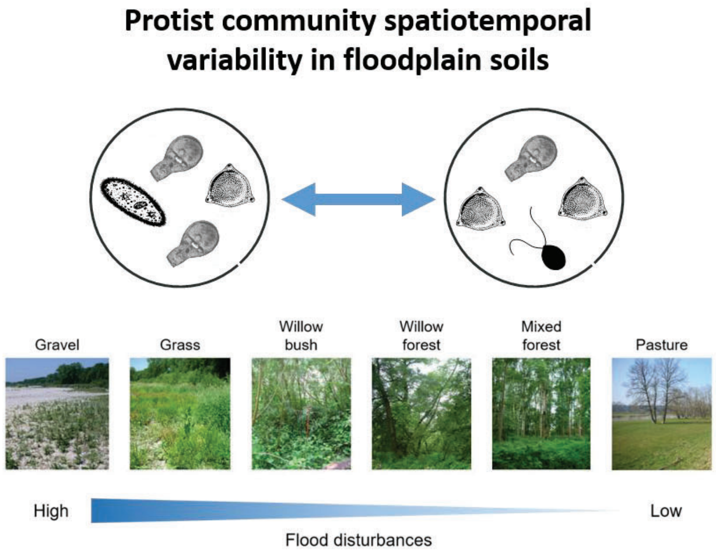
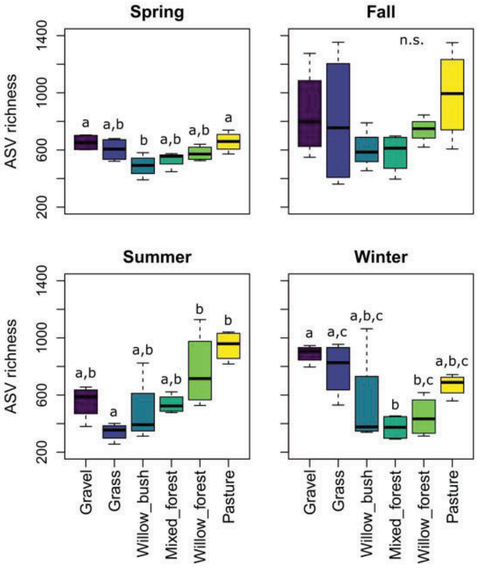
Graphical Abstract (for review)





Highlights

We studied floodplain soil micro-eukaryotic diversity by Illumina sequencing of e-DNA

Alpha and beta diversity varied more spatially among habitats than seasonally

The edaphic drivers of α and β diversities changed among habitats and seasons

These patterns reflect seasonal flood dynamics and soil biogeochemical cycles

Thus, microbial ecology of dynamic systems should best be studied over time and space

Higher spatial than seasonal variation in floodplain soil eukaryotic microbial communities

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Abstract

Beta diversity is a key component of biodiversity with implications ranging from species dynamics to ecosystem functioning. However, β-diversity and its drivers have received little attention, especially for micro-eukaryotes which play key roles in soil functioning. We studied the diversity of soil micro-eukaryotes in a Swiss lowland floodplain using high-throughput Illumina sequencing of soil DNA. We determined the temporal *vs*. spatial patterns of soil micro-eukaryotic α- and β-diversity in six contrasted habitats sampled over one year. We identified the drivers of these patterns among soil conditions and functions and identified indicator taxa of habitats in each season. We found higher spatial than temporal variability and a strong space-time interaction in soil micro-eukaryotic diversity patterns as well as in their edaphic drivers, which contrasts with previous observation of bacterial diversity patterns. Our results show that, although soil micro-eukaryotic diversity indeed varies seasonally, it is correlated most strongly with edaphic variables and vegetation but the strength of correlations with individual drivers varied seasonally. Microbial diversity patterns and their drivers can thus differ quite substantially among seasons and taxa. Despite the dominance of spatial patterns, the temporal component of microbial diversity should not be ignored to accurately estimate the diversity and the complexity of soil microbial community assembly processes. Given the importance of soil microbial diversity for ecosystem functioning such knowledge is relevant for land management.

Keywords: soil protist; beta diversity; spatiotemporal dynamics; soil physico-chemical conditions; soil ecosystem functions; riparian ecosystem.

1. Introduction

Soil microbial eukaryotes (here including protists and fungi, but excluding micro-metazoans) are highly diverse and play central roles in soil food webs (Geisen 2016). They are thus key actors of biogeochemical cycling: they influence nutrient cycling and energy fluxes in soils via the microbial loop (Bonkowski 2004), thus contributing to soil fertility (Barrios 2007; de Vries *et al.* 2013). However, there are still significant gaps in basic knowledge of the diversity and ecology of soil microbial eukaryotic communities. Notably, analyses of seasonal dynamics of soil microbial eukaryotic communities are rare and limited to a relatively small range of ecosystem types.

With the development of high-throughput multi-taxa identification using environmental DNA, hereafter eDNA metabarcoding (Taberlet *et al.* 2012), more efficient and complete characterization of microbial eukaryotic diversity is possible. Indeed, macroecological patterns such as the latitudinal biodiversity gradient, which are well documented in macro-organisms, were also observed in several major groups of soil fungi, but not in ectomycorrhizal fungi (Tedersoo *et al.* 2014). Similarly, morphological and molecular studies showed an increase of diversity toward the equator for euglyphid testate amoebae (Lara *et al.* 2016) or towards mid-latitudes where the water-energy balance is maximal for all testate amoebae (Fernández *et al.* 2016). There is compelling evidence that at least some micro-eukaryote taxa are not globally distributed, and that the composition of communities diverges considerably across large geographic distances (Foissner 2006; Heger *et al.* 2013). Furthermore, environmental distance-decay similarity in diatom communities were shown to be comparable to that observed in macro-organisms (Astorga *et al.* 2012). Morphological- and DNA-based fine-scale analyses of the spatial variations of soil microbial eukaryotic communities are scarce (Mitchell *et al.* 2000; Acosta-Mercado & Lynn 2002). But, Fiore-Donno *et al.* (2019) have shown, using a metabarcoding approach, that the spatiotemporal heterogeneity of edaphic factors can drive the changes in microbial eukaryotic community compositions highlighting the importance of deterministic niche-based processes. Despite the methodological progress, studies investigating the patterns of diversity distribution and biogeography of microbial eukaryotes are less numerous than for bacteria and often do not consider temporal changes. The few studies that have investigated the temporal changes of soil microbial eukaryotic communities have revealed a marked seasonality (Lamentowicz *et al.* 2013; Fiore-Donno *et al.* 2019; Levy-Booth *et al.* 2019; Zhao *et al.* 2019). A better characterization of the relative importance of seasonal as compared to spatial variability in soil microbial eukaryotic communities can improve our understanding of community assembly processes, and is necessary to clarify the patterns and drivers of diversity and biogeography of soil microbial communities.

