# Effects of short-term flooding on aquatic and terrestrial microeukaryotic communities: a mesocosm approach

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ABSTRACT: Freshwater and soil are not strictly isolated habitats. In particular, floods may facilitate the exchange of organisms and nutrients. Flooding can have both a stimulating and a harmful effect on the organisms of the respective habitats. The effects of short-term flooding on microeukaryotic communities in the aquatic and terrestrial habitat have so far been scarcely studied. Here, we investigated the effect of a 24 h artificial inundation on the microeukaryotic community composition in AquaFlow mesocosm systems. We investigated the shift of community composition based on molecular amplicon diversity both on soil and water during flooding and for a period of 12 d after flooding. Community composition was, as expected, strongly different between soil and water. Flooding had a significant effect on the freshwater community, whereas the soil community was hardly affected. In particular, we observed a transfer of nutrients from the terrestrial habitat into the aquatic habitat and identified ~50 taxa that were transferred by the flooding event. This effect of flooding was, however, overlaid by shifts of the communities with time, presumably reflecting an acclimatization to the conditions in the AquaFlow systems.

KEY WORDS:  Flooding · Inundation · Microbial diversity · Streams · Nutrients · Protists · Fungi · Mesocosm experiment

## INTRODUCTION

Life at the water–soil boundary is exposed to recurring floods, in particular due to seasonal rain and thaw (Blom & Voesenek 1996, Power et al. 2008) but also due to summer inundations (Antheunisse & Verhoeven 2008) or other weather extremes. Flooding events are one trigger of biodiversity in aquatic and interlinked terrestrial ecosystems as they provide ways for the exchange of nutrients (Baldwin & Mitchell 2000, Beltman et al. 2007, Schulz et al. 2015) and organisms (Shearer et al. 2007, Schulz et al. 2015) between both habitat types. The river continuum concept of Vannote et al. (1980) describes soil as the resource donor and considers the biotic change along the river. In contrast, the flood pulse concept of Junk et al. (1986) described the flood pulse as a 'major force controlling biota in river floodplains' of large tropical lowland rivers. This concept considers the vertical connectivity of the river and the floodplain and the resulting possible exchange between both habitats. The extension of Tockner et al. (2000) adapted this concept to the temperate zones with shorter and less predictable flood pulses. Concepts developed later, like the meta-ecosystem concept by Loreau et al. (2003), emphasize the reciprocal influ-

ence of 'a set of ecosystems connected by spatial flows of energy, materials and organisms across ecosystem boundaries' (p. 673). Aside from such potential stimulating effects, flooding events can also disturb the community of organisms (Lake 2000).

Microeukaryotes, predominantly protists and fungi, are an important component in both habitats. In aquatic habitats, protists are the dominant planktonic primary producers and effectively feed upon bacteria and thereby contribute to the self-purification of water bodies (Fenchel 1986, Šimek et al. 1997, Finlay & Esteban 1998). In terrestrial habitats, protists and microfungi are involved in soil respiration and decomposition processes (Coûteaux & Darbyshire 1998, Barrios 2007, Rousk & Frey 2015). Rønn et al. (2012, p. 229) pointed out that most soil protists are 'fundamentally aquatic creatures visiting a terrestrial world'. Many protist and microfungi taxa, e.g. chrysophytes, ciliates, oomycetes and chytridiomycetes, indeed occur in both habitat types (Wong et al. 1998, Boenigk et al. 2005, Shearer et al. 2007, Foissner et al. 2008, Findenig et al. 2010, Blackwell 2011). Nevertheless, microeukaryotic communities distinctly differ between freshwater and terrestrial habitats (Grossmann et al. 2016). Flooding events are one potentially important route for the dispersal of organisms (Weisse 2008) and consequently may drive the distribution of these taxa between the different habitat types (Shearer et al. 2007, Crump et al. 2012, Schulz et al. 2015). Taxa can be transferred from water to the soil where the soil pores may provide an appropriate habitat for their survival. Vice versa, taxa that occur in soil are washed out of the soil matrix into the water when substrate adhesion is not strong enough. Aside from the exchange of vegetative cells, a flooding event may transfer cysts and spores between habitats and stimulate the germination of cysts (Shearer et al. 2007, Weisse 2008, Foissner 2011). Such exchanges of organisms or immigration may change the community composition or the abundances of taxa which in turn can affect ecosystem functions (Litchman 2010). Identifying the extent of this exchange and the persistence of introduced microeukaryotes is therefore one objective of our study.

Most studies of flooding effects have so far focused on altered flow regimes within streams (see e.g. Bunn & Arthington 2002, Poff & Zimmerman 2010 for review), the flux of nutrients (Bardgett et al. 2001) and changes in nutrient cycles (see e.g. Baldwin & Mitchell 2000 for review), floodplains and riparian zones (see e.g. Naiman & Decamps 1997, Ward et al. 1999 for review) as well as on artificial long-term inundations, e.g. agricultural studies and mesocosm experiments leading to anoxic conditions in soil (Liesack et al. 2000, Unger et al. 2009b). These studies investigated plant and microbial communities in floodplain or riparian zones (Blom & Voesenek 1996, Beltman et al. 2007, Kobayashi et al. 2009, Baldwin et al. 2013, Simoes et al. 2013), effects of altered hydraulic conditions on fishes, invertebrates and the algae community in streams (Growns & Growns 2001, Anderson et al. 2006, McKay & King 2006) or the effect of abiotic changes on the microbial communities in soils (Bossio & Scow 1995). However, most studies focused on one habitat type. The effect of short-term inundation is less well studied — particularly for streams but also for the interconnected soils (but see Mentzer et al. 2006, Muylaert & Vyverman 2006, Antheunisse & Verhoeven 2008, Unger et al. 2009a, Wilson et al. 2011). Mentzer et al. (2006), Unger et al. (2009a) and Wilson et al. (2011) studied the effects of short-term inundations on soil microbial communities with regard to the community composition, microbial biomass and activity by terminal restriction fragment length polymorphism analysis (T-RFLP), phospholipid fatty acid analysis (PLFA) and activity analysis of several enzymes. All studies identified a change in community structure in soil caused by flooding, e.g. Unger et al. (2009a) found a decrease of fungal PLFA markers compared to anaerobic bacterial PLFA markers. Muylaert & Vyverman (2006) studied the effect of a short-term inundation on the aquatic community composition. They found shifts in community assemblage caused by the flooding event and the return to the original community composition after >14 d.

Here, we analyze the effect of 1 short, 24 h ongoing flooding event on the eukaryotic community (excluding Metazoa and Embryophyta) of both freshwater and the interconnected soil using experimental mesocosm systems, i.e. the AquaFlow systems in the greenhouses at the botanical garden of the University Duisburg-Essen (Fig. 1). We investigated the shifts in community structure for a time period of 14 d. Further, we analyzed the rate of exchange of organisms caused by the flooding event and the persistence of immigration of individual taxa.

