# DNA metabarcoding reveals impact of local recruitment, dispersal, and hydroperiod on assembly of a zooplankton metacommunity

Katrin Kiemel<sup>1</sup>, Guntram Weithoff<sup>2</sup>, and Ralph Tiedemann<sup>1</sup>

 $^{1}\mathrm{University}$  of Potsdam Institute of Biochemistry and Biology  $^{2}\mathrm{University}$  of Potsdam

February 1, 2022

#### Abstract

How the environment impacts the assembly of local communities as well as their spatial and temporal connection in a metacommunity has remained a largely unresolved question in community ecology. This study aims to unravel the underlying metacommunity dynamics and environmental factors that result in observed zooplankton communities. In extension to most studies concerning metacommunity dynamics, we jointly examine zooplankton communities both in open water and in the sediment where zooplankton resting stages/dormant communities are stored. We used a two-fragment DNA metabarcoding approach (COI and 18S) to monitor zooplankton communities of 24 kettle holes over a two-year period to unravel (I) how the community is spatially and temporally connected, (II) what are the environmental factors influencing local communities, and (III) what are the underlying metacommunity dynamics in this system. We found a strong separation of zooplankton communities from kettle holes of different hydroperiods (ephemeral vs. permanent) throughout the season, while the community composition within single kettle holes did not differ between years. Species richness was primarily dependent on pH and hydroperiod, while species diversity was influenced by kettle hole location. Community composition was further impacted by kettle hole size, water temperature and pH. Soil samples showed a separate community composition compared to water samples, but did not differ between ephemeral and permanent kettle holes. Our results suggest that communities are mainly structured by environmental filtering based on pH, water temperature, kettle hole size and hydroperiod. Species sorting is a dominant driver in community assembly in the studied kettle hole zooplankton metacommunity.

DNA metabarcoding reveals impact of local recruitment, dispersal, and hydroperiod on assembly of a zooplankton metacommunity

Kiemel K.<sup>1</sup>, Weithoff G.<sup>2,3</sup> & Tiedemann R.<sup>11</sup> Unit of Evolutionary Biology/Systematic Zoology, Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany<sup>2</sup> Unit of Ecology and Ecosystem Modelling, Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany<sup>3</sup> Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

- 1. Kiemel, Katrin : kiemel.katrin@gmail.com, kiemel@uni-potsdam.de
- 2. Weithoff, Guntram : weithoff@uni-potsdam.de
- 3. Tiedemann, Ralph : tiedeman@uni-potsdam.de (corresponding author)

Keywords: DNA-metabarcoding, eDNA, Zooplankton, metacommunity, dispersal, environmental filtering

#### Abstract

How the environment impacts the assembly of local communities as well as their spatial and temporal connection in a metacommunity has remained a largely unresolved question in community ecology. This study aims to unravel the underlying metacommunity dynamics and environmental factors that result in observed zooplankton communities. In extension to most studies concerning metacommunity dynamics, we jointly examine zooplankton communities both in open water and in the sediment where zooplankton resting stages/dormant communities are stored. We used a two-fragment DNA metabarcoding approach (COI and 18S) to monitor zooplankton communities of 24 kettle holes over a two-year period to unravel (I) how the community is spatially and temporally connected, (II) what are the environmental factors influencing local communities, and (III) what are the underlying metacommunity dynamics in this system. We found a strong separation of zooplankton communities from kettle holes of different hydroperiods (ephemeral vs. permanent) throughout the season, while the community composition within single kettle holes did not differ between years. Species richness was primarily dependent on pH and hydroperiod, while species diversity was influenced by kettle hole location. Community composition was further impacted by kettle hole size, water temperature and pH. Soil samples showed a separate community composition compared to water samples. but did not differ between ephemeral and permanent kettle holes. Our results suggest that communities are mainly structured by environmental filtering based on pH, water temperature, kettle hole size and hydroperiod. Species sorting is a dominant driver in community assembly in the studied kettle hole zooplankton metacommunity.

### Introduction

Understanding the species composition of local communities and its dynamics over larger regional scales is a central goal in community ecology and an essential prerequisite to predict how environmental change (e.g. habitat fragmentation) may affect the assembly of local and regional communities (Cardoso et al., 2017; Schlägel et al., 2020; Thompson et al., 2020). A metacommunity comprises a set of local communities that are linked by dispersal of multiple potentially interacting species (Leibold et al., 2004; Wilson, 1992). Regarding the prevalent interactions and processes in a metacommunity, Leibold et al. (2004) have formulated four paradigms, reflecting species dispersal abilities and/or environmental conditions: Neutral model, patch dynamics, species sorting, and mass effects. The neutral model assumes community composition to be controlled by the probabilities of random species loss and gain from the regional species pool. According to the patch dynamics model, local patches are identical, but not always occupied by identical communities due to dispersal limitations. Species sorting and mass effects imply that communities are structured by a combination of local habitat conditions and species' dispersal traits, but differ in the relative importance of dispersal. Species sorting occurs when dispersal is sufficient to allow species to reach all suitable habitats across heterogeneous landscapes, resulting in local communities varying predominantly due to differences in local environmental conditions (Chesson, 2000: Leibold et al., 2004). Mass effects stress the mass effect of dispersal, i.e., a local community may also contain species that are poor competitors, if immigration is substantial from habitats where these species are abundant. Metacommunity structure is thus a function of both the dispersal rate and the respective habitat characteristics (Leibold et al., 2004).

One of the current challenges in community ecology is to test for these theoretical expectations by observational evidence, as only a few attempts have been made to apply the metacommunity concept to natural communities (Almeida-Gomes et al., 2020; Logue et al., 2011). Wetland metacommunity studies based on ponds (Declerck et al., 2011), rockpools (Kulkarni et al., 2019), river systems (Dias et al., 2015), and flood-plains (Chaparro et al., 2018) have been conducted, but are often limited to a momentary perspective of a particular month/season or year. Furthermore, studies on metacommunity dynamics usually rely solely on the analysis of spatial patterns, and often neglect temporal aspects of community dynamics (Jabot et al., 2020). Only a few studies are available that also consider the temporal scale, by analysing consecutive years (Cottenie & De Meester, 2005; Horvath et al., 2016) or an environmental gradient (hydroperiod) over

consecutive years (Kulkarni et al., 2019). Such a detailed connection of field observations to metacommunity theory is important because it would allow a conceptual reduction of observed complexity to simpler models with higher explanatory power for the observed dynamics (Konopka, 2009; van Vliet et al., 2015).

Ponds (defined as lacustrine areas < 1 ha) are the most widespread water bodies on Earth (Meerhoff & Jeppensen, 2009). Their ecological value has been long time neglected (Céréghino et al., 2007), but has recently attracted increasing attention (Céréghino et al., 2014). These small ecosystems contribute substantially to regional diversity. Kettle holes are ponds that comprise remnant water bodies formed by retreating glaciers after the last glacial maximum. They frequently experience pronounced wet-dry cycles or show a tendency to high water overflows (Kalettka & Rudat, 2005). Nowadays, they are often surrounded by agricultural land and can be viewed as "inverse islands" supporting high biodiversity and often encompassing endangered species (Gerke et al., 2010; Ungaro et al., 2014).

Zooplankton kettle hole metacommunities represent an ideal model system to test predictions of the metacommunity theory, as they include a variety of phylogenetically unrelated groups, i.e., rotifers and crustaceans (Cladocera, ostracods and copepods), with different life history and dispersal capabilities (Caceres & Soluk, 2002; Cottenie & De Meester, 2005; Dias et al., 2015; Frisch et al., 2012). Importantly, rotifers, ostracods, cladocera, and copepods can all produce resting stages.

