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Aquatic Hyphomycete Species Are Screened by the Hyporheic Zone of Woodland Streams

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Aquatic hyphomycetes strongly contribute to organic matter dynamics in streams, but their abilities to colonize leaf litter buried in streambed sediments remain unexplored. Here, we conducted field and laboratory experiments (slow-filtration columns and stream-simulating microcosms) to test the following hypotheses: (i) that the hyporheic habitat acting as a physical sieve for spores filters out unsuccessful strategists from a potential species pool, (ii) that decreased pore size in sediments reduces species dispersal efficiency in the interstitial water, and (iii) that the physicochemical conditions prevailing in the hyporheic habitat will influence fungal community structure. Our field study showed that spore abundance and species diversity were consistently reduced in the interstitial water compared with surface water within three differing streams. Significant differences occurred among aquatic hyphomycetes, with dispersal efficiency of filiform-spore species being much higher than those with compact or branched/tetraradiate spores. This pattern was remarkably consistent with those found in laboratory experiments that tested the influence of sediment pore size on spore dispersal in microcosms. Furthermore, leaves inoculated in a stream and incubated in slow-filtration columns exhibited a fungal assemblage dominated by only two species, while five species were codominant on leaves from the stream-simulating microcosms. Results of this study highlight that the hyporheic zone exerts two types of selection pressure on the aquatic hyphomycete community, a physiological stress and a physical screening of the benthic spore pool, both leading to drastic changes in the structure of fungal community.

Shade by the riparian vegetation considerably depresses the *in situ* primary productivity of small headwater woodland streams. Therefore, the important input of allochthonous organic matter from the riparian vegetation is the major source of carbon and energy for these aquatic ecosystems (1–3). A substantial part of leaf litter entering running waters may become buried in the streambed during storms, as a consequence of flooding and sediment movement (4–6). Thus, the hyporheic zone of streams (i.e., water-saturated sediment area below and beside running waters [constituting the benthic zone] where ground water and surface water mix [7]) is often a major site of organic matter storage (8). Aquatic heterotrophic microorganisms, especially aquatic hyphomycetes, are key mediators in energy and nutrient transfers to higher trophic levels within headwater streams (9–13).

During plant litter decomposition, fungal biomass on decaying leaves may reach 17% of total detrital mass (14). Several studies estimated that ca. 50% of the total fungal production is allocated to the formation of conidia (15–17), which are asexual reproductive spores. Therefore, spore concentrations in headwater streams can reach several thousands per liter during the autumn season, when deciduous leaves fall into the stream (18–20). Gessner (14) estimated that 20 g of spore dry mass was transported daily through a cross-section of a small stream (discharge of 60 liters s^{-1}). Similarly, on an annual basis, Suberkropp (21) calculated that 375 g of spore dry mass was exported from a low-nutrient stream with a base flow of 4 to 5 liters s^{-1}. The release of spores is the predominant mechanism involved in the rapid spreading of aquatic fungi after the annual leaf fall in temperate streams (22). The ability of spores to be transported and to colonize leaf patches would therefore be crucial for the organic matter processing in streams (23). However, these transport and colonization processes remain understudied in hyporheic zone, while they are probably essential for organic matter dynamics in sedimentary environments (24).

Unlike most terrestrial fungi, aquatic hyphomycetes produce spores with a variety of shapes and sizes (25), which reflects their adaptation to stream habitats (26). Many of these spores are either tetraradiate, branched, or filiform and exceed 100 μm in length (although small ovoid spores also exist). As a consequence, differences in spore shapes may influence the transport of aquatic hyphomycetes and their ability to colonize leaf litter patches (27), particularly those buried within the hyporheic sediments. Interactions between spore shape and sediment properties (notably, porosity) may influence the dynamics of aquatic fungi in stream sediments.

In addition to this physical-biological interaction, aquatic fungi and their spores may be vulnerable to prevailing chemical conditions within the hyporheic zone. Dissolved oxygen (DO) availability in interstitial water is a major factor influencing fungal biomass and microbial activities (28), and low DO concentrations have been shown to markedly affect the community structure of aquatic hyphomycetes (29). For example, Rajashekhar and Kaveriappa (30) found a positive correlation between DO concentration and aquatic hyphomycete species richness in rivers of the

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TABLE 1 Physical-chemical characteristics of water from the benthic and hyporheic zones of the three streams, measured at late autumn (25 November 2009) and at early spring (21 March 2010)[a]

Stream	Altitude (m a.s.l.)	Width (m)	Depth (m)	Slope (%)	Discharge (liter/s)	Value for indicated physical-chemical variable								
						Zone	Temp (°C)	pH	Dissolved oxygen (% sat.)	Conductivity (μS/cm)	Alkalinity (mg $CaCO_3$/liter)	P-PO_4 (μg/liter)	N-NO_3 (mg/liter)
Béal	551	1.40	0.17	2.10	75–88	Benthic	9.2 ± 0.7	6.7 ± 0.3	99.3 ± 1.4	56.8 ± 3.2	9.4 ± 0.2	3.7 ± 1.63	1.25 ± 0.50
						Hyporheic	9.5 ± 0.7	6.4 ± 0.2	56.2 ± 10.8	58.3 ± 3.4	10.9 ± 1.4	2.79 ± 1.26	0.96 ± 0.43
Bergnassonne	700	2.20	0.13	3.40	79–122	Benthic	7.6 ± 0.1	6.3 ± 0.3	98.3 ± 1.7	35.2 ± 1.2	3.8 ± 0.7	2.01 ± 0.04	1.13 ± 0.35
						Hyporheic	8.2 ± 0.2	6.2 ± 0.3	69.3 ± 21.1	56.9 ± 12.1	14.7 ± 7.2	8.01 ± 5.38	0.96 ± 0.51
Orbiel	780	2.20	0.22	3.20	108–166	Benthic	6.2 ± 0.6	6.4 ± 0.3	99.3 ± 2.7	28.8 ± 2.9	3.3 ± 0.5	2.90 ± 1.10	0.71 ± 0.37
						Hyporheic	7.6 ± 0.6	5.8 ± 0.1	61.2 ± 12.4	32.6 ± 2.8	5.3 ± 1.0	5.82 ± 3.64	0.56 ± 0.34

[a] Values are means ± SE, $n = 6$ per stream and per compartment. m a.s.l., meters above sea level; sat., saturation.

Western Ghats of India. Furthermore, studies by Field and Webster (31) demonstrated that some aquatic hyphomycete species are able to survive and to grow under anaerobic conditions, which suggests a species-specific sensitivity of aquatic hyphomycetes to hypoxic conditions.

Consequently, physical and chemical constraints should influence aquatic hyphomycete dynamics in hyporheic sediments. The aim of the present study was to determine how the hyporheic habitat can filter out unsuccessful strategists from the pool of benthic aquatic hyphomycete species and thereby control fungal community composition within sediment. For this purpose, the abundance and identity of aquatic hyphomycete spores circulating in interstitial water were examined within the hyporheic zone of different streams and compared to the spore pool in surface water. This field approach was coupled with laboratory experiments to quantify the influences of sedimentary and chemical (e.g., DO concentration) conditions on spore production and transport in the hyporheic zone. Because the circulation of spores in the hyporheic zone may depend both on their intrinsic dispersal ability (i.e., ability to cope with physical filtering) and on the intensity of endogenous spore production (i.e., ability to cope with physiological filtering), we addressed these two potential filtering mechanisms in dedicated microcosms. The following hypotheses have been tested: (i) the density and diversity of aquatic hyphomycete spores should be reduced in the hyporheic zone in comparison with the surface water; (ii) the dispersal ability of spores through the sedimentary matrix should be influenced both by sediment grain size and by spore traits; (iii) the ability of aquatic hyphomycetes to develop and sporulate within the hyporheic zone should be reduced compared to that observed in benthic habitats and should differ among species.

MATERIALS AND METHODS

A first study was carried out in the field to compare the abundance and diversity of aquatic hyphomycete spores in streams between benthic and hyporheic zones. Two others were designed in laboratory microcosms (i.e., slow-filtration column and stream-simulating microcosm, both designed to simulate the physical and chemical conditions occurring in the hyporheic and benthic zones, respectively) to evaluate (i) the spore dispersal efficiency of several aquatic hyphomycete species in contrasted sedimentary habitats and (ii) the influence of hyporheic physicochemistry on spore production by aquatic hyphomycetes. These three studies were based on the quantification of spore production and/or transport.

Field assessment of aquatic hyphomycete spores' abundance and diversity. (i) Study sites. The experiment was carried out in the Montagne Noire, southwestern France, a 1,450-km^2 region covered by a mixed broadleaf forest with an altitudinal range of 250 to 1,211 m above sea level (m a.s.l.). Two first-order (Béal and Bergnassonne) and one second-order (Orbiel) permanent streams with similar physical and chemical characteristics were selected in forested areas, situated between 02°09′E and 02°20′E longitude and 43°21′N and 43°27′N latitude (8). In all catchments, forestry was the only anthropogenic disturbance, although limited within the study area (32).

(ii) Stream characterization. Physical and chemical characteristics of the three streams are detailed in Table 1. Briefly, the streams had very similar geomorphologic characteristics, especially in terms of water depth and channel width (more details can be found in reference 8). Physical and chemical conditions in the hyporheic zone (i.e., at 15 cm below the sediment surface; interstitial water was pumped from a specific sampling device using a hand-held vacuum pump—see below; more details, as well as additional information regarding measurement protocols, can be found in reference 8) were rather comparable to those at the surface level, except for DO concentration, which was substantially lower in the hyporheic zone.

(iii) Construction and placement of sampling devices. Fifty-four cylinders of polyvinyl chloride (PVC; 10-cm length, 12-mm inner diameter) were cut and pierced (50 holes with a diameter of 2.5 mm) and capped at the bottom with a plastic stopper. A length of plastic tubing (5-mm inner diameter; Tygon) was hermetically sealed at the top of each cylinder to allow sampling of interstitial water for spores and water chemistry and kept isolated from surface water with a clamp. We selected three riffles per stream. At the head of each riffle, four cylinders were carefully introduced in the stream sediment to a 20-cm depth (see details in reference 33). One of them was used to determine water chemistry at two sampling dates (i.e., 25 November 2009 and 21 March 2010), and the three others were used to characterize circulating spore assemblages in late autumn.

