterogeneity (Samaritani et al., 2011) and dominated by tall herbs (*Phalaris arundinacea*). The third habitat (WILLOW BUSH) was characterised by soils of average depth (FLUVIOSOLS TYPIQUES) and patches of planted willow bushes. The last two habitats were forests growing on deep soils (FLUVIOSOLS TYPIQUES), subjected to limited influence of flooding, dominated either by old willows (*Salix alba* – WILLOW FOREST) or composed of mixed deciduous tree species (*Acer pseudoplatanus*, *Fraxinus excelsior* – FOREST). These two forest sites were present before restoration but were increasingly influenced by the fluvial dynamics following the restoration. In addition, a pasture (PASTURE) located directly upstream from the restored site in an area still protected from floods by levees was sampled as a reference of the state of the ecosystem before restoration. This habitat was replaced by GRAVELS, HERBS and WILLOW BUSH in the restored section.

We first analysed the general patterns of earthworm communities in the six habitats. To assess the impact of river restoration, we then compared GRAVELS, HERBS, and WILLOW BUSH to PASTURE. FOREST and WILLOW FOREST were not considered in this comparison because (1) they existed prior to the restoration, (2) they were only marginally influenced by the restoration, and (3) no comparable habitats were available in the reference area (Fig. 1). Given the absence of natural ecosystems comparable to the study site in the region, we selected the PASTURE habitat as reference. The advantage of this approach is that all sites share the same climate, geology, river flow rate, and potential species pool. The selected habitats are exposed to different flood dynamics (ranging from 24 floods per year to one flood every 2 years; www.hydrodaten.admin.ch/d/2044.htm) and different water table levels (high at both extremes and low in the middle of the gradient; lowest in PASTURE), but are otherwise all exposed to the same climatic, geological, and river flow conditions.

2.2. Sampling

Targeting a snapshot of the ongoing ecological processes, earthworms were sampled in September 2008 using the mustard extraction method after a period of two weeks without flood and rain (Lawrence and Bowers, 2002). This method was preferred over electrical or formalin solution extractions because of safety (proximity of the watertable), environmental (pollution of the aquifers), and legal (it is illegal to use formalin in Switzerland) issues. The mustard extraction method preferentially targets anecic species (Chan and Munro, 2001; Lawrence and Bowers, 2002). Indeed, endogeic species may either not be reached by the solution or may escape laterally rather than toward the soil surface. However, the importance of this bias is determined by soil permeability, being strongest for the less permeable soils with high clay content and minimal for well-drained sandy to loamy soils such as the FLUVIOSOLS studied here. Furthermore, should this bias still affect our sites, it may affect the absolute results, but probably not the interpretation of patterns among habitats, which is the main goal of our study.

The sampling design consisted of 36 plots distributed among six habitats. The habitats exposed to more than one flood per year (GRAVELS, HERBS, WILLOW BUSH) were sampled using six replicates, whereas the habitats exposed to less than one flood per year (WILLOW FOREST, PASTURE) were sampled using four replicates (www.hydrodaten.admin.ch/d/2044.htm). Ten replicates were used in the forest (FOREST) to cover a gradient in topography and vegetation within this otherwise relatively homogeneous area.

Each plot consisted of circle of four meters radius disposed regularly in each habitat, avoiding highly heterogeneous areas. Within each plot, two homogeneous areas of one squared meter were

delimited and watered with ~36 l of mustard powder solution [10 g l^{-1}]. On sloping plots, more solution was used in order to compensate for runoff and thus ensure soil saturation. Individuals were sampled within the delimited areas, stored in formaldehyde 4% and brought back to the lab for species level identification (Bouché, 1972; Sims and Gerard, 1999). Juveniles classified as individuals with tanylobic or epilobic prostomium (Bouché, 1972) were not included in the final matrix (sites × species), but were used for overall density and biomass calculations.

All individuals were measured (see supplementary material) and weighed. Information on other traits such as species length (type of variable: continuous), number of segments (continuous), pH ecological optima and range of tolerance (continuous), prostomium type (binary; tanylobic or epilobic shaped prostomium), ecological type (qualitative ordinal; epigeic, anecic; and endogeic), and preference for given C/N ratios (binary; low = 0 and high = 1) was gathered in the literature (Bouché, 1972, 1977; Sims and Gerard, 1999).

Geographical coordinates and elevation of sample sites were measured at the centre of the plots with a differential GPS. Relative covers of the tree, bush, and herbaceous strata, as well as litter, dead wood, and mosses were expressed as percentage of the total plot area following Braun-Blanquet (1964). Soil variables focused on the structure and chemical composition of the uppermost layer of the soil profile (topsoil). The coarse material size distribution (*i.e.* gravels of various sizes; large > 5 cm, medium > 2 cm, small) of the uppermost 5 cm of soils were visually estimated *in situ* following the key of Baize and Jabiol (1995). For organic (OC), total carbon (C) and total nitrogen (N) measurements, three cores of 10 cm depth and 6 cm diameter were extracted at each sampling site, homogenised and sieved at 2 mm, and measured following the methods of Walthert et al. (2010). The minimum flow rate required to flood each habitat was determined by Samaritani et al. (2011) from inundation maps produced by digital terrain modelling based on river cross section measurements. The average number of floods per year was calculated for each habitat using river flow measurement data covering the period from 2003 to 2008 (www.hydrodaten.admin.ch/d/2044.htm). Water table depth was best estimated as the difference between habitat elevation and river level (Dr. Tobias Vogt, personal communication) (Table 1).

2.3. Numerical analyses

We first structured the data into three matrices: *L* (*sites* × *species*), *Q* (*species* × *traits*), and CWM (*sites* × *traits*). The two earthworm sub-samples for each plot were summed to build the matrix *L*. For matrix *Q*, binary traits were treated as continuous variables, and all other variables were continuous or ordinal. To assess the changes in trait composition at the community level, we calculated the community weighted means (CWMs) of traits using the following formula for each trait:

$$\mathrm{CWM} = \sum^{n} p_i \times \mathrm{trait}_i \quad (1)$$

where p is the relative contribution of species$_i$ to the community and trait$_i$ is the value of the considered trait for species$_i$. CWMs were scaled prior analyses.

To assess the changes of earthworm communities in species composition, species mean density and biomass, species richness and evenness as well as Shannon diversity were calculated for each plot. Deltas were then calculated for the density and biomass of each species, as the difference between the dynamic-restored and reference area as follows:

$$\Delta_x = X_{\mathrm{dynamic\_restored}} - X_{\mathrm{reference}} \quad (2)$$

where X = mean abundance [ind m^{-2}] or mean biomass [g m^{-2}].

Species that increased both in density and biomass were considered as "species that benefit most from the restoration" whereas species that decreased in density and biomass were considered as "most dramatically impacted by the restoration".

We then analysed the internal structure of *L* and CWM matrices using principal component analyses (PCA) and between class analysis (BCA), and their relationships with environmental variables using redundancy analyses (RDA). Earthworm species data were Hellinger transformed before PCA, BCA, and RDA analyses (Legendre and Gallagher, 2001). We used PCA to characterise the distribution patterns of earthworm species and traits and BCA Monte Carlo tests (Dolédec and Chessel, 1987) were performed to discriminate the different habitats and areas. Functional dispersion (FDis) was calculated for each plot (Anderson, 2006; Laliberté and Legendre, 2010). We assessed whether biomass, density, species richness, Shannon diversity, CWM and FDis values differed among habitats and between the two areas using Mann–Whitney tests.

We used redundancy analyses (RDA) to determine the impact of environmental variables on earthworm community composition and functioning. The environmental dataset was scaled and centred and then used as explanatory matrix in the RDA models. The CWM and species per site matrices were alternatively used as response matrices. For each RDA model, we calculated the cumulated proportion of explained variance (EV) by all constrained axes as well as the EV of the two first RDA axes. The significance of RDA models, RDA axes, and variable contributions were then tested using ANOVA permutation tests. The relative goodness of fit of each model was then assessed by calculating the Akaike's Information Criterion (AIC; Sakamoto et al., 1986).

All analyses were performed with the R statistical software (R Development Core Team, 2010) using the "vegan" (Oksanen et al., 2010), "FD" (Laliberté and Shipley, 2010), and "ade4" (Dray and Dufour, 2007) packages.

3. Results

In total, 3707 earthworms were sampled representing an overall biomass of 1126 g. The average biomass was 28 g m^{-2} and 35 g m^{-2} in the restored area and the reference area, respectively, and the average abundances were respectively 93 and 65 individuals per square meter (ind m^{-2}) with maximal values of 394 ind m^{-2} in HERBS and minimal values below 5 ind m^{-2} in GRAVELS. Earthworm biomass was the highest in FOREST with up to 70 g m^{-2} and the lowest close to the river (GRAVELS) with values below 5 g m^{-2}.

A total of 15 species and subspecies were identified (Table 2) of which 10 benefitted from the restoration whereas five and the juveniles with a tanylobic prostomium were negatively impacted. The former accounted for 9.5% of the total biomass and 17.8% of the total density, and the latter 42% and 15.5% respectively. Of the species that benefitted from the restoration, four were epigeic, two were endogeic, and two more were epiendogeic, but none was anecic. Of the five negatively impacted species, four were anecic species and one was endogeic.

Clear differences in community composition, biomass, and density were observed among habitats and especially between the most dynamic habitats and the more stable forest and pasture (Fig. 2). Earthworm abundance was similar across all habitats except for HERBS where the highest average number of individuals per square meters (260 ind m^{-2}) was recorded. The percentage of juveniles within the community was highest in HERBS (75%) and lowest in GRAVELS (56%).

All species were present in the restored area whereas nine were found in the non-restored area (PASTURE). Within the restored area, none of the habitats hosted all the species, the maximal total richness (*i.e.* total number of species and subspecies present in a

Table 1
Summary of the numbers of earthworm individuals caught in the Thur River study site for each species within each habitat. Flood related variables are also given for each habitat.

	GRAVELS	HERBS	WILLOW BUSH	FOREST	WILLOW FOREST	PASTURE
Allolobophora chlorotica	48 ± 0.6	312 ± 4.3	19 ± 1	55 ± 2.7	13 ± 0.6	14 ± 0.4
Allolobophora georgii	0 ± 0	0 ± 0	0 ± 0	2 ± 0.1	0 ± 0	0 ± 0
Aporrectodea caliginosa caliginosa	0 ± 0	18 ± 1.2	7 ± 0.3	38 ± 1.1	13 ± 0.9	1 ± 0.1
Aporrectodea c. nocturna	0 ± 0	3 ± 0.1	9 ± 0.1	48 ± 0.8	12 ± 0.8	13 ± 0.7
Aporrectodea c. tuberculata	0 ± 0	3 ± 0.2	4 ± 0.2	14 ± 0.5	22 ± 0.2	24 ± 0.7
Aporrectodea giardi	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.1	1 ± 0.1
Aporrectodea longa	10 ± 0.4	21 ± 0.7	29 ± 0.8	31 ± 0.6	16 ± 0.7	43 ± 1
Aporrectodea rosea	0 ± 0	0 ± 0	2 ± 0.1	33 ± 0.7	21 ± 1.1	0 ± 0
Dendrodrilus rubidus	0 ± 0	2 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Eiseniella tetraedra	14 ± 0.6	18 ± 0.4	0 ± 0	1 ± 0.1	0 ± 0	0 ± 0
Lumbricus castaneus	0 ± 0	3 ± 0.1	2 ± 0.1	2 ± 0.1	7 ± 0.3	1 ± 0.1
Lumbricus meliboeus	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 0.2	0 ± 0
Lumbricus rubellus	5 ± 0.3	21 ± 0.9	9 ± 0.4	6 ± 0.2	5 ± 0.3	2 ± 0.2
Lumbricus terrestris	0 ± 0	1 ± 0.1	4 ± 0.2	34 ± 0.4	13 ± 0.3	8 ± 0.4
Octolasion tyrtaeum tyrtaeum	0 ± 0	1 ± 0.1	1 ± 0.1	5 ± 0.3	1 ± 0.1	0 ± 0
Juveniles epilobiques	51 ± 4.5	67 ± 1	57 ± 1.7	58 ± 1.4	53 ± 1.5	53 ± 2.6
Juveniles tanylobiques	5 ± 1.6	8 ± 1.4	14 ± 1.2	12 ± 0.9	13 ± 2.5	14 ± 0.6
Number of floods per habitat in 2008	24	17	3	1	1	1
Minimum river flow for inundation [$m^3 s^{-1}$]	175	190	300	630	415	415
Depth of the water table [m]	1.41	1.65	1.75	1.71	0.93	3.15

Table 2
Summary statistics of the redundancy analyses (RDA) of earthworm data from the Thur River site. Explained variances are given in percent. *p*-values result from ANOVA permutation tests. AIC is the Akaike Information Criterion (Sakamoto et al., 1986).