According to the *neutral model* of metacommunity assembly, one could consider kettle holes as equivalent patches, each containing a random assemblage of the regional species pool, irrespective of local environmental conditions and geographic proximity. However, if dispersal is limited among these otherwise equivalent quasiinsular habitats, the assembly should follow the *patch dynamics* model. Here, geographic proximity among kettle holes could translate into similarity in species composition. In contrast, if the individual kettle holes are heterogeneous regarding their abiotic conditions and the local species composition is driven by these conditions (Cottenie et al., 2001; Cottenie & De Meester, 2005), the system would neither follow patch dynamics nor neutral model dynamics (Cottenie & De Meester, 2005). Then, under the *species sorting* model, we should expect the species composition of two kettle holes to be the more alike, the more similar they are, irrespective of their geographic proximity. In comparison, the *mass effects* model would predict a less pronounced fit of species composition with environmental conditions and some correlation between geographic proximity and species composition among kettle holes.

Because kettle holes have been a persistent part of the landscape for so long, each kettle hole could theoretically harbour its peculiar biodiversity based on actual specific environmental conditions. Many zooplankton species produce an extensive egg bank (Brendonck & De Meester, 2003; De Stasio, 1989; Hairston, 1996) that serves within a pond as an insurance against unfavourable conditions and a source for the pelagic population (Bell & Weithoff, 2003; Hairston et al., 2000), but also as an effective dispersal pathway even for long distance dispersal (Fontaneto, 2019; Incagnone et al., 2015; Pinceel et al., 2015). Dispersed resting stages can distribute many species in a certain region independent from the actual prevalent environmental conditions. We argue that metacommunity assembly is not only related to interactions among extant communities (i.e., *horizontal dispersal* of zooplankton among open water habitat patches), but could also be driven by *vertical dispersal* from the egg bank into the local waterbody. Hence, a comprehensive assessment of a local zooplankton community should consider all living specimens and viable resting stages, both those present in the water body and those in active egg banks, an aspect rarely considered in previous zooplankton metacommunity analyses (Cottenie & De Meester, 2005; Declerck et al., 2011), but whose importance has recently been emphasized (Wisnoski et al. 2019).

Traditionally, species determination has been predominantly relied on morphological analysis to identify species (Pilgrim et al., 2011; Souza et al., 2019). This approach was often limiting biomonitoring programs, as morphological species determination in zooplankton communities is tedious. Furthermore, it is challenging even for experts, because of cryptic species and phenotypic plasticity, relative to ecosystem conditions (Karlsson & Winder, 2018; Xiong et al., 2019). As an alternative, species identification by DNA sequence analysis of specific genetic regions (Hebert et al., 2003) has been increasingly applied throughout the last decades, an approach commonly known as DNA barcoding. While traditional DNA barcoding uses short generic sequences to identify individual taxa, DNA metabarcoding supports the simultaneous identification of entire assemblages using high-throughput sequencing (Taberlet et al., 2012). This approach provides consistency, accuracy, and high taxonomic resolution in species identification (Baird & Hajibabaei, 2012).

To overcome limitations of previous studies in terms of sampling frequency, seasonal/annual resolution, and habitat type (sediment and open water), we applied the following approach: Over a two-year period, 24 kettle holes in the northeastern German Uckermark region were sampled and a multi-marker DNA metabarcoding approach using a combination of two markers (fragments of the nuclear 18S and the mitochondrial cytochrome oxidase subunit I gene; COI) was used to determine the overall composition of the entire zooplankton community. The objectives of this study were (I) to assess the connectivity of the kettle holes over a regional (spatial) and temporal (water body vs. soil egg bank) scale; (II) to identify local environmental parameters (e.g., kettle hole type and size, pH, water temperature) affecting the individual community structure; and (III) to infer the underlying metacommunity dynamics in the study system, relative to expectations derived under the different theoretical models.

Methods

## Sampling

Over a period of two years (2019 and 2020), samples from the open water were collected from 24 kettle holes in northeastern Germany (Quillow catchment, Uckermark, figure 1, table S1). In the first year, a total of eight sampling campaigns with a sampling interval of one month were carried out from March to October. In the second year, the sampling period was the same, but the sampling interval was once every two months. To collect water samples, we filtered 10 L of water from each kettle hole (taken from different parts of the water body) through a 30 µm plankton net (Hydrobios, acc. Apstein). We transferred the remaining 15 mL of water to a 50 mL centrifuge tube, to which 1.5 mL of sodium acetate and 33 mL of EtOH were added (Ladell et al., 2016). The samples were stored in -20 °C until DNA extraction took place. This resulted in a total number 121 samples for the two years (note that water samples could only be taken if a pond had not dried out at the time of sampling; cf. table S1). Environmental parameters, i.e., pH, water temperature, and surrounding field crops, were determined during each sampling event. Wind data was recorded using an anemometer (Vantage Pro2, Davis) during the whole campaign period. Soil samples were collected from all accessible kettle holes (n=17) at the end of the first sampling year (2019) to capture the total post-season resting stages deposited in the soil. Therefore, four soil samples were randomly taken within a  $1 \text{ m}^2$  rectangle using a Gardena(r) bulb planter, the first 5 cm of the active egg bank were taken, pooled, and stored in 4 degC until processing.

#### Hatching experiment

To discriminate between viable resting stages (to be included into our assessment) and dead animals/eggs (to be excluded from further analysis), we performed a hatching experiment. Soil samples were manually homogenized and transferred to a O 19 cm glass petri dish. The samples were dried at 30 degC in a drying oven (BINDER FD 115-E2) for approx. four days. Once the samples were completely dry, they were again manually homogenized, weighed, and distributed into four replicates, each with 14 g soil in a 165 mL vessel. 50 mL of tap water was added to the soil of each replicate and samples were incubated at 15 degC (16/8 cycle) for four weeks in a climate chamber (RU|MED Rubarth Apparate GmbH). Twice per week, 15 mL of the supernatant (hence comprising hatched live specimens) were collected and stored in 1.5 mL sodium acetate and 35 mL 95% EtOH. These samples were further processed as described in the DNA extraction section and pooled for metabarcoding.

#### **DNA** extraction

To extract total DNA, samples were centrifuged at 5000 g, 0 degC for 35 minutes. Pellet DNA extraction was performed with a commercial DNeasy Tissue Kit (QIAGEN) following the standard protocol for human and animal tissues (QIAGEN, user manual p.28-30). To ensure a sufficient final yield of DNA, we doubled the amounts of chemicals during the step 1-3 and performed the elution step twice with 30  $\mu$ L AE-buffer. After a control of the concentration and purity via a spectral photometer (NanoDrop ND-1000, Thermo Scientific(R)), the DNA was stored at -20 °C.

### Species identification via DNA metabarcoding

We used a combined fragment approach to increase the accuracy of the species identification (Zhang et al. 2018). To identify species present in each sample, we targeted the mitochondrial cytochrome oxidase subunit I (Leray fragment) and the nuclear 18S gene using primers of Leray et al. (2013) (mlCOIintF 5' - GGW ACW GGW TGA ACW GTW TAY CCY CC - 3', jgHco 5' - TAA ACT TCA GGG TGACCA AAR AAY CA—3') and Zhang et al. (2018) (UNI18s 5'-AGG GCA AKY CTG GTG CCA GC-3', UNI18SR 5'-GRC GGT ATC TRA TCG YCT T-3'), respectively. Amplification of the fragments, library preparation, next generation sequencing and blasting against reference databases were performed by a commercial company (AIM GmbH, Leipzig, Germany) based on the following procedure (Morinière et al., 2016, 2019):

From each sample, 5 µL of extracted genomic DNA was used together with the MyTaq PCR Kit (Bioline, Luckenwalde, Germany) and barcode primers adapted to High Throughput Sequencing (HTS). Amplification for COI and 18S was conducted under the PCR conditions described by Morinière et al. (2019) and Zhang et al. (2018), respectively. Amplification success and fragment length was assessed by gel electrophoresis and amplified DNA was purified and resuspended in 50 µL molecular water for each sample before proceeding. Illumina Nextera XT (Illumina Inc., San Diego, USA) indices were ligated to the PCR-product in a second PCR reaction using the same annealing temperature as in the first PCR reaction, but with only 7-9 cycles. Ligation success was confirmed by gel electrophoresis. DNA concentrations were measured using a Qubit fluorometer (Life Technologies, Carlsbad, USA) and samples were combined into 40 µL pools containing equimolar concentrations of 100 ng each. To remove unwanted smaller fragments and residual primer dimers, the amplicon pools were combined with NGS magnetic beads (MagSi-NGSPrep Plus, Magtivio) and purified using a 0.8 to 1.0 ratio of beads to amplicon PCR product. A final elution volume of 20  $\mu$ L was used. HTS was performed on an Illumina MiSeq using v3 chemistry (2\*300bp, 600 cycles, maximum of 25mio paired end reads). The COI run of all 138 samples resulted in a total number of 26715829 reads, while 18S resulted in 16576630 reads. Sequences were quality filtered (minimum length COI: 100bp, 18S: 300bp) using vsearch 2.9.1 and trimmed using the software cutadapt 1.18 (Martin, 2011) in Python 2.7.15. After dereplication non-singleton sequences were clustered using using vsearch (v.2.9.1) (Rognes et al., 2016) and after chimera detection the total number of operational taxonomic units (OTUs) were blasted against NCBI, BOLD (COI only), and Silva (18S only). 9950 OTUs matched in the reference databases for COI and 3336 for 18S.