Beta diversity measures the differences in community composition among sites within a region of interest (Whittaker 1960). Many different definitions and metrics of beta diversity exist (Tuomisto 2010). In its additive form (i.e. $\beta = \gamma - \alpha$), beta diversity can be defined as the amount by which the species richness of the entire (regional) dataset exceeds that of a single sampling unit of mean species richness (Tuomisto 2010). This approach allows quantifying the relative importance of alpha and beta diversity for total gamma diversity. While it is widely used for the study of macro-organisms (Wagner *et al.* 2000; Gering *et al.* 2003; Tylianakis *et al.* 2005; Schmidt *et al.* 2017), such an approach has, to our knowledge, never been used to assess the variability of soil microbial eukaryotic communities.

We focus on the spatiotemporal variability of soil microbial eukaryotes in riparian soils. Floodplains, at the interface between the riverbed and the surrounding upland terrestrial ecosystems (Sedell et al., 1989), are among the most diverse environments on earth. They are characterized by sharp environmental gradients and are strongly influenced by the seasonal dynamic of the river. Riparian soils contain a high diversity of eukaryotic microorganisms (Foissner, Chao & Katz 2007; Baldwin *et al.* 2013), that is very heterogeneously distributed among the various floodplain habitats (Binkley *et al.* 1997). Previous studies about the seasonal variations of soil microbial eukaryotic communities were conducted in habitats with relatively low spatial variability in environmental conditions such as temperate rainforests (Levy-Booth *et al.* 2019), agricultural soils (Zhao *et al.* 2019), and grasslands (Fiore-Donno *et al.* 2019) which might lead to a biased view of the relative importance of spatial and seasonal beta diversity. In the few cases where protist communities of temporarily flooded systems were investigated, and where the temporal dimension was included, marked seasonal patterns were observed (Simon *et al.* 2015; Sisson *et al.* 2018). A better knowledge on the patterns and drivers of soil micro-eukaryotic diversity in a broader range of ecosystems types is needed to improve our understanding of the structure and functions of soil ecosystems in general. Dynamic systems such as floodplains characterized by irregular perturbations have to this date not been studied for soil micro-eukaryotes. And, virtually nothing is known about the seasonal variability of floodplain soil microbial eukaryotic communities despite strong and well-known seasonal changes in climate and flood dynamic. Riparian soils are thus ideal model ecosystems to investigate the spatiotemporal variability of soil microbial eukaryotic communities.

We present the results of a field survey of soil microbial eukaryotic communities in a Swiss lowland floodplain. The survey was conducted in six contrasted characteristic floodplain habitats with four seasonally replicated sampling campaigns. The purpose of this study was to examine the richness and turnover (beta diversity) of microbial eukaryote taxa in relation to the spatial, temporal, and edaphic characteristics of the selected habitats. As our focus is on taxa richness and turnover to quantify the spatiotemporal changes of soil micro-eukaryotic assemblages, we decomposed the total diversity into spatial and temporal components. Finally, we related these indices to measured variables reflecting soil conditions and functions. We expected a stronger contribution of habitats to total beta diversity because of the marked differences in soil conditions and functions (Samaritani *et al.* 2011), vegetation type (Fournier *et al.* 2013), and soil organism groups (Fournier et al. 2012a, Fournier et al. 2012b, Fournier et al. 2015) at the study site. However, we expected the important seasonal changes in soil and climate to induce significant temporal changes of soil microbial eukaryotic communities. Our results confirmed these expectations by showing that beta diversity (spatial and temporal) contributes to a greater extent to total gamma diversity than local alpha diversity. And, although important, seasonal changes in soil microbial eukaryotic communities were less marked than spatial ones. These results highlight the importance of considering both the spatial and temporal changes for understanding soil microbial eukaryotic diversity and functions.

2. Experimental procedure

2.1. Study site

The study was conducted in a restored section of the River Thur in north-western Switzerland. River Thur flows through the Swiss Plateau from Mount Säntis to the Rhine. Its regime is similar to that of alpine rivers, with peak flow generally occurring in spring after snowmelt and in summer and fall after large storms. The study site is a 1.5 km long recently restored section of the river located in a peri-urban / agricultural region of Switzerland (long-term maximum, mean, and minimum flow rates are 1130, 50, and 2 $m^3 s^{-1}$, respectively; 1904–2005: http://www.hydrodaten.admin.ch/en/index.html). More information about the study site can be found in Schirmer *et al.* (2014) and Woolsey *et al.* (2007).

