THESIS

Meiofauna in river epilithic biofilm:

Dynamics and trophic relationships

Nabil Majdi

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JURY

Evelyne Buffan-Dubau,	MCF, Université Paul Sabatier, Toulouse (FR)
Alain Dauta,	CR, Université Paul Sabatier, Toulouse (FR)
Rutger De Wit,	DR, Université Montpellier 2, Montpellier (FR)
Tom Moens,	Pr., Universiteit Gent, Gent (BE)
Anne L. Robertson,	Pr., University of Roehampton, London (GB)
Michèle Tackx,	Pr., Université Paul Sabatier, Toulouse (FR)
Loïc Ten-Hage,	MCF, Université Paul Sabatier, Toulouse (FR)
Walter Traunspurger,	Pr., Universität Bielefeld, Bielefeld (DE)

Ecole doctorale : **Sciences de l'Univers, de l'Environnement et de l'Espace (SDUEE)**

Unité de recherche : **EcoLab UMR 5245**

Directrices de Thèse : **Michèle Tackx et Evelyne Buffan-Dubau**

Rapporteurs : **Rutger De Wit et Tom Moens**

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Avant-propos / Foreword

English readers should be aware that this thesis was not entirely translated (for formatting convention reasons). I apologize for this inconvenience, besides this thesis includes four articles in English preceded by a French summary.

Cette thèse a été financée par le Ministère délégué à l'Enseignement Supérieur et à la Recherche sous forme d'une allocation de recherche doctorale (numéro 31381-2008). Cette thèse a aussi bénéficié du soutien financier du programme national CNRS EC2CO-CYTRIX.
Les prélèvements et les expériences ont été réalisés en milieu naturel sur un même site de la Garonne situé à 36 km en amont de Toulouse (commune de Le Fauga). Aussi, certaines méthodes sont répétées dans les différents chapitres. Pour éviter trop de redondance, les méthodes sont particulièrement détaillées dans le **chapitre II**, et les autres chapitres s'y réfèrent. Les références bibliographiques sont listées à la fin de ce mémoire. Ce mémoire est articulé autour de 4 articles, dont l'état d'avancement au 10 Janvier 2012 est :

I. Majdi, N., B. Mialet, S. Boyer, M. Tackx, J. Leflaive, S. Boulêtreau, L. Ten-Hage, F. Julien, R. Fernandez & E. Buffan-Dubau (**2012**). The relationship between epilithic biofilm stability and its associated meiofauna under two patterns of flood disturbance. **Freshwater Science** 31: 38–50.

II. Majdi, N., W. Traunspurger, S. Boyer, B. Mialet, M. Tackx, R. Fernandez, S. Gehner, L. Ten-Hage & E. Buffan-Dubau (**2011**). Response of biofilm-dwelling nematodes to habitat changes in the Garonne River, France: influence of hydrodynamics and microalgal availability. **Hydrobiologia** 673: 229–244.

III. Majdi N., M. Tackx, W. Traunspuger & E. Buffan-Dubau (**2012**). Feeding of biofilm-dwelling nematodes examined using HPLC-analysis of gut pigment contents. **Hydrobiologia** 680: 219–232.

IV. Majdi N., M. Tackx & E. Buffan-Dubau (**soumis**). Trophic positionning and microphytobenthic carbon uptake of biofilm-dwelling meiofauna in a temperate river. **Freshwater Biology**.

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Liste des abréviations

AFDM	:	Ash-free dry mass
AI	:	Autotrophic index
AU	:	Arbitrary units
C	:	Carbon (ou carbone)
C1	:	Sampling campaign (2004–2006)
C2	:	Sampling campaign (2008–2010)
Chl	:	Chlorophyll (ou chlorophylle)
Chl *a*-eq	:	Chlorophyll a-equivalents
CMPB	:	Carbone microphytobenthique
DAD	:	Daily assimilation demand
DAF	:	Days after flood
DIREN	:	Direction régionale de l'environnement
DM	:	Dry mass
EPS	:	Exopolymeric substances
FPOM	:	Fine particulate organic matter
GPT	:	Gut passage time
HPLC	:	High performance liquid chromatography
ind	:	Individual(s)
MPB	:	Microphytobenthos / Microphytobenthic
MPBC	:	Microphytobenthic carbon
MDD	:	Mean daily discharge
MI	:	Maturity index
MWD	:	Mean weekly discharge
N	:	Nitrogen (ou azote)
RDA	:	Redundancy analysis
SIA	:	Stable isotope analysis
TEF	:	Trophic enrichment factor
V	:	Streambed flow velocity
WW	:	Wet weight

Introduction

1. Le biofilm épilithique

1.1. Définitions

Les biofilms sont des assemblages diversifiés et connexes d'organismes enchâssés dans une matrice d'exopolymères (EPS, *exopolymeric substances*). L'organisation en biofilms permet aux micro-organismes d'adhérer efficacement à des substrats pour y fonder une architecture biologique complexe leur assurant—notamment grâce à la matrice d'EPS—protection, cohésion, sorption des nutriments et régulation physiologique (Neu *et al.*, 2003). De plus, les fonctionnalités écologiques des différents micro-organismes sont souvent complémentaires, permettant une exploitation optimale des ressources disponibles (Marsh & Bowden, 2000; Kreft, 2004). Stoodley *et al.* (2002) et Webb *et al.* (2003) suggèrent que l'organisation des micro-organismes en biofilms soit un premier pas vers la multi-cellularité, de par le développement de comportements complexes et proto-coopératifs (*e.g.* communication cellulaire par *quorum sensing*, création d'un environnement extra-cellulaire homéostatique). D'ailleurs la majeure partie des micro-organismes sont capables de former des biofilms, et ce depuis très longtemps puisque les plus anciens fossiles de biofilms (*e.g.* stromatolithes) datent de l'Archéen, il y a ~3,4 milliards d'années (Westall, 2011).

Tous les milieux aquatiques sont concernés par la formation de biofilms. Dans les zones euphotiques des lacs et des rivières, un biofilm qualifié de « phototrophe » colonise roches, constructions, bois flottés, sédiment superficiel, *etc.* Ces biofilms sont densément peuplés par des organismes photo-autotrophes comme les micro-algues (Peterson, 1996; Romaní, 2010). Du coup, la production primaire assurée par ces biofilms est importante (Vadeboncoeur & Steinman, 2002), notamment lorsque l'hydrodynamique ne permet pas un temps de résidence propice pour le développement du phytoplancton (Reynolds *et al.*, 1994). C'est typiquement le cas dans la Garonne entre sa source et sa confluence avec le Tarn (Fig. 1a), où la canopée est largement ouverte et où de nombreux bancs de galets permettent un développement important de ces biofilms en eau peu profonde (Ameziane *et al.*, 2003; Leflaive *et al.*, 2008). Lorsqu'il colonise ce type de substrats durs on qualifie le biofilm d' « épilithique » (Fig. 1b).

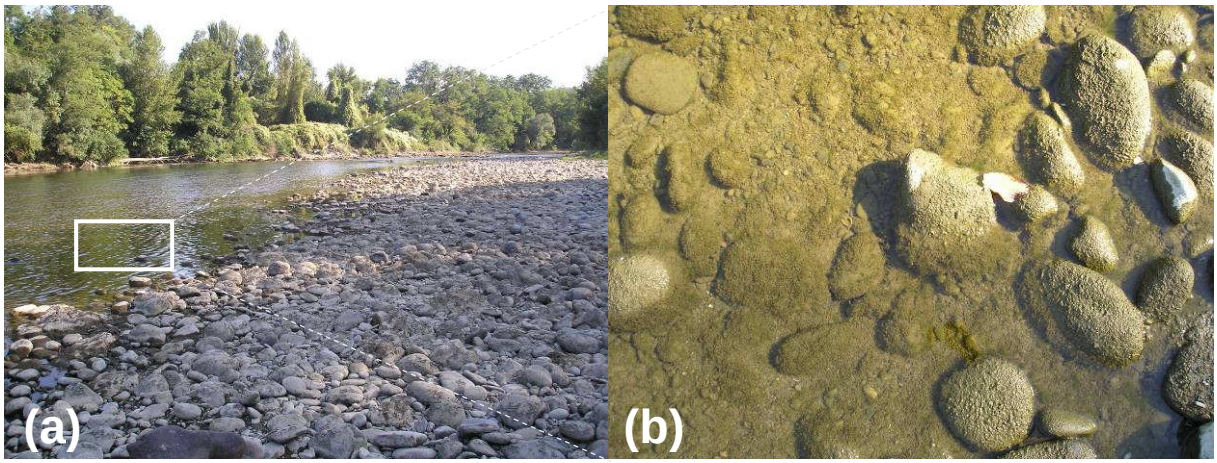

Figure 1. (a) Tronçon de la Garonne situé 36 km en amont de Toulouse, où **(b)** les galets en eau peu profonde sont recouverts d'une épaisse couche de biofilm épilithique.

1.2. Développement

Le biofilm épilithique, de par sa position, est très exposé aux perturbations, surtout dans les systèmes lotiques qui sont particulièrement dynamiques et instables. De ce fait, il est soumis à une alternance entre phases de croissance et de décrochage définies principalement par des contraintes abiotiques comme l'hydrodynamique (Biggs & Close, 1989; Peterson & Stevenson, 1992; Biggs, 1996). Ces perturbations influencent la dynamique de la succession des micro-organismes au cours de la maturation et de l'épaississement du biofilm. Ainsi, après une perturbation induisant un arrachage important du biofilm, le concept de succession écologique s'applique aux microphytes et aux bactéries qui vont recoloniser le biofilm selon leurs caractéristiques morphologiques et physiologiques (Korte & Blinn, 1983; Peterson & Stevenson, 1992; Biggs *et al.*, 1998; Jackson *et al.*, 2001; Lyautey *et al.*, 2005). La stabilité (pérennité) du substrat face à l'hydrodynamique va donc aussi définir les potentialités de développement du biofilm (Cardinale *et al.*, 2002).

Le développement du biofilm épilithique est également lié à l'intensité lumineuse disponible : la présence de canopée, la hauteur d'eau et la turbidité vont fortement influencer la nature de l'assemblage épilithique (Velasco *et al.*, 2003; Roeselers *et al.*, 2007). La disponibilité en nutriments indispensables au métabolisme algal (*e.g.* silicates pour les diatomées) est aussi un facteur déterminant la nature de l'assemblage, au même titre que le pH et la température. Parmi les facteurs biotiques qui vont influencer la composition et le développement du biofilm épilithique, on peut citer : l'allélopathie, le *quorum sensing*, le détachement autogène

ou encore l'activité de broutage et/ou de bioturbation par les invertébrés (Stevenson, 1997; Lawrence *et al.*, 2002; Sabater *et al.*, 2002; Stanley & Lazazzera, 2004; Boulêtreau *et al.*, 2006; Gaudes *et al.*, 2006; Leflaive & Ten-Hage, 2007; Leflaive *et al.*, 2008).

1.3. Rôle fonctionnel

La formation de biofilms peut poser certains problèmes sociétaux : *e.g.* bio-fouling des coques de navires, contamination des réseaux de distribution d'eau potable et des équipements médicaux, *etc.* Les biofilms épilithiques de rivière sont en revanche des auxiliaires utiles, par exemple pour la séquestration des métaux lourds et des polluants xénobiotiques (Kaplan *et al.*, 1987; Wolfaardt *et al.*, 1995; Beck *et al.*, 2011), ainsi que pour le recyclage des nutriments (Burkholder *et al.*, 1990; Mulholland, 1992; Flemming, 1995; Teissier *et al.*, 2007). Naturellement, les microphytes et les bactéries de ces biofilms assimilent et transforment les matières organiques particulaires et dissoutes de la colonne d'eau. Ainsi, le carbone exogène dérivant, en provenance du bassin versant, est partiellement retenu et recyclé par les organismes du biofilm mais aussi au sein même de la matrice d'EPS grâce à une importante activité enzymatique extracellulaire (Lock *et al.*, 1984; Sinsabaugh *et al.*, 1991; Romaní *et al.*, 2004). Les biofilms épilithiques peuvent donc réguler les flux biogéochimiques verticalement : en arbitrant les transferts du milieu pélagique vers le milieu hyporhéique, et horizontalement : en accroissant la connectivité entre zones ripariales et continuum fluvial (Pusch *et al.*, 1998; Battin *et al.*, 2003). La structuration et la diversité de la communauté épilithique joue bien sûr un rôle dans l'efficacité de ces fonctions (Sabater *et al.*, 2002; Romaní *et al.*, 2004; Cardinale, 2011).

La production primaire et la rétention de la matière organique en suspension fait des biofilms épilithiques une base pour le réseau trophique, pouvant ainsi soutenir une importante production secondaire (McIntire, 1973; Fuller *et al.*, 1986; Feminella & Hawkins, 1995; Hillebrand, 2002, 2009). La matrice d'EPS alimentée par les exsudats des microphytes et le piégeage de la matière organique représente la majeure partie de la biomasse du biofilm (Romaní, 2010). Ces EPS sont pour la plupart des composés à faible poids moléculaire, qui sont facilement assimilés et recyclés par la boucle microbienne (Nold & Ward, 1996; Romaní & Sabater, 1999). Les biofilms sont donc une source de nourriture microbienne conséquente et diverse soutenant un réseau trophique complexe mettant en scène nano-flagellés, ciliés et invertébrés (Weitere *et al.*, 2005; Wey *et al.*, 2008; Hillebrand, 2009). L'activité de broutage

des invertébrés peut influencer fortement le fonctionnement des biofilms épilithiques (Sabater *et al.*, 2002). Cependant, la plupart des études trophiques ne considèrent pas les plus petits invertébrés qui pullulent dans ces biofilms : la méiofaune.

2. La méiofaune

2.1. Définitions

Le terme méiofaune a été introduit par Mare (1942) pour désigner les plus petits invertébrés benthiques retenus par une maille de 42 µm, mais passant au travers d'une maille de tamis de 1 mm. Par la suite, la définition de cet intervalle de taille définissant la méiofaune a quelque peu varié selon les auteurs. Cette étude considère un intervalle de taille de 40–500 µm d'après Fenchel (1978) et Giere (2009). Certains invertébrés passent toute leur vie dans cet intervalle de taille : c'est la méiofaune permanente (Fig. 2). D'autres ne passent que les premiers stades de leur développement dans cet intervalle de taille : c'est la méiofaune temporaire. En eau douce, la méiofaune temporaire comprend principalement les premiers stades larvaires d'insectes. Dans cette étude, les invertébrés retenus par une maille de 500 µm sont considérés comme faisant partie de la macrofaune.