	Total explained variance [%]	Variance explained by the first constrained axis [%]	Variance explained by the second constrained axis [%]	Model *p*-value	First axis *p*-value	Second axis *p*-value	AIC
Species	63.99	19.22	11.23	0.62	0.22	0.9	104.02
CWM	77.72	49.01	15.81	0.04	0.03	0.73	69.34
Species richness	71.79	71.79	NA	0.27	0.27	NA	0.69
Shannon diversity	79.63	79.63	NA	0.08	0.08	NA	−10.69
FDis	78.56	78.56	NA	0.17	0.09	NA	−8.91

habitat) being reached in the forest habitats (FOREST and WILLOW FOREST) with 12 species, and the minimal close to the river (GRAVELS) with four species. The indices accounting for the variance of species and CWMs matrices revealed a trend toward increasing functional and taxonomic diversity with decreasing perturbation (Fig. 3). GRAVELS and HERBS had relatively low values for all indices whereas the contrary occurred in WILLOW FOREST. WILLOW BUSH and FOREST showed a higher variation, although this variation was relatively small for functional dispersion in FOREST.

In both PCAs based on density and on trait data (Fig. 3), the habitats were distributed along the first axis according to their position along the fluvial dynamic gradient. Monte Carlo permutation tests gave strong evidence against the hypothesis that all habitats were similar in the species or trait ordination space (p-value < 0.01 in both cases). The samples were organised in two clusters: the first was composed by the habitats most prone to flooding (*i.e.* GRAVELS and HERBS) and occupying little ordination space, and the second included the habitats influenced to a lesser extent by fluvial dynamism and covering much more ordination space. *A. chlorotica* and *Eiseniella tetraedra*, and to a lesser extent, *Lumbricus rubellus* and *Dendrodrilus rubidus* were associated with GRAVELS and HERBS whereas *L. terrestris*, *A. caliginosa nocturna*, and *A. c. tuberculata* were associated with the most stable conditions. This pattern was identical for abundance data (shown here) as well as biomass data (not shown). In stable habitats, earthworms were large and heavy. Communities contained an important proportion of anecic species and they differed from those of dynamic habitats in their pH optima and C/N ratio preference.

The RDA model on CWMs was significant ($P = 0.04$) and revealed strong correlation to environmental variables. In this model,

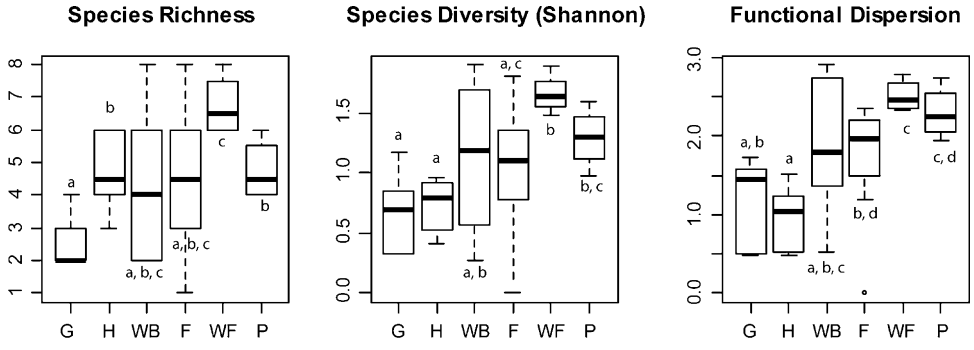

Fig. 2. Boxplots of earthworm species richness, diversity, and functional dispersion (*alpha* values) for all habitats of the Thur River site (G: GRAVELS, H: HERBS, WB: WILLOW BUSH, F: FOREST, WF: WILLOW FOREST, P: PASTURE). Error bars represent standard errors.

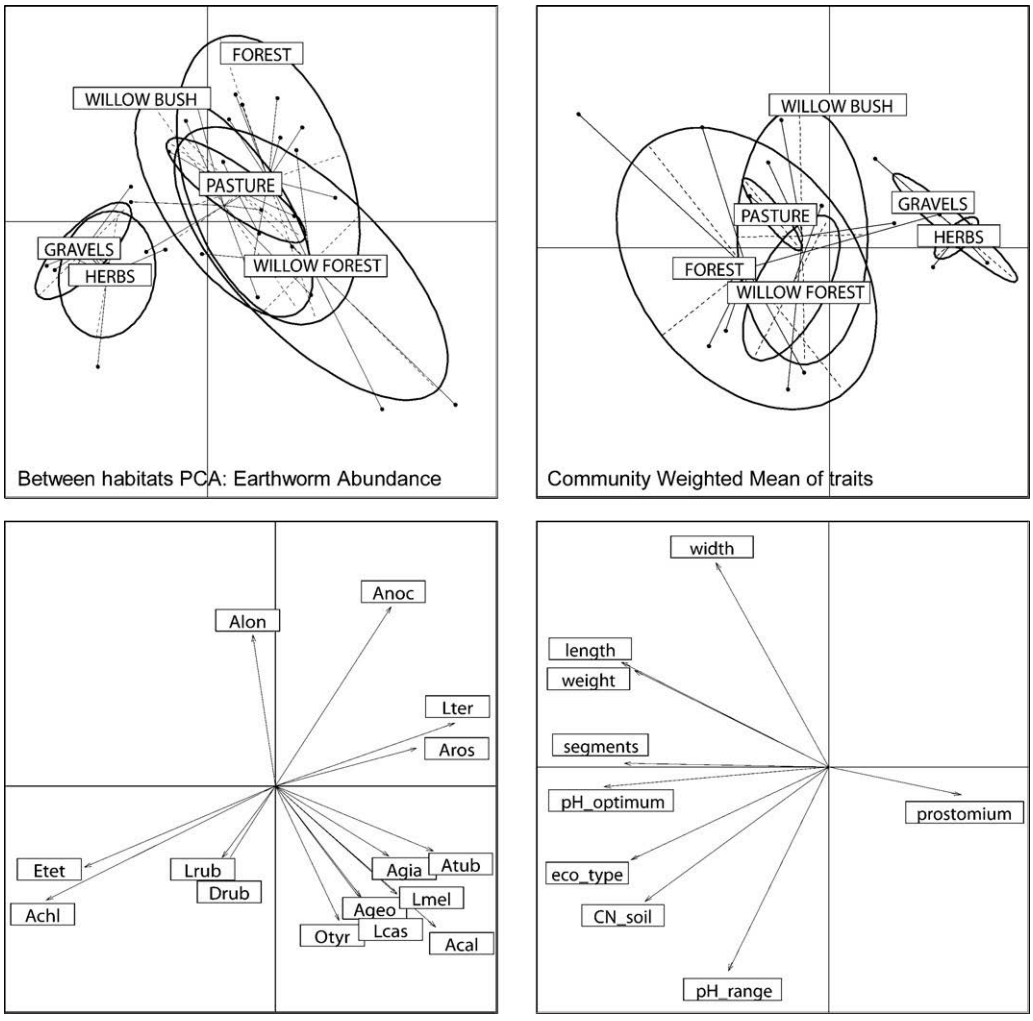

Fig. 3. Principal component analyses (PCA) of earthworm abundance and community weighted means of traits data from the Thur River site. Ellipses highlight the different habitats; and arrows the position of species or traits within the ordination space. Species name abbreviations are composed of the first letter of the genera and the three first letters of the species name.

earthworm communities were distributed along the first axis, which corresponded to the influence of flood regime (Fig. 4) and was significantly correlated with the average number of floods per year and the relative cover of woody debris. By contrast, in RDAs based on species composition, diversity, or functional diversity the correlation was weaker and the models non-significant (Table 2).

4. Discussion

At the floodplain scale, the observed values for biomass, abundance, species richness, and diversity were similar to those recorded in comparable settings (Ivask et al., 2007; Plum and Filser, 2005; Salomé et al., 2011; Zorn et al., 2005) and testify from well-developed earthworm communities. The PCA and Monte Carlo tests (Fig. 3) clearly showed that the investigated habitats could be separated into two groups.

Earthworm communities of the first group – GRAVELS and HERBS – were dominated by relatively small and epigeic taxa of low biomass, more specifically by species characterised by an epilobic type prostomium, preferring high C/N ratios, more acid conditions, and having relatively low tolerance to pH variations. These adaptations reflect the *in situ* conditions encountered by earthworms in our study. Caution must however be taken talking about relative acid conditions considering that geological substrate consists of carbonates. Moreover, in these habitats, total soil carbon content and litter input (constituted almost exclusively of *P. arundinacea*) were high; soils were thin and poorly developed because of the regular impact of floods (Guenat et al., unpublished results). Indeed, dynamic processes such as sedimentation, aggradation, and – predominantly in our case – erosion did not allow sufficient time for *in situ* pedogenesis to occur. The preference of earthworms for more acidic conditions could be explained by the deposition by the river of exogenous acidic material such as soil layers eroded from upstream banks, vegetation, mineral aggregates of various sizes, and organic matter. At the species level, *A. chlorotica*, *E. tetraedra*, and *L. rubellus* – three epigeic *r*-strategists with fast maturation and high reproduction rates (Bouché, 1972; Gerard, 1967; Satchell, 1967) – dominated the communities in GRAVELS and HERBS. *E. tetraedra* is considered as characteristic of river banks (Bouché, 1972) and indeed this species was among the species that benefitted most from the restoration. *A. chlorotica* is characteristic of perturbed environment (*e.g.* building sites) that are returning to their equilibrium states (Bouché, 1972). This species likely took advantage of the perturbation generated by the restoration process to increase in density and biomass. *L. rubellus* is a successful coloniser (Eijsackers, 2010) well adapted to flooded soils (Roots, 1956) such as those found in the newly created habitats (GRAVELS and HERBS). However, *L. rubellus* was shown to be more sensitive to flooding than *A. chlorotica*; and its response to such perturbations consists mainly of escaping to more favourable habitats (Simonsen

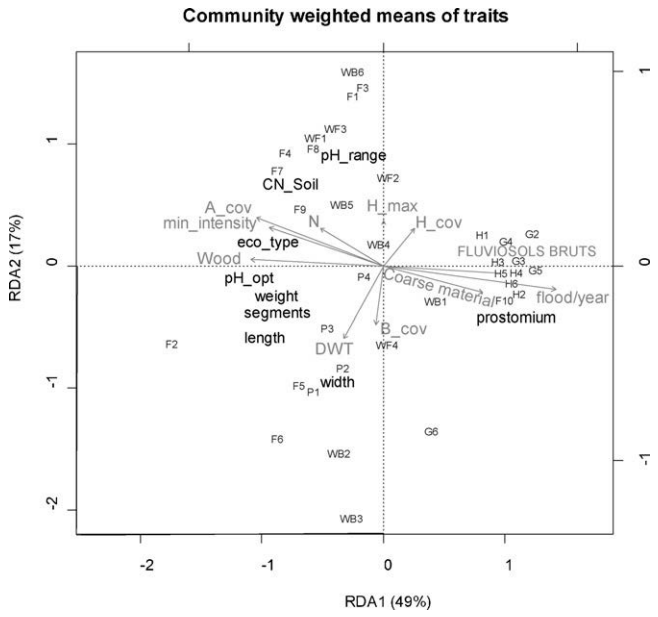

Fig. 4. RDA triplot of earthworm community weighted means of traits (black) and environmental variables (grey) from the Thur River site. Explained variance is given in brackets for each axis. Site abbreviations (black, smaller characters) are composed by the first letter(s) of the habitat and the replicate number (G: GRAVELS, H: HERBS, WB: WILLOW BUSH, F: FOREST, WF: WILLOW FOREST, P: PASTURE).

and Klok, 2010; Zorn et al., 2008). In agreement with this, *L. rubellus* was less abundant and reached lower biomass than *A. chlorotica* and *E. tetraedra* in flood prone sites. We therefore conclude that the optimal strategy for earthworms to colonise habitats submitted to high flood dynamics consists of being epigeic and having fast growth and high reproduction rates and good dispersal ability together with a propensity to tolerate flood. Moreover, the abundance and distribution (including patchiness) of dynamic flood-prone habitats along rivers is likely to play a crucial role in the dispersal of these species.

