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doi:10.1128/AEM.00971-09

Microbial Decomposer Communities Are Mainly Structured by Trophic Status in Circumneutral and Alkaline Streams[▽]†

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In streams, the release of nitrogen and phosphorus is reported to affect microbial communities and the ecological processes they govern. Moreover, the type of inorganic nitrogen (NO_3, NO_2, or NH_4) may differently impact microbial communities. We aimed to identify the environmental factors that structure aquatic microbial communities and drive leaf litter decomposition along a gradient of eutrophication. We selected five circumneutral (Portuguese) and five alkaline (French) streams differing in nutrient concentrations to monitor mass loss of alder leaves, bacterial and fungal diversity by PCR-denaturing gradient gel electrophoresis, fungal biomass and reproduction, and bacterial biomass during 11 weeks of leaf immersion. The concentrations of inorganic nutrients in the stream water ranged from 5 to 300 μg liter^{-1} soluble reactive phosphorus, 0.30 to 5.50 mg liter^{-1} NO_3-N, 2 to 103 μg liter^{-1} NO_2-N, and <4 to 7,100 μg liter^{-1} NH_4-N. Species richness was maximum in moderately anthropized (eutrophic) streams but decreased in the most anthropized (hypertrophic) streams. Different species assemblages were found in subsets of streams with different trophic statuses. In both geographic areas, the limiting nutrient, either nitrate or phosphate, stimulated the microbial activity in streams of intermediate trophic status. In the hypertrophic streams, fungal biomass and reproduction were significantly lower, and bacterial biomass dramatically decreased at the site with the highest ammonium concentration. The limiting nutrients that defined the trophic status were the main factor structuring fungal and bacterial communities, whatever the geographic area. A very high ammonium concentration in stream water most probably has negative impacts on microbial decomposer communities.

There is evidence that increases in nitrate and phosphate concentrations stimulate microbial respiration and fungal and bacterial activity (biomass buildup, sporulation, and/or productivity) on plant litter, leading to faster leaf decomposition in freshwaters (16, 17, 26, 34). However, fungal demands of nitrate and phosphate are reported to be fulfilled at relatively low levels (1, 12), and further increases in these nutrients in the stream water do not necessarily result in enhanced fungal activity. Besides, the form in which inorganic nutrients are present in streams, their biological availability, and even their toxicity have different ecological consequences. In densely anthropized hypertrophic streams, high levels of nitrate and phosphate were associated with decreased fungal biomass and leaf breakdown, most probably because of the high concentrations of ammonium and ammonia (2). On the other hand, the positive effects of nutrients on biomass and productivity of leaf-associated fungi can be offset by other factors, such as low oxygen concentration and sedimentation, leading to retarded decomposition (26, 33, 34).

Changes in inorganic nutrient concentrations in the stream water were reported to alter the structure of fungal communities on plant litter (16, 36). Nutrient additions to moderate levels increased the diversity of fungal communities in circumneutral soft-water Appalachian mountain streams (18) but not in a Mediterranean alkaline stream (1). Moreover, fungal diversity was lower in circumneutral eutrophic streams than in reference streams (10, 35). Fungal diversity has been assessed mostly through the morphological analysis of produced conidia, not taking into account nonsporulating fungi. This raises the question of whether the differential impacts of eutrophication on fungal diversity could be due partly to difficulties in measuring actual diversity. Besides, the study of bacterial diversity on decomposing leaves has been strongly restricted to a few cultivable bacteria (<1%). Molecular typing, such as denaturing gradient gel electrophoresis (DGGE) of a specific rRNA gene region, has proved useful for assessing diversity in both leaf-associated fungi and bacteria (7, 8, 9, 11, 30).

We aimed to identify the environmental factors that drive the ecological processes in freshwaters impacted by eutrophication through examination of leaf litter decomposition and associated microbial communities along a gradient of nutrient enrichment. Specifically, we addressed the following two questions: (i) which are the environmental factors that mainly structure the fungal and bacterial communities and (ii) what are the relationships between concentrations of inorganic nutrients in the stream water, leaf litter decomposition, and the activity of associated microbes? We selected 10 stream sites spanning wide concentration ranges of dissolved inorganic nitrogen (NO_3-N, NO_2-N, NH_4-N, and NH_3-N) and soluble reactive phosphorus (SRP), including 5 in northwestern Portugal with circumneutral pH and 5 in southwestern France with an alkaline pH. With these two groups of stream sites, we assessed the

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† Supplemental material for this article may be found at http://aem.asm.org/.

TABLE 1. Characteristics of stream water at two groups of stream sites[a]

Stream site	Longitude	Latitude	Elevation (m)	pH ($n = 7$)	Water temp (°C) ($n = 7$)	Conductivity (μS cm^{-1}) ($n = 7$)	Oxygen level (mg liter^{-1}) ($n = 7$)
Circumneutral stream sites (Portugal)							
Maceira	41°46′07.64″N	8°08′49.07″W	857	7.0 ± 0.3	9.0 ± 0.4	14 ± 0.5	10.7 ± 0.5
Algeriz	41°35′24.56″N	8°22′36.96″W	220	7.1 ± 0.04	13.0 ± 0.3	42 ± 0.9	12.4 ± 0.6
Este 2	41°35′05.50″N	8°21′05.93″W	406	6.7 ± 0.06	13.0 ± 0.3	44 ± 0.7	11.0 ± 0.3
Este 3	41°31′36.83″N	8°26′08.50″W	148	7.0 ± 0.06	13.0 ± 0.5	161 ± 2.3	9.4 ± 0.6
Souto	41°31′34.43″N	8°17′16.03″W	138	7.1 ± 0.1	11.0 ± 0.7	295 ± 85	9.9 ± 0.7
Alkaline stream sites (France)							
Tescou	43°54′41″N	1°45′45″E	180	8.0 ± 0.2	8.5 ± 2.2	604 ± 25	12.3 ± 2.0
Seye	44°15′04″N	1°52′01″E	290	8.0 ± 0.1	9.4 ± 2.0	628 ± 14	12.9 ± 1.8
Lère	44°09′15″N	1°31′29″E	107	7.8 ± 0.1	9.9 ± 1.7	614 ± 17	11.9 ± 1.8
Lupte	44°15′34″N	1°21′48″E	128	7.9 ± 0.2	9.7 ± 1.9	667 ± 28	11.8 ± 1.7
Tauge	44°03′07″N	1°26′43″E	98	7.7 ± 0.1	9.0 ± 2.3	722 ± 69	7.1 ± 2.3

[a] Data are means ± SEM.

structure of and diversity in both sporulating and nonsporulating fungal communities, using asexual spore morphology and DGGE fingerprints of the ITS2 region, and in bacterial communities, using DGGE fingerprints of the 16S rRNA gene region. Additionally, we examined leaf mass loss and microbial activity on decomposing leaves by determining bacterial and fungal biomass and fungal reproduction.

MATERIALS AND METHODS

Study areas. Five sampling sites were selected in streams of northwestern Portugal, where granitic rocks dominate the geological substratum. Three sampling sites, Este 2, Este 3, and Souto, are in the Ave River basin, in an area with a high population density, intensive agriculture, and industrial activities, while the other two sampling sites, Algeriz and Maceira, belong to the Cávado River basin (36, 39). The dominant riparian vegetation includes *Alnus glutinosa* (L.) Gaertn. at Este 2, Este 3, and Souto, *Quercus robur* L. at Este 3 and Souto, and *Eucalyptus globulus* Labill and *Pteridium aquilinum* Khun. at Este 2 and Algeriz. Maceira is located in the Peneda-Gerês National Park and is bordered by *Quercus pyrenaica* Willd., *Q. robur*, *Chamaecyparis* sp., and *Ilex aquifolium* L. Five other streams were selected in southwestern France, between Cahors, Montauban, and Gaillac (2, 21). All of the streams flow through intensively farmed landscapes. The dominant vegetation bordering the five streams consists primarily of *A. glutinosa*, *Q. robur*, and *Fraxinus excelsior* L. The bedrock is composed mainly of limestone and clay. Boulders and pebbles are dominant in Seye, while gravel and sand dominate in the other four streams, i.e., Tescou, Lère, Lupte, and Tauge.