## MATERIALS AND METHODS

### Experimental setup

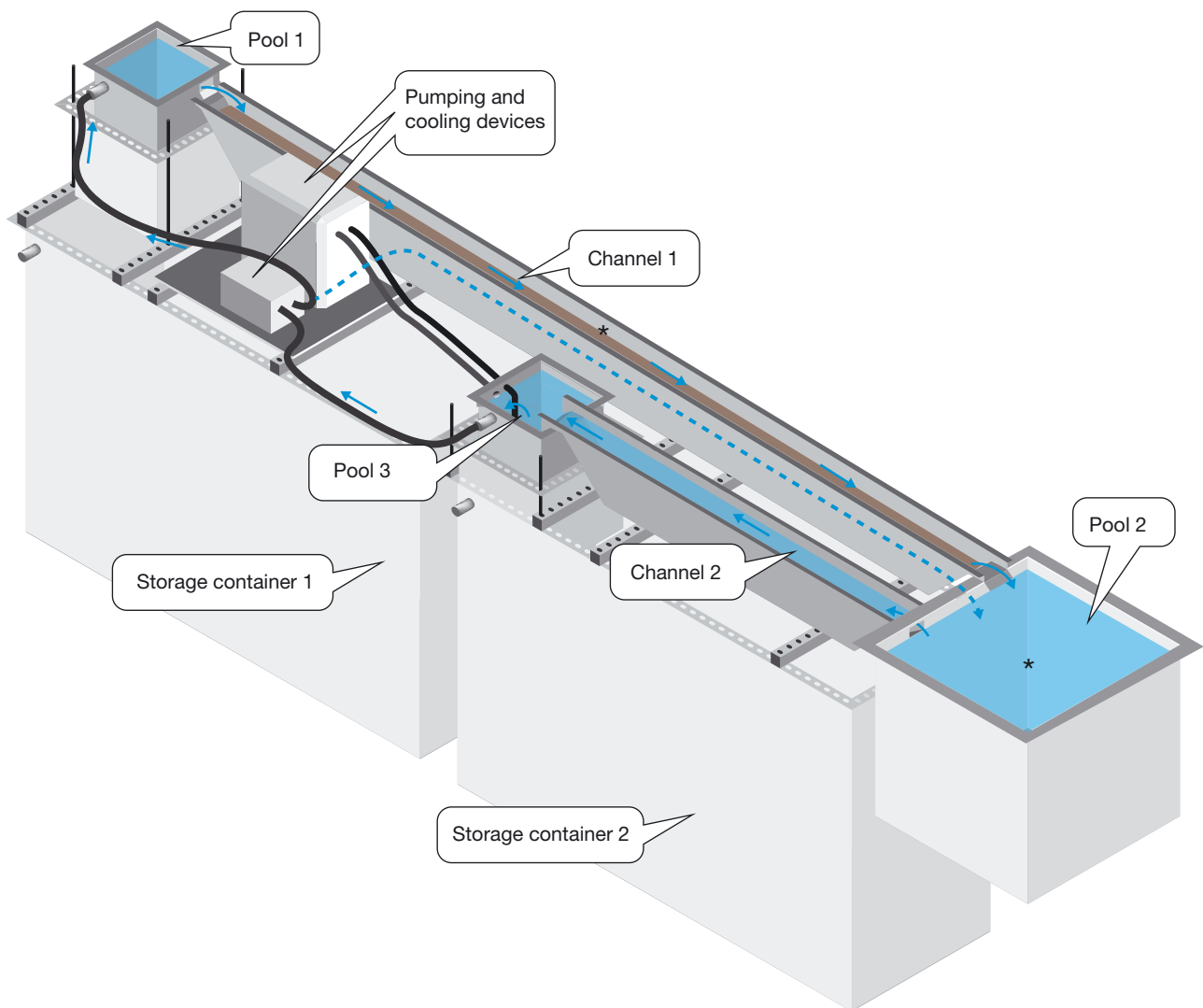Experiments were performed in 6 experimental stream systems, i.e. the AquaFlow systems at the

Fig. 1. Schematic illustration of one 'AquaFlow'. The brown area shows the soil-filled part of the AquaFlow; the blue area shows the water-filled part of the AquaFlow. Blue arrows highlight the water cycle of the flooded treatments, whereas the dashed line shows the shortcut water cycle of the control treatments (without Pool 1 and Channel 1). The marked positions (*) in Channel 1 and Pool 2 show the positions of sampling

University of Duisburg-Essen (Fig. 1). Each system, constructed as a circulatory system, comprises a series of tanks and channels simulating the riffle and pool sequence of streams. Three tanks (~40 l, ~270 l, ~40 l) are connected by 2 channels (4 m and 2 m) which are exchangeable for channels with different profiles ranging from 5 to 10 cm width and a depth between 5 and 20 cm. Two large storage containers (~1600 l) are used for defined media. The systems can therefore be operated in circular flow or uniflow mode. These systems allow for manipulation of water volumes, flow velocity, slope of the channels and water temperature. Further, the systems allow alternatively for natural illumination and/or artificial illu-

mination. Parameters such as room temperature, maximal light intensity of sunlight (up to a defined limit; beyond this limit, the AquaFlows are automatically shaded) or artificial illumination, as well as the water temperature (by use of water-refrigerators), can be adjusted.

For our experiment, the systems were used for simulating soil–water interactions in the course of a flooding event. The channels simulate the soil habitats, whereas the water tanks simulate the aquatic habitat. We used flow channels with 10 cm width and 10 cm sediment depth so that total soil volume per system was ~40 l. For the aquatic habitat, we used the small water cycle excluding the containers, i.e.

with Pools 1 to 3 and both channels so that the total water volume per system was ~350 l.

Water originated from the river Emscher (51°29′ 19.8″ N, 7°31′ 51.7″ E, Germany) and soil from an untreated meadow (51°28′ 33.9″ N, 7°15′ 18.6″ E, Germany). Soil, i.e. the A-horizon, was taken and homogenized (sieved, mesh size 1 cm$^2$) to remove remnants of plant roots, stones and visible invertebrates. Immediately after transport, the homogenized soil was filled into Channel 1 of each of the 6 AquaFlow systems. There, it was stored under experimental conditions for 5 d until the experiment started. Water was sampled from the river Emscher by a generator driven pump MP 120/5A/ GJ (Güde, Wolpertshausen,) including a filter (mesh size 1 mm$^2$) to remove plant particle and invertebrates. Water was transported in a 1000 l IBC water tank (Barrel Trading, Gaildorf) and placed in Pools 1 to 3 of each of the 6 AquaFlow systems immediately after sampling. There, it was stored under experimental conditions for 2 d until the experiment started. The experiment took 14 d and was conducted from 10 to 23 September 2014. During the experiment, evaporating water was replaced with deionized UV-irradiated water to maintain the water-levels.

Natural illumination up to 900 µE (at higher light intensities, the green house units were shaded) was used for the experiment. Air temperature in the greenhouse units was adjusted to 20°C, and water temperature was adjusted to 17.3 ± 0.4°C (SD) by cooling with the water-refrigerator TC20 (TECO SRL, Ravenna, ITA). The inclination of the channels was set to 2°, and the rotation of the peristaltic pump Hei-FLOW advantage (Heidolph Instruments, Schwabach) was 350 rpm, corresponding to a water discharge of 150 l h$^{-1}$.