(iv) Spore sampling. Aquatic hyphomycete spores were collected from the benthic (water column) and hyporheic (interstitial water) zones of the riffles studied. Three replicate samples per riffle and per zone (i.e., benthic and hyporheic) were taken in late autumn 2009. Spores were collected by filtering 250 ml of stream water through a membrane filter (5-μm pore size, 47-mm diameter; Millipore Corporation, Bedford, MA, USA). The filters were fixed and stained with a 60% lactic acid and 0.1% trypan blue solution (18). All filters were examined microscopically at a magnification of ×200, and spores present were counted and identified to the species level (34, 35).

Spore dispersal efficiency across the sediment. (i) Preparation of spore suspensions. To encompass the variety of size and shape in spores produced by aquatic hyphomycetes, seven species from fungal assemblages reported in studied streams (Table 2, showing data from the field experiment) were selected. Strains were isolated from neighboring streams of the Montagne noire and the Pyrénées (southwestern France): *Articulospora tetracladia* Ingold (tetraradiate conidia, arms up to 70 μm long), *Flagellospora curvula* Ingold (sigmoid conidia, up to 120 μm long and 2 μm wide), *Heliscus lugdunensis* Saccardo & Thérry (subclavate or clove-shaped conidia, up to 40 μm long and 5 μm wide), *Lemonniera*

TABLE 2 Relative abundance of the aquatic hyphomycete species in the benthic and hyporheic zones of the three streams[a]

Species	Relative abundance in:					
	Benthic zone			Hyporheic zone		
	Bergnassonne	Béal	Orbiel	Bergnassonne	Béal	Orbiel
Alatospora acuminata Ingold	5.58 ± 0.49	2.33 ± 0.40	3.16 ± 0.47	1.45 ± 0.91	2.45 ± 1.85	1.90 ± 1.19
Alatospora constricta Dyko	3.57 ± 0.18	2.00 ± 0.30	2.37 ± 0.39	0.61 ± 0.27	0.07 ± 0.05	0.54 ± 0.37
Alatospora flagellata (Gönczöl) Marvanová	0.04 ± 0.03		0.02 ± 0.02			
Anguillospora crassa Ingold	0.25 ± 0.14	0.03 ± 0.03	0.31 ± 0.06	1.66 ± 0.84		5.62 ± 3.34
Anguillospora filiformis Greathead	0.39 ± 0.13	11.26 ± 1.15	0.80 ± 0.16	4.15 ± 2.20	6.65 ± 3.42	1.62 ± 1.25
Anguillospora furtiva Webster & Descals		0.04 ± 0.04				
Anguillospora longissima (Sacc. & Syd.) Ingold	0.86 ± 0.05	1.48 ± 0.18	1.06 ± 0.16	2.70 ± 2.20	6.57 ± 2.81	1.24 ± 0.71
Articulospora tetracladia Ingold	3.27 ± 0.30	1.76 ± 0.22	0.78 ± 0.14	0.39 ± 0.29		0.19 ± 0.19
Casaresia sphagnorum Gonz. Fragoso						0.03 ± 0.03
Clavariopsis aquatica De Wild.	0.49 ± 0.10	0.60 ± 0.19	0.12 ± 0.03	0.07 ± 0.07		
Clavatospora longibrachiata (Ingold) Marvanová & Nilsson	5.41 ± 0.52	3.00 ± 0.27	1.65 ± 0.20	0.52 ± 0.29	0.26 ± 0.20	1.73 ± 0.86
Crucella subtilis Marvanová & Suberkropp	2.82 ± 0.44	0.96 ± 0.16	2.63 ± 0.31	2.58 ± 2.19		2.09 ± 1.25
Culicidospora aquatica Petersen	0.10 ± 0.04	0.06 ± 0.04	0.10 ± 0.04			
Dendrospora erecta Ingold	0.02 ± 0.02	0.04 ± 0.04		0.11 ± 0.11		
Flagellospora curvula Ingold	22.99 ± 1.08	36.49 ± 1.73	9.16 ± 0.77	47.04 ± 9.83	43.73 ± 11.13	21.78 ± 8.80
Fontanospora alternibrachiata Dyko		0.37 ± 0.09	0.19 ± 0.03		0.07 ± 0.05	
Goniopila monticola (Dyko) Marvanová & Descals	0.02 ± 0.02	0.29 ± 0.12	0.07 ± 0.03		0.43 ± 0.34	
Heliscella stellata (Ingold & Cox) Marvanová & Nilsson	0.61 ± 0.20	0.20 ± 0.06	0.11 ± 0.04			
Heliscina campanulata Marvanová						0.18 ± 0.18
Heliscus lugdunensis Sacc. & Thérry	2.73 ± 0.13	0.93 ± 0.11	1.43 ± 0.18	0.29 ± 0.24		0.57 ± 0.41
Lateriramulosa uni-inflata Matsushima	0.15 ± 0.05					
Lemonniera aquatica De Wild.		0.72 ± 0.13				
Lemonniera cornuta Ranzoni		0.06 ± 0.06				
Lemonniera terrestris Tubaki	0.04 ± 0.04	3.48 ± 0.54			1.05 ± 0.82	
Lunulospora curvula Ingold	5.96 ± 0.29	0.81 ± 0.15		4.09 ± 1.68	0.33 ± 0.25	
Mycocentrospora sp. 1 cf. *angulatum* R.H. Petersen	4.46 ± 0.35	1.03 ± 0.12	1.55 ± 0.18	0.82 ± 0.47	0.63 ± 0.35	0.06 ± 0.06
Mycocentrospora sp. 2	0.10 ± 0.07	0.28 ± 0.09	0.03 ± 0.03		0.07 ± 0.05	
Mycofalcella calcarata Marvanová, Om-Kalth. & Webster	0.02 ± 0.02	0.15 ± 0.04	0.10 ± 0.04			
Stenocladiella neglecta (Marvanová & Descals) Marvanová & Descals		0.14 ± 0.08				
Taeniospora gracilis Marvanová	35.91 ± 1.02	29.97 ± 2.31	74.12 ± 1.08	33.21 ± 6.86	34.99 ± 10.53	52.07 ± 10.07
Tetrachaetum elegans Ingold	2.39 ± 0.22	0.48 ± 0.10	0.06 ± 0.02	0.29 ± 0.22	2.51 ± 1.84	
Tetracladium marchalianum De Wild.		0.18 ± 0.08				
Tricelophorus monosporus Ingold		0.20 ± 0.06				
Tricladium chaetocladium Ingold	1.66 ± 0.21	0.60 ± 0.12	0.17 ± 0.04		0.20 ± 0.15	0.12 ± 0.12
Tricladium splendens Ingold		0.05 ± 0.03	0.01 ± 0.01			
Tumularia tuberculata (Gönczöl) Descals & Marvanová	0.13 ± 0.05		0.02 ± 0.02			
Varicosporium elodeae Kegel	0.02 ± 0.02	0.04 ± 0.04				
Unknown filiform, ≤60 μm						9.03 ± 9.03
Unknown tetraradiate						1.23 ± 1.23
Total no. of species	27	32	24	16	15	17

[a] Values are percentages ± SD.

terrestris Tubaki (tetraradiate conidia, conoid or obclavate arms, up to 45 μm long), *Tetrachaetum elegans* Ingold (tetraradiate conidia, arms up to 150 μm long), *Tetracladium marchalianum* De Wild. (tetraradiate conidia with 2 globose elements, arms up to 60 μm long), and *Tricladium chaetocladium* Ingold (tetraradiate conidia, arms up to 120 μm long) (34, 35). Growing colonies were kept at 15°C in petri dishes with 10 ml of 2% malt agar. Sporulation of aquatic hyphomycetes was induced by incubation of agar plugs of 7- to 14-day-old colonies in stream-simulating microcosms, as described in reference 16. Each microcosm consisted of a 50-ml glass chamber connected from the bottom to an aeration tube, which provides a continuous cotton-filtered airflow (100 ml min^{-1}) and creates turbulence, keeping the agar plugs in permanent agitation. A tap at the bottom allowed for the aseptic drainage of the chamber and recovery of the spore suspension. Fresh mineral salt solution (40 ml) consisting of 100 mg $CaCl_2 \cdot 2H_2O$, 10 mg $MgSO_4 \cdot 7H_2O$, 10 mg KNO_3, 0.055 mg K_2HPO_4, and 0.5 g MOPS (3-morpholinopropanesulfonic acid) buffer in 1 liter of

water (pH adjusted to 7.0) was added to microcosms through the open top, which was otherwise closed with a glass lid. The microcosms and mineral salt solution were autoclaved before inoculation.

The incubation was performed in a constant-temperature room at 15 ± 0.5°C in the dark. Spores produced after 24 to 48 h were used in experiments. An aliquot of each specific spore suspension was used to prepare multiple- or single-species spore suspensions to inoculate each slow-filtration column (see below). Owing to differences in spore production rates between aquatic hyphomycete species, the initial densities of spores were different.

(ii) Microcosms for the study of spore dispersal through sediment. Experiments were carried out in slow-filtration columns designed to simulate the physical and chemical conditions occurring in water-saturated sediments of the hyporheic zone of gravel-dominated streams, similar to those described in references 36, 37, and 38. Each column was 40 cm high and 10 cm in diameter. To investigate the influence of sediment physical characteristics on the dispersal efficiency of aquatic hyphomycete spores, we tested three ranges of grain size, 4 to 8 mm (S1), 2 to 4 mm (S2) and 1 to 2 mm (S3). These grain sizes were in the range of streambed sediments of the three streams studied in a previous phase (see details in reference 8). For each range of grain size (S1, S2, or S3), experimental columns were filled to a height of 30 cm with the corresponding sediment. A water column (10 cm) was left at the surface of the sediment to simulate stream overlying water. After sediment installation, the columns were supplied with the same mineral salt solution as that used to induce spore production (see "Preparation of spore suspensions" above) using a peristaltic pump. Interstitial water velocity was fixed at 0.5×10^{-3} m s^{-1} in columns, in accordance with reported values in the hyporheic zone of streams (38, 39).

