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OASES IN TREES: PATTERNS OF AQUATIC HYPHOMYCETES DIVERSITY IN DENDROTELMATA AND STREAMS OF A MEDITERRANEAN FOREST

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ABSTRACT.- Freshwater ecosystems are vital oases for biodiversity across Mediterranean landscapes. Acquiring basic knowledge on how aquatic biodiversity is distributed across Mediterranean landscapes is pivotal to anticipate how aquatic ecosystems are responding to various stressors, such as drought intensification. Fungi – a very diverse group – are involved in important ecosystem functions and services, such as organic matter decomposition. Aquatic hyphomycetes (a polyphyletic group of fungi) are particularly relevant to forested aquatic ecosystems because of their capacity to decompose wood and leaf litter. So far, this group received little attention outside of their preferred habitat, known as oxygenated forest streams. In the Massane forest, an old-growth beech forest in southwestern France, we explored the patterns of alpha, beta and gamma diversity of aquatic hyphomycetes in a mosaic of two main freshwater habitats (water-filled tree holes – so called dendrotelmata –, and streams). Dendrotelmata harboured locally-rich communities of aquatic hyphomycetes (sometimes as much as adjacent streams), but their composition (beta diversity) was more heterogeneous in comparison to streams. The spatially discrete nature of dendrotelmata across a forested landscape (compared to vectorised, dendritic networks of permanent streams), and/or the environmental singularity of each dendrotelmata, might explain why beta diversity was higher in dendrotelmata than streams. Differences in community composition between stream and dendrotelmata habitats were as high as the differences between the two main types of dendrotelmata (pan vs. rot holes). A considerable proportion of species occurred in both dendrotelmata and streams (29 %) which, in addition to the strong environmental

differences observed in both habitats, suggests that a considerable proportion of species we identified may have broad ecological niches. The high beta diversity of aquatic hyphomycetes among dendrotelmata could foster fine-scale monitoring of dendrotelmata communities at the individual-tree level rather than at the forest plot level, offering new opportunities to improve current management and conservation of old-growth forests and their biodiversity. Our study suggests that we need to expand our knowledge on the distribution of aquatic hyphomycetes outside their preferred habitat to fully understand their biodiversity and ecological roles.

INTRODUCTION

Mediterranean ecosystems are hotspots of biodiversity including many endemic species (Cuttelod *et al.* 2008), but they are increasingly threatened by anthropogenic stressors (Dudgeon *et al.* 2006, Reid *et al.* 2019, Tierno De Figueroa *et al.* 2013). Key ecosystem processes and services sustained by Mediterranean freshwater ecosystems and their biodiversity include decomposition of allochtonous organic matter (Jabiol *et al.* 2014) and supply of water for animals (Kirsch *et al.* 2021). Understanding how freshwater biodiversity is structured across space and time is thus critical to Mediterranean regions. This understanding is needed to forecast and mediate the adverse effects of global change, such as the increasing frequency of drought events (Bonhomme *et al.* 2021, Bruder *et al.* 2011, Céréghino *et al.* 2020, Humphries & Baldwin 2003). However, most studies addressing the effects of drought focused on streams (Bonada & Resh 2013, Elias *et al.* 2015), often neglecting other freshwater ecosystems, which can be abundant at the landscape level and could act as refugia and/or support patch dynamics among a mosaic of freshwater habitats (e.g. stream networks, forested ponds, rock pools, etc). Advancing this knowledge might also lead to a better understanding of the distribution of the various organisms in different freshwater ecosystems that can compose aquatic ecosystems at the landscape scale, including fungi (Arias-Real *et al.* 2023, Gómez-Gener *et al.* 2016).

Aquatic fungi colonise a wide range of habitats, where they contribute to fundamental ecosystem processes and contribute to biogeochemical cycles (Seena *et al.* 2023). Studying how fungal communities are structured across freshwater habitats throughout Mediterranean landscapes can improve our understanding of how freshwater ecosystems respond to multiple stressors (Arias-Real *et al.* 2023, Bruder *et al.* 2019, Réveillon *et al.* 2022, Seena *et al.* 2023). Aquatic hyphomycetes – a polyphyletic group of filamentous fungi –, are particularly important for forested freshwater ecosystems (Bärlocher 1992). Their high diversity, productivity, and functional capabilities to breakdown recalcitrant organic compounds such as lignin and cellulose, make them vital contributors to the ecosystem functioning of forested headwater streams and other freshwater ecosystems (Gessner *et al.* 2010, Gessner & Chauvet 1994, Mariz *et al.* 2021, Suberkropp & Arsuffi 1984). Although aquatic hyphomycetes are best known in well-oxygenated lotic habitats, they have also regularly been found in other habitats (Chauvet *et al.* 2016), including water-filled tree holes, also called dendrotelmata (e.g. Magyar *et al.* 2017).