To investigate the effect of flooding, we simulated a flooding event with a duration of 24 h in 3 of the 6 AquaFlow systems. The other 3 AquaFlow systems were used as controls. In the control treatments, water circulation was shortcut by connecting Pool 3 and Pool 2 with an 8 mm silicon tube bypassing Pool 1 and Channel 1. The soil in these channels stayed therefore separate from the water flow. During the flooding, water was pumped from Pool 3 into Pool 1 through an 8 mm silicon hose. From there, water could flow through Channel 1 into Pool 2 where it was mixed by an aquarium pump (EHEIM, Deizisau) and further flow through Channel 2 into Pool 3. Water flow in the flooded treatments was shortcut after the end of the flooding event as described for the control treatment.

## Sampling strategy and sample preparation

The experiment ran for a total time-period of 14 d. Each of the 6 AquaFlow systems was sampled at 7 different times (Table 1). At each time point of sampling, water samples were collected, whereas soil samples were only collected when the AquaFlow systems were not flooded (no collection of samples at Day 1.1 and Day 1.2). Water samples were collected near the surface in Pool 2 (except the samples of Day 1.1; here, we collected the 'first water' from the output of channel 1) and soil samples in the center of Channel 1.

At each sampling, we measured various physicochemical parameters in the water: nitrate, nitrite, phosphate, ammonium, and chloride concentration, as well as carbonate hardness, temperature, conductivity, and pH. The levels of nitrite, nitrate, phosphate, ammonium, carbonate hardness and chloride were measured using test kits of Spectroquant® following the instructions of the manufacturers. Therefore, 100 ml subsamples were taken and filtered through 0.45 µm PES membrane filters type 154 (Sartorius, Göttingen). Temperature, conductivity and pH were measured within the AquaFlows using the combi-measuring instrument Combo pH & EC (Hanna, Vöhringen). In soil, we measured temperature, soil moisture (via suction tension of soil water) and oxygen availability (via the redox potential). To conduct measurements, we used a probe thermometer, tensiometers and redox electrodes (Maßfeld, ecoTech Umwelt-Meßsysteme, Bonn). Measurements were performed within the AquaFlow systems. The tensiometers and the redox electrodes were permanently installed during the whole experiment in the center of Channel 1. Chemical parameters were determined once, and all physical parameters in water and soil were determined twice independently. Mean values and standard deviations were calculated amongst the control treatments and the flooded treatments.

Further, we collected water and soil samples. Water samples were collected in sterile 1 l glass bottles. From each sample, 10 ml subsamples were taken and dark-adapted (30 min.) for measurement of the photosynthetic activity via chlorophyll *a* fluorescence using the AquaPen-C AP100 instrument (Photon System Instruments, Brno). We performed the OJIP protocol to measure the '$F_V/F_M$' ratio and the 'Fix Area' value. The '$F_V/F_M$' ratio measures the performance of the photo system II and thus indicates the extent of stress and vitality (Strasser et al. 2000, Thwe & Kasemsap 2014). The 'Fix Area' value meas-

ures the area under the induction curve of fluorescence during a saturating light flash (duration: 1 s) which represents indirectly the chlorophyll *a* content (Strasser et al. 2000, Thwe & Kasemsap 2014). Additional 10 ml subsamples were preserved with formaldehyde (final concentration: 5.4%) and another subsample of 200 ml was filtered through a 0.2 µm polycarbonate Isopore™ membrane filter (Merck Millipore®) at ~350 mbar by a LABOPORT® N820 FT.18 pump (KNF Neuberger, Freiburg) for enrichment of biomass. We collected 0.3 g soil per sample via sterile spatula. Biomass filters and soil samples were stored on ice until freezing at −80°C on the same day. To avoid cross contamination, we used for each AquaFlow separate or ethanol-cleaned measuring instruments and sterile laboratory equipment.

## Laboratory procedures

Bacteria from formol fixed samples were quantified by staining with DAPI (4′6-diamidino-2-phenylindole) and visualization by fluorescence microscopy.

DNA from water samples was extracted using the extraction kit my-Budget DNA Mini Kit (BioBudget Technologies, Krefeld) according to the manufacturer s instructions, except for the following steps: Biomass filters were transferred to lysis tubes E (MP Biomedicals); 800 µl TLS lysis buffer was added and incubated (55°C, 10 min); then mechanical lysis (3 × 6 m s$^{-1}$, 45 s) was performed with the FastPrep24 instrument (MP Biomedicals) followed by 3 centrifugation steps (10 000 × *g*, 1 × 15 min, 2 × 2 min). Prior to the additional washing steps (at least 2) with the MS wash buffer, we incubated the sample in the first MS washing for 15 min. Elution was performed in 30 µl elution buffer. Extraction of DNA from 0.3 g soil samples was performed according to the protocol developed by Peršoh et al. (2008) with a modified amount of aluminium sulfate and elution buffer according to Röhl et al. (2017). The isolated DNA of all samples was stored at −20°C.

PCR amplification targeting a ~600 bp long fragment including the SSU V9 region including the ITS1. Forward primers are based on the Euk1391F primer (5′-GTA CAC ACC GCC CGT C-3′) (Lane 1991, Lange et al. 2015). The reverse primers are based on the design by White et al. (1990) and were modified by J. Nuy (pers. comm.). To cover a broad taxonomic spectrum, 2 primers with different wobble positions were combined in a ratio of 10%:90%: 5′-GCT GCG CCC TTC ATC GKT G-3′(ITS2_Dino; 10%) and 5′-GCT GCG TTC TTC ATC GWT R-3′

(ITS2_broad; 90%). PCR was carried out in accordance to the protocol published by Röhl et al. (2017). For each sample, 2 separate PCR products were amplified with diverging primers (A & B variant) for the Amplicon-Duo pipeline (Lange et al. 2015, short description below). Paired-end HiSeq 2500 sequencing with 2× 300 bp reads using the 'rapid run' mode was carried out using the Illumina platform of a sequencing provider (Fasteris, Geneva). Barcode sorting of samples, as well as adapter and quality trimming, was performed by the sequencing company.

## Data processing and statistical analyses

Raw sequence data are available at the GenBank sequencing read archive under the accession number PRJNA388564. Quality of the demutiplexed sequence reads was checked using the FastQC software (v0.11.3; Andrews 2015). Subsequently, the Amplicon-Duo analysis pipeline (Lange et al. 2015) was used to filter reads for quality using PRINSEQ-lite (v0.20.4, parameters: basequality = 15, meanquality = 25; Schmieder & Edwards 2011), assemble the paired-end reads with PANDAseq (v2.7, parameters: threshold = 0.9, minoverlap = 20; Masella et al. 2012), detect chimera sequences with UCHIME (v7.0.1090, parameters: default; Edgar et al. 2011) and remove OTUs which did not occur in subsample A and B. Reads remaining after A-B filtering were clustered via the software Swarm (v2.2.9, default parameters; Mahé et al. 2014). The resulting OTUs were additionally clustered by identical V9 sequences (160 bp, ident = 100%; R Script 'V9_Clust.R' by M. Jensen available at https://github.com/manfred-uni-essen/V9-cluster) and subsequently taxonomically assigned by searching the NCBI database (NCBI BLAST-2.5.0+; parameter: ident = 85%, e-value ≤ 10$^{-12}$, max_target_seqs 10, representative OTU = maximum of log(seq_length) × log(taxlevel) × ident(blast)). This resulted in 7545 OTUs and 27 266 168 reads, whereby 60 OTUs and 6245 reads were unassigned. The remaining read counts ranged between 195 030 and 755 629 per sample (except for 1 outlier with 22 232 reads; this water sample from Day 14 was excluded from further analyses). All reads assigned to Metazoa (114 OTUs, 118 601 reads) and Embryophyta (136 OTUs, 795 896 reads) were excluded from the dataset because microeukaryotes are the target organisms of this study. The remaining read counts ranged between 88 779 and 751 215 per sample. To balance differences in sequencing depth

between the samples, we performed 'Hellinger' normalization (square root from the relative abundance) and trimmed mean of M-values (TMM) normalization for the statistical analyses in edgeR (v3.16.0; Robinson et al. 2010). Finally, we excluded 1 replicate of the soil sample of Day 1 that was an outlier with regard to the OTU richness (5-fold OTU richness compared to the average OTU richness of all other soil samples). Taxonomic affiliation at species or genus level of OTUs were mentioned if it was >~98%. All OTU sequences and the abundance matrix (number of reads per OTU and samples) used in this study are deposited at Figshare under the doi 10.6084/m9.figshare.5277751.

