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BDELLOID ROTIFERS FROM AQUATIC AND LIMNO-TERRESTRIAL HABITATS IN THE MASSANE FOREST RESERVE, FRANCE

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ABSTRACT.- We report the results of a faunistic survey of bdelloid rotifers in the Massane forest reserve, France, covering 47 samples from aquatic and limno-terrestrial habitats. In total, 41 species were found, of which 22 represented new records for continental France. All species are widespread and their occurrence in the various habitats follows what was already known for them. No differences were found in species richness for each sample between aquatic and limno-terrestrial habitats, but community composition was significantly different between the two habitats, with no species found in both aquatic and limno-terrestrial habitats.

INTRODUCTION

The Massane Forest Reserve protects an old-growth European beech (*Fagus sylvatica* L.) forest situated close to the Mediterranean Sea in the Eastern part of the Pyrénées, in l'Albera massif (Travé 2000). It has been classified as a UNESCO World Heritage in 2021. It was one of the most northerly glacial refugia during the Holocene in western Europe for populations of European beech (Magri 2008). The forest is considered as following a natural evolution trajectory, meaning it suffered no or very sporadic land-use at least over the last 150 years. Nevertheless, the forest is still impacted from the indirect consequences of anthropogenic activities (e.g. climate change, aerosol pollution, invasive species). The Massane forest reserve is working towards completing an All-Taxa Biodiversity Inventory (Lawton *et al.* 1998) for the protected area. To achieve this goal, the reserve organised a sampling workshop targeting microscopic aquatic animals (also known as meiofauna: Giere 2019) in spring 2023. The aim for the reserve is to become the first non-marine protected area with a detailed list of meiofaunal organisms. This initiative is part of the reserve's efforts to celebrate its 50th anniversary (Majdi *et al.* 2024). This report contains detailed information and analyses on the survey of bdelloid rotifers (Rotifera, Bdelloidea) during the workshop, and with species lists already listed in a datapaper summarising the species of rotifers, tardigrades, nematodes, gastrotrichs and micro-flatworms found in the forest (Majdi *et al.* 2024) (GBIF dataset: https://doi.org/10.15468/96fy2a). The results we will report here deal with the ecological analyses of the potential drivers of occurrence of bdelloid rotifer species in the area.

Bdelloid rotifers are microscopic aquatic animals (Fig. 1), usually less than a millimeter in length, notorious in evolutionary ecology for their ancient asexuality and the ability to evolve and speciate in the apparent absence of sexual recombination (Simion *et al.* 2021), with an extremely high level of horizontal gene transfer from other non-metazoan organisms (Gladyshev *et al.* 2008).

Fig. 1.- Photographs of some of the bdelloid species found during the survey of meiofauna in the Massane forest reserve: A, *Philodina flaviceps*, B, *Rotaria rotatoria*, C, *Mniobia russeola*, D, *Habrotrocha constricta*. Photo courtesy of Michael Plewka (www.plingfactory.de). The photographed animals did not come from the Massane survey but are mostly from Germany.

In addition, they are able to survive desiccation and frost, entering a dormant state at any stage of their life (Ricci & Fontaneto 2009). Such ability may thus allow them to persist and thrive also in limno-terrestrial habitats (any terrestrial habitat where a film of water is temporarily available), including very harsh conditions like polar regions and deserts (Hespeels *et al.* 2023). Given their desiccation ability, propagules of dry bdelloids can be easily dispersed passively across long distances and colonise any available habitat (Artois *et al.* 2011).

This study aims to analyse and discuss the distribution of the bdelloid species found in the Massane forest reserve from a biogeographical and an ecological perspective. First, we explore the biogeographical patterns of the species that were found in the survey; then, we analyse the occurrence of the species across the reserve, testing for differences in species richness and species composition between aquatic and limno-terrestrial habitats. Our main hypotheses are that, firstly, the species observed should align with those typically found in the investigated habitats across Europe and other continents. Secondly, we anticipate a discernible ecological filtering effect, whereby the local habitat influences which species can be found in a particular sample.

MATERIALS AND METHODS

Samples were gathered during a meiofauna sampling workshop organised by the Massane forest reserve in March-April 2023 (Table I, Supplementary Fig. S1). Samples covered as many microhabitats as possible in the Massane forest reserve to obtain the largest diversity of freshwater and limno-terrestrial habitats available at this period of the year within the perimeter of the protected beech forest.

Freshwater samples included running and standing waters, focusing on submerged mosses, wet sediments, layers of fallen leaves on the bottom of scours in the streambed, macrophytes, *Fontinalis* moss patches in running waters, epilithic biofilms, and plankton samples.

Limno-terrestrial samples included moss and lichen patches on different substrates (e.g. rock, tree trunk, soil), in addition to water-filled tree-holes (dendrotelmata) and ivy rootlets, which are common tree-related microhabitats (TreMs) in the area (Larrieu *et al.* 2018).

Samples were collected using various methodologies (detailed in Majdi *et al.* 2024), immediately stored in plastic containers or zip-lock bags, and brought to the laboratory for microscopic inspection. Water samples were kept at 4°C in the dark and extracted within a few days or dried for long-term storage. Dry samples of limno-terrestrial habitats were stored dry for longer periods of times in paper envelopes and inspected within the following few months. Taxonomic identifications were performed to species-level whenever possible, following Donner (1965) and all the most recent taxonomic literature on the group.

The main rationale of the statistical analyses included whether differences in species richness or in species composition between samples were related to habitat type, namely with differences between samples collected in proper aquatic habitats or in limno-terrestrial habitats associated with the beech forest ecosystem (e.g. mosses, lichens, TreMs).

The models used to test the effect on species richness between samples (α-diversity) were generalised linear models (GLM) with negative binomial error structure, because species richness was represented by count data, which may not fit the assumption of normally distributed model residuals in a linear model: fit to model assumptions could be improved by a negative binomial approximation (Crawley 2012).

The models were run using the glm.nb function in the MASS package v7.3.60 (Venables & Ripley 2002) of the R statistical software v4.3.2 (R Core Team 2023). We checked model assumptions (e.g. homogeneity of variance, normality of residuals, influential observations, overdispersion) with the R package performance v0.10.4 (Lüdecke *et al.* 2021). Comparisons between habitats were visually represented by boxplots with overlayed jittered data.

To test the effect on the differences between habitats in species composition (β-diversity), we first calculated the pairwise Bray-Curtis dissimilarity matrix based on presence/absence data and then applied a permutational multivariate analysis of variance (PERMANOVA, function adonis2) in the R package vegan v2.6.4 (Oksanen *et al.* 2022), using only habitats that have homogeneous variances, tested with the R vegan function betadisper for multivariate homogeneity of group dispersions (variances). Comparisons were visually represented by non-metric Multi-Dimensional Scaling (NMDS) plots.

As a comparison between the total number of species for each habitat, we computed cumulative curves with Coleman approximation and obtained Chao and jackknife estimates of expected richness (Palmer 1990) using the R package vegan. In addition, we obtained the indicator species for each habitat (Dufrene & Legendre 1997) with the indval function of the R package labdsv v2.1.0 (Roberts 2023).