(iii) Experiments on single- and multiple-species spore suspensions. In a first experiment, to assess the dispersal efficiency of aquatic hyphomycete spores with extreme shapes and sizes within the interstitial pores of sediment and to suppress possible interspecific interactions, slow-filtration columns were inoculated with single-species spore suspensions from four aquatic hyphomycete species. For this purpose, ca. 8,000 spores for *F. curvula*, 11,000 for *A. tetracladia*, 1,500 for *H. lugdunensis*, or 6,500 for *T. elegans* were used. Three replicated columns were used per aquatic hyphomycete species.

In a second experiment, to assess the dispersal efficiency of aquatic hyphomycete spores within the interstitial pores of sediment and taking into account the possible species interactions, the same protocol as the previous one was followed, except that slow-filtration columns were inoculated simultaneously with spores of seven aquatic hyphomycete species. Each of three replicate microcosms received ca. 39,000 spores divided as follows: 25,000 spores for *F. curvula*, 1,500 for *A. tetracladia*, 3,500 for *H. lugdunensis*, 2,500 for *T. elegans*, 2,000 for *L. terrestris*, 1,000 for *T. marchalianum*, and 3,500 for *T. chaetocladium*.

In these two experiments, the spore suspensions were introduced as a single pulse from the top of each of nine slow-filtration columns. Aquatic hyphomycete spores were counted in samples collected at the column outlets at 5, 15, 25, 35, 45, 55, 65, 80, 95, 150, and 270 min after the introduction of spore suspensions. For this purpose, 40 ml of water was collected and the spore suspensions were stored in 50-ml centrifuge tubes and preserved with 2 ml of 37% formalin. A 0.5-ml solution of Triton X-100 (0.5% in distilled water) was added to spore suspensions, and the resulting suspensions were gently stirred to ensure uniform distribution of spores. A 10-ml aliquot was then filtered through a membrane filter (5-μm pore size, 25-mm diameter; Millipore Corporation), and spores were counted and identified (see "Spore sampling" above).

For each grain size treatment, the dispersal efficiency of aquatic hyphomycete spores was calculated as the cumulative number of spores (expressed as the percentage of spores introduced in the inoculum).

Influence of water physicochemical conditions on spore production. (i) Stream inoculation. Alder leaves were conditioned in fine (1-mm) nylon mesh bags, 12 by 12 cm, immersed in the Montaud stream on 22 February 2008 (see details of the stream in reference 40). Leaves were conditioned in the stream for 17 days, a time sufficient for effective microbial colonization (41).

(ii) Microcosms, medium, and experimental setup. Conditioned leaves were individually rinsed with stream water and cut into discs (15-mm diameter) with a cork borer. Three batches of 15 alder leaf discs were placed in stream-simulating microcosms with 40 ml of a mineral salt solution (see "Preparation of spore suspensions" above). Other randomly chosen batches of 15 alder leaf discs were enclosed in circular fine-mesh bags (9.6-cm diameter, 1-mm mesh size) and were placed either at the sediment surface or at 10 or 30 cm below the sediment surface in slow-filtration columns (50-cm height, 10-cm diameter, $n = 3$). Mesh bags with leaf discs were inserted into columns during their filling with a 40-cm height of gravel (4 to 10 mm) following a procedure similar to that used previously (see "Microcosms for the study of spore dispersal through sediment" above). Interstitial water velocity was fixed at 3.48 cm per hour in columns, in accordance with reported values in the hyporheic zone of streams (42, 43). Supplied water was aerated to maintain DO concentrations of 9 ± 0.5 mg $liter^{-1}$ at the inlet of the columns throughout the experiment. The experiments in stream-simulating microcosms and slow-filtration columns were performed at a constant temperature of 15 ± 0.5°C and in the dark to suppress photoautotrophic processes for the whole duration of the experiment (57 and 56 days, respectively).

(iii) Sampling and assessment of relative spore production by aquatic hyphomycete species. Every 2 days, during the renewal of mineral salt solution in stream-simulating microcosms, suspensions of spores released from leaf discs were collected. A 45-ml volume of spore suspensions was transferred into a 50-ml centrifuge tube and fixed with 2 ml of 37% formalin.

Every 4 days, water circulation in the slow-filtration columns was shunted at each treatment depth, just below the leaf litter fine-mesh bags to collect 45 ml of water into a 50-ml centrifuge tube, and preserved with 2 ml of 37% formalin. The spore suspensions of each microcosm, resulting from the multiple changes of the medium (stream-simulating microcosm) or from the multiple spore suspension samplings (slow-filtration column), were combined, and then spores were counted and identified (see "Spore sampling" above).

(iv) Dissolved O_2 measurement in slow-filtration columns. DO concentrations were measured every 4 days in the slow-filtration columns to characterize the influence of O_2 availability on spore production. Similarly to spore collection, water circulation in the slow-filtration columns was shunted at each treatment depth by openings situated just below and above the litter bags. Measurements were also done at the entrance and exit of the columns to characterize the vertical profiles of O_2 in hyporheic sediments.

An oxygen microsensor probe (Unisense minisensor OX500 mounted in T-piece [with a flowthrough cell]) plugged into a picoammeter (model Unisense PA2000) was connected to the water derivation to measure dissolved O_2 without contact with atmospheric oxygen.

Statistical analyses. (i) Field assessment of aquatic hyphomycete spores' abundance and diversity. Simpson's diversity and dominance indices were computed for spore assemblages associated with each of the two zones (i.e., benthic and hyporheic) for the three streams. Analysis of variance (ANOVA) was used to test for the effects of the zone, the riffle, and the stream on spore density, aquatic hyphomycete species richness, and Simpson's diversity and dominance indices. We used a nested design with stream, riffle nested in streams, and zone as main effects.

As the total number of spores varied largely across samples, and particularly between hyporheic and benthic ones, it was necessary to correct the species richness observed to account for a possible "sampling effect" (44, 45). This was done using a Monte Carlo procedure consisting in computing spore subsamples with an equal size. For this purpose, the data corresponding to all the samples from the same zone and from the same stream were pooled. From each of these pools (containing between 277 and 6,806 spores), 200 spores were randomly selected, and the number of

species within these subgroups were determined. This procedure was repeated 999 times to be able to calculate an average ± standard deviation (SD) of the species richness for each zone in each stream. The significance of the differences in fungal species richness between the benthic and hyporheic zones was tested using Student's *t* test on paired samples.

(ii) Spore dispersal efficiency across the sediment. In the single-species test, the relation between spore dispersal efficiency and sediment grain size was tested using a two-way ANOVA with aquatic hyphomycete species identity (conditioning spore shape and size) and sediment grain sizes as main effects, followed by Tukey's honestly significant difference (HSD) tests for *post hoc* pairwise comparisons.

Owing to the nonhomogeneity of variances when studying the dispersal efficiency of spores with the multiple-species inoculum, the nonparametric Kruskal-Wallis test was used to examine the effect of sediment grain size on spore dispersal efficiency. In addition, the nonindependence among treatments (i.e., aquatic hyphomycete species) required the use of the Friedman test to compare the dispersal efficiency among species for the three sediment grain sizes. Normality and homoscedasticity were tested using Shapiro-Wilk's and Levene's tests, respectively.

Possible relationships between the spore dispersal ability and spore traits (i.e., biovolume and minimal obstruction size) were investigated using Spearman's correlation tests. Biovolume and minimal obstruction size were calculated for each species from spore descriptions obtained from the literature (34).

(iii) Influences of water physicochemical conditions on spore production. The similarities in the species composition of aquatic hyphomycete spore outputs under the various exposition treatments were measured with the Steinhaus index (46). Standard deviations for these indices were computed using a bootstrap procedure ["boot()" function from the "boot" package in R; 1,000 bootstrap replicates]. Differences in DO concentrations among depths in slow-filtration columns were analyzed using one-way repeated-measures analysis of variance (RM-ANOVA) with depth as main factor and time ($n = 15$) as repeated factor. Because the sphericity assumption (tested with Mauchly's test) was met, no correction was applied. When significant differences in DO concentrations were detected between depths, Tukey's HSD tests were then carried out for *post hoc* pairwise comparisons.

Statistica 6.0 (47) was used for all statistical analyses. Differences were considered significant when *P* values were <0.05. Monte Carlo and bootstrap procedures were performed using R software 2.15.2 (48).

RESULTS

Field assessment of aquatic hyphomycete spores' abundance and diversity. Spore densities were similar among riffles within streams (nested ANOVA, $F_{6,44} = 0.55$, $P = 0.77$) but differed between streams, with similar total spore densities measured in the Bergnassonne and the Béal contrasting with higher values measured in the Orbiel (nested ANOVA, $F_{2,6} = 18.71$, $P < 10^{-2}$). Spores were significantly less abundant in hyporheic water than in surface water (nested ANOVA, $F_{1,44} = 183.05$, $P < 10^{-3}$). Spore densities averaged 2,794 liter^{-1} (range, 2,222 to 3,680) in the benthic zone and 404 liter^{-1} (range, 185 to 617) in the hyporheic zone. The average spore density in the hyporheic zone was thus almost 7-fold lower than in the surface water (Fig. 1a).

A total of 39 aquatic hyphomycete species were recorded from the three streams and the two zones (Table 2). There was no difference in species richness among streams and among riffles within streams (nested ANOVAs, $F_{2,6} = 2.32$ and $P = 0.19$ for streams and $F_{6,44} = 0.53$ and $P = 0.78$ for riffles). The number of aquatic hyphomycete species from the benthic zone was significantly higher than the hyporheic one (Student's *t* test on paired samples, $t = 7.82$, $P = 0.016$ [Fig. 1b]). The Simpson dominance index slightly differed among streams (nested ANOVA, $F_{2,6} = 6.87$, $P = 0.03$) but not among riffles within streams (nested ANOVA, $F_{6,44} = 1.53$, $P = 0.19$). The Simpson diversity index was significantly higher in the benthic than in the hyporheic zone (nested ANOVA, $F_{1,44} = 19.52$, $P < 10^{-3}$), denoting lower evenness in the spores circulating within sediment (Fig. 1c). The fungal community was dominated by two species (*Taeniospora gracilis* and *F. curvula*), which accounted for 59 to 83% of the total spore production in the benthic zone and 74 to 80% in the hyporheic zone. Although the other species often individually accounted for less than 5% of the total spore densities, some differences in relative abundances of aquatic hyphomycete species between the two zones were observed. Spores of *Alatospora acuminata*, *Alatospora constricta*, *A. tetracladia*, *Clavatospora longibrachiata*, *H. lugdunensis*, *Lunulospora curvula*, *Mycocentrospora* sp. 1 and *T. chaetocladium* (either tetraradiate/branched or arched filiform/sigmoid) were more abundant in the surface water than in the interstitial one. In contrast, the relative abundance of scolecoid or filiform spores from species such as *Anguillospora crassa*, *Anguillospora filiformis*, *F. curvula*, and an undetermined filiform spore (with a length of ≤60 μm) was increased in the interstitial water compared to the surface water.