OTU richness and abundance were computed for each time of sampling and treatment. Therefore, mean values and standard deviations (SD) of replicates were calculated. Community structure analyses were based on the normalized read abundance of OTUs. To investigate the most important variables explaining differences in community composition, hierarchical clustering analysis (Ward) combined with principal coordinate analysis (PCoA) was performed based on the normalized read abundance of OTUs and percentage difference dissimilarity matrix (Bray) using the R-package vegan (v2.4-1; Oksanen et al. 2016). Further, beta-diversity indices (Sørensen: presence-absence of OTUs, Dice 1945, Sørensen 1948; Renkonen: 'Hellinger' normalized read-abundance of OTUs, Renkonen 1938) were calculated between water and soil samples, between non-flooded control and flooded samples as well as between different sampling times.

OTUs were classified as invasive if they fulfilled all of the following 3 criteria: (1) at the start of the experiment, the OTU was exclusively present in 1 habitat type; (2) while/after the flooding event, the OTU was present in both habitat types; (3) the OTU did not occur in the control throughout the whole experiment. Further, we investigated the effect of the flooding event by statistical testing for significant differential OTU abundances between non-flooded control samples and flooded samples with the R-package edgeR (v3.16.0; Robinson et al. 2010).

All plots were created in R using the package ggplot2 (Wickham 2009).

## RESULTS

We investigated the effect of flooding on protist diversity and community composition within experimental stream systems called AquaFlows (Fig. 1)

using molecular sampling. At the start of the experiment, the soil community and aquatic community differed decisively.

### Initial community structure

We detected on average 1071 ± 100 OTUs in the samples from the aquatic community (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a080p257_supp.pdf). Based on read abundance, green algae (i.e. Chlorophyta and Steptophyta) (17.7% ± 1.5%), ascomycetes (15.5% ± 1.9%), chrysophytes (10.2% ± 2.6%), chytridiomycetes (10.2% ± 1.8%) and diatoms (7.7% ± 0.8%) were the dominating groups (Fig. 2). The most abundant sequences were affiliated with a chrysophyte, a Chlorophyceae, the saccharomycete *Phaeosphaeria* sp. (99.6% identity), the chrysophyte *Poteriospumella lacustris* (100% identity), an uncultured basidiomycete, ascomycete and glomeromycete, as well as a diatom, a cercozoan and an agaricomycete. These 10 OTUs accounted for ~10% of the reads.

In contrast, the soil community initially comprised only 247 ± 69 OTUs. Based on read abundance, ascomycetes (41.9% ± 6.9%), cercozoans (9% ± 0.9%) and basidiomycetes (6% ± 1.5%) were the dominating groups (Fig. 2). The most abundant sequences were affiliated with 2 uncultured ascomycetes, the fungi *Mortierella* sp. (100% identity), an uncultured zygomycete, a cercozoan, 2 saccharomycetes, the Chlorophyceae *Coelastrella terrestris* (97.96% identity) as well as the oomycetes *Pythium heterothallicum* (100% identity) and *Pythium pleroticum* (100% identity). These 10 OTUs accounted for ~20% of the reads.

### Non-flooded control treatment

In the aquatic community, taxon richness decreased steadily over time to an OTU richness of 290 ± 30 towards the end of the experiment (Table 1, Fig. S1). The overall decrease in OTU richness was paralleled by a decrease of primary production as reflected by a decreasing 'Fix Area' value of chlorophyll *a* fluorescence (Fig. 3) which is to be expected for bottom-up controlled communities.

The relative abundance of ascomycetes sharply decreased during the course of the experiment (Fig. 2). In contrast, the relative abundance of ciliates, choanoflagellates and diatoms increased during the experiment (Fig. 2). The relative abundance of
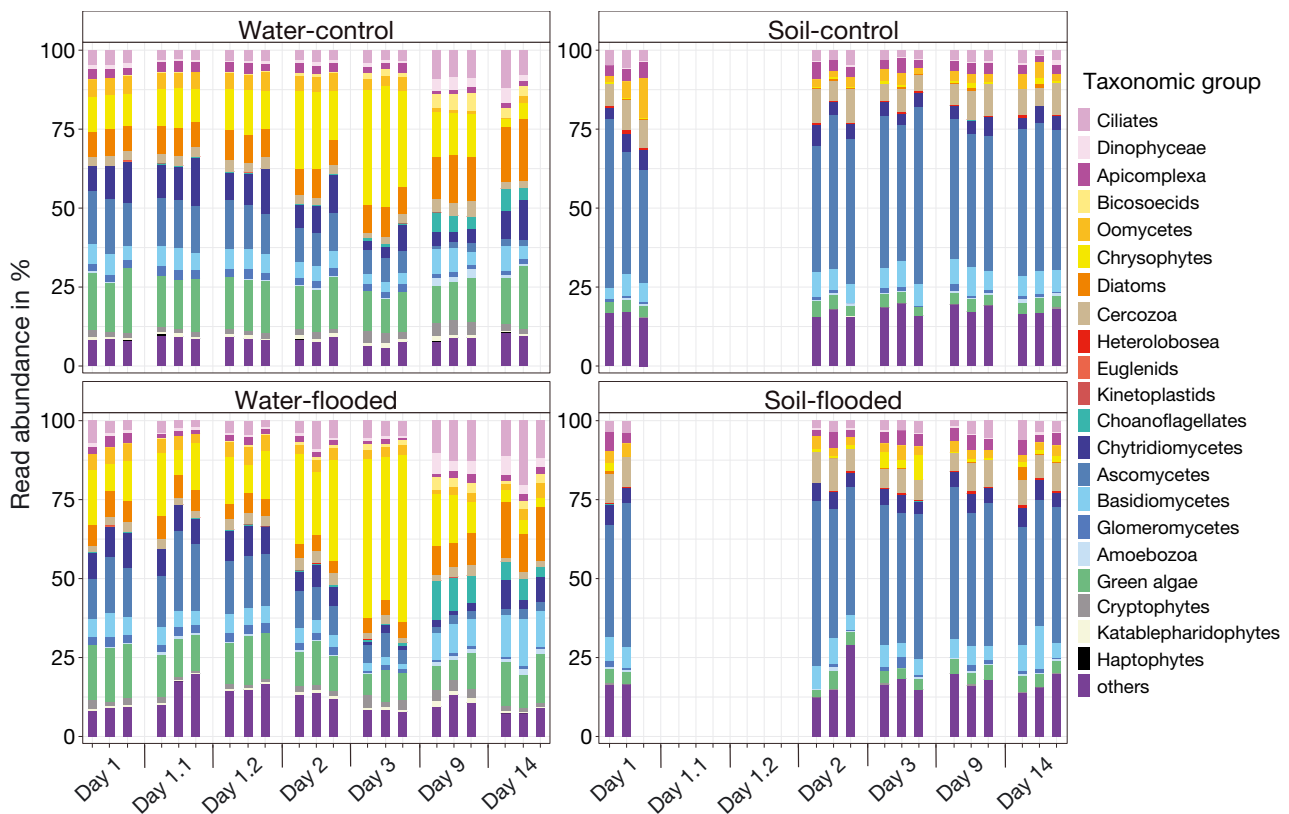
Fig. 2. Community composition based on read abundance for aquatic non-flooded control samples and aquatic flooded samples (left) as well as terrestrial non-flooded samples and terrestrial flooded samples (right)