Earthworm communities in the second group of habitats (WILLOW FOREST, FOREST, WILLOW BUSH and PASTURE) were dominated by longer and heavier anecic species such as *Aporrectodea longa*, *A. caliginosa nocturna*, and *L. terrestris*, and species with a tanylobic type prostomium (most likely young individuals of *L. terrestris*) that showed greater tolerance for variation in pH values. Previous works showed that anecic species are strongly related to soil depth (Bouché, 1972; Guenat et al., 1999; Phillipson et al., 1976). Our study confirmed that *A. c. nocturna* and *L. terrestris* prefer thick soils as already shown by Salomé et al. (2011) and, by extension, drier conditions; and provide evidence that *A. longa* adopts a similar behaviour. Among the three species, *A. longa* reached the highest abundance in HERBS and GRAVELS thus showing the greatest tolerance to flooding. Moreover, the three species were present in HERBS and GRAVELS, whereas *A. giardi* and *L. meliboeus* were absent, most likely because they are less tolerant to inundation than the three previously mentioned species. However, *A. giardi* and *L. meliboeus* were found in only two sites and in low abundance. Such difference in flooding tolerance can tentatively be explained by changes in behaviour according to age class or environmental factors. For example we observed that juveniles of *L. terrestris* adopt a more active behaviour relatively similar to epigeic earthworms whereas they are less active and behave as anecic species do when mature.

Endogeic earthworms are generally not tolerant to water saturation (Bouché, 1972). In agreement, *A. rosea* preferentially occupied the driest places within habitats rarely flooded (FOREST and WILLOW FOREST). However, among the exceptions is *A. c. caliginosa*, a relatively small species tolerant to inundation (Zorn et al., 2008). This species was the only endogeic earthworm present in relatively large number in HERBS where the influence of floods is pre-dominant. It was also characteristic of WILLOW FOREST where the influence of floods was relatively low, but where water table was high. The influence of water table most likely prevented species that tolerate water saturation to a lesser extent (*e.g.* anecic species) to develop in large numbers in this habitat.

The observed distribution patterns of individual species across the six studied habitats agree well with their known biological and ecological characteristics. The differences between the dynamic and stable habitats are in line with the decrease of biomass expected by Thonon and Klok (2007) in response to river restoration and illustrate the potential of earthworms as bioindicators.

As a result, different tolerance for flooding within anecic and endogeic species may help discriminating soils less prone to flooding and with no to low hydromorphy, and, by extension, indicating the initial development of alluvial terraces (either by erosion of the river bed leading to a general lowering of the water table, or by deposition of material). It remains to be determined how fast communities adapt to changing conditions, during shifts to either wetter or drier conditions and increasing or decreasing exposure to floods.

Our study confirmed that flood dynamics have a predominant influence on earthworm communities. Most of the patterns observed can indeed be explained by changes in the frequency of flooding along the gradient. Moreover, the linear increase of all indices with decreasing perturbation agreed with hypotheses of increasing belowground diversity with decreasing perturbations (Wardle, 2002). However, high variation in WILLOW BUSH and FOREST complicated the interpretation of the patterns.

Beside this main effect, our results highlighted the impact of litter quality on earthworm traits. Woody debris can enhance water residence time or trap fallen leaves and seeds thus increasing the food resource for earthworm. Moreover they can constitute hot spots of biological interaction among species (*e.g.* predation) because of the large number of small species (*e.g.* arthropods, mammals, and birds) that preferentially live in woody debris. In forest ecosystems, the relation between litter quality (*i.e.* relative cover of woody debris) and earthworm communities suggest possible positive feedbacks leading to spatial differentiation of ecological conditions through time (*e.g.* Ponge et al., 1999). The significant effect of woody debris on earthworm traits suggests that such processes are likely to occur also at the Thur River and contribute toward maintaining forest communities.

The Thur site, despite its small size, provides a good experimental setting to understand the changes that occurred following restoration at a fine scale. Although generalisation of the present results may be difficult, the agreement with findings of other studies confirmed the pertinence of this approach. Moreover the present study is the first, to our knowledge, that deals with earthworm species traits in floodplains. The results showed that this approach is indeed relevant and confirms the potential of earthworms as bioindicators. Moreover, ecological traits revealed more pertinent than anatomical ones, with the exception of earthworm body length. In addition, our results suggest that the ratio of the relative abundances of epigeic and anecic species, and the differences in species composition within anecic and endogeic ecological categories could be used as indicators of soil development and functioning in floodplains. The next steps would require the improvement of the spatio-temporal variability covered by the data, for example, through comparisons with other (natural) floodplains, together with modelling and manipulative mesocosm or field experiments to calibrate bioindication tools usable for management in general.

5. Conclusion

Restoration created habitats (GRAVELS and HERBS) that imposed strong constraints on earthworms mainly related to flood perturbations. This process was the main driver of changes within earthworm communities at the floodplain scale. Epigeic species that are able to live in thin soil (*r*-selected or able to cope with flooding/inundation) rapidly colonised this area (*i.e.* within 5 years) possibly by hydrochory along the river whereas anecic species that dig vertical galleries prone to inundation were rare or absent. The change in species composition of endogeic communities can be interpreted as a shift toward more flood-tolerant species.

As a result, in the context of floodplains, high abundance of epigeic species at the community scale can be considered as indicative of pioneer conditions and early soil developmental stages, while dominance of anecic species indicates low influence of floods and good soil development. Moreover differences in species composition of the anecic and endogeic communities can help in further discriminating local conditions. In the context of river restoration, these results provide environmental management authorities with a potential new tool for monitoring and assessing soil development.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2012.03.015.

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WETLANDS AND AQUATIC PROCESSES

Soil Nitrogen Dynamics in a River Floodplain Mosaic

J. Shrestha, P.A. Niklaus, E. Frossard, E. Samaritani, B. Huber, R. L. Barnard, P. Schleppi, K. Tockner, and J. Luster*

In their natural state, river floodplains are heterogeneous and dynamic ecosystems that may retain and remove large quantities of nitrogen from surface waters. We compared the soil nitrogen dynamics in different types of habitat patches in a restored and a channelized section of a Thur River floodplain (northeast Switzerland). Our objective was to relate the spatiotemporal variability of selected nitrogen pools (ammonium, nitrate, microbial nitrogen), nitrogen transformations (mineralization, nitrification, denitrification), and gaseous nitrogen emission (N_2O) to soil properties and hydrological processes. Our study showed that soil water content and carbon availability, which depend on sedimentation and inundation dynamics, were the key factors controlling nitrogen pools and processes. High nitrogen turnover rates were measured on gravel bars, characterized by both frequent inundation and high sediment deposition rates, as well as in low-lying alluvial forest patches with a fine-textured, nutrient-rich soil where anaerobic microsites probably facilitated coupled nitrification–denitrification. In contrast, soils of the embankment in the channelized section had comparatively small inorganic nitrogen pools and low transformation rates, particularly those related to nitrate production. Environmental heterogeneity, characteristic of the restored section, favors nitrogen removal by creating sites of high sedimentation and denitrification. Of concern, however, are the locally high N_2O efflux and the possibility that nitrate could leach from nitrification hotspots.

River floodplains are among the most dynamic, diverse, and productive ecosystems on earth (Keddy, 2000; Tockner and Stanford, 2002). They provide a wide range of ecosystem services (Naiman and Decamps, 1997; Ward et al., 2002), including nutrient retention and removal (Brunet et al., 1994; Olde Venterink et al., 2006). Specifically, nitrogen is retained by physical (sedimentation) and biological (plant uptake and microbial immobilization) processes or removed through denitrification. These processes improve the surface and groundwater quality (Naiman and Decamps, 1997; Pinay et al., 2002). In floodplains, physicochemical and biological factors typically vary greatly in space and time. Because nitrogen transformations are extremely sensitive to these factors, floodplains cannot be considered as homogeneous buffers (Pinay et al., 1992; Naiman et al., 2005).

Interactions between hydrogeomorphic (flood dynamics) and ecological (biological succession) processes create a dynamic mosaic of habitat patches. These patches differ in their age of formation, inundation regime, and soil properties, as well as in their productivity, organic matter dynamics, and community composition, and are arranged along distinct succession gradients in a floodplain (Naiman and Decamps, 1997; Naiman et al., 2005). The patches expected to differ in their functional performance are defined as functional process zones (FPZs; Thorp et al., 2006; Samaritani et al., 2011). The different FPZs in a floodplain may act as sources or sinks of nitrogen, depending on their soil physicochemical properties, their biological characteristics (vegetation, microbial population), and the micro-environmental conditions (Johnston et al., 2001; Ruckauf et al., 2004; Mentzer et al., 2006). Floods affect the environmental drivers of soil nitrogen transformations (namely, soil moisture, organic carbon, and nitrogen substrate availability) differently, depending on the FPZ (Pinay et al., 2002; Samaritani et al., 2011).

Microbial nitrogen transformations have been studied intensively in floodplain soils. A strong focus has been on

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*Corresponding author (joerg.luster@wsl.ch).

5585 Guilford Rd., Madison, WI 53711 USA

J. Shrestha, B. Huber, P. Schleppi, and J. Luster, Swiss Federal Institute for Forest, Snow, and Landscape Research, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland; P.A. Niklaus, Institute of Evolutionary Biology and Environmental Studies, Univ. of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland; J. Shrestha and E. Frossard, Institute of Agricultural Sciences, ETH Zurich, Eschikon 33, 8315 Lindau, Switzerland; E. Samaritani, Institute of Biology, Univ. of Neuchatel, Rue Emile Argand 11, CH-2000 Neuchatel, Switzerland; R.L. Barnard, Dep. of Agroecology, INRA Dijon, 17 rue Sully, BP 86510, Dijon, France; K. Tockner, Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), and Institute of Biology, Freie Universität Berlin, Müggelseedamm 310, D-12587, Berlin, Germany. Assigned to Associate Editor Jake Beaulieu.

Abbreviations: C_{mic}, microbial carbon; DEA, denitrifying enzyme activity; DEA_{ns}, denitrifying enzyme activity without substrate addition; FPZ, functional process zone; GM, gross mineralization; GN, gross nitrification; N_{mic}, microbial nitrogen; PN, potential nitrification; SWC, soil water content; WEOC, water extractable organic carbon.

denitrification as the process of permanent nitrogen removal, including the effects of the hydrogeomorphic gradient (Clément et al., 2002), soil depth (Hill et al., 2000), carbon availability (Hill and Cardaci, 2004), and vegetation (Hernandez and Mitsch, 2007). Denitrification has frequently been studied together with nitrous oxide emissions, with particular emphasis on spatiotemporal variability at different scales (Dhondt et al., 2004; van den Heuvel et al., 2009). In contrast, studies considering the entire nitrogen cycle are rare (Pinay et al., 1995; Hefting et al., 2005), although in nutrient-rich floodplains all processes of the nitrogen cycle can be very rapid, particularly nitrification during unsaturated conditions (Hefting et al., 2004).

We investigated nitrogen cycling in a mosaic of different FPZs of the River Thur floodplain, Switzerland. There, the frequency and average duration of flooding vary with elevation and distance from the river, and floodplain soils are unsaturated at base flow, i.e., most of the time (Samaritani et al., 2011). We quantified the effect of the spatiotemporal heterogeneity, inherent to the mosaic of FPZs, on the major processes of the nitrogen cycle, including nitrogen mineralization, nitrification, and denitrification. We quantified soil nitrogen pools (extractable ammonium, nitrate, and microbial nitrogen), nitrogen transformation rates based on laboratory incubations (gross mineralization, gross and potential nitrification, and denitrification enzyme activity with and without substrate addition), and the surface efflux of nitrous oxide (N_2O) seasonally from April to October 2009. The study was performed mainly in a restored section of the Thur River reflecting a successional gradient of FPZs, from bare gravel areas to mature alluvial forests, and additionally on the embankment in the channelized section. Our objectives were (i) to relate the spatiotemporal variability of nitrogen pools, transformation rates, and emissions to soil properties and degree of flood-related disturbances, and (ii) to examine the linkages between the various nitrogen transformation processes. We hypothesized that flood-related variability in soil moisture and the available carbon would drive changes in nitrogen dynamics and that the magnitude of change would differ between FPZs with increasing degrees of flooding frequency and duration.