2.2. La méiofaune d'eau douce : une communauté injustement négligée

La méiofaune d'eau douce, et notamment les rotifères ont été des sujets d'études populaires depuis que le développement de la microscopie les ont rendus visibles (Van Leewenhoeck, 1677; Hudson & Gosse, 1886). Cet enthousiasme précoce s'est traduit par d'importants travaux taxonomiques durant le 19ème et la première moitié du 20ème siècle. Cependant, après ces débuts prometteurs, l'étude de la méiofaune d'eau douce (et notamment de son écologie) est restée pour le moins marginale relativement à l'avancée de la compréhension du rôle de la macrofaune dans le fonctionnement des écosystèmes. Le même phénomène s'est déroulé en milieu marin, bien que la méiofaune y soit comparativement beaucoup plus étudiée qu'en eau douce (Robertson *et al.*, 2000). Un intérêt croissant s'est manifesté à l'égard de la méiofaune d'eau douce depuis le début des années 90, avec notamment comme point d'orgue la publication d'un numéro spécial de la revue *Freshwater Biology* (2000, volume 44) et la publication des livres *Freshwater Meiofauna* (Rundle *et al.*, 2002) et *Freshwater nematodes:*

ecology and taxonomy (Abebe *et al.*, 2006a). Cependant, de nombreuses zones d'ombre persistent, poussant récemment Giere (2009) à plaider : « *the meiofauna ecology of large rivers requires urgent investigation* ». Gaudes (2011) a très récemment recensé >120 articles se concentrant spécifiquement sur la méiofaune d'eau douce depuis la parution du numéro spécial de *Freshwater Biology* (*i.e.* depuis 12 ans). Elle souligne également que certains thèmes, comme par exemple la dynamique de la méiofaune face aux contraintes environnementales, restent encore très peu explorés. De plus, il est intéressant de noter que, parmi toutes les études menées sur la méiofaune des lacs et des rivières, la vaste majorité ne considère que la méiofaune des milieux interstitiels. Ainsi, les études incluant ou se concentrant sur la méiofaune dans des milieux épibenthiques comme les biofilms épilithiques restent particulièrement rares (Peters, 2005).

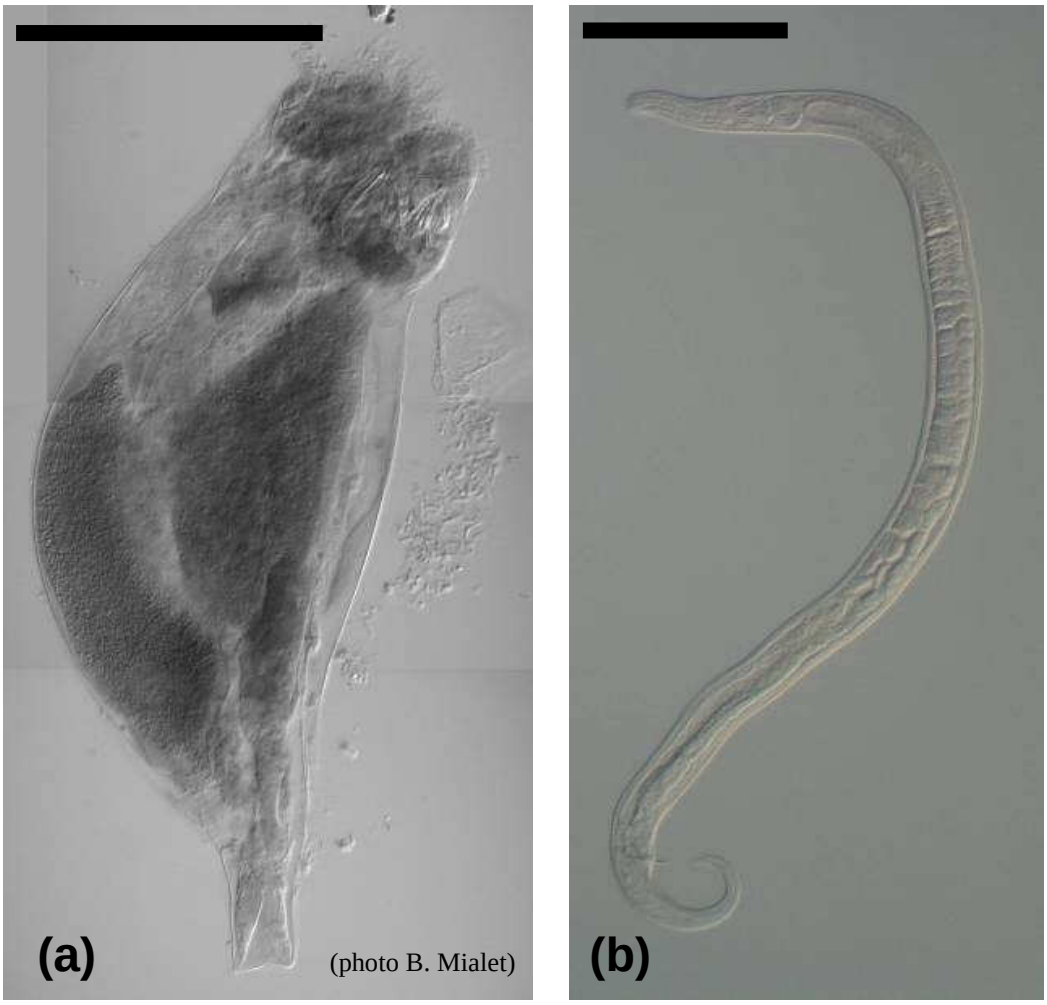

Figure 2. Exemples des 2 principaux groupes de la méiofaune permanente observés dans les biofilms épilithiques de la Garonne : **(a)** Rotifera *Proales sp*., **(b)** Nematoda *Chromadorina sp*. *La barre d'échelle représente 100 µm.*

Cette désaffection pour la méiofaune est parfois expliquée par la petite taille et par l'identification taxonomique relativement délicate de ces invertébrés qui peut compliquer la conduite de certains protocoles expérimentaux. Pourtant, c'est justement du fait de leur petite taille et de leur diversité que ces organismes abondants sont particulièrement utiles pour l'examen de théories écologiques générales : *e.g.* la relation entre la biodiversité et le fonctionnement des écosystèmes, la théorie métabolique de l'écologie, *etc.* (Reiss *et al.*, 2010). La méiofaune est également reconnue pour son rôle d'intermédiaire important entre la production microbienne et les consommateurs de plus grande taille dans les réseaux trophiques d'eau douce (Perlmutter & Meyer, 1991; Schmid-Araya & Schmid, 2000; Schmid-Araya *et al.*, 2002; Beier *et al.*, 2004; Dineen & Robertson, 2010; Reiss & Schmid-Araya, 2011; Spieth *et al.*, 2011). Ainsi quand la méiofaune est considérée, la résolution du réseau trophique est considérablement améliorée et complexifiée (Fig. 3).

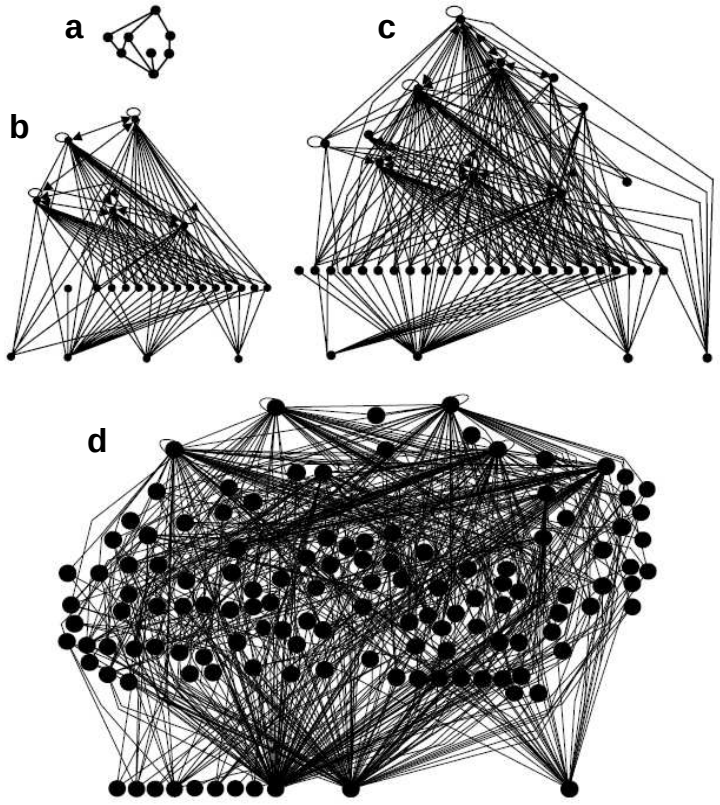

Figure 3. Niveau croissant de complexité d'un réseau trophique de rivière schématisé dans Woodward *et al.* (2005b) : **(a)** première représentation (d'après Cohen, 1978). Rivière Broadstone, UK : **(b)** réseau trophique initial (d'après Hildrew *et al.*, 1985), **(c)** réseau trophique après l'arrivée d'un super prédateur—la larve de libellule *Cordulegaster boltonii* (d'après Woodward & Hildrew, 2001), **(d)** réseau trophique après inclusion de la méiofaune (d'après Schmid-Araya *et al.*, 2002).

3. La méiofaune dans les biofilms épilithiques

3.1. Description et dynamique

Les processus écologiques à l'œuvre dans les biofilms épilithiques sont de plus en plus étudiés. Par exemple Hillebrand (2009) recense 835 études examinant le broutage de la macrofaune sur le biofilm. Cependant, il est surprenant qu'aussi peu d'attention ait été portée aux organismes de la méiofaune qui pullulent littéralement dans ces biofilms (Fig. 4). Durant le siècle dernier, quelques études ont néanmoins contribué à la description de la méiofaune des biofilms épilithiques et épiphytiques (Micoletzky, 1914; Schneider, 1922; Meschkat, 1934; Meuche, 1938; Young, 1945; Pieczynska, 1964; Traunspurger, 1992). Ces études rapportent notamment que des nématodes de la famille Chromadoridae : *e.g. Chromadorina bioculata*, *Chromadorina viridis* et *Punctodora ratzeburgensis* dominent bien souvent les populations de nématodes dans ces biofilms.

Un intérêt écologique croissant s'est tout de même manifesté à l'égard de la méiofaune des biofilms épilithiques depuis quelques années, particulièrement avec les travaux de Peters en milieu lentique qui en a précisé la distribution spatiale, les processus de développement et les interactions avec la macrofaune (Peters & Traunspurger, 2005; *in press*; Peters *et al.*, 2005, 2007a, 2007b). En milieu lotique, le suivi de biofilms cyanobactériens suggère que l'activité de la méiofaune puisse influencer la libération de métabolites secondaires comme la géosmine (Sabater *et al.*, 2003) et le décrochage du biofilm (Gaudes *et al.*, 2006). En cultivant des biofilms de diatomées de la Garonne en laboratoire en présence de différentes densités de nématodes, Mathieu *et al.* (2007) ont mis en évidence que des densités >50 ind cm^{-2} influencent le cycle de l'oxygène au sein de ces biofilms expérimentaux—ces densités sont souvent atteintes en milieu naturel. Toutes ces études indiquent que le biofilm peut-être perçu comme un habitat favorable pour la méiofaune, et qu'en retour l'activité de la méiofaune influence probablement l'organisation et les fonctionnalités du biofilm. De plus, les très fortes densités observées en lien avec la disponibilité des microphytes plaident aussi pour une utilisation du biofilm comme ressource trophique par la méiofaune (Peters & Traunspurger, 2005; Gaudes *et al.*, 2006).

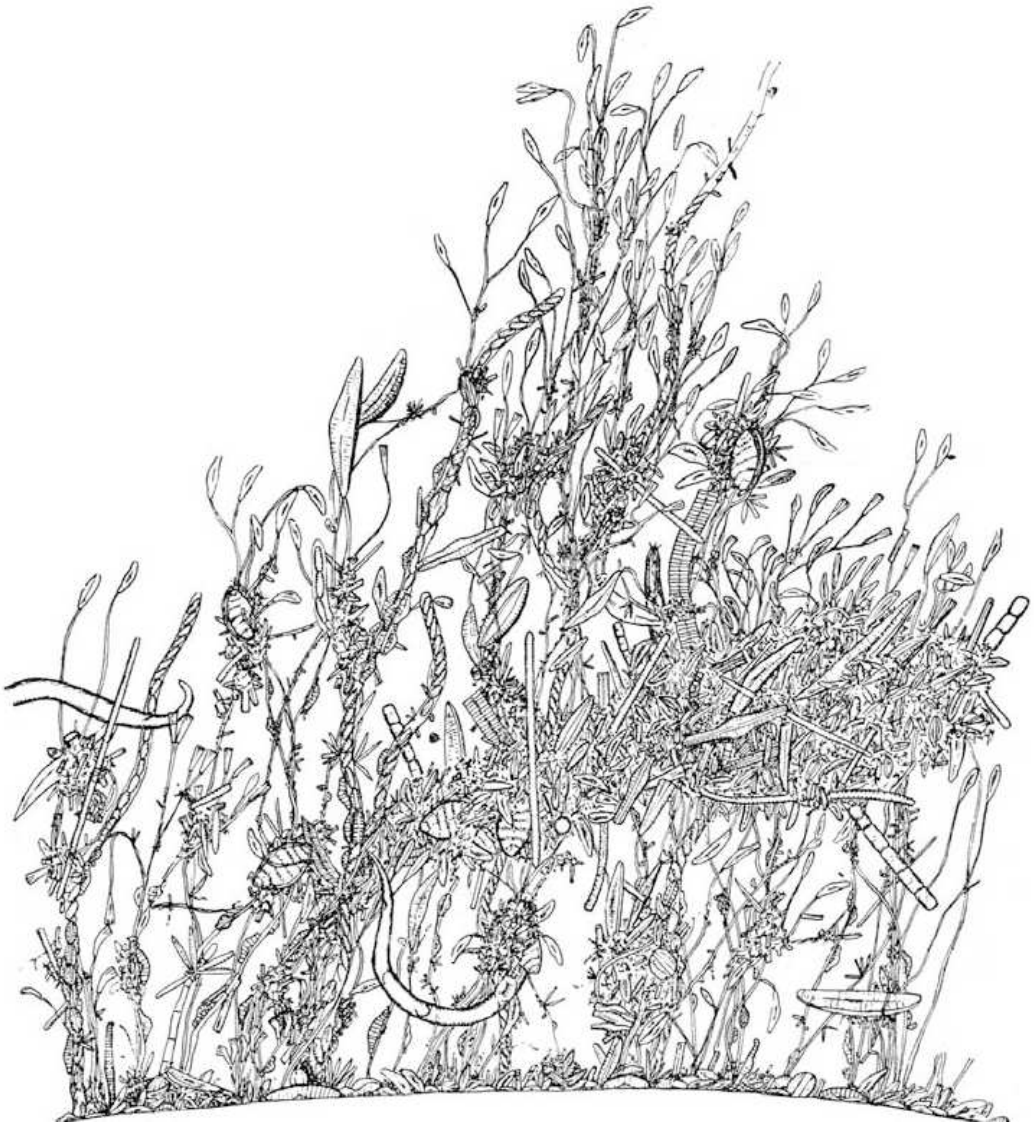

Figure 4. Illustration détaillée d'une communauté épilithique lentique dominée par des diatomées, et où évoluent des nématodes (Meschkat, 1934)

3.2. Interactions trophiques

Les biofilms épilithiques représentent un habitat adapté aux besoins nutritionnels de la méiofaune : Palmer *et al.* (2000) ont montré que l'abondance de la méiofaune était supérieure dans des habitats avec une forte biomasse microbienne. En milieu côtier, les biofilms épilithiques possèdent des qualités nutritionnelles tout à fait compatibles avec les besoins de la méiofaune (Da Silva *et al.*, 2007). En laboratoire, les nématodes montrent des comportements subtils (chimiotactisme positif et négatif) vis-à-vis des métabolites secondaires produits par des biofilms cyanobactériens (Höckelmann *et al.*, 2004). De

nombreuses études en milieu interstitiel montrent également des couplages et/ou une certaine sélectivité de la méiofaune envers les ressources benthiques disponibles (Schmid & Schmid-Araya, 2002; Moens *et al.*, 2006; Schroeder *et al.*, 2010; Reiss & Schmid-Araya, 2011; Ristau & Traunspurger, 2011). Les travaux récents de Kathol *et al.* (2011) montrent aussi que les rotifères du biofilm épilithique du Rhin filtrent des quantités considérables d'organismes planctoniques (bacterioplancton, phytoplancton et nano-flagellés). Ainsi, ces rotifères du biofilm contribuent significativement au couplage trophique pélagos–benthos, une fonction qui a longtemps été seulement attribuée à la macrofaune (bivalves) dans les milieux lotiques.