2.2. Sampling design

Six habitats were distinguished based on flood dynamics (assessed using topographic conditions: distance to the river and elevation) as well as soil and vegetation characteristics (*Gravel, Grass, Willow bush, Mixed forest, Willow forest,* and *Pasture*; Fig. S1). *Gravel* is characterized by frequent floods (average = 24 floods y^{-1}), patches of poorly developed soils (Calcaric Regosols), and pioneer vegetation. *Grass* is characterized by frequent floods (average = 17 floods y^{-1}), more developed soils (Calcaric Regosols) with a high spatial and temporal heterogeneity (Samaritani et al., 2011), and dense vegetation dominated by tall herbs (*Phalaris arundinacea*). *Willow Bush* experiences on average three floods per year and has soils of average depth (Calcaric Fluvisols) where willow bushes (mostly *Salix viminalis*) were planted. *Mixed Forest* is subjected to limited influence of flooding (0.2 flood y^{-1}), has deep soils (Calcaric Fluvisols), and is dominated by mixed deciduous tree species (*Acer pseudoplatanus, Fraxinus excelsior*). *Willow Forest* occurs at a similar distance to the river than *Mixed Forest*, but at a slightly lower elevation. It experiences on average 0.5 flood per year, has deep soils with more hydromorphic features than *Mixed forest* (Calcaric Gleyic Fluvisols), and the vegetation is dominated by old willows (*Salix alba*). Finally, *Pasture* lies outside the restored section of the river and is still protected from floods by levees (number of floods per year < 0.2), has the most developed soils (Calcaric Fluvisols), and harbors the typical vegetation of a grassland. The number of floods per year per habitat was calculated in Fournier et al. (2015). The soil taxonomy was assessed by Fournier et al. (2013) according to the World reference base for soil resources (IUSS Working Group 2006). Four plots were sampled in each habitat and each plot was sampled four times (spring = 09.04.2008; summer = 08.07.2008; autumn = 08.10.2008; and winter = 09.01.2009; N = 24 * 4 = 96 samples). This design aimed at capturing a maximum of the fine-scale spatial and temporal heterogeneity within the study site without *a priori* knowledge of environmental conditions.

2.3. Soil physico-chemical conditions

Soil physico-chemical variables were presented by Samaritani *et al.* (2011). *Soil texture* (sand; silt; clay) was measured on dried samples using the pipette method (Gee & Bauder 1986). The percentage of *total organic carbon (TOC)* of dried, homogenized soils was measured using a TOC analyzer (Shimadzu, Tokyo, Japan) after HCl (10%) acid digestion to remove carbohydrates. *Total carbon and nitrogen contents* were measured using an automatic element analyzer (Shimadzu, Tokyo, Japan). The Olsen P method was used as a proxy of *available P* (Kuo 1996). *Soil temperature* (T) at 5 cm depth was continuously measured during this study in each plot at 30 min resolution with

TidBit v2 temperature loggers (Bourne, MA, USA). *Soil Moisture (SM)* was estimated at each sampling time by measuring the weight loss upon drying 20 g of fresh soil at 105 °C for 24 h. See Samaritani et al. (2011) for further details about the measurements of soil conditions.

2.4. Ecosystem functioning proxies

Four variables indicative of soil functioning were selected and measured in each plot and season: basal respiration, microbial biomass (carbon and nitrogen), and enzymatic activity. These variables are ecosystem functioning proxies that can be linked to ecosystem services such as decomposition, nutrient cycling and carbon storage. *Basal respiration* (BR) was measured in a closed soil-chamber system connected to a Li-8100 infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA) (Samaritani *et al.* 2017). The gas flow and the CO_2 concentration were recorded and the BR was calculated according to Rieder *et al.* (2013). Fluxes are reported as mmol CO_2 h^{-1} g^{-1} soil dry weight. *Microbial biomass Carbon* (MC) and *Nitrogen* (MN) were determined by chloroform fumigation-extraction (Vance *et al.* 1987; Frey *et al.* 2006; Samaritani *et al.* 2011). MC and MN data were expressed in mg kg^{-1} soil dry weight. *Enzymatic activity* (EA) was estimated by fluorescein diacetate analysis (see Samaritani *et al.* 2017 for more information). Fluorescein diacetate (FDA) is hydrolysed by proteases, lipases and esterases and can therefore be used to determine the microbial activity (Söderström, 1977; Schnürer and Rosswall, 1982; Adam and Duncan, 2001). The formation of a yellow color was assessed at 490 nm by spectrophotometer. The intensity of the resulting yellow color is indicative of the amount of enzymatic cleavage of the FDA molecule (colorless) and the overall enzymatic activity in the sample. The results were expressed in mg of degraded FDA h^{-1} g^{-1} soil dry weight.

2.5. DNA extraction

We used aliquot of soil DNA from the study of Samaritani et al. (2017). Briefly, a 0.5 g subsample of fresh soil and 0.75 g glass beads (0.1 mm diameter) were suspended in 1 ml extraction buffer (0.2 M Na_3PO_4 [pH 8], 0.1 M NaCl, 50 mM EDTA, 0.2% CTAB). The DNA was purified by chloroform extraction with 2 ml chloroform. The DNA was precipitated by the addition of 3 ml of precipitation solution (20% PEG 6000, 2.5 M NaCl). The supernatant was removed and the samples were air dried for 20 min, and re-suspended in AE buffer (10 mM TrisCl, 0.5 mM EDTA, pH9; Qiagen, Hilden, Germany) at 1 ml AE per g of extracted soil (dry weight equivalent). The extracted DNA was examined by electrophoresis on agarose gels (1% w/v in Tris-Borate-EDTA buffer), quantified using PicoGreen and stored at -20 °C.

2.6. 18S rRNA amplification and Illumina sequencing

The microbial eukaryotic communities were investigated using high-throughput Illumina sequencing. The V9 SSU rRNA hypervariable region was amplified with the general eukaryotic primer pair 1380f/1510r (Amaral-Zettler *et al.* 2009). The forward primers were tagged with 96 different 9 nucleotides long keys. 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 was run for each differently tagged primer combination was run. Amplification conditions followed Amaral-Zettler et al. (2009) protocol: 3 minute denaturation at 94°C, followed by 30 cycles of 30s at 94°C, 60s at 57°C, and 90s at 72°C and final extension at 72°C for 10 minutes.

The three PCR products from each sample were combined and purified through Zymo columns. Then, approximately 4 ng of amplicons for each sample were pooled together. Amplicons were quantified by fluorometry with the QuBit HS dsDNA kit (Life Technologies, Carlsbad, CA). A DNA library was prepared for Illumina sequencing following New England Biolabs's kit NEBNext DNA Sample Prep Master Mix Set 1. The 100bp paired-end sequencing run was performed with the Illumina HiSeq2000 platform at the Genomics Core Facility of Brown University (Providence, USA). The sequencing provided a total of 221,625,392 barcoded reads. The amplicon data are available on EMBL European Nucleotide Archive under project number: PRJEB35438 (ERP118478).