Materials and Methods

Study Site

The study was performed at the main study site of the interdisciplinary project RECORD (Restored Corridor Dynamics, http://www.cces.ethz.ch/projects/nature/Record) in the Thur River corridor at Niederneunforn (northeast Switzerland, 8°77′12″ E; 47°59′10″ N). During the study year (2009), the total precipitation was 908 mm, and the average monthly temperature ranged from 0.9°C (January) to 19.0°C (July) (Meteoswiss, https://gate.meteoswiss.ch/idaweb). The Thur River is characterized by a flashy flow regime with frequent floods throughout the year (average discharge: 50 m^3s^{-1}; range: 2–1130 m^3s^{-1}; Federal Office for the Environment, Switzerland, recording period 1904–2005, http://www.hydrodaten.admin.ch/d/2044.htm; Fig. 1). The main stem of the Thur River was channelized in the 1890s to protect the valley against flooding. However, since 1993, several river sections have been restored, including the 2-km-long study section. Widening of the channel has allowed gravel bars to form, and lowering the levees has increased hydrological connectivity between the main channel and the fringing alluvial forest. Overall, restoration has led to the formation of a succession gradient composed of different FPZs (see below).

A detailed description of the study site and the characterization of the various FPZ are given in Samaritani et al. (2011). Briefly, the six FPZs were classified according to their vegetation, distance from the river, and topography and then combined into three types: (i) dynamic FPZs in the restored section on and next to a frequently flooded gravel bar: a mosaic of bare gravel and patchy vegetation (GRAVEL); gravel covered by up to 1m of fine sediments (GRASS); the banks connecting the gravel bar with the forest (WILLOW BUSH); (ii) stable FPZs in the restored section: two types of forest communities characteristic of floodplains (MIXED FOREST, WILLOW FOREST); and (iii) the embankment in the adjacent channelized section (PASTURE). Table 1 gives an overview of

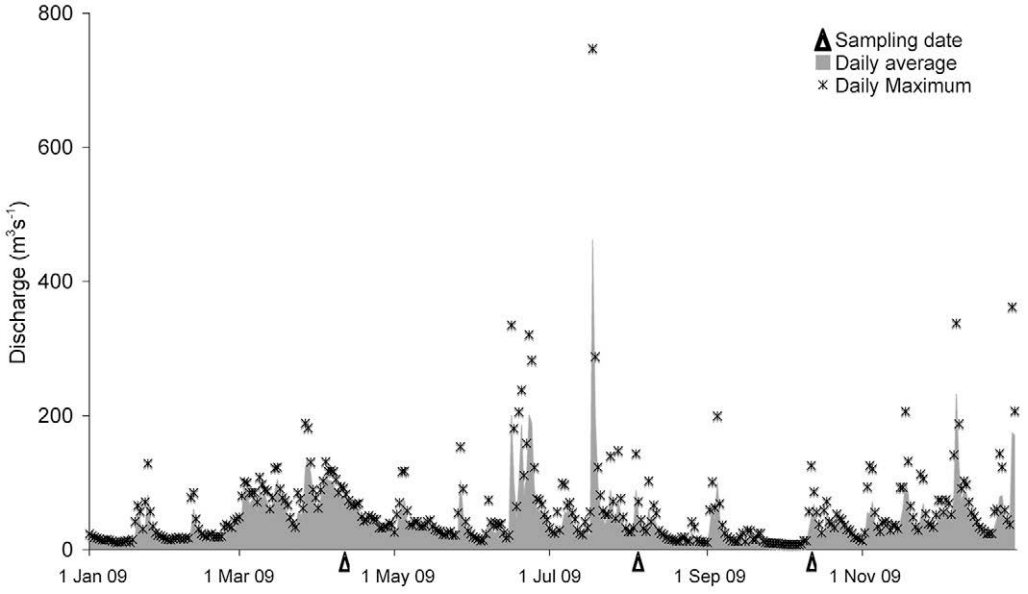

Fig. 1. Daily average and maximum of the Thur River discharge at the study site in 2009. Sampling dates are marked as triangles on the *x* axis (Source: Federal Office for the Environment, Switzerland).

important characteristics of the FPZs. Within each FPZ, four plots 8 m in diameter were selected to assess spatial variability (Fig. 2). The upstream half-circle of each plot was used for biodiversity monitoring and gas sampling, and the downstream half-circle was used for destructive soil sampling. During the sampling period, GRASS and WILLOW BUSH were inundated during three consecutive flood events in June 2009, and one major flood on 18 July 2009 inundated the entire floodplain except for one high-lying plot in MIXED FOREST. GRAVEL was inundated more frequently, i.e., at discharge > 100 $m^3 s^{-1}$ (Fig. 1).

Table 1. General characteristics of the different functional process zones (FPZs) of the study site in the Thur River floodplain. For details, see Samaritani et al. (2011).

		GRAVEL	GRASS	WILLOW BUSH	MIXED FOREST	WILLOW FOREST	PASTURE
Implication of restoration		Gravel bar exposed during restoration and further developed later	Formed after restoration through sediment trapping and colonization by *Phalaris arundinacea*	Levee forming the new bank after widening; planted with willow saplings for stabilization	Hydrologically reconnected with river; inundated during high floods	Hydrologically reconnected with river; inundated during high floods	Nonrestored channelized section occasionally used for sheep grazing and grass harvesting
Location within floodplain		Next to river, areal extension changes with river discharge and after each major flood	Within gravel bar, area expanding due to sediment accumulation and plant colonization	The levee separating the gravel bar from the forest	High lying forest separated by the levee from the river	Low lying forest separated by the levee from the river; next to side channel collecting runoff from hill slope and river back flow	Next to the river but separated by high embankments
Flooding frequency	yr^{-1}	>10	>10	4–6	1–2	1–2	1–2
Flooding duration	$d\ yr^{-1}$	>10	~10	~ 2	< 1	~1	~1
Texture†	Sand/silt/clay (%)	81/14/5	66/26/8	44/44/12	38/47/15	25/58/18	65/27/8
pH† (0.01 M $CaCl_2$)		7.6 ± 0.1	7.4 ± 0.1	7.5 ± 0.0	7.5 ± 0.0	7.4 ± 0.0	7.5 ± 0.0
N_{tot}†	$g\ N\ kg^{-1}$ soil	0.7 ± 0.2	1.0 ± 0.4	1.1 ± 0.3	1.6 ± 0.3	1.8 ± 0.1	0.9 ± 0.2
C_{org}/N_{tot}†	$g\ g^{-1}$	15.2 ± 0.5	16.2 ± 1.6	15.2 ± 0.7	13.4 ± 0.6	14.0 ± 0.2	14.0 ± 0.4
Soil type	(IUSS Working Group WRB, 2006)		Haplic fluvisol (calcaric, humic)	Haplic fluvisol (calcaric, humic)	Haplic fluvisol (calcaric, humic)	Haplic or gleyic fluvisol (calcaric, humic, siltic)	Haplic fluvisol (calcaric, humic)
Dominant vegetation		Various seasonal herbs, patchy	*Phalaris arundinacea*	Tree: *Salix viminalis* Understory: *Rubus* sp.	Tree: A*cer pseudoplatanus* & *Fraxinus excelsior* Understory: see Samaritani et al., 2011	Tree: *Salix alba* Understory: *Urtica dioica* & *Ranunculus ficaria*	Managed grassland dominated by grasses and forbs, mainly *Trifolium* sp.
FPZ type		Dynamic	Dynamic	Dynamic	Stable	Stable	Channelized

† Top soil (10 cm); except for texture, mean ± SD (n = 4) is given. C_{org}, organic carbon; N_{tot}, total nitrogen.

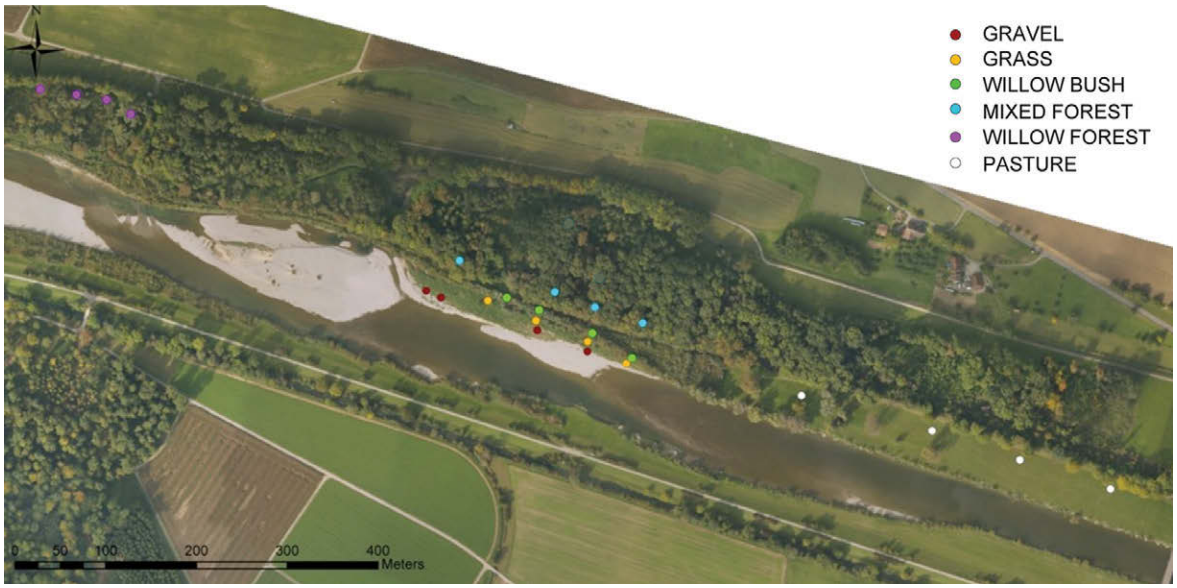

Fig. 2. Aerial picture of the study site near Niederneunforn, northeast Switzerland, in 2009, showing the sampling plots in the six functional process zones.

Soil Sampling

Topsoil sampling was performed in April, August, and October 2009. In each plot, three soil cores (6.5 cm diameter, 10 cm deep) were collected and pooled. In GRAVEL, soil was collected by digging out pits. Half of the field-moist soil was sieved (4 mm mesh) and stored at 4°C, and the other half was dried (40°C) and then sieved (2 mm mesh).

Soil Environmental Conditions

Soil temperature (T) at 10 cm depth was measured with a hand-held thermometer at the time of sampling. Soil water content (SWC) was determined as weight loss during drying of 20 g of fresh soil at 105°C.

Water Extractable Organic Carbon

Water extractable organic carbon (WEOC) was extracted from dried soils with 10 mM $CaCl_2$ at a soil:extractant ratio of 1:2 for 10 min on an end-over-end shaker (Embacher et al., 2007). The soil slurry was then centrifuged for 10 min at 1335 *g* and filtered through a 0.45-μm membrane filter (ME 25, Whatman). The filtered extract was measured for nonpurgeable organic carbon using a TOC analyzer (Formacs HT, Skalar Analytical, Breda, the Netherlands).

Microbial Carbon and Nitrogen

Microbial carbon (C_{mic}) and nitrogen (N_{mic}) were determined by the chloroform fumigation-extraction method (Brookes et al., 1985; Beck et al., 1997). Fresh soil samples equivalent to 10 g dry mass were placed in a desiccator containing chloroform. The desiccator was evacuated and left in the dark for 24 h. The fumigated soil samples and another set of fresh soil samples were extracted for 1 h with 0.5 M K_2SO_4 at a 1:5 soil-to-extractant ratio. The filtered extracts (0.45 μm, ME 25, Whatman) were measured for organic carbon using a TOC analyzer (TOC-V CPH/CPN, Shimadzu, Kyoto, Japan) and for total Kjeldahl N (Kjehldahl, 1883). Microbial carbon and nitrogen were calculated as difference between the concentrations measured for fumigated and non-fumigated soils. Because our main goal was to compare the different FPZs in our study, we did not determine extraction efficiencies (k_{EC} and k_{EN}) for our soils, nor did we use extraction efficiencies from the literature.

Inorganic Nitrogen Pools (Ammonium and Nitrate)

Ammonium and nitrate were measured in extracts prepared from fresh soil using 1 M KCl (soil-to-extractant ratio of 1:4; 1.5 h on an end-over-end shaker; filtration through 0790½, Whatman). Ammonium in the extracts was measured by flow injection analysis (alkalinization of the sample, followed by diffusion of NH_3 into a receiver stream and colorimetric detection via color change of an indicator dye; PerkinElmer UV/VIS Spectrometer Lambda 2S, Autosampler AS 90, FIAS 300). Nitrate was measured colorimetrically at 210 nm (Varian Cary 50) as the difference in absorbance between nonreduced and reduced (using H_2SO_4 and copperized zinc) extracts (Navone, 1964).