Experimental field setup. In October 2006, freshly fallen *A. glutinosa* leaves were collected from local trees in a riparian zone and air dried for 1 week at room temperature. Two hundred eighty sets of 4 ± 0.05 g of alder leaves were immersed for 5 min in deionized water (to prevent breakup) and placed in 0.5-mm-pore-size mesh bags (20 by 20 cm). Twenty-eight leaf bags were immersed at each sampling site at the end of November 2006. Four replicate leaf bags were retrieved after 1, 2, 3, 4, 6, 8, and 11 weeks. On each sampling date, leaf bags were transferred to ziplock plastic bags containing stream water and transported in a cool box to the laboratory. Two sets of four additional leaf bags were immersed in 0.2-μm filtered deionized water for 4 h and processed as controls (time zero, before submersion in stream water).

Physical, chemical, and microbial analyses of stream water. On each sampling date, the pH, dissolved oxygen, conductivity, and temperature were measured in situ with field probes (Multiline 340i; WTW, Weilheim, Germany). The current velocity was determined with a flow meter (model 2030R [General Oceanics Inc., Miami, FL] or Flo-Mate model 200 [Marsh-McBirney, Inc., Frederick, MD]). Stream water samples were collected in sterile glass bottles, transported on ice, and analyzed within 24 h. The nitrate concentration was quantified with a Hach DR/2000 photometer (Hach Company, Loveland, CO) by cadmium reduction (Hach kit, program 355) or by UV spectral deconvolution (41) with an Anhtelie 70MI instrument (Secomam, Ales, France), the nitrite concentration by the diazotization method (Hach kit, program 371) or UV spectral deconvolution, the ammonium concentration by the salicylate method (Hach kit, program 385) or indophenol blue method (5), and the SRP concentration by the ascorbic acid method (Hach kit, program 490) or the molybdate and malachite green method (28). Alkalinity determination was performed by titration at pH 4.5 ± 0.05. Fecal coliform numbers were determined by the membrane filter technique (5).

Leaf bag processing and leaf mass loss. In the laboratory, leaves were rinsed with deionized water to remove sediments. From each replicate bag, leaf disks were cut with a 1.2-cm cork borer. Four leaf disks were stored in 10 ml of a 2% formaldehyde solution at 4°C for bacterial counts, eight disks were freeze-dried for ergosterol quantification, four disks were also freeze-dried for DNA extraction, and eight disks were used for conidial production. The remaining leaves were dried at 80°C for 3 days and weighed to the nearest 0.01 g. About 1 g of dry leaves was ground, ashed at 550°C for 6 h, and weighed to determine ash-free dry mass (AFDM).

Fungal biomass and sporulation. Fungal biomass on leaves was estimated by the ergosterol concentration according to the method of Gessner (13). Briefly, lipids were extracted from sets of eight leaf disks by heating (80°C for 30 min) in 0.8% (wt/vol) KOH-methanol, purified by solid-phase extraction (Oasis HLB 30-μm cartridge; Waters, Milford, MA), and quantified at 262 nm by high-performance liquid chromatography on a C_{18} column with methanol as the mobile phase. Ergosterol was converted to fungal biomass by using a factor of 5.5 μg ergosterol mg^{-1} fungal dry mass (4, 14).

To induce sporulation, sets of eight leaf disks from each replicate bag were incubated at 10°C (France) or 14°C (Portugal) with shaking (140 rpm) in 100-ml Erlenmeyer flasks with 40 ml of filtered (0.45-μm membrane pore size) stream water from each respective site. After 48 h, conidial suspensions were mixed with 35 μl of 15% (vol/vol) Triton X-100 and 4 ml of 37% formaldehyde in 50-ml polypropylene tubes. For conidial identification and counting, samples of conidial suspensions were filtered on cellulose nitrate membranes (5-μm pore size; Millipore, Billerica, MA) and stained with 0.05% (wt/vol) cotton blue in lactic acid. At least 300 conidia were identified and counted per replicate (magnification, ×100 to ×400; Leica Biomed, Heerbrugg, Switzerland).

Bacterial biomass. Bacterial cells were detached from four leaf disks with a 12.7-mm flat-tip ultrasonic probe connected to a digital Sonifier 250 machine (Branson Ultrasonics, Danbury, CT) operating continuously for 1 min at a 50% amplitude (2, 3). The bacterial suspension was resuspended and allowed to settle for 10 s, and a 200-μl sample was taken about 5 mm below the surface and mixed with 0.2-μm-filtered deionized water to a final volume of 2 ml. Appropriate dilutions of the bacterial suspensions were stained for 10 min after the addition of 288 μl of 40-mg liter^{-1} 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO). Bacterial numbers were determined by epifluorescence microscopy (Axioplan; Carl Zeiss, Le Pecq, France) at a magnification of ×1,000. At least 300 cells were counted per filter in a total of 10 fields. Bacterial numbers were converted to bacterial carbon by using a conversion factor of 20 fg bacterial carbon per cell (31) and to bacterial biomass by assuming that carbon was 50% of bacterial dry mass.

Molecular analyses. DNAs were extracted from four freeze-dried leaf disks by use of a soil DNA extraction kit (MoBio Laboratories, Solana Beach, CA)

TABLE 1—*Continued*

NO_3-N concn (mg liter^{-1}) (n = 7)	NO_2-N concn (μg liter^{-1}) (n = 7)	NH_4-N concn (μg liter^{-1}) (n = 7)	NH_3-N concn (μg liter^{-1}) (n = 7)	SRP concn (μg liter^{-1}) (n = 6)	Alkalinity (mg $CaCO_3$ liter^{-1}) (n = 4)	Current velocity (cm s^{-1}) (n = 6)	Fecal coliform level (CFU ml^{-1}) (n = 3)
0.3 ± 0.04	2 ± 0.3	<4	<0.004	20 ± 7	1.6 ± 0.3	31 ± 10	<1
0.6 ± 0.1	3 ± 0.9	4 ± 2	0.01 ± 0.007	40 ± 10	2.0 ± 0.2	37 ± 8	<1
0.9 ± 0.1	2 ± 0.2	4 ± 3	0.004 ± 0.003	20 ± 5	4.0 ± 0.5	28 ± 11	<1
5.4 ± 0.2	20 ± 3	730 ± 90	1.8 ± 0.5	70 ± 20	26.0 ± 0.2	44 ± 4	360 ± 75
3.3 ± 0.5	60 ± 10	7,100 ± 4,200	36.8 ± 25.9	60 ± 20	16.0 ± 3.7	43 ± 4	<1
1.9 ± 0.2	6 ± 5	20 ± 7	0.3 ± 0.1	5 ± 2	297 ± 4.0	17 ± 8	2 ± 1
5.5 ± 0.5	5 ± 2	13 ± 4	0.2 ± 0.1	6 ± 5	296 ± 5.3	15 ± 11	1 ± 1
3.4 ± 0.2	17 ± 6	32 ± 5	0.4 ± 0.1	31 ± 24	283 ± 4.9	36 ± 26	40 ± 30
4.3 ± 0.2	54 ± 22	208 ± 10	4.3 ± 1.5	76 ± 40	308 ± 11.8	34 ± 11	105 ± 80
3.2 ± 0.2	103 ± 29	1,800 ± 200	15.6 ± 9.2	300 ± 87	358 ± 2.5	3 ± 1	99 ± 103

according to the manufacturer's instructions. The ITS2 region of fungal ribosomal DNA was amplified with the primer pair ITS3GC and ITS4 (43). For PCR of fungal DNA, 1× *Taq* buffer [KCl–$(NH_4)_2SO_4$], 3 mM of $MgCl_2$, a 0.2 mM concentration of each deoxynucleoside triphosphate, 0.4 μM of each primer, 1.5 U of DNA *Taq* polymerase (Fermentas, Vilnius, Lithuania), and 1 μl containing 50 ng DNA were used in a final volume of 50 μl.

The V6-to-V8 region of the bacterial 16S rRNA gene was amplified with the primer pair 984GC (32) and 1378 (20). For PCR of bacterial DNA, 30 μl of reaction mix containing 1× colorless GoTaq Flexi buffer (Promega Corporation, Madison, WI), 1.5 mM $MgCl_2$, a 0.2 mM concentration of each deoxynucleoside triphosphate, 0.8 μM of each primer, and 2.5 U of DNA *Taq* polymerase was added to 20 μl containing 50 ng DNA (24, 25).