2.7. *Sequence data processing and taxonomic assignment*

The absence of sequencing primers in the dataset was verified using cutadapt (Martin 2011). The analysis of the reads was then done with the DADA2 package (version 1.12) in *R version* 3.5.0 (R Development Core Team 2018). The DADA2 pipeline includes the following steps: filtering, dereplication, sample inference, chimera identification, and merging of paired-end reads. DADA2 infers exact amplicon sequence variants (ASVs) from sequencing data (Callahan *et al.* 2016). The ASVs were then taxonomically assigned with QIIME2 (Bolyen *et al.* 2018) using a pre-trained Naive Bayes classifiers (Silva Ref NR 99, release 132) (Quast *et al.* 2012). All ASVs which were not assigned to Nucletmycea or a protist group were removed from the dataset. After this all ASVs representing less than 1% of the average sampling depth were removed (from 14900 ASVs to 13909 ASVs). This was done to remove potential technical artifacts.

2.8. Spatial and temporal changes of ASV richness

The analyses were first focused on the changes in ASV richness among habitats and seasons. The importance of seasons, habitat types, and their interaction as potential drivers of changes in soil micro-eukaryotic ASV richness was assessed. A linear model using ASV richness as a response and habitats and seasons as the two explanatory variables (i.e. without interaction, adjusted R^2= 0.31, P <

0.001) was computed. A second linear model using the same variables was then computed, but, this time, considering an interaction between seasons and habitats (adjusted R^2= 0.54, $P < 0.001$). An ANOVA showed that including the interaction improved the model ($P < 0.001$). Because the interaction between seasons and habitats was significant, these variables (and their interaction) were considered together instead of individually.

The extent to which spatiotemporal, soil condition, and soil function variables explained the variation in ASV richness was then assessed using a variation partitioning analysis (Peres-Neto *et al.* 2006; Legendre & Legendre 2012). More specifically, the variation of ASV richness was partitioned into a spatiotemporal fraction (i.e. interaction between seasons and habitats), a soil function fraction, and a soil condition fraction. Generalized Linear Models (GLM) with ASV richness as response and six variables reflecting soil conditions alongside four variables reflecting soil functions as descriptors were used to assess the importance of soil condition and soil function variables within each season. Soil silt and clay content were removed before analyses because of collinearity.

2.9. Beta diversity: ASV turnover among habitats and seasons

In order to estimate the relative importance of the spatial and temporal turnover of ASV per habitat as well as at the floodplain scale (i.e. within and among habitats), an additive partitioning of total ASV diversity (gamma) into alpha, beta spatial, and beta temporal components was conducted. Through additive decomposition, β-diversity is explicitly an average amount of diversity just as is α-diversity (Veech et al. 2002). This approach thus allows direct comparison of alpha and beta diversities which is particularly relevant for testing theoretical concepts and developing conservation and/or management applications. The additive partitioning of diversity was done following the method of Tylianakis et al. (2005) (see also: Veech *et al.* 2002; Crist *et al.* 2003). Alpha diversity (α) was defined as the mean number of ASV per plot per season. The temporal turnover in ASV between seasons was calculated for each plot (β_{TPlot}) within a given habitat type as: the total number of ASV found within that plot (over the entire year) minus the mean number of ASV per season for that plot (α). Overall β_T was calculated as the mean β_{TPlot} for a given habitat type. Spatial turnover (β_S) was calculated as the total number of ASV found within a habitat type over the entire year minus the mean number of ASV per plot of that habitat type (over the entire year). Therefore, the overall diversity of a habitat type can be described as $\gamma = \alpha + \beta_T + \beta_S$.

The drivers of community compositional changes were then investigated using PERMANOVA applied on a Bray-Curtis dissimilarity matrix (function ADONIS, R package “vegan”; Oksanen *et al.* 2015). Seasons (time), habitat (space), and their interaction (space-time) were used as explanatory variables. As for ASV richness, there was a significant space-time interaction (whole model: adjusted $R^2 = 0.34$, $P < 0.001$; interaction term: adjusted $R^2 = 0.2$, $P = 0.001$). The variation in ASV dissimilarity was then partitioned into a spatiotemporal fraction (i.e. interaction between seasons and habitats), a soil function fraction, and a soil condition fraction (Peres-Neto *et al.* 2006; Legendre & Legendre 2012). Finally, the importance of soil condition and soil function variables within each season was explored using PERMANOVA with ASV dissimilarity as response and six soil condition and four function variables as descriptors. As for ASV richness, soil silt and clay content were removed before analyses because of collinearity.

2.10. Indicator ASV

Indicator ASVs were searched for each habitat in each season. The indicative value (IndVal) of each ASV for each habitat per season was assessed using the function “multipatt” of the R package “indicspecies” (De Cáceres, Legendre & Moretti 2010). This approach calculates an Indicator Value (IndVal) index to measure the association between a species (here ASV) and a group of sites (here habitats; Dufrêne & Legendre 1997). The statistical significance of this relationship was assessed using a permutation test.

All statistical analyses were performed in *R version* 3.6.1 (R Development Core Team 2016).

3. Results

3.1. Metabarcoding of riparian soil microbial eukaryote environmental DNA.

A total of 11,280,627 microbial eukaryote reads belonging to 14,900 distinct amplicon sequence variants (ASVs) were identified in the studied riparian soils. Overall, the dominant taxonomic groups in proportion of sequences were Fungi (36%), Cercozoa (15%), Ciliophora (8%), Bacillariophyta (diatoms) (6%), Chrysophycaea (3%), and Peronosporomycetes (=”Oomycetes”) (3%), while 30% of all ASVs belonged to less abundant taxonomic groups.

3.2. Spatiotemporal changes in ASV richness

ASV richness varied considerably over space and time (Fig. 1A). The most striking differences among habitats were observed in summer and winter. ASV richness was least variable among habitats in autumn where a large intra-habitat variation was observed. In agreement, the interactions of seasons and habitats explained an important part of the variation in ASV richness (adjusted $R^2 = 0.36$; Fig. 1B). Part of this variation can be explained by changes in soil conditions (adjusted $R^2 = 0.1$) and soil functions (adjusted $R^2 = 0.08$). We also found strong difference in the importance and effect of soil conditions and functions within each season (Table 1).