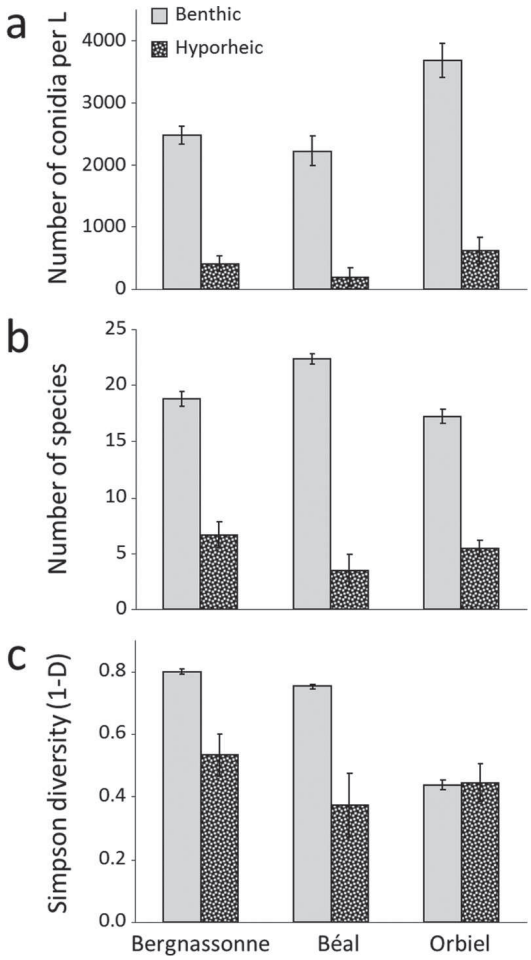

FIG 1 Mean spore density (a), species richness (b), and Simpson diversity index (c) of the fungal communities in the benthic and hyporheic zones of the Bergnassonne, Béal, and Orbiel. Bars denote ± 1 standard error (SE).

Spore dispersal efficiency across the sediment. In the single-species experiment, the average dispersal efficiency measured dur-

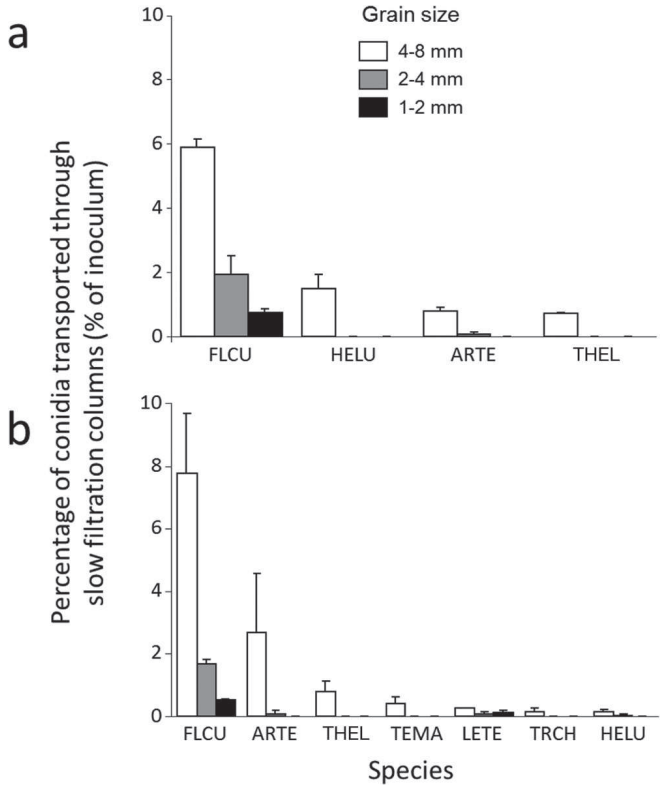

FIG 2 Cumulative number of spores from aquatic hyphomycete single-species (a) or multiple-species (b) spore suspensions, recovered after 270 min at the outlet of slow-filtration columns for different sediment grain sizes (mean ± 1 SE; $n = 3$). FLCU, *Flagellospora curvula*; ARTE, *Articulospora tetracladia*; THEL, *Tetrachaetum elegans*; TEMA, *Tetracladium marchalianum*; LETE, *Lemonniera terrestris*; TRCH, *Tricladium chaetocladium*; HELU, *Heliscus lugdunensis*.

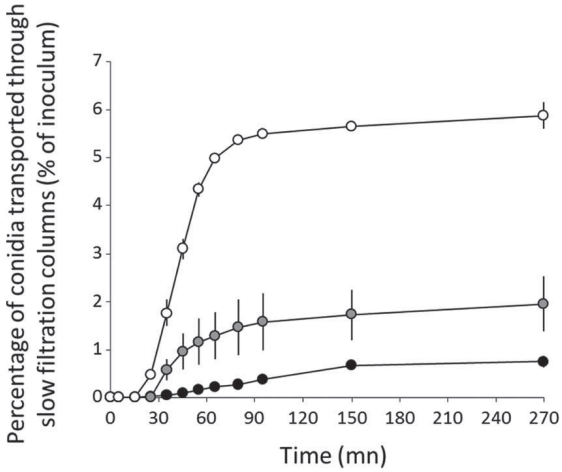

FIG 3 Cumulative number of spores from *Flagellospora curvula* monospecific spore suspensions recovered at the outlet of slow-filtration columns, for different sediment grain sizes and as a function of time (mean ± 1 SE; $n = 3$). Grain size is denoted by color: white, 4 to 8 mm; gray, 2 to 4 mm; black, 1 to 2 mm.

ing the experiment was significantly influenced by sediment grain size (ANOVA, $F_{2,24} = 156.44$, $P < 10^{-6}$) and species identity (ANOVA, $F_{3,24} = 118.06$, $P < 10^{-6}$) (Fig. 2a). The influence of spore identity on the dispersal efficiency depended on sediment characteristics, as indicated by the significant interaction between grain size and species identity (ANOVA, $F_{6,24} = 4.18$, $P < 10^{-2}$). The dispersal efficiency of *F. curvula*, an aquatic hyphomycete species producing filiform/sigmoid spores, was always significantly higher than that of the three other species with compact and branched/tetraradiate spores, whatever the sediment grain size (*post hoc* HSD tests, $P < 10^{-3}$).

In the coarsest sediment, dispersal efficiency varied greatly among the four fungal species, with a total dispersal efficiency of 5.88% ± 0.47% (mean ± SD) for the small filiform/sigmoid spore of *F. curvula*, 1.48% ± 0.80% for the compact spore of *H. lugdunensis*, 0.81% ± 0.21% for the small tetraradiate spore of *A. tetracladia* and 0.71% ± 0.11 for the large tetraradiate spore of *T. elegans*. As a consequence, the dispersal efficiency of *F. curvula* was 4-, 7-, and 8-fold higher than those of *H. lugdunensis*, *A. tetracladia*, and *T. elegans*, respectively. *F. curvula* spores were able to disperse in all sediment treatments (Fig. 3). In contrast, the three other species did not disperse within the finest sediment, and from these species, only *A. tetracladia* was able to disperse within the medium-size sediment (Fig. 2a). From data obtained in the single-species experiment, Spearman correlation analyses between the two morphological traits of spores and their dispersal efficiency revealed a significant relationship for minimal obstruction size of spores ($r = -0.78$; $P < 10^{-2}$) but not for spore biovolume ($r = -0.50$; $P = 0.10$).

In the multiple-species test, dispersal efficiency was also influenced by sediment grain size (Kruskal-Wallis test, $H = 7.2$, df = 2, $P < 0.05$). The proportion of spores able to disperse within the sediment dramatically decreased with decreasing grain size, thereby reducing interstitial voids (Fig. 2b). As a consequence, the number of spores passing the sediment matrix was reduced by almost 85% between the intermediate and the coarsest grain size and by almost 95% between the coarsest and the finest one.

In addition, highly significant differences existed among aquatic hyphomycete species (Friedman tests, $Q = 33.19$, df = 6, $P < 10^{-4}$), indicating that spore features greatly influenced dispersal efficiency whatever the sediment grain size (Fig. 2b). These results were consistent with those from the single-species experiment, showing large differences in dispersal efficiency among aquatic hyphomycete species.

Three aquatic hyphomycetes of the seven species tested showed differences in their ability to disperse along the gradient of sediment grain size (by the Kruskal-Wallis test; df = 2; for *F. curvula*, $H = 7.2$, $P < 0.05$; for *A. tetracladia*, $H = 6.7$, $P < 0.05$; for *T. elegans*, $H = 7.6$, $P < 0.05$; for *T. marchalianum*, $H = 4.5$, $P = 0.10$; for *L. terrestris*, $H = 5.7$, $P = 0.06$; for *T. chaetocladium*, $H = 4.5$, $P = 0.10$; and for *H. lugdunensis*, $H = 3.3$, $P = 0.20$). It is worth noting that *H. lugdunensis* exhibited a higher dispersal rate than *A. tetracladia* and *T. elegans* in the single-species experiment but the opposite result was obtained in the multiple-species experiment.

The relationships between dispersal efficiency and spore traits obtained in the experiment with the multiple-species inoculum were consistent with those measured in the experiment with single-species inocula, although less pronounced (dispersal efficiency versus minimal obstruction size of spore: $r = -0.44$; $P < 0.05$; dispersal efficiency versus spore biovolume: $r = -0.17$; $P = 0.46$).