Nitrogen Transformation Rates

Gross and potential nitrogen transformation rates were measured on sieved and homogenized fresh soil samples in the laboratory. This method was preferred to in situ incubations of soil cores for two reasons. First, the high sand content precluded setting up in situ incubations in GRAVEL, GRASS, and WILLOW BUSH as cores fell apart when the soil was too dry. Second, with our method, it was possible to determine different parameters on the same soil sample, which allowed direct comparison of different nitrogen pools, nitrogen transformation rates, and soil properties. However, homogenization of soil samples can lead to artifacts (Luxhoi et al., 2005), and thus the measured rates have to be considered as potential rather than actual rates, which limits comparability with other studies. Nevertheless, the results allowed us to compare different FPZs and sampling times in our study.

Gross Mineralization

Gross mineralization (GM) was measured by ^{15}N isotope dilution (Davidson et al., 1991; Luxhoi et al., 2008). Fresh soil, equivalent to 100 g dry matter, was thinly spread on a tray and sprayed with 1 mL of 0.02 μmol L^{-1} NH_4Cl (99.5 atom% ^{15}N). The samples were thoroughly mixed, transferred into a glass container, covered with perforated parafilm, and incubated at the average soil temperature measured during the sampling (12°C for April, 18°C for August, and 13°C for October). Aliquots of 20 g of soil were taken after 2 and 26 h of incubation, and then extracted and analyzed for ammonium as described above. We determined ^{15}N-NH_4^+ in the extracts using the ammonia diffusion technique (Davidson et al., 1991; Schleppi et al., 2006). An aliquot of extract containing approximately 40 μg of N-NH_4^+ was transferred into a polyethylene bottle. A calcinated (6 h at 450°C) and acidified (30 μL of 2 M citric acid) glass microfiber filter (GF/F 25 mm, Whatman) of 5 by 12 mm, wrapped in polytetrafluorethylene band, was added along with MgO (1.5 mg L^{-1} of extract). The tightly closed bottle was gently shaken on a horizontal shaker for 5 d. The filter paper was taken out, dried in a desiccator over concentrated H_2SO_4, unwrapped, packed in a tin capsule, and measured for the $^{15}N/^{14}N$ ratio with an elemental analyzer (Euro EA 3000, Hekatech GmbH, Germany), coupled with an isotope ratio mass spectrometer (Delta V Advantage, Thermo, Germany).

Gross mineralization was calculated following Kirkham and Bartholomew (1954):

$$GM = [(M_0 - M_1)/t] \times \{\log[(H_0 M_1)/(H_1 M_0)]/\log(M_0/M_1)\} \quad [1]$$

where GM = gross mineralization rate per unit mass of soil per unit time, M_0 = total NH_4^+ (tracer + nontracer) per unit mass of soil at 2 h, H_0 = $^{15}NH_4^+$ from tracer per unit mass of soil at 2 h, M_1 = total NH_4^+ (tracer + nontracer) per unit mass of soil at 26 h, H_1 = $^{15}NH_4^+$ from tracer per unit mass of soil at 26 h, and t = time between 2 h and 26 h

Gross Nitrification

The incubation and extractions for gross nitrification (GN) were performed as described for GM, except that the soil samples were incubated with KNO_3 (99.5 atom% ^{15}N). Nitrate in the extracts was measured as described above. The $^{15}N/^{14}N$ ratio of nitrate was also analyzed using the ammonia diffusion technique (Davidson et al., 1991; Schleppi et al., 2006). However, in the first step, only MgO was added to the extracts and shaken for 5 d without closing the lid. After removing all NH_4^+ as NH_3, microfiber filter paper (treated as described above) and 0.5 g Devarda's alloy were added, the lids tightly closed, and the

extracts shaken for another 5 d. Nitrate reduced to ammonium and transformed to ammonia was trapped on the acidified filter paper. The filter paper was processed and measured for $^{15}N/^{14}N$ ratio as described for GM. Gross nitrification was calculated in a similar way as GM, but substituting NO_3^- for NH_4^+.

Potential Nitrification

Potential nitrification (PN) of the soil samples was measured using the shaken soil slurry method (Hart et al., 1994). Fresh soil samples were suspended in a 1 mM phosphate buffer (K_2HPO_4 and KH_2PO_4, adjusted to pH 7.2 by adding NaOH) at a 1:9 soil-to-solution ratio. Ammonium sulfate at 140 mg N-NH_4^+ per kg soil was added and the slurry incubated at 25°C on an orbital shaker at 2.5 Hz. Aliquots of 10 mL each were taken after 1, 4, 6, and 22 h. The aliquots were immediately mixed with 2.5 M KCl at a 1:1.5 ratio to stop nitrification, centrifuged for 3 min at 854 g, filtered (0790½, Whatman) and analyzed for nitrate. Potential nitrification was calculated as the slope of linear regression of nitrate concentrations vs. time.

Potential Denitrification (DEA and DEA_{ns})

Denitrifying enzyme activity (DEA) in the soil samples was measured using the short period acetylene inhibition assay (Smith and Tiedje, 1979; Patra et al., 2005). Fresh soil samples equivalent to 5 g dry soil were placed in 150-mL plasma flasks, and an aqueous solution containing 12.5 mg glucose, 14.1 mg sodium glutamate, and 7.2 mg KNO_3 was added. The flask was evacuated and the headspace was replaced with 9:1 (v/v) helium:acetylene mixture and incubated at 26°C. During incubation, 3-mL aliquots of gas samples were taken from the overhead space of the flask at 1, 1.5, and 2 h. Nitrous oxide concentrations in the gas samples were measured using a gas chromatograph with electron-capture detector (Agilent 6890, Santa Clara, USA). Denitrifying enzyme activity without substrate addition (DEA_{ns}) was determined like DEA, but without adding carbon and nitrogen substrates. Comparing DEA with DEA_{ns} provides indications about the substrate limitation for denitrification.

Nitrous Oxide Efflux

Polyvinyl chloride rings (30 cm diameter, 30 cm long, inserted 20 cm deep into the soil) were installed in each plot. Immediately before sampling, the vegetation within the ring was clipped and the chamber closed with an air-tight lid. Headspace air samples were collected after 5, 25, and 45 min, injected into pre-evacuated exetainers, and analyzed for N_2O concentration on a gas chromatograph with an electron-capture detector (Agilent 6890, Santa Clara, CA). The efflux of N_2O from soil to atmosphere was calculated as the slope of linear regression of headspace concentration vs. time (Yanai et al., 2003).

Statistical Analyses

Data were analyzed by fitting linear mixed-effects models by maximum likelihood in R (R 2.15, http://www.r-project.org). Functional process zone, sampling date (April, August, or October), and their interaction were fixed effects, and site was a random effect. We tested for a correlation of residuals between sampling dates, e.g., by fitting a model with compound symmetric variance–covariance structure. However, serial correlations were smaller than expected by chance. If there was a trend toward an effect, then covariances were even negative, i.e., the statistical power for tests of FPZ would tend to be smaller (in the standard pseudoreplicated case, an inflated type I error rate occurs). We also tested for spatial autocorrelation of residuals between plots, but Moran's I did not indicate a correlation exceeding effects than are to be expected by chance. We therefore did not include corresponding terms in the final analysis.

Significant effects of FPZ (or FPZ × date) were investigated in detail by seeking for linear contrasts splitting FPZ into groups so that significances remained between but not within groups. For example, a significant interaction FPZ×month might be analyzed by splitting FPZ into two groups. The first may contain GRASS, whereas the second (called OTHERS) may contain all remaining FPZs; a significant effect of the contrasts (GRASS vs. OTHERS)×month but no effect of OTHERS × month will then indicate that the effect of FPZ × month is driven by a different temporal response pattern of GRASS compared with the other FPZs (which all respond similarly). Such a partitioning of the FPZ effect was quite obvious in most cases. If no such explanation was found, we calculated all pairwise contrasts, applying a Bonferroni-correction to control the groupwise type I error rate.

Finally, we fitted a linear model with sequential sum of squares containing the terms FPZ, site, month, and FPZ × month to calculate how much of the total variance each model term explained.

All data were log-transformed except for soil temperature, soil water content, and potential nitrification (the residual distribution was normal without transformation, and log-transformation would have resulted in some extreme residuals due to a few nitrification rates close to zero).

The coefficients of variation (CV) for nitrogen pools, transformation rates, and efflux were calculated to quantify within-FPZ variability. They were calculated as the mean of the CVs of each sampling date.

To assess the relation between nitrogen transformations and potential controls, we calculated Pearson correlation coefficients between soil environmental conditions, nitrogen pools, nitrogen transformation rates, and N_2O efflux. In addition, (multiple) linear regressions were performed to derive mixed linear models with which transformation rates could be predicted based on environmental conditions and substrate availability. Parameter elimination was performed stepwise considering nonsignificant correlations and autocorrelation between parameters.

Results

Spatiotemporal Variability

The effects of FPZ, sampling date, and differences in temporal behavior of different FPZs (FPZ × date interaction) on the variability of soil environmental conditions, available organic carbon, microbial biomass, inorganic nitrogen pools, nitrogen transformation rates, and N_2O emissions are summarized in Table 2; the data are presented in Table 3, Fig. 3, and Fig. 4. The three following characteristic patterns emerge when considering the most prominent effects by FPZ or FPZ × time interaction (in terms of significance and variance explained). Soil water content, WEOC, C_{mic}, and N_{mic}, as well as PN, exhibit the same pattern as soil texture and soil organic matter content; that is, in the restored river section, they increase with the gradients of decreasing grain size and increasing soil organic carbon content along the transect from GRAVEL to WILLOW FOREST, and

the values of PASTURE are similar to those in GRAVEL or GRASS (Table 1; Samaritani et al., 2011). Nitrate, GN, DEA, and DEA_{ns} are maximum either in WILLOW FOREST or in both GRASS and WILLOW FOREST. In addition, GRASS is

Table 2. Significance and percentage of variance explained from ANOVA showing effects of functional process zone (FPZ), sampling date, and their interaction (FPZ × date), as well as variance not explained. Results are shown for individual ANOVAs for temperature (T), soil water content (SWC), water extractable organic carbon (WEOC), microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), NH_4^+, NO_3^-, gross mineralization (GM), gross nitrification (GN), potential nitrification (PN), denitrifying enzyme activity (DEA), denitrifying enzyme activity without substrate (DEA_{ns}) and N_2O efflux rate. Significance and variance explained are calculated using different models (for details see text). All data except for T, SWC, and PN were log transformed to fulfill the requirement of normal distribution.

	Significance (main effect)†			Variance explained				
	FPZ	Date	FPZ × date	FPZ	Date	FPZ × date	Replicate plot	Residual
				%				
T	***	*** (August)	**	12.0	78.4	2.8	4.3	2.5
SWC	*** (texture)	***	*	54.4	10.4	5.0	23.8	6.4
WEOC	*** (texture)	*** (August)	ns‡	25.4	58.2	3.8	5.3	7.2
C_{mic}	*** (texture)	***	*** (August)	30.4	12.1	33.8	16.2	7.5
N_{mic}	*** (texture)	**	***	53.4	2.6	22.6	13.9	7.6
NH_4^+	ns	***	ns	3.2	22.0	8.5	36.6	29.8
NO_3^-	*** (GS, WF)	*	ns	39.8	7.0	13.3	18.6	21.3
GM	*	**	*** (GS)	13.5	10.5	39.5	12.7	23.8
GN	*** (GS, WF)	ns	ns	31.3	1.9	16.9	12.0	38.0
PN	*** (texture)	*	ns	67.7	2.9	3.2	14.6	11.6
DEA	** (WF)	***	**	35.3	12.4	16.9	18.8	16.6
DEA_{ns}	** (GS, WF)	*	ns	29.5	7.5	6.8	26.6	29.6
N_2O	ns	*** (August)	ns	11.9	21.3	13.9	30.1	22.7

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

† Main effects are abbreviated as follows: (texture)—parameter exhibits the same pattern as soil texture and soil organic matter content (for details see text); (GS), (WF)—FPZ or FPZ × date effect driven by different values or different temporal pattern in GRASS or WILLOW FOREST, respectively; (August)—values or FPZ effect in August different from April and October.

‡ ns = not significant.

Table 3. Mean ± SD of soil physicochemical properties in top 10 cm of soil in the six functional process zones (FPZs) on three sampling dates ($n = 4$).