Dendrotelmata are small freshwater habitats forming in tree hollows. They are parts of two important classifications; tree-related microhabitats (Larrieu *et al.* 2018) and phytotelmata, or so-called natural aquatic microcosms (Srivastava *et al.* 2004). Dendrotelmata can be classified in two types. ‘Pan’

holes are hollows forming within joint elements of a tree, such as in tree roots or branch axils. This type of tree hole leaves the bark intact. On the contrary, 'rot' holes are forming in tree injuries such as branch breaks, where often active rotting of the wood occurs. The release of organic nutrients and carbon from microbial activity in rot holes is likely to exacerbate environmental differences between pan and rot holes (Kitching 1971). As abundant and discrete freshwater units distributed in forests from local to global scale, and exhibiting large environmental gradients, dendrotelmata provide an ideal ecological model to study biodiversity, meta-community dynamics, and detritus-based food webs and processes (Petermann & Gossner 2022). Very few studies have explored community assembly patterns of aquatic hyphomycetes in dendrotelmata, but some important findings were made by studies in Indian tropical rainforests and European temperate forests (Gönczöl & Révay 2003, Karamchand & Sridhar 2008, Magyar *et al.* 2017, Sridhar 2021, Sridhar & Karamchand 2009, Sridhar *et al.* 2013). Dendrotelmata in Mediterranean forests have, to our knowledge, not yet been studied. However, their diversity and composition could strongly differ from those in tropical and temperate forests. Exploring these differences could help to complete the picture of the natural range of freshwater habitats where aquatic hyphomycetes naturally occur, thereby deepening our appraisal of biodiversity patterns of this group across different habitats and biomes (Chauvet *et al.* 2016).

In this study, we investigated alpha-, beta- and gamma diversity patterns of aquatic hyphomycete communities in the National Nature Reserve of the Massane Forest (UNESCO world heritage), a 100-year-old unmanaged and 50-yo protected old-growth forest dominated by European Beech (*Fagus sylvatica*). We did so in a mosaic of patchy habitats (i.e. dendrotelmata) and compared diversity patterns to spatially-vectorised, continuous freshwater habitats (i.e. streams). We a priori defined the streams flowing through the Massane forest as reference habitats for aquatic hyphomycetes, since streams are considered so far as the preferred habitat for aquatic hyphomycetes (Chauvet *et al.* 2016, Sridhar, 2021) and because their aquatic hyphomycete communities are better described. First, we identified species that occurred only in streams or dendrotelmata versus those shared by both habitats. As aquatic hyphomycetes are known to occur in very diverse habitats (Sridhar *et al.* 2013), we expected (*i*) a significant overlap of species sharing both dendrotelmata and stream habitats (i.e. more than 5 % of species). Secondly, we investigated alpha, beta, and gamma diversity of aquatic hyphomycete communities among stream and dendrotelmata habitats, expecting that (*ii*) streams would express the highest alpha diversity, as they are thought to be the most suitable habitat for this group. Finally, we explored differences in community composition (beta diversity turnover) between streams and dendrotelmata, but also between the two main types of dendrotelmata (i.e. pan and rot holes). Here we expected that (*iii*) differences in community composition between streams and dendrotelmata could be as high as between the two main types of dendrotelmata.

MATERIALS AND METHODS

Study site

Our study site was located in the Massane forest, an unmanaged and protected old-growth forest dominated by European beech (*F. sylvatica*), in France (N 42° 28' 58'', E 3° 1' 45''). The Massane forest

is located near the Mediterranean Sea in the Oriental Pyrenees, at an altitude of *ca.* 600–800 m a-s-l (Mansourian *et al.* 2013). The Massane forest exhibits a Temperate–Mediterranean climate (Charles *et al.* 2022), with a trend towards a Mediterranean climate with global warming. Our sampling took place from April to May 2022.

Dendrotelmata mapping and sampling

We selected an area of *ca.* 75 ha (1500 m × 500 m) bordered by the Massane river in the west. We mapped Beech trees holding dendrotelmata by recording their coordinates with a GPS (Garmin GPSMAP65) and measured basic morpho-ecological features (i.e. size, height on the tree, and pan *vs.* rot holes). We selected 35 among the *ca.* 200-mapped dendrotelmata to cover the range of environmental characteristics and the study area. We also sampled four sites spread along the Massane stream, and one site in each of the two tributaries in the southeast part of the study area (n = 6). From the selected dendrotelmata, we recorded oxygen concentration (mg l^{-1}), oxygen saturation (%), water temperature (°C), pH, and conductivity (µS cm^{-1}), using a multi-parameter probe (HACH HQ Series). Then, with sterile gloves, we collected three to five items of coarse organic debris (e.g. leaves of different tree species, bark pieces, beech fruits, small branches, etc.) from the dendrotelmata and stream sites, stored them in plastic bags, with some water from the same habitat. These items represented the diversity of coarse organic debris present at each site and maximized the diversity of hyphomycete species detected in each sampling unit. We kept samples in a cool box during the transport to the laboratory.

Sporulation assay

We conducted sporulation assays following Bruder *et al.* (2011), but with some adaptations for the present study. We placed three to five pieces of each debris item (not exceeding 80 mm in length) in a pre-labelled Petri dish with 20 ml of deionized water. The Petri dishes were then placed on an orbital shaker at 50 rpm, incubated at 10 ± 2°C, and exposed to a light:dark cycle of 12:12 h (artificial light in the incubator). After seven days, we transferred the spore suspension to 50 ml plastic vials and fixed it with formaldehyde (4 % final concentration). The items of coarse organic material were then stored frozen at -20°C, freeze-dried, and weighed to the nearest 0.001 mg.

To account for slight variations in water volume among sporulation samples, we adjusted the volume of each individual sample with deionized water to the maximal value found among the samples and noted the dilution factor. Subsequently, we transferred each sample to a 100 ml glass beaker and rinsed each sample vial of 50°ml with 10 ml of Milli-Q water, which we added to the beaker. We then added 100 ml of Triton X-100 to the beaker and mixed the samples for 15 min on a stirrer plate at 120 rpm, to prevent conidia conglomeration. We then filtered 20 ml of each sample (MF-MilliporeTM 5.0 µm MCE membrane, 47 mm diameter), placed the filters on a Petri dish, and stained them with Trypan Blue (0.05 % in 60 % lactic acid) on each side. Then, we mounted the filters on microscope slides and observed them under a microscope at 500 × magnification (Leica ATC 2000). We counted and identified conidia (hereafter spores) using taxonomic keys (Descals *et al.* 1989, Gulis *et al.* 2020). If more than 300 spores were encountered per slide, only one filter-half was analysed, otherwise both. We then multiplied the spore counts by the dilution factor, merged counts for each dendrotelmata or stream site, and standardised by gram of the corresponding organic matter items and by day of sporulation.