Table 1. Timing scheme of sampling including description of experimental phases for the 2 treatments 'flooded' and 'control'. OTU richness and the number of taxa with significantly different read abundances (p < 0.05) between the non-flooded control treatment and flooded treatment of water and soil samples. Data reported as mean ± SD; –: no sampling done

| | Day 1 | Day 1.1 | Day 1.2 | Day 2 | Day 3 | Day 9 | Day 14 |
|---|---|---|---|---|---|---|---|
| **Description** | | | | | | | |
| Flooded samples | Start value | While flooding-direct after start of flooding | While flooding-5 h after start of flooding | Drying period direct after flooding | Drying period-1 d drying | Drying period-7 d drying | Drying period-12 d drying |
| Control samples | Start value | Sampling 2 | Sampling 3 | Sampling 4 | Sampling 5 | Sampling 6 | End sampling |
| **OTU richness** | | | | | | | |
| Water-control | 978 ± 48 | 1327 ± 290 | 1042 ± 164 | 927 ± 169 | 664 ± 104 | 370 ± 72 | 290 ± 42 |
| Water-flooded | 1163 ± 45 | 323 ± 125 | 967 ± 364 | 1038 ± 546 | 808 ± 276 | 628 ± 108 | 391 ± 66 |
| Soil-control | 205 ± 39 | – | – | 312 ± 59 | 321 ± 27 | 425 ± 77 | 369 ± 122 |
| Soil-flooded | 310 ± 86 | – | – | 246 ± 59 | 239 ± 101 | 270 ± 29 | 322 ± 118 |
| **No of taxa with significantly different read abundance** | | | | | | | |
| Water-control vs. Water-flooded | 4 | 19 | 62 | 142 | 65 | 11 | 0 |
| Soil-control vs. Soil-flooded | 0 | – | – | 0 | 0 | 1 | 0 |

green algae decreased and the relative abundance of chrysophytes increased from Day 1.1 until the second day. From the second day onwards, this trend was reversed, i.e. the relative abundance of green algae

increased while the relative abundance of chrysophytes decreased (Fig. 2). Diatoms and green algae were among the dominating groups at the end of the experiment. This was reflected by the most abundant

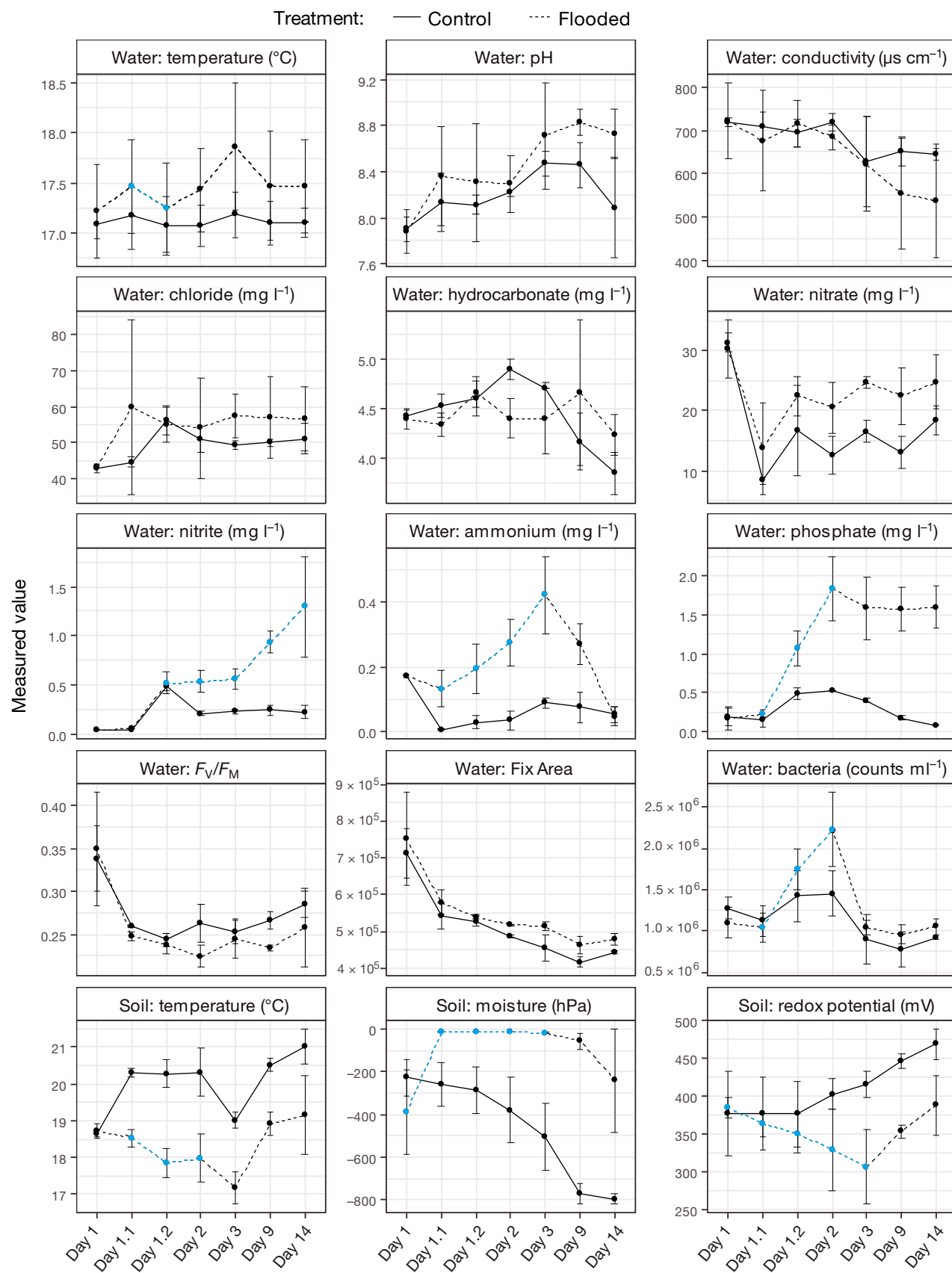Treatment: —— Control ······ Flooded

Fig. 3. Physico-chemical parameters, chlorophyll *a* fluorescence and bacteria counts of aquatic samples for both treatments (non-flooded control treatment and flooded treatment) as well as physical parameters of terrestrial samples (non-flooded control treatment and flooded treatment) for all times of sampling. Parameters that were affected by the flooding event are highlighted in blue

OTUs at the end of the experiment: 2 diatoms (one affiliated to *Cyclotella meneghiniana*, 100% identity), a Chlorophyceae and a choanoflagellate were the most abundant taxa. These community shifts were accompanied by changes in abiotic parameters. Most pronounced were shifts in inorganic nitrogen concentrations, e.g. nitrate concentration dropped from ~30 mg l$^{-1}$ at the start of the experiment to ~18 mg l$^{-1}$ towards the end (Fig. 3).

