Response of biofilm-dwelling nematodes to habitat changes in the Garonne River, France: influence of hydrodynamics and microalgal availability

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Abstract Lotic epilithic biofilms are submitted to seasonal disturbances (e.g. flood events, self-detachment), which influence the biomass, diversity and viability of their algal and bacterial communities. The objective of this study is to examine whether (1) biofilm-dwelling nematodes respond to such seasonal changes in terms of diversity and community structure, (2) nematode species and feeding-types distribution respond to the varied trophic situations within the biofilm, since variations in biofilm microalgal composition may represent a variation in available food. The biofilm-dwelling nematode community was monitored in a temperate river over an 18 month period with a high sampling frequency. These data were linked to environmental abiotic and biofilm biotic factors. Nematode density was positively correlated to biofilm and microalgal biomass, but was dampened by floods. A clear seasonal pattern of the community was detected (summer shift), so that two nematode groups stand out: (1) the epistrate-feeders *Chromadorina bioculata* (Schultze *in* Carus, 1857) and *Chromadorina viridis* (Linstow, 1876) were primarily related to diatom availability, and dominated the nematode assemblage most of the time, (2) seven species from various feeding types (deposit-feeders, suction-feeders and chewers) grew mainly under summer conditions concomitantly to a change of biofilm trophic status and microalgal composition. Overall, the results suggested that, in addition to abiotic disturbances, the availability of potential preys in the biofilm might represent an important driver of nematode community patterns.

Keywords Nematodes · Periphyton · Diversity · Feeding types · Algae · Environmental factors

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Introduction

In rivers, any hard submerged substrate can be coated by a complex assemblage of organisms (e.g. bacteria, fungi, algae, heterotrophic protozoans, meiofauna and macrofauna) embedded in a mucous matrix of exopolymeric substances (Costerton, 2000; Leflaive et al., 2008). This organic layer which is named either epilithic biofilm, epilithon, 'Aufwuchs' or periphyton can comprise more than 30% of microalgae in terms of biomass (Peterson, 1996). Consequently, epilithic

biofilms can constitute the main site of primary production in shallow water rivers harbouring hard substrates such as the Garonne in its middle part (Ameziane et al., 2003). These biofilms contribute substantially to benthic food web functioning (Liess & Hillebrand, 2004) and to biogeochemical processes such as decomposition and nutrient retention (e.g. Ford & Lock, 1987; Battin et al., 2003; Teissier et al., 2007). However, epilithic biofilms are unstable habitats, well-exposed to environmental perturbations. Hence they are strongly influenced by seasonal disturbances such as floods (Biggs & Close, 1989) and self-detachment, a temperature-dependent bacterial degradation of the mat (Biggs, 1996; Boulêtreau et al., 2006). These disturbances are recognized to shape the biomass, diversity and viability of the algal and bacterial communities inhabiting the mat (e.g. Peterson & Stevenson, 1992; Lyautey et al., 2010), implying important consequences on the functioning of biofilm processes (Cardinale, 2011).

Free-living nematodes are important protagonists within biofilm communities: on the one hand, epilithic biofilms represent both a habitat and a probable important food resource for them (e.g. Peters & Traunspurger, 2005; Gaudes et al., 2006; Traunspurger et al., 2006; Caramujo et al., 2008). On the other hand, it has been suggested that nematode activity (e.g. through bioturbation and grazing) could affect key biofilm processes: for instance, Mathieu et al. (2007) indicate that nematodes influence the oxygen turnover of artificial diatom biofilms, and Sabater et al. (2003) and Gaudes et al. (2006) highlight that meiofauna (mainly nematodes) can influence the release of unpleasant odorous metabolites (e.g. geosmin) by cyanobacterial biofilms, implying high economic relevance for fishing industry and drinking water production.

Despite their important presence within these habitats, biofilm-dwelling nematodes still remain poorly considered as most nematological studies focus rather on sediment-dwelling nematodes (Traunspurger et al., 2006). As a matter of fact, most information on biofilm-dwelling nematodes has issued from lentic environments: e.g. spatial distributional patterns and colonization pathways (Traunspurger, 1992; Peters & Traunspurger, 2005; Peters et al., 2005). So far, only two previous studies have examined temporal distribution of biofilm-dwelling nematodes in running waters during relatively short periods (Gaudes et al., 2006; Caramujo et al. 2008). But, long-term studies of biofilm-dwelling nematodes are still lacking, which hampers the assessment of how epilithic nematode communities react and adapt to recurrent (seasonal) abiotic disturbances and/or to fluctuations of food resources over time.

In this context, the questions put forward in this study are: (1) In temperate areas, epilithic biofilms are subject to seasonal temperature changes and hydrological events, which, as mentioned above, change their biomass and the composition of the algal and bacterial communities. Is the biofilm-dwelling nematode community influenced by such seasonal changes of their habitat? (2) As variations in composition of the microalgal community may represent a variation in available food within the mat (in terms of amount, availability and quality), do the nematode species and feeding-types distribution match with the biofilm trophic situation at a given time? With these objectives, density, biomass, diversity, age, sex and feeding types of the biofilm-dwelling nematode community was monitored over an 18 month field survey in a large temperate river: the Garonne (SW France). These data were analysed to detect potential seasonal changes, then the nematode species distribution was examined through the influence extent of both environmental abiotic drivers and biofilm biotic conditions.

Methods

Study site and sample collection

The Garonne is the largest river of south-western France with a drainage basin of 57,000 km^2 and a length of 647 km. The Garonne River displays a pluvio-nival flow regime with relatively short flash-floods caused by heavy rainfall (occurring mainly between November and January) and a long annual flood period due to snow-melt (April to June). In the Garonne, alternate cobble bars are frequently found even in channel up to the seventh-order. Between floods (i.e. low-water periods), a high epilithic biomass can grow on cobbles, being favoured by low-water velocities on the river bed and low turbidity (Boulêtreau et al., 2006). The study site was situated on one of these cobble bars located at 36 km upstream