Statistical analyses

We conducted data analysis with the R software version 4.3.3 (R Development Core Team, 2023). First, with a Venn diagram, we visualised the number of species belonging to dendrotelmata and

stream habitats, respectively, and the one shared by both habitats. We used the function "iNEXT" of the eponym package (Hsiech *et al.* 2024) to plot accumulation curves of gamma species richness and to calculate the number of species in each habitat for 75 % and 100 % of sample coverage, therefore allowing for comparisons of community assembly patterns among different habitats (Chao *et al.* 2014, Hsiech *et al.* 2024). We explored alpha diversity patterns between habitats with rarefied species richness, Shannon diversity, and Chao's adjusted index ('vegan' package, Borcard *et al.* 2011, Oksanen *et al.* 2007). We assumed that the relative abundances of species were species-specific sporulation rates rounded to integers (conidia $g_{\text{organic matter DW}}^{-1}$ d^{-1}). To test for differences in beta-diversity between habitats, we calculated Whittaker's beta-diversity as the ratio between gamma and alpha diversity for each dendrotelmata or stream site (Whittaker 1972). Due to the unbalanced design between dendrotelmata and stream sample sizes (n=35 and n=6, respectively), we used a non-parametric Kruskal-Wallis test to compare alpha and beta diversity values in both habitats.

For the analysis of community composition, we normalized the matrix of species abundance using log(abundance + 1) and then applied an Hellinger transformation, using the function 'decostand' ('vegan' package; Oksanen *et al.* 2007), in order to avoid the double-zero issue (Legendre 1998). We then computed a pairwise Bray-Curtis distance matrix with the function 'vegdist' (Oksanen *et al.* 2007). We performed a permutational multivariate analysis of variance (PERMANOVA) with orthogonal and planned contrasts on the Bray-Curtis distance matrix to test for differences in community composition (i.e. community turnover) between the two freshwater habitats (streams; n = 6 vs. dendrotelmata; n = 35), as well as between the two types of dendrotelmata (rot-holes; n = 5 vs. pan-holes; n = 30). We tested the assumption of homoscedasticity of this PERMANOVA with a permutation test (Oksanen *et al.* 2007). We displayed graphically the results of the PERMANOVA with a non-metric multidimensional scaling (NMDS) analysis (Oksanen *et al.* 2007). We set the statistical significance threshold at $\alpha = 0.05$. Finally, we mapped spatial patterns of alpha and beta diversity in both habitats using the packages 'terra' and 'geodata' (Hijmans *et al.* 2023, 2024).

RESULTS

Physico-chemical parameters

The two freshwater habitats we sampled (35 dendrotelmata and 6 stream sites) showed contrasting physico-chemical conditions (Table I). Oxygen concentration was on average two-fold lower, and the conductivity seven-fold higher in dendrotelmata than in streams (Table I). Among dendrotelmata, conductivity, dissolved oxygen, and oxygen saturation varied greatly, with coefficients of variation (CVs) of 168 %, 91 % and 87 %, respectively. This high variation of oxygen and especially conductivity values among dendrotelmata was in part related to differences *between* pan- and rot-holes, with pan-holes showing higher oxygen concentration (by 59 %) and lower conductivity (by 134 %) than rot-holes (Table I). However, it is noteworthy here that similarly, the environmental variation *within* both dendrotelmata habitats showed great variability, with CVs for conductivity of 188% and 80% within pan- and rot-hole categories, respectively (Table I). On the contrary, the variability among stream sites was dramatically lower for the same parameters (CVs: 2 %, 3 %, and 6 % for conductivity, oxygen concentration and oxygen saturation, respectively; Table I). The differences in pH and temperature

among habitats were negligible (Table I).

Parameters	Dendrotelmata	Pan holes	Rot holes	Streams
Temperature (°C)	11.46 (± 2.71)	11.45 (± 2.53)	11.54 (± 4.03)	11.9 (± 1.59)
Conductivity (µS cm^{-1})	676.09 (± 1134.96)	429.17 (± 809.73)	2157.60 (±1730.5)	95.1 (± 2.02)
Dissolved oxygen (mg l^{-1})	3.5 (± 3.17)	3.74 (± 3.34)	2.04 (± 1.26)	9.54 (± 0.30)
Oxygen saturation (%)	34.40 (± 29.93)	36.72 (± 31.42)	20.49 (± 13.19)	96.1 (±6.01)
pH	6.95 (± 0.70)	6.79 (± 0.46)	7.90 (± 1.13)	6.78 (± 0.44)

Table I.- Average values (± standard deviation) of physico-chemical parameters measured in dendrotelmata and streams of the Massane Forests

Aquatic hyphomycetes diversity

In total, 37 species of aquatic hyphomycetes were reported in this study. Among them, 21 species were identified as known species, leaving the percentage of unidentified species to 43 % (Table II). The five most frequent species in the sampled dendrotelmata were *Alatospora accuminata* (in 23 samples), *Dimorphospora foliicola* (in 21 samples), cf. *Sigmoidea aurantiaca* (in 14 samples), "Unidentified Species 3" (in 11 samples) *and Flagellospora fusarioides* (in nine samples). In streams, the most abundant species were "Unidentified Species 11", *Neonectria lugdunensis* (in four samples), *Tricladium chaetocladium*, A*nguillospora rosea*, *Tricladium splendens* and *Flagellospora fusarioides* (in three samples; see Table II, Fig. 1).