Subsequently, species lists were separately analysed. OTUs which had a [?] 97% hit identity were extracted and only hits to zooplankton species were retained. Two soil samples had to be excluded from further analysis (Meta\_37\_p, Meta\_1598\_p) because no zooplankton species were detected. From the remaining 136 samples, relative read numbers were calculated by dividing the read number per species in the sample by the total read number of that sample (Deagle et al., 2019; Zamora-Terol et al., 2020) which is a reliable proxy for biomass (Schnek et al., 2019). For each species, fragment-specific relative read number values were averaged, in an attempt to balance potential primer/fragment-specific species detection biases. The resulting combined species list across amplified fragments with mean relative read numbers as a proxy for relative abundance (Krueger et al., 2021) was used for all subsequent analyses.

#### Statistical analysis

All statistical analyses were performed using R version 4.0.5 with the packages: vegan v.2.5-7 (Oksanen et al., 2020), ade4 v. 1.7-18 (Dray et al., 2007), lme4 v.1.1-27.1 (Bates et al., 2015), glmmADMB v.0.8.3.3. (Bolker et al., 2012), MuMIn v.1.43.17 (Barton, 2016) statMatch 1.4.0 (D'Orazio) and ape v.5.5 (Paradis et al., 2021). Kettle holes were classified into two hydroperiod-types based on the timespan of water containment, i.e., permanent (kettle holes which contained water during more than half of the sampling campaigns) and ephemeral (kettle holes which contained water during half or less of the sampling campaigns). Temporal stability of the species composition of a kettle hole was assessed over two subsequent years (2019 and 2020), by comparing data from water samples collected in the same month across both years (n=60 from 15 kettle holes) using diversity indices (Species richness S, Shannon Index H', and Simpson Index D<sub>1</sub>). A non-metric multidimensional scaling (NMDS) approach based on Bray-Curtis dissimilarities in combination with a Permanova was used to assess the difference in community species compositions within kettle holes across years. As these analyses did not reveal significant differences among 2019 and 2020, further analyses focused on the more densely sampled year 2019.

Seasonality in species numbers and composition of the open water zooplankton communities within a year was assessed across the eight sampling campaigns in 2019 (n= 91 metabarcoded samples from 24 kettle holes). Species richness (S) and Shannon Index (H') were related to environmental parameters (pH, water temperature, surrounding field crops, kettle hole size, kettle hole location, numbers of neighbouring kettle holes in a 500 m radius, average wind direction/wind speed) with a generalized linear mixed effects model (Gelman & Hill, 2006; Zuur et al., 2009), using the lme4 package for Species richness (S) and glmmADMB package for Shannon Index (H'). Kettle hole ID, referring to repeated observations from the same kettle hole, was used as a random effect. The diversity as response variable (H') and predictor variables (pH, water temperature, kettle hole size etc.) were Tukey-transformed to obtain a distribution approximate to normal. To identify the model that explains most of the variance, model selection was conducted based on the Akaike information criterion (AIC), using the dedgre function in the R package MuMIn (Barton, 2016). A NMDS approach based on Bray-Curtis dissimilarities in combination with Permanovas and environmental parameter correlation fitting (package vegan, function: envfit) was used to assess the difference in species composition and putative drivers of community assembly. To evaluate the influence of the individual kettle hole within a season on community composition, a Permanova based on the Bray-Curtis dissimilarities was conducted. To assess the impact of geographic/environmental proximity/distance based on km and Gower's distance (Gower, 1971), a Mantel test was performed to correlate community dissimilarities with geographic/environmental distances among all pairs of kettle holes. To assess potential for dispersal from the resting stages located in the sediment ("vertical dispersal"), we compared soil and water samples from the same year (2019), using the same diversity indices and the NMDS approach described above.

#### Results

### Species detection and identification

For 2422847 and 2768328 reads had a blast hit for COI and 18S, respectively. After filtering out all blast hits that had a hit rate of [?] 97%, 2422708 and 960645 reads remained for COI and 18S, respectively. Filtering for zooplankton species resulted in a final read count of 1761637 for COI and 348851 for 18S. We detected a total number of 65 zooplankton species in the 136 metabarcoded samples (full data available at Dryad XXX). 10 species were detected by both fragments, while 27 resp. 28 species were only detected by CO1 resp. 18S. All 65 species were found in the water samples (n=121). When comparing open water and soil (2019 only), we found 58 species in the water samples (n=75), while only 9 of them were detected in our 15 sediment samples (figure 2). Among the zooplankton-specific hits of all open water samples, the species-specific percentage of hits (as a proxy of relative abundances; Krueger et al., 2021) revealed copepods and cladocerans (Cyclops insignis : 25.8 %, Cyclops strenuus : 16.7 %, Thermocyclops crassus 16.1 %, Daphnia

magna: 4.8 %) and some rotifers (*Polyarthra dolichoptera*: 7.1 % and *Synchaeta pectinata*: 5.2 %) to be the most frequent hits, the soil was dominated by *Brachionus calyciflorus*: 34.7 % and *Daphnia magna*: 20.0 %.

#### Reproducibility over subsequent years

Comparing kettle hole specific barcoding results for the same month of two subsequent years (2019 and 2020), there was no difference in species composition between the two years, neither for ephemeral nor permanent kettle holes (*figure S1*, *table 1*). The reproducibility in species composition across subsequent years was also reflected in the diversity indices (S, H', D<sub>1</sub>; *figure S2*).

Community composition in the open water in relation to environmental parameters and season

NMDS of the 2019 water samples shows a significant difference in community composition between ephemeral and permanent kettle holes (F-value: 7.50, R<sup>2</sup>: 0.078, adjusted p-value 0.001) (figure S3). Correlations of environmental vectors and factors on the ordination reveal the vectors for "water temperature", "pH", and "kettle hole size" to have a greater influence on species composition in permanent than in ephemeral kettle holes. Further, the factors "location" and "season" had an influence on the observed community composition (figure 3, table 2). The communities in larger kettle holes were more similar to each other than those in smaller or medium-sized kettle holes (figure S4). A high proportion of variability in species composition (38.5 %) was explained by the individual kettle hole, while the season explained a further 9.2 % of the variability (table 3). The performed Mantel test showed no significant correlation (R: 0.037, p-value: 0.225) between the pairwise geographic distance among kettle holes and the observed Bray-Curtis dissimilarities of the respective communities (figure 4), but indicated a correlation between the Gowers's distance (as a measure for environmental difference) and observed Bray-Curtis dissimilarities (R: 0.224, p-value: 0.0003) (figure 5).

#### Species richness and Diversity

Kettle hole-specific temporal dynamics of species richness (S) over all eight sampling campaigns of 2019 did not follow a clear common trend, neither for permanent nor for ephemeral kettle holes (*figure 6*). GLMMs revealed that species richness (S) was related to kettle hole type and pH (*table S2, table S3*), with a generally higher species richness at intermediate pH (figure 7) and in permanent rather than in ephemeral kettle holes (*figure S5*). This trend was also observed for the Shannon (H') and Simpson Index (D<sub>1</sub>), but not yield statistical significance in a Wilcoxon test (*figure S5*). The GLMM based on Shannon Index (H') reveals this diversity measure to be dependent on the kettle hole location (*table S4, table S5*).