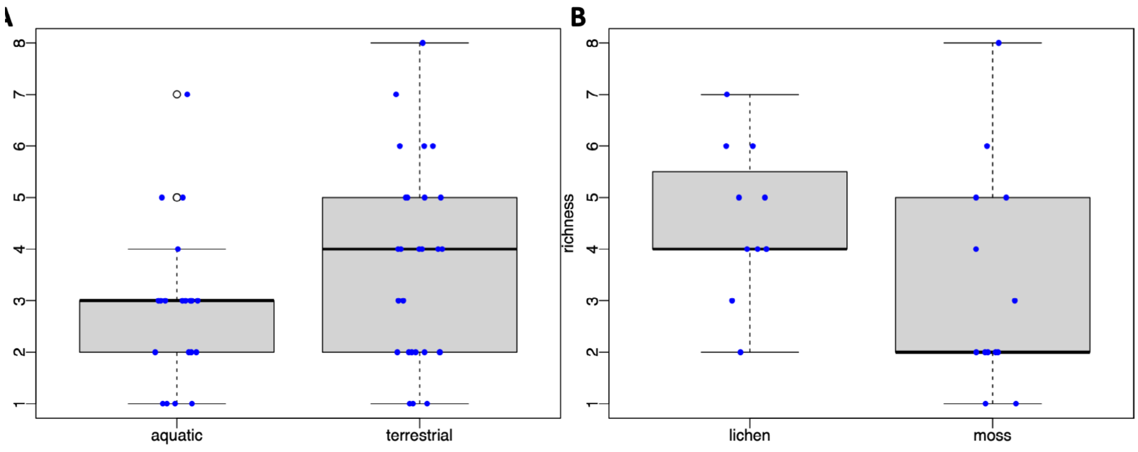

Fig. 2.- Distribution of the number of species found in each sample for A, aquatic and limno-terrestrial habitats, and for B, lichen and moss samples within the limno-terrestrial habitats. The horizontal thick line represents the median of the distribution, the boxes group data from the first to the third quartile, the whiskers extend to the lowest and highest value within the interquartile range, the empty circles represent the few values outside the interquartile range. The actual data is overlayed to the boxplots as jittered blue dots, in order to represent sample size.

Sample ID	latitude	longitude	main habitat	habitat type
386A	42.49056	3.02898	aquatic	epibiont on caddisfly larva
386B	42.49056	3.02898	terrestrial	moss
386C	42.49056	3.02898	terrestrial	lichen
387A	42.49066	3.02956	aquatic	moss
387B	42.49066	3.02956	aquatic	moss
387E	42.49066	3.02956	aquatic	algae
387G	42.49066	3.02956	aquatic	floating *Lemna*
388B	42.49279	3.03237	terrestrial	dendrotelmata
389B	42.48983	3.02899	terrestrial	moss
390A	42.48931	3.02893	terrestrial	lichen
390C	42.48931	3.02893	terrestrial	moss
391A	42.48866	3.0287	terrestrial	moss
391B	42.48866	3.0287	terrestrial	moss
392A	42.4883	3.02869	aquatic	moss
392B	42.4883	3.02869	aquatic	moss
393A	42.49099	3.03179	terrestrial	moss
393B	42.49099	3.03179	terrestrial	lichen
394A	42.48081	3.03662	terrestrial	moss
394C	42.48081	3.03662	terrestrial	lichen
395A	42.47725	3.0361	aquatic	sediment
395B	42.47725	3.0361	aquatic	moss
396A	42.47572	3.03084	aquatic	moss
396B	42.47572	3.03084	aquatic	sediment
397A	42.47649	3.03016	aquatic	sediment
398A	42.47968	3.0319	aquatic	moss
399A	42.48171	3.03303	aquatic	moss
400A	42.48205	3.03253	aquatic	algae
401A	42.48415	3.03146	terrestrial	lichen
402A	42.48456	3.03096	aquatic	moss
402B	42.48456	3.03096	aquatic	algae
403A	42.47073	3.01505	terrestrial	lichen
403B	42.47073	3.01505	terrestrial	moss
403C	42.47073	3.01505	terrestrial	moss
403D	42.47073	3.01505	terrestrial	lichen
404A	42.47072	3.01442	aquatic	moss
404B	42.47072	3.01442	terrestrial	moss
404C	42.47072	3.01442	terrestrial	ivy rootlets
404D	42.47072	3.01442	terrestrial	dendrotelmata
405A	42.47028	3.00942	aquatic	sediment
406A	42.47066	3.00689	aquatic	sediment
407A	42.49796	3.02834	terrestrial	lichen
407C	42.49796	3.02834	terrestrial	lichen
407D	42.49796	3.02834	terrestrial	moss

408A	42.49529	3.03062	terrestrial	moss
408B	42.49529	3.03062	terrestrial	lichen
408C	42.49529	3.03062	terrestrial	lichen
408D	42.49529	3.03062	terrestrial	moss

Table I.- List of 47 samples collected from the Massane forest reserve in spring 2023, reporting sample ID, geographical coordinates in WGS84 reference system, main habitat (whether aquatic or limno-terrestrial) and habitat type (e.g. moss, lichen, sediment).

RESULTS

The survey of bdelloid rotifers (Fig. 1) in different habitats of the Massane forest reserve produced a list of 41 species from 47 samples (GBIF https://doi.org/10.15468/96fy2a, Fig. 1, Table II, Supplementary Fig. S1). All species are already known from palearctic areas and from several other biogeographic regions (Table III), with no species that has a biogeographic distribution limited to one continent only.

The 20 aquatic samples revealed on average (± standard deviation) 2.85 ± 1.53 species (median = 3, range = 1-7); the 27 limno-terrestrial samples revealed on average 3.70 ± 1.92 species (median = 4, range = 1-8). No significant differences were revealed in species richness between aquatic and limno-terrestrial samples (GLM: $z = 1.6$, $p = 0.114$; Fig. 2A). Of the 27 limno-terrestrial samples, 11 were from lichens, with 4.55 ± 1.44 species (median = 4, range = 2-7) and 13 from mosses, with 3.31 ± 2.14 species (median = 2, range = 1-8). No difference in species richness was found between lichen and moss samples (GLM: $z = 1.5$, $p = 0.126$; Fig. 2B). The other three limno-terrestrial samples were from tree-related microhabitats (TreM), each of them with one, two, and four bdelloid species.

Species composition (β-diversity) was significantly different between aquatic and limno-terrestrial habitats (PERMANOVA: $F_{1,45} = 8.3$, $p = 0.001$, $R^2 = 0.15$; test for homogeneity of variance: $p = 0.7580$). The NMDS visual representation of the similarities in species composition between samples confirmed that the composition of bdelloid communities from aquatic habitats did not overlap with those from limno-terrestrial habitats (Fig. 3). The analysis on indicator species revealed *Philodina flaviceps* ($p = 0.001$), *Rotaria rotatoria* ($p=0.007$) and *Macrotrachela quadricornifera* ($p = 0.023$) as characteristic of aquatic habitats whereas *Mniobia russeola* ($p = 0.001$), *Macrotrachela plicata* ($p = 0.004$), *Macrotrachela habita* ($p = 0.014$) and *Habrotrocha constricta* ($p = 0.029$) as characteristic of limno-terrestrial habitats (Fig. 1). Within limno-terrestrial habitats, no comparisons between lichens, mosses and TreM were possible for community composition because variances were not homogeneous ($p = 0.0047$), not even for the comparison between the two most sampled habitats: lichens and mosses ($p = 0.0091$). Visually, it is anyways possible to notice that communities from lichens and TreMs did not overlap but were both included in the NMDS space of the communities from mosses (Fig. 3).