Species	Dendrotelmata	Stream
Alatospora acuminata Ingold	66	33
Anguillospora crassa Ingold	0	33
Anguillospora rosea J. Webster & Descals	3	50
Articulospora tetracladia Ingold	3	17
Clavatospora longibrachiata (Ingold) Sv. Nilsson ex Marvanová & Sv. Nilsson	6	33
Dimorphospora foliicola Tubaki	60	17
Flagellospora curvula Ingold	3	0
Flagellospora fusarioides S. H. Iqbal	26	50
Heliscella stellata (Ingold & V. J. Cox) Marvanová	14	17
Mycocentrospora clavata S. H. Iqbal	20	0
Neonectria lugdunensis (Saccardo & Therry) L. Lombard & Crous	0	67
cf. *Sigmoidea aurantiaca* Descals	40	17
Taeniospora gracilis Marvanová	0	17
Tetracladium setigerum (Grove) Ingold	3	0
Tricellula aquatica J. Webster	3	0
Tricladium attenuatum S.H. Iqbal	3	0
Tricladium castaneicola B. Sutton	9	0
Tricladium chaetocladium Ingold	0	50
Tricladium splendens Ingold	11	50
Tripospermum camelopardus Ingold, Dann & P.J. McDougall	3	0
Varicosporium elodeae W. Kegel	3	0
Unidentified Species 1	3	0
Unidentified Species 2	2	0
Unidentified Species 3	31	0
Unidentified Species 4	9	0
Unidentified Species 5	11	0
Unidentified Species 6	14	33
Unidentified Species 7	3	0
Unidentified Species 8	0	17
Unidentified Species 9	0	17
Unidentified Species 10	3	0
Unidentified Species 11	3	67
Unidentified Species 12	3	0
Unidentified Species 13	0	17
Unidentified Species 14	0	17
Unidentified Species 15	0	17
Unidentified Species 16	3	0

Table II.- List of the 37 aquatic hyphomycete species found in the study area. Percentage of occurrence for each species across all the sample locations are given for each habitat type (%).

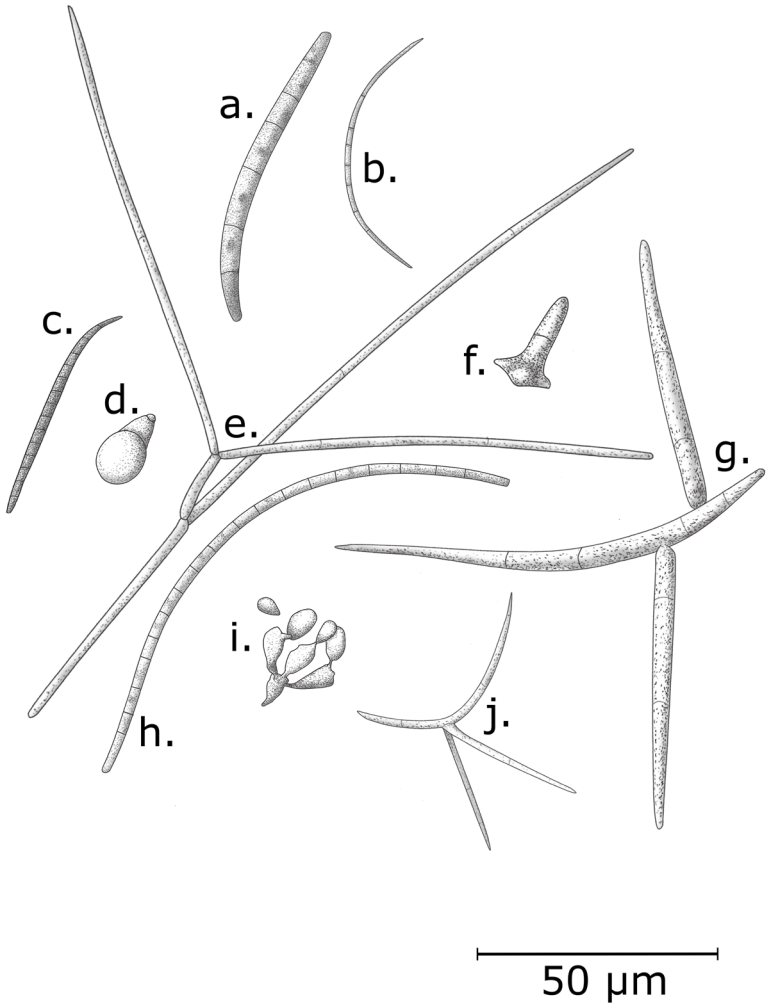

Fig. 1.- Illustrations of the five most abundant aquatic hyphomycete spores found in the streams (c, e, f, g & b/h) and dendrotelmata (a, b, d, i & j). a.; cf. *Sigmoidea aurantiaca*; b. *Flagellospora fusarioides*; c. *Unidentified species 11*; d. *Unidentified species 3*; e. *Hydrocina chaetocladia*; f. *Neonectria lugdunensis*; g. *Tricladium splendens*; h. *Anguillospora sea*; i; *Dimorphospora foliicola*; j. *Alatospora acuminata*.