[Here Fig. 1 and Table 1]

3.3. Spatiotemporal variability in soil microbial eukaryotic assemblages

The turnover of species was higher among habitats (β_S) than seasons (β_T), (Fig. 2A) indicating that the community composition of soil microbial eukaryotes varied more spatially than seasonally. The temporal turnover of species was higher than alpha diversity in all habitats. However, the temporal turnover was about half of the spatial turnover within each habitat. Furthermore, the relative importance of the spatial turnover of species increased at the floodplain scale highlighting the heterogeneity among habitats. We found a similar pattern for the six most abundant taxonomic groups with little variability among groups (Fig. S2). We further found that the interaction between habitat and seasons explained 14 % of the variation in community composition whereas soil conditions and functions each explained about 2 % of this variation (Fig. 2B). We also found strong changes in the importance of soil conditions and functions within each season (Table 2).

[Here Fig. 2 and Table 2]

3.4. Indicator ASVs

We found several indicator ASVs for each habitat in each season, but with important differences in the number and type of indicators (Fig. 3). In spring and autumn, the number of indicator ASVs was clearly higher in *Pasture*. In winter, however, the highest number of indicators was observed in *Gravel*. We also observed differences among taxonomic groups. For example, diatoms were associated to *Gravel* in winter and Cercozoa were more frequently associated to the three habitats further away from to the river (*Mixed forest, Willow forest,* and *Pasture*).

[Here Fig. 3]

4. Discussion

Soil microbial eukaryotes are highly diverse and play key roles in soil functioning (Geisen et al. 2018). Currently, however, the spatial and, especially, temporal patterns of soil microbial eukaryotic beta diversity remains poorly known and our main goal was to characterize these patterns and understand their drivers in a highly dynamic natural and complex ecosystem. Characterizing soil microbial eukaryotic spatiotemporal beta diversity will improve our understanding of community dynamics as well as our capacity to anticipate future changes in soil ecosystem structure and functions.

The present study is, to our knowledge, the first that presents a detailed assessment of soil microbial eukaryote ASV richness and spatiotemporal beta diversity in riparian soils. Soil microbial eukaryotic richness and beta diversity were surveyed using a DNA-based approach over an entire year along a gradient of very heterogeneous habitats encompassing strong changes in flood dynamics, vegetation, and soil conditions and functions. Our results highlight a strikingly important contribution of beta diversity to total microbial eukaryotic diversity within the floodplain. Specifically, the spatial and, to a lesser extent, temporal turnover of ASVs are the main sources of microbial eukaryotic diversity within the floodplain. This contrasts with previous studies based on microscopic observations highlighting a high local diversity of microbial eukaryotes and a relatively high local/global species ratio of soil microbial eukaryotes (e.g. Finlay 2002; Fenchel & Finlay 2004). Such studies, however, characterized a limited fraction of the total diversity by lumping large numbers of genetically very different organisms into single morphospecies (Bass *et al.* 2007; Heger *et al.* 2013) which likely greatly underestimates the importance of beta diversity (Singer *et al.* 2018). The advent of high throughput metabarcoding studies now allows a more complete and objective assessment of biodiversity patterns and a reassessment of existing knowledge. In a study of Neotropical rainforest soils, Lentendu et al. (2018) found a high alpha and low beta diversity for several groups of microbial eukaryotes. However, this study was done in rainforest habitats only (i.e. it does not include a gradient of habitats ranging from bare soils to relatively dense forests contrary to our study) and was based on a single sampling campaign, thus not considering the temporal aspect of beta diversity, which might explain the low beta diversity observed. In contrast, Benke et al. (2010), studying the spatiotemporal changes of protist communities in marine environments, found that the temporal variation was as pronounced as the spatial differences between depths. And Chénard et al. (2019) observed a higher seasonal variation in coastal waters exposed to the influence of the monsoon. In our case, the temporal variation is less pronounced than the spatial one. We attribute this difference as reflecting the higher spatial heterogeneity of soils as compared to marine ecosystems.

We observed a high spatiotemporal complexity in the edaphic drivers of soil microbial eukaryotic alpha and beta diversities. Indeed, a strong interaction between seasons and habitats determines ASV richness and beta diversity. And, seasonal analyses of the drivers of ASV richness and beta diversity show important shifts in variable importance (R^2) as well as shifts in the direction of the relationship (Table 1 and 2). While other studies, in agreement with our results, have highlighted the importance of soil water availability (Bates *et al.* 2013; Geisen *et al.* 2014; Fiore-Donno *et al.* 2019), C and N cycling (Krashevska *et al.* 2010), and soil temperature (Tsyganov *et al.* 2011) as drivers of microbial eukaryote taxa, to our knowledge no study has assessed the importance of proxies for soil ecosystem functioning alongside soil conditions and the seasonal shifts in these drivers. These shifts are likely to have important consequences for biogeochemical processes in soil ecosystems (Levy-Booth *et al.* 2019). For example, soil moisture has negative effect on richness in spring and summer and a positive effect on richness in winter. In agreement with these results, Geisen et al. (2014) showed a non-linear effect of soil water availability on soil protists suggesting maximum abundance of soil protists at intermediate levels of soil water availability. Most of the other studied soil variables showed complex seasonal shifts in importance and/or effects that likely reflects flooding dynamics, and biogeochemical cycles in the soil ecosystems. Interestingly, we observed a floodplain-scale homogenization of soil microbial eukaryotic communities in autumn (i.e. no significant differences in composition among habitats). This homogenization likely resulted from floods occurring shortly before the sampling campaign. Floods can bring propagules from outside the floodplain and move soil material among habitats resulting in increased dispersal at the floodplain scale. Dispersal in metacommunities has indeed the potential to increase immigration in local communities resulting in homogenization at the metacommunity scale (Fodelianakis *et al.* 2019).