#### Soil egg bank vs. open water

There was a consistent difference in species composition between soil and water samples, both for ephemeral and permanent kettle holes (figure 8, table 4). The among kettle hole variation in species composition was higher in the soil than in the open water (figure 8). The differences in species composition among soil samples did not correlate to the geographic distance among the sampled kettle holes (R: -0.042, p-value: 0.652) (figure S7). We found differences in species composition between the ephemeral and permanent kettle holes in the open water, but not in the soil samples (figure 8, table 4). When comparing species richness and diversity indices, a large difference between soil and open water became evident, with a significantly lower number of species in soil. In fact, only species capable of forming resting stages (Cladocera, copepods, ostracods, and rotifers) were detected in the soil samples (figure 9). The Shannon Index (H') was significantly different among all cohorts. The Simpson Index  $(D_1)$  generally resembled the same pattern of difference, but the pairwise comparisons involving ephemeral water samples did not yield statistical significance (*figure* 9).

### Discussion

# Multi-marker DNA barcoding for zooplankton community inference

We present an analytical pipeline for time-efficient DNA-metabarcoding species identification and diversity inference in zooplankton communities, based on total DNA extracted from water samples. These water samples were either taken directly from the environment (eDNA) or resulted from hatching experiments on resting stages from the sediment. We used a two-fragment DNA metabarcoding approach (COI and 18S) which significantly improved species recognition by offsetting some of the errors that may have occurred due to incompleteness of reference databases, but potentially also due to primer bias, i.e., biased amplification successes among different taxonomic groups (Cicala et al., 2021; Clarke et al., 2017; Stefanni et al., 2018; Leite et al., 2021; Zhang et al., 2018). Indeed, without the use of two markers, a fair proportion of species in our kettle holes would have remained undetected, potentially reducing the power of our biodiversity assessment.

# Influence of environmental parameters on zooplankton communities

We examined zooplankton occurrence relative to regional (spatial) and local (environmental) parameters in a kettle hole community over two entire seasons. Our analyses indicate species composition and numbers within a kettle hole hydroperiod type (permanent vs. ephemeral) to remain relatively stable in subsequent years, suggesting little between-year variability in composition. Our dense within-year sampling over eight months exhibited pronounced seasonal variation of the zooplankton community, as earlier described for rotifers, based on just two sampling campaigns (Spring and Autumn; Onandia et al., 2021). According to our analyses, the community composition is further strongly affected by the environmental factors of the local habitat, such as water temperature, pH (as in Onandia et al., 2021; Tavernini, 2008), location, kettle hole size, and hydroperiod (permanent vs. ephemeral) (as in Kulkarni et al., 2019). Differences in water persistence, as among the ephemeral and permanent ponds, can influence species exchange (Ripley & Simovich, 2009; Florencio et al., 2015; Dulić et al., 2014). Ephemeral kettle holes are more dynamic due to dryfall and subsequent rewetting, which might lead to a faster succession of species, sometimes correlated with pond size (Basińska et al., 2014), and allows for a restart of otherwise less competitive species from the egg bank (Incagnone et al., 2015; Serrano & Fahd, 2005; Tilman, 1994). Additionally, dryfall of kettle holes can reduce the occurrence of predator species like amphibians and macroinvertebrates which potentially affect the zooplankton community (Taylor & Mahoney, 1990). In contrast, the permanent kettle holes are more stable and less variable in species composition (Vagaggini et al., 2011) because they are filled with water (most of the time) and therefore tend to harbour species adapted to a stable environment (Brendonck et al., 2017; De Block et al., 2008). For these species, direct competitive abilities are key to persist. Indeed, different local (environmental) factors appear to play a predominant role in permanent, relative to ephemeral kettle holes. Permanent kettle hole communities were stronger determined by water temperature and pH. As these parameters fluctuate less in stable water bodies, there may be stronger environmental filtering towards species adapted to these specific conditions (Wellborn et al., 1996). In comparison, season was a dominating factor in community assembly in ephemeral kettle holes, evidently linked to the seasonally fluctuating water availability. Species and lineages adapted to more dynamic environments may tolerate such a wider range of perturbation in environmental conditions, as they may exhibit adaptations such as higher resting stage production, faster development, and resistance to dryfall and associated conditions (Both et al., 2011; Wellborn et al., 1996). It has been observed in *Daphnia carinata* that juveniles from temporary ponds have a faster growth rate than those from stable permanent ponds (Drapes et al., 2021). The different pace in pond succession is also reflected in the number of species observed in the kettle holes, with higher numbers in permanent than in ephemeral kettle holes, a finding supported by a long term mesocosm study (Zokan & Drake, 2015), but contrary to findings of Kuczyńska-Kippen & Pronin (2018) which report temporary ponds of shorter hydroperiods to generally exhibit a higher zooplankton diversity. The diversity of kettle hole zooplankton communities (measured by H') depends on kettle hole location. We further found a significant proportion of the variation in species composition to be attributed to the individual kettle hole, as found in a comparable study of Montaña et al. (2021). Indeed, the location of any specific kettle hole could be peculiar in further environmental parameters not considered here, such as geological characteristics or hidden connectivity among kettle holes (Vyse et al., 2020). A possibly unique set of environmental parameters in each kettle holes could also explain the very different seasonal dynamics of community composition and species richness in our individual kettle holes. This environmental impact is further supported by the correlation of Gower's distance (as proxy for heterogeneity of kettle holes) and community composition differences, such that the more similar local environments are, the more similar is their species composition (see also Kulkarni et al., 2019).

#### Spatial impacts on zooplankton communities

Kulkarni et al. (2019) have reported geographic distance to correlate with zooplankton community dissimilarity on a very small geographic scale (1.5 km). In our study, however, there was no such relation between community composition and geographic proximity, arguing against isolation-by-distance as a significant factor in community assembly in our system, at least on the geographic scale we analysed (~14.0 km). Passively dispersed organisms, as zooplankton, are dependent on dispersal vectors (e.g., Fontaneto, 2019). If they are wind-dependent, their dispersal effectiveness strongly depends on the geographic scale (Horvath et al., 2016; Vanschoenwinkel et al., 2009). They can also depend on animals, so called mobile links (Jeltsch et al., 2013; Lundberg & Moberg, 2003) to get dispersed on a landscape scale (Brochet et al., 2009; Frisch et al., 2007; Moreno-Linares et al., 2016; Vanschoenwinkel et al., 2008, 2009). These mobile links often do not necessarily create an isolation-by-distance related dispersal framework, as they may not explore habitats in a purely distance-related manner and may have other selective criteria, such as landscape configuration (habitat quality, connectivity, competition etc.). In addition, these factors may change temporally, especially in agricultural landscapes (due to farming/cultivation activities). These complex and confounding factors may result in dynamic and non-linear dispersal dynamics for passive disperses (like zooplankton), which makes it difficult to detect any distance-based patterns, should they exist on the scale of the analysed metacommunity (Burel & Baudry, 2005; Kleyheeg et al., 2017; Kloskowski et al., 2010). Furthermore, individual zooplankton species might have different dispersal rates/abilities (Caceres & Soluk et al., 2002; Frisch et al., 2007; Vanschoenwinkel et al., 2009) and pathways (Lopes et al., 2016), a pattern potentially masked in our community approach.