Fungal PCRs were carried out in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA), using the following program: initial denaturation at 95°C for 2 min, followed by 36 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 1 min. The final extension was done at 72°C for 5 min (9, 30). For bacteria, PCR was carried out in an MJ Research PTC-200 thermal cycler (Bio-Rad Laboratories, Hercules, CA), using the following program: initial denaturation at 94°C for 5 min; 10 touchdown cycles of denaturation at 94°C for 1 min, annealing at 65 to 55°C for 1 min (decreasing 1°C each cycle), and extension at 72°C for 3 min; and 20 standard cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 3 min. Final extension was done at 72°C for 5 min (24, 25). Fungal and bacterial DNAs were amplified, and 125-ng samples of PCR amplicons from each of the four replicates were pooled for DGGE analysis.

DGGE analysis was performed using a DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, CA). For fungal DNA, 700-ng samples of amplification products of 380 to 400 bp were loaded on an 8% (wt/vol) polyacrylamide gel in 1× Tris-acetate-EDTA buffer with a denaturing gradient from 25 to 70% (100% denaturant corresponds to 40% formamide and 7 M urea). The gels were run at 55 V and 56°C for 16 h and stained with 1 μg ml^{-1} of ethidium bromide (Bio-Rad Laboratories, Hercules, CA) for 5 min. The gel images were captured under UV light in a ChemiDoc XRS molecular imager (Bio-Rad). For bacterial DNA, 700-ng samples of amplification products of 433 bp were loaded on a 6% (wt/vol) polyacrylamide gel in 1× Tris-acetate-EDTA with a denaturing gradient from 30 to 60%. The gels were run at 100 V and 60°C for 16 h and stained with SYBR green (Sigma-Aldrich) diluted ×2,000 for 45 min. The gel images were captured by a charge-coupled device camera under UV light, using Vision-Capt software (Vilber Lourmat, Marne-la-Vallée, France).

In each DGGE gel for fungal fingerprinting, a DNA mixture of the taxa *Anguillospora* sp., *Anguillospora filiformis* Greath. (UMB-225.02), *Articulospora tetracladia* Ingold (UMB-22.01), *Clavariopsis aquatica* De Wild. (UMB-19.99), *Dimorphospora foliicola* Tubaki (UMB-30.01), *Lemonniera aquatica* De Wild. (UMB-143.01), *Tetracladium marchalianum* De Wild. (UMB-94.01), *Tricladium chaetocladium* Ingold (UMB-163.01), and *Varicosporium elodeae* W. Kegel (UMB-20.01) was used to calibrate the gels in further analyses. For bacterial DNA fingerprinting, one of the DNA samples (from site Seye at 4 weeks) was included in all DGGE gels to allow calibration in further analyses.

Data analyses. AFDM of alder leaves was fit to the exponential model $m_t = m_0 \times e^{-kt}$, where m_t is the AFDM remaining at time t, m_0 is the initial AFDM, and k is the rate of leaf decomposition. Regression lines of ln-transformed values of AFDM were compared by analysis of covariance (ANCOVA) followed by Tukey's test (45).

Two-way analysis of variance (ANOVA) was used to test if stream site and time significantly affected fungal and bacterial biomass and fungal sporulation (45). To achieve normal distributions, data for fungal biomass were ln transformed, while data for fungal sporulation and bacterial biomass were $\ln(x + 1)$ transformed (45).

The relationships between stream water variables, leaf mass loss, and fungal and bacterial parameters were examined by the Spearman rank correlation.

DGGE gels were aligned and normalized. A DGGE band was considered an operational taxonomic unit (OTU), taking into account that DNAs from more than one species can comigrate to the same position in the gel. The relative intensities of bands in the fingerprints were analyzed in Gelcompar II (Applied Maths, Sint-Martens-Latem, Belgium).

Shannon's diversity (H') and Pielou's equitability (J') indices were used to assess the diversity in aquatic fungi as follows:

$$H' = -\sum_{i=1}^{s} P_i(\ln P_i) \quad (1)$$

$$J' = H'/\ln S \quad (2)$$

where P_i is the relative abundance of conidia of taxon i or the relative intensity of OTU i and S is the total number of sporulating taxa or OTUs (22).

A principal component analysis (PCA) was used to ordinate sites according to inorganic nutrients, after standardization. Canonical correspondence analysis (CCA) was used to determine the relationships between inorganic nutrients and fungal sporulating species or DNA fingerprints of microbial communities (40). Monte Carlo tests based on 499 permutations were used to test the null hypothesis that microbial communities were unrelated to environmental variables (23). The resulting ordination biplots approximated the weighted average of each species (abundance of conidia or relative intensity of each OTU) with respect to each environmental variable, represented by an arrow.

Univariate analyses were done in Statistica 6.0 for Windows (Statsoft, Inc., Tulsa, OK), multivariate analyses were done in CANOCO, version 4.5, for Windows (Microcomputer Power, Ithaca, NY), and Shannon's and Pielou's indices were calculated with the Primer v6 software package (Primer-E Ltd., United Kingdom).

RESULTS

Stream water characteristics. Higher water temperatures were found in the Portuguese (9.0 to 13.0°C) than in the French (8.5 to 9.9°C) streams (Table 1). In contrast, lower values for pH (6.7 to 7.1 versus 7.7 to 8.0), conductivity (14 to 295 versus 604 to 722 μS cm^{-1}), and alkalinity (1.6 to 26 versus 296 to 358 mg $CaCO_3$ liter^{-1}) were observed in the Portuguese than in the French streams. In circumneutral streams, the non-

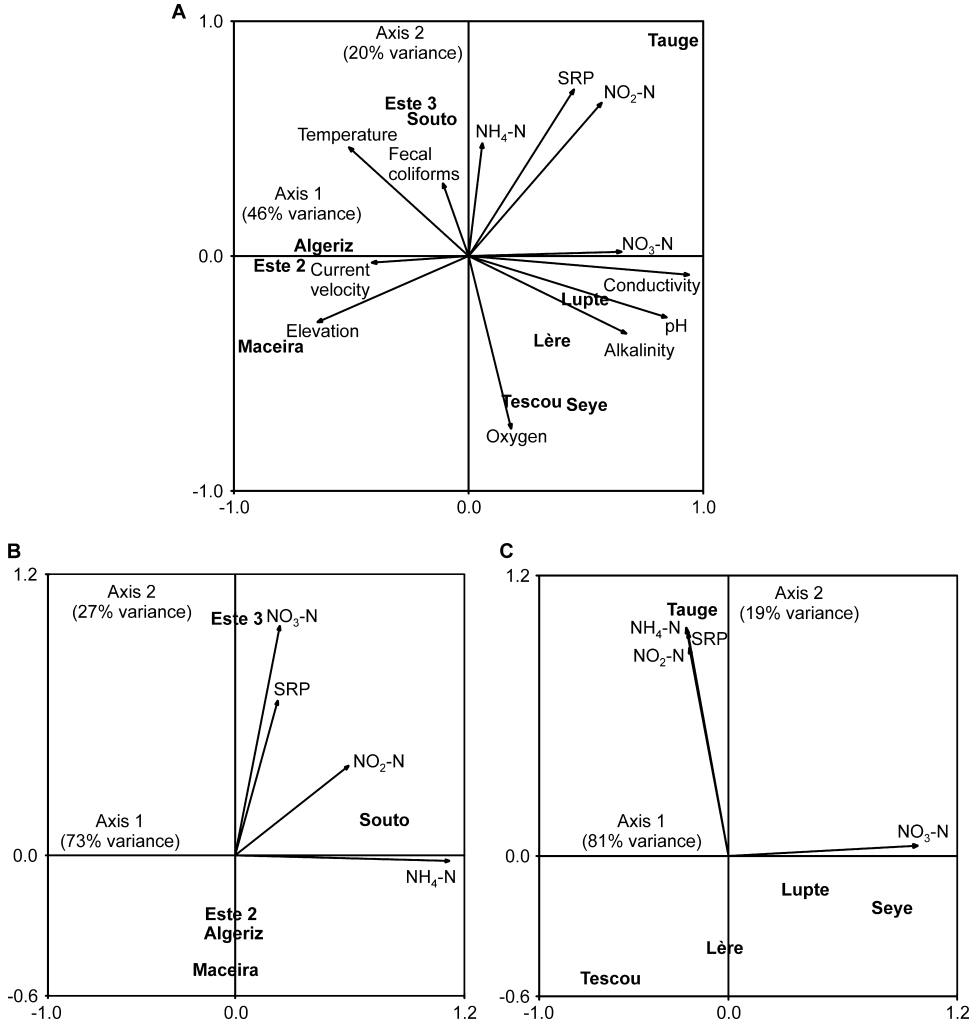

FIG. 1. PCA of chemical, physical, and microbial stream water parameters of 10 stream sites, Maceira, Algeriz, Este 2, Este 3, Souto, Tescou, Seye, Lère, Lupte, and Tauge (A), and PCA based only on the inorganic nutrient concentrations found in circumneutral (B) and alkaline (C) streams. The direction of the arrow indicates the direction in which the corresponding variable increases most, and the length of the arrow reflects the rate of change in that direction.