Gamma diversity

Aquatic hyphomycetes diversity differed significantly between dendrotelmata and streams (Fig. 2, Table III), although they shared a substantial number of species (11 species out of 37, or 29 %; Fig. 2B). Gamma diversity was slightly higher in dendrotelmata (n = 28) than in streams (n = 20), but our sample coverage analysis showed that gamma diversity values at 75 % and 99 % coverage were roughly identical in streams and dendrotelmata (Table III). However, compared to stream habitats, the accumulation of species as the number of identified spores increased was more progressive in dendrotelmata than in streams, while in streams, we rather found a steeper accumulation of species which saturated at a very small number of spores identified (Fig. 2A). The inset in Fig. 2A shows further that fewer samples were needed to cover the gamma diversity in streams than dendrotelmata.

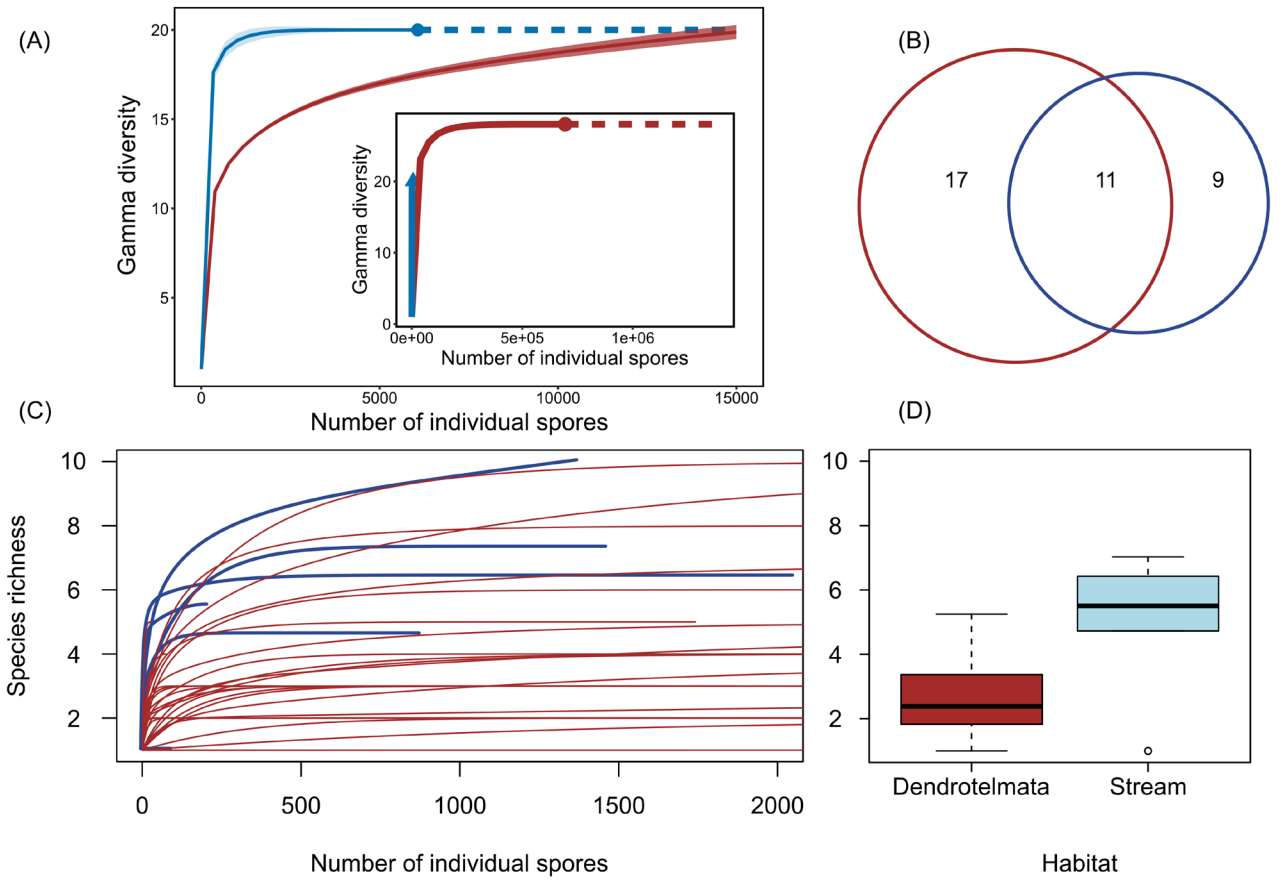

Fig. 2.- A, Species accumulation curves as the number of identified spores increases for each habitat. We set a maximum of 15000 spores for the sake of clarity. The inset shows the full accumulation curve to show the point where dendrotelmata reached their total gamma diversity (n = 28). B, Venn diagrams of the number of species unique to each habitat and those shared by both habitats. C, Rarefaction curves of species richness (alpha diversity) for each sampled unit by habitat type as the number of identified spores increases for each habitat. D, Boxplots of rarefied alpha diversity per habitat type. Dendrotelmata is shown in dark brown, and stream in blue, respectively.

Diversity index	Dendrotelmata	Streams
Total number of species *(γ-diversity)*	28	20
Number of species for 75 and 99% coverage *(γ-diversity)*	13/23	13/18
Rarefied richness *(α-diversity)*	2.62 (± 1.21)	5.03 (± 2.14)
Shannon diversity *(α-diversity)*	0.40 (± 0.36)	1.00 (± 0.59)
Sporulation *(abundance)*	19826.7 (± 40231.6)	1011.8 (±764.8)
Chao's adjusted index *(α-diversity))*	3.6 (± 2.35)	6.5 (± 3.62)
Whittaker's index *(β-diversity)*	13.46 (± 7)	6.24 (± 6.76)

Table III:- Gamma, alpha and beta diversity in dendrotelmata and stream habitats (± SD).