### Proposed Metacommunity dynamics

Our study shows kettle holes within a certain area (here, the analyzed Uckermark region) to harbour similar species in the active soil egg banks, irrespective of local environmental parameters (such as hydroperiod or pH) and geographic distance. We cannot rule out the possibility that our hatching conditions acted as an unconscious filter, as we may have overlooked some species that did not hatch under our experimental conditions. However, this was an unavoidable trade-off because we intended to examine "true" dispersal (vertical/horizontal), which only occurs when viable resting stages are present/transported. An investigation

of the whole soil without a previous hatching test would not have allowed for a distinction between dead and living organisms. We found higher species richness in the resting egg bank of ephemeral, relative to permanent kettle holes, in line with findings of Olmo et al. (2020). Yet, there is a similar community of resting stages in the soils of different kettle holes, implying that there is sufficient dispersal at the landscape scale to homogenize occurrence of those zooplankton groups which generate resting stages (ostracods, cladocerans, copepods, and rotifers). This similarity of the species found as viable resting stages in the sediment of different kettle holes, irrespective of distance, points towards no current or previous dispersal limitation within our metacommunity (Kleyheeg et al., 2017; Vanschoenwinkel et al., 2008). It appears – on the geographic scale of our study - that potentially any species could reach any kettle hole. In so far, our studied kettle hole system is indeed a true metacommunity, in which dispersal limitations (paradigm of *patch dynamics*) and random loss and gain of species from the regional species pool (*neutral model*) most likely can be excluded as major determinants of community assembly (Cottenie & De Meester, 2005). The overall similarity in species composition locally present as resting stages would have gone undetected, if only the open water community composition had been analysed. This further underlines that the underlying dynamics of a zooplankton metacommunity can only be understood, if dormant stages are included (Wisnoski et al., 2019).

Despite of an apparent lack of dispersal limitations among open water bodies and soil egg banks, we found a high species heterogeneity among the different kettle holes. Here, the positive correlation of environmental distance and community dissimilarity renders environmental filtering */species sorting* the most likely driver of the observed zooplankton communities. Consequently, distinct local communities reflect the differences of the heterogeneous environments in the studied metacommunity system. Species sorting may be particularly pronounced in permanent systems because those might be more stable through time (Cottenie et al., 2003), exhibiting more competition and potentially competitive exclusion.

The prevalence of species sorting over dispersal limitations for metacommunity assembly may however be related to the geographic scale of our study. It might change across spatial scales, with species sorting as the main process on the smaller spatial scale we had focussed on, while dispersal limitations potentially becoming a structuring force over a larger spatial scale (Declerck et al., 2011; Heino et al., 2015).

The data on which our study is based meet the criteria proposed for the analysis of metacommunity dynamics (Louge et al., 2011) by providing species abundances, spatial data, and environmental data. In addition, by providing a "temporal" component, we were able to compare different seasonal stages, as well as to gain insight into the active egg bank and thus putative vertical dispersal in these kettle holes. By focusing on only two years, however, we cannot yet draw conclusions about evolutionary (Pillar & Duarte, 2010) or historical community assembly processes (Fukami et al., 2010).

### Conclusions

We were able to demonstrate that a multi-marker metabarcoding approach can significantly improve species detection by balancing the effect of primer bias/differential amplification success and incompleteness of reference databases for zooplankton species. DNA metabarcoding enabled as to assess zooplankton communities in two years at 12 different dates, yielding altogether 136 eDNA samples, in a time-efficient manner. Using traditional specimen-specific morphological determination instead of eDNA, we would not have been able to conduct such a comprehensive study with the same time-dense sampling setup, potentially reducing the power of our analysis. Our study of local zooplankton communities in quasi-insular ponds embedded in an unfavourable agricultural landscape matrix indeed confirmed our studied kettle hole system to comprise a metacommunity, as we find strong connectivity between the observed communities (active in the water body and passive in the soil egg banks), without apparent dispersal limitation. We were able to identify environmental drivers such as pH, water temperature, and hydroperiod together with temporal effects, i.e., season, explaining a major part of the differences among the individual kettle hole zooplankton communities. This suggests that the underlying dynamics shaping zooplankton communities in this metacommunity are most likely based on environmental filtering/species sorting.

Our focus of the study was on identifying the underlying dynamics and influences (spatial/temporal), based on established metacommunity theory (Leibold et al., 2004). We however did not address coexistence theory. Future studies should put more emphasis on the internal structure of metacommunities, applying the recently proposed new conceptual framework of Leibold et al. (2021). This would advance our comprehension of how specific species and sites contribute to the global system of metacommunities, thus expanding our knowledge of the complex and interactive relationships between processes and patterns in metacommunities.

#### Acknowledgements

We thank the farmers and landowners for their cooperation in permitting the sampling of the kettle holes. The authors would also like to acknowledge Dr. Binia De Cahsan and Dr. Michael Westbury for their assistance in analysing the metabarcoding data. Maxi Tomowski, Jonas Stiegler and Victor Parry are thanked for their help with statistics and writing the code in R, and Maxi Tomowski for her assistance in the field. We also thank Dr. Marijke Autenrieth for support with data visualization. This work was supported by Deutsche Forschungsgemeinschaft (DFG) in the framework of the BioMove Research Training Group (DFG-GRK 2118).

#### References

Almeida-Gomes, M., Valente-Neto, F., Pacheco, E. O., Ganci, C. C., Leibold, M. A., Melo, A. S., & Provete, D. B. (2020). How does the landscape affect metacommunity structure? A quantitative review for lentic environments. *Current Landscape Ecology Reports*, 5 (3), 68-75. Baird, D. J., Hajibabaei, M. (2012). Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. *Molecular Ecology*, 21 (8), 2039–2044.

Basińska, A. M., Antczak, M., Świdnicki, K., Jassey, V. E., & Kuczyńska-Kippen, N. (2014). Habitat type as strongest predictor of the body size distribution of *Chydorus sphaericus* (OF Müller) in small water bodies. *International Review of Hydrobiology*, 99 (5), 382-392.

Barton, K. (2016). MuMIn: Multi-model inference. R Package. Version 1.15.6.

Bates, D., Mächler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67 (1). DOI: 10.18637/jss.v067.i01.

Bell, E. M., & Weithoff, G. (2003). Benthic recruitment of zooplankton in an acidic lake. *Journal of Experimental Marine Biology and Ecology*, 285, 205-219.

Bolker, B., Skaug, H., Magnusson, A., & Nielsen, A. (2012). Getting started with the glmmADMB package.

Both, C., Cechin, S. Z., Melo, A. S., & Hartz, S. M. (2011). What controls tadpole richness and guild composition in ponds in subtropical grasslands? *Austral Ecology*, 36(5), 530-536.

Brendonck, L., Pinceel, T., & Ortells, R. (2017). Dormancy and dispersal as mediators of zooplankton population and community dynamics along a hydrological disturbance gradient in inland temporary pools.*Hydrobiologia*, 796(1), 201-222.

Brendonck, L., & De Meester, L. (2003). Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment.*Hydrobiologia*, 491(1), 65-84. Brochet, A. L., Gauthier-Clerc, M., Guillemain, M., Fritz, H., Waterkeyn, A., Baltanás, Á., Green, A. J. (2010). Field evidence of dispersal of branchiopods, ostracods and bryozoans by teal (*Anas crecca*) in the Camargue (southern France). *Hydrobiologia*, 637 (1), 255–261.

Burel, F., & Baudry, J. (2005). Habitat quality and connectivity in agricultural landscapes: the role of land use systems at various scales in time. *Ecological Indicators*, 5(4), 305-313.

Cáceres, C. E., Soluk, D. A. (2002). Blowing in the wind: a field test of overland dispersal and colonization by aquatic invertebrates. *Oecologia*, 131 (3), 402–408.

Cardoso, S. J., Nabout, J. C., Farjalla, V. F., Lopes, P. M., Bozelli, R. L., Huszar, V. L. M., Roland, F. (2017). Environmental factors driving phytoplankton taxonomic and functional diversity in Amazonian floodplain lakes. *Hydrobiologia*, 802 (1), 115–130.

Céréghino, R., Biggs, J., Oertli, B., Declerck, S. (2008). The ecology of European ponds: defining the characteristics of a neglected freshwater habitat. *Hydrobiologia*, 597 (1), 1–6.

Céréghino, R., Boix, D., Cauchie, H. M., Martens, K., & Oertli, B. (2014). The ecological role of ponds in a changing world. *Hydrobiologia*, 723(1), 1-6.