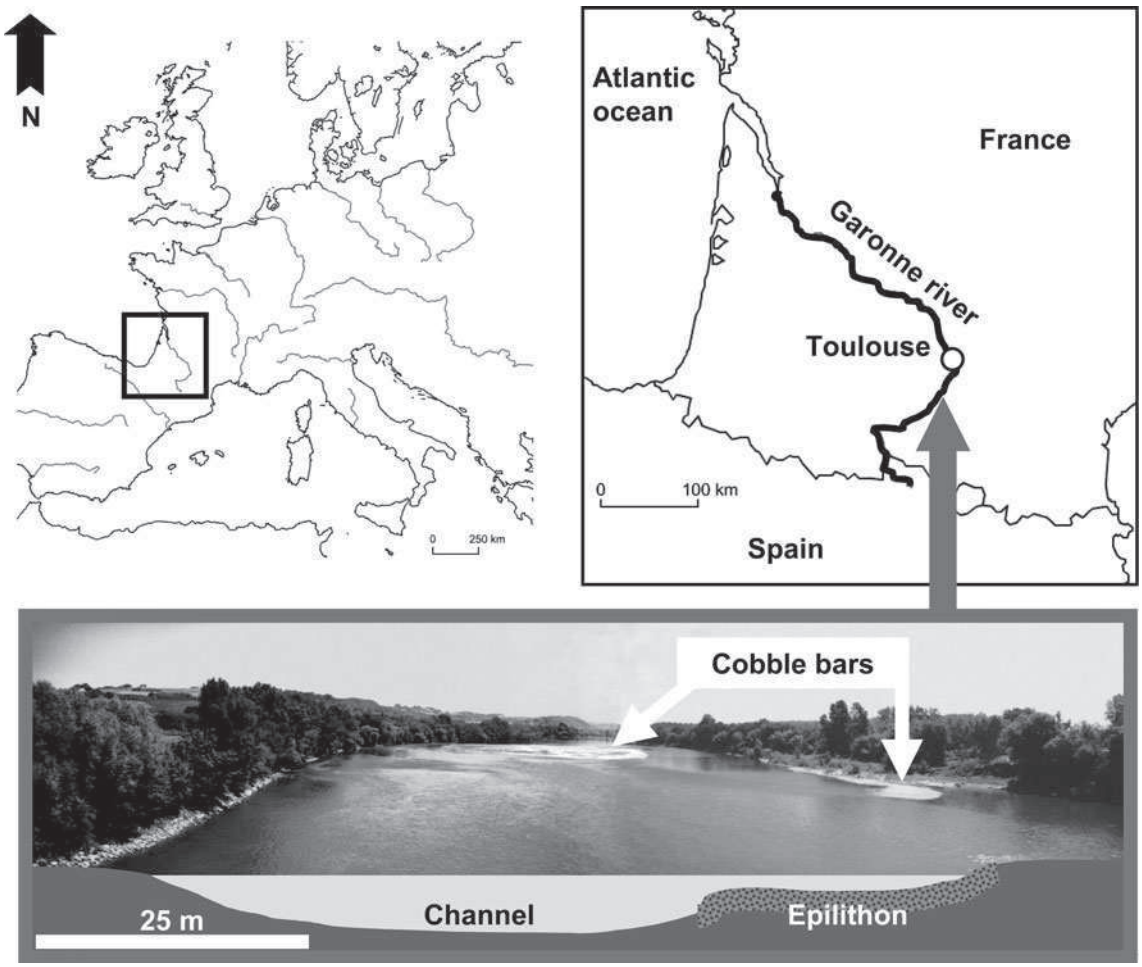

Fig. 1 Location of the sampling site and cross-section view of the Garonne River at the sampling site

the city of Toulouse (01°17′53″E, 43°23′45″N; elevation 175 m a.s.l.), where the Garonne is of sixth-order (Fig. 1).

Samplings ($N = 51$) were regularly performed from September 2008 to March 2010 when hydrological conditions permitted it (sampling was only possible when discharge was lower than 175 $m^3\ s^{-1}$). On each sampling occasion, 12 immerged cobbles (mean diameter: 10 cm) were collected underwater using plastic bags to prevent any biofilm detachment during removal. To consider water level changes and depth where the biofilm typically develops (Amezianne et al., 2002), cobbles were collected on a cross-section from a reference point in the riverside so that water height above cobbles remained between 30 and 50 cm. Collected cobbles were transported to the laboratory within 2 h in cool boxes with minimal disturbance. The biofilm was gathered by scraping the upper surface of each cobble with a scalpel and a toothbrush. Biofilm samples were finally suspended in MilliQ water to obtain 12 biofilm suspensions (25 ml each), in which algal aggregates were carefully crumbled with scissors. These 12 biofilm suspensions were used for the three following treatments: (1) nematode species identification and density and biomass measurements, (2) HPLC analyses of microalgal pigments and (3) epilithic ash-free dry mass (AFDM) measurements. Four replicate suspensions were used for each treatment. Scraped cobbles were photographed, and the surface of biofilm which had been removed was clearly visible and measured using ImageJ software version 1.38 (Abramoff et al., 2004). Removed

biofilm surfaces were then reported to corresponding biofilm suspension volumes, so as densities, biomass and pigment concentrations were quantitatively expressed per area unit.

Nematode processing

Nematodes were extracted from four replicate biofilm suspensions using a modified gravity gradient centrifugation technique involving Ludox HS-40 after Pfannkuche & Thiel (1988). Nematodes so extracted were cleaned from Ludox by sieving through a 40 µm sieve, then preserved in formaldehyde (5% final concentration) and stained with 1% Rose Bengal. Nematodes were counted in a Dolfuss cell (Elvetec services, Clermont-Ferrand, France) under a Leica MZ 9.5 stereomicroscope (9×–90×) and their density was expressed per cm^2. According to nematode density, between 12 and 25 individuals were randomly picked up from each replicate while counting, transferred to glycerol solution (Seinhorst, 1959), mounted on slides and identified to the best species level using a Leitz Dialux microscope at 1250× magnification.

Nematodes were classified according to their age (juveniles, fourth stage juveniles and adults), their sexual category (females, gravid females and males), and their feeding type (epistrate-feeders, deposit-feeders, suction-feeders and chewers) after Traunspurger (1997). The Maturity Index (MI) was calculated on each sampling occasion as the weighted mean frequency of individual colonizer–persister values (cp) after Bongers (1990). MI ranged from 1 to 5. Nematode species with a cp = 1 were considered r-strategists (colonizers) with short-generation times, high fecundity and extreme population changes whereas those with a cp = 5 were defined as K-strategists (persisters) with lower breeding efficiency. The MI is expected to decrease during disturbed periods, when opportunistic nematodes are favoured (Bongers & Bongers, 1998). Over a 1-year period from September 2008 to September 2009 ($N = 37$), at least 100 individual nematode body dimensions (length and maximum width) were measured on each sampling occasion from microscopic pictures taken while counting. Mean individual wet weight (WW) was then determined after Andrássy (1956).

Abiotic environmental factors

Mean Daily Discharge (MDD) was supplied by a gauging station of the French water management authority (DIREN Midi-Pyrénées, Marquefave station) located at 10 km upstream the study site—with no tributary and no dam between the gauging station and the study site. The Mean Weekly Discharge (MWD) before each sampling occasion was considered in statistical analysis. To better reflect the effect of flood disturbance, days after flood (DAF), which were effective days after the last flood (MDD > 300 $m^3\ s^{-1}$), were calculated for each sampling occasion and considered in statistical analysis. Water temperature, conductivity, pH and dissolved oxygen concentration were measured every 30 min during the whole study period with an automated multi-parameter probe (YSI 6000, YSI inc., Yellow springs, OH, USA) which was permanently settled at 5 cm above the streambed at the study site.