limiting concentration of phosphate ranged from 20 to 70 μg SRP liter^{-1}, while in the alkaline streams it varied more, with low values for the Tescou and Seye streams (5 and 6 μg SRP liter^{-1}, respectively), intermediate values for the Lère and Lupte streams (31 and 76 μg SRP liter^{-1}, respectively), and a very high value for the Tauge stream (300 μg SRP liter^{-1}). The concentration of nitrate varied less in the alkaline streams (1.9 to 5.5 mg NO_3-N liter^{-1}) than in the circumneutral ones. Three of the circumneutral stream sites (Maceira, Algeriz, and Este 2) had low nitrate concentrations (0.3 to 0.9 mg NO_3-N liter^{-1}), while the other two sites (Souto and Este 3) reached values at the upper limit of those found in the alkaline streams (3.3 and 5.4 mg N-NO_3 liter^{-1}, respectively). A gradient of NO_2-N was observed in both circumneutral (Maceira = Este 2 < Algeriz < Este 3 < Souto) and alkaline (Seye < Tescou < Lère < Lupte < Tauge) streams. The NH_4-N concentration in the hypertrophic circumneutral Souto stream was fourfold higher than that found in the hypertrophic alkaline Tauge stream. A slight decrease in oxygen concentration was found in the streams of high trophic status (Este 3, Souto, and Tauge).

PCA ordination of the 10 sites according to the stream water variables showed that axes 1 and 2 explained 70% of the total variance (Fig. 1A). The first PC axis separated Portuguese from French streams mainly due to differences in pH, conductivity, and alkalinity (Fig. 1A; Table 1), while the second PC axis described the nutrient enrichment. Subsequent PCA ordinations of the stream sites in each geographic region based on the concentration of inorganic nutrients in the stream water indicated that for circumneutral streams, axis 1 explained 73% of the variance and separated the most anthropized stream (Souto) from the others (Algeriz, Este 2, Maceira, and Este 3) according to the ammonium concentration gradient, while axis 2 explained 27% of the variance and separated eutrophized Este 3 from the other streams (Fig. 1B). For the alkaline streams, axis 1 explained 81% of the total variance and separated the streams along the nitrate concentration gradient,

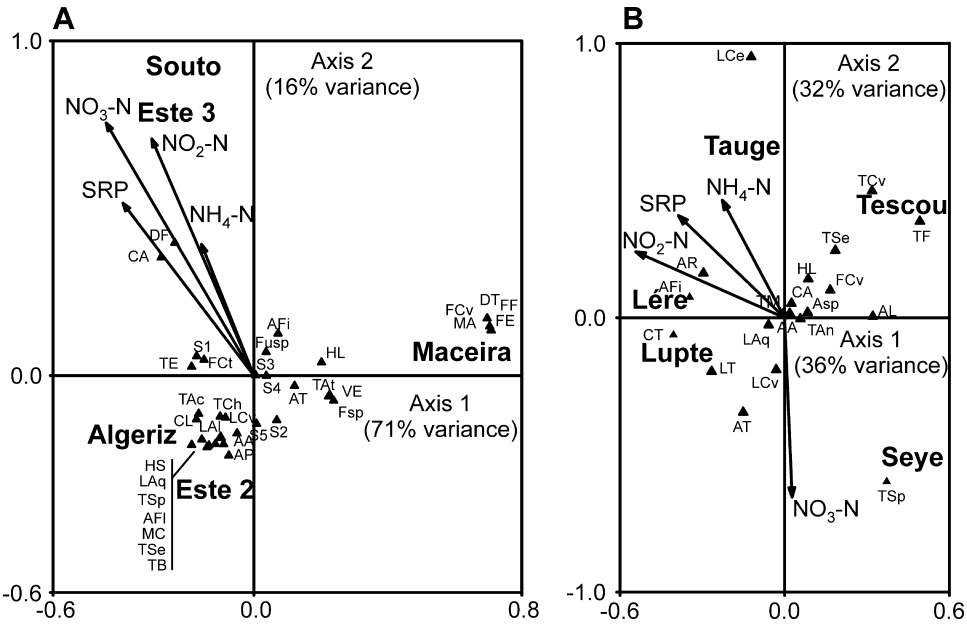

FIG. 2. CCA diagrams for ordination of inorganic nutrients in stream water and of fungal taxa on decomposing leaves at the 10 sites, based on conidial morphology, for circumneutral (A) and alkaline (B) streams. The percentages of variance explained by the first two axes were significant (Monte Carlo permutation tests; $P < 0.05$). Fungal taxa were as follows: AA, *Alatospora acuminata* Ingold; AFl, *Alatospora* cf. *flagellata* (Gönczol) Marvanová; AP, *Alatospora pulchella* Marvanová; AFi, *Anguillospora filiformis* Greath.; AL, *Anguillospora longissima* Saccardo and Sydow; AR, *Anguillospora rosea* J. Webster and Descals; Asp, *Anguillospora* sp.; AT, *Articulospora tetracladia* Ingold; CA, *Clavariopsis aquatica* De Wild.; CL, *Clavatospora longibrachiata* (Ingold) Marvanová and Sv. Nilsson; CT, *Clavatospora tentaculata* (Ingold) Marvanová and Sv. Nilsson; DT, *Dendrospora tenella* Descals and J. Webster; DF, *Dimorphospora foliicola* Tubaki; FCt, *Flagellospora curta* J. Webster; FCv, *Flagellospora curvula* Ingold; Fsp, *Flagellospora* sp.; FE, *Fontanospora eccentrica* (R. H. Petersen) Dyko; FF, *Fontanospora fusiramosa* Marvanová, P. J. Fisher, and Descals; Fusp, *Fusarium* sp.; HS, *Heliscella stellata* (Ingold and V. J. Cox) Marvanová; HL, *Heliscus lugdunensis* Sacc. and Thérry; LAl, *Lemonniera* cf. *alabamensis* R. C. Sinclair and Morgan-Jones; LAq, *Lemonniera aquatica* De Wild.; LCe, *Lemonniera centrosphaera* Marvanová; LT, *Lemonniera terrestris* Tubaki; LCv, *Lunulospora curvula* Ingold; MA, *Mycocentrospora acerina* (R. Hartig) Deighton; MC, *Mycofalcella calcarata* Marvanová; TE, *Tetrachaetum elegans* Ingold; TB, *Tetracladium breve* A. Roldán; TF, *Tetracladium furcatum* Descals; TM, *Tetracladium marchalianum* De Wild.; TSe, *Tetracladium setigerum* (Grove) Ingold; TAn, *Tricladium angulatum* Ingold; TAt, *Tricladium attenuatum* S. H. Iqbal; TCh, *Tricladium chaetocladium* Ingold; TCv, *Tricladium curvisporum* Descals; TSp, *Tricladium splendens* Ingold; TAc, *Triscelosphorus* cf. *acuminatus* Nawawi; Tsp, *Triscelosphorus* sp.; S1, sigmoid 1 (10 to 20 by 2 to 3 μm); S2, sigmoid 2 (5 to 10 by 0.5 to 1 μm); S3, sigmoid 3 (70 to 80 by 2 to 3 μm); S4, sigmoid 4 (80 to 120 by 1 to 2 μm); S5, sigmoid 5 (20 to 25 by 2.5 to 3 μm).

while axis 2, explaining 19% of the variance, separated Tauge from all the others (Fig. 1C).