	Property†		GRAVEL	GRASS	WILLOW BUSH	MIXED FOREST	WILLOW FOREST	PASTURE
Apr. 2009	T	°C	15.2 ± 2.8	11.4 ± 0.7	11.0 ± 0.7	10.3 ± 0.5	11.7 ± 0.5	10.7 ± 0.8
	SWC	g kg^{-1}	215 ± 139	259 ± 68	330 ± 13	281 ± 42	383 ± 44	180 ± 19
	WEOC	mg kg^{-1}	98 ± 38	187 ± 20	159 ± 41	170 ± 36	195 ± 34	151 ± 9
	C_{mic}	mg kg^{-1}	67 ± 13	61 ± 22	94 ± 12	100 ± 13	200 ± 33	93 ± 7
	N_{mic}	mg kg^{-1}	21.5 ± 6.8	24.9 ± 7.8	35.0 ± 6.0	49.0 ± 8.3	50.3 ± 3.8	39.4 ± 4.0
	C_{mic}:N_{mic}	g g^{-1}	3.5 ± 1.6	2.4 ± 0.5	2.7 ± 0.4	2.1 ± 0.4	4.0 ± 0.4	2.4 ± 0.4
Aug. 2009	T	°C	19.3 ± 1.0	18.4 ± 0.8	16.6 ± 0.2	16.3 ± 0.1	16.6 ± 0.6	18.8 ± 0.2
	SWC	g kg^{-1}	181 ± 70	388 ± 117	348 ± 41	365 ± 30	493 ± 44	276 ± 15
	WEOC	mg kg^{-1}	155 ± 60	324 ± 168	418 ± 103	480 ± 44	608 ± 131	297 ± 34
	C_{mic}	mg kg^{-1}	150 ± 41	158 ± 21	138 ± 21	162 ± 16	118 ± 30	86 ± 21
	N_{mic}	mg kg^{-1}	27.6 ± 9.8	32.8 ± 9.2	47.4 ± 6.4	50.3 ± 0.6	45.2 ± 4.4	16.2 ± 6.0
	C_{mic}:N_{mic}	g g^{-1}	5.6 ± 0.5	5.0 ± 1.0	3.0 ± 0.7	3.2 ± 0.3	2.7 ± 0.8	5.7 ± 1.8
Oct. 2009	T	°C	13.1 ± 0.9	12.5 ± 0.3	11.9 ± 0.4	12.0 ± 0.2	11.6 ± 0.1	12.8 ± 0.5
	SWC	g kg^{-1}	182 ± 34	244 ± 156	318 ± 44	323 ± 19	437 ± 44	174 ± 77
	WEOC	mg kg^{-1}	43 ± 24	111 ± 17	128 ± 25	138 ± 25	173 ± 9	111 ± 10
	C_{mic}	mg kg^{-1}	48 ± 15	101 ± 39	107 ± 13	112 ± 7	146 ± 12	82 ± 11
	N_{mic}	mg kg^{-1}	10.5 ± 6.1	19.2 ± 6.2	33.1 ± 5.0	45.8 ± 4.9	54.3 ± 6.1	32.3 ± 5.4
	C_{mic}:N_{mic}	g g^{-1}	5.6 ± 3.1	5.3 ± 1.5	3.3 ± 0.6	2.5 ± 0.3	2.7 ± 0.4	2.6 ± 0.5
Significant differences between FPZs‡	SWC		a	ab	ab	ab	b	a
	WEOC		a	ab	ab	b	b	ab
	C_{mic}		a	ab	ab	ab	b	a
	N_{mic}		a	ab	bc	c	c	ab

† T, temperature; SWC, gravimetric soil water content; WEOC, water extractable organic carbon; C_{mic}, microbial carbon; N_{mic}, microbial nitrogen; C_{mic}:N_{mic}, microbial carbon to nitrogen ratio.

‡ Significant differences between FPZs are indicated by different letters (pairwise contrasts using Bonferroni correction; $p < 0.05$).

characterized by a different temporal pattern of GM compared with the other FPZs.

The spatiotemporal variability is described in detail below for soil environmental conditions, WEOC and microbial biomass, nitrogen pools, transformation rates, and N_2O efflux.

Soil Environmental Conditions

Temperatures were significantly higher in August than at the two other dates (linear contrasts, $F_{1,33} = 850$, $p < 0.001$), but the small differences between April and October were also significant (linear contrasts, $F_{1,33} = 15$, $p < 0.001$). Although there were no significant differences in temperature between any two of the FPZs, the significant FPZ effect on temperature (Table 2) can be explained by GRAVEL being warmer than all other FPZs (linear contrasts, $F_{1,18} = 67$, $p < 0.001$). The texture and soil organic carbon pattern was expressed mainly as significantly higher SWC in WILLOW FOREST than in GRAVEL and PASTURE (pairwise contrasts, Table 3). The date effect on SWC with higher values in August than on the other dates was significant (linear contrasts, $F_{1,34} = 52$, $p < 0.001$), although with large differences between the means only in GRASS, WILLOW FOREST, and PASTURE.

Water Extractable Organic Carbon and Microbial Biomass

The texture and soil organic carbon pattern was expressed mainly as significantly higher WEOC contents in MIXED FOREST and WILLOW FOREST soils than in GRAVEL (pairwise contrasts, Table 3). Water extractable organic carbon was significantly higher in soils sampled in August than in soils sampled in April and October (linear contrasts, $F_{1,33} = 288$; $p < 0.001$). Considering the significance of differences between individual FPZs, the texture and soil organic carbon pattern was more strongly expressed in N_{mic} than in C_{mic} (pairwise contrasts, Table 3). This can be explained by similar mean values of C_{mic} in August along the entire transect in the restored part.

Inorganic Nitrogen Pools

There was no significant difference in ammonium content among the FPZs. Although the overall time effect was significant (Table 2), differences between sampling dates were small except for some high April values in GRAVEL (Fig. 3). Differences in nitrate content among FPZs were significant and explained about 40% of the variance (Table 2). This was mainly driven by higher NO_3 contents in GRASS and WILLOW FOREST than in the other FPZs (linear contrasts, $F_{1,18} = 26$, $p < 0.001$; Fig. 3). The largest within-FPZ variability for both ammonium and nitrate occurred in GRAVEL, followed by GRASS (Table 4).

Nitrogen Transformation Rates

The significant FPZ × time interaction in GM, which explains about 40% of the variance, was driven mainly by the strong increase in rates in GRASS in August, while the other FPZs exhibited a rather opposite behavior (linear contrasts, $F_{2,33} = 19$, $p < 0.001$; Fig. 4). Whereas GN was significantly higher in GRASS and WILLOW FOREST (linear contrasts, $F_{1,17} = 23$, $p < 0.001$), PN followed mainly the texture and soil organic carbon pattern (pairwise contrasts; Fig. 4). The latter FPZ effect explained almost 70% of the variance in PN (Table 2). Differences in both DEA and DEA_{ns} among FPZs were significant and explained 35 and 32% of the variance, respectively. This was driven by higher

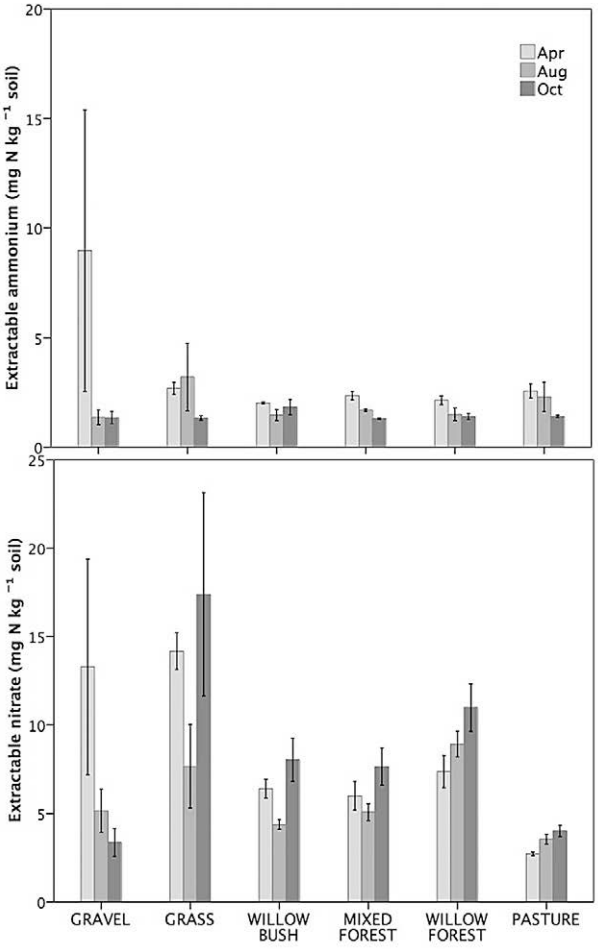

Fig. 3. Extractable ammonium and nitrate in the top 10 cm of soil in the six functional process zones of the Thur River floodplain on three sampling dates in 2009. Bars indicate mean ± SE (*n* = 4).

DEA values in WILLOW FOREST than in the other FPZs (linear contrasts, $F_{1,18} = 19$, $p < 0.001$) and higher DEA_{ns} in both GRASS and WILLOW FOREST (linear contrasts, $F_{1,18} = 13$, $p = 0.002$) (Fig. 4). The average within-FPZ variability of the transformation rates over time was higher in dynamic FPZs than in stable ones (Table 4), with exceptionally high values occurring predominantly in GRASS during the August sampling.

Nitrous Oxide Efflux

Nitrous oxide emissions did not differ significantly among FPZs, and their within-FPZ variability was large in all FPZs with CV > 70% (Table 4). However, the N_2O efflux was generally higher in August than on the other sampling dates (linear contrasts, $F_{1,30} = 27$, $p < 0.001$; Fig. 4).

Nitrogen Turnover and Ratios between Nitrogen Transformation Rates

Table 5 shows turnover of NH_4^+, calculated as ratio between NH_4^+ concentration and GM, and of NO_3^-, calculated as ratio between NO_3^- concentration and GN, as well as ratios between different rates. With a few exceptions in August, mean NH_4^+ turnover was faster than mean NO_3^- turnover. Mean GN/GM was about equal to or greater than 1 in most cases, while mean DEA_{ns}/GN was much greater than 1 in all cases. As observed for GM, NH_4^+ turnover showed a different temporal behavior in

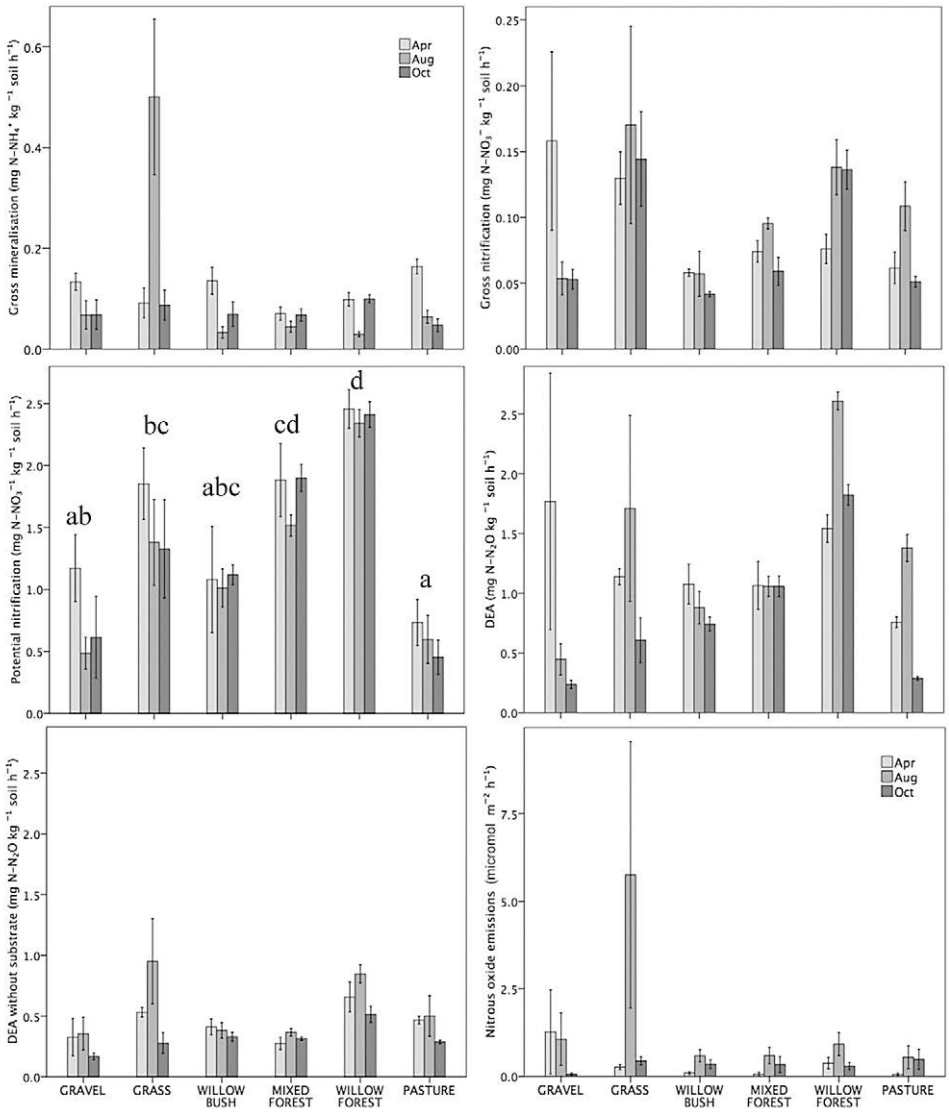

Fig. 4. Nitrogen transformation rates (gross mineralization, gross nitrification, potential nitrification, denitrifying enzyme activity [DEA], and denitrifying enzyme activity without substrate) in the top 10 cm of soil, as well as nitrous oxide emissions in the six functional process zones (FPZs) on three sampling dates in 2009. Bars indicate mean ± SE (n = 4). For potential nitrification, significant differences between FPZs are indicated by different letters (pairwise contrasts using Bonferroni correction; $p < 0.05$)

GRASS than in the other FPZs (linear contrasts, $F_{2,33} = 11$, $p < 0.001$), as did GN/GM (linear contrasts, $F_{2,32} = 13$, $p < 0.001$). Nitrate turnover was generally faster in August than on the other dates (linear contrasts, $F_{1,32} = 13$, $p < 0.001$).