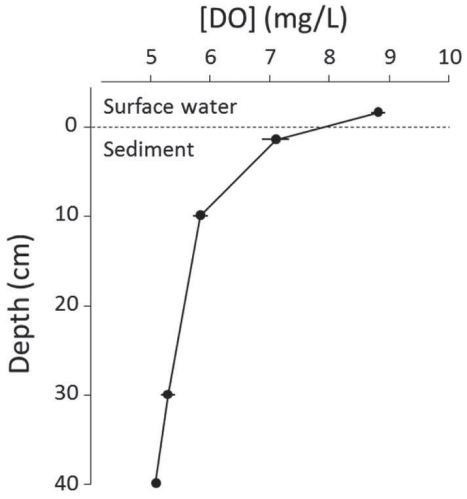

FIG 4 Vertical distribution of dissolved O_2 concentration in water circulating in the slow-filtration columns (mean ± 1 SE).

Influences of water physicochemical conditions on spore production. In the stream-simulating microcosms, the constant water agitation kept DO concentrations at saturation throughout the experiment. In the slow-filtration columns, DO concentrations presented a sharp drop with depth (Fig. 4), from 8.8 ± 0.1 mg liter^{-1} at the sediment surface to 5.1 ± 0.1 mg liter^{-1} in the deepest sediment layer (RM-ANOVA, $F_{4,10} = 114.45$, $P < 10^{-6}$; for HSD tests for comparison of DO concentrations between surface layer and other layers, $P < 10^{-3}$) (Fig. 4).

After 57 days of incubation, the mean number of fungal species associated with alder leaf discs from stream-simulating microcosms was markedly higher than that from the slow-filtration columns (i.e., sediment surface and 10 and 30 cm below the sediment surface), with 14, 7, 8, and 7 species found in the four exposure treatments, respectively (Table 3).

The species composition of spore outputs was more comparable between the three exposure treatments in slow-filtration columns (Steinhaus similarity index ranging from 0.445 to 0.736) than between any of these treatments and the stream-simulating microcosm treatment (Steinhaus similarity index ranging from 0.307 to 0.400 [Table 3]). The relative contributions of *F. curvula*, *A. tetracladia*, and *L. terrestris* to the spore production were lower in the stream-simulating microcosms than in the slow-filtration columns. The most striking differences among treatments were the disappearance of *A. acuminata*, *Clavariopsis aquatica*, *C. longibrachiata*, *Culicidospora aquatica*, and *T. chaetocladium* and the low contribution of *Anguillospora longissima* in the spore pool collected in slow-filtration columns. The spore output from slow-filtration columns was dominated by only two species (*F. curvula* and *A. tetracladia*), whereas five species were codominant in the spore output from stream-simulating microcosms (Fig. 5). The greatest difference was observed for *F. curvula*, which contributed up to 70% of the overall spore production in the slow-filtration column treatments, whereas its contribution was <17% in the stream-simulating microcosms. This strong dominance markedly increased with depth in slow-filtration columns, averaging 59%, 69%, and 81% at the sediment surface and at 10 and 30 cm below the sediment surface, respectively (Table 3).

DISCUSSION

Abundance and diversity of aquatic hyphomycetes in the hyporheic zone. Several authors have previously suggested that aquatic hyphomycetes and other fungi readily disperse within the hyporheic zone (49, 50, 51). Smith and Lake (52) observed fungal hyphae on leaf litter incubated either above or below the surface of sediment, while Krauss et al. (53) reported that sterile alder leaves incubated in groundwater wells could support an unexpectedly high fungal diversity. Surprisingly, and despite the considerable amount of plant matter buried in sediment (8), little is known about the role of aquatic hyphomycetes on the decomposition of leaf litter in the hyporheic zone of headwater streams. Some insights have been brought by Cornut et al. (24), who have recently examined the role of aquatic hyphomycetes in processing allochthonous organic matter in streambed sediments. They demonstrated that the relative contributions of fungi varied considerably depending on the location within the streambed (i.e., benthic versus hyporheic zone). Mycelial and conidial production in the hyporheic environment accounted for 12% of the initial carbon transformed after 80 days, i.e., roughly 3 times more than under benthic conditions, suggesting that the role of fungi is particularly important in the hyporheic zone. However, a significant gap persists in our knowledge concerning the pathways of vertical dispersal and colonization of new substrates by aquatic hyphomycetes into the hyporheic habitat (Fig. 6b). Colonization of leaves and other substrates by aquatic hyphomycetes may occur through various mechanisms: by direct contact as a result of hyphal outgrowth from a colonized leaf to a contiguous leaf or at distance by either detached hyphal fragments (54, 55) or spores (56) landing on a leaf surface. The last mechanism is predominant for the colonization of distant leaf patches (23). Bärlocher et al. (50) gave support to the hypothesis suggesting that spores or other fungal propagules move relatively easily through the streambed sediments and establish colonies on the substrates they encounter. However, this hypothesis remained to be tested.

Our results indicated that the spore assemblages found in the interstitial water from the hyporheic zone differed markedly from the assemblages found in the benthic zone. Spore density and

TABLE 3 Mean numbers of leaf-associated aquatic hyphomycete species, contribution of *F. curvula* spore production to the total conidial production by all species, and Steinhaus similarity index for the four exposure treatments, determined from the cumulative spore production over the whole experimental period[a]

Characteristic	SSM	SFC-surface	SFC-10	SFC-30
Total no. of species	14.00 ± 0.58	7.00 ± 0.58	8.00 ± 0.58	7.00 ± 0.58
Contribution of *F. curvula* conidial production (%)	16.50 ± 1.31	59.12 ± 3.73	68.71 ± 7.64	81.26 ± 3.91
Steinhaus similarity index				
SSM		0.400 ± 0.171	0.341 ± 0.099	0.307 ± 0.092
SFC-surface			0.610 ± 0.073	0.445 ± 0.168
SFC-10				0.736 ± 0.058
SFC-30				

[a] Values are means ± 1 SE ($n = 3$); standard deviations for the Steinhaus similarity indices have been computed using a bootstrap procedure (1,000 bootstrap replicates) and are indicated. SSM, stream-simulating microcosms; SFC-surface, slow-filtration columns at the water-sediment interface surface; SFC-10, slow-filtration columns 10 cm below the water-sediment interface; SFC-30, slow-filtration columns 30 cm below the water-sediment interface.

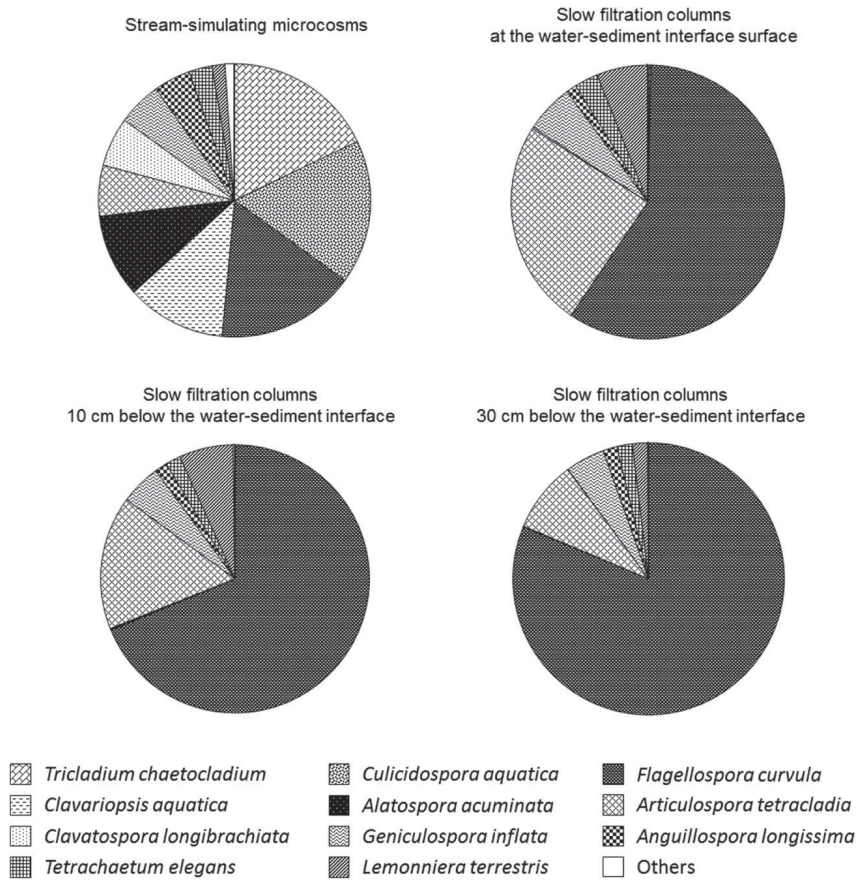

FIG 5 Relative abundance (%) of leaf-associated aquatic hyphomycete species for the four exposure treatments: stream-simulating microcosms, slow-filtration columns at the water-sediment interface surface, slow-filtration columns 10 cm below the water-sediment interface, and slow-filtration columns 30 cm below the water-sediment interface, determined from the cumulative spore production over the whole experimental period (3 replicates combined).

species richness were strongly lower in the hyporheic than in the benthic habitat for the three streams: spore density was reduced by 83 to 92%, while species richness was reduced by 65 to 85%. The higher values of Simpson dominance index observed in the hyporheic zone in comparison with those from the benthic zone suggest that some aquatic hyphomycetes species were favored, relatively to other species, by the physical and chemical conditions prevailing in the hyporheic zone.

Bärlocher et al. (50) underlined the crucial role played by the presence of suitable substrates within stream sediments, but water chemistry and especially DO availability were also predominant for controlling fungal growth (31) (Fig. 6b). The differences in the composition of spore assemblages observed in our field study between the benthic and the hyporheic zones may therefore be due both to (i) spore dispersal efficiency, likely to vary among aquatic hyphomycete species as a result of contrasting spore traits, and (ii) the physiological constraints of the hyporheic habitat, particularly the low DO supply, modulating the establishment of the different aquatic hyphomycete species and the endogenous spore production (Fig. 6b).