Fungal diversity on decomposing leaves. During the study, a total of 45 aquatic fungal taxa were found sporulating on leaves decomposing at the 10 stream sites. The highest values for fungal species richness (28 taxa) and Shannon's ($H' = 1.75$) and Pielou's ($J' = 0.53$) indices were found for the Este 2 stream, and the lowest ones were found for the Souto stream (8 taxa; $H' = 0.16$ and $J' = 0.078$). Hence, the community diversity varied more in the circumneutral streams (3.5-fold difference in species richness between Este 2 and Souto) than in the alkaline streams (1.4-fold difference between Lère or Lupte and Tauge). Among circumneutral streams, *Articulospora tetracladia* had the highest contribution (49 to 72%) to the total conidium production on decomposing leaves in streams of lower trophic status (Maceira, Algeriz, and Este 2), whereas *Dimorphospora foliicola* was the dominant species in streams of higher trophic status (Este 3 and Souto), contributing more than 90% of the total conidium production. In alkaline streams, the taxon *Anguillospora* sp. contributed 28 to 59% of the total conidium production, with lower values for the eutrophic streams (Lère and Lupte). In the latter streams, we observed the highest contributions of *Tetracladium marchalianum* (36% in Lupte) and *Lemonniera aquatica* (29% in Lère). The mean percentages of each aquatic fungal species sporulating on decomposing leaves at the two groups of stream sites are provided in the supplemental material.

DNA fingerprints of fungal communities on decomposing leaves showed that among circumneutral streams, Algeriz had the most diversity (37 OTUs) and Este 3 had the least (28 OTUs), while among alkaline streams Lère had the most diversity (30 OTUs) and Tauge the least (21 OTUs). Hence, the variations in community diversity were very similar for the circumneutral and alkaline streams (1.3- and 1.4-fold differences in taxon richness between the most and the least diverse communities, respectively). However, differences in Shannon's and Pielou's indices were found only for the French streams (1.8- and 1.2-fold differences, respectively). Among circumneutral streams, OTU 59, which migrated to the same position as a pure culture of *D. foliicola*, was associated with streams with the highest trophic status (Este 3 and Souto). Also, OTU 34, which appeared in most fingerprints of alkaline streams, migrated to the same position as the ubiquitous sporulating species *Anguillospora* sp. Lastly, OTU 42, corresponding to *A. tetracladia*, was specifically detected in Maceira, Algeriz, and Este 2, while OTU 41, corresponding to *T. marchalianum*, was present at several dates in Lupte and Tauge, where the highest

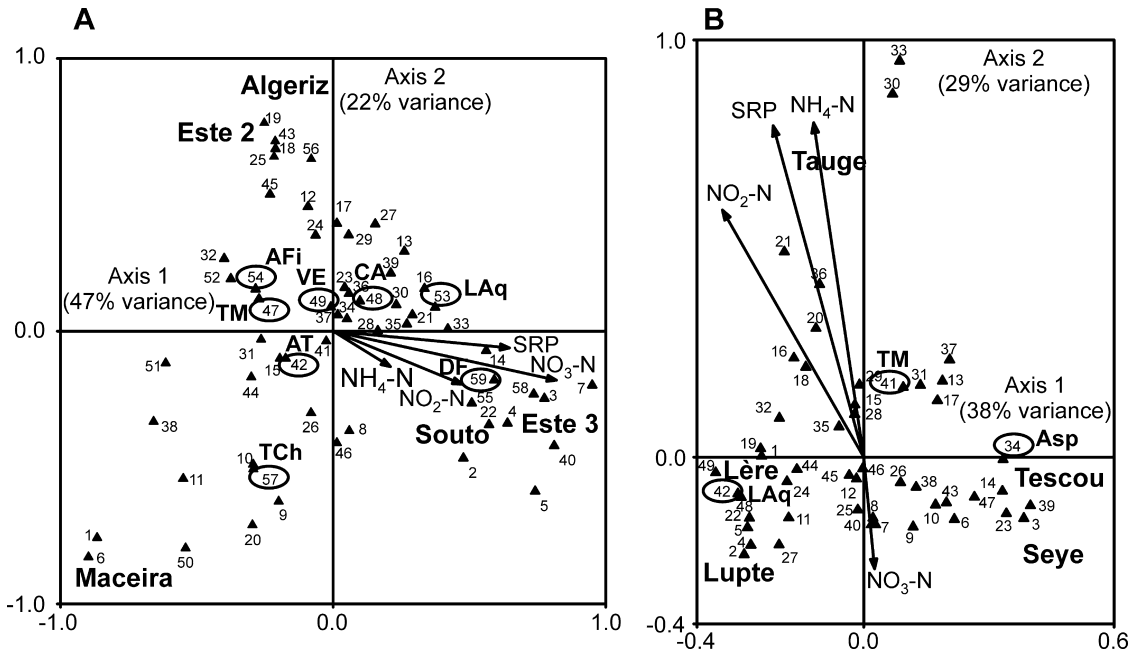

FIG. 3. CCA diagrams for ordination of inorganic nutrients in stream water and of the 59 and 49 fungal OTUs from DGGE fingerprints in the circumneutral (A) and alkaline (B) streams, respectively. The percentages of variance explained by the first two axes were significant (Monte Carlo permutation tests; $P < 0.05$). Circled numbers in the biplots correspond to OTUs that migrated to the same positions as OTUs from pure cultures. Asp, *Anguillospora* sp.; AT, *Articulospora tetracladia*; TM, *Tetracladium marchalianum*; CA, *Clavariopsis aquatica*; VE, *Varicosporium elodeae*; LAq, *Lemonniera aquatica*; AFi, *Anguillospora filiformis*; TCh, *Tricladium chaetocladium*; DF, *Dimorphospora foliicola*.

abundances of its conidia were recorded. Pictures of fungal DGGE gels are provided in the supplemental material.

CCA of the relationships between dissolved inorganic nutrients and the structure of fungal communities based on conidial morphology showed that the first two axes explained 87% and 68% of the total variance in circumneutral streams (Fig. 2A) and alkaline streams (Fig. 2B), respectively. CCA discriminated three groups of sites (Maceira site, Algeriz and Este 2 sites, and Este 3 and Souto sites) among circumneutral streams and four groups (Seye site, Tescou site, Lère and Lupte sites, and Tauge site) among alkaline streams. Nitrate concentration was the variable that best correlated with the structure of sporulating fungal communities in circumneutral streams (67%), followed by NO_2-N concentration (15%), SRP concentration (15%), and NH_4-N concentration (3%). On the other hand, in alkaline streams, the variable that most explained the differences in fungal communities was SRP concentration (32%), followed by NH_4-N concentration (25%), NO_3-N concentration (25%), and NO_2-N concentration (18%).

CCA of DGGE fingerprints of fungal communities showed that the first two axes explained 69% of all variance in circumneutral streams (Fig. 3A) and 67% of the variance in alkaline streams (Fig. 3B). Stream sites grouped similarly to the groups based on sporulating fungal data (Fig. 2A versus Fig. 3A and Fig. 2B versus Fig. 3B). Also, the variable that most explained the community structure in circumneutral streams was NO_3-N concentration (48%), followed by NO_2-N concentration (21%), NH_4-N concentration (17%), and SRP concentration (15%), while in alkaline streams NH_4-N (37%) was the nutrient that better correlated with fungal communities, followed by SRP (28%), NO_2-N (22%), and NO_3-N (12%).

Bacterial diversity on decomposing leaves. Among circumneutral streams, the most bacterial diversity was found in Algeriz (50 OTUs) and the least diversity was found in Este 3 (44 OTUs), while among alkaline streams the most bacterial diversity was found in Tescou (38 OTUs) and the least diversity was found in Tauge (24 OTUs). Among the circumneutral streams, OTUs 3 and 39 were associated with streams with the highest trophic status (Este 3 and Souto), while OTUs 10 and 14 were detected in streams with low trophic status (Maceira, Algeriz, and Este 2). Among alkaline streams, OTUs 21, 25, and 28 appeared at all sites except Tauge, while OTU 27 appeared only at Lupte and Tauge. Pictures of bacterial DGGE gels are provided in the supplemental material.