D'autre part, Hillebrand *et al.* (2002) ont examiné les effets *top-down* (broutage de la macrofaune) et *bottom-up* (concentration des nutriments) sur les différents compartiments du biofilm épilithique en zone lentique (dont la méiofaune). Un effet positif de la présence de macro-invertébrés brouteurs (macro-brouteurs) sur la méiofaune est observé dans le lac Erken (Suède). Ces auteurs expliquent que le broutage de la macrofaune pourrait être sélectivement orienté vers certaines algues (*e.g.* algues vertes filamenteuses) et que la digestion partielle de ces algues de grande taille pourrait profiter à la méiofaune environnante, qui autrement aurait plus de difficultés à consommer ces ressources. Cependant, dans ce même lac une étude plus récente de Peters & Traunspurger (*in press*) montre que les macro-brouteurs—notamment les gastéropodes *Theodoxus fluviatilis*—peuvent aussi fortement réduire les densités de méiofaune en raison d'une intense pression de broutage non-sélective. D'autres macro-invertébrés prédateurs pourraient aussi se nourrir spécifiquement de la méiofaune du biofilm, comme mis en évidence dans les milieux interstitiels (*e.g.* Schmid & Schmid-Araya, 1997; Beier *et al.*, 2004).

4. Objectifs et organisation du mémoire

4.1. Structuration de la communauté méiobenthique

Compte-tenu du manque d'information sur la dynamique de la méiofaune face aux contraintes environnementales, et ce tout particulièrement dans les biofilms épilithiques de rivière. Le premier objectif de ce travail de thèse est d'analyser la distribution temporelle de la méiofaune en relation avec les facteurs abiotiques et biotiques rencontrés dans un biofilm épilithique de rivière.

Il est postulé que les perturbations hydrologiques (entraînant l'instabilité du biofilm) et la composition de la communauté microphytobenthique pourraient être les facteurs principaux en lien avec la structure de la communauté méiobenthique.
Dans cette optique, un suivi régulier sur le long terme avec une fréquence d'échantillonnage élevée a été mis en place sur un même site de la Garonne.

Le **chapitre I** est dédié à l'analyse de la dynamique des grands groupes de la méiofaune du biofilm épilithique au cours de deux longues périodes (15 et 18 mois) à l'hydrodynamique contrastée. L'influence de l'hydrodynamique y est examinée au travers de son impact sur la stabilité et le développement de la communauté épilithique. Les capacités de résistance et de recolonisation de la méiofaune y sont décrites, et les facteurs structurant identifiés au moyen d'analyses multi-variées.

Le **chapitre II** est inscrit dans le même contexte que le chapitre I, en se concentrant sur la structuration fine de la communauté de nématodes (en termes d'assemblage d'espèces, de types trophiques, *etc.*) en relation avec les changements du biofilm (et notamment de son contenu en microphytes).

4.2. Interactions trophiques biofilm–méiofaune–macrofaune

Dans la bibliographie, l'utilisation du biofilm épilithique comme ressource trophique pour la méiofaune est fortement suspectée, mais pas encore démontrée ni quantifiée. Le second objectif de ce travail de thèse est de préciser et de quantifier les interactions trophiques entre biofilm, méiofaune et macrofaune en condition naturelles—en se focalisant notamment sur le comportement de broutage de la méio- et macrofaune sur les microphytes du biofilm.

Dans cette optique, trois approches analytiques complémentaires ont été appliquées :
(1) Caractérisation et quantification des contenus pigmentaires intestinaux des nématodes par chromatographie liquide à haute performance (HPLC).
(2) Comparaison des rapports isotopiques de l'azote 15 (^{15}N) et du carbone 13 (^{13}C) des ressources trophiques basales et des invertébrés du biofilm.
(3) Marquage au ^{13}C des microphytes pour un suivi et une quantification de leur incorporation par les invertébrés du biofilm (expérience *pulse-chase*).

Le **chapitre III** est focalisé sur le broutage des nématodes en utilisant l'analyse et la quantification par HPLC des pigments microphytiques contenus dans les intestins des nématodes issus d'échantillons de biofilms épilithiques naturels. Ces résultats ont été confrontés avec les contraintes environnementales et les variations de la disponibilité du microphytobenthos au sein du biofilm afin de caractériser et de quantifier le comportement de broutage des nématodes en conditions naturelles.

Le **chapitre IV** est axé sur l'analyse et la comparaison des rapports isotopiques naturels ^{15}N et ^{13}C de la méiofaune, de la macrofaune et des ressources trophiques potentielles pour définir le positionnement des invertébrés au sein du réseau trophique du biofilm. Ces données sont complétées par une expérience de *pulse-chase* réalisée *in situ* en utilisant un marquage du carbone microphytobenthique (CMPB) du biofilm au ^{13}C. Les dynamiques d'incorporation du CMPB sont mesurées chez les invertébrés méio- et macrobenthiques pour quantifier le transfert d'énergie entre producteurs primaires et invertébrés du biofilm.

4.3. Discussion générale et conclusion

Les objectifs principaux de ce travail de thèse, les hypothèses initiales, les aspects méthodologiques et les résultats exposés dans les chapitres précédents sont synthétisés, assemblés puis discutés dans le **chapitre V**. Les hypothèses émergentes ainsi que quelques perspectives expérimentales pour tester ces hypothèses y sont aussi exposées.

Chapitre I

Relation entre la stabilité du biofilm épilithique et de sa méiofaune associée lors de deux types de régime de crues

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I.1. Résumé de l'article

I.1.1. Contexte et objectifs

Les biofilms épilithiques de rivière sont un assemblage complexe et connexe d'organismes (bactéries, champignons, microphytes, protozoaires hétérotrophes et métazoaires de la méio- et macrofaune) enchâssés dans une matrice de substances exo-polymériques (Lock *et al.*, 1984; Costerton, 2000). Ces biofilms sont fortement déstabilisés par les perturbations hydrodynamiques en milieu lotique (Biggs & Close, 1989), mais peuvent également être impactés par des processus biologiques tels que le détachement autogène, le broutage par la macrofaune et la bioturbation (*e.g.* Lawrence *et al.*, 2002; Boulêtreau *et al.*, 2006; Gaudes *et al.*, 2006; Peters *et al.*, 2007a). Ces perturbations peuvent façonner les communautés microbiennes (Peterson & Stevenson, 1992; Leflaive *et al.*, 2008; Lyautey *et al.*, 2010), modifiant de ce fait les modalités fonctionnelles du biofilm (Cardinale, 2011). Les invertébrés de la méiofaune sont peu étudiés en eau douce en général, et dans les biofilms en particulier. Ce sont pourtant des organismes abondants, dont les caractéristiques biologiques (petite taille, cycles de vie généralement courts) en font d'importants acteurs du fonctionnement des écosystèmes benthiques (Hakenkamp & Morin, 2000; Schmid-Araya & Schmid, 2000; Schmid-Araya *et al.*, 2002; Stead *et al.*, 2005; Bergtold & Traunspurger, 2005; Reiss *et al.* 2010). Les biofilms de rivière, de par les perturbations récurrentes qui les déstabilisent, offrent un cadre approprié pour étudier la capacité de résilience de la méiofaune après les perturbations, ainsi que l'influence des périodes de stabilité sur la composition de la communauté méiobenthique du biofilm.

Cette étude a déterminé la distribution de la méiofaune du biofilm épilithique au cours de deux séries temporelles étendues montrant des régimes hydrologiques contrastés sur un même site de la Garonne. L'objectif de cette étude était d'examiner la dynamique et la structuration de la communauté méiobenthique entre ces deux périodes en fonction des paramètres environnementaux pour déterminer si les scénarios hydrologiques (déterminant l'instabilité du biofilm) étaient les facteurs essentiels conditionnant, directement ou indirectement (*e.g.* via une modification des caractéristiques du biofilm), la distribution de la méiofaune.

I.1.2. Principaux résultats et discussion

La première campagne d'échantillonnage (2004–2006) différait de la seconde (2008–2010) principalement en termes de fréquence des perturbations hydrologiques. Parmi les paramètres testés, la durée des périodes post-crues était le facteur significatif essentiel déterminant la distribution de la densité des organismes méiobenthiques associés au biofilm. La vitesse du courant, la biomasse du biofilm, des algues vertes et des cyanobactéries, et enfin la conductivité étaient aussi des facteurs significativement influents mais à un bien moindre degrés. Ainsi, cette étude permet de valider l'hypothèse de l'influence prépondérante de la stabilité du biofilm sur l'abondance de la méiofaune dans les biofilms épilithiques.

Les nématodes ont été plus affectés par la fréquence des crues que les rotifères. La densité des rotifères était davantage reliée à la dynamique de la biomasse du biofilm, ce qui suggère que les scénarios hydrologiques influencent directement les nématodes et indirectement les rotifères (*i.e.* à travers la réduction de l'épaisseur du biofilm).

Un seuil de vitesse de courant (à 5 cm du fond) a été estimé d'après la méthode de Palmer (1992) à 30 cm s^{-1}. Pour des vitesses supérieures, les densités de rotifères et de nématodes (groupes de méiofaune les plus abondants) apparaissaient nettement réduites. Cependant, l'impact du courant touchait plus les nématodes que les rotifères. Ce seuil dépasse le seuil observé dans les sédiments fins (12 cm s^{-1}) par Palmer (1992). Ainsi, sachant que des vitesses de courant comprises entre 12 et 30 cm s^{-1} représentaient 54% des cas de figure dans la Garonne durant cette étude, il est envisageable que les biofilms puissent servir de refuge à la méiofaune du sédiment fin dérivant dans la colonne d'eau, ainsi que de sources pour la recolonisation des zones de dépôt sédimentaire.

La résilience des rotifères (50–58 jours après les crues dues aux précipitations) était légérement plus rapide que celle des nématodes (58–65 jours). Ceci peut s'expliquer par les caractéristiques morphologiques et physiologiques des rotifères qui font d'eux des colonisateurs précoces (Ricci & Balsamo, 2000). Durant la phase de recolonisation intervenant après les crues de débâcle (Avril–Juin), la proportion des nématodes vis-à-vis de la densité totale de méiofaune était positivement corrélée à la durée de la période d'étiage, tandis que la proportion des rotifères y était négativement corrélée. Ce résultat corrobore la

succession rotifères–nématodes observée lors de la colonisation de substrats artificiels immergés en milieu lentique (Peters *et al.*, 2007b).

Lors des deux campagnes d'échantillonnage, une réduction importante de la biomasse du biofilm ainsi que des densités de rotifères a été observée au mois de Juillet. L'hypothèse que cette réduction pourrait être due à une forte pression de broutage de la part de larves d'insectes qui pullulent en Juillet dans le biofilm est discutée dans la limite des informations disponibles.

The relationship between epilithic biofilm stability and its associated meiofauna under two patterns of flood disturbance

N. Majdi, **B. Mialet**, **S. Boyer**, **M. Tackx**, **J. Leflaive**, **S. Boulêtreau**, **L. Ten-Hage**, **F. Julien**, **R. Fernandez and E. Buffan-Dubau**

Université de Toulouse-UPS-INP, EcoLab UMR 5245, 118 route de Narbonne, 31062 Toulouse, France
CNRS, EcoLab UMR 5245, 31062 Toulouse, France

I.2. Abstract

Habitat stability is an important driver of ecological community composition and development. River epilithic biofilms are particularly unstable habitats for the establishment of benthic communities because they are regularly disturbed by floods. Our aim was to determine the influence of habitat instability on meiobenthic organisms. We hypothesized that hydrologic variables are the most important predictors of meiofauna distribution. We monitored epilithic communities (meiofauna and microalgae) with a high sampling frequency during 2 sampling periods with contrasting hydrodynamic patterns in a temperate river (the Garonne, France). Nematodes and rotifers dominated meiofaunal assemblages. The critical flow velocity threshold for their maintenance in the biofilm was ~30 cm/s, a result suggesting that meiofauna can resist higher flow velocity within the biofilm than within sediments. Nematode distribution was primarily influenced by the duration of undisturbed periods, whereas rotifer distribution was also correlated with the thickness of the biofilm. During the periods after floods, rotifers were faster colonizers than nematodes. Collectively, our results show that flow regime was an essential driver for biofilm community development.

Keywords: habitat stability, resilience, recolonization, flow velocity, meiobenthos, rotifers, nematodes, periphyton.

I.3. Introduction

Biotope stability is an important driver of community composition and development in both terrestrial and aquatic systems (*e.g.* Cobb *et al.*, 1992; Death & Winterbourn, 1995; Villenave *et al.*, 2001; Van der Wurff *et al.*, 2007). Instability in aquatic systems can result from natural variations in flow regime (Death, 2002; Lake, 2003; Cardinale *et al.*, 2005) or from human-induced perturbations, such as acute pollution, introduced species, and flushing of reservoirs (*e.g.* Charlebois & Lamberti, 1996; Lai & Shen, 1996; Carpenter *et al.*, 1998).

River biofilms are a complex assemblage of organisms (bacteria, fungi, algae, heterotrophic protozoans, meiofauna, and macrofauna) embedded in a mucous matrix of exopolymeric substances. These biofilms grow on any hard submerged substrate (Lock, 1993; Costerton, 2000) and generally are copiously inhabited by microalgae (Peterson, 1996). Consequently, they can constitute the main site of primary production in shallow-water rivers with hard substrates, such as the middle reaches of the Garonne (Ameziane *et al.*, 2003). These biofilms are an important food resource for stream consumers (Fuller *et al.*, 1986; Lawrence *et al.*, 2002; Liess & Hillebrand, 2004). Moreover, they play a key role in biogeochemical processes, such as nutrient retention (Battin *et al.*, 2003; Teissier *et al.*, 2007). Nevertheless, these complex habitats are particularly unstable because they can be partially or entirely removed from their substrates—with their inhabitant fauna—on a regular basis by flood events, bacterial degradation processes, grazing, and bioturbation (Biggs & Close, 1989; Lawrence *et al.*, 2002; Boulêtreau *et al.*, 2006; Gaudes *et al.*, 2006; L. Peters *et al.*, 2007a). This instability can shape the biomass, diversity, and viability of algal and bacterial communities inhabiting the mats (*e.g.* Peterson & Stevenson, 1992; Lyautey *et al.*, 2010) and can affect biofilm processes (Cardinale, 2011).