Spore traits and dispersal efficiency in the hyporheic zone. In our microcosm experiment, the sedimentary matrix had a strong filtering effect on aquatic hyphomycete spores. Spore dispersal ability was positively related to sediment grain size. However, only ca. 15% of the inoculum was transported through the column filled with the coarsest sediment. Our results also demonstrated significant differences among aquatic hyphomycete species, showing the great influence played by spore traits on the dispersal efficiency within stream sediment. Our first laboratory experiment using single-species inocula showed the higher dispersal efficiency of *F. curvula*, a species with sigmoid spore shape, compared with species having tetraradiate (*A. tetracladia* and *T. elegans*) or compact (*H. lugdunensis*) spore shapes. This significant influence of spore shape on dispersal ability was partly corroborated by our second laboratory experiment from the multiple-species inoculum, in which *F. curvula* outperformed species with large tetraradiate (*T. elegans* and *T. chaetocladium*), small tetraradiate (*A. tetracladia*, *T. marchalianum*, and *L. terrestris*), and compact (*H. lugdunensis*) forms. Not surprisingly, the main factor determining dispersal ability was the spore size, expressed in terms of minimal obstruction size, while there was no significant relationship between spore biovolume and dispersal efficiency. The spore minimal obstruction sizes of *F. curvula* and *H. lugdunensis* were by far the smallest of all the species. We cannot exclude that other characteristics of spores also play a decisive role in their dispersal ability, alone or in combination with spore minimal obstruction size. In the present study, we considered only two spore morphological traits, while other characteristics, such as the quantity and type of mucilage at the tip of spore arms (56, 57), the speed with which attached spores form appressoria (56), or the altera-

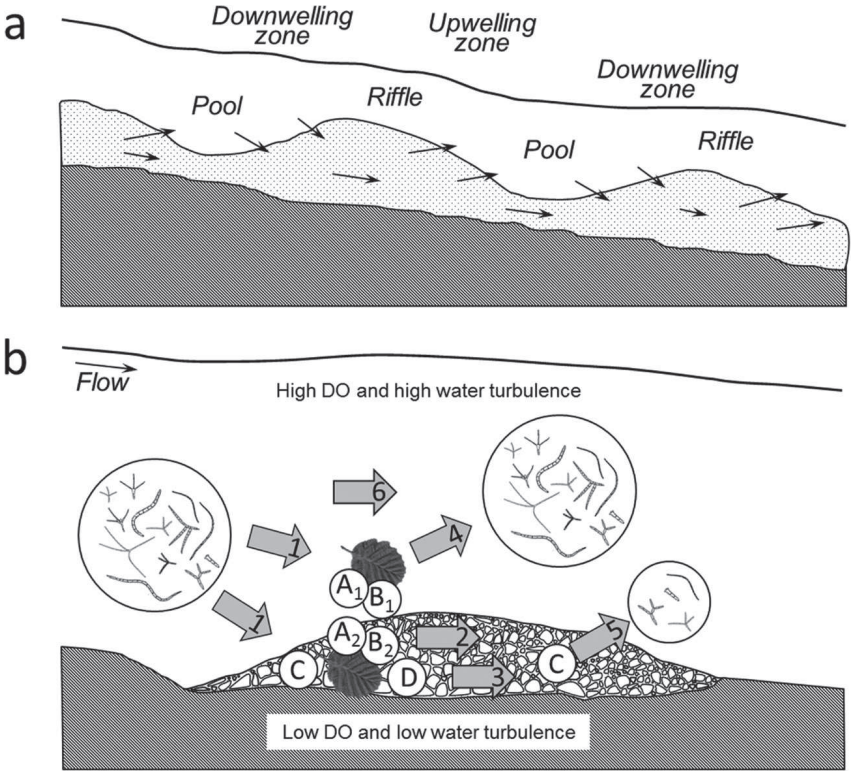

FIG 6 (a) Reach-scale surface-subsurface exchange flows. (b) Conceptual scheme of how hyporheic habitat affects spore dispersal and identity. The hyporheic zone exerts two types of selection pressure on the aquatic hyphomycete community, a physiological stress and a physical screening of the benthic spore pool, both inducing changes in the species composition. A_1, A_2, fungal colonization; B_1, B_2, spore production; C, physical screening; D, physiological stress (e.g., low dissolved O_2 concentration and low water turbulence); 1, flux of spore inoculum; 2, spore dispersal from benthic to hyporheic zone; 3, spore dispersal within the hyporheic zone; 4, release of spore; 5, spore dispersal from the hyporheic to the benthic zone; 6, spore dispersal within the benthic zone.

tion of spore shape by germination, might have negatively influenced dispersal efficiency by improving attachment to various substrates (58). Interspecific interactions among spores could also reduce dispersal, as indicated by the different hierarchy across species in terms of dispersal ability observed between experiments with single- and multiple-species inocula. It may also be the reason for the large differences in dispersal patterns of *H. lugdunensis* depending on whether it was as a single species or in mixture with other species.

Our results highlight the importance of pore size in streambed sediments, as well as spore shape, spore size, and additional properties of aquatic hyphomycete spores, in determining the dispersal efficiency of fungi within streambed sediments. The present study suggests that a decrease in the pore size in hyporheic habitats, due to physical and/or biological clogging, for instance, would impair the density and diversity of aquatic hyphomycetes.

Pore size characterization plays a major role in the prediction of the subsurface distributions of biota, chemistry, and water movements. However, pore size is not easy to define and even much less to measure (59). There are several ways by which pore size measurements can be done. The most straightforward measurements of pore size are with geometric-image-based techniques (60). Although we did not assess the pore opening features of our three types of sediment, we can use the estimations of Frost (59), who gave the pore openings' distribution within a porous medium constructed under gravity and made of monosize spheres. Accordingly, the most likely pore opening size is about $0.3 \times D$, with D being the sphere diameter with which the porous medium is built. For simplicity, it was assumed that our sediment grains fulfilled these assumptions. Therefore, considering our finest sediment grain size condition (i.e., S3 = 1 to 2 mm), we calculated that pore openings in our slow-filtration columns would reach a size of 300 μm, which fairly corresponded to the span of the largest tetraradiate spores for the species studied (i.e., >200 μm for *T. chaetocladium* and *T. elegans* [34, 35]). These pore opening size calculations seem realistic and are in accordance with our findings (Fig. 2) showing a marked trapping threshold for these species exhibiting tetraradiate spores under the finest sediment grain size conditions. In contrast, *F. curvula*, exhibiting a shorter and also simpler spore shape (i.e., filiform, 120 μm long and 2 μm wide), was relatively less trapped within the sediment.

Our results lead us to conclude that the spore shape and size are at least as important for controlling hyporheic dispersal ability of spores as other site-specific factors such as sediment characteristics (Fig. 6b).

Although spore morphology is usually less variable for terrestrial than for aquatic fungi (25), some studies have however also highlighted the significance of spore features (e.g., size, shape, and weight) as crucial components in the dispersal strategy of some fungal species in terrestrial ecosystems (61–64). Such spore traits are obviously involved in the adaptation to specific spore dispersal modes, with wind dispersal as the predominant one. The morphology of fungal spores is highly species specific, and several correlations have been found between spore characteristics, eco-

logical species traits, and species' dispersal strategy (64). For instance, the shape of a spore directly influences its aerodynamic properties: spherical spores have the ability to gain higher speed and thus better insert themselves into an object (e.g., tree bark or branches) than do narrow spores; in contrast, high impaction efficiency might not be compatible with extensive dispersal due to relative spore weight (62). Therefore, species with small spores, and more generally with spores exhibiting a high surface/volume ratio, might rather be adapted to colonize widely distributed, nonspecific resources. These examples illustrate some general tradeoffs in fungal dispersal strategies that ultimately concern both terrestrial and aquatic species.

Differential spore production among aquatic hyphomycete species in the hyporheic zone. Laboratory experiments showed that spore release by fungal assemblages was strongly affected by exposure treatments. This suggests that some aquatic hyphomycete species exhibit a better ability to develop and/or vegetatively reproduce than others under the physical and chemical conditions prevailing in the hyporheic zone (Fig. 6b). Our microcosm system, designed to mimic hyporheic conditions, reproduced a clear vertical gradient leading to a rapid decrease of DO concentrations with depth in the sediment, which was comparable with downwelling zones in streams (65–68) (Fig. 6a). The lower DO concentrations measured in the slow-filtration columns compared with concentrations measured in stream-simulating microcosms probably explained changes in fungal assemblages. DO is a fundamental factor for biological colonization and activity in the interstitial habitats because its availability determines the suitability of the environment for macroinvertebrates and the biogeochemical processes occurring in sediments (28, 69).

Although it is difficult to assess the effects of DO concentrations on the structure of fungal communities in streams due to confounding factors (e.g., nutrient availability), several studies (29, 30, 70, 71, 72) underlined the potential significance of DO availability on fungal diversity. For instance, Chergui and Pattee (70) suggested that the lower number of aquatic hyphomycete species found in a side arm of the Rhône River compared with the main channel was attributable to differences in DO concentrations. Many studies conducted in rivers also showed that a reduction of the DO concentration eliminated several aquatic hyphomycete species, resulting in impoverished communities under hypoxic conditions (30, 71, 72). All these results confirm the theory of Field and Webster (31) stating that the tolerance of aquatic fungi to hypoxic conditions in streams is due to few species growing under low-oxygenated conditions.

Medeiros et al. (29) recently reported strong changes in the structure of the fungal community associated with decomposing alder leaves exposed to low DO concentrations. They found a substantial decrease in the community evenness under hypoxic stress (i.e., 54% of O_2 saturation, equivalent to ca. 5 mg liter^{-1}), with *F. curvula* being the dominant species under such conditions. In line with these results, we demonstrated a marked decrease in fungal community evenness in our study, both from alder leaf-discs incubated in the slow-filtration columns and from leaves exposed in the interstitial water of the three studied streams. *F. curvula* was also one of the two dominant species associated with alder leaves incubated under low DO concentrations (i.e., 5.3 mg liter^{-1}) in our slow-filtration columns (i.e., relative contribution, 81%) and in the hyporheic habitat of the three streams (i.e., average abundance for the 3 streams, 38%). These findings show that some species respond differently to DO depletion, confirming the assumption of Field and Webster (31).