Alpha diversity

Stream habitats exhibited *ca.* 2-fold higher rarefied species richness than dendrotelmata (Kruskal-Wallis, $\chi^2 = 6.45$, df = 1, P = 0.01; Fig. 2C-D and Table III). Differences in Chao's adjusted alpha diversity between both habitats corroborated the rarefied richness result, while for Shannon diversity, the difference between both habitats was higher (2.5-fold; Table III).

Beta diversity

Both rot vs. pan holes (PERMANOVA: $F_{1,38} = 2.2$; P = 0.037), and dendrotelmata vs. stream comparisons (PERMANOVA: $F_{1,38} = 4.8$; P = 0.001) showed contrasted communities (Fig. 3). Based on the F-values given above, the difference in community composition was about 2-fold stronger between stream and dendrotelmata than between rot- and pan-holes, which can be considered of the same order of magnitude (Fig. 3).

Contrarily to alpha diversity which was higher in streams than dendrotelmata (see Figs. 2C, D), beta diversity was higher among dendrotelmata than among stream habitats (Kruskal-Wallis, $\chi^2 = 7.87$, P = 0.005; Table III and Fig. 4A). Finally, beta and alpha diversity of aquatic hyphomycetes exhibited patterns which were spatially dependent across the study area (Figs. 4A and 4B, respectively). These patterns suggest a lower beta diversity in the southern part, while alpha diversity showed an opposite pattern.

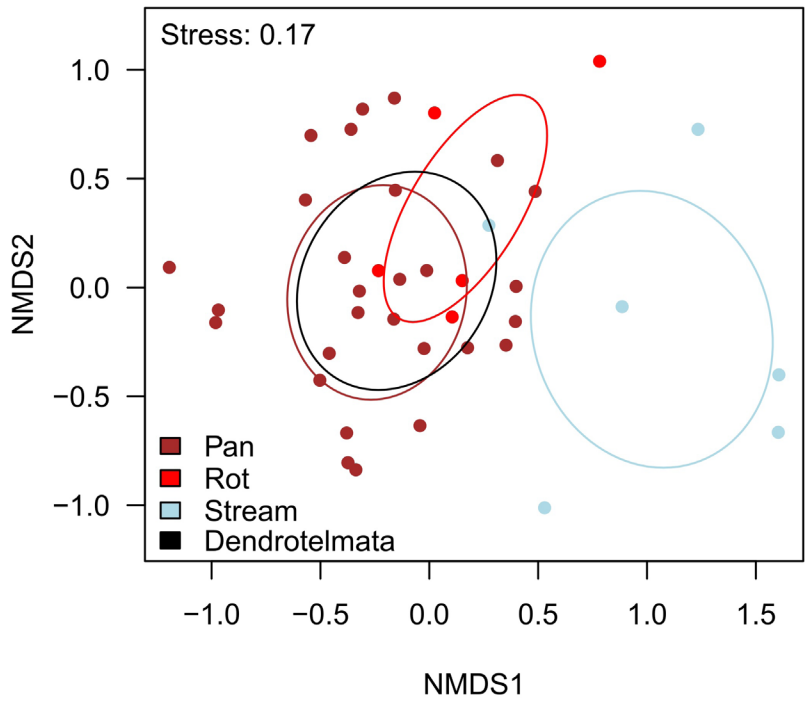

Fig. 3.- NMDS of aquatic hyphomycete assemblages in the two main types of dendrotelmata (pan and rot, brown and red ellipses, respectively), and the stream sites (blue ellipse). The ellipse for all dendrotelmata is given in black