Meiobenthic invertebrates (40–500-µm size range; Giere, 2009) are particularly abundant within these biofilms (Peters & Traunspurger, 2005; Gaudes *et al.*, 2006; Kathol *et al.*, 2011). They are a highly diverse and abundant component of stream communities (*e.g.* Schmid-Araya, 1997; Beier & Traunspurger, 2003b) and are important foodweb intermediates between micro- and macrofauna (Schmid-Araya & Schmid, 2000; Schmid-Araya *et al.*, 2002; Spieth *et al.*, 2011). Moreover, given their often short generation times and high fecundity rates (Bergtold & Traunspurger, 2005; Stead *et al.*, 2005; Reiss & Schmid-Araya, 2010), they

are particularly relevant model organisms for testing general ecological theories, especially those relating to population dynamics and community stability (Reiss *et al.*, 2010).

Post-flood periods present an opportunity to study recolonization processes and resilience of lotic meiofauna. In most of the few studies of this topic in rivers, investigators focused on sediment recolonization after catastrophic disturbances (reviewed in Robertson, 2000). In a few studies, investigators examined the influence of noncatastrophic flow on the dynamics of sediment-dwelling meiofauna. Palmer (1992) showed that sediment-dwelling meiofauna are frequently found above the sediment–water interface even under low-flow conditions, and Smith & Brown (2006) found that meiofauna can rapidly recolonize sediments in artificial stream channels. However, data are lacking on resistance and resilience of biofilm-dwelling meiofauna to variations of flow in rivers. Sediment-dwelling meiofauna also can respond to environmental constraints, such as temperature, that change seasonally (Stead *et al.*, 2003) and to habitat characteristics, such as sediment grain size distribution or organic matter availability (Swan & Palmer, 2000; Beier & Traunspurger, 2003b; Reiss & Schmid-Araya, 2008; Tod & Schmid-Araya, 2009). Studies addressing the temporal dynamics of meiofauna in river biofilms are rare. Gaudes *et al.* (2006) and Caramujo *et al.* (2008) considered only relatively short time periods, Kathol *et al.* (2011) highlighted pelagic–benthic coupling via biofilm-dwelling consumers, and Majdi *et al.* (2011) focused on temporal patterns of nematode assemblages. Therefore, how the complete meiobenthic community responds to the instability of their biofilm habitat is unclear.

Our objective was to determine how biofilm stability influences the composition of biofilm-dwelling meiofauna. We examined the temporal evolution of this relationship and the factors driving its development during 2 periods with contrasting patterns of flood disturbance in a temperate river (the Garonne, France). We hypothesized that hydrologic factors are the most important predictors of meiofauna distribution directly or via modification of biofilm status.

I.4. Methods

I.4.1. Study site and epilithic biofilm sampling

The Garonne is the largest river of southwestern France and has a drainage basin of 57 000 km^2 and a length of 647 km. The Garonne is a physically active river (Chauvet & Décamps, 1989) with a pluvio-nival flow regime characterized by an intense spring-flood period caused by snowmelt in the Pyrenees Mountains followed by a long low-water period that can continue for the rest of the year. Flash floods caused by heavy rainfall can occur (mostly during autumn and winter) in some years. The river bed consists mainly of cobble and gravel, and large alternating cobble bars are found frequently even in channels up to 7^{th} order. During low-water periods, a thick biofilm favored by low flow velocities on the river bed and low turbidity typically coats the upper surfaces of cobbles (Boulêtreau *et al.*, 2006; Leflaive *et al.*, 2008).

We sampled the epilithic biofilm at a cobble bar 36 km upstream the city of Toulouse where the Garonne is 6^{th} order (lat 01°17'53"E, long 43°23'45"N; elevation: 175 m asl). The epilithic microbial community has been previously described at this site (Lyautey *et al.*, 2005; Leflaive *et al.*, 2008). In this stretch of the Garonne, total P and total N concentrations in the water column vary over a year from 0.01 to 0.05 and 0.4 to 1.4 mg L^{-1}, respectively. Dissolved organic C and SiO_4^{4-} vary from 1 to 5 and 2 to 6 mg L^{-1}, respectively (Leflaive *et al.*, 2008). The canopy is widely open but the residence time is too short to allow substantial phytoplankton development, so benthic biofilms are assumed to provide most of the primary production (Ameziane *et al.*, 2002, 2003).

The 1^{st} sampling period (C1) lasted from November 2004 to March 2006 and had 44 sampling occasions. The 2^{nd} sampling period (C2) lasted from September 2008 to March 2010 and had 51 sampling occasions. We sampled weekly when possible, but sample collection was possible only when discharge was <175 m^3 s^{-1}. On each sampling occasion, we collected 12 randomly selected cobbles (mean diameter = 10 cm) by sliding them into a plastic bag underwater (depth = 30–50 cm) to prevent any detachment of the epilithic biofilm during removal. Within 2 h of collection, we transported the cobbles to the laboratory in a cool box to reduce pigment degradation. There, we removed the biofilm by scraping the total upper

surface of each cobble with a scalpel and a toothbrush. We cut long algal filaments into short segments with scissors and then suspended biofilm samples in ultrapure water (MilliQ filtration; Millipore, Billerica, Massachusetts) to obtain 12 biofilm suspensions (25 mL each). We divided these 12 suspensions into 3 groups of 4 replicates to be used for meiofaunal counts, algal pigment analyses, and estimation of epilithic ash-free dry mass (AFDM). We photographed scraped cobbles and measured the area of the surface from which biofilm had been removed (clearly visible on the cobble) and measured the scraped area (ImageJ software, version 1.38; Abramoff *et al.*, 2004). We expressed meiofauna counts, algal pigments, and AFDM quantitatively per unit area. During C1, AFDM was determined on all 44, algal pigments on 24, and meiofauna on 17 sampling occasions. During C2, AFDM, algal pigments, and meiofauna were determined on all 51 sampling occasions.

I.4.2. Abiotic environmental factors

Mean daily discharge (MDD) was supplied by a gauging station of the French water management authority (DIREN Midi-Pyrénées, Marquefave station) 10 km upstream the study site. No tributaries or dams occur between the gauging station and the study site. We measured stream flow velocity 5 cm above the streambed (mean of 3 measurements flanking the sampling area) on each sampling occasion with a Flow-meter Flo-Mate 2000 (Flow-Tronic, Welkenraedt, Belgium). We quantified stability as the number of days between a given sampling occasion and the last critical flood (days after flood [DAF]). Our long-term field observations (including periods during which most of the biofilm had been removed from the cobbles) allowed us to deduce that MDD of critical floods inducing a detachment of the major part of the epilithic biofilm is >300 $m^3 s^{-1}$.

During C1, we measured temperature, conductivity, pH, and dissolved O_2 in the water column on each sampling occasion with a LF95 conducti-meter, a pH320 pH meter, and an OXI320 oxi-meter, respectively (WTW, Weilheim, Germany). During C2, we measured these variables every 30 min with an automated multiparameter probe (YSI 6000; Yellow Springs Instruments, Yellow Springs, Ohio), which was permanently set 5 cm above the stream bed. We cleaned and calibrated probes monthly to avoid loss of accuracy.

I.4.3. Density, biomass, and resilience of biofilm-dwelling meiofauna

On each sampling occasion, we extracted the organic fraction from the 4 replicate biofilm suspensions with a modified gravity-gradient centrifugation technique (Pfannkuche & Thiel, 1988). We used Ludox® HS-40 colloidal silica (Sigma–Aldrich, St. Louis, Missouri) and poured the extract through stacked 500-µm and 40-µm meshes. We preserved the organisms retained on the 40-µm mesh (including meiofauna) in formaldehyde (5% final concentration) with 1% rose Bengal. We counted >200 meiobenthic organisms per replicate in a Dolfuss cell (Elvetec Services, Clermont-Ferrand, France) under a stereomicroscope (9–90×) to measure their density.

On each sampling occasion, we isolated >10 meiofaunal chironomid larvae in small aluminium cups and dried them for 48 h at 50°C to determine their dry mass (DM). We processed meiofaunal oligochaetes and water mites as described for chironomids, but because of their low occurrence in some samples, their DM was not obtained on each sampling occasion. For these organisms, we pooled DM measurements to obtain a mean DM value for each sampling campaign. For nematodes, rotifers, harpacticoid copepods, and tardigrades, we assessed individual DM from biometric conversions of their body dimensions (Giere, 2009).

We estimated resilience of nematodes and rotifers (time required for population densities to reach maximum preflood densities; Schmid-Araya, 1994) during C2 after 2 critical flash floods caused by rainfall (23 January 2009 and 15 January 2010, both MDD = 462 $m^3 s^{-1}$) and after the last critical flood of the spring snowmelt flood period (12 April 2009, MDD = 330 $m^3 s^{-1}$). We did not estimate resilience for chironomid larvae because it can be biased by emergence.

I.4.4. Biofilm biomass and extraction of microalgal pigments for high-performance liquid chromatography and chemotaxonomic analysis

On each sampling occasion, we dried (105°C, 18 h), weighed, and combusted (450°C, 8 h) 4 replicate biofilm suspensions to measure the AFDM content of the biofilm.

We centrifuged the 4 remaining suspensions (3220 × *g*, 20 min) and freeze-dried and thoroughly homogenized the pellets. We removed 250-mg subsamples from each pellet and

extracted algal pigments from each subsample 3 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) by sonication (Buffan-Dubau & Carman, 2000b). We filtered 1 mL of the pigment solution through a 0.2-µm polytetrafluoroethylene (PTFE) syringe filter and analyzed the filtrate with 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, Santa Clara, California). We prepared and programmed the mobile phase according to the analytical gradient protocol given by Barlow *et al.* (1997). We identified algal pigments by comparing their retention time and absorption spectra with those of pure standards (DHI LAB products, Hørsholm, Denmark; see Majdi *et al.*, 2011 for further details).

We coupled HPLC-analysis of algal pigments with a chemotaxonomic analysis using CHEMTAX software (version 1.95; Mackey *et al.* 1996) to estimate the biomass of microphytobenthic groups in the biofilm in terms of contribution to total chlorophyll *a* (Chl *a*) biomass. We used the biomarker pigment ratios of biofilm microalgal groups reported in Majdi *et al.* (2011) to supply the initial matrix needed to run the chemotaxonomic analysis.

I.4.5. Data analysis

We used Mann–Whitney *U* tests to compare values of abiotic (DAF, conductivity, pH, O_2, temperature, and flow velocity) and biotic (AFDM, Chl *a*, biomass of algal groups, density and individual biomass of meiofaunal groups) variables between the 2 study periods. We used Spearman rank correlation analysis to examine correlations between biofilm AFDM and Chl *a* and between proportions of meiofaunal groups and DAF. We used STATISTICA software (version 8.0; Statsoft, Tulsa, Oklahoma) for these analyses.

We used canonical ordination analyses (CANOCO, version 4.5; Biometris, Wageningen, The Netherlands) to assess the influence of biotic and abiotic factors on the density distribution of main meiofaunal groups (rotifers, nematodes, chironomid larvae, and oligochaetes) in the biofilm. We did not consider tardigrades, harpacticoid copepods, and water mites in this analysis because of their low occurrence in samples. We applied the canonical ordination analyses to $\log(x + 1)$-transformed meiofaunal density data for C1 and C2 separately and for pooled C1 and C2 data. First, we used a detrended correspondence analysis (DCA). The total

inertia observed was <2.6, so a predominance of linear group response curves could be expected (Ter Braak, 1987, 1994). Therefore, we used a redundancy analysis (RDA) in which the ordination axes were constrained to be linear combinations of abiotic and biofilm biotic factors to investigate the relationships between these factors and the distribution of main meiofaunal groups. We chose streambed flow velocity (V) over MDD in the RDA because these factors covaried strongly. We listed factors (conditional effects) according to the variance they explained singly (*i.e.* without eventual covariability with other factors), given by their eigenvalues (λ). We tested for statistical significance with Monte Carlo permutations (499 unrestricted permutations, $\alpha = 0.05$).

I.5. Results

I.5.1. Abiotic background

The 2 study periods contrasted hydrologically. Eight critical floods (MDD >300 m^3 s^{-1}) occurred during C2, and only 4 occurred during C1 (Fig. I.1a, b). Three of the critical floods during C2 and 1 during C1 were flash floods caused by heavy rainfall. The durations of the low-water period were 9 mo (June 2005–February 2006) in C1 and 5 mo (June–October 2009) in C2. DAF of sampling occasions differed significantly between periods (Mann–Whitney *U*, $P < 0.05$). Thus, C1 can be considered less disturbed than C2. Among the other abiotic factors, only conductivity and pH differed significantly between periods (Mann–Whitney *U*, conductivity: $P < 0.001$, pH: $P < 0.01$).

I.5.2. Epilithic biofilm and associated microphytes

AFDM and Chl *a* were strongly correlated (pooled C1 and C2; Spearman rank correlation, $N = 75$, $r = 0.72$, $P < 0.001$). They both decreased drastically after critical floods and tended to increase during low-water periods (Fig. I.1a, b). Sudden decreases of AFDM and Chl *a* also were observed in July during C1 and C2, but these decreases were not linked to floods.

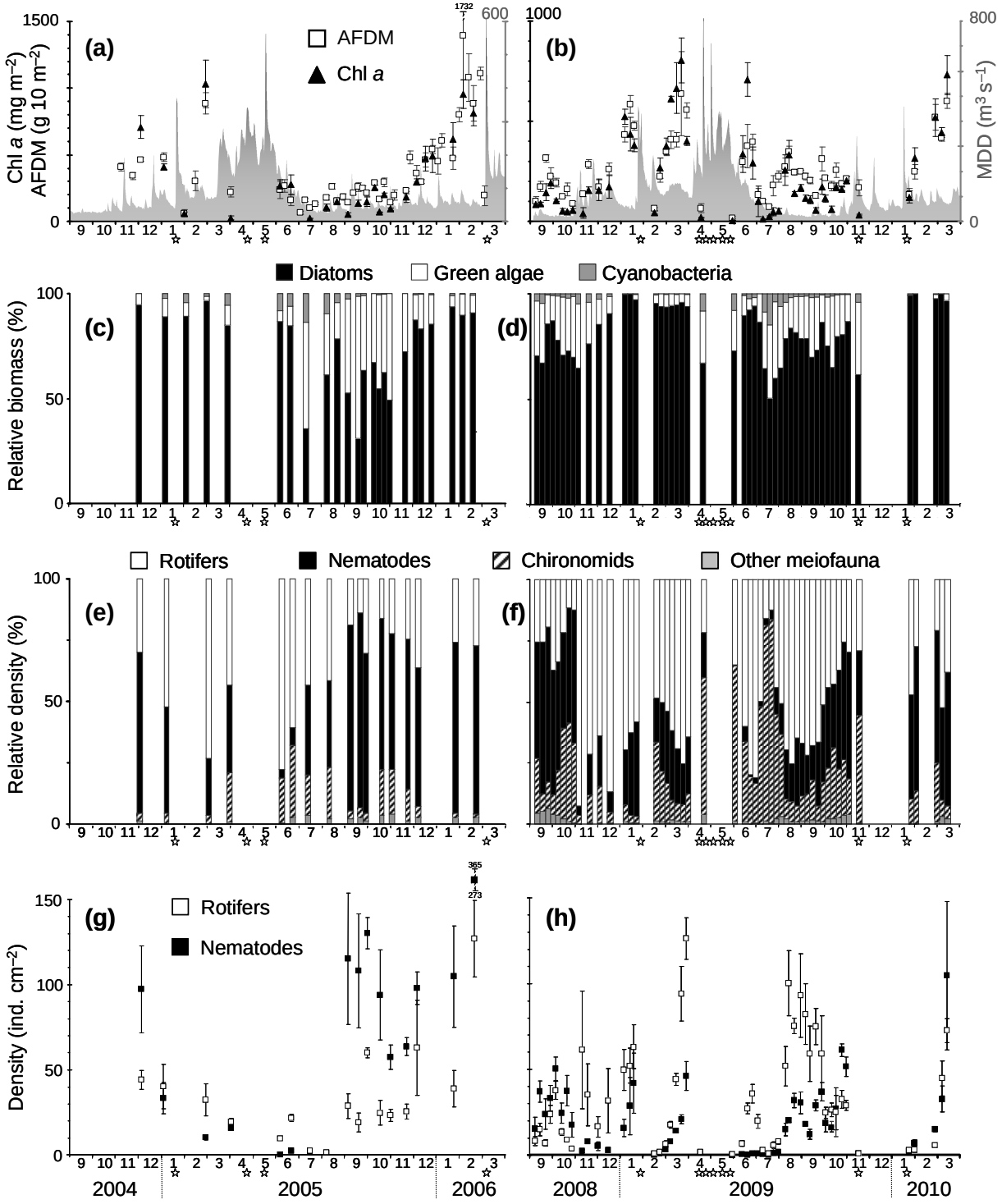

Figure I.1. Mean (±1 SE, N = 4) ash-free dry mass (AFDM) of the epilithic biofilm, epilithic chlorophyll *a* concentration (Chl *a*), and daily discharge (MDD) during the 1st (C1) **(a)** and 2nd (C2) **(b)** sampling periods; relative biomass of biofilm microalgal groups during C1 **(c)** and C2 **(d)**; relative density of biofilm-dwelling meiofauna during C1 **(e)** and C2 **(f)**; and density of biofilm-dwelling nematodes and rotifers during C1 **(g)** and C2 **(h)**. Critical floods, during which mean daily discharge was >300 m^3 s^{-1}, are indicated by stars on *x* axes. Numbers on the *x*-axis represent months of the year.