In parallel, an examination of the composition of leaf-associated aquatic hyphomycete species for the slow-filtration columns at the water-sediment interface (Fig. 5) reveals that DO concentrations may only partly explain the observed differences between the benthic and hyporheic habitats. Indeed, not only DO concentration but also the related physical parameters such as water turbulence may explain the observed patterns as supported by experimental data from references 73, 74, and 75 (Fig. 6b). For instance, Webster and Towfik (73) found that sporulation could be considerably enhanced by mechanical agitation or by forced aeration. The findings from the present study are likely a manifestation of the same effect. We suspect the reduced water turbulence in the slow-filtration columns (i.e., in the layer of water left above the sediment surface as well as in the interstitial water) compared to that in the stream-simulating microcosms to be partly responsible for the observed reduction of fungal diversity. Therefore, higher water turbulence would promote a higher diversity of sporulating species due to stimulation of additional pools of fungal species. Practically, water turbulence might affect the sporulation process via a greater density of conidiophore produced or more branched conidiophores, thus susceptible to release more spores, than under conditions of low agitation (73).

Sanders and Webster (75) distinguished two groups of aquatic hyphomycete species based on their sporulation response to water turbulence, with the first one being able to release spores at null flow rate without enhancement by increased flow, and the second one exhibiting a sporulation rate that is very low at null flow rate but enhanced as water flow increases.

Although sporulation responses for most identified aquatic hyphomycete species in this study were consistent with the suggestions made by Sanders and Webster (75), some species such as *A. longissima*, *A. tetracladia*, *L. terrestris*, and *T. elegans* did not fit into any of these groups. These patterns are more likely due to the converging effects of DO concentrations and water turbulence, which led to drastic reduction in fungal diversity and unusual sporulation responses. Environmental filtering of aquatic hyphomycete species assemblages through DO concentration and water turbulence may therefore act synergistically (Fig. 6b), even though individual responses, perhaps exacerbated by interspecific interactions, differed among species.

Overview. Aquatic hyphomycetes are key mediators in energy and nutrient transfers to higher trophic levels in woodland stream ecosystems. Although critical to an overall assessment of their ecological role, our understanding of the processes that structure aquatic hyphomycete communities within the hyporheic habitat remained very fragmentary. Our findings from field and laboratory experiments consistently showed that the density and diversity of aquatic hyphomycete spores were reduced in hyporheic compared to surface waters. The manipulation of sediment properties and hyphomycete spore assemblages highlighted the importance of sediment pore opening size, as well as aquatic hyphomycete spore morphology, in determining the fungal dispersal ability within streambed sediments. In addition to this physical-biological interaction modulating their dispersal efficiency, some aquatic hyphomycete species exhibited a better ability to develop and/or vegetatively reproduce, relatively to others, under the physical and chemical conditions prevailing in the hyporheic zone. Not only DO concentration, but also related physical parameters such as

water turbulence, may explain the observed drastic reduction in fungal diversity and sporulation rates. Based on the outcomes of our field and laboratory experiments, we propose a conceptual scheme describing the multiple screenings of aquatic hyphomycetes in the hyporheic zone of woodland streams (Fig. 6b), likely to cause important shifts in the structure of fungal decomposer communities within this zone, and thus potentially altered enzymatic capacities and performance in litter breakdown, as reported by recent studies (24, 76, 77).

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REFERENCES

1. **Fisher SG, Likens GE.** 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecol. Monogr. **43:**421–439. http://dx.doi.org/10.2307/1942301.
2. **Kaushik NK, Hynes HBN.** 1968. Experimental study on the role of autumn shed leaves in aquatic environments. J. Ecol. **52:**229–243.
3. **Webster JR, Meyer JL.** 1997. Organic matter budgets for streams: a synthesis. J. N. Am. Benthol. Soc. **16:**141–161. http://dx.doi.org/10.2307/1468247.
4. **Herbst GN.** 1980. Effects of burial on food value and consumption of leaf detritus by aquatic invertebrates in a lowland forest stream. Oikos **35:**411–424. http://dx.doi.org/10.2307/3544658.
5. **Metzler GM, Smock LA.** 1990. Storage and dynamics of subsurface detritus in a sandbottomed stream. Can. J. Fish. Aquat. Sci. **47:**588–594. http://dx.doi.org/10.1139/f90-067.
6. **Naegeli MW, Hartmann U, Meyer EI, Uehlinger U.** 1995. POM-dynamics and community respiration in the sediments of a floodprone prealpine river (Necker, Switzerland). Arch. Hydrobiol. **133:**339–347.
7. **Orghidan T.** 1959. Ein neuer Lebensraum des unterirdischen Wassers: der hyporheische Biotop. Arch. Hydrobiol. **55:**392–414.
8. **Cornut J, Elger A, Greugny A, Bonnet M, Chauvet E.** 2012. Coarse particulate organic matter in the interstitial zone of three French headwater streams. Ann. Limnol. Int. J. Limnol. **48:**303–313. http://dx.doi.org/10.1051/limn/2012019.
9. **Kaushik NK, Hynes HBN.** 1971. The fate of the dead leaves that fall into streams. Arch. Hydrobiol. **68:**465–515.
10. **Bärlocher F, Kendrick B.** 1976. Hyphomycetes as intermediaries of energy flow in streams, p 435–446. *In* Jones EBG (ed), Recent advances in aquatic mycology. Elek, London, United Kingdom.
11. **Bärlocher F.** 1985. The role of fungi in the nutrition of stream invertebrates. Bot. J. Linn. Soc. **91:**83–94. http://dx.doi.org/10.1111/j.1095-8339.1985.tb01137.x.
12. **Suberkropp K.** 1992. Interaction with invertebrates, p 118–134. *In* Bärlocher F (ed), The ecology of aquatic hyphomycetes. Springer-Verlag, New York, NY.
13. **Gulis V, Kuehn KA, Suberkropp K.** 2009. Fungi, p 233–243. *In* Likens G (ed), Encyclopedia of inland waters, vol 3. Elsevier, Oxford, United Kingdom.
14. **Gessner MO.** 1997. Litter breakdown in rivers and streams. Limnética **13:**33–44.
15. **Findlay SE, Arsuffi TL.** 1989. Microbial growth and detritus transformations during decomposition of leaf litter in a stream. Freshw. Biol. **21:**261–269. http://dx.doi.org/10.1111/j.1365-2427.1989.tb01364.x.
16. **Suberkropp K.** 1991. Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. Mycol. Res. **95:**843–850. http://dx.doi.org/10.1016/S0953-7562(09)80048-8.
17. **Sridhar KR, Bärlocher F.** 2000. Initial colonization, nutrient supply, and fungal activity on leaves decaying in streams. Appl. Environ. Microbiol. **66:**1114–1119. http://dx.doi.org/10.1128/AEM.66.3.1114-1119.2000.
18. **Iqbal SH, Webster J.** 1973. The trapping of aquatic hyphomycete spores by air bubbles. Trans. Br. Mycol. Soc. **60:**37–48. http://dx.doi.org/10.1016/S0007-1536(73)80057-9.
19. **Webster J, Descals E.** 1981. Morphology, distribution, and ecology of spore fungi in freshwater habitats, p 295–355. *In* Cole GC, Kendrick B (ed), Biology of spore fungi. Academic Press, London, United Kingdom.
20. **Bärlocher F.** 1992. Research on aquatic hyphomycetes: historical background and overview, p 1–11. *In* Bärlocher F (ed), The ecology of aquatic hyphomycetes. Springer-Verlag, New York, NY.
21. **Suberkropp K.** 1997. Annual production of leaf-decaying fungi in a woodland stream. Freshw. Biol. **38:**169–178. http://dx.doi.org/10.1046/j.1365-2427.1997.00203.x.
22. **Bärlocher F.** 2009. Reproduction and dispersal in aquatic hyphomycetes. Mycoscience **50:**3–8. http://dx.doi.org/10.1007/S10267-008-0449-X.
23. **Sridhar KR, Bärlocher F.** 1997. Water chemistry and sporulation by aquatic hyphomycetes. Mycol. Res. **101:**591–596. http://dx.doi.org/10.1017/S0953756296003024.
24. **Cornut J, Elger A, Lambrigot D, Marmonier P, Chauvet E.** 2010. Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. Freshw. Biol. **55:**2541–2556. http://dx.doi.org/10.1111/j.1365-2427.2010.02483.x.
25. **Dang CK, Gessner MO, Chauvet E.** 2007. Influence of spore traits and leaf structure on attachment success of aquatic hyphomycetes on leaf litter. Mycologia **99:**24–32. http://dx.doi.org/10.3852/mycologia.99.1.24.
26. **Webster J.** 1987. Convergent evolution and the functional significance of spore shape in aquatic and semi-aquatic fungi, p 191–201. *In* Rayner ADM, Brasier CM, Moore D (ed), Evolutionary biology of the fungi. University Press, Cambridge, United Kingdom.
27. **Kearns SG, Bärlocher F.** 2008. Leaf surface roughness influences colonization success of aquatic hyphomycete spore. Fungal Ecol. **1:**13–18. http://dx.doi.org/10.1016/j.funeco.2007.07.001.
28. **Navel S, Mermillod-Blondin F, Montuelle B, Chauvet E, Simon L, Marmonier P.** 2011. Water-sediment exchanges control microbial processes associated with leaf litter degradation in the hyporheic zone: a microcosm study. Microb. Ecol. **61:**968–979. http://dx.doi.org/10.1007/s00248-010-9774-7.
29. **Medeiros AO, Pascoal C, Graça MAS.** 2009. Diversity and activity of aquatic fungi under low oxygen conditions. Freshw. Biol. **54:**142–149. http://dx.doi.org/10.1111/j.1365-2427.2008.02101.x.
30. **Rajashekhar M, Kaveriappa KM.** 2003. Diversity of aquatic hyphomycetes in the aquatic ecosystems of the Western Ghats of India. Hydrobiologia **501:**167–177. http://dx.doi.org/10.1023/A:1026239917232.
31. **Field JI, Webster J.** 1983. Anaerobic survival of aquatic fungi. Trans. Br. Mycol. Soc. **81:**365–369. http://dx.doi.org/10.1016/S0007-1536(83)80088-6.
32. **Laitung B, Pretty JL, Chauvet E, Dobson M.** 2002. Response of aquatic hyphomycete communities to enhanced stream retention in areas impacted by commercial forestry. Freshw. Biol. **47:**313–324. http://dx.doi.org/10.1046/j.1365-2427.2002.00801.x.
33. **Baxter C, Hauer RF, Woessner WW.** 2003. Measuring groundwater-stream water exchange: new techniques for installing minipiezometers and estimating hydraulic conductivity. Trans. Am. Fish. Soc. **132:**493–502. http://dx.doi.org/10.1577/1548-8659(2003)132<0493:MGWENT>2.0.CO;2.
34. **Chauvet E.** 1990. Hyphomycètes aquatiques du sud-ouest de la France. Gaussenia **6:**3–31.
35. **Gulis V, Marvanová L, Descals E.** 2005. An illustrated key to the common temperate species of aquatic hyphomycetes, p 185–188. *In* Graça MAS, Bärlocher F, Gessner MO (ed), Methods to study litter decomposition—a practical guide. Springer, Dordrecht, Netherlands.
36. **Griebler C.** 1996. Some applications for the DMSO-reduction method as a new tool to determine the microbial activity in water-saturated sediments. Arch. Hydrobiol. Suppl. **113:**405–410.
37. **Mermillod-Blondin F, Mauclaire L, Montuelle B.** 2005. Use of slow filtration columns to assess oxygen respiration, consumption of dissolved organic carbon, nitrogen transformations and microbial parameters in hyporheic sediments. Water Res. **39:**1687–1698. http://dx.doi.org/10.1016/j.watres.2005.02.003.
38. **Navel S, Mermillod-Blondin F, Montuelle B, Chauvet E, Simon L, Piscart C, Marmonier P.** 2010. Interactions between fauna and sediment control plant matter breakdown in river sediments. Freshw. Biol. **55:**753–766. http://dx.doi.org/10.1111/j.1365-2427.2009.02315.x.
39. **Claret C, Marmonier P, Bravard J-P.** 1998. Seasonal dynamics of nutri-