CCA ordinations of bacterial communities and environmental variables show that the first two axes explain 67% of the total variance in circumneutral streams (Fig. 4A) and 75% of that in alkaline streams (Fig. 4B). Similar to the case for CCA applied to fungal communities from circumneutral streams, NO_3-N was the variable that most correlated with differences in bacterial communities among sites (38%), followed by NO_2-N (23%), NH_4-N (21%), and SRP (18%). In alkaline streams, SRP explained most of the differences between bacterial communities (56%), followed by NO_3-N (18%), NO_2-N (14%), and NH_4-N (11%).

Microbial activity on decomposing leaves. Among circumneutral streams, fungal sporulation rates peaked earlier at Maceira and Este 2 (3 weeks; 5.8×10^5 and 9.8×10^5 conidia g^{-1} AFDM day^{-1}, respectively) and later at Algeriz and Este 3 (4 weeks; 12×10^5 and 9.3×10^5 conidia g^{-1} AFDM day^{-1}, respectively) (Fig. 5A). In the Souto stream, the sporulation rate did not exceed 1.1×10^5 conidia g^{-1} AFDM day^{-1} over

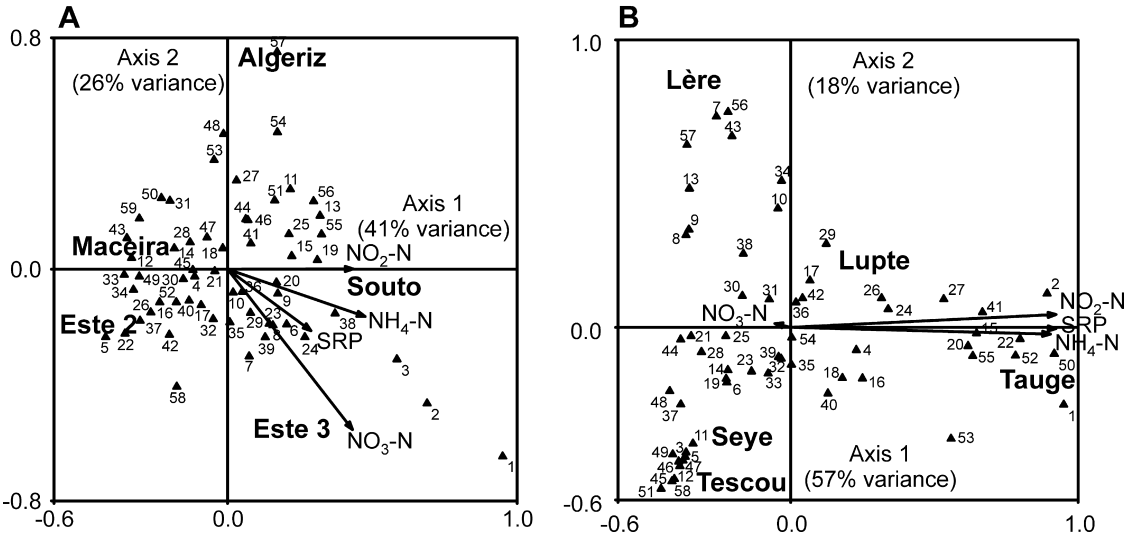

FIG. 4. CCA diagrams for ordination of inorganic nutrients in the stream water and of the 59 and 58 bacterial OTUs from DGGE fingerprints in the circumneutral (A) and alkaline (B) streams, respectively. The percentages of variance explained by the first two axes were significant (Monte Carlo permutation tests; $P < 0.05$).

11 weeks. Among alkaline streams, sporulation peaks occurred after 1 week in leaves submerged in the Lère and Lupte streams (11×10^5 conidia g^{-1} AFDM day^{-1} for both streams) and after 3 weeks in the Seye and Tescou streams (8.1×10^5 and 6.1×10^5 conidia g^{-1} AFDM day^{-1}, respectively) (Fig. 5B). For the Tauge stream, the maximum sporulation rate was 7.2×10^4 conidia g^{-1} AFDM day^{-1}. Fungal sporulation differed between sites and times, and the interaction between the two factors was significant for both groups of streams (two-way ANOVA; $P < 0.001$). Mean sporulation rates were significantly lower on leaves decomposing in the Este 3 and Souto streams than in the other circumneutral streams (Tukey's test; $P < 0.01$), while the Tauge stream had the lowest values among the alkaline streams (Tukey's test; $P < 0.01$). Mean sporulation rates were negatively correlated with NO_2-N ($r = -0.43$; $P = 0.02$), NH_4-N ($r = -0.52$; $P = 0.003$), and NO_3-N ($r = -0.43$; $P = 0.02$) concentrations in the stream water in circumneutral streams, while in alkaline streams they were negatively correlated with the NO_2-N ($r = -0.40$; $P = 0.03$) and NH_4-N ($r = -0.45$; $P = 0.01$) concentrations.

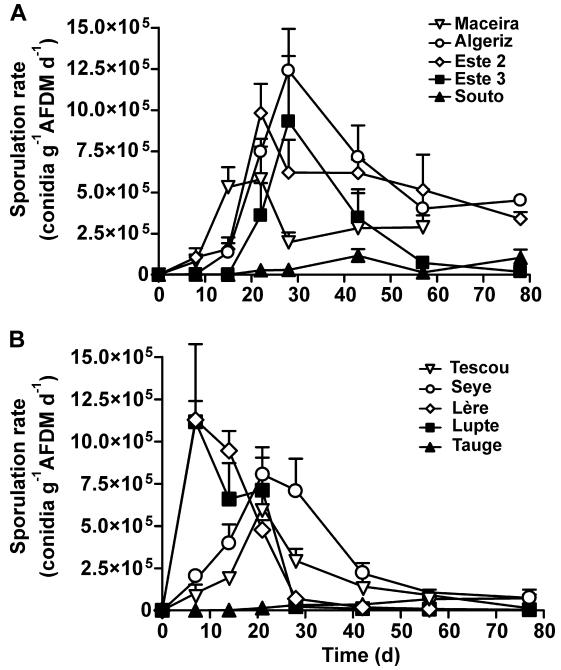

FIG. 5. Sporulation rates of aquatic fungi on leaves decomposing at the five circumneutral (A) and five alkaline (B) streams. Data are means plus standard errors of the means (SEM) ($n = 4$).

In circumneutral streams of higher trophic status (Este 2, Este 3, and Souto), leaf-associated fungal biomass followed a bimodal curve, with a short peak after 1 week and a more pronounced and wider peak spanning the last 9 weeks (72, 46, and 50 mg g^{-1} AFDM at Este 2, Este 3, and Souto, respectively) (Fig. 6A). For the Algeriz stream, only a wide peak was observed (69 mg g^{-1} AFDM), whereas for the Maceira stream a plateau was observed between the second and sixth weeks (32 to 46 mg g^{-1} AFDM), followed by an increase after 8 weeks (82 mg g^{-1} AFDM). In the eutrophic alkaline streams, Lère and Lupte, fungal biomass peaked after 2 weeks (74 mg g^{-1} AFDM) and 3 weeks (96 mg g^{-1} AFDM), respectively (Fig. 6B). In the remaining alkaline streams, fungal biomass reached a plateau after 1 (Tauge), 2 (Seye), and 4 (Tescou) weeks, corresponding to 11, 30, and 35 mg g^{-1} AFDM, respectively. Fungal biomass differed between sites and times of leaf immersion, and site and time interactions were significant for both stream groups (two-way ANOVA; $P < 0.001$). Mean fungal biomass on leaves was lower in the streams of higher trophic status (Este 3 and Souto) than in the circumneutral sites of lowest trophic status (Tukey's test; $P < 0.001$). Among alkaline streams, mean fungal biomass was lower in the hypertrophic Tauge stream, intermediate in the low-trophic-status Tescou and Seye streams, and higher in the eutrophic Lère and Lupte streams (Tukey's test; $P < 0.001$ for all comparisons), and it increased with the SRP gradient from the Tescou to Lère streams and then