Diatoms dominated the algal community of the biofilm (especially during winter) in C1 and C2 (Fig. I.1c, d). Their relative biomass was lower during C1 than during C2 (71 *vs* 82%, respectively; Mann–Whitney *U*, $P < 0.01$). In contrast, the relative biomass of green algae was higher during C1 than during C2 (26 *vs* 15.5%, respectively; Mann–Whitney *U*, $P < 0.05$). The proportion of green algal biomass was highest during the summer–autumn low-water period. Cyanobacteria generally were minor contributors to total microphytobenthic biomass. However, they peaked up to 14–15% in July during both sampling periods (Fig. I.1c, d).

During the recolonization periods after the spring snowmelt floods (June 2005–February 2006 and June–November 2009), diatom relative biomass was highest during early (10–40 DAF) and late (>200 DAF) successional stages. Relative cyanobacterial biomass peaked at 50–60 DAF, and relative green algal biomass peaked between 50 and 170 DAF, when diatom biomass was relatively low (Fig. I.2a).

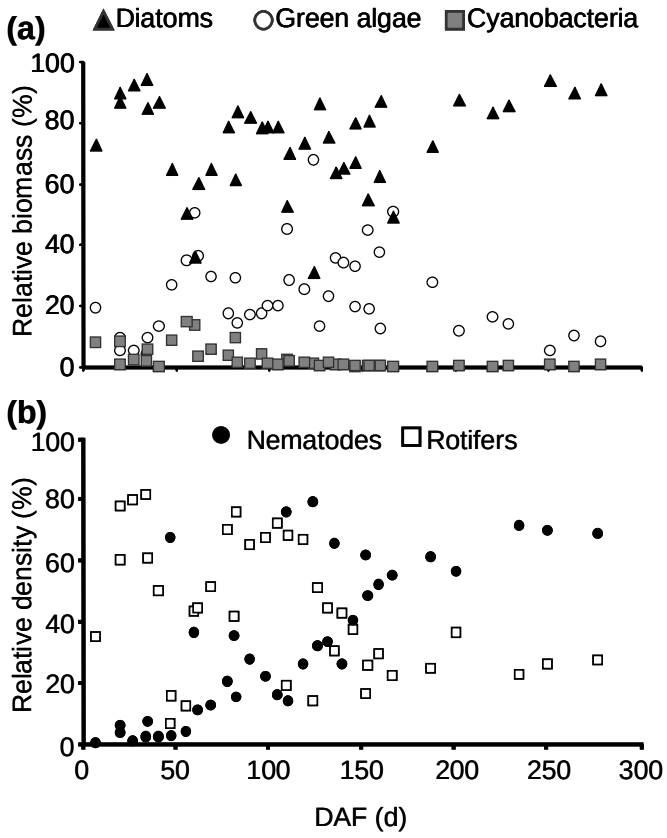

Figure I.2. Relative biomass ($N = 41$) of diatoms, green microalgae, and cyanobacteria **(a)**, and relative density ($N = 37$) of nematodes and rotifers **(b)** in the biofilm relative to the duration of the undisturbed period (days after flood = DAF) after the spring snowmelt floods (pooled data from June 2005–February 2006 and June–November 2009).

Nematodes and rotifers dominated meiofaunal assemblage density and, on average, accounted for 88% of the total meiofaunal density during C1 and C2 (Fig. I.1e, f, Table I.1). However, they contributed little to biomass. On average, they accounted for 3.3% of the total meiofaunal biomass, which was dominated by chironomid larvae (66%) and oligochaetes (27%). The means and ranges of density and biomass of meiofaunal-sized chironomid larvae were similar between periods, a result that indicated common patterns of larval development between periods. Chironomid density peaked in October (means ± SE: 28 ± 9, 32 ± 4, 29 ± 9 individuals [ind.] cm^{-2} in October 2005, 2008, and 2009, respectively). Chironomid biomass peaked in February 2006 and March 2009 and 2010 because larval DM was high (up to 18 µg $ind.^{-1}$) during these periods (Fig. I.3a, b). Tardigrades, harpacticoid copepods, and water mites were rarely found (Table I.1). Nematode and rotifer densities and meiofaunal-sized oligochaete and chironomid biomass decreased drastically after critical floods. However, during July, rotifer density and chironomid biomass decreased suddenly and in the absence of any flood (Figs I.1g, h and I.3a, b).

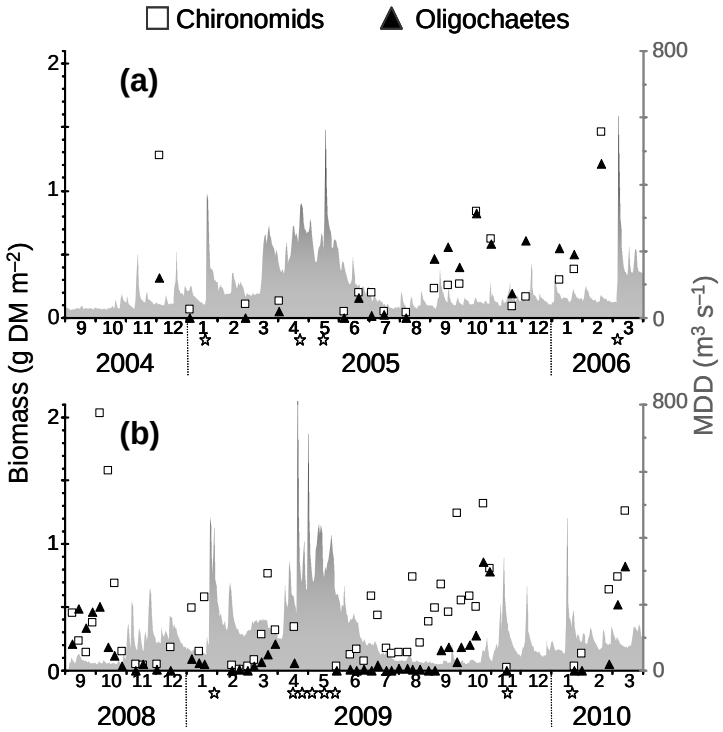

Figure I.3. Dry mass (DM) of meiofaunal chironomid larvae and oligochaetes and mean daily discharge (MDD) during the 1st (C1) **(a)** and 2nd (C2) **(b)** sampling periods. Critical floods, during which mean daily discharge was >300 m^3 s^{-1}, are indicated by stars on *x* axes.

Table I.1. Mean (N = 17 for C1 and N = 51 for C2) and maximum (Max) density, mean biomass, and relative contribution (%) of each meiofaunal group to the total biofilm-dwelling meiofauna community on cobbles in the Garonne River during 2 study periods (C1 and C2). The resilience times (days to recovery) following winter flash floods (Winter) and spring snowmelt floods (Spring) are for nematodes and rotifers during C2. ind. = individuals, DM = dry mass

Meiofauna	C1 period (2004–2006)					C2 period (2008–2010)						
	Density (ind cm^{-2})			Biomass (µgDM ind^{-1})		Density (ind cm^{-2})			Biomass (µgDM ind^{-1})		Resilience (d)	
	Mean	Max	%	Mean	%	Mean	Max	%	Mean	%	Winter	Spring
Nematodes	78	319	65	0.10	8	20	104	32	0.07	2	58–65	148–156
Rotifers	32	127	27	0.02	1	33	126	53	0.03	1	50–58	>340
Chironomids	8	28	6	6.75	56	9	32	14	6.77	82	–	–
Oligochaetes	2	8	2	15.65	32	<1	4	<1	20.16	11	–	–
Harpacticoids	<1	1	<1	0.39	<1	<1	2	<1	0.37	<1	–	–
Tardigrades	<1	4	<1	0.28	<1	<1	1	<1	0.21	<1	–	–
Water mites	<1	<1	<1	81.83	3	<1	1	<1	87.50	4	–	–

I.5.4. Influence of abiotic and biotic factors

The factors (DAF, flow velocity, and conductivity) that significantly influenced the density of the main meiofaunal groups were mainly linked to hydrodynamics (RDA on pooled data from C1 and C2). DAF, which can be viewed as an indicator of habitat stability, was the most important predictor of meiofaunal density distribution (Table I.2). AFDM and green algal and cyanobacterial biomass, factors related to biofilm status, also significantly influenced meiofaunal density distribution. All meiofaunal groups were on the right side of the biplot (Fig. I.4). Axis 1 was correlated mainly with DAF, flow velocity, and cyanobacterial and green algal biomass. Thus, meiofauna were more abundant during stable, undisturbed periods than during disturbed periods. Densities of chironomids, oligochaetes, and particularly nematodes were correlated with DAF (stability), whereas density of rotifers was more strongly correlated with AFDM. RDA analyses done on data C1 and C2 separately gave essentially the same results as the analysis of the total set (not shown).

Table I.2. Results of the redundancy analysis (RDA) testing the effects of biotic and abiotic factors on the density distribution of biofilm-dwelling meiofauna. Factors are listed by their eigenvalues (λ), *i.e.* the relative contribution of each factor to the explanation of meiofaunal density variance, without covariability (see Methods). * indicates factors that were statistically significant (Monte Carlo permutation test, $P < 0.05$)

RDA conditional effects		
Factors	λ	*p*-value
Days after flood	0.15	0.002 *
Streambed flow velocity	0.07	0.014 *
Biofilm ash-free dry mass	0.06	0.014 *
Green algae	0.06	0.012 *
Cyanobacteria	0.04	0.024 *
Conductivity	0.03	0.020 *
pH	0.01	0.268
Temperature	0.01	0.420
Diatoms	0.01	0.492
Dissolved O_2	0	0.972
Sum of all λ	0.44	

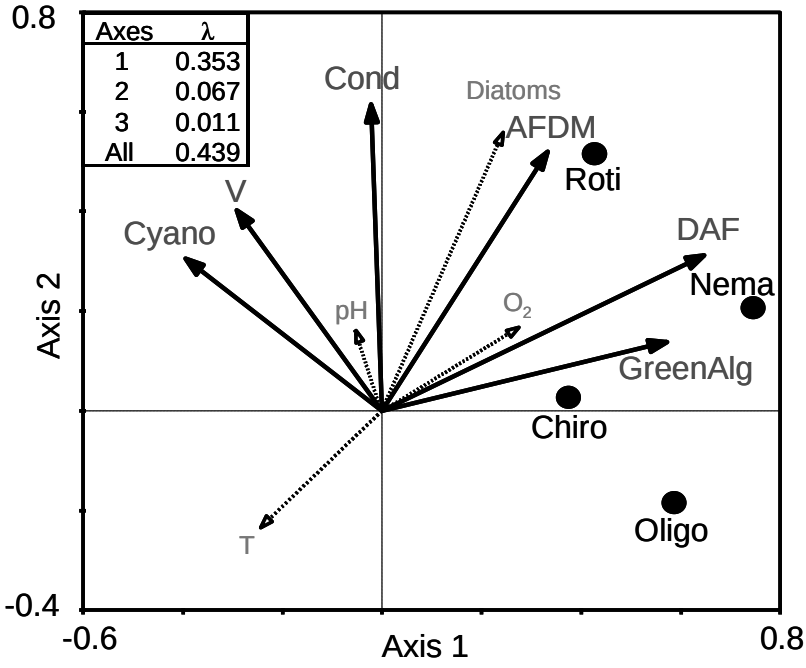

Figure I.4. Redundancy analysis (RDA) biplot showing the density distribution of major meiofaunal taxa under the influence of environmental factors over both sampling periods (C1 and C2). Bold arrows represent statistically significant factors (Monte Carlo permutation test, $P < 0.05$). Slim dotted arrows represent nonsignificant factors. Black points show meiofaunal group positions. The eigenvalues (λ) are indicated for main ordination axes. AFDM = ash-free dry mass of biofilm, GreenAlg = green algae, Cyano = cyanobacteria, DAF = days after flood, V = streambed flow velocity, Cond = conductivity, T = water temperature, Nema = nematodes, Rot = rotifers, Chiro = chironomid larvae, Oligo = oligochaetes.

I.5.5. Response to flood disturbance

Nematodes reached higher average density and biomass during the less-disturbed C1 (Table I.1) than during the frequently perturbed C2 (Mann–Whitney *U*, $P < 0.01$, $P < 0.001$, respectively). Mean rotifer density did not differ between C1 and C2, but rotifer biomass was significantly greater during C2 than C1 (Mann–Whitney *U*, $P < 0.05$). These results suggest that nematodes were more negatively affected by the frequency of critical floods than rotifers (Fig. I.1e–h). Therefore, flood frequency was the main driver of changes in community composition.

Nematodes and rotifers had different resilience times depending on flood type (Table I.1). Nematode and rotifer assemblages required more time to recover their preflood densities after snowmelt floods than after flash floods. Mean resilience times after flash floods tended to be lower for rotifers (50–58 d) than for nematodes (58–65 d). During the recolonization periods following spring snowmelt floods (June 2005–February 2006 and June–November 2009), the proportion of nematodes to total meiofauna density was positively correlated with DAF (Spearman rank correlation, $N = 37$, $r = 0.729$, $P < 0.001$), whereas the proportion of rotifers to total density was negatively correlated with DAF ($r = –0.3$, $P < 0.05$). Thus, nematodes and rotifers had different recolonization patterns in the biofilm (Fig. I.2b).