Biofilm microalgal composition and biomass

Microalgal pigments extraction and HPLC-analysis

On each sampling occasion, four replicate biofilm suspensions were centrifuged (3,220 g, 20 min). Pellets were freeze-dried and thoroughly homogenized. Then, 250 mg aliquots were removed from each pellet. Algal pigments from each pellet aliquot were then extracted three times (15 min at −20°C) with a total of 25 ml (10, 10 and 5 ml) 98% cold-buffered methanol (with 2% of 1 M ammonium acetate) following Buffan-Dubau & Carman (2000b). Algal pigment release was favoured at each extraction step by an ultrasonication probe (Sonifier 250A, Branson Ultrasonics corp., Danbury, CT, USA).

One millilitre of the pigment solution so obtained was then filtered on 0.2 µm PTFE syringe filter and analyzed using a high-performance liquid chromatograph (HPLC) consisting of a 100 µl loop autosampler and a quaternary solvent delivery system coupled to a diode array spectrophotometer (LC1200 series, Agilent Technologies inc., Santa Clara, CA, USA). The mobile phase was prepared and programmed according to the analytical gradient protocol described in Barlow et al. (1997). Pigment separation was performed through a C8, 5 µm column (MOS-2

Table 1 CHEMTAX pigment ratio matrix

Algal group	Species	Biomarker pigment ratios to Chl *a*								
		Fuco	Lut	Viola	Diad	Zea	*β*-car	Chl *a*	Chl *b*	Chl *c*
Green algae	*P. borianum*		0.143	0.049		0.014	0.043	1	0.088	
Diatoms	*N. palea*	0.477			0.102		0.002	1		0.121
Cyanobacteria	*S. leopoliensis*					0.411	0.011	1		

Ratios were calculated considering the relative concentrations of fucoxanthin (Fuco), lutein (Lut), violaxanthin (Viola), diadinoxanthin (Diad), zeaxanthin (Zea), *β*-carotene (*β*-car), chlorophyll *b* (Chl *b*) and chlorophyll *c* (Chl *c*) versus chlorophyll *a* (Chl *a*) concentrations from corresponding microalgal cultures. For green algae and diatoms these ratios were obtained from pure cultures of, respectively, *Pediastrum boryanum* and *Nitzschia palea*. For cyanobacteria, pigment ratios were obtained from *Synechococcus leopoliensis* (Schlüter et al., 2006)

HYPERSIL, Thermo Fisher Scientific inc., Waltham, MA, USA). The diode array detector was set at 440 nm to detect carotenoids, and at 665 nm to detect chlorophylls and pheopigments (Wright et al., 1991). Data analysis was performed using ChemStation software (version A.10.02, Agilent Technologies inc.). Pigments were identified by comparing their retention time and absorption spectra with those of pure standards pigments (DHI LAB products, Hørsholm, Denmark). Each pigment concentration was calculated by relating its chromatogram's peak area with the corresponding area of calibrated standard.

Microalgal cultures and chemotaxonomy

Algal pigment analysis by HPLC coupled with chemotaxonomic analysis using CHEMTAX program (Mackey et al., 1996) has proven to be a fast and precise method to determine the biomass of phytoplanktonic and microphytobenthic groups in marine and freshwater environments (e.g. Schlüter et al., 2006; Caramujo et al., 2008; Lionard et al., 2008). As reported by Leflaive et al. (2008), microalgal groups inhabiting epilithic biofilms of the Garonne River are diatoms, green algae and cyanobacteria. The biomarker pigment composition found in the biofilm can be used to estimate the biomass of each of these microalgal groups by chemotaxonomy. Prior to the chemotaxonomic analysis, biomarker pigment ratio to chlorophyll *a* (Chl *a*) for each microalgal group has to be obtained. Thus, a green algae species, *Pediastrum boryanum* (Turpin) Meneghini (strain Pedbo01) and a diatom species, *Nitzschia palea* (Kützing) W. Smith (strain Nitpa01) were isolated from the biofilm of the Garonne River and maintained on Combo medium (Kilham et al., 1998) at 18°C (light:dark 16:8, 45 µmol m^{-2} s^{-1}). An aliquot of each algal culture (10 mL) was filtered on 0.7 µm glass fibre filter (GF/F, Whatman, Clifton, NJ, USA) and algal pigments were extracted and analysed from the filters following the same procedure than biofilm samples. Concerning cyanobacteria, pigment ratios calculated by Schlüter et al. (2006) for *Synechococcus leopoliensis* (Raciborski) Komrek (University of Toronto Culture Collection strain 102) were considered.

The biomarker pigment ratio to Chl *a* so obtained were used to supply the initial matrix needed for CHEMTAX analysis (Table 1). Then, CHEMTAX version 1.95 software (Mackey et al., 1996) was run to estimate the biomass of diatoms, green algae and cyanobacteria which were expressed as Chl *a* equivalents and considered as environmental biotic factors in further statistical analysis.

Total epilithic biomass and autotrophic index

On each sampling occasion, four biofilm suspensions were dried at 105°C for 18 h, weighted and then combusted at 450°C for 8 h to weight the ash-free dry mass (AFDM) of the biofilm. The Autotrophic Index (AI) was determined as the ratio AFDM/Chl *a*. This index is commonly used to describe the trophic status of biofilm communities, e.g. higher AI values are found in biofilms with higher proportions of heterotrophs and/or organic detritus (Biggs & Close, 1989).

Statistical analysis

To investigate seasonal changes of the nematode community structure, the differences in biomass, diversity, age, sex, feeding types and MI were

analysed between samples assigned to their corresponding sampling season (i.e. summer: 21 June–21 September, $N = 15$; autumn: 21 September–21 December, $N = 18$; winter: 21 December–21 March, $N = 15$ and spring: 21 March–21 June, $N = 3$). The homogeneity of variance was assessed with Levene's test, and differences were examined either by one-way ANOVA followed by a post-hoc Tukey HSD test or by Kruskal–Wallis ANOVA. The same statistical procedures were applied to investigate seasonal changes of biofilm and microalgal biomass. The correlations between total nematode density and biotic and abiotic factors were investigated by Spearman's rank correlation test. These tests were performed with STATISTICA software (version 8.0, Statsoft inc., Tulsa, OK, USA).