Chaparro, G., Horváth, Z., O'Farrell, I., Ptacnik, R., Hein, T. (2018). Plankton metacommunities in floodplain wetlands under contrasting hydrological conditions. *Freshwater Biology*, 63 (4), 380–391.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics, 31(1), 343-366.

Cicala, F., Arteaga, M. C., Herzka, S. Z., Hereu, C. M., Jimenez-Rosenberg, S. P., Saavedra-Flores, A., ... & Galindo-Sanchez, C. E. (2021). Environmental conditions drive zooplankton community structure in the epipelagic oceanic water of the southern Gulf of Mexico: A molecular approach. *Molecular Ecology*, 31 (2), 546-561.

Clarke, L. J., Beard, J. M., Swadling, K. M., & Deagle, B. E. (2017). Effect of marker choice and thermal cycling protocol on zooplankton DNA metabarcoding studies. *Ecology and Evolution*, 7(3), 873-883.

Cottenie, K., Michels, E., Nuytten, N., & De Meester, L. (2003). Zooplankton metacommunity structure: regional vs. local processes in highly interconnected ponds. *Ecology*, 84(4), 991-1000.

Cottenie, K., Nuytten, N., Michels, E., De Meester, L. (2001). Zooplankton community structure and environmental conditions in a set of interconnected ponds. *Hydrobiologia*, 442 (1), 339–350.

Cottenie, K., & De Meester, L. (2005). Local interactions and local dispersal in a zooplankton metacommunity. Metacommunities. University of Chicago Press, Chicago, 189-211.

Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., ... & Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, 28(2), 391-406.

De Block, M., McPeek, M. A., & Stoks, R. (2008). Life history plasticity to combined time and biotic constraints in *Lestes* damselflies from vernal and temporary ponds. *Oikos*, 117(6), 908-916.

Declerck, S. A., Coronel, J. S., Legendre, P., & Brendonck, L. (2011). Scale dependency of processes structuring metacommunities of cladocerans in temporary pools of High-Andes wetlands. *Ecography*, 34(2), 296-305.

De Stasio, B. T. (1989). The seed bank of a freshwater crustacean: copepodology for the plant ecologist. Ecology, 70(5), 1377-1389.

D'Orazio, M. (2012) "Statistical Matching and Imputation of Survey Data with the Package StatMatch for the R Environment".

Dias, J. D., Simoes, N. R., Meerhoff, M., Lansac-Toha, F. A., Velho, L. F. M., Bonecker, C. C. (2016). Hydrological dynamics drives zooplankton metacommunity structure in a Neotropical floodplain. *Hydrobiologia*, 781 (1), 109–125.

Drapes, S., Hall, M. D., & Phillips, B. L. (2021). Effect of habitat permanence on life-history: extending the Daphnia model into new climate spaces. *Evolutionary Ecology*, 1-13.

Dray, S., Dufour, A. B. (2007). The ade4 Package: Implementing the Duality Diagram for Ecologists. *Journal of Statistical Software*, 22 (4), 1-20. Dulić, Z., Marković, Z., Živić, M., Ćirić, M., Stanković, M., Subakov-Simić, G., & Živić, I. (2014). The response of phytoplankton, zooplankton and macrozoobenthos communities to change in the water supply from surface to groundwater in aquaculture ponds. International Journal of Limnology, 50 (2).131-141.

Florencio, M., Díaz-Paniagua, C., & Serrano, L. (2016). Relationships between hydroperiod length, and seasonal and spatial patterns of beta-diversity of the microcrustacean assemblages in Mediterranean ponds. *Hydrobiologia*, 774(1), 109-121.

Fontaneto, D. (2019). Long-distance passive dispersal in microscopic aquatic animals. Movement ecology, 7(1), 1-10.

Frisch, D., Cottenie K., Badosa A., Green A. J. (2012). Strong spatial influence on colonization rates in a pioneer zooplankton metacommunity. *PloS one*, 7 (7), e40205.

Frisch, D., Green A. J., Figuerola J. (2007). High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquatic Sciences*, 69 (4), 568–574.

Fukami, T., Dickie, I. A., Paula Wilkie, J., Paulus, B. C., Park, D., Roberts, A., ... & Allen, R. B. (2010). Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. *Ecology Letters*, 13(6), 675-684.

Gelman, A., Hill, J. (2006). Data Analysis Using Regression and Multilevel/Hierarchical Models. Cambridge University Press, Cambridge.

Gerke, H. H., Koszinski, S., Kalettka, T., Sommer, M. (2010). Structures and hydrologic function of soil landscapes with kettle holes using an integrated hydropedological approach. *Journal of Hydrology*, 393 (1-2), 123–132. Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, (27), 857-871. Hairston, Jr, N. G., Hansen, A. M., & Schaffner, W. R. (2000). The effect of diapause emergence on the seasonal dynamics of a zooplankton assemblage. *Freshwater Biology*, 45(2), 133-145. Hairston, Jr, N. G. (1996). Zooplankton egg banks as biotic reservoirs in changing environments. Limnology and Oceanography, 41(5), 1087-1092. Hebert, P. D. N., Ratnasingham, S., de Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings. Biological Sciences*, 270. 96-99.

Heino, J., Melo, A. S., Siqueira, T., Soininen, J., Valanko, S., & Bini, L. M. (2015). Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. *Freshwater Biology*, 60(5), 845-869.

Horváth, Z., Vad, C. F., & Ptacnik, R. (2016). Wind dispersal results in a gradient of dispersal limitation and environmental match among discrete aquatic habitats. *Ecography*, 39 (8), 726-732.

Incagnone, G., Marrone, F., Barone, R., Robba, L., & Naselli-Flores, L. (2015). How do freshwater organisms cross the "dry ocean"? A review on passive dispersal and colonization processes with a special focus on temporary ponds. *Hydrobiologia*, 750(1), 103-123.

Jabot, F., Laroche, F., Massol, F., Arthaud, F., Crabot, J., Dubart, M., ... & Datry, T. (2020). Assessing metacommunity processes through signatures in spatiotemporal turnover of community composition. *Ecology Letters*, 23 (9), 1330-1339.

Jeltsch, F., Bonte, D., Peer, G., Reineking, B., Leimgruber, P., Balkenhol, N., ... & Bauer, S. (2013). Integrating movement ecology with biodiversity research-exploring new avenues to address spatiotemporal biodiversity dynamics. *Movement Ecology*, 1(1), 1-13.

Kalettka, T., Rudat, C. (2006). Hydrogeomorphic types of glacially created kettle holes in North-East Germany. *Limnologica*, 36 (1), 54–64.

Karlsson, K., Winder, M. (2018). Ecosystem effects of morphological and life history traits in two divergent zooplankton populations. *Frontiers in Marine Science*, 5. DOI: 10.3389/fmars.2018.00408. Kleyheeg, E.,

Treep, J., de Jager, M., Nolet, BA., & Soons, MB. (2017). Seed dispersal distributions resulting from landscape-dependent daily movement behaviour of a key vector species, Anas platyrhynchos. *Journal of Ecology*, 105(5), 1279-1289. Kloskowski, J., Nieoczym, M., Polak, M., & Pitucha, P. (2010). Habitat selection by breeding waterbirds at ponds with size-structured fish populations. *Naturwissenschaften*, 97(7), 673-682.

Konopka, A. (2009). What is microbial community ecology? The ISME journal, 3 (11), 1223–1230.