The present study also highlights the role of deterministic processes for soil microbial eukaryotic diversity. The high beta diversity and the identification of several ASVs as specific to a particular season and habitat (Fig. 3) suggests a direct response to environmental heterogeneity, as reported for testate amoebae (Fournier *et al.* 2012) and macro-invertebrates at the same site (Fournier *et al.* 2015). Previous studies have already highlighted that different habitat types (e.g., seawater versus soils) harbour different suites of microbial eukaryotes (Bates *et al.* 2013). And the role of a variety of environmental factors in determining soil microbial eukaryotic community structure and richness is well established (Krashevska *et al.* 2010; Bates *et al.* 2013; Geisen *et al.* 2014; Fiore-Donno *et al.* 2019). As such, it is likely that species sorting is a major driver of soil microbial eukaryotic diversity patterns (Leibold *et al.* 2004; Pandit *et al.* 2009; Singer *et al.* 2018). However, at a finer scale, soil microbial eukaryotes were also shown to have a stochastic distribution in boreal forest soils (Bahram *et al.* 2016), a result most likely due to the short environmental gradient sampled. Thus, the degree of environmental heterogeneity covered in a study likely determines the inferred strength of stochastic versus deterministic processes for the assembly of soil microbial eukaryotic communities with the importance of deterministic niche-based processes increasing together with heterogeneity (e.g. along environmental gradients) (Jassey *et al.* 2011).

Our results contrast with a previous study at the same study site showing a much higher temporal than spatial variation of bacterial communities (Samaritani *et al.* 2017). Studies showing a higher spatial than temporal beta diversity of soil bacteria also exists (e.g. Lauber *et al.* 2013).

However, these studies were conducted over larger spatial extent and the importance of dispersal limitation is thus likely to be higher than in our study. Two interrelated hypotheses can explain the observed differences in spatiotemporal beta diversity between soil microbial eukaryotes and bacteria. Microbial eukaryotes typically have longer life span and generation time than bacteria. Despite large variation in life strategies within micro-eukaryotes and within bacteria, microbial eukaryotes can, in general, be considered as K-strategists while bacteria would then be r-strategists, and thus the diversity patterns of microbial eukaryotes should be driven (and explained) by local adaptation (r-K selection; MacArthur & Wilson 1967; Pianka 1970). In addition, microbial eukaryotes might have a lower passive dispersal capacity (e.g. by wind and flood) than bacteria due to their larger (by microbial standard) size (Wilkinson *et al.* 2012). It can thus be expected that local niche-based processes and dispersal limitations play a larger role for protists than for bacteria. In agreement with this, a recent study in marine ecosystems highlighted lower sorting/dispersal effect ratios for bacterial communities as compared to protist communities (Wu *et al.* 2018).

Our study, however, did not consider some potentially important factors for soil microbial eukaryotic diversity. For example, seasonal shifts from bacteria-based to fungal-based decomposition pathways should modify the availability of food resources potentially causing shifts in the composition of the phagotrophic component of the soil microbial eukaryotic communities. Indeed, previous studies have identified highly specialized fungal feeder microbial eukaryote taxa (Petz *et al.* 1985; Foissner 1999) that would be negatively impacted by a decrease in the abundance of fungi in the soil. However, some protists are generalist feeders that are also capable of facultative mycophagy (Geisen *et al.* 2016). Furthermore, some protists are autotrophs, mixotrophs or osmotrophs and are thus not or only marginally affected by the relative abundance of fungi vs bacteria. In any case, the lower temporal than spatial variation suggest that eukaryotes can cope with potential seasonal shifts in prey availability either by being flexible in their feeding source or by encysting. More specific investigations are needed to understand the implications of our results for the whole microbial foodweb. In addition, studies at larger spatial scale or experimental approaches might complement our results about the role of dispersal for beta diversity and its changes across spatial scales. Similarly, our study does not consider the inter-annual or intra-seasonal variability in soil microbial communities which are expected to be smaller than the inter-seasonal variability but might still represent a significant fraction of the total diversity. And, because our approach is based on eDNA, our data might include ASVs derived from extracellular DNA, encysted, or inactive organisms. Finally, our study raises the question of the role of floods for microbial taxa dispersal within the floodplain. Indeed, floods can bring propagules from outside the floodplain and move soil material among habitats thus increasing dispersal and stochasticity potentially leading to homogenization of community composition at the floodplain scale. As such, one can expect low spatial structuring of communities. However, our results do not support this view and rather point toward the importance of local environmental filtering for community structure.

5. Conclusions

We demonstrated the existence of both spatial and temporal turnover in floodplain soil microbial eukaryotic diversity mirroring the spatiotemporal patterns of biogeochemical processes in these ecosystems. Our results also illustrate the value of spatial monitoring of soil biodiversity across habitats within a complex ecosystem. The observed temporal dynamics illustrate the value of seasonal sampling for biodiversity assessment to accurately estimate the diversity and the complexity of assembly processes of soil microbial communities. Sound measurements of the patterns and drivers of soil microbial diversity is needed to understand the relationships between biodiversity and ecosystem functions. This, in turn, allows to assess the impact of environmental changes and management options including restoration efforts on a range of ecosystems.

Conflict of interests

The authors declare no conflict of interest regarding the publication of this article.

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Table and figure legends

Table 1. Predictors in generalized linear models for floodplain soil micro-eukaryotic amplicon sequence variants (ASV) richness per season, their coefficient (Z), and significance (P). GLMs were fitted assuming a Poisson error distribution and using the logarithm as the link function. Bolded characters highlight significance ($\alpha < 0.05$).