The soil communities were much more stable. Taxon richness as well as community structure hardly changed over time. Communities were dominated by ascomycetes and other fungi, as well as by cercozoans throughout the course of the experiment. This trend was reflected by the most abundant OTUs: 2 uncultured ascomycetes and the fungi *Mortierella* sp. (100% identity) were the most abundant OTUs at the end of the experiment. In contrast to the community composition, the abiotic parameters changed considerably in soil during the course of the experiment; in particular, the redox potential and soil moisture changed over time (Fig. 3).

### Flooded treatment

In addition to the time-dependent shifts in the non-flooded control samples, which were also present in the flooded treatment, we observed a clear effect of flooding: flooding obviously resulted in nutrient discharge from soil to the aquatic phase. This was particularly pronounced for phosphorus but also distinct for nitrogen (Fig. 3). This fertilization of water was accompanied by an increase of bacteria (Fig. 3). Soil temperature was generally higher than water temperature, but in the flooded treatments, the temperature of soil and water converged during flooding (Fig. 3).

Flooding also affected community composition, although this effect was only pronounced in the water, whereas flooding-related community changes were minor in the soil. In the water, flooding also resulted in an immediate decrease of OTU richness to 323 ± 102 OTUs (Table 1, Fig. S1). However, diversity recovered quickly after the end of the flooding and subsequently followed the general decreasing trend observed in the non-flooded control treatment. At the end of the experiment, the OTU richness in the water was 391 ± 54 OTUs (Table 1, Fig. S1). The com-

munity structure in the water was similar to the non-flooded control samples, but shifts were more pronounced in the flooded treatment (Fig. 2). In particular, ciliates, chrysophytes, choanoflagellates and basidiomycetes increased during the experiment. At the end of the experiment, diatoms, ciliates and green algae were the dominating groups. However, the most abundant OTUs were affiliated with 2 uncultured basidiomycetes, 2 diatoms (one affiliated to *Cyclotella meneghiniana*, 100% identity), a choanoflagellate and a ciliate.

Further, flooding resulted in an organism exchange between both habitat types. We identified 41 OTUs that were definitively introduced into the water by the flood (Table S1). In particular, several fungi including strains related to e.g. an unidentified *Mucoromycote* (99.7% identity), *Rhizophlyctis rosea* (99.3% identity), as well as some cercozoans and ciliates, were initially only present in soil but were observed in water after flooding. However, most of these immigrating taxa failed to establish a stable population. A total of 11 OTUs were only found in the water during flooding, whereas 30 taxa were also found after the flooding event. Already 1 d after the flooding event (at Day 3), we detected only 15 of the introduced taxa, and only 6 of these taxa were detected 1 wk after the flooding, e.g. 1 strain related to the peronosporomycete *Lagenidium* sp. (100% identity), the ascomycete *Aspergillus versicolor* (100% identity) and 1 OTU affiliated with Mucoromycotina (100% identity); the latter 2 OTUs were detected at almost each sampling until the end of the experiment. Only 8 OTUs originating from water were detected in the soil after flooding. Of these, an uncultured chytridiomycete and one basidiomycete were detectable in soil at a later sampling, i.e. at day 14.

### Comparison

The most important factors explaining differences in community composition as derived from PCoA were the habitat type followed by the duration of the experiment, whereby the duration of the experiment only affected the water community (Fig. 4). The habitat type was clearly the variable with the strongest explanatory power. This was further confirmed by a generally low community similarity between soil and
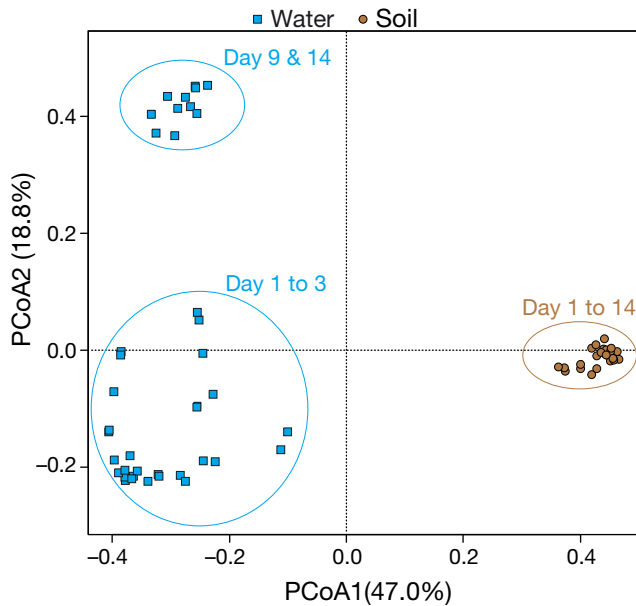
Fig. 4. Principal coordinate analysis (PCoA) combined with Ward cluster analysis of aquatic and terrestrial samples for all time-points of sampling

water as reflected by low values of the Renkonen and Sørensen indices (Fig. 5A). Our study revealed that within the experimental set-up a temporal development of water communities was pronounced, presumably due to adaptions to the mesocosm systems. This community succession was reflected by decreasing Renkonen and Sørensen indices over time (Fig. 5C).

Nevertheless, flooding had a significant impact on the water community. Several taxa differed significantly (p < 0.05) in their read abundance between the treatments (Table 1, Fig. S2). The effect of the flooding was most pronounced directly after the flooding event (at Day 2). At this time, 142 taxa differed significantly in their read abundance between the water treatments. Approximately 35% of these taxa were fungi. Soil biodiversity was much less affected by flooding: for the whole duration of the experiment, we identified only 1 *Mortierella* sp. that differed significantly in read abundance between the treatments. The similarity of the soil community even increased after the initial flooding between treatments, as indicated by increasing Renkonen and Sørensen indices (Fig. 5B).

Further, flooding initially decreased OTU richness in water, but richness recovered after this initial breakdown (followed by the general decrease towards the end of the experiment). This initial decrease of OTU richness in water resulted in a strong drop of community similarity between the

flooded treatment and control, as reflected by the Renkonen and Sørensen index (Fig. 5B). While OTU richness recovered after the initial decrease, the Renkonen index remained low, which indicated shifts in the abundances of the water community.