I.5.6. Resistance threshold to flow velocity

The method described by Palmer (1992) can be used to deduce critical flow-velocity thresholds from Fig. I.5. AFDM reached values >50 g m^{-2} only at flow velocities <30 cm s^{-1} (Fig. I.5a). At higher velocities, AFDM never reached values >43.4 ± 3.8 g m^{-2}. The mean AFDM reached when velocity was >30 cm s^{-1} represented 74% of the mean AFDM reached when velocity was <30 cm s^{-1}. A similar resistance threshold of ~30 cm s^{-1} was observed for nematodes (Fig. I.5b). At velocities >30 cm s^{-1}, their density was limited to a maximum of 15 ± 5 ind. cm^{-2}. Rotifer density also tended to be reduced when velocity was >~30 cm s^{-1} (Fig. I.5c). However, their densities still reached between 7 ± 3 to 50 ± 24 ind. cm^{-2} at velocities >30 cm s^{-1}. Above this flow-velocity threshold, the mean density of nematodes reached only 13.8% of the mean value observed at velocities <30 cm s^{-1} (cf. 60.6% for rotifers; Fig. I.5b, c). Moreover, nematodes reached their maximum densities only during the less-disturbed C1, but this pattern was not found for rotifers (Figs I.5b and I.1e–h).

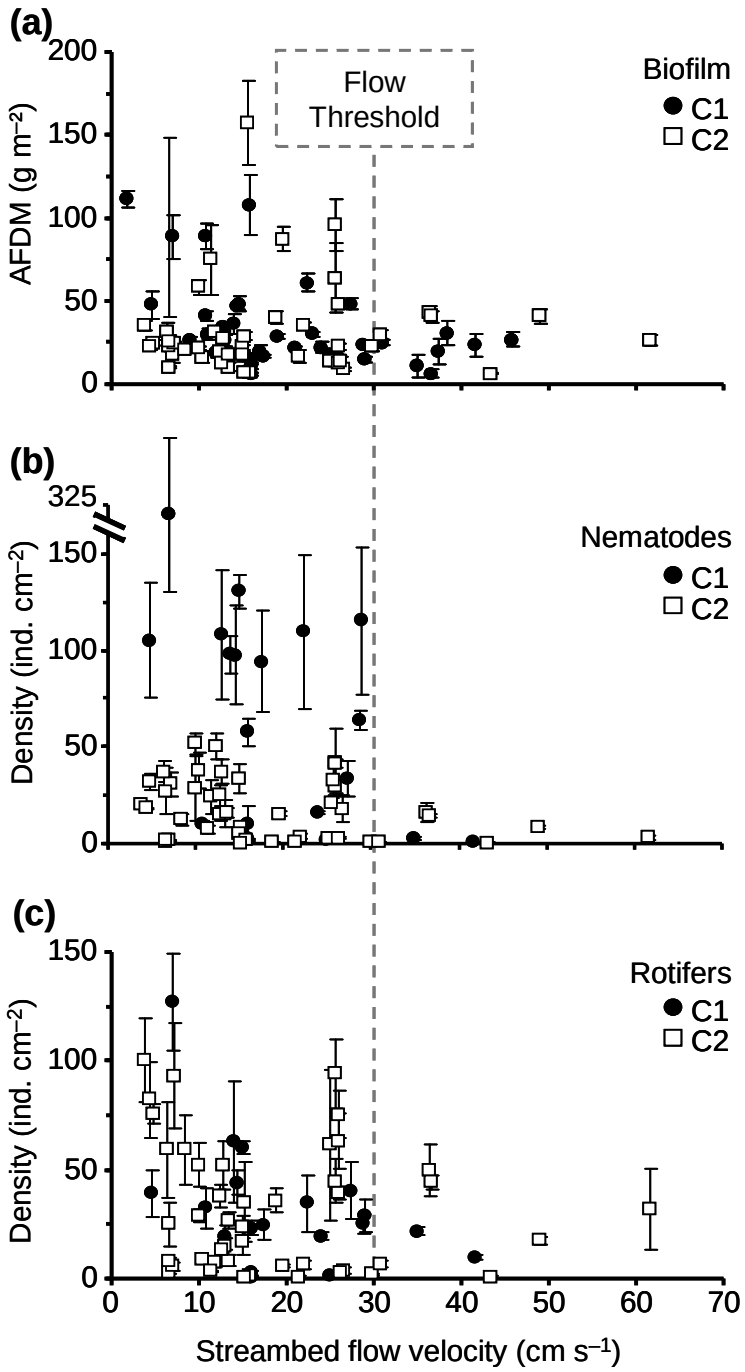

Figure I.5. Mean (±1 SE, *N* = 4) ash-free dry mass (AFDM) of the epilithic biofilm **(a)**, and density of biofilm-dwelling nematodes **(b)** and rotifers **(c)** relative to streambed flow velocity during sampling periods (C1 and C2). The vertical dotted line shows the critical flow velocity threshold visually deduced from our data.

I.6. Discussion

We addressed the interaction between hydrological regime and development of biofilm community, considering both its algal and meiofaunal constituents. These factors are clearly linked, but for clarity, we will discuss the aspects essentially related to abiotic factors before discussing the more biotic aspects.

I.6.1. Abiotic factors

Meiofauna were abundant in the epilithic biofilm of the Garonne River, a finding that corroborates the results of the few other studies considering biofilm-dwelling meiofauna in other temperate rivers (Sabater *et al.*, 2003; Gaudes *et al.*, 2006; Kathol *et al.*, 2011). The density distribution of meiofaunal groups depended primarily on biofilm (in)stability imposed by flood disturbance. The frequency of floods and DAF affected the biofilm and its associated meiofauna. Development of biofilm biomass (AFDM), its main microalgae (diatoms and green algae), and its meiofauna are associated with DAF and flow velocity (Fig. I.4). The significance of the conductivity vector seems odd, but can be explained by the covariation of conductivity and temperature. During colder periods, which more or less coincide with flood periods, import of solute ions from the upper drainage basin of the Garonne surpasses the dilution effect of increased runoff and conductivity increases substantially (Probst & Bazerbachi, 1986). The hydrological regime in the Garonne River is probably the major determinant of the seasonal distribution of biofilm-dwelling meiofauna because we observed density maxima in winter. In other words, meiofauna peaked when biofilm biomass peaked, and biofilm biomass peaks occurred with increasing periods of stability (DAF). In contrast, density maxima have been reported for spring and summer in most long-term monitoring studies of meiofauna in rivers (Beier & Traunspurger, 2003b; Stead *et al.*, 2003; Tod & Schmid-Araya, 2009). In general, our results agreed with those of other studies pointing out floods as a major shaping force in lotic environments (Lake, 2000).

Nematodes were more affected than rotifers by the frequency of critical floods (*i.e.* habitat instability). Overall, rotifer density was closely coupled to biofilm AFDM, a result suggesting that hydrological scenarios probably influenced rotifer density through biofilm status (*e.g.* its thickness). Most rotifers consume small algae, protozoans, and bacteria by filtering, scraping,

or browsing (Ricci & Balsamo, 2000; Kathol *et al.*, 2011). Thus, the abundance of potential food resources within the biofilm might favor rotifer development.

The resilience period of rotifer populations (50–58 d) was slightly shorter than that of nematodes (58–65 d) after flash floods. Gaudes *et al.* (2010) showed that meiofauna with worm-shaped bodies (*e.g.* nematodes) have high resilience during recolonization of sediments after a flood. In epilithic biofilms, we showed that rotifers also had high resilience. Rotifers have cilia, short life cycles, parthenogenetic reproduction, and can produce resting eggs or form dormant stages to overcome harsh habitat conditions (Ricci & Balsamo, 2000). They can feed on suspended organisms (Kathol *et al.*, 2011) and may be able to take advantage of the increased drifting material shortly after periods of high flow, whereas nematodes would not profit from these circumstances. Moreover, most benthic rotifers found in our study (Bdelloidea and *Proales* spp.) have pedal adhesive glands that secrete a sticky cement used for temporary attachment to the substrate (Ricci & Balsamo, 2000). These characteristics apparently render rotifers particularly efficient at recolonizing cobble surfaces cleaned by floods. This ability to colonize early was also observed on submerged wood surfaces (Golladay & Hax, 1995). In sediments of artificial stream channels, Smith & Brown (2006) reported very short recolonization periods: *i.e.* 0.5 and 5 d for rotifers and nematodes respectively, *vs* >50 d in our study. This difference may be caused by differences in flow velocities between the studies. Smith & Brown (2006) used a maximum flow velocity of 12 cm s^{-1}, whereas during our study it ranged from 4–62 cm s^{-1}, and was >12 cm s^{-1} on 62% of the sampling occasions. Palmer *et al.* (1992) found that meiofaunal density in azoic chambers placed in a 4^{th} order temperate stream reached values that were 70% of natural stream density within 12 d. The resilience values deduced in our study were within the range of values observed for meiofauna recolonizing sediments of a 3^{rd} order stream after a flood (42–60 d; Gaudes *et al.*, 2010) and for microcrustaceans recolonizing sediments of a headwater stream after a flood (<54–>243 d; reviewed in Robertson, 2000).

Flow velocity also was a significant predictor of the distribution of biofilm-dwelling meiofauna. Palmer (1992) used flume experiments to determines a critical threshold velocity (9–12 cm s^{-1}), above which meiofauna (rotifers, oligochaetes, chironomids, and copepods) were removed from the sandy substrate and entered the water column. However, Smith & Brown (2006) reported that a flow velocity = 12 cm s^{-1} did not remove meiofauna from gravel substrates in artificial channels. Smith & Brown (2006) suggested that this difference was the

result of differences in shear stress needed to displace meiofauna from sand *vs* from gravel substrates. We found a critical threshold flow velocity ~30 cm s^{-1} for nematodes, rotifers, and their biofilm habitat. This suggests that biofilm-dwelling meiofauna might be more resistant to higher flow velocity than fine-sediment-dwelling meiofauna. However, meiofauna entered the water column when flow velocity was >30 cm s^{-1}, probably directly via erosion as particles and indirectly because in detached biofilm fractions. Gaudes *et al.* (2006) reported that nematodes can be particularly abundant in free-floating biofilm fractions. Streambed flow velocities between 12 and 30 cm s^{-1} occurred frequently in the Garonne River (54% of the sampling occasions). Thus, biofilms could serve as refugia for drifting sediment-dwelling meiofauna and could be source of colonizers for soft-sediment patches in the river bed. Our results also provide support for the idea that epilithic biofilms serve as a refuge for meiofauna (Höckelmann *et al.*, 2004; Mathieu *et al.*, 2007). Interstitial meiofauna can partly resist removal by making small-scale vertical migrations in response to flow variations (Dole-Olivier *et al.*, 1997; Swan & Palmer, 2000). Thus, interstitial- and drifting-meiofauna might be important sources for biofilm recolonization processes after critical floods (*i.e.* when biofilm is almost totally removed).

I.6.2. Biotic factors

Our results show a close linkage between biofilm biomass (AFDM) and algal biomass (Chl *a*), as is commonly found in the Garonne River (Ameziane *et al.*, 2002; Boulêtreau *et al.*, 2006). Biofilm biomass averaged 34 g AFDM m^{-2} and 260 mg Chl *a* m^{-2} over the 2 sampling campaigns. These values are high compared to values of 22 g AFDM m^{-2} and 77 mg Chl *a* m^{-2} from epilithic biofilms of 7 nutrient-rich streams in New Zealand (Biggs, 1995). This result strengthens the conclusion that benthic biofilms are important primary producers in the middle reaches of the Garonne and they probably are an important food source for consumers. However, biofilm biomass suffered when streambed flow velocity exceeded 30 cm s^{-1}. Biggs *et al.* (1998) reported important biofilm biomass losses when flow velocity exceeded 20 cm s^{-1} in 5 New Zealand streams, and Poff *et al.* (1990) reported 30–40× lower biofilm biomass under high (29–41 cm s^{-1}) *vs* slow (<1–17 cm s^{-1}) flow velocities in troughs connected to the Colorado River.

Flow constraints also determined algal species distribution and succession (Fig. I.2a). Diatom relative biomass was highest during early (10–40 DAF) and late succession (>200 DAF).

Both green algae and cyanobacteria occurred during middle succession (50–170 DAF), but cyanobacteria were relatively abundant only during a short period (50–60 DAF). Ecological succession of lotic biofilm on artificial substrates occurs after preconditioning by bacteria and organic matter. Early colonists are small diatom species with attachment mechanisms, such as raphes, and succession is completed (>21 d) by filamentous diatoms and green algae (Korte & Blinn, 1983; Peterson & Stevenson, 1992).

At present, our data do not allow us to determine whether the correlations among microalgal and meiofaunal groups are the result of specific trophic relationships or a common development pattern. Diatoms were the main constituent of the biofilm microalgae during most of both study periods. Diatom abundance was correlated with biofilm biomass (AFDM) and meiofauna (especially rotifer) density. Green algal biomass also was a significant predictor of meiofauna distribution, particularly for nematodes and chironomids. Majdi *et al.* (2011) examined nematode species distribution in the biofilm during C2 and found correlations between diatom biomass and the distribution of the dominant nematode species *Chromadorina bioculata* (Schultze *in* Carus, 1857) and *Chromadorina viridis* (Linstow, 1876). In our study, the correlations among green algae, nematodes, and chironomids might be explained by the late development of green algae, nematodes, and chironomids after spring snow-melt floods. In contrast, cyanobacteria abundance was negatively correlated with meiofaunal density. Cyanobacteria peaked during July, when rotifer and nematode densities were at their lowest. This negative correlation could be a consequence of a seasonal development cycle of cyanobacteria concomitant with other influences, such as grazing and predation (see below) or temperature-induced self-detachment of the biofilm (Boulêtreau *et al.*, 2006). Moreover, cyanobacteria can produce and release secondary metabolites (Sabater *et al.*, 2003; Leflaive & Ten-Hage, 2007) that attract or repel benthic invertebrates, *e.g.* nematodes (Höckelmann *et al.*, 2004). Thus, we cannot exclude the possibility that cyanobacteria could have a repellent effect on meiofauna. However, considering the relatively minor contribution of cyanobacteria to the biofilm community on most of the sampling occasions, we consider a strong repellent effect unlikely. Both diatoms and green algae are potentially good food sources for meiobenthic organism (Buffan-Dubau & Carman, 2000b).

Biofilm and its meiofauna collapsed suddenly in July 2005 and 2009 even though flow was low. Concomitantly, macrofauna crowded the cobbles. In July 2005, mean macroinvertebrate density on cobbles was 12 059 ind. m^{-2}. Large (~5 mm) *Psychomyia pusilla* (Fabricius 1781)

(Trichoptera: Psychomyiidae) larvae contributed 71% of the total density (NM, *unpublished data*). In July 2009, mean density was 11 650 ind. m^{-2}. Psychomyiidae larvae contributed 40% and Ephemeroptera larvae (mainly Baetidae and Ameletidae) contributed 28% of the total density (NM, *unpublished data*). Psychomyiid larvae construct retreat tubes of small particles held by silk, and they graze the surrounding biofilm by extending their tubes to reach new areas (Wiggins, 2004). Their high density and biomass suggests that they could have reduced biofilm biomass directly by grazing or indirectly by destabilizing (bioturbation) deeper biofilm layers. Macrofaunal grazers strongly affect biofilm biomass and community structure (*e.g.* Feminella & Hawkins, 1995; Hillebrand, 2009), and meiofauna embedded in biofilm patches can be ingested incidentally by these grazers. However, meiofauna and rotifers can actively migrate from sediment to the water column presumably to avoid predation or habitat disturbances (Palmer *et al.*, 1992; Schmid & Schmid-Araya, 1997; Smith & Brown, 2006). We speculate that low densities of meiofauna in July could have resulted from indirect predation or from migration of meiofauna subsequent to depletion of their habitat and resources by macrofaunal competitors.