The cumulative curves for aquatic and limno-terrestrial habitats largely overlapped (Fig. 4) and the estimated overall richness for the two habitats (ɣ-diversity) did not differ substantially (Table IV).

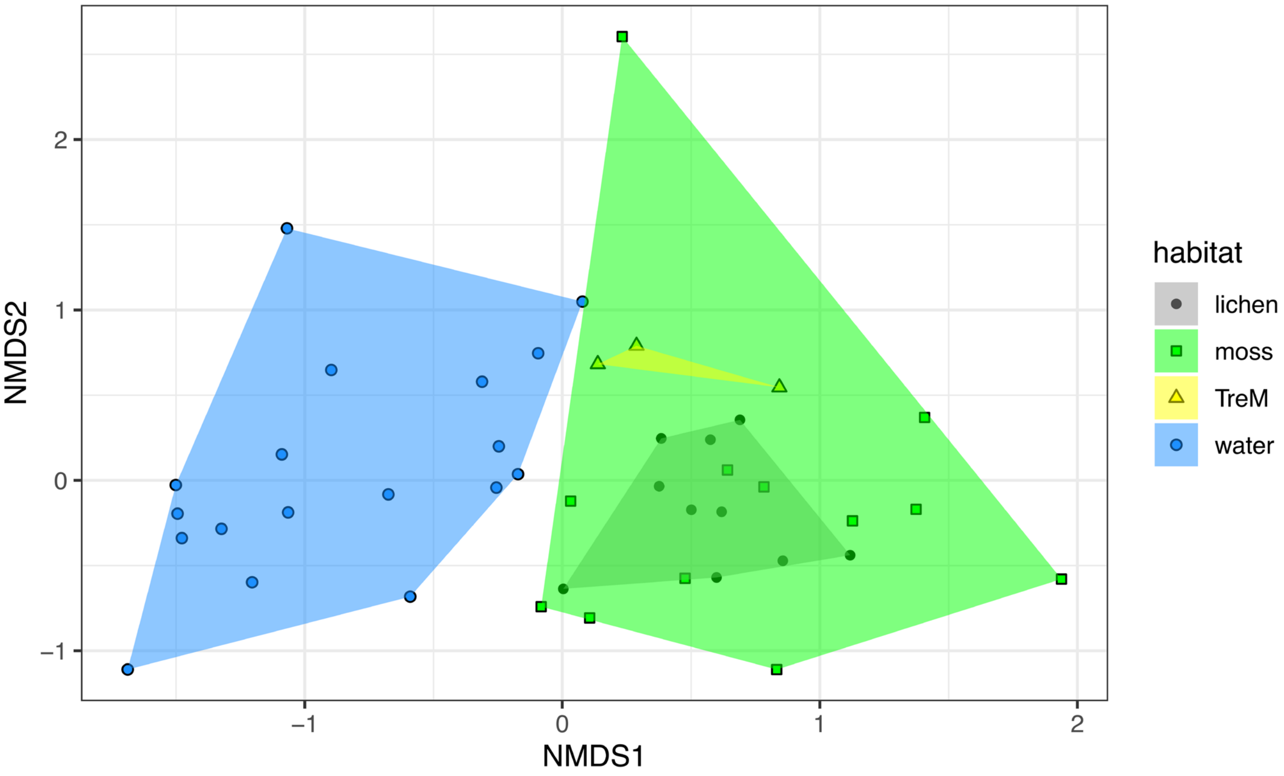

Fig. 3.- Non-metric Multi-Dimensional scaling (NMDS) biplot representing similarities in community composition between samples on two axes. Samples from aquatic (water) and limno-terrestrial habitats (lichen, moss, TreM) are presented with different shapes and colours and grouped by coloured polygons representing the convex hulls for each of the four habitat types. Supplementary Fig. S2 reports sample ID for each of the samples.

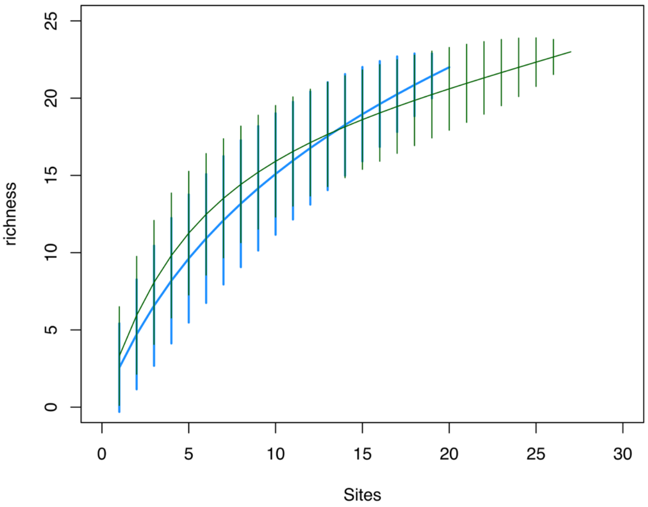

Fig. 4.- Cumulative curves of the number of species (richness) for a growing number of samples (sites), with confidence intervals for the aquatic habitat (blue, 20 samples) and the limno-terrestrial habitat (dark green, 27 samples). The extrapolated estimates of the potential number of species for each of the two habitats is reported in Table IV.

Sample ID	*Adineta barbata* Janson, 1893	*Adineta vaga* (Davis, 1873)	*Didymodactylos carnosus* Milne, 1916	*Dissotrocha macrostyla* (Ehrenberg, 1838)	*Embata hamata* (Murray, 1906)	*Embata laticeps* (Murray, 1905)	*Habrotrocha aspera* (Bryce, 1892)	*Habrotrocha bidens* (Gosse, 1851)	*Habrotrocha collaris* (Ehrenberg, 1832)	*Habrotrocha constricta* (Dujardin, 1841)	*Habrotrocha lata* (Bryce, 1892)	*Habrotrocha pulchra* (Murray, 1905)	*Habrotrocha rosa* Donner, 1949	*Habrotrocha* sp.	*Habrotrocha spicula* Bryce, 1913	*Habrotrocha sylvestris* Bryce, 1915	*Macrotrachela ehrenbergii* (Janson, 1893)	*Macrotrachela habita* (Bryce, 1894)	*Macrotrachela musculosa* Milne, 1886	*Macrotrachela nana* (Bryce, 1912)	*Macrotrachela papillosa* Thompson, 1892	*Macrotrachela plicata* (Bryce, 1892)	*Macrotrachela quadricornifera* Milne, 1886	*Macrotrachela* sp.	*Mniobia magna* (Plate, 1889)	*Mniobia russeola* (Zelinka, 1891)	*Mniobia scarlatina* (Ehrenberg, 1853)	*Philodina citrina* Ehrenberg, 1832	*Philodina flaviceps* Bryce, 1906	*Philodina plena* (Bryce, 1894)	*Philodina roseola* Ehrenberg, 1832	*Philodina* sp.	*Philodina vorax* (Janson, 1893)	*Philodinavus paradoxus* (Murray, 1905)	*Pleuretra brycei* (Weber, 1898)	*Pleuretra humerosa* (Murray, 1905)	*Pleuretra lineata* Donner, 1962	*Rotaria quadrioculata* (Murray, 1902)	*Rotaria rotatoria* (Pallas, 1766)	*Rotaria sordida* (Western, 1893)	*Rotaria tardigrada* (Ehrenberg, 1830)
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408D	x	x																x								x									x						

Table II.- List of species of bdelloid rotifers found in the 47 samples collected in the Massane forest reserve in spring 2023.