Relations of Nitrogen Transformation and Flux Rates to Soil Properties and Substrate Availability, and among Each Other

Table 6 shows Pearson correlations between soil environmental conditions, labile organic C, microbial biomass, inorganic nitrogen pools, nitrogen transformation rates, and N_2O emissions, considering all data irrespective of sampling date and FPZ. Nitrogen transformation rates were not correlated to their respective nitrogen substrates; that is, neither GN nor PN correlated with NH_4^+, and neither DEA nor DEA_{ns} correlated with NO_3^-. Gross mineralization and GN exhibited a significant correlation mainly with their product, i.e., NH_4^+ and NO_3^-, respectively.

By contrast, PN, DEA, and DEA_{ns} correlated strongly with SWC, and to various degrees also with WEOC and microbial biomass. However, WEOC, C_{mic}, and N_{mic} correlated as well with SWC. In addition, PN correlated negatively with T,

Table 4. Coefficient of variation for ammonium, nitrate, gross mineralization (GM), gross nitrification (GN), potential nitrification (PN), denitrifying enzyme activity (DEA), denitrifying enzyme activity without substrate (DEA_{ns}) , and N_2O efflux rate in the six functional process zones over three sampling times in the Thur River floodplain (n = 12).

	GRAVEL	GRASS	WILLOW BUSH	MIXED FOREST	WILLOW FOREST	PASTURE
NH_4^+	0.76	0.44	0.25	0.10	0.25	0.31
NO_3^+	0.60	0.47	0.20	0.25	0.22	0.13
GM	0.56	0.65	0.59	0.41	0.26	0.38
GN	0.46	0.56	0.26	0.22	0.27	0.28
PN	0.61	0.44	0.41	0.18	0.10	0.59
DEA	0.66	0.55	0.26	0.23	0.10	0.13
DEA_{ns}	0.63	0.50	0.29	0.21	0.27	0.30
N_2O	1.25	0.80	0.69	1.24	0.77	1.27

DEA positively with NH_4^+, and both DEA and DEA_{ns} with mineralization and nitrification rates. Furthermore, PN and DEA can be reasonably well predicted by a mixed linear model using SWC and T or NH_4^+, respectively:

$$PN = 1.06\ (0.35) + 0.0052\ (0.0006) \times SWC - 0.088\ (0.022) \times T\ (R^2 = 0.55) \quad [2]$$

$$DEA = -0.61\ (0.14) + 0.0049\ (0.0004) \times SWC + 0.147\ (0.014) \times NH_4^+\ (R^2 = 0.80) \quad [3]$$

Units are g kg^{-1} (SWC), °C (T), mg N kg^{-1} (NH_4^+), and mg N kg^{-1} h^{-1} (PN, DEA). Standard errors of the coefficients are given in parentheses. All relations are significant at $p < 0.001$.

The apparent high correlation between N_2O efflux and GM was strongly influenced by the August data in GRASS, and the correlation was not significant without these data.

Discussion

Comparison with Nitrogen Pools and Transformation Rates in Other Floodplains

With up to 1.8 g kg^{-1}, the total nitrogen content in the Thur River floodplain soils is in the lower range of values reported for mineral soils in European riverine floodplains (Pinay et al., 1992, 1995, 2000; Clément et al., 2002; Dhondt et al., 2004; Antheunisse et al., 2006;Olde Venterink et al., 2006; Sgouridis et al., 2011). Organic carbon-to-total nitrogen ratios of 13 to 16 are in the upper range of these reference studies but still indicate high nitrogen availability and favorable conditions for organic matter degradation (Samaritani et al., 2011). The relatively low total nitrogen contents and relatively high C-to-N ratios were reflected in NH_4^+ contents that were also in the lower range of reported values (Pinay et al., 1992, 1995, 2000; Clément et al., 2002). In contrast, NO_3^- contents were well in the average range reported in these studies.

Methodological bias and differences between studies need to be considered when comparing transformation rates. In particular, the homogenization of soil samples by sieving, as in our study, precludes the effects of soil aggregates on substrate and oxygen availability. Luxhoi et al. (2005) compared methods with and without aggregate destruction for the determination of GM and GN rates. Studying sandy soils, they found rather good agreement between the two methods for GM and a lower but still acceptable agreement for GN. The differences can, however, be expected to be larger for more finely textured soils, such as in the forest FPZs of the Thur River floodplain. Because it appears that

Table 5. Turnover of ammonium (NH_4^+/GM) and nitrate (NO_3^-/GN), as well as ratios between nitrification and mineralization (GN/GM), and between denitrification and nitrification (DEA_{ns}/GN) in the top 10 cm of soil. Shown are mean and SD (n = 4).†

			GRAVEL	GRASS	WILLOW BUSH	MIXED FOREST	WILLOW FOREST	PASTURE
Apr. 2009	NH_4^+ turnover	d	0.76 ± 0.07	1.40 ± 0.50	0.68 ± 0.24	1.51 ± 0.61	0.90 ± 0.18	0.63 ± 0.15
	GN/GM		1.11 ± 0.71	1.69 ± 0.81	0.49 ± 0.23	1.20 ± 0.61	0.81 ± 0.30	0.41 ± 0.20
	NO_3^- turnover	d	4.21 ± 1.62	4.93 ± 2.57	4.42 ± 0.54	3.32 ± 1.12	4.30 ± 2.19	1.88 ± 0.67
	DEA_{ns}/GN		2.59 ± 1.23	4.36 ± 1.23	7.14 ± 2.46	3.77 ± 1.31	9.37 ± 3.97	8.51 ± 2.58
Aug. 2009	NH_4^+ turnover	d	1.65 ± 2.04	0.37 ± 0.39	3.08 ± 2.93	1.94 ± 1.09	2.40 ± 1.55	2.32 ± 2.94
	GN/GM		1.63 ± 1.91	0.69 ± 1.03	2.44 ± 2.64	2.64 ± 1.46	5.09 ± 1.94	2.16 ± 1.64
	NO_3^- turnover	d	3.89 ± 1.10	2.34 ± 1.42	7.74 ± 10.54	2.14 ± 0.43	2.74 ± 0.79	1.46 ± 0.62
	DEA_{ns}/GN		6.32 ± 3.87	10.63 ± 12.47	12.45 ± 12.82	3.88 ± 0.84	6.42 ± 1.68	5.44 ± 4.04
Oct. 2009	NH_4^+ turnover	d	2.30 ± 2.49	0.80 ± 0.37	1.53 ± 0.91	0.83 ± 0.24	0.57 ± 0.13	1.81 ± 1.61
	GN/GM		2.60 ± 2.82	2.13 ± 1.35	1.10 ± 1.04	0.93 ± 0.38	1.36 ± 0.10	1.51 ± 1.10
	NO_3^- turnover	d	3.42 ± 0.50	7.16 ± 8.65	7.68 ± 2.17	5.41 ± 1.41	3.32 ± 1.02	3.23 ± 0.67
	DEA_{ns}/GN		3.55 ± 0.68	2.69 ± 3.00	7.88 ± 1.43	5.87 ± 2.13	3.76 ± 0.23	5.79 ± 1.53

† GM, gross mineralization; GN, gross nitrification; DEA_{ns}, denitrifying enzyme activity without substrate.

Table 6. Pearson correlation coefficients between environmental conditions, nitrogen pools, nitrogen transformation rates, and N_2O emissions (n = 72); only significant correlations are given ($P < 0.05$); nonsignificant relations are marked as ns.†

	T	SWC	WEOC	C_{mic}	N_{mic}	NH_4^+	NO_3^-	GM	GN	PN	DEA	DEA_{ns}	N_2O
T	1.00												
SWC	ns	1.00											
WEOC	0.50	0.62	1.00										
C_{mic}	ns	0.56	0.36	1.00									
N_{mic}	–0.25	0.56	0.37	0.62	1.00								
NH_4^+	ns	ns	ns	ns	ns	1.00							
NO_3^-	–0.25	ns	ns	ns	ns	ns	1.00						
GM	ns	ns	ns	ns	ns	0.44	ns	1.00					
GN	ns	0.26	ns	ns	ns	ns	0.49	ns	1.00				
PN	–0.29	0.66	0.32	0.38	0.56	ns	0.38	ns	0.26	1.00			
DEA	ns	0.69	0.47	0.25	0.29	0.64	ns	ns	0.31	0.50	1.00		
DEA_{ns}	ns	0.54	0.55	0.32	0.24	ns	ns	0.35	0.42	0.47	0.55	1.00	
N_2O	0.29	ns	ns	ns	ns	ns	ns	0.59	0.31	ns	ns	ns	1.00

† T, soil temperature; SWC, soil water content; WEOC, water extractable organic carbon; C_{mic}, microbial carbon; N_{mic}, microbial nitrogen; GM, gross mineralization; GN, gross nitrification; PN, potential nitrification; DEA, denitrifying enzyme activity; DEA_{ns}, denitrifying enzyme activity without substrate addition.

neither method yields systematically larger values, the following comparison seems justified. There are very few reports on gross mineralization and nitrification in riparian soils. The main range of GM in our soils of 0.05 to 0.15 mg N kg^{-1} h^{-1} is similar to values recently reported for mineral soils in riparian forests (Koyama et al., 2012) and coastal wetlands (Jin et al., 2012), while in wetland soils with a higher organic matter content, GM values can reach up to one order of magnitude higher (Clein and Schimel, 1995; Bédard-Haughn et al., 2006; Chen et al., 2012;). By contrast, our GN rates of 0.05 to 0.15 mg N kg^{-1} h^{-1} are within or slightly above the range reported for other wetlands, irrespective of the organic matter content, and are even similar to GN in coarse-textured agricultural soils (Luxhoi et al., 2005).

The different conditions during nitrogen mineralization and nitrification are also illustrated by a comparison with turnover rates found in seasonal wetlands by Bédard-Haughn et al. (2006). Whereas the NH_4^+ turnover rates we measured are comparable to those in cultivated wetlands (wheat, fertilized), our NO_3^- turnover rates are similar to those in the uncultivated wetlands (natural grassland).

The strong interest in nitrogen removal has spurred a tremendous amount of work on denitrification in floodplains, usually using a variation of the acetylene inhibition method (Groffman et al., 2006). Although denitrification rates obtained at otherwise natural conditions (e.g., Hefting et al., 2004; 2006; Olde Venterink et al., 2006) are not directly comparable to our study, there are sufficient data from anaerobic incubations of soil slurries with and without substrate addition. This is a well-established method to estimate the DEA as a measure of the denitrification potential. Groffman et al. (2006) noted that this method "is still widely (and validly) used for comparison of sites and experimental treatments." Both our DEA_{ns} (mainly between 0.2 and 1 mg N kg^{-1} h^{-1}) and DEA values (0.2–2.5 mg N kg^{-1} h^{-1}) are above values measured in other mineral riparian soils (Groffman et al., 1992; Pinay et al., 1995, 2000; Clément et al., 2002; Dhondt et al., 2004; Hernandez and Mitsch, 2007) and thus indicate a relatively high denitrification potential. Similar DEA_{ns} rates as in our study were observed in the Cole River floodplain soils characterized by particularly low C-to-N ratios of 4 to 10 (Sgouridis et al., 2011), and significantly larger rates were measured for organic riparian soils (Ambus, 1993).