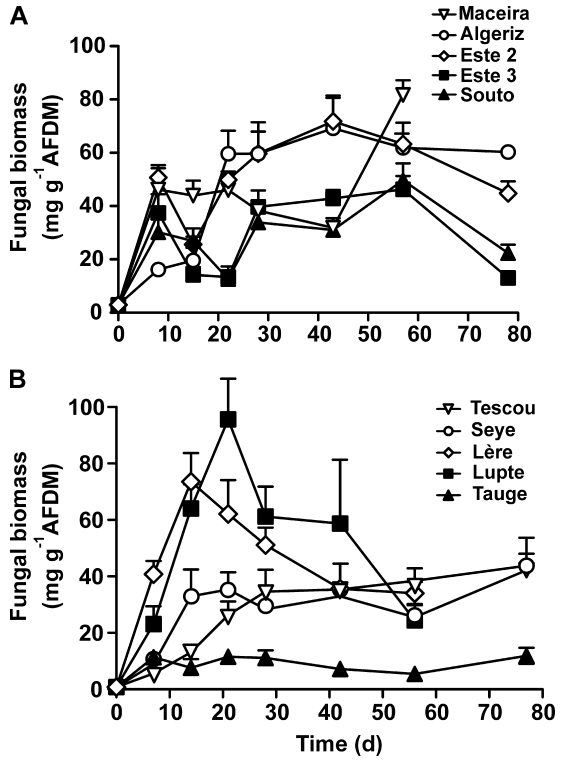

FIG. 6. Biomasses of fungi on leaves decomposing at the five circumneutral (A) and five alkaline (B) streams. Data are means plus SEM ($n = 4$).

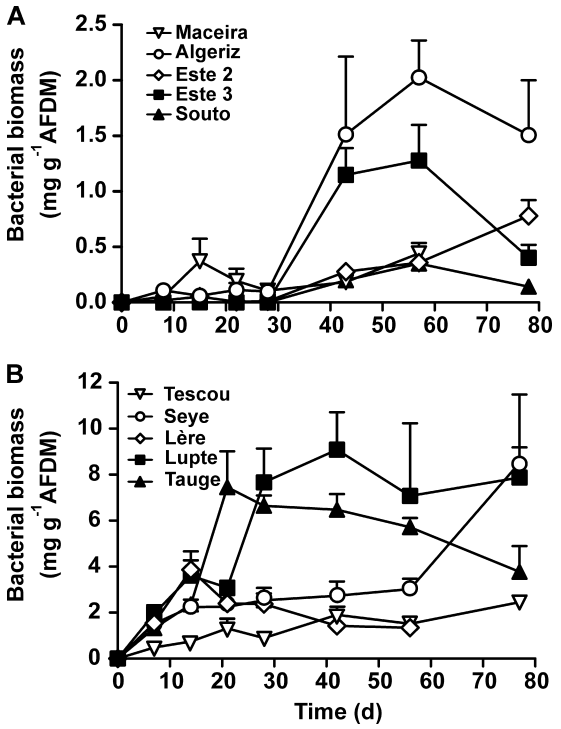

FIG. 7. Biomasses of bacteria on leaves decomposing at the five circumneutral (A) and five alkaline (B) streams. Data are means plus SEM ($n = 4$).

decreased in the Tauge stream. Fungal biomass was negatively correlated with concentrations of NO_3-N ($r = -0.52$; $P = 0.003$), NO_2-N ($r = -0.47$; $P = 0.008$), and NH_4-N ($r = -0.48$; $P = 0.007$) in circumneutral streams, while in alkaline streams no significant correlations were found.

In circumneutral streams, leaf-associated bacterial biomass peaked after 8 weeks, with the highest value for the Algeriz stream (2.0 mg g^{-1} AFDM) and the lowest one for the Souto stream (0.3 mg g^{-1} AFDM), except for the Este 2 stream, in which maximum bacterial biomass (0.8 mg g^{-1}AFDM) was attained after 11 weeks (Fig. 7A). Bacterial biomass on leaves was much higher in alkaline than in circumneutral streams and peaked earlier in the Lère (2 weeks; 3.9 mg g^{-1} AFDM), Tauge (3 weeks; 7.5 mg g^{-1} AFDM), and Lupte (6 weeks; 9.1 mg g^{-1} AFDM) streams than in the Seye and Tescou streams (11 weeks; 8.5 and 2.5 mg g^{-1} AFDM, respectively) (Fig. 7B). Bacterial biomass was affected by time of leaf immersion, stream site, and time-site interactions for both groups of streams (two-way ANOVA; $P < 0.001$). Mean bacterial biomass was higher in the Algeriz and Este 3 streams than in the other circumneutral streams (Tukey's test; $P < 0.05$). Among alkaline streams, mean bacterial biomass increased along the SRP gradient, to reach a plateau in the Lupte and Tauge streams (Tukey's test; $P < 0.05$). Positive correlations were found between bacterial biomass and all nutrient concentrations for alkaline streams ($r = 0.45$ to 0.56; $P = 0.01$ to 0.001), while for circumneutral streams a negative correlation was found between bacterial biomass and NO_3-N ($r = -0.52$; $P = 0.003$).

Mass loss of alder leaves followed a negative exponential model in all streams (not shown). Rates of leaf decomposition differed significantly between sites for both circumneutral and alkaline streams (ANCOVA; $P < 0.0001$) (Table 2). Among the former group of streams, leaf decomposition rates attained the highest value for the Algeriz stream (Tukey's test; $P < 0.05$), while among alkaline streams the highest rates were found for the Seye, Lère, and Lupte streams (Tukey's test; $P < 0.05$). Mass loss was positively correlated with fungal and bacterial biomasses in both circumneutral ($r = 0.58$ and 0.53, respectively; $P < 0.0001$) and alkaline ($r = 0.49$ [$P < 0.0001$] and $r = 0.25$ [$P = 0.006$], respectively) streams. No significant correlations were found between leaf breakdown rates and any of the inorganic nutrients in the stream water.

TABLE 2. Decomposition rates (k) of alder leaves immersed at two groups of stream sites[a]

Stream site	Mean k (day^{-1}) ± SE	W_0 (%)	r^2
Portuguese streams			
Maceira	−0.0111 ± 0.0017[a]	75.8	0.62
Algeriz	−0.0179 ± 0.0013[b]	80.9	0.87
Este 2	−0.0144 ± 0.0021[a,b]	70.2	0.63
Este 3	−0.0115 ± 0.0017[a]	74.4	0.64
Souto	−0.0114 ± 0.0017[a]	72.5	0.62
French streams			
Tescou	−0.0127 ± 0.0021[a]	76.9	0.59
Seye	−0.0263 ± 0.0019[b]	82.8	0.89
Lère	−0.0274 ± 0.0032[b]	72.5	0.73
Lupte	−0.0276 ± 0.0029[b]	75.8	0.77
Tauge	−0.0095 ± 0.0012[a]	80.6	0.70

[a] W_0, intercept; r^2, coefficient of determination. Identical superscript letters indicate no significant differences in each group (ANCOVA and Tukey's test; $P > 0.05$) ($n = 28$).

DISCUSSION

Trophic status and structure of microbial communities. A gradient of eutrophication from oligotrophy to hypertrophy was found in the selected streams, with nitrate and phosphate concentrations defining the trophic status in circumneutral and alkaline streams, respectively. However, the effects of trophic status on ecological processes and associated communities can be modified severely due to the anthropogenic release of possible toxic compounds, such as ammonia and nitrite. Our results showed that inorganic nutrients in the stream water were correlated with changes in the structure of aquatic microbial communities, as indicated by CCA ordination of fungal and bacterial communities on decomposing leaves. In the set of sites that we retained in both geographic areas, remarkable differences were found in the sporulating fungal taxa, even between the two sites in the same stream that differed in trophic status. In circumneutral streams, nitrate concentration, which defined the trophic status, was also the variable that best described the structures of fungal and bacterial communities on leaves, assessed either as sporulating fungi or as DNA fingerprints. Similarly, in alkaline streams, phosphate was the variable that most contributed to explaining the differences in the structures of bacterial and sporulating fungal communities. Hence, the limiting nutrient, either nitrate or phosphate, that defines the trophic status is the main factor that structures the fungal and bacterial communities associated with leaf litter.