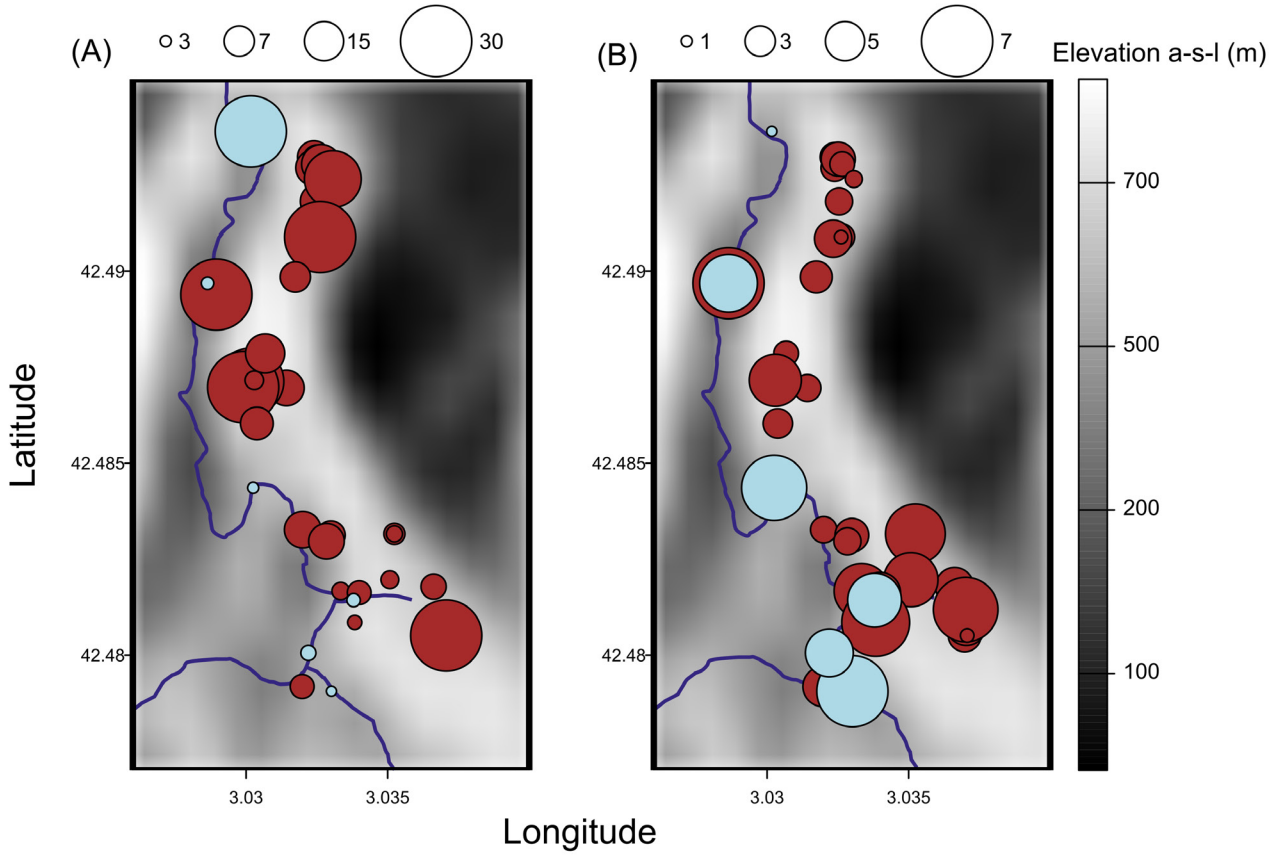

Fig. 4.- A, Map of the distribution of beta diversity (γ/α) of aquatic hyphomycetes in the Massane forest. B, Map of alpha diversity (rarefied richness). Blue circles represent stream sites, and brown ones show dendrotelmata, respectively. The size of circles is proportional to diversity. The streams are in dark blue. The grey gradient corresponds to the elevation above sea level (m).

DISCUSSION

With our study, we aimed to explore patterns of diversity in aquatic hyphomycete communities in streams and dendrotelmata in the Massane forest. Both habitats showed diverse communities, with 37 morpho-species reported in total (21 identified at the species level). This diversity is within the range of what has been found by other authors. For instance, Sridhar *et al.* (2013), found 18 species in the dendrotelmata of a tropical rainforest of the Western Ghats Mountains in India, and Gönczöl & Révay (2003), found 31 species in the dendrotelmata of a beech forest in Hungary. The most frequent species we found was *Alatospora acuminata*, which is among the globally most widely distributed aquatic hyphomycete species (Duarte *et al.* 2016). This species was also found in the dendrotelmata of the Hungarian beech forest by Gönczöl & Révay (2003), but not in a five-year long-term study of a maple tree hole in Norway, where other fungal species were more common (Magyar *et al.* 2017). The low overlap between the communities detected in our study with the ones described in other studies in dendrotelmata suggests a high heterogeneity of community composition at the continental scale, but

also emphasizes our limited knowledge of aquatic hyphomycete biogeography in other habitats than forested streams (Chauvet *et al.* 2016, Gönczöl & Révay 2003, Magyar *et al.* 2017).

We found that 29 % of species were occurring in both streams and dendrotelmata. This result adds to the growing evidence that not all species of aquatic hyphomycetes are habitat specialists (Chauvet *et al.* 2016). However, the overlap we report here is much lower than the one observed by Sridhar *et al.* (2013), where all but one species occurred in both habitats. One explanation could be that Sridhar *et al.* (2013) focused on riparian trees only, which probably facilitates the colonisation by aquatic hyphomycetes from the stream (Sridhar & Bärlocher 1993, Webster 1977). Tropical fungi, including the ones described by Sridhar *et al.* (2013), might also be more often habitat generalists than temperate/ Mediterranean species, or perhaps the high humidity of tropical rain forests is favouring the dispersal of aquatic hyphomycetes between streams and dendrotelmata. Deciphering hyphomycete species habitat preferences among biomes requires more coordinated studies.

In the present study, we found that some species were restricted to the stream habitats, which likely reflects their habitat preference. Among them, *Taeniospora gracilis* and *Hydrocina chaetocladia* have both been described in mountain streams (Chauvet 1991), and the environmental conditions in dendrotelmata (e.g. low dissolved oxygen) could be particularly restrictive for them (Medeiros *et al.* 2009). However, dendrotelmata specialists were more difficult to identify from our data. Several species that were classified as dendrotelmata specialists based on our analysis have been previously found to be common in stream habitats (such as in Descals & Moralejo 2001) and were also found in other surveys of the Massane stream (*Flagellospora curvula*, *Tricladium castaneicola*, *Varicosporium elodeae*; J. Jabiol, *pers. com.*). This, together with the significant difference in community composition between rot- and pan-dendrotelmata, suggests that while many species are able to disperse across and develop within different habitats, environmental factors remain a strong filter driving local community assembly, and this irrespectively of the habitat considered.

On average, we found that stream sites showed locally *ca.* twice the number of species than dendrotelmata, and they were less dominated by single species (Shannon diversity was 2.5-fold higher in streams than dendrotelmata). Stream environmental conditions such as their high oxygenation levels are suitable for a large range of aquatic hyphomycete species. Moreover, streams are complex ecosystems that contain patches of diverse habitats and resources (e.g. litter of different types, species and degradation stages), but connected by a unidirectional flow of water dispersing aquatic hyphomycete spores (Bärlocher 2009). Water flow might contribute to a homogenisation of communities among connected stream sites. This could have resulted to a lower beta diversity and a faster saturation of the species accumulation curve in stream habitats compared to dendrotelmata. On the contrary, dendrotelmata communities were much more spatially heterogeneous, and thus harboured a higher beta diversity than stream communities. This might be because the species pool in each dendrotelmata is restricted to the species able to reach the dendrotelmata through dispersal across the terrestrial matrix, survive to harsher environmental conditions, and grow and reproduce on the available resources. Higher beta-diversity in dendrotelmata communities compared to those in streams is thus likely a combination

of the heterogeneity in environmental factors among dendrotelmata (as shown here within and between pan and rot holes) and their discrete spatial distribution, which can be seen as aquatic islands within a forested landscape (Kitching 2000, Petermann & Gossner 2022).