Krueger, J., Foerster, V., Trauth, M. H., Hofreiter, M., & Tiedemann, R. (2021). Exploring the past biosphere of Chew Bahir/southern Ethiopia: Cross-species hybridization capture of ancient sedimentary DNA from a deep drill core. Frontiers in Earth Science, 775. DOI: 10.3389/feart.2021.683010. Kuczyńska-Kippen, N., & Pronin, M. (2018). Diversity and zooplankton species associated with certain hydroperiods and fish state in field ponds. Ecological Indicators, 90, 171-178. Kulkarni, M. R., Padhye, S. M., Rathod, R. B., Shinde, Y. S., & Pai, K. (2019). Hydroperiod and species-sorting influence metacommunity composition of crustaceans in temporary rock pools in India. Inland Waters, 9(3), 320-333. Ladell, B. A., Walleser, LR., McCalla, S. G., Erickson, R. A., Amberg, J. J. (2019). Ethanol and sodium acetate as a preservation method to delay degradation of environmental DNA. Conservation GeneticsResources, 11 (1), 83–88. Leibold, M. A., Rudolph, F. J., Blanchet, F. G., De Meester, L., Gravel, D., Hartig, F., ... & Chase, J. M. (2021). The internal structure of metacommunities. Oikos. DOI: 10.1111/oik.08618. Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., Holt, RD., Shurin, JB., Law, R., Tilman, D., Loreau, M., Gonzalez, A. (2004). The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters, 7 (7), 601–613.

Leite, B. R., Vieira, P. E., Troncoso, J. S., & Costa, F. O. (2021). Comparing species detection success between molecular markers in DNA metabarcoding of coastal macroinvertebrates. *Metabarcoding and Metagenomics*, 5, e70063.

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., ... & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, 10 (1), 1-14.

Logue, J. B., Mouquet, N., Peter, H., Hillebrand, H. (2011). Empirical approaches to metacommunities: a review and comparison with theory. *Trends in Ecology & Evolution*, 26 (9), 482–491.

Lopes, P. M., Bozelli, R., Bini, L. M., Santangelo, J. M., & Declerck, S. A. (2016). Contributions of airborne dispersal and dormant propagule recruitment to the assembly of rotifer and crustacean zooplankton communities in temporary ponds. *Freshwater Biology*, 61(5), 658-669.

Lundberg, J., & Moberg, F. (2003). Mobile link organisms and ecosystem functioning: implications for ecosystem resilience and management. Ecosystems, 6(1), 0087-0098.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, 17(1), 10-12.

Meerhoff, M., & Jeppesen, E. (2009). Shallow lakes and ponds. *Earth Systems and Environmental Sciences*, 645-655.

Montaña, C. G., Keppeler, F. W., Laughrey, C. P., & Schalk, C. M. (2021). Community assembly within ponds: the roles of space, time, and environmental gradients. *Aquatic Ecology*, 1-20. DOI: 10.1007/s10452-021-09902-0.

Moreno, E., Pérez-Martínez, C., Conde-Porcuna, J. M. (2016). Dispersal of zooplankton dormant propagules by wind and rain in two aquatic systems. *Limnetica*, (35):323–36.

Morinière, J., Balke, M., Doczkal, D., Geiger, M. F., Hardulak, L. A., Haszprunar, G., Hausmann, A., Hendrich, L., Regalado, L, Rulik, B., Schmidt, S., Wägele, J. W., Hebert, P. D. N. (2019). A DNA barcode

library for 5,200 German flies and midges (Insecta: Diptera) and its implications for metabarcoding-based biomonitoring. *Molecular Ecology Resources*, 19 (4), 900–928.

Morinière, J., Cancian de Araujo, B., Lam, A. W., Hausmann, A., Balke, M., Schmidt, S., Hendrich, L., Doczkal, D., Fartmann, B., Arvidsson, S., Haszprunar, G. (2016). Species Identification in Malaise Trap Samples by DNA Barcoding Based on NGS Technologies and a Scoring Matrix. *PloS one*, 11 (5), e0155497.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H. (2020) Package 'vegan'. Version 2.5-7.

Olmo, C., Antón-Pardo, M., Ortells, R., & Armengol, X. (2020). Influence of restoration age on egg bank richness and composition: an ex-situ experiment. *Journal of Plankton Research*, 42(5), 553-563.

Onandia, G., Maassen, S., Musseau, C. L., Berger, S. A., Olmo, C., Jeschke, J. M., & Lischeid, G. (2021). Key drivers structuring rotifer communities in ponds: insights into an agricultural landscape. *Journal of Plankton Research*, 43(3), 396-412.

Paradis, E., Claude, J., Strimmer, K. (2004). APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics*(Oxford, England), 20 (2), 289–290.

Pilgrim, E. M., Jackson, SA., Swenson, S., Turcsanyi, I., Friedman, E., Weigt, L., Bagley, M. J. (2011). Incorporation of DNA barcoding into a large-scale biomonitoring program: opportunities and pitfalls. *Journal* of the North American Benthological Society, 30 (1), 217–231. Pillar, V. D., & Duarte, L. D. S. (2010). A framework for metacommunity analysis of phylogenetic structure. *Ecology Letters*, 13(5), 587-596.

Pinceel, T., Brendonck, L., & Vanschoenwinkel, B. (2016). Propagule size and shape may promote local wind dispersal in freshwater zooplankton—a wind tunnel experiment. *Limnology and Oceanography*, 61 (1), 122-131.

Ripley, B. J., & Simovich, M. A. (2009). Species richness on islands in time: variation in ephemeral pond crustacean communities in relation to habitat duration and size. Hydrobiologia, 617(1), 181-196. Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. PeerJ, 4, e2584. Schenk, J., Geisen, S., Kleinbölting, N., & Traunspurger, W. (2019). Metabarcoding data allow for reliable biomass estimates in the most abundant animals on earth. Metabarcoding and Metagenomics, 3, e46704. Schlägel, U. E., Grimm, V., Blaum, N., Colangeli, P., Dammhahn, M., Eccard, J. A., ... & Jeltsch, F. (2020). Movement-mediated community assembly and coexistence. *Biological Reviews*, 95(4), 1073-1096. Serrano, L., Fahd K. (2005). Zooplankton communities across a hydroperiod gradient of temporary ponds in the Donana National Park (SW Spain). Wetlands, 25 (1), 101–111. Souza, C. A. de, Machado, K. B., Nabout, J. C., Muniz, D. H. dF., Oliveira-Filho, E. C., Kraus, C. N., Da Ribeiro, R. J. C., Vieira, L. C. G. (2019). Monitoring simplification in plankton communities using different ecological approaches. Acta Limnologica Brasiliensia, 31. Stefanni, S., Stanković, D., Borme, D., de Olazabal, A., Juretić, T., Pallavicini, A., & Tirelli, V. (2018). Multi-marker metabarcoding approach to study mesozooplankton at basin scale. Scientific Reports, 8(1), 1-13. Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology, 21(8), 2045-2050. Tavernini, S. (2008). Seasonal and inter-annual zooplankton dynamics in temporary pools with different hydroperiods. Limnologica, 38(1), 63-75. Taylor, B. E., Mahoney, D. L. (1990). Zooplankton in Rainbow Bay, a Carolina Bay pond: population dynamics in a temporary habitat. Freshwater Biology, 24 (3), 597-612. Thompson, P. L., Guzman L. M., Meester L. de, Horváth Z., Ptacnik R., Vanschoenwinkel B., Viana D. S., Chase J. M. (2020). A process-based metacommunity framework linking local and regional scale community ecology. Ecology Letters, 23 (9), 1314–1329. Tilman, D. (1994). Community diversity and succession: the roles of competition, dispersal, and habitat modification. 327–344. Springer, Berlin, Germany. Ungaro, F., Zasada, I., Piorr, A. (2014). Mapping landscape services, spatial synergies and trade-offs. A case study using variogram models and geostatistical simulations in an agrarian landscape in North-East Germany. Ecological Indicators, 46, 367–378. Vagaggini, D., Ulisse, G., Seminara, M., & Margaritora, F. G. (2002).

Zooplankton communities in two astatic basins in the natural reserve of Castelporziano (Central Italy): composition and temporal succession. *Journal of Freshwater Ecology*, 17(1), 27-36. van Vliet, J., Magliocca, N. R., Büchner, B., Cook, E., Rey Benayas, J. M., Ellis, EC., Heinimann, A., Keys, E., Lee, T. M., Liu, J., Mertz, O., Meyfroidt, P., Moritz, M., Poeplau, C., Robinson, B. E., Seppelt, R., Seto, K. C., Verburg, P. H. (2016). Meta-studies in land use science: Current coverage and prospects. *Ambio*, 45 (1), 15–28.