Microbial diversity and trophic status. Decreased diversity in sporulating fungi was found on leaves decomposing at sites of higher trophic status for both groups of streams, as generally reported (35, 36, 38). The diversity in fungal mycelia in leaf litter was also affected by nutrient enrichment, although the differences between streams of low and high trophic statuses were less pronounced when assessed by the number of OTUs of colonizing fungi. Fungal sporulation is often reported as the parameter most sensitive to eutrophication (12, 35, 36), and sporulation rates were negatively correlated with nitrite and ammonium concentrations in streams in both geographic areas. Hence, the use of fingerprinting techniques, such as DGGE, that do not rely on fungal reproduction appears to be advantageous to detect dominant and subdominant fungal species on leaves. Both conidium production and molecular typing allowed detection of changes in the structure of fungal communities for both groups of streams. The dominance of *D. foliicola* among sporulating fungi on leaves decomposing at the Este 3 and Souto sites was corroborated by a corresponding OTU, while other correspondences were observed with the ubiquitous *Anguillospora* sp. in alkaline streams and specific species for a subset of sites for both groups of streams. The dominance of *Anguillospora* sp. in all alkaline streams suggests that its dominance is related to the alkalinity and/or the high nitrate concentration. In contrast, the presence of other dominant species for a subset of sites for both groups of streams is probably related to the trophic status of these sites. For example, *D. foliicola*, which has consistently been found in polluted streams of northwest Portugal (10, 35–37), and *T. marchalianum* were dominant species at the most anthropized sites in circumneutral and alkaline streams, respectively. The presence of different species in each subset of streams within each group may be related to other environmental factors, such as the higher temperature found in circumneutral Portuguese streams. Our results suggest that some species may tolerate or develop adaptation to high nutrient concentrations in the stream water, allowing them to prevail over other species in colonizing leaf litter and/or by producing larger numbers of conidia. This also points to the potential application of aquatic hyphomycetes as indicators of water pollution, as suggested by others (15, 34, 38). Fungal colonization of downstream substrates and the subsequent decomposition process depend on conidium production. This parameter could be used as a bioindicator of water quality in combination with species identification to discriminate between streams of low and high trophic statuses. However, seasonal fluctuations of aquatic hyphomycetes on decomposing leaves occur (30), and further studies are needed to assess the potential use of this group of fungi in water quality assessment.

Bacterial taxa are less known than fungal taxa for lotic systems, but bacterial abundance and activity on decomposing leaves are often reported to be stimulated by increased nutrient concentrations in the stream water (2, 10, 33–35). In our study, differences in bacterial community structure were also found between sites of low and high trophic statuses in both circumneutral and alkaline pH streams. Although we did not identify the bacterial species on leaves decomposing at the different streams, there was clear evidence of the presence of unique OTUs at specific stream sites. Modifications in the composition of the bacterial plankton community in a lake have been attributed to water chemistry, including the eutrophication status (44). Because bacteria and fungi interact during leaf decomposition (27), more studies are needed to determine the effects of microbial interactions and nutrient enrichment on the community structures of both groups of microbes.

Microbial activity, microbial diversity, and dissolved inorganic nutrients. In both geographic areas, microbial biomass was positively correlated with leaf mass loss. In addition, the highest level of diversity in fungi was found in circumneutral streams of intermediate trophic status, according to the disturbance-based model (6, 42). In contrast, fungal diversity levels were similar in all alkaline streams, but assemblages with different dominant species were present in streams of intermediate trophic status, as the abundances of two dominant species followed two reverse gradients over the trophic status range and two other species were present only in eutrophic streams. This strongly suggests that the different combinations of species selected by the anthropogenic release of nutrients (see above) have an impact on the decomposition process. Fungal biomass was stimulated in eutrophic alkaline streams (Lère and Lupte), but the hypertrophic sites (Souto and Tauge) had depressed fungal biomasses for both groups of streams. Similar to the observations of the current study, nitrate and phosphate concentrations have been reported to differently affect fungal biomass (2, 26, 34, 35). High concentrations of other compounds, namely, nitrite and ammonia, at hypertrophic sites were probably responsible for the reduction in fungal activity, as suggested by Baldy et al. (2). Indeed, we found that fungal biomass and reproduction correlated negatively with nitrite and ammonia concentrations in the stream water. However, fungal biomasses (almost 100% of *D. foliicola* within the sporulating community) were similar at the Este 3 and Souto sites,

despite the fact that the mean nitrite, ammonium, and ammonia concentrations were 3-, 10-, and 20-fold higher, respectively, in the latter stream, suggesting that *D. foliicola* is somewhat insensitive to these compounds. In contrast, at the Lupte stream, where six dominant sporulating species were present, fungal biomass was almost sixfold higher than that in the Tauge stream (with four dominant sporulating species, of which 60% were *Anguillospora* sp. conidia), where nitrite and ammonia concentrations were two- and sixfold higher, respectively, suggesting that in alkaline streams the fungal species were quite sensitive to these compounds.

Leaf decomposition was faster at sites with moderate concentrations of inorganic N and P and slower at sites with higher or lower N and P concentrations (Table 2). The range of limiting nutrient concentrations corresponding to higher breakdown rates was wider for alkaline streams than for circumneutral streams. This could be related to the dominance of *Anguillospora* sp. in all alkaline streams and the absence of a ubiquitous dominant species in circumneutral streams. When no confounding factors are present, leaf decomposition rates follow linear or Michaelis-Menten models as a function of the nitrate (12) or phosphorus (15) concentration in the stream water. However, such conditions are rarely found in nature due to the spreading of human activities. In the present study, the high concentrations of ammonia and nitrite could modulate the positive effects of phosphorus or nitrate on leaf decomposition and microbial activity in the hypertrophic sites. In a previous study, a decrease in abundance and diversity of invertebrate shredders that feed on coarse particulate organic matter was associated with retarded leaf decomposition. This result was attributed to the deleterious effects of ammonia, but microbial decomposing activity on leaf litter was not affected by the highest ammonium concentration (21). In the current study, concentrations of ammonium were 5 to 32 times higher than those found in the same alkaline streams (2, 21) and 16 to 21 times higher than those found in Portuguese circumneutral streams (33, 37). Ammonium did not appear to be toxic to *Corynebacterium glutamicum* (*Actinobacteria*), *Escherichia coli* (*Proteobacteria*), and *Bacillus subtilis* (*Firmicutes*) (29). However, species that play a significant role in plant polymer decomposition belong to other genera (7) and could be sensitive to ammonium. Besides, Hess et al. (19) reported that ammonium toxicity limits the growth of *Saccharomyces cerevisiae* at low concentrations of potassium. In the alkaline streams, the potassium concentration was 1 to 3 mg liter^{-1} (unpublished data), which is about 1/250 of the physiological concentration at which yeast growth was not impaired by ammonium (19). Although aquatic hyphomycetes that are the main fungal decomposers of leaf litter belong to other groups, the high concentrations of ammonium in the hypertrophic Souto and Tauge streams might be toxic for fungal decomposers in streams with low potassium concentrations. Such a toxic effect would decrease their degradation capacity and could participate in reducing their diversity.

In conclusion, we present the following answers to the questions raised in this study. First, nitrate or phosphate, which defined the trophic status, was the main factor that structured the communities of microbial decomposers that use leaf litter as a carbon resource. Second, anthropogenic increases of the limiting nutrient stimulated microbial activity, which combined with either high community diversity or unique species assemblages resulted in increased leaf mass loss. The absence of simple relationships between trophic status and the litter breakdown rate could be related to the toxic effect of ammonium in low-potassium waters.

ACKNOWLEDGMENTS

We thank Y. Nicaise, D. Lambrigot, and D. Dalger for expert technical assistance and I. Fernandes, D. Duarte, and L. Soares for field assistance.

This research was supported by the Centre National de la Recherche Scientifique and by the Centre of Molecular and Environmental Biology. S. Duarte was supported by the Portuguese Foundation for Science and Technology (SFRH/BD/13482/2003).

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