Spatiotemporal Variability of Nitrogen Pools and Nitrogen Transformation Rates

Low spatial variation in the total soil organic carbon-to-total nitrogen ratio of the soils indicates that nitrogen retention in the study site is linked with organic matter deposition of sediments (Stoeckel and Miller-Goodman, 2001; Noe and Hupp, 2005). Topographical differences in the sedimentation conditions at the study site have led to a characteristic distribution of grain size and soil organic matter among the different FPZs, with decreasing grain size and increasing soil organic matter content from GRAVEL to WILLOW FOREST in the restored section (Samaritani et al., 2011). Samaritani et al. (2011) showed that the carbon dynamics in the three types of FPZs investigated in this study were influenced by the physicochemical soil properties and by the degree of flood disturbance. The effects of the flood disturbance were attributed to (i) changes in soil moisture and (ii) local and temporary stimulation of microbial activity from the input of nonstructured allochthonous soil material. Although the sampling dates in the present study were somewhat different from those in Samaritani et al. (2011; October 2009 instead of October 2008, no data from January 2009), both the generally higher WEOC values in August than in April and October and the higher microbial biomass values in August in the FPZs with high sediment deposition support these conclusions.

The spatial pattern of PN following the successional gradient in the restored section, and the maximum of DEA in WILLOW FOREST suggest that the activities of nitrifiers and denitrifiers are strongly related to soil organic carbon, total nitrogen, and soil texture. Potential nitrification and DEA are not measures of actual rates but represent the soil potential under optimal conditions that reflect the environmental constraints on the respective processes (Groffman et al., 1992; Hart et al., 1994). The two maxima of NO_3^-, GN, and DEA_{ns}, in GRASS and WILLOW FOREST suggest an overlap of the soil property gradient by flood disturbance. The generally similar values of NH_4^+ and GM in all FPZs suggest that the soil physicochemical properties have little effect on NH_4^+ producers (Jackson et al., 2008).

The PASTURE FPZ is used extensively for grazing and fodder production, which directly influences soil nitrogen pools. On the one hand, organic nitrogen (plants) is removed through grazing and grass harvesting, which reduce the litter input. On the other hand, organic nitrogen (animal waste) is added by the grazing animals. More than 30% of vegetation cover in PASTURE was attributed to clover, while this plant was almost completely absent in the restored section (Bertrand Fournier, personal communication, 2011). These factors indicate that soil nitrogen dynamics in PASTURE differ from those in the other FPZs. In addition, PASTURE is also different from the restored section in terms of topography and hydrology. All this may explain why PASTURE and GRASS, despite having similar physicochemical soil properties, exhibited very different nitrogen transformation rates. The potential activity of nitrifiers and denitrifiers in PASTURE was comparatively low, as indicated by the low values of PN, DEA, and C_{mic}, whereas the effective rates, as indicated by GM, GN, and DEA_{ns}, did not differ much between PASTURE and the restored section. This suggests that the substrate may be less limiting in PASTURE soils.

In general, variance analysis suggests that the spatial variability of the nitrogen pools and transformation rates is higher than the temporal variability, except for mineralization. The three sampling dates were chosen to represent three distinct phases of the growing season. From previous studies, it can be expected that mineralization would show maxima in spring and autumn (Jamieson et al., 1999; Morecroft et al., 1992), which can be explained by the time-dependent abundance of available plant-derived organic nitrogen in the rhizosphere and topsoil. Indeed, such a seasonal trend can explain the temporal variability of GM in all forested FPZs, whereas the summer maximum in GRASS is most likely due to flood-related organic nitrogen release by sediment input or litter production due to destruction of the *Phalaris* cover.

Spatiotemporal Variability and Source of Nitrous Oxide Efflux

Denitrification (DEA and DEA_{ns}) and SWC in the topsoils were not found to correlate with N_2O efflux. This can be

explained by (i) the production of N_2O through processes other than denitrification, and (ii) N_2O production in the subsoil and the aquifer.

Under drier conditions, the major source of N_2O emissions from soils is expected to be nitrification, while under wetter conditions, it is expected to be denitrification (Webster and Hopkins, 1996). The generally higher N_2O emissions during the August sampling, when soils exhibited the highest moisture content, suggest that denitrification is a major source of N_2O in our soils. On the other hand, a study by Kool et al. (2011) indicates that below 70% water-filled pore space, a major fraction of N_2O originates from nitrification. Since this is the case in our topsoils most of the time (except during flooding; data not shown), we can assume that at least in the topsoils, a major proportion of N_2O is produced through nitrification. This is supported by Ambus (1998), who found that more than 60% of N_2O in riparian grasslands can be produced through nitrification.

Nitrous oxide efflux from the soil surface is the net result of production, consumption, and transport throughout the soil column (Davidson et al., 2000). Thus, contributions by denitrification in deeper soil layers or groundwater, where redox conditions are optimum for denitrification, cannot be excluded. This could also explain the high N_2O efflux rates in the low-lying dynamic FPZs and in WILLOW FOREST, where the average water table is closer to the surface than in the other FPZs.

Within-FPZ Spatial Variability of Nitrogen Pools, Transformations, and Nitrous Oxide Efflux

Comparing different FPZs, the relative variability of nitrogen dynamics within the FPZs corresponded to the respective relative variability of soil physicochemical properties, environmental conditions, and carbon dynamics (Samaritani et al., 2011), as Watts and Seitzinger (2000) also found. Comparing the results of the present study and of Samaritani et al. (2011) shows that the within-FPZ variability of nitrogen pools and transformation rates was higher than the one of carbon pools and fluxes. The particularly high variability in the low-lying dynamic FPZs (GRAVEL and GRASS) can be attributed to them being severely and frequently disturbed by flooding, with a comparatively long average inundation period. According to Pinay et al. (1999), this can create variability in the biogeochemical patterns on a meter scale or smaller. Also notable is the co-occurrence of high rates of both denitrification and nitrification in these two FPZs. The very high within-FPZ spatial variability of N_2O emissions in all the FPZs is consistent with other studies (Hefting et al., 2006; Yanai et al., 2003) and can be related to the additional variability of conditions in the entire soil column and the aquifer below.

Controls of Nitrogen Transformations

Soil nitrogen transformations are microbially driven and mainly controlled by soil moisture, which influences soil aeration and redox conditions and the availability of organic carbon and nitrogen substrate (Ponnamperuma, 1972, Jackson et al., 2008). The spatiotemporal variability of these factors is therefore expected to govern the variability of nitrogen transformations. However, GM correlated with neither SWC nor WEOC, which is consistent with Pinay et al. (2002), who maintained that SWC plays only a minor role in mineralization as this process can occur under both aerobic and anaerobic conditions.

The PN rates were 10 times higher than the GN rates, which suggests that despite high extractable nitrate concentrations, nitrification is often limited in our study site. The strong correlation between PN and SWC indicates that the activity of nitrifiers is limited by low soil moisture (Stark and Firestone, 1995). This is supported by continuous in situ measurements of volumetric water content (Huber et al., 2012), which showed that plots in most FPZs are often below the optimum soil moisture required for nitrification, i.e., 60% water-filled pore space (Bollmann and Conrad, 1998). Although no correlation between GN and GM or NH_4^+ was observed, ammonium limitation of nitrification is indicated by generally shorter NH_4^+ than NO_3^- turnover and by often higher GN than GM. Furthermore, extractable ammonium represents ions in soil solution plus the ammonium sorbed on the cation exchange complex and thus tends to overestimate the ammonium available for nitrification (Robertson, 1989).

The strong correlation between potential denitrification (DEA and DEA_{ns}) and both SWC and WEOC is in accordance with earlier findings that soil moisture and available organic carbon are major controlling factors for denitrification (Ambus, 1993; Hill and Cardaci, 2004). The strong additional correlation of DEA with NH_4^+ indicates nitrogen substrate limitation. This is further supported by the correlation between DEA_{ns} and both GM and GN. However, DEA was only twice as high as DEA_{ns}, which suggests that substrate limitation was not severe. Furthermore, we cannot tell from our data whether substrate limitation is due to current conditions or caused by past denitrification that has used up the available substrate. Indeed, past conditions of soil saturation could have been important for substrate consumption in the bulk soil, particularly for the August sampling that took place a few weeks after a major flood. However, because of the mostly good aeration of the bulk soils at all sampling dates—except for a few low-lying sites in GRASS and WILLOW FOREST in August—effective denitrification should have been limited to anaerobic microsites.

Nitrogen Turnover

The following indicate that biological processes at the study site were not nitrogen-limited: (i) the GN rates were often higher than the GM rates (Chapin et al., 2002), (ii) denitrification was only weakly substrate limited, (iii) microbial C-to-N ratios were in the lower range of bacterial C-to-N ratios (Martin, 1991), and (iv) extractable nitrate concentrations were high in the FPZs of the restored section.

The fast NH_4^+ turnover in all FPZs, the often higher GN than GM, and the always higher DEA_{ns} than GN indicate that the overall nitrogen turnover was governed by mineralization. This means that under the predominant aerobic conditions, ammonium that is not immobilized or taken up by plants is generally quickly nitrified, except during conditions that are too dry for nitrification (Huber et al., 2012). The resulting nitrate accumulation is indicated by high concentrations and slow turnover rates of nitrate when compared to ammonium, and also by often high nitrate concentrations in the soil solution (Huber et al., 2012). The high denitrification potentials and higher DEA_{ns} than GN suggest that during water saturation, and during drying phases in anoxic microsites, accumulated or freshly produced nitrate is quickly denitrified.

The maximum GN and DEA_{ns} in GRASS and WILLOW FOREST indicate that these FPZs can be considered hot zones of overall nitrogen cycling and turnover. In GRASS, this can be attributed to regular flooding, which makes the soil sufficiently moist for microbial processes most of the time (Pinay et al., 2002). In addition, it leads to regular inputs of available organic carbon that stimulates microbial activity (Pinay et al., 2000; Mentzer et al., 2006; Samaritani et al., 2011). In contrast, the fast nitrogen turnover in WILLOW FOREST can be explained mainly by the relatively fine soil texture, with some additional effects of flooding. The fine texture leads to anoxic conditions at a lower water content than in coarse-textured soils (Bollmann and Conrad, 1998; Pinay et al., 1999), so that anaerobic microsites are formed even under unsaturated soil conditions. The existence of adjacent nitrification and denitrification microsites under such conditions facilitates coupled nitrification–denitrification and thus increases nitrogen turnover and loss (Baldwin and Mitchell, 2000). The notion of a generally fast nitrogen turnover in the Thur River floodplain is further supported by nitrogen budget calculations (Huber et al., 2012). These show that soils in MIXED FOREST, where nitrogen input by sedimentation is low, experience a net loss of 30 to 40 kg N ha^{-1} yr^{-1}, even without considering denitrification (i.e., by leaching of NO_3^- and dissolved organic nitrogen only).

Conclusions

The overall dependence of the nitrogen dynamics in our floodplain soils on both soil physicochemical properties and flood disturbance is in agreement with earlier findings (Clément et al., 2003; van den Heuvel et al., 2009), indicating a strong influence of landscape position. A comparison between the restored and channelized sections of the Thur River corridor suggests that the development of near-natural floodplains following river widening has drastically increased nitrogen turnover. The specific properties of PASTURE, such as the presence of nitrogen fixers and extensive land use, may play a role. However, we argue that it is mainly the environmental heterogeneity, characteristic of the restored section, that favors nitrogen removal, as it creates locations with high sedimentation and nitrogen turnover and hence, permanent nitrogen removal through denitrification. Although our measurements were restricted to the topsoil, we can speculate that similar conditions also occur deeper in the soil since basic physicochemical soil properties change little with depth at our site (Samaritani et al., 2011). Independent of soil depth, this has a positive effect on groundwater quality because denitrification in anoxic microsites reduces the accumulation of nitrate during times of maximum nitrate production—that is, well-aerated but sufficiently moist conditions in the bulk soil—and thus the amount leached. On the other hand, Huber et al. (2012) showed that nitrate accumulation can be high at all soil depths and that major leaching occurs with the percolating rain water in winter and during floodings. In addition, the occurrence of locally high emissions of N_2O, a potent greenhouse gas, is a matter of concern.

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