The influence of biotic and abiotic environmental factors on the nematode species distribution was analyzed through canonical ordination analysis with CANOCO software (version 4.5, Biometris, Wageningen, The Netherlands). Rare species (with relative occurrence <0.1%) were not considered in this analysis. Species densities were square-root transformed prior to the analysis. The distribution of nematodes was first analyzed by a detrended correspondence analysis (DCA). As the total inertia observed was less than 2.6, a predominance of linear species response curves could be expected (Ter Braak, 1987, 1994). Therefore, a redundancy analysis (RDA) in which the ordination axes were constrained to be linear combinations of provided environmental factors was used to investigate the relationships between these factors and the distribution of main nematode species. Environmental factors were also listed (conditional effects) according to the variance they explained singly (i.e. without eventual co-variability with other factors). The statistical significance was tested with Monte Carlo permutation test (499 unrestricted permutations) with applying Bonferroni's correction (significance level set at $P < 0.005$).

Results

Dynamics of the epilithic biofilm

The range and annual mean values of each measured abiotic and biotic factor are listed in Table 2. AFDM and Chl *a* content of the epilithic biofilm were significantly positively correlated (Spearman rank: $R = 0.75$; $P < 0.001$) and showed considerable variations throughout the sampling period, being particularly dampened after floods (Fig. 2a). The AI was significantly higher during summer than during the other seasons (ANOVA: $F = 60.2$; $P < 0.001$), implying globally a lower availability of microalgae within summer biofilm communities. Diatoms dominated the epilithic microalgal assemblage over the whole sampling period (Fig. 2b, Table 2). The diatom biomass was significantly higher during winter than during the other seasons (ANOVA: $F = 16.1$; $P < 0.001$). Conversely, cyanobacterial biomass was significantly higher during summer (ANOVA: $F = 4.6$; $P < 0.01$), and green algal biomass was

Table 2 Measured abiotic and biofilm biotic factors

	Annual mean ± SE	Min	Max
Temperature (°C)	14.6 ± 0.05	1.7	27.3
O_2 (mg l^{-1})	11.5 ± 0.02	7.4	22.1
pH (–)	7.6 ± 0.004	6.7	9.1
Conductivity (µS cm^{-1})	270.9 ± 0.001	154	493
Mean daily discharge (m^3 s^{-1})	124.7 ± 6.0	18	814
Days after flood (day)	89.4 ± 11.1	7	233
AFDM (g m^{-2})	27.4 ± 2.7	4.4	79.7
Chlorophyll *a* (mg m^{-2})	321.5 ± 50	10.7	1012.8
Green algae (%)	17.1 ± 2.3	0	36.3
Cyanobacteria (%)	2.2 ± 0.6	0	14.6
Diatoms (%)	80.7 ± 2.7	50.6	100

Annual means refer to 2009. For temperature, O_2, pH and conductivity ($N = 17507$). For days after flood and the biotic factors ($N = 31$). Minimum and maximum values refer to the whole sampling period (i.e. September 2008–March 2010)

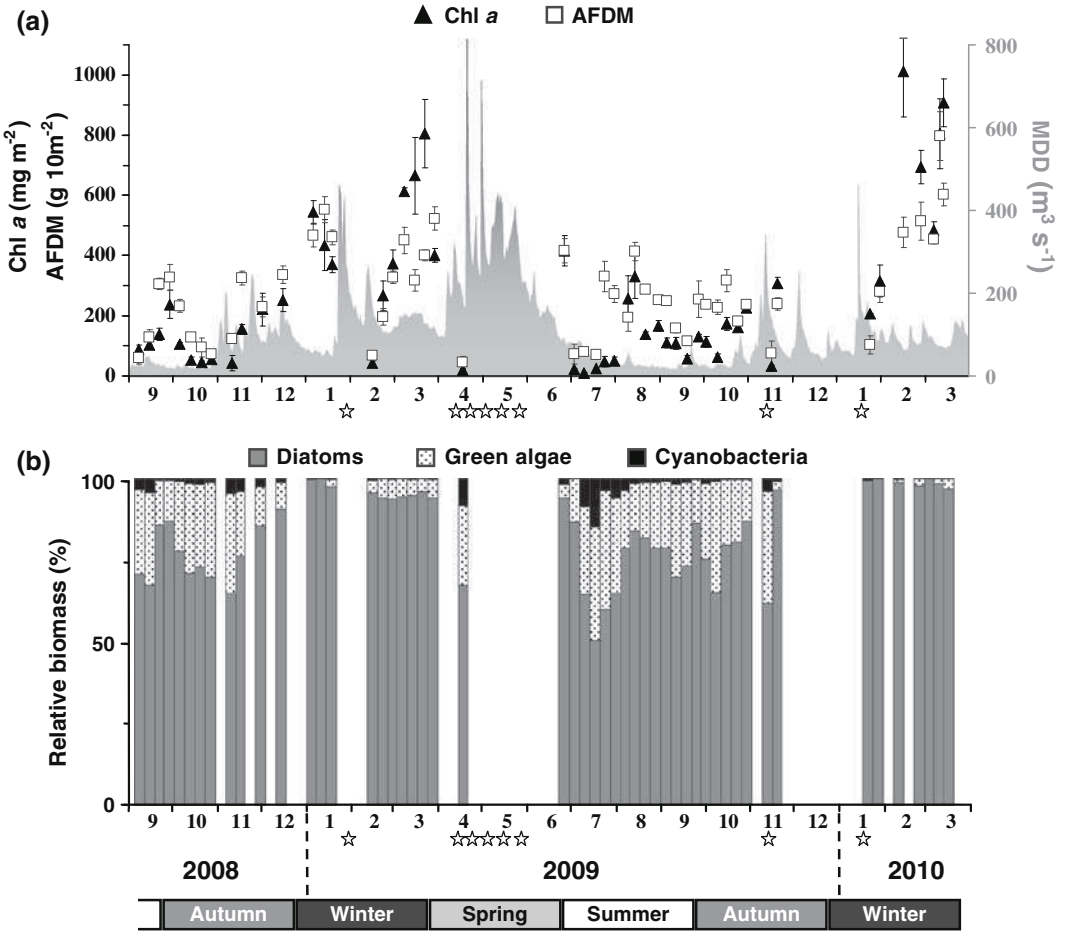

Fig. 2 Temporal dynamics of **a** epilithic chlorophyll *a* (Chl *a*) concentration (±SE, $N = 4$), ash-free dry mass (AFDM) of the biofilm (±SE, $N = 4$) and mean daily discharge (MDD), and **b** the relative proportion (%) of epilithic microalgal groups to total Chl *a* biomass ($N = 4$). Months, years, seasons and floods during which MDD > 300 m^3 s^{-1} (represented by *stars*) are indicated on the *X* axis

significantly higher during summer and autumn (ANOVA: $F = 2.8$; $P < 0.05$) than during the remainder of the year.