## DISCUSSION

### Habitat-specific community structure

Microeukaryotic community composition differed between water and soil during the whole experiment. Based on read abundances, the community in stream waters was composed of algae (in particular green algae, chrysophytes and diatoms), phagotrophs (in particular ciliates and heterotrophic chrysophytes) and fungi (in particular chytridiomycetes and ascomycetes). The soil community was strongly dominated by fungi, in particular ascomycetes, but cercozoans, ciliates and some algae were also part of the community. Community composition was therefore dominated by taxa typically reported for rivers (Gamier et al. 1995, López-Archilla et al. 2001, Weitere & Arndt 2003, Muylaert & Vyverman 2006, Shearer et al. 2007, Kiss et al. 2009, Crump et al. 2012) and soils (O'Brien et al. 2005, Barrios 2007, Crump et al. 2012, Baldwin et al. 2013, Geisen et al. 2015, Grossmann et al. 2016).

In the water, the most abundant OTUs reflected the community composition. Amongst these, the ascomycete *Phaeosphaeria* sp. and the chrysophyte *Poteriospumella lacustris* could be identified in the initial community. *Phaeosphearia* spp. are distributed from the Arctic to the tropics; they are common endophytic decomposers which can be found in soils (Connell et al. 2006) and on submerged materials in marine or freshwater environments (Bucher et al. 2004, Sakayaroj et al. 2011). The chrysophyte *Poteriospumella lacustris* was previously described as a *Spumella*-like flagellate or reported under heterotrophic nanoflagellates (HNFs). Both 'groups' were reported to be abundant in freshwater by several authors (e.g. Nolte et al. 2010, Weitere & Arndt 2003). *Phaeosphaeria* sp. and *Poteriospumella lacustris* seem to be generalists and thus can occur in high abundances after transfer from their original habitat to the experimental systems. At the end of the experiment, independent from flooding, the diatom *Cyclotella meneghiniana* was amongst the most abundant OTUs. High abundances or blooms in rivers of *Cyclotella meneghiniata* have been reported several times (Mallin et al. 1991, Rojo et al. 1994,
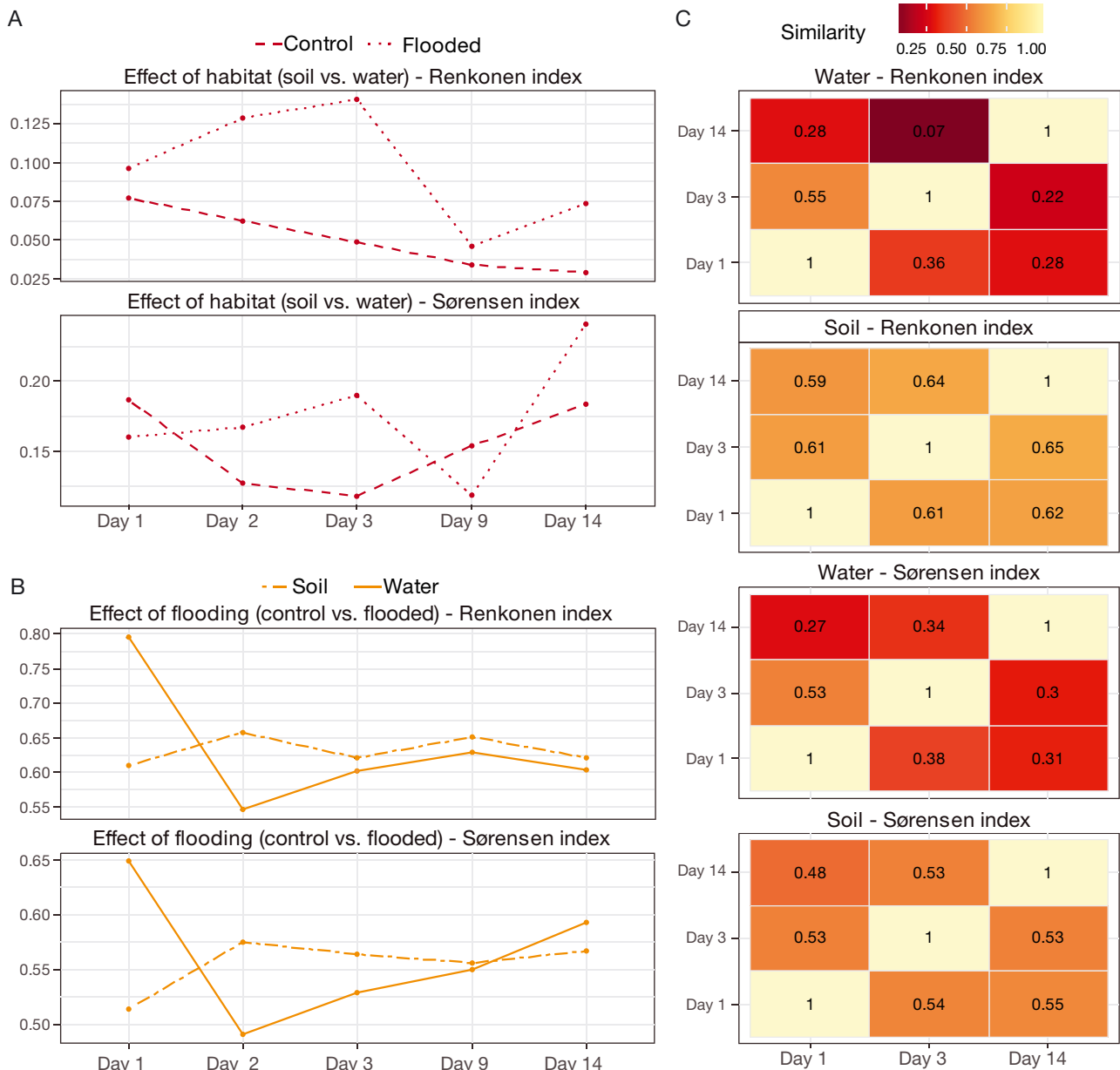
Fig. 5. Renkonen and Sørensen diversity indices of water and soil samples. (A) The effect of the habitat (soil vs. water) on the relative abundance of OTUs (Renkonen) and on the presence-absence of OTUs (Sørensen) was calculated for the non-flooded control treatments and the flooded treatments. (B) The effect of flooding (non-flooded control treatment vs. flooded treatment) on the relative abundance of OTUs (Renkonen) and on the presence-absence of OTUs (Sørensen) was calculated for water and soil. (C) The effect of time on the relative abundance of OTUs (Renkonen) and on the presence-absence of OTUs (Sørensen) was calculated for both treatments (non-flooded control treatments: upper left triangle of matrix; flooded treatments: lower right triangle of matrix) of water and soil for the sampling times Day 1 vs. Day 3, Day 1 vs. Day 14 as well as Day 3 vs. Day 14

Mitrovic et al. 2008). In soil, the fungi *Mortierella* sp., the green algae *Coelastrella terrestris* and the oomycetes *Pythium heterothallicum* and *Pythium percoticum* could be identified amongst the most abundant OTUs in the initial community. *Coelastrella terrestris*, also known under several synonyms e.g. *Scotiellopsis terrestris*, was reported as rarely

occurring but globally distributed terrestrial green algae (Hoffmann & Darienko 2005). *Pythium* spp. are common plant pathogens that cause the disease root rot (Schroeder et al. 2006, Broders et al. 2009). The abundance of *Pythium* spp. varies and depends on the type of land use, but it is not reported as highly abundant (Mukundi et al. 2009, Xu et al. 2012). In

contrast, *Mortierella* spp. are typical decomposers and reported to be very abundant in soils (Warcup 1951) but also in freshwaters (Gonçalves et al. 2012). Likewise, at the end of the experiment, independent of flooding, we found the fungi *Mortierella* sp. amongst the most abundant OTUs. Hence, the most abundant OTUs are in agreement with previously reported taxa, their abundances and habitat types.