Overall, the distribution of meiofauna depended primarily on biofilm (in)stability related to flood disturbance. Algal and biofilm biomass were strongly shaped by flow. Densities of nematodes, chironomid larvae, and oligochaetes were related to stability of the biofilm, whereas rotifer density was related to biofilm thickness. These divergences could imply different trophic strategies regarding biofilm resources (*e.g.* selectivity) that deserve further examination. High grazing activity of macrofaunal insect larvae in early summer could deplete biofilm and weaken meiofauna, but this speculation also needs further examination.

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Chapitre II

Réponse des nématodes du biofilm aux changements de leur habitat dans la Garonne : influence de l'hydrodynamique et de la disponibilité micro-algale

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II.1. Résumé de l'article

II.1.1. Contexte et objectifs

Les biofilms épilithiques de rivière sont des habitats instables (**chapitre I**), dont la composition microbienne varie selon les forçages environnementaux *e.g.* l'hydrodynamique (Biggs & Close, 1989; Biggs *et al.*, 1998; Lyautey *et al.*, 2005; Leflaive *et al.*, 2008). La méiofaune qui peuple densément ces biofilms reste peu étudiée (**chapitre I**), bien que les biofilms jouent à la fois un rôle d'habitat et de source de nourriture probable pour ces petits métazoaires (Peters & Traunspurger, 2005; Caramujo *et al.*, 2008). Les nématodes libres font partie des plus importants acteurs (en termes de densité) de la méiofaune (Traunspurger, 2002). Ils sont potentiellement capables de consommer le panel de ressources trophiques à leur disposition dans le biofilm (Traunspurger *et al.*, 1997; Hamels *et al.*, 2001; Riemann & Helmke, 2002; Ruess *et al.*, 2002; Höckelmann *et al.*, 2004). De plus, leur activité au sein du biofilm (*e.g.* bioturbation) semble influencer des processus clés comme le cycle de l'oxygène (Mathieu *et al.*, 2007), soulignant leur probable importance dans le fonctionnement de ces communautés. Cependant, peu d'études ont examiné les relations et la réponse de la communauté d'espèces de nématodes aux perturbations et au statut trophique des biofilm épilithiques de rivière, si ce n'est celle de Gaudes *et al.* (2006), mais dont la dimension temporelle et la fréquence d'échantillonnage reste limitée.

Cette étude décrit la distribution temporelle de la communauté de nématodes en termes de densité, diversité, biomasse, âge, sexe et type trophique (morphologie buccale) dans le biofilm épilithique de la Garonne moyenne sur une période de 18 mois avec une haute fréquence d'échantillonnage. En reliant ces données avec les conditions abiotiques et biotiques du biofilm, cette étude a pour objectifs :

(1) de préciser l'influence des changements saisonniers du biofilm sur la communauté de nématodes

(2) de déterminer si la distribution des groupes fonctionnels de nématodes correspond au statut trophique du biofilm (*e.g.* disponibilité des groupes de microphytes).

II.1.2. Principaux résultats et discussion

Au cours de cette étude, 28 espèces de nématodes ont été recensées dans le biofilm épilithique de la Garonne. Toutefois, deux espèces de la famille Chromadoridae : *Chromadorina bioculata* et *Chromadorina viridis* dominent très fortement la communauté de nématodes, représentant 86% des 2875 individus identifiés dans cette étude. Ces deux espèces sont du type trophique « epistrate-feeders », impliquant une certaine affinité avec les ressources microphytobenthiques (Traunspurger, 1997, 2000). Ces deux espèces sont corrélées à la disponibilité des diatomées au sein du biofilm et les nématodes de cette famille consomment les diatomées en milieu marin (Tietjen & Lee, 1977; Deutsch, 1978; Jensen, 1982). Les diatomées sont d'ailleurs le groupe microphytobenthique dominant dans le biofilm de la Garonne étudié. Ainsi, ce résultat plaide pour une correspondance entre la stratégie alimentaire des nématodes et la disponibilité en proies potentielles dans le biofilm.

Concernant la composition de la communauté de nématodes, un changement saisonnier significatif a été mis en évidence : une diversité plus importante est observée en été, tandis que la biomasse individuelle des nématodes et la proportion des deux espèces dominantes est moindre. Ce contraste estival se traduit par un groupe d'espèces (principalement des nématodes de la famille Monhysteridae) se retrouvant distribué en corrélation avec le gradient de température. Parallèlement, la proportion d'organismes autotrophes dans le biofilm (en termes d'index d'autotrophie) est réduite. Les nématodes Monhysteridae sont du type trophique « deposit-feeders » impliquant un régime principalement bactérivore (Traunspurger, 1997, 2000). Ainsi, ces nématodes bactérivores semblent bénéficier des conditions estivales qui profitent également au développement bactérien dans les biofilms de la Garonne (Lyautey *et al.*, 2010). Cependant, les conditions estivales sont généralement contraignantes pour la méiofaune de ces biofilms (**chapitre I**). Il est donc possible que des nématodes opportunistes comme les Monhysteridae, qui ont des cycles de vie courts et une reproduction parthénogénétique (Traunspurger, 1991), puissent mieux profiter de ces conditions globalement contraignantes que les Chromadoridae.

Cette étude suggère l'importance des ressources du biofilm et notamment de son contenu en diatomées pour la structuration de la communauté de nématodes (en termes de diversité et de types trophiques). Une étude ciblée du comportement trophique des nématodes serait avisée pour confirmer ce résultat, et démêler l'implication de la saisonnalité dans ce couplage.

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

N. Majdi[1,2], W. Traunspurger[3], S. Boyer[1,2], B. Mialet[1,2], M. Tackx[1,2], R. Fernandez[1,2], S. Gehner[3], L. Ten-Hage[1,2] and E. Buffan-Dubau[1,2]

[1] Université de Toulouse-UPS-INP, EcoLab UMR 5245, 118 route de Narbonne, 31062 Toulouse, France

[2] CNRS, EcoLab UMR 5245, 31062 Toulouse, France

[3] University of Bielefeld, Animal Ecology, Morgenbreede 45, 33615 Bielefeld, Germany

II.2. 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

II.3. 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 were 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.

II.4. Methods

II.4.1. Study site and sample collection

The Garonne is the largest river of south-western France with a drainage basin of 57 000 km² 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 the city of Toulouse (01°17'53"E, 43°23'45"N; elevation 175 m asl), where the Garonne is of sixth order (Fig. II.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 (Ameziane *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.

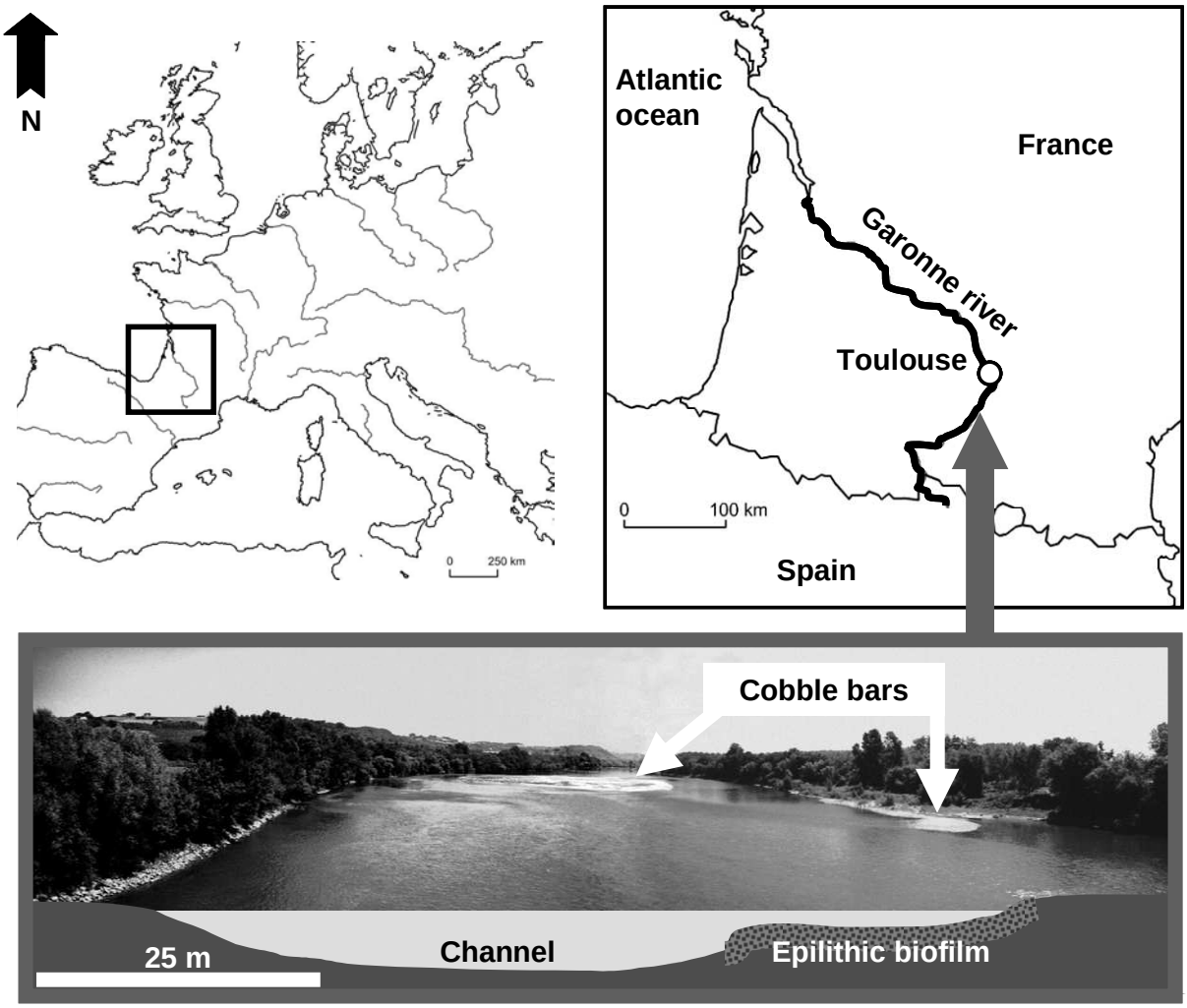

Figure II.1. Location of the sampling site and cross section view of the Garonne River at the sampling site.

II.4.2. 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 and shortly after 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).

II.4.3. 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.

II.4.4. Biofilm microalgal composition and biomass

II.4.4.1. *Microalgal pigments extraction and HPLC-analysis*

On each sampling occasion, four replicate biofilm suspensions were centrifuged (3220 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 1M 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 a 0.2 µm PTFE syringe filter and analyzed using a high-performance liquid chromatograph (HPLC) consisting of a 100 µL loop auto-sampler 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 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 pigment standards (DHI LAB products, Hørsholm, Denmark). Each pigment concentration was calculated by relating its chromatogram's peak area with the corresponding area of calibrated standard.

II.4.4.2. *Microalgal cultures and chemotaxonomy*

Algal pigment analysis by HPLC coupled with chemotaxonomic analysis using the 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 as for 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 II.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.

Table II.1. CHEMTAX pigment ratio matrix. 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)

		Biomarker pigment ratios to Chl *a*								
Algal group	Species	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		

II.4.4.3. *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 weigh 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).

II.4.5. 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 $P < 0.005$).

II.5. Results

II.5.1. Dynamics of the epilithic biofilm

Table II.2. Measured abiotic and biofilm biotic factors. Annual means refer to 2009. For temperature, O_2, pH and conductivity (N = 17 507). For days after flood and the biotic factors (N = 51). Minimum and maximum values refer to the whole sampling period (*i.e.* September 2008–March 2010)

		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

The range and annual mean values of each measured abiotic and biotic factor are listed in Table II.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. II.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. II.2b, Table II.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 significantly higher during summer and autumn (ANOVA: F = 2.8; P < 0.05) than during the remainder of the year.

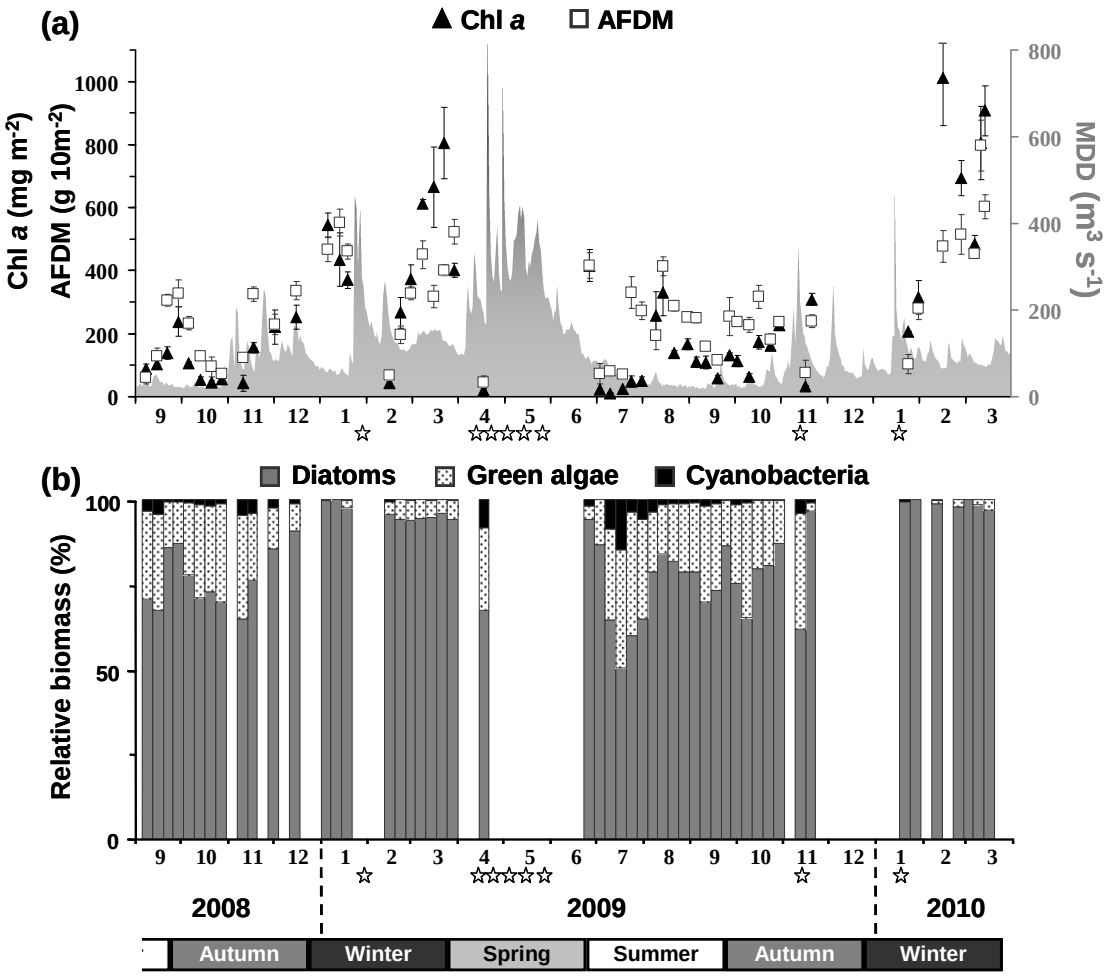

Figure II.2. Temporal dynamics of **(a)** epilithic chlorophyll *a* (Chl *a*) concentration (± SE, *N* = 4), ash-free dry mass (AFDM) of the biofilm (± SE, N = 4) and mean daily discharge (MDD), and **(b)** the relative proportion (%) of epilithic microalgal groups to total Chl *a* biomass (*N* = 4).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

II.5.2. 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. II.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. II.3a).