Adineta barbata Janson, 1893: AFR, ANT, AUS, NEA, NEO, PAL
Adineta vaga (Davis, 1873): AFR, ANT, AUS, NEA, NEO, ORI, PAL
Didymodactylos carnosus Milne, 1916: AFR, AUS, PAL
Dissotrocha macrostyla (Ehrenberg, 1838): AFR, AUS, NEA, NEO, ORI, PAL
Embata hamata (Murray, 1906): AUS, PAL
Embata laticeps (Murray, 1905): NEA, ORI, PAL
Habrotrocha aspera (Bryce, 1892): AFR, AUS, NEA, NEO, ORI, PAC, PAL
Habrotrocha bidens (Gosse, 1851): AFR, AUS, NEA, NEO, ORI, PAL
Habrotrocha collaris (Ehrenberg, 1832): AFR, AUS, NEA, PAL
Habrotrocha constricta (Dujardin, 1841): AFR, ANT, AUS, NEA, NEO, PAC, PAL
Habrotrocha lata (Bryce, 1892): AFR, AUS, NEA, NEO, ORI, PAL
Habrotrocha pulchra (Murray, 1905): AFR, ANT, AUS, NEA, NEO, PAL
Habrotrocha rosa Donner, 1949: AFR, AUS, NEA, NEO, PAL
Habrotrocha spicula Bryce, 1913: AFR, AUS, ORI, PAL
Habrotrocha sylvestris Bryce, 1915: AUS, NEO, PAL
Macrotrachela ehrenbergii (Janson, 1893): AFR, AUS, NEA, NEO, ORI, PAC, PAL
Macrotrachela habita (Bryce, 1894): AFR, ANT, AUS, NEA, NEO, ORI, PAL
Macrotrachela musculosa Milne, 1886: AFR, ANT, AUS, NEA, ORI, PAL
Macrotrachela nana (Bryce, 1912): AFR, AUS, NEA, NEO, PAL
Macrotrachela papillosa Thompson, 1892: AFR, AUS, NEA, NEO, ORI, PAL
Macrotrachela plicata (Bryce, 1892): AFR, AUS, NEA, NEO, PAL
Macrotrachela quadricornifera Milne, 1886: AFR, ANT, AUS, NEA, NEO, ORI, PAL
Mniobia magna (Plate, 1889): AUS, NEA, NEO, PAL
Mniobia russeola (Zelinka, 1891): AFR, ANT, AUS, NEA, NEO, PAL
Mniobia scarlatina (Ehrenberg, 1853): AFR, AUS, NEO, PAL
Philodina citrina Ehrenberg, 1832: AFR, AUS, NEA, NEO, ORI, PAL
Philodina flaviceps Bryce, 1906: AFR, AUS, NEA, NEO, ORI, PAL
Philodina plena (Bryce, 1894): AFR, ANT, AUS, NEA, NEO, PAL
Philodina roseola Ehrenberg, 1832: AFR, AUS, NEA, NEO, PAL
Philodina vorax (Janson, 1893): AFR, AUS, NEA, NEO, ORI, PAL
Philodinavus paradoxus (Murray, 1905): AUS, NEA, PAL
Pleuretra brycei (Weber, 1898): AFR, AUS, NEA, NEO, PAL
Pleuretra humerosa (Murray, 1905): AFR, AUS, NEA, NEO, PAC, PAL
Pleuretra lineata Donner, 1962: AUS, PAL
Rotaria quadrioculata (Murray, 1902): AUS, PAL
Rotaria rotatoria (Pallas, 1766): AFR, AUS, NEA, NEO, ORI, PAL
Rotaria sordida (Western, 1893): AFR, AUS, NEA, NEO, ORI, PAC, PAL
Rotaria tardigrada (Ehrenberg, 1830): AFR, AUS, NEA, NEO, ORI, PAL

Table III.- Known biogeographical distribution of each of the bdelloid taxa in the Massane forest reserve identified to species level, according to Segers (2007). Acronyms of biogeographical regions are: AFR: Afrotropical; ANT: Antarctic; AUS: Australian; NEA: Nearctic; NEO: Neotropical; ORI: Oriental; PAC: pacific; PAL: Palearctic.

DISCUSSION

The 41 species of bdelloid rotifers found during the survey of different aquatic and limno-terrestrial habitats of the Massane forest reserve represent biological communities that are typical of these types of habitat in Europe (Donner 1965, Fontaneto *et al.* 2006, 2011). All the animals identified to species level belong to widespread species (Table III), found in the palearctic region but also in other regions worldwide, making most of these species as cosmopolitan. Bdelloid rotifers are already notorious for the extremely wide geographical distribution of most of their species (Artois *et al.* 2011), according to the remits of the ubiquity hypothesis for microscopic organisms (Fenchel & Finlay 2004). Our results tend to support this hypothesis, as well. One caveat to such a support could be seen in the occurrence of cryptic species, a common feature in bdelloid rotifers (Fontaneto *et al.* 2009), with each cryptic species within a complex showing a narrower geographical range than that of the whole complex; yet, we have no information on the genetic diversity of the morphological species we found and we cannot speculate on the effect of cryptic species on our results.

The ubiquity hypothesis states that "everything is everywhere, but the environment selects" (De Wit & Bouvier 2006): indeed, the bdelloid species of the Massane forest reserve are all widespread, but each species was found either in aquatic or in limno-terrestrial habitats, with no overlap in community composition, β-diversity, between the two main types of habitat (Fig. 3). Further differences were found also in community composition between lichen and moss patches, at least in terms of multivariate homogeneity of variances. Such a scenario of widespread global biogeographic distribution coupled with local occurrence linked to ecological differences is already known for bdelloid species (Fontaneto *et al.* 2006). Bdelloid species usually have clear ecological requirements, with different species that evolved to be adapted to different ecological niches, notwithstanding the absence of sexual recombination in this notorious group of ancient asexual animals (Fontaneto *et al.* 2007). The species that are highlighted as characteristic of aquatic or limno-terrestrial habitats are also already known to live in such habitats (Donner 1965).