ent and biofilms in interstitial habitats of two contrasting riffles in a regulated large river. Aquat. Sci. **60:**33–55. http://dx.doi.org/10.1007/s000270050025.
40. **Laitung B, Chauvet E.** 2005. Vegetation diversity increases species richness of leaf decaying fungal communities in woodland streams. Arch. Hydrobiol. **164:**217–235. http://dx.doi.org/10.1127/0003-9136/2005/0164-0217.
41. **Suberkropp K, Chauvet E.** 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology **76:**1433–1445. http://dx.doi.org/10.2307/1938146.
42. **Triska FJ, Duff JH, Avanzino RJ.** 1993. The role of water exchange between a stream channel and its hyporheic zone in nitrogen cycling at the terrestrial-aquatic interface. Hydrobiologia **251:**167–184. http://dx.doi.org/10.1007/BF00007177.
43. **Morrice JA, Dahm CN, Valett HM.** 2000. Terminal electron accepting processes in the alluvial sediments of a headwater stream. J. N. Am. Benthol. Soc. **19:**593–608. http://dx.doi.org/10.2307/1468119.
44. **Peet RK.** 1975. Relative diversity indices. Ecology **56:**496–498. http://dx.doi.org/10.2307/1934984.
45. **Wolda H.** 1981. Similarity indices, sample size and diversity. Oecologia **50:**296–302. http://dx.doi.org/10.1007/BF00344966.
46. **Legendre P, Legendre L.** 1998. Numerical ecology, 2nd English ed. Elsevier Science BV, Amsterdam, Netherlands.
47. **StatSoft Inc.** 2001. Statistica 6.0, electronic statistics textbook. http://www.statsoft.com/textbook/stathome.html.
48. **R Development Core Team.** 2007. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.
49. **Bärlocher F, Murdoch JH.** 1989. Hyporheic biofilms—a potential food source for interstitial animals. Hydrobiologia **184:**61–67. http://dx.doi.org/10.1007/BF00014302.
50. **Bärlocher F, Nikolcheva LG, Wilson KP, Williams DD.** 2006. Fungi in the hyporheic zone of a springbrook. Microb. Ecol. **52:**708–715. http://dx.doi.org/10.1007/s00248-006-9102-4.
51. **Bärlocher F, Seena S, Wilson K, Williams DD.** 2008. Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. Freshw. Biol. **53:**368–379. http://dx.doi.org/10.1111/j.1365-2427.2007.01899.x.
52. **Smith JJ, Lake PS.** 1993. The breakdown of buried and surface-placed leaf-litter in an upland stream. Hydrobiologia **271:**141–148. http://dx.doi.org/10.1007/BF00005411.
53. **Krauss G, Sridhar KR, Jung K, Wennrich R, Ehrman J, Bärlocher F.** 2003. Aquatic hyphomycetes in polluted groundwater habitats of central Germany. Microb. Ecol. **45:**329–339.
54. **Park D.** 1974. Accumulation of fungi by cellulose exposed in a river. Trans. Br. Mycol. Soc. **63:**437–447. http://dx.doi.org/10.1016/S0007-1536(74)80090-2.
55. **Knudsen GR, Stack JP.** 1991. Modeling growth and dispersal of fungi in natural environments, p 625–645. *In* Arora DK, Rai B, Mukerji KG, Knudsen G (ed), Handbook of applied mycology. Marcel Dekker, New York, NY.
56. **Read SJ, Moss ST, Jones EBJ.** 1992. Attachment and germination of spore, p 135–151. *In* Bärlocher F (ed), The ecology of aquatic hyphomycetes. Springer-Verlag, New York, NY.
57. **Au DWT, Jones EBJ, Moss ST, Hodgkiss IJ.** 1996. The role of mucilage in the attachment of spore, germ tubes, and appressoria in the saprobic aquatic hyphomycetes Lemonniera aquatica and Mycocentrospora filiformis. Can. J. Bot. **74:**1789–1800. http://dx.doi.org/10.1139/b96-216.
58. **Cox PA.** 1983. Search theory, random motion and the convergent evolution of pollen and spore morphology in aquatic plants. Am. Nat. **121:**9–31. http://dx.doi.org/10.1086/284037.
59. **Frost H.** 1978. ONR technical report no. 6. Division of Applied Sciences, Harvard University, Cambridge, MA.
60. **Nimmo JR.** 2004. Porosity and pore size distribution, p 295–303. *In* Hillel D (ed), Encyclopedia of soils in the environment. Elsevier, London, United Kingdom.
61. **Parmasto E, Parmasto I.** 1992. Size and shape of basidiospores in the Hymenomycetes. Mycol. Helv. **5:**47–78.
62. **Kauserud H, Colman JE, Ryvarden L.** 2008. Relationship between basidiospore size, shape and life history characteristics: a comparison of polypores. Fungal Ecol. **1:**19–23. http://dx.doi.org/10.1016/j.funeco.2007.12.001.
63. **Fischer MWF, Stolze Rybczynski JL, Cui YL, Money NP.** 2010. How far and how fast can mushroom spores fly? Physical limits on ballisto spore size and discharge distance in the Basidiomycota. Fungal Biol. **114:**669–675.
64. **Hussein T, Norros V, Hakala J, Petäjä T, Aalto PP, Rannik U, Vesala T, Ovaskainen O.** 2013. Species traits and inertial deposition of fungal spores. Aerosol Sci. **61:**81–98. http://dx.doi.org/10.1016/j.jaerosci.2013.03.004.
65. **Valett HM, Fisher SG, Stanley EH.** 1990. Physical and chemical characteristics of the hyporheic zone of a Sonoran Desert stream. J. N. Am. Benthol. Soc. **9:**201–215. http://dx.doi.org/10.2307/1467584.
66. **Boulton AJ, Findlay S, Marmonier P, Stanley EH, Valett HM.** 1998. The functional significance of the hyporheic zone in streams and rivers. Annu. Rev. Ecol. Sys. **29:**59–81. http://dx.doi.org/10.1146/annurev.ecolsys.29.1.59.
67. **Mermillod-Blondin F, Creuzé des Chatelliers M, Marmonier P, Dole-Olivier M-J.** 2000. Distribution of solutes, microbes and invertebrates in river sediments along a riffle-pool-riffle sequence. Freshw. Biol. **44:**255–269. http://dx.doi.org/10.1046/j.1365-2427.2000.00562.x.
68. **Marmonier P, Luczyszyn H, Creuzé des Châtelliers M, Landon N, Claret C, Dole-Olivier M-J.** 2010. Hyporheic flowpaths and interstitial invertebrates associated with stable and eroded river sections: interactions between micro- and meso-scales. Fund. Appl. Limnol. **176:**303–317. http://dx.doi.org/10.1127/1863-9135/2010/0176-0303.
69. **Strayer DL, May SE, Nielsen P, Wollheim W, Hausam S.** 1997. Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. Arch. Hydrobiol. **140:**131–144.
70. **Chergui H, Pattee E.** 1988. The dynamics of Hyphomycetes on decaying leaves in the network of the River Rhône (France). Arch. Hydrobiol. **114:**3–20.
71. **Pascoal C, Pinho M, Cássio F, Gomes P.** 2003. Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. Freshw. Biol. **48:**2033–2044. http://dx.doi.org/10.1046/j.1365-2427.2003.01130.x.
72. **Pascoal C, Cássio F.** 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Appl. Environ. Microbiol. **70:**5266–5273. http://dx.doi.org/10.1128/AEM.70.9.5266-5273.2004.
73. **Webster J, Towfik FH.** 1972. Sporulation of aquatic hyphomycetes in relation to aeration. Trans. Br. Mycol. Soc. **59:**353–364. http://dx.doi.org/10.1016/S0007-1536(72)80117-7.
74. **Webster J.** 1975. Further studies of sporulation of aquatic hyphomycetes in relation to aeration. Trans. Br. Mycol. Soc. **64:**119–127. http://dx.doi.org/10.1016/S0007-1536(75)80082-9.
75. **Sanders PF, Webster J.** 1980. Sporulation responses of some 'aquatic hyphomycetes' in flowing water. Trans. Br. Mycol. Soc. **74:**601–605. http://dx.doi.org/10.1016/S0007-1536(80)80062-3.
76. **Danger M, Cornut J, Elger A, Chauvet E.** 2012. Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa. Freshw. Biol. **57:**1017–1030. http://dx.doi.org/10.1111/j.1365-2427.2012.02762.x.
77. **Cornut J, Clivot H, Chauvet E, Elger A, Pagnout C, Guérold F.** 2012. Effect of acidification on leaf litter decomposition in benthic and hyporheic zones of woodland streams. Water Res. **46:**6430–6444. http://dx.doi.org/10.1016/j.watres.2012.09.023.