Dynamics of biofilm-dwelling nematodes

Over the whole study period, the nematode density averaged 25.4 ± 4.3 ind cm^{-2} and varied greatly throughout the year: the lowest density (0.36 ± 0.14 ind cm^{-2}) occurred in early summer 2009 whereas the highest density (161.36 ± 52.5 ind cm^{-2}) was attained during late winter 2010. As AFDM and Chl *a*, the nematode density was clearly dampened after flood events (Fig. 3a). Nematode density was positively correlated with DAF (Spearman rank: $R = 0.36$; $P < 0.01$), AFDM (Spearman rank: $R = 0.41$; $P < 0.01$) and Chl *a* (Spearman rank: $R = 0.47$; $P < 0.001$). From September 2008 to September 2009, the nematode individual wet weight averaged 0.3 µg. The individual biomass was significantly lower during summer (ANOVA: $F = 14.1$; $P < 0.001$) than during the other seasons (Fig. 3a).

From the 2,875 nematodes identified, 28 species belonging to 11 families were found (see species list in Table 3). Two species: *Chromadorina bioculata* and *Chromadorina viridis* (family Chromadoridae) strongly dominated the assemblage accounting for 86% of all identified nematodes. Although the family Monhysteridae—particularly with species *Eumonhystera dispar*, *Eumonhystera vulgaris* and *Monhystrella*

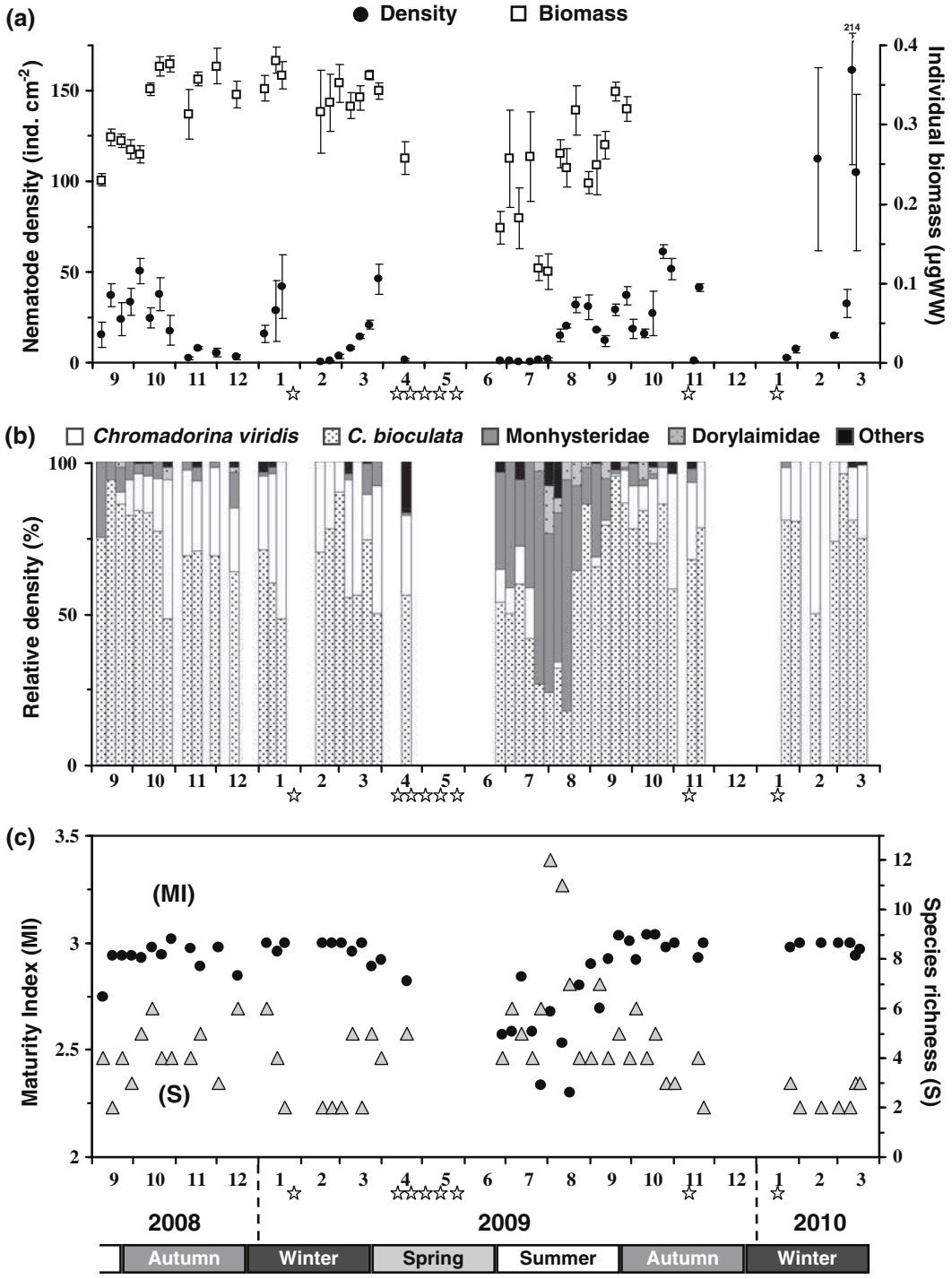

Fig. 3 Temporal dynamics of **a** nematode density (±SE, $N = 4$) and individual wet weight (WW) biomass (±SD, $N \geq 100$), **b** relative density of main nematode taxa, and **c** Maturity index (MI) and species richness (S) in the epilithic biofilm. Months, years, seasons and floods during which MDD > 300 $m^3 s^{-1}$ (represented by *stars*) are indicated on the *X* axis

Table 3 Biofilm-dwelling nematode species in the study site between September 2008 and March 2010