Vanschoenwinkel, B., Waterkeyn, A., Vandecaetsbeek, T. I., Pineau, O., Grillas, P., Brendonck, L. U. (2008). Dispersal of freshwater invertebrates by large terrestrial mammals: a case study with wild boar (*Sus scrofa*) in Mediterranean wetlands. *Freshwater Biology*, 53, 2264-2273.

Vanschoenwinkel, B., Gielen, S., Seaman, M., & Brendonck, L. (2009). Wind mediated dispersal of freshwater invertebrates in a rock pool metacommunity: differences in dispersal capacities and modes. Hydrobiologia, 635(1), 363-372. Vyse, S. A., Taie Semiromi, M., Lischeid, G., Merz, C. (2020). Characterizing hydrological processes within kettle holes using stable water isotopes in the Uckermark of northern Brandenburg, Germany. Hydrological Processes, 34 (8), 1868–1887. Wellborn, G. A., Skelly, D. K., & Werner, E. E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics, 27(1), 337-363. Wilson, D. S. (1992). Complex Interactions in Metacommunities, with Implications for Biodiversity and Higher Levels of Selection. Ecology, 73, 1984–2000. Wisnoski, N. I., Leibold, M. A., & Lennon, J. T. (2019). Dormancy in metacommunities. The American Naturalist, 194(2), 135-151. Xiong, W., Huang, X., Chen, Y., Fu, R., Du, X., Chen, X., Zhan, A. (2020). Zooplankton biodiversity monitoring in polluted freshwater ecosystems: A technical review. Environmental Science and Ecotechnology, 1, 100008. Zamora-Terol, S., Novotny, A., & Winder, M. (2020). Reconstructing marine plankton food web interactions using DNA metabarcoding. Molecular Ecology, 29(17), 3380-3395. Zhang, G. K., Chain, F. J. J., Abbott, C. L., Cristescu, M. E. (2018). Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. Evolutionary Applications, 11 (10), 1901–1914. Zokan, M., & Drake, J. M. (2015). The effect of hydroperiod and predation on the diversity of temporary pond zooplankton communities. Ecology and Evolution, 5(15), 3066-3074. Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., Smith, G. M. (2009). Mixed effects models and extensions in ecology with R. Springer New York, New York, NY, 580 p.

#### Data availability statement

Sample metadata, taxonomic assessment and relative read counts per sample per species are available from the Dryad repository (XXX). DNA amplicon sequences are available from the Genbank of NCBI at https://www.ncbi.nlm.nih.gov/ under Sequence Read Archive (SRA) under the accession numbers: XXX.

#### Author Contributions

K.K., R.T. and G.W. designed the research. K.K. coordinated the fieldwork, collected samples, performed laboratory processing of samples, and analysed the data with input from R.T. and G.W. K.K. produced the graphics and wrote the manuscript with the support of R.T. and G.W. All authors approved the final version of the manuscript.

 Table 1: Permanova performed with 999 permutations based on Bray-Curtis dissimilarities on water samples from permanent and ephemeral kettle holes of two different years.

Pairwise PERMANOVA	$\mathbf{F}_1$	$\mathbb{R}^2$	Adjusted p
2019 ephemeral vs. 2020 ephemeral	0.877	0.052	1.000
2019 permanent vs. 2020 permanent	1.403	0.034	0.936

Table 2: Permanova performed with 999 permutations on community composition based on Bray-Curtis
dissimilarities of water samples from permanent and ephemeral kettle holes to infer drivers of community
structure. Asteriks indicate significant results.

Environmental parameter fit	$\mathbb{R}^2$	р	
Vectors			
Kettle hole size	0.1512	0.022*	
pH	0.2247	0.002*	
Water temperature	0.1362	$0.038^{*}$	
Factors			
Seasons	0.1503	0.003*	
Locations	0.2489	$0.001^{*}$	

PERMANOVA	Df	SumsOfSqs	MeanSqs	$\mathbf{F}_1$	$\mathbb{R}^2$	р
Season	2	2.9110	1.45549	5.9889	0.09210	0.001*
Pond ID	23	12.1722	0.52923	2.1776	0.38510	$0.001^{*}$
Season*Pond	17	4.8589	0.28582	1.1761	0.15373	0.074
ID						
Residuals	48	11.6655	0.24303		0.36907	
Total	90	31.6076			1.00000	

**Table 3:** Permanova performed with 999 permutations on community composition based on Bray-Curtis dissimilarities of water samples from permanent and ephemeral kettle holes. The asteriks indicate significant results.

**Table 4:** Permanova performed with 999 permutations based on Bray-Curtis dissimilarities on water andsoil samples from permanent and ephemeral kettle holes. Asteriks indicate significant results.

Pairwise PERMANOVA	F <sub>1</sub>	$\mathbb{R}^2$	Adjusted p
Soil ephemeral vs. water ephemeral	9.243464	0.4351204	0.006*
Soil ephemeral vs. soil permanent	1.934268	0.1295188	0.198
Water ephemeral vs. water permanent	3.501158	0.2121765	0.048*
Water permanent vs. soil permanent	3.904207	0.2180609	0.006*















2
18.1
÷Ĕ
Ξ.
re.
24
ğ
25
na
1
ta
õ
ě.
-16
Le.
2
e e
ġ,
en
ě
Ξ.
9
18
Ξ.
'n
II.
1.e
9
3
bis
E
5
2
<u> </u>
36
36
69
16.
40
74
12
164
Ξ.
a
$\geq$
7
5
2
6.0
0
01
2
1
ğ
E.
Ĩ
n. – ]
ion. — 1
ission. — 1
mission. — 1
ermission. — 1
permission. — 1
ut permission. — 1
nout permission 1
ithout permission. — 1
without permission. — 1
se without permission. — 1
use without permission. — 1
reuse without permission. — l
Io reuse without permission 1
No reuse without permission. — 1
d. No reuse without permission. — l
ved. No reuse without permission. — l
erved. No reuse without permission. $-1$
'eserved. No reuse without permission. $-1$
s reserved. No reuse without permission. $-\!-\!1$
hts reserved. No reuse without permission. $-\!-\!1$
·ights reserved. No reuse without permission. $-1$
] rights reserved. No reuse without permission. $-1$
All rights reserved. No reuse without permission. $-1$
$\sim$ All rights reserved. No reuse without permission. — l
ler. All rights reserved. No reuse without permission. $-1$
nder. All rights reserved. No reuse without permission. $-\!-\!1$
funder. All rights reserved. No reuse without permission. $-1$
$\mathrm{r}/\mathrm{funder}.$ All rights reserved. No reuse without permission. — l
hor/funder. All rights reserved. No reuse without permission. $-1$
uthor/funder. All rights reserved. No reuse without permission. $-1$
author/funder. All rights reserved. No reuse without permission. $-\!-\!1$
1e author/funder. All rights reserved. No reuse without permission. $-\!-\!1$
the author/funder. All rights reserved. No reuse without permission. $-\!-\!1$
is the author/funder. All rights reserved. No reuse without permission. $-\!-\!1$
er is the author/funder. All rights reserved. No reuse without permission. $-1$
lder is the author/funder. All rights reserved. No reuse without permission. $-1$
holder is the author/funder. All rights reserved. No reuse without permission. $-1$
t holder is the author/funder. All rights reserved. No reuse without permission. $-1$
ght holder is the author/funder. All rights reserved. No reuse without permission. $-1$
right holder is the author/funder. All rights reserved. No reuse without permission. $-1$
pyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
e copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
- The copyright holder is the author/funder. All rights reserved. No reuse without permission. — 1
— The copyright holder is the author/funder. All rights reserved. No reuse without permission. — 1
2- The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
022 - The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
2022 - The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
$\pm 0.022 - $ The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
Feb $2022 - $ The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
a 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
we a 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
horea 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — 1
uthorea 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
Authorea 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — 1
n Authorea 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. $-1$
t on Authorea 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — 1
ed on Authorea 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — 1
sted on Authorea 1 Feb 2022 — The copyright holder is the author/funder. All rights reserved. No reuse without permission. — 1