Notwithstanding the widespread distribution of the species found in the Massane forest reserve, 22 species are new to continental France according to Fauna Europaea (Jong *et al.* 2014). The currently known species list of bdelloids for continental France increased from 54 (Jong *et al.* 2014) to 76. Overall, 29 % of the known freshwater bdelloids from continental France are so far only known from the Massane forest reserve stressing the need to include bdelloids in routine survey. Although bdelloid identification can be seen as a challenging task at first, in fact, in comparison with other meiofaunal groups like nematodes, bdelloid sampling and identification procedures are relatively cheap and straightforward (small amounts of samples can be kept dry or frozen for long periods of time and without using any fixatives before identifying living specimens under the microscope). Moreover, the relatively predictable distribution of species worldwide (depending on habitat) makes it relatively straightforward to identify specimen from expected assemblages to species-level.

Species richness in each sample, α-diversity, was within the range of what is already known from surveys in similar habitats across the palearctic region (Fig. 5). No differences were found in richness in each sample between aquatic and limno-terrestrial habitats, as already found in previous studies (Fontaneto *et al.* 2006). No differences were found also in richness between moss and lichen patches, contrary to what was found in a previous survey in the Alps (Fontaneto & Ricci 2006), when mosses were found to be richer than lichens.

Overall, more limno-terrestrial than aquatic species and genera exist in the group (Donner 1965), with a higher resistance to desiccation in limno-terrestrial species (Hespeels *et al.* 2023); a higher diversity and heterogeneity is also usually found in local surveys from limno-terrestrial than from aquatic habitats (Fontaneto *et al.* 2006). In our study, estimates of overall richness, ɣ-diversity, revealed that aquatic and limno-terrestrial habitats could have similar number of species (Table IV), in contrast to previous studies and the overall diversity of bdelloids.

Overall, biodiversity surveys for understudied organisms in the light of the All-Taxa Biodiversity Inventories for protected areas like the one performed on meiofauna to celebrate the 50 years of the Massane forest reserve (Majdi *et al.* 2024) produce valuable data that can be used to plan conservation activities, and improve our basic knowledge of biodiversity, even in relatively well-studied regions of the world like continental France.

habitat	number of samples	observed species	Chao ± s.e.	jackknife ± s.e.
aquatic	20	22	36.37 ± 11.88	32.45 ± 3.71
terrestrial	27	23	57.67 ± 24.72	31.67 ± 3.49

Table IV. - Estimated number of species according to Chao and jackknife algorithms, together with standard errors (s.e.), in addition to number of samples and number of observed species.

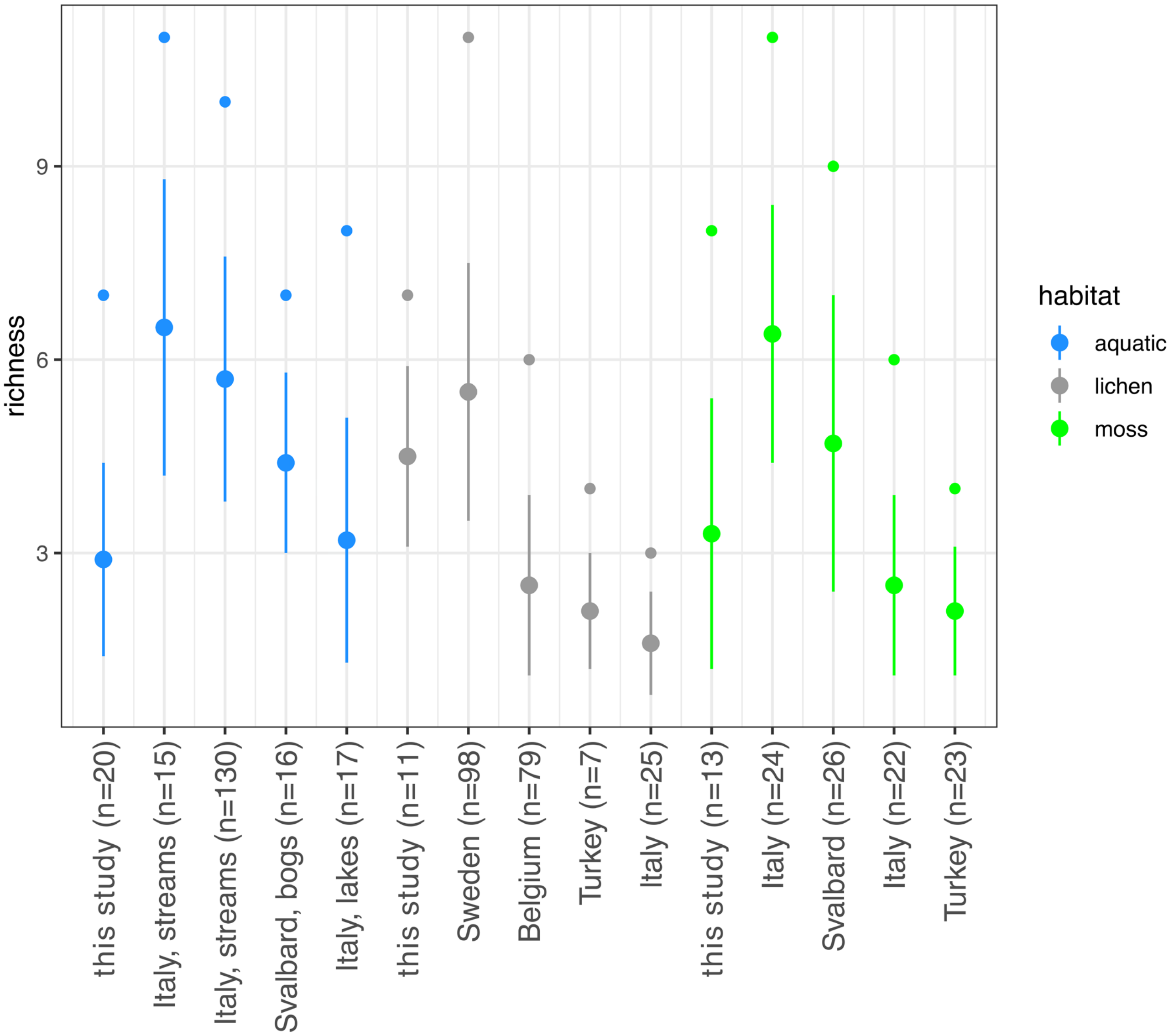

Fig. 5.- Distribution of richness of bdelloid rotifers for each sample in aquatic habitats, lichen and moss patches, in comparison to previously published studies. The results of the survey in the Massane forest reserve (this study, in bold) are compared with other surveys in the Palaearctic region, in decreasing order for each habitat. The large dot represents the average value, the whiskers extend to ± 1 standard deviation, the small dot represents the maximum value. The labels of the X axis report the geographic area with sample size in parentheses, ordered from the richest to the poorest within each of the three habitat types. Data from Fontaneto & Ricci (2006), Fontaneto *et al.* (2006, 2008, 2011), Kaya (2013), Kaya *et al.* (2010), Partemi *et al.* (submitted).

DATA AVAILABILITY

The occurrence data of bdelloid rotifers in the Massane forest reserve is part of a larger survey of meiofauna diversity, available through GBIF: https://doi.org/10.15468/96fy2a. The R script and the dataset to run the analyses are available as supplementary material.