		Spring		Summer		Autumn		Winter	
		(R^2 = 0.56)		(R^2 = 0.61)		(R^2 = 0.51)		(R^2 = 0.8)	
		Z	P	Z	P	Z	P	Z	P
Soil conditions	Soil moisture	**-2.446**	**0.014**	**-12.461**	**>0.001**	1.837	0.066	**5.745**	**>0.001**
	Soil temperature	**2.082**	**0.037**	**21.63**	**>0.001**	-1.459	0.145	**2.149**	**0.032**
	Sand	1.802	0.071	**-10.511**	**>0.001**	**-10.102**	**>0.001**	0.177	0.859
	Organic carbon	**-4.215**	**>0.001**	**-13.692**	**>0.001**	**-12.639**	**>0.001**	**-3.73**	**>0.001**
	Available phosphorus	**3.118**	**0.002**	1.183	0.237	**13.147**	**>0.001**	**12.863**	**>0.001**
	Total nitrogen	**3.327**	**0.001**	**13.936**	**>0.001**	**4.845**	**>0.001**	-1.447	0.148
Soil functions	Basal respiration	**-2.292**	**0.022**	**12.225**	**>0.001**	**9.432**	**>0.001**	**-4.565**	**>0.001**
	Enzymatic activity	**2.684**	**0.007**	**3.735**	**>0.001**	0.657	0.511	**-8.354**	**>0.001**
	Microbial carbon	**5.432**	**>0.001**	**9.289**	**>0.001**	**-16.266**	**>0.001**	**4.241**	**>0.001**
	Microbial nitrogen	**-2.167**	**0.030**	-0.531	0.596	**12.318**	**>0.001**	0.613	0.540

Table 2. Predictors in PERMANOVA for floodplain soil micro-eukaryotic community dissimilarity (Bray-Curtis) per season, their partial R-squared (R^2), and significance (P). Bolded characters highlight significance ($\alpha < 0.05$).

		Spring		Summer		Autumn		Winter	
		(R^2 = 0.49)		(R^2 = 0.47)		(R^2 = 0.5)		(R^2 = 0.49)	
		R^2	P	R^2	P	R^2	P	R^2	P
Soil conditions	Soil moisture	**0.055**	**0.044**	0.047	0.205	**0.064**	**0.014**	0.037	0.583
	Soil temperature	**0.075**	**0.002**	0.058	0.073	**0.074**	**0.007**	0.038	0.49
	Sand	**0.060**	**0.018**	0.056	0.099	0.044	0.192	**0.099**	**0.001**
	Organic carbon	0.043	0.249	0.039	0.463	0.041	0.302	0.040	0.384
	Available phosphorus	0.038	0.536	0.044	0.294	0.048	0.13	0.040	0.366
	Total nitrogen	0.042	0.273	0.045	0.265	0.046	0.153	0.037	0.61
Soil functions	Basal respiration	0.034	0.84	0.049	0.197	0.053	0.064	**0.051**	**0.052**
	Enzymatic activity	**0.053**	**0.042**	0.036	0.663	0.038	0.444	**0.055**	**0.033**
	Microbial carbon	0.039	0.452	0.060	0.071	0.046	0.169	0.046	0.147
	Microbial nitrogen	0.049	0.078	0.037	0.543	0.042	0.292	0.049	0.086

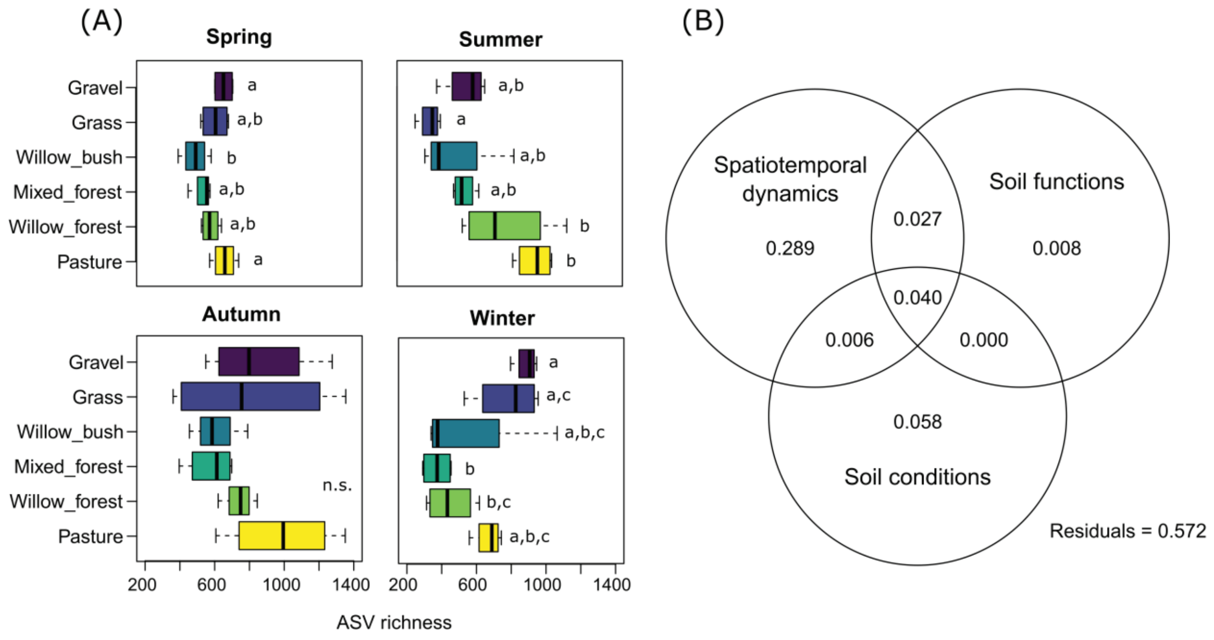

Fig. 1. (A) Changes in floodplain soil micro-eukaryotic amplicon sequence variants (ASV) richness among habitats per season. Letters indicate pairwise differences in mean (Tukey honest differences). Colors highlight the six different habitats. (B) Partitioning of the variation in ASV richness among spatiotemporal (36.2%), soil condition (10.4%), and soil function (7.5%) components.