The discrete distribution of dendrotelmata across forested landscapes probably makes their communities subject to pronounced ecological drift and patch dynamics, both mechanisms being expected to favour community turnover across space according to meta-community and island biogeography theories (Mouquet & Loreau 2003, Petermann & Gossner 2022). Probably as a result of their higher beta diversity, and despite a lower alpha diversity than in streams, dendrotelmata habitats contained overall a slightly higher number of species (n = 28) than streams (n = 20). As a result of the unbalanced design, we refrained from statistically comparing gamma diversity between both habitats. Nevertheless, our sample coverage analysis acknowledged for this bias and led us to conclude that gamma diversity would be similar in stream and dendrotelmata habitats. The unbalanced design between stream and dendrotelmata, and between the two types of dendrotelmata in our study could also have weaken our ability to detect rare species. However, in using species accumulation curves and rarefied richness in most of our inferences of diversity patterns, and in using a permutational multivariate analysis of variance (PERMANOVA) for community composition, we accounted for most of the bias inherent to the unbalanced design.

CONCLUSIONS AND PERSPECTIVES

We found that aquatic hyphomycete communities were roughly as diverse in dendrotelmata of beech trees as in the Massane stream, but that community assembly processes are likely to be very different in those two habitats. With our case study, we are adding to the growing evidence that dendrotelmata can be an important freshwater habitat for aquatic hyphomycetes in forested landscapes. Because of their discrete nature (i.e. as aquatic islands) and patchy distribution, and since they exhibit very heterogeneous environmental conditions, the dendrotelmata of the Massane forest harbour singular and diverse aquatic hyphomycete assemblages. To further advance our knowledge of these communities, we need to better understand dispersal abilities of auatic hyphomycetes and their environmental niches outside of streams, by tackling a meta-community approach between stream and non-stream habitats. To this end and given the relatively high percentage of unidentified species that we report, further efforts need to be made to improve our taxonomic and functional knowledge of this group of aquatic fungi, for instance by developing trait-based approaches and molecular tools such as meta-barcoding in addition to taxonomy in general. To this end, we consider it important to build trait databases, and to expand the genetic databases of aquatic hyphomycete species from different habitats (Franco-Duarte *et al.* 2022), as well as to complement morphological taxonomic keys.

At the forest level, the presence of dendrotelmata may enhance the resilience of aquatic hyphomycete communities in the face of extreme climatic events (e.g. droughts, floods), which are common and increasing Mediterranean phenomena and have consequences on aquatic hyphomycete

communities and processes (Bruder *et al.* 2011). Dendrotelmata could act as reservoirs, in collecting spores during throughfall and stemflow (Chauvet *et al.* 2016). It is thus possible that dendrotelmata store aquatic hyphomycete spores during drying events, and that these spores disperse to other dendrotelmata and to surrounding streams through wind and rain. They might thus play a role in maintaining complex patch dynamics among stream and non-stream habitats. Moreover, the environmental stress that can induce the ephemeral and variable nature of abiotic conditions in dendrotelmata (fast changes in hydrology, aerobic and anaerobic phases, large gradients in conductivity), could trigger the growth and/or sexual reproduction of some aquatic hyphomycete species, or might on the contrary lead to the local extinction of others. These different responses could lead to complex meta-community dynamics, from local habitat filtering to storage and mass effects among dendrotelmata. In this context, one key aspect to explore is how dendrotelmata can serve as a refugia and/or a source of organisms for adjacent freshwater habitats, before, during, and after such disturbances.

Dendrotelmata are unique but poorly known oases of biodiversity in trees. These ecosystems should be explicitly considered in research and conservation plans from global (i.e. forests in different biomes) to local scales (i.e. individual trees in old-growth forests or in urban areas). At the global and regional scales, conservation of forests hosting high densities of dendrotelmata, as it is usually the case in old-growth forests, would help conserving global and regional pools of aquatic hyphomycete species and other taxonomic groups. At the local scale, we encourage management and conservation plans that focus on individual trees, as conducted by the team of the National Nature Reserve of the Massane forest. Monitoring individual trees (e.g. tree size, presence of dendro-microhabitats including dendrotelmata and their features etc.) and promoting this functional microhabitat diversity would help promoting the alpha and beta diversity of aquatic hyphomycetes and of other species. Within managed forests, this approach and the recognition of the individual value of trees, could preclude any managerial decisions to remove trees hosting tree-related microhabitats such as dendrotelmata and their biodiversity. Future research should thus evaluate how forest ecological and managerial status are affecting the diversity of aquatic hyphomycetes and other organisms groups in dendrotelmata and other understudied habitats.

Declaration of conflict of interests

The authors of this study have no conflict of interest to declare.

Authorship' statement

R. C. A.B. M.G. and T.R.: Study design and compilation of main ideas. R. C. F.C. A.B. M.G. and T.R.: Fieldwork, and sample analysis in the laboratory. R. C. and T.R.: Design of the statistical analyses and writing of a first draft. R.C. J.J. F.C. I.F. I.S. A.B. M.G. and T.R.: Interpretation of the results and writing of the final version of the manuscript.

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