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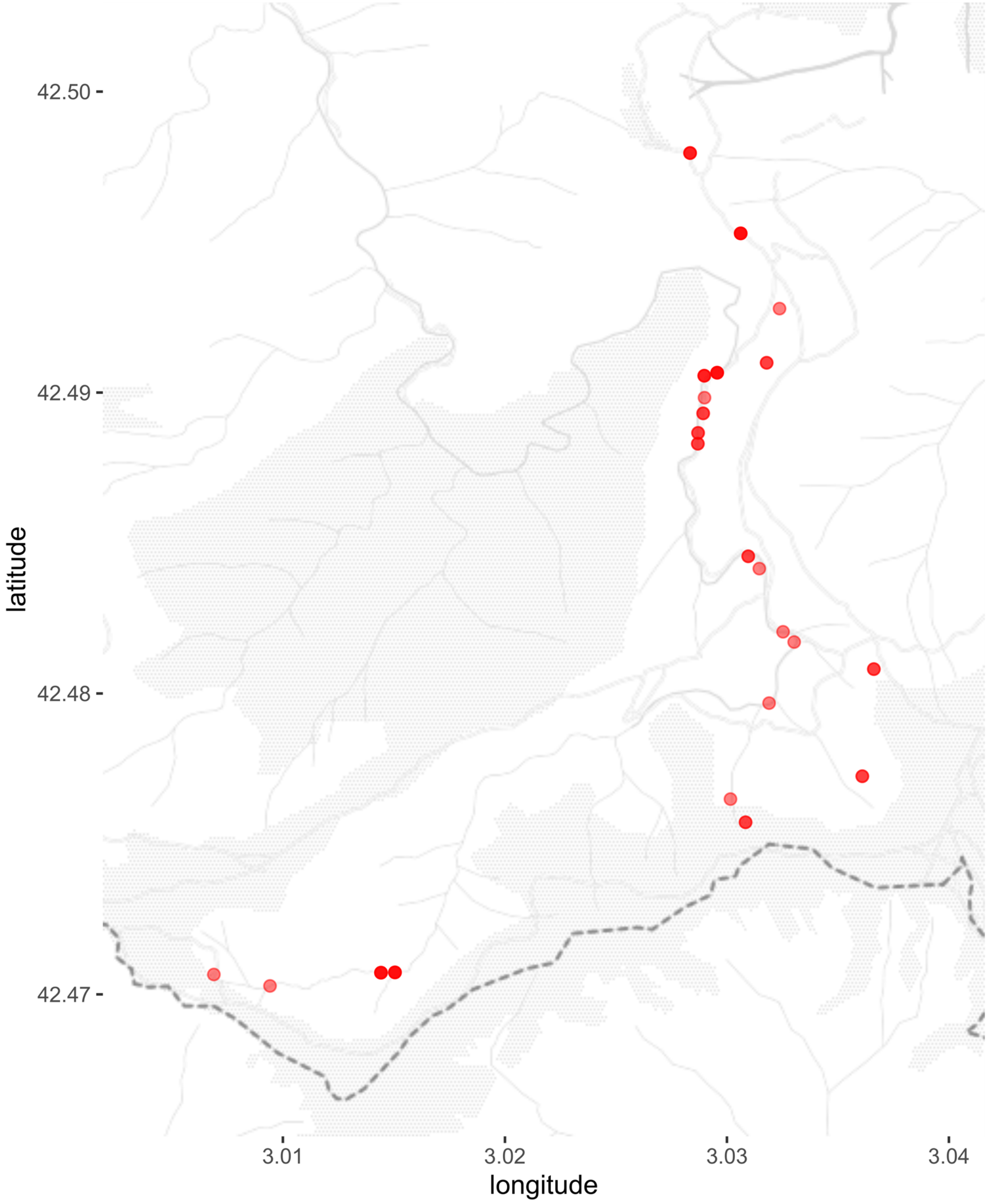
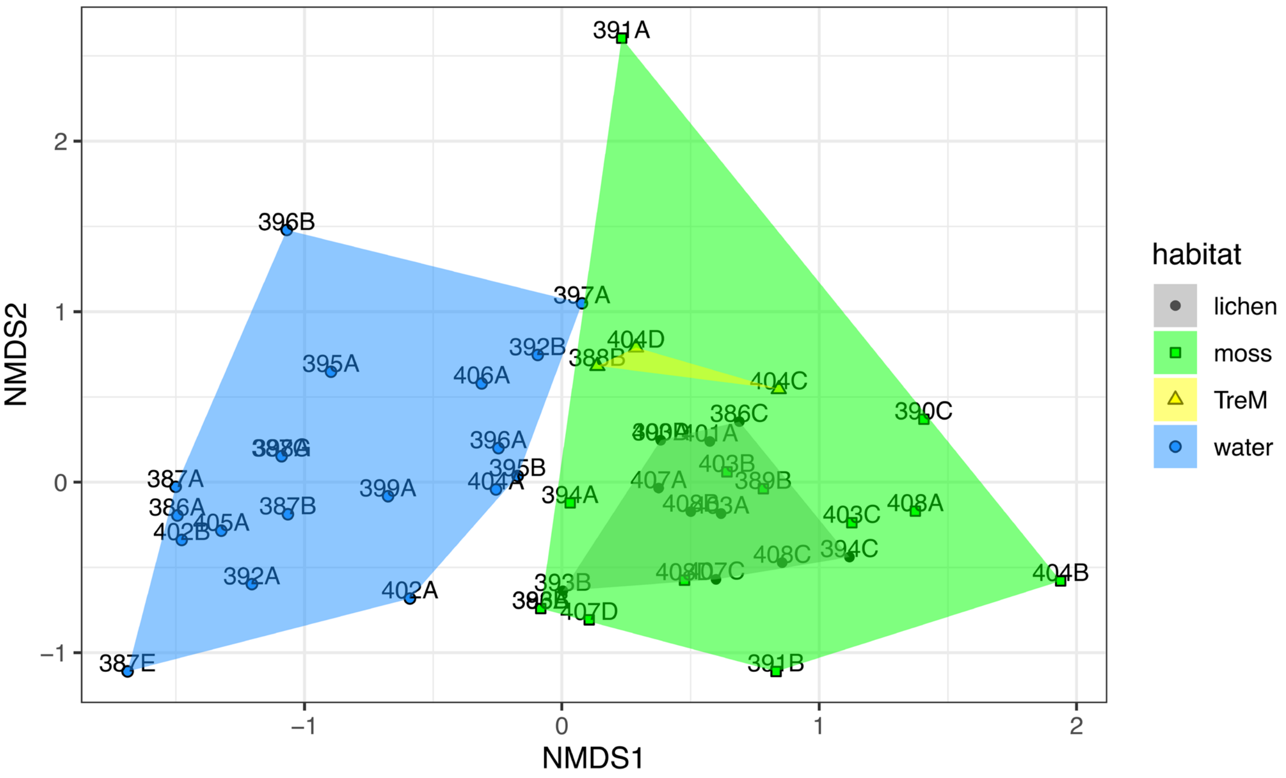


Supplementary Fig. S1.- Geographic localisation of the 47 samples with bdelloids collected in the Massane forest reserve during the survey. Latitude and longitude are expressed in WGS84 reference system; the thick dashed line represents the border between France and Spain.