Nematode taxa	%	cp	FT
CHROMADORIDA Filipjev, 1929			
Chromadoridae Filipjev, 1917			
Chromadorina bioculata (Schultze *in* Carus, 1857)	68.87	3	E
Chromadorina viridis (Linstow, 1876)	17.15	3	E
Plectidae Örley, 1880			
Plectus opisthocirculus Andrássy, 1952	0.59	2	D
Plectus aquatilis Andrássy, 1985	0.14	2	D
Plectus rhizophilus de Man, 1880	<0.1	2	D
Plectus cirratus Bastian, 1865	<0.1	2	D
Prismatolaimidae Micoletzky, 1922			
Prismatolaimus cf. *intermedius* (Bütschli, 1873)	<0.1	3	E
Rhabdolaimidae Chitwood, 1951			
Rhabdolaimus aquaticus de Man, 1880	<0.1	3	D
MONHYSTERIDA Filipjev, 1929			
Monhysteridae de Man, 1876			
Eumonhystera dispar (Bastian, 1865)	6.92	2	D
Eumonhystera vulgaris (de Man, 1880)	1.84	2	D
Eumonhystera simplex (de Man, 1880)	0.35	2	D
Eumonhystera barbata Andrássy, 1981	0.31	2	D
Eumonhystera cf. *filiformis* (Bastian, 1865)	<0.1	2	D
Eumonhystera longicaudatula (Gerlach & Riemann, 1973)	<0.1	2	D
Eumonhystera sp.	<0.1	2	D
Monhystrella paramacrura (Meyl 1954)	1.04	2	D
DORYLAIMIDA Pearse, 1942			
Dorylaimidae de Man, 1876			
Mesodorylaimus cf. *subtiliformis* (Andrássy, 1959)	1.04	4	S
Mesodorylaimus sp.	<0.1	4	S
Eudorylaimus sp.	<0.1	4	S
Dorylaimus stagnalis Dujardin, 1845	<0.1	4	S
Mermithidae Braun, 1883			
Mermithidae	<0.1	1	P
ENOPLIDA Filipjev, 1929			
Tobrilidae Filipjev, 1918			
Brevitobrilus stefanskii (Micoletzky, 1925)	0.56	3	C
Tobrilus gracilis (Bastian, 1865)	<0.1	3	C
Tripylidae de Man, 1876			
Tripyla cf. *filicaudata* de Man, 1880	<0.1	3	C
Tripyla glomerans Bastian, 1865	<0.1	3	C
Alaimidae Micoletzky, 1922			
Paramphidelus sp.	<0.1	2	D
TYLENCHIDA Thorne, 1949			
Aphelenchoididae Skarbilovich, 1947			
Aphelenchoides sp.	0.24	2	S
Tylenchidae Örley, 1880			

Table 3 continued

Nematode taxa	%	cp	FT
Coslenchus sp.	<0.1	3	S

The proportion (%) of each species to the total number of identified nematodes ($N = 2875$) is provided. Each species is assigned to its corresponding colonizer–persister value (cp) after Bongers & Bongers (1998) and to its corresponding feeding type (FT) after Traunspurger (1997): epistrate-feeders (E), deposit-feeders (D), suction-feeders (S) chewers (C) and insect parasites (P)

paramacrura—represented only 10% of all identified nematodes over the whole period, they clearly dominated the assemblage from mid-July to mid-August (Fig. 3b). Sixteen species were rare, accounting for <0.1% of all identified nematodes (Table 3). The species richness (S) varied from 2 to 12 species averaging $S = 4.23$ over the whole study period. S was significantly higher during summer (ANOVA: $F = 6.5$; $P < 0.001$) than during the other seasons. Conversely, the Maturity Index (MI) was significantly lower (MI = 2.67) during summer (Kruskal–Wallis ANOVA: $H = 31.5$; $P < 0.001$) than during the other seasons. This summer shift in S and MI is illustrated in Fig. 3c.

Epistrate-feeders—mainly represented by *C. bioculata* and *C. viridis*—dominated representing 86% of nematodes identified over the whole sampling period. Deposit-feeders were the second most observed group representing 12% while suction-feeders and chewers were less common representing, respectively, 1.5 and 0.5%. Insect parasites (i.e. Mermithidae) represented <0.1%. During summer, the epistrate-feeders were significantly less represented (ANOVA: F = 28.5; $P < 0.001$) while deposit-feeders were significantly more represented (Kruskal–Wallis ANOVA: H = 38.7; $P < 0.001$) than during the other seasons (Fig. 4a).

The seasonal proportion of juveniles, fourth stage juveniles, females, gravid females and males is presented in Fig. 4b. Concerning the age structure of the community, adult nematodes averaged 70% of all identified nematodes, while fourth stage juveniles and early instar juveniles contributed, respectively, to 14 and 16%. Early instar juveniles were significantly more represented during spring (ANOVA: $F = 2.8$; $P < 0.05$) than during the other seasons. Concerning the sex structure of the community, females represented 28% (non-gravid females) and 14% (gravid females) against 28% for males. Males contributed

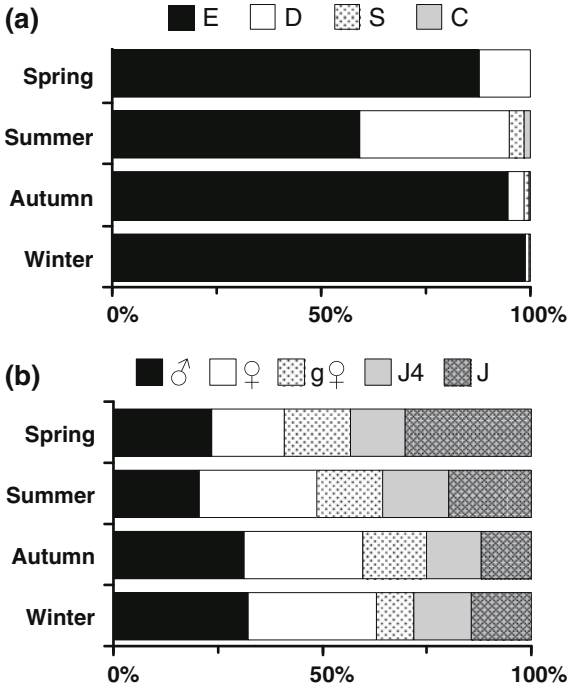

Fig. 4 Seasonal variations of the nematode community structure in the biofilm: **a** seasonal proportion of epistrate-feeders (E), deposit-feeders (D), suction-feeders (S) and chewers (C), and **b** seasonal proportion of males (♂), females (♀), gravid females (g♀), fourth stage juveniles (J4) and juveniles (J)

significantly less during summer (ANOVA: $F = 3.2$; $P < 0.05$) than during winter.