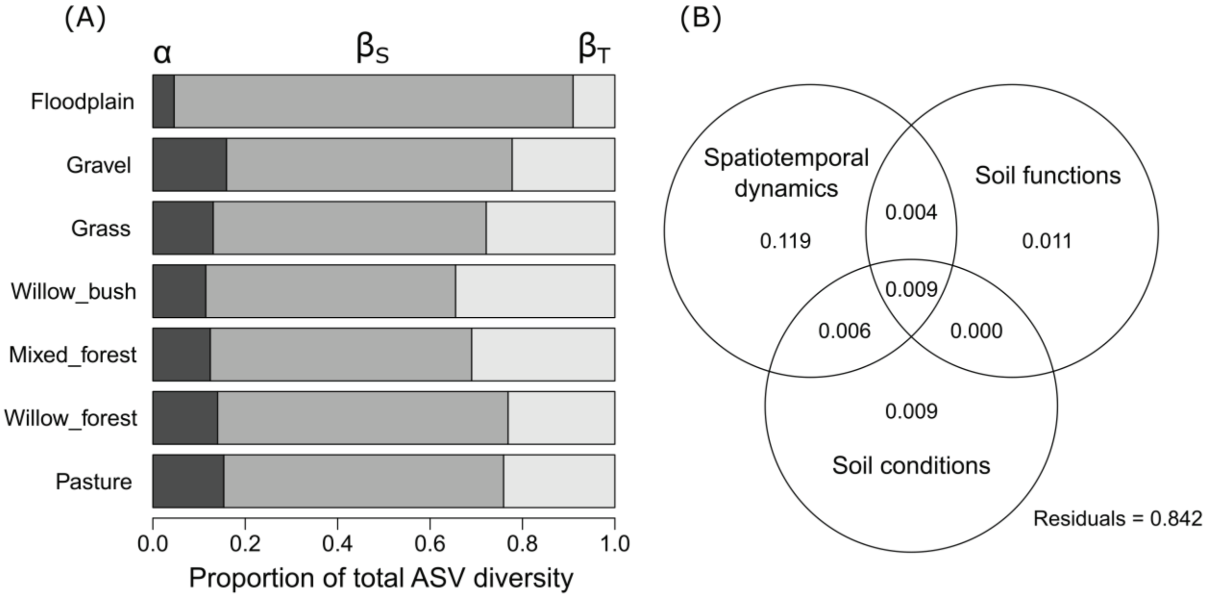

Fig. 2. Spatiotemporal turnover of floodplain soil micro-eukaryotic taxa (amplicon sequence variants - ASV). (A) Partitioning of total diversity (γ) into local diversity (α), temporal species turnover (β_T), and spatial species turnover (β_S) per habitat ($\gamma = \alpha + \beta_T + \beta_S$). This analysis shows a higher spatial than temporal turnover of soil microbial eukaryotic taxa both at the floodplain scale and within habitats. (B) Partitioning of the variation in ASV community composition among spatiotemporal (13.8%), soil condition (2.4%), and soil function (2.4%) components.

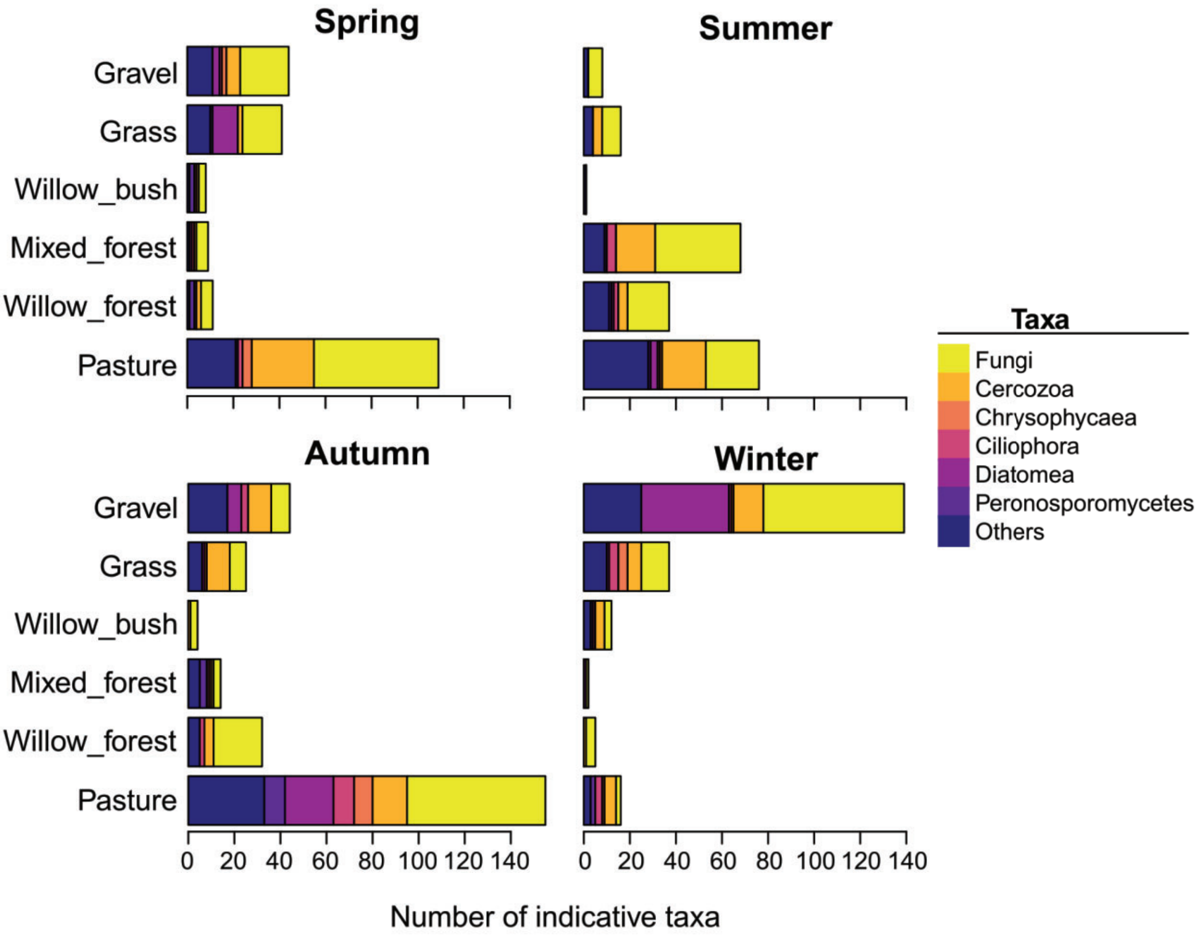

Fig. 3. Indicator ASVs (amplicon sequence variants) of floodplain soil micro-eukaryotes for each habitat at each season. Only ASVs with significant IndVal values (indicator values; De Cáceres, Legendre & Moretti 2010) are shown. Colors show the different taxonomic groups. Other are ASVs belonging to less abundant taxonomic groups.