However, we suspect that fungi are overrepresented in the sequence libraries because specifically the reverse primers have originally been designed for fungi. Because fungi were expected to be a dominant component specifically in soil (O'Brien et al. 2005, Barrios 2007, Baldwin et al. 2013), a certain bias towards this group and a presumably good taxon coverage for this group was considered advantageous when selecting the primer set. In contrast, a low read abundance for e.g. kinetoplastids and amoebozoans, which are among the typical soil protists (von der Heyden & Cavalier-Smith 2005, Fiore-Donno et al. 2016), indicates a certain underestimation of these taxa due to the selected primers. This general underrepresentation of specific groups is known for broad primers because the ribosomal sequences of e.g. amoebozoans are particularly rich in inserts and thus underperform during PCR and the subsequent sequencing step (Geisen et al. 2015, Fiore-Donno et al. 2016). Further, the highly variable ITS1 can lead to a length bias for groups with long ITS1 sequences which may not be sequenced by the Illumina technology and thus be underrepresented in the sequencing libraries. Thus, as known for basically any broad primer set, the sequence read abundance does not reflect the real taxon abundance (Pawlowski et al. 2012). Nevertheless, despite a certain taxon bias, the analyses allow for comparative community analyses as has been demonstrated in numerous studies (e.g. Taib et al. 2013, de Vargas et al. 2015, Grossmann et al. 2016).

### Shifts in aquatic community structure over time

Our results demonstrate a strong succession of the aquatic microeukaryotic community. The temporal shift of the community composition is presumably reflecting 2 events: natural taxon turnover and the acclimation to the conditions in the AquaFlow mesocosms. The chrysophyte read abundance increased during the first days of the experiment but decreased later on to be replaced by reads affiliated with green algae, diatoms, ciliates and choanoflagellates. This development is in accordance with expectations: heterotrophic chrysomonad flagellates are r-strategists and among the first taxa which respond to environmental stimuli and food availability, reflected by increasing relative read abundances (Šimek et al. 1997, Auer & Arndt 2001, Boenigk et al. 2006). Important predators of chrysomonads are ciliates (Auer & Arndt 2001) and thus maybe responsible for the later decline of chrysomonads during the experiment. Choanoflagellates may benefit from the decline of chrysomonads and become the dominant bactivores after the decline of heterotrophic chrysomonads (Carrias et al. 1998). Chlorophyceae and diatoms tend to be the dominating phytoplankton taxa and increase during later stages of succession (Gamier et al. 1995).

Further, some taxa initially present in the aquatic community were presumably allochthonous. These taxa decreased considerably during the first few days of the experiment. For instance, ascomycetes were the second most abundant group in water at the experiment start. Around 3000 aquatic fungi are known in the temperate region, with most taxa belonging to ascomycetes and chytridiomycetes (Goh & Hyde 1996, Shearer et al. 2007). They often occur in streams on submerged material, such as leaves and wood, where they degrade these plant materials (Wong et al. 1998, Shearer et al. 2007). However, the massive decrease of ascomycetes during the experiment presumably indicates a terrestrial origin for many of the observed taxa that may have entered the water as spores or on plant material (Röhl et al. 2017).

The soil community was much more stable. This agrees with the expectations as 'soils are buffered against environmental change by biophysical properties' (Bardgett et al. 2001). However, the extent of stability was unexpected. Maybe this is due to the dominance of fungi. There is, however, evidence for natural taxon turnover in fungal communities (Voríšková et al. 2014), but these studies investigated the communities on longer temporal scales compared to ours.

### Flooding as a factor for organism exchange and shifts in community

We were able to demonstrate that short flooding events cause inter-habitat dispersal of microeukaryotes. In water, a few allochthonous taxa originating from soil were observed throughout the whole experiment. Two fungi, one taxa affiliated to Mucoromycotina and the saccharomycete *Aspergillus versi-*

*color,* seemed to establish a stable population in the new habitat. *Aspergillus versicolor* is a resistant and ubiquitous fungus. It is known from several habitats, e.g. soil (Baakza et al. 2004), marine environments (Baakza et al. 2004, Nazareth et al. 2012) and freshwater (Kinsey et al. 1998). Thus, it is a good candidate for persisting immigration. But several taxa were only observed during the actual flooding or for a short time period after the flooding event, indicating that they were only transferred from soil into the water (and vice versa) but unable to establish stable populations in the new habitat. These results correspond to those of Crump et al. (2012) who demonstrated that freshwater was inoculated with soil taxa but the persistence of soil taxa was minor for *Eukaryota* compared to *Bacteria* and *Archaea.*

Upon flooding, nutrient discharge from soil to water resulted in a massive change in physico-chemical conditions in the water. This nutrient pulse and the accompanied increase of bacteria resulted in a shift in community composition and relative abundance of taxa. The shifts in community composition were similar in the flooded and the non-flooded treatment. This temporal succession was, however, more pronounced in the flooded treatment — in particular for heterotrophic chrysomonads, choanoflagellates and ciliates, which may be due to the higher abundance of bacteria (Šimek et al. 1997, Carrias et al. 1998). The strong decrease of OTU richness in the water during the initial phase of the flooding may be related to a retention of taxa by the soil due to a 'filter' effect (Abu-Ashour et al. 1994). However, if this assumption is true, the aquatic taxa hardly survive in the soil as we did not find evidence for a successful colonization of soil by aquatic taxa. The subsequent increase of OTU richness in the water may be a result of germination of taxa of the seedbank (Lennon & Jones 2011) and potentially of an introduction of alien taxa by air ventilation in the greenhouse chambers. The airborne introduction of species is in accordance with theoretical considerations and experimental evidence of e.g. Rogerson & Detwiler (1999), Altenburger et al. (2010) and Genitsaris et al. (2011).

In the soil, contrary to the studies of Wilson et al. (2011) and others, we found only minor changes in the community structure caused by flooding. In Wilson et al. (2011), the duration of the flood was considerably longer, so we suspect that short-term floods as in our study do not allow for successful establishment of taxa, whereas longer flooding periods may support the colonization and establishment of allochthonous taxa. Interestingly, the similarity between control and flooded treatment of soil even increased after the initial flooding. This finding was unexpected but may reflect the immigration of some airborne taxa. Even though temporal succession was hardly detectable in soil, this low temporal succession seems to override the effect of immigration, holding the communities relatively stable.

## CONCLUSIONS

The effect of short-term flooding on the microeukaryotic community was analyzed in mesocosm systems, called AquaFlows. The strongest effect on community structure was caused by the habitat type followed by the temporal succession and by flooding. The effects of flooding were pronounced in the aquatic phase but not in the soil. Further, the onset of flooding was accompanied by a strong shift of nutrient concentrations in the water and a pronounced decrease of OTU richness. Richness recovered later on, but the community did not develop towards the initial community composition as indicated by the similarity indices. Flooding had therefore a lasting effect on the aquatic community composition, whereas soil biodiversity was hardly affected. We demonstrate that even short floods significantly affect abiotic conditions and microeukaryotic community structure. However, short-term floods do not allow for a successful establishment of allochthonous species: only a handful of taxa persisted for more than a few days.

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