Supplementary Fig. S2.- Similar to Fig. 3, Non-metric Multi-Dimensional scaling (NMDS) biplot representing similarities in community composition between samples on two axes. Samples from aquatic (water) and limno-terrestrial habitats (lichen, moss, TreM) are presented with different shapes and colours and grouped by coloured polygons representing the convex hulls for each of the four habitat types. Sample 400A is excluded from the biplot because it had only one species, not found in any other sample.

Code for statistical analyses. The R script, “La_Massane_bdelloids.R” together with the dataset in csv format, “La_Massane_bdelloids.csv”. (annexes)

Annexes

```
########################################################
##       Bdelloid rotifers from La Massane, France      ##
## Diego Fontaneto, Thiago Quintão Araújo & Nabil Majdi ##
##            Verbania, Italy, 3 January 2024           ##
##                  revised 18 March 2024               ##
##########################################################

# load libraries
library(ggplot2)
library(ggvegan)
library(labdsv)
library(mark)
library(MASS)
library(performance)
library(tidyverse)
library(vegan)


# read the data
bdello <- read.csv("La_Massane_bdelloids.csv", header=T, as.is=F)


##### richness #####

# model for species richness
m1 <- MASS::glm.nb(richness~water_terrestrial, data=bdello)
performance::check_model(m1)
summary(m1)

# plot the results
plot(richness~water_terrestrial, data=bdello, xlab="")
stripchart(richness~water_terrestrial, vertical=TRUE, data=bdello,
           method="jitter", add=TRUE, pch=20, col="blue")

# summary statistics
bdello %>%
  group_by(water_terrestrial) %>%
  summarise(
    n = n(),
    median = median(richness),
    min = min(richness),
    max = max(richness),
    mean = mean(richness),
    sd = sd(richness)
  )

# only terrestrial samples
bdelloT <- droplevels(subset(bdello, water_terrestrial=="terrestrial"))
bdelloT <- droplevels(subset(bdelloT, three_habitats!="TreM"))
```

```
# model for species richness
m1T <- MASS::glm.nb(richness~three_habitats, data=bdelloT)
performance::check_model(m1T)
summary(m1T)


# plot the results
plot(richness~three_habitats, data=bdelloT, xlab="")
stripchart(richness~three_habitats, vertical=TRUE, data=bdelloT,
           method="jitter", add=TRUE, pch=20, col="blue")

# summary statistics
bdelloT %>%
  group_by(three_habitats) %>%
  summarise(
    n = n(),
    median = median(richness),
    min = min(richness),
    max = max(richness),
    mean = mean(richness),
    sd = sd(richness)
  )


##### community composition #####

# read and prepare data
bdello_data <- read.csv("La_Massane_bdelloids.csv", header=F)

bdello_comm <- data.matrix(droplevels(bdello_data[-1,7:47]))-1
row.names(bdello_comm) <- bdello[,1]
colnames(bdello_comm) <- colnames(bdello[7:47])

# distance matrices
bdello_J <- vegan::vegdist(bdello_comm, "jaccard", diag=T, upper=T)
bdello_BC <- vegan::vegdist(bdello_comm, "bray", binary=T, diag=T, upper=T)

# betadisper, adonis and anosim for aquatic-terrestrial comparison
anova(vegan::betadisper(bdello_BC, bdello$water_terrestrial))

vegan::adonis2(bdello_BC~water_terrestrial, data=bdello)

bdello_anosim <- vegan::anosim(bdello_BC, bdello$water_terrestrial)
summary(bdello_anosim)
plot(bdello_anosim)
autoplot(bdello_anosim, notch = FALSE) +
  theme_bw()

# betadisper, adonis and anosim for the limno-terrestrial habitats
bdello_betadisp <- vegan::betadisper(bdello_BC, bdello$three_habitats)
anova(bdello_betadisp)
plot(TukeyHSD(bdello_betadisp))
TukeyHSD(bdello_betadisp)
plot(bdello_betadisp)
```

```
bdello_data3 <- bdello_data[-1,]
bdello_data3 <- droplevels(bdello_data3[bdello$three_habitats!="water",])
bdello_comm3 <- data.matrix(droplevels(bdello_data3[,7:47]))-1
row.names(bdello_comm3) <- bdello_data3[,1]
colnames(bdello_comm3) <- bdello_data[1,7:47]
bdello_BC3 <- vegan::vegdist(bdello_comm3, "bray", binary=T, diag=T, upper=T)
three_habitats3 <- mark::remove_na(droplevels(bdello$three_habitats, "water"))
bdello_betadisp3 <- vegan::betadisper(bdello_BC3, three_habitats3)
anova(bdello_betadisp3)
plot(TukeyHSD(bdello_betadisp3))
TukeyHSD(bdello_betadisp3)
plot(bdello_betadisp3)

bdello_data2 <- bdello_data[-1,]
bdello_data2 <- droplevels(bdello_data2[! bdello$three_habitats %in%
c('water','TreM'),])
bdello_comm2 <- data.matrix(droplevels(bdello_data2[,7:47]))-1
row.names(bdello_comm2) <- bdello_data2[,1]
colnames(bdello_comm2) <- bdello_data[1,7:47]
bdello_BC2 <- vegan::vegdist(bdello_comm2, "bray", binary=T, diag=T, upper=T)
three_habitats2 <- mark::remove_na(droplevels(bdello$three_habitats,
c("water","TreM")))
bdello_betadisp2 <- vegan::betadisper(bdello_BC2, three_habitats2)
anova(bdello_betadisp2)
plot(TukeyHSD(bdello_betadisp2))
TukeyHSD(bdello_betadisp2)
plot(bdello_betadisp2)

bdello_anosim_3 <- vegan::anosim(bdello_BC3, three_habitats3)
summary(bdello_anosim_3)
plot(bdello_anosim_3)
autoplot(bdello_anosim_3, notch = FALSE) +
  theme_bw()

# indicator species
summary(labdsv::indval(bdello_comm, bdello$water_terrestrial))

# plot NMDS scatterplot with polygons for the four groups
bdello_NMDS <- vegan::metaMDS(bdello_comm)
# not good... the reason is that sample 400A has one species only,
# not found anywhere else
# thus, I removed sample 400A
row.names(bdello_comm)
# it is sample 27
bdello_comm_reduced <- bdello_comm[-27,]

bdello_NMDS <- vegan::metaMDS(bdello_comm_reduced)
stressplot(bdello_NMDS)
plot(bdello_NMDS, type="t")

data.scores <- as.data.frame(vegan::scores(bdello_NMDS)$sites)
data.scores$habitat <- bdello$three_habitats[-27]
data.scores$site <- rownames(data.scores)

hull_hab <- data.scores %>%
  group_by(habitat) %>%
```

```
  slice(chull(NMDS1, NMDS2))

ggplot2::ggplot(data.scores, aes(NMDS1, NMDS2)) +
  aes(fill=habitat, shape=habitat) +
  geom_point() +
  scale_shape_manual(values=c("water"=21, "lichen"=16, "moss"=22, "TreM"=24))
+
  # geom_text(data=data.scores,aes(x=NMDS1,y=NMDS2,label=site),size=3,vjust=0)
+
  geom_polygon(data=hull_hab, alpha = 0.5) +
  scale_fill_manual(values=c("water"="dodgerblue", "lichen"="grey60",
"moss"="green", "TreM"="yellow")) +
  theme_bw()


##### cumulative curves #####
bdello_cumul <- vegan::specaccum(bdello_comm,"coleman")
bdello_cumul_water <- vegan::specaccum(bdello_comm[which(bdello$water_
terrestrial=="aquatic"),],"coleman")
bdello_cumul_terre <- vegan::specaccum(bdello_comm[which(bdello$water_
terrestrial=="terrestrial"),],"coleman")

plot(bdello_cumul_water, col="dodgerblue", lwd=2, ylim=c(0,25), xlim=c(0,30),
ylab="richness")
plot(bdello_cumul_terre, add=T, col="darkgreen")

vegan::specpool(bdello_comm, bdello$water_terrestrial)


##### MAP OF SAMPLES #####

library("ggmap")
register_stadiamaps("YOUR-API-KEY-HERE", write = TRUE)

bbox <- c(left=(min(locs$longitude)-0.005), bottom=(min(locs$latitude)-0.005),
right=(max(locs$longitude)+0.005), top=(max(locs$latitude)+0.005))

massane <- get_stadiamap(bbox, zoom=14, maptype = "stamen_toner_lite")
ggmap(massane)

ggmap(massane) +
      labs(y="latitude", x="longitude") +
      geom_point(aes(x=longitude,y=latitude),data=locs, color="red", size=2,
alpha=0.5)

# qmplot(longitude, latitude, data=locs, maptype = "stamen_toner_lite",
color=I("red"))
```