Influence of environmental factors on nematode species distribution

The results of the redundancy analysis (RDA) testing the influence of biotic and abiotic factors on nematode species and feeding-types distribution are presented in Fig. 5 and Table 4. The temporal distribution of nematode species was significantly influenced by

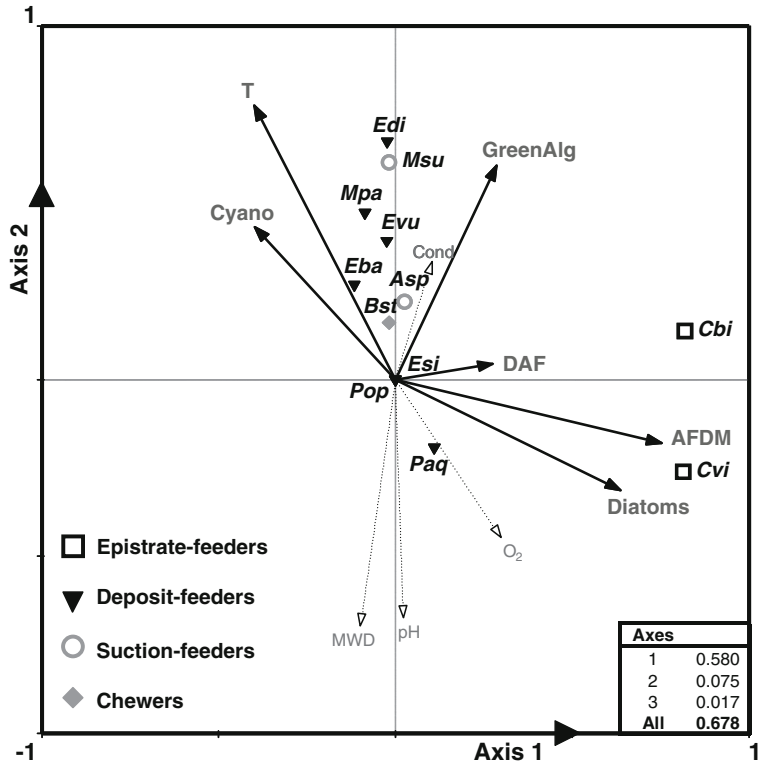

Fig. 5 Biplot from the redundancy analysis (RDA) explaining the distribution of nematode species densities according to environmental factors. Ordination axes were rescaled to range from −1 to 1. *Slim dotted arrows* are non-significant factors. *Bold arrows* are significant factors (Monte Carlo permutation test with Bonferroni's correction, $P < 0.005$). The eigenvalues (λ) are indicated for main ordination axes. Environmental factor abbreviations: biomass of diatoms (Diatoms), green algae (GreenAlg) and cyanobacteria (Cyano), epilithic ash-free dry mass (AFDM), water temperature (*T*), pH, dissolved O_2 (O_2), conductivity (Cond), mean weekly discharge (MWD) and days after flood (DAF). Nematode species abbreviations: *Aphelenchoides* sp. (*Asp*), *Chromadorina bioculata* (*Cbi*), *C. viridis* (*Cvi*), *Eumonhystera barbata* (*Eba*), *E. dispar* (*Edi*), *E. simplex* (*Esi*), *E. vulgaris* (*Evu*), *Brevitobrilus stefanskii* (*Bst*), *Monhystrella paramacrura* (*Mpar*), *Mesodorylaimus* cf. *subtiliformis* (*Msub*), *Plectus aquatilis* (*Paq*) *and P. opisthocirculus* (*Pop*)

temperature, AFDM, DAF and biomass of cyanobacteria, green algae and diatoms. The sum of all significant factor eigenvalues explained 64.1% of the variance. This analysis allowed to clearly distinguish two groups of nematode species: The first group comprised the two dominant epistrate-feeder species *C. bioculata* and *C. viridis*. These two species are situated along axis 1, scoring towards the middle right side of the biplot. Since axis 1 involved mainly factors AFDM, DAF and diatom biomass, this indicated that both species were more abundant during prolonged undisturbed periods with a high biofilm and diatom biomass. The second group comprised deposit-feeders (i.e. *Eumonhystera dispar*, *E. vulgaris, E. barbata*, *Plectus aquatilis* and *Monhystrella paramacrura*), suction-feeders (i.e. *Mesodorylaimus* cf. *subtiliformis* and *Aphelenchoides sp*.) and chewers (i.e. *Brevitobrilus stefanskii*). These species are distributed along axis 2, scoring towards the upper part of the biplot (except for *P. aquatilis*). Since axis 2 involved mainly factors temperature and biomass of cyanobacteria and green microalgae, and since these both microalgal groups were significantly more represented during summer, this indicated that these nematode species were more abundant under summer conditions. No clear trend was observed for the distribution of *Plectus opisthocirculus* and *Eumonhystera simplex*.

Discussion

To the best of our knowledge, the present study is the first long-term monitoring of nematode assemblages inhabiting lotic epilithic biofilms. Although the

Table 4 Conditional effects from the redundancy analysis (RDA)

Factors	λ	P
Diatoms	0.149	0.002**
T	0.138	0.002**
DAF	0.104	0.002**
AFDM	0.102	0.002**
Cyano	0.084	0.004**
GreenAlg	0.064	0.004**
Cond	0.015	0.122
pH	0.013	0.154
MWD	0.006	0.502
O_2	0.003	0.786

Each environmental factor is listed by its eigenvalue (λ) indicating the importance of its own contribution (i.e. without co-variabiliy, see "Methods") to explain the distribution variance of nematodes species. Significant factors (**) at $P < 0.005$ (see "Methods"). Biomass of diatoms (Diatoms), green algae (GreenAlg) and cyanobacteria (Cyano), epilithic ash-free dry mass (AFDM), water temperature (T), pH, dissolved O_2 (O_2), conductivity (Cond), mean weekly discharge (MWD) and days after flood (DAF)

biofilm-dwelling nematode community was not diversified, two groups of species showing different dynamics were clearly distinguished and seemed to adapt to biofilm composition and seasonality: the first group, consisting of the strongly dominating *Chromadorina bioculata* and *C. viridis*, was mainly related to biofilm composition (i.e. age, thickness and diatom content) whereas the second group of species mainly grew under summer conditions.

The nematode density averaged 25.4 ind cm^{-2} and ranged from 0.4 to 161.4 ind cm^{-2} in the epilithic biofilm over the whole study period. This result lies within the range of values reported for lake epilithic biofilms, i.e. 2.8–161.5 ind cm^{-2} (Peters & Traunspurger, 2005) and for river epilithic biofilms, i.e. 10–100 ind cm^{-2} (Gaudes et al., 2006). In our study, the nematode community constituted a permanent component of river epilithic biofilms. Mathieu et al. (2007) suggested that nematode activity could affect the oxygen turnover of diatom biofilms at density values $\geq$50 ind cm^{-2}. This threshold value of density was reached on several occasions during the study period suggesting that this influence was substantial in the epilithic biofilms of the Garonne River.

Nematode density positively correlated with AFDM and Chl *a*. This strengthens the hypothesis that the amount of microalgae and organic matter favour meiobenthic organisms—such as nematodes—in epilithic biofilms (Hillebrand et al., 2002; Peters & Traunspurger, 2005). However, nematode density and biofilm biomass were both clearly dampened after floods (Figs. 2a, 3a). Moreover, the positive relation found between nematode density and DAF pointed out the negative impact of floods on nematode populations. It is well-known that epilithic biofilms are detached by shear stress, substratum instability and abrasive effects of suspended solids during flood events (Biggs & Close, 1989; Boulêtreau et al., 2006). It is thus obvious that nematodes were swept away with the biofilm when flood occurred. This corroborates the studies of Robertson et al. (1997) and Palmer et al. (1996) showing that floods are important factors shaping meiobenthic communities in rivers.