sample_ID	386A	386B	386C	387A	387B	387E	387G	388B	389B	390A	390C	391A	391B	392A	392B	393A	393B	394A	394C	395A	395B	396A	396B	397A	398A	399A	400A	401A	402A	402B	403A	403B	403C	403D	404A	404B	404C	404D	405A	406A	407A	407C	407D	408A	408B	408C	408D
Adineta barbata																												1														1					1
Adineta vaga		1														1	1	1			1	1							1						1						1	1	1			1	1
Didymodactylos carnosus																																1															
Dissotrocha macrostyla																																								1							
Embata hamata				1																																											
Embata laticeps	1																																														
Habrotrocha aspera																																				1											
Habrotrocha bidens																																														1	
Habrotrocha collaris																													1																		
Habrotrocha constricta									1										1														1			1								1		1	
Habrotrocha lata														1								1																									
Habrotrocha pulchra																			1																		1					1		1			
Habrotrocha rosa																														1																	
Habrotrocha sp.			1					1	1	1					1			1			1	1		1				1				1		1	1		1	1		1	1						
Habrotrocha spicula														1																																	
Habrotrocha sylvestris																																														1	
Macrotrachela ehrenbergi																																												1			
Macrotrachela habita									1									1	1												1	1										1			1		1
Macrotrachela musculosa													1																			1	1								1		1				
Macrotrachela nana									1		1																					1															
Macrotrachela papillosa												1																																			
Macrotrachela plicata			1								1																	1			1	1	1				1				1	1		1		1	
Macrotrachela quadricornifera																				1		1		1											1												
Macrotrachela sp.															1							1																									
Mniobia magna																																					1										
Mniobia russeola			1						1	1									1									1			1		1	1							1				1	1	1
Mniobia scarlatina										1																1		1			1	1		1	1						1				1		
Philodina citrina																											1																				
Philodina flaviceps	1			1	1		1							1											1	1			1	1					1				1								
Philodina plena															1																																
Philodina roseola					1	1																							1																		
Philodina sp.																																						1									
Philodina vorax			1																									1			1																
Philodinavus paradoxus																													1																		
Pleuretra brycei							1																		1	1																					
Pleuretra humerosa									1				1																			1															1
Pleuretra lineata										1							1											1						1										1			
Rotaria quadrioculata																								1																							
Rotaria rotatoria					1		1													1	1				1															1							
Rotaria sordida												1												1																							
Rotaria tardigrada																						1	1																								
richness	2	1	4	2	3	1	3	1	6	4	2	2	2	3	3	1	2	3	4	2	3	7	1	4	3	3	1	7	5	2	5	8	4	4	5	2	4	2	1	3	6	5	2	5	3	6	5

sample_ID	latitude	longitude	water_terres	habitat_type	three_habitats
386A	42.49056	3.02898	aquatic	epibiont	water
386B	42.49056	3.02898	terrestrial	moss	moss
386C	42.49056	3.02898	terrestrial	lichen	lichen
387A	42.49066	3.02956	aquatic	moss	water
387B	42.49066	3.02956	aquatic	moss	water
387E	42.49066	3.02956	aquatic	algae	water
387G	42.49066	3.02956	aquatic	Lemna	water
388B	42.49279	3.03237	terrestrial	dendrotelma	TreM
389B	42.48983	3.02899	terrestrial	moss	moss
390A	42.48931	3.02893	terrestrial	lichen	lichen
390C	42.48931	3.02893	terrestrial	moss	moss
391A	42.48866	3.0287	terrestrial	moss	moss
391B	42.48866	3.0287	terrestrial	moss	moss
392A	42.4883	3.02869	aquatic	moss	water
392B	42.4883	3.02869	aquatic	moss	water
393A	42.49099	3.03179	terrestrial	moss	moss
393B	42.49099	3.03179	terrestrial	lichen	lichen
394A	42.48081	3.03662	terrestrial	moss	moss
394C	42.48081	3.03662	terrestrial	lichen	lichen
395A	42.47725	3.0361	aquatic	sediment	water
395B	42.47725	3.0361	aquatic	moss	water
396A	42.47572	3.03084	aquatic	moss	water
396B	42.47572	3.03084	aquatic	sediment	water
397A	42.47649	3.03016	aquatic	sediment	water
398A	42.47968	3.0319	aquatic	moss	water
399A	42.48171	3.03303	aquatic	moss	water
400A	42.48205	3.03253	aquatic	algae	water
401A	42.48415	3.03146	terrestrial	lichen	lichen
402A	42.48456	3.03096	aquatic	moss	water
402B	42.48456	3.03096	aquatic	algae	water
403A	42.47073	3.01505	terrestrial	lichen	lichen
403B	42.47073	3.01505	terrestrial	moss	moss
403C	42.47073	3.01505	terrestrial	moss	moss
403D	42.47073	3.01505	terrestrial	lichen	lichen
404A	42.47072	3.01442	aquatic	moss	water
404B	42.47072	3.01442	terrestrial	moss	moss
404C	42.47072	3.01442	terrestrial	ivy rootlets	TreM
404D	42.47072	3.01442	terrestrial	dendrotelma	TreM
405A	42.47028	3.00942	aquatic	sediment	water
406A	42.47066	3.00689	aquatic	sediment	water
407A	42.49796	3.02834	terrestrial	lichen	lichen
407C	42.49796	3.02834	terrestrial	lichen	lichen
407D	42.49796	3.02834	terrestrial	moss	moss
408A	42.49529	3.03062	terrestrial	moss	moss
408B	42.49529	3.03062	terrestrial	lichen	lichen
408C	42.49529	3.03062	terrestrial	lichen	lichen
408D	42.49529	3.03062	terrestrial	moss	moss