The species richness observed in the present study (i.e. 28 species over the whole study period) agreed with those observed for several lake epilithic biofilms, i.e. 29 and 8–34 species (in, respectively, Traunspurger, 1992; Peters & Traunspurger, 2005). However, higher species richness values were often reported for sediment-dwelling nematodes (see review of Traunspurger, 2002). As previously shown in lakes (Peters & Traunspurger, 2005), our results suggest that, also in rivers, nematode diversity is lower in biofilms than in sediments. Reasons for this diversity difference remain complex and unclear (Hodda et al., 2009). A possible explanation might be that, in the Garonne river, nematodes had to totally re-colonize the biofilm after critical floods several times a year (e.g. in January, April–May and November 2009, Fig. 3a). Conversely, in sediments, meiobenthic organisms can migrate deeper towards less disturbed sediment layers to shelter against increasing discharge conditions (Dole-Olivier et al., 1997). Thus, biofilm-dwelling nematodes could be more exposed than sediment-dwelling nematodes to flood disturbances, which are known to decrease benthic invertebrate diversity (Death & Winterbourn, 1995).

While diatoms dominated biofilm algal assemblages in terms of biomass, two epistrate-feeder species *Chromadorina bioculata* and *Chromadorina viridis* dominated strongly the nematode assemblage. This observation supports the trend previously hypothesized that, in freshwater benthic environments,

nematode communities are generally dominated by few species (e.g. Zullini & Ricci, 1980; Michiels & Traunspurger, 2005a; Peters & Traunspurger, 2005). Furthermore, this corroborates a previous study indicating that the epistrate-feeder *Chromadorita leuckarti* (de Man, 1876) dominates the nematode assemblages in diatom-dominated biofilms of the Llobregat River, Spain (Gaudes et al., 2006). *C. bioculata* and *C. viridis* were clearly segregated from the other nematode species (Fig. 5) and primarily positively related to diatom biomass. Due to their high content of polyunsaturated fatty acids (Phillips, 1984), diatoms are known to represent a high-quality food resource often selected by benthic primary consumers (e.g. Goedkoop & Johnson, 1996; Buffan-Dubau & Carman, 2000a). Furthermore, it has been evidenced that a marine nematode belonging to the *Chromadorina* genus: *Chromadorina germanica* (Bütschli, 1874) feeds on benthic diatoms (e.g. Tietjen & Lee, 1977; Deutsch, 1978). Therefore, it is likely that the presence of large amounts of a potential food resource may favour *C. bioculata* and *C. viridis*. This finding strengthens that nematode feeding strategies match with the availability of their preys within the biofilm.

Our results indicate that a clear shift of the nematode community occurred during summer (Fig. 3b). Such seasonal variations of species composition were previously reported for sediment-dwelling nematode communities in lakes (Traunspurger, 1991; Michiels & Traunspurger, 2005c) and in rivers (Beier & Traunspurger, 2003). In our study, the summer nematode community is more diversified with a higher proportion of deposit-feeders: e.g. Monhysteridae (Figs. 3c, 4a). Concomitantly, the proportion of microalgae in the biofilm (AI) was reduced, but the microalgal community became more diversified. Several hypotheses can be advanced to account for this summer shift:

Firstly, the RDA analysis (Fig. 5) evidenced that a diversified group of nematode species (mainly deposit-feeding species) grew under summer conditions. It is known that summer temperatures enhance the proportion of diversified bacterial assemblages inside epilithic biofilms of the Garonne River (Boulêtreau et al., 2006; Lyautey et al., 2010). Deposit-feeding nematodes can show species-specific feeding response to bacterial and cyanobacterial diversity and availability (Moens et al., 1999; Höckelmann et al., 2004; Schroeder et al., 2010). Therefore, it can be suggested that the higher nematode diversity observed during summer could result from a decrease of interspecific competition while the microbial food resources are more diversified (e.g. cyanobacteria, green microalgae and potentially bacteria), confirming that resource availability can structure nematode species composition and diversity (Michiels & Traunspurger, 2005b; Ristau & Traunspurger, 2011).

Secondly, Michiels & Traunspurger (2003, 2004) observed that the density of predators can increase the number of co-existing nematode species by preventing competitive exclusion due to dominant species. In the present study, the density of the predatory nematode *Brevitobrilus stefanskii* was positively linked to summer conditions (Fig. 5). However, preventing competitive exclusion could also have resulted from macrobenthic predators and grazers (e.g. insect larval stages of Plecoptera, Trichoptera and Ephemeroptera), which are particularly abundant during summer (peaking in early July) in the Garonne River (Leflaive et al., 2008, Majdi et al., unpubl. data).

Thirdly, temperature is known to strongly influence benthic communities in running waters (Hawkins et al., 1997; Stead et al., 2003). When temperature is high, the biomass of the epilithic biofilm remains severely controlled by self-generated detachment processes and grazers (Boulêtreau et al., 2006; Hillebrand, 2009). Moreover, Lawrence et al. (2002) experimentally showed that grazing of phototrophic biofilm by macrobenthic invertebrates resulted in a significant reduction of autotrophic biomass with an increase of bacterial biomass within grazed regions, corroborating the first hypothesis described above. Thus, these disturbances can lead to a thin summer biofilm layer with a high proportion of heterotrophic organisms where intensive competition for space and resources may create harsh life conditions for epibenthic invertebrates. This suggestion is supported by the decrease of the algal proportion in the biofilm observed during this period. Therefore, it makes sense that typical opportunistic and bacterial-feeding nematodes with a small body size and a low MI (e.g. Monhysteridae) could benefit from these harsh conditions. Moreover, Monhysteridae species—especially genus *Eumonhystera*—are known to reproduce parthenogenetically (Traunspurger, 1991). This reproductive strategy probably accounted for the significant reduction of the male proportion observed during summer (Fig. 4b). Overall, summer nematode species

lifestyle fits well with corresponding biofilm biotic conditions, suggesting that a close coupling occurs between nematode assemblage functional structure and biofilm characteristics.

Conclusion

Biomass of epilithic microalgae constituting potential food sources for nematodes was plainly identified as an important predictor of nematode community dynamics. Overall, our results strongly suggest that variations in microalgal composition and proportion in the biofilm might drive the observed changes in nematode diversity and functional feeding group composition. This supports the hypothesis that nematodes are involved in a strong trophic coupling with their microbial habitat and should be taken into consideration in further studies on biofilm dynamics and functioning. Notably, studies of nematode feeding behaviour could disentangle trophic interactions in epilithic biofilms and their potential feedback on biofilm's structure and composition.

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