From the 2875 nematodes identified, 28 species belonging to 11 families were found (see species list in Table II.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 paramacrura*—represented only 10% of all identified nematodes over the whole period, they clearly dominated the assemblage from mid-July to mid-August (Fig. II.3b). Sixteen species were rare, accounting for <0.1% of all identified nematodes (Table II.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. II.3c.

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

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			
Coslenchus sp.	<0.1	3	S

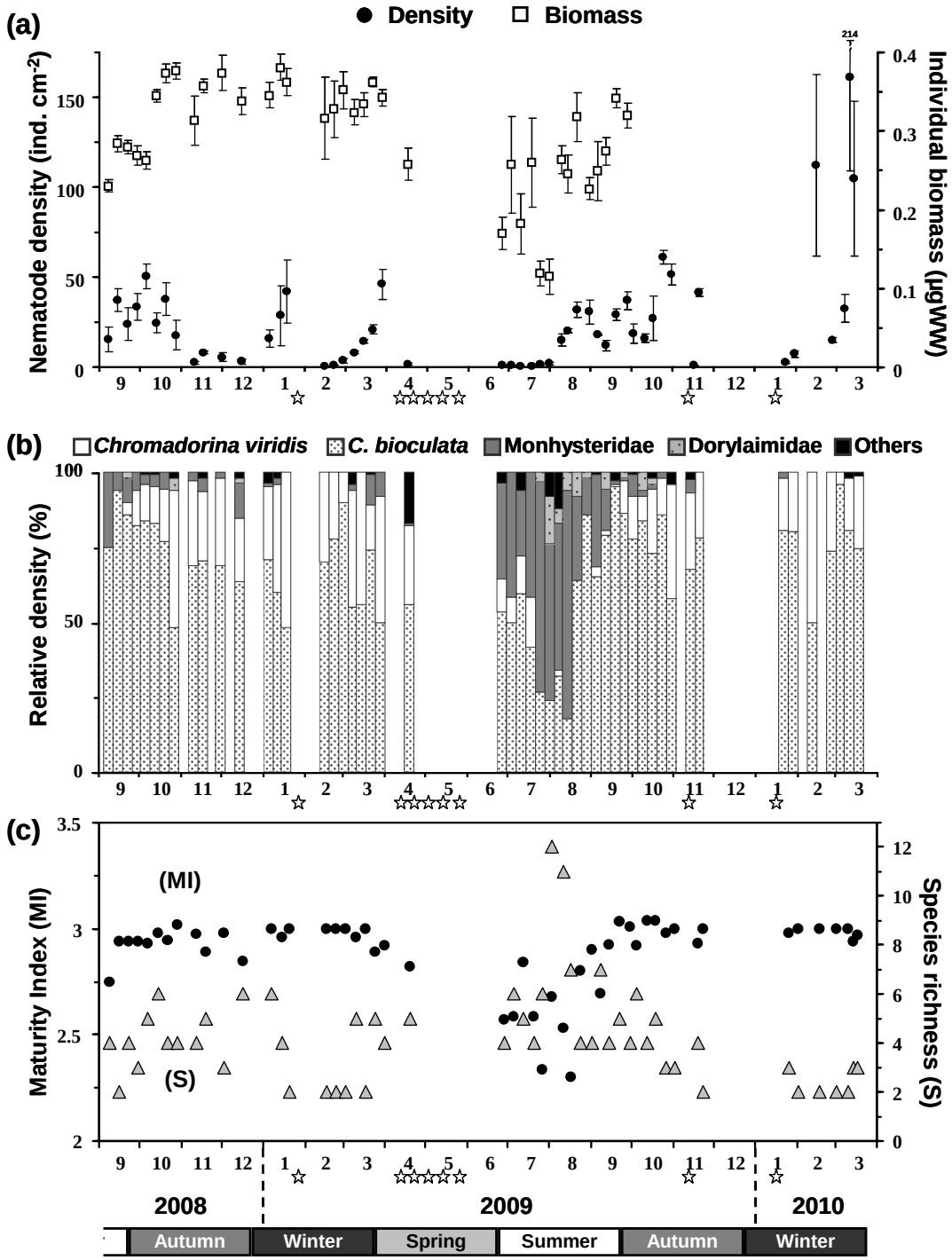

Figure II.3. Temporal dynamics of **(a)** nematode density (±SE, N = 4) and individual wet weight (WW) biomass (±SD, $N \geq 100$), **(b)** relative density of main nematode taxa, and **(c)** Maturity index (MI) and species richness (S) in the epilithic biofilm. Months, years, seasons and floods during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

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. II.4a).

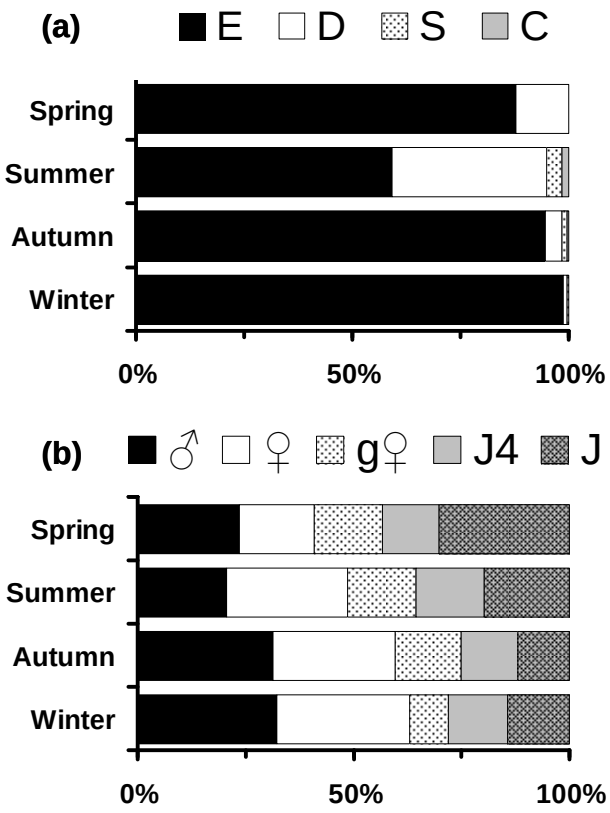

Figure II.4. Seasonal variations of the nematode community structure in the biofilm: **(a)** seasonal proportion of epistrate-feeders (E), deposit-feeders (D), suction-feeders (S) and chewers (C), and **(b)** seasonal proportion of males (♂), females (♀), gravid females (g♀), fourth stage juveniles (J4)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

The seasonal proportion of juveniles, fourth stage juveniles, females, gravid females and males is presented in Fig. II.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 significantly less during summer (ANOVA: $F = 3.2$; $P < 0.05$) than during winter.

II.5.3. 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. II.5 and Table II.4. The temporal distribution of nematode species was significantly influenced by 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 both these 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*.

Table II.4. Conditional effects from the redundancy analysis (RDA). 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)

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	

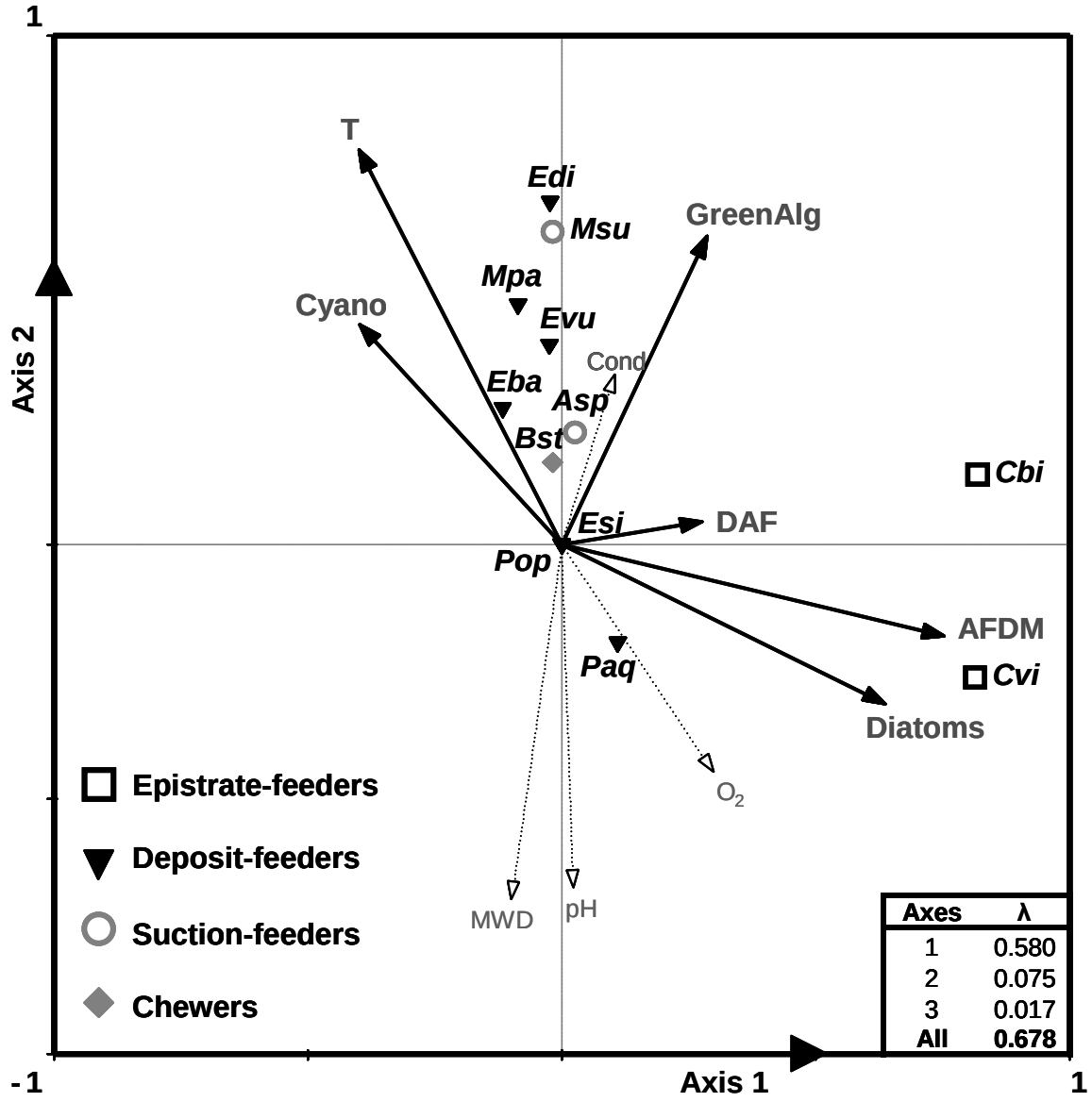

Figure II.5. Biplot from the redundancy analysis (RDA) explaining the distribution of nematode species densities according to environmental factors. Ordination axes were rescaled to range from –1 to 1. Slim dotted arrows are non-significant factors. Bold arrows are significant factors (Monte Carlo permutation test with Bonferroni's correction, $P < 0.005$). Eigenvalues (λ) are indicated for main ordination axes. Environmental factor abbreviations (see Table II.4). 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*).

II.6. 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 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 >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 II.2a and II.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. II.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, 2005c; 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. II.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 (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. II.3b). Such seasonal variations of species composition were previously reported for sediment-dwelling nematode communities in lakes (Traunspurger, 1991; Michiels & Traunspurger, 2005a) and in rivers (Beier & Traunspurger, 2003a). In our study, the summer nematode community is more diversified with a higher proportion of deposit-feeders: *e.g.* Monhysteridae (Figs II.3c and II.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. II.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.*, 1999a; 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. II.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.*, 2012a*).*

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. II.4b). Overall, summer nematode species lifestyle fits well with corresponding biofilm biotic conditions, suggesting again that a close coupling occurs between nematode assemblage functional structure and biofilm characteristics.

II.7. 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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Chapitre III

L’alimentation des nématodes du biofilm examinée par l’analyse HPLC de leurs contenus pigmentaires intestinaux

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III.1. Résumé de l'article

III.1.1. Contexte et objectifs

Les biofilms épilithiques de rivière sont des habitats complexes ou coexistent de nombreuses proies potentielles pour les nématodes. Lors du suivi des populations de nématodes associés au biofilm de la Garonne, un couplage a été mis en évidence entre la disponibilité des diatomées et les densités des deux espèces de nématodes dominantes : *Chromadorina bioculata* et *Chromadorina viridis* (**chapitre II**). Bien que le comportement trophique des nématodes d'eau douce soit peu documenté dans les biofilms (Höckelmann *et al.*, 2004), de nombreuses études conduites en condition contrôlées ou en milieu naturel avec des nématodes marins illustrent la complexité et la diversité du comportement trophique des nématodes (voir *e.g.* Moens & Vincx, 1997; Moens *et al.*, 2006). Examiner les habitudes alimentaires des nématodes en milieu naturel apparaît pertinent pour mieux comprendre l'organisation des assemblages microbiens dans des milieux complexes comme les biofilms. En effet, si les nématodes consomment sélectivement leurs proies microbiennes, ils contraindront la contribution des taxas microbiens sélectionnés au fonctionnement de l'écosystème (*e.g.* Traunspurger *et al.*, 1997; Moens *et al.*, 1999a). Ainsi, pouvoir spécifier et quantifier l'impact du broutage des nématodes sur les microorganismes phototrophes benthiques (le microphytobenthos, MPB) peut permettre de mieux appréhender leur influence sur la capacité de production primaire du biofilm. En milieu marin, le broutage du zooplancton et de la méiofaune peut-être caractérisé et quantifié en utilisant l'analyse par chromatographie liquide à haute performance (HPLC) des pigments contenus dans leur tube digestif (*e.g.* Buffan-Dubau *et al.*, 1996; Gasparini *et al.*, 1999; Buffan-Dubau & Carman, 2000a; Goldfinch & Carman, 2000; Irigoien *et al.*, 2000; Tackx *et al.*, 2003). A notre connaissance, cette technique n'a pas encore été appliquée aux nématodes (Moens *et al.*, 2006). L'avantage de cette technique est qu'elle renseigne sur le comportement alimentaire des invertébrés en conditions naturelles. Dans ce contexte, les contenus pigmentaires intestinaux des nématodes ont été analysés pour la première fois par HPLC en comparaison avec les concentrations pigmentaires du biofilm, avec pour objectifs de tester l'hypothèse d'une sélectivité trophique de *C. bioculata* et *C. viridis* sur les diatomées épilithiques de la Garonne et de quantifier cette pression de broutage.

III.1.2. Principaux résultats et discussion

Les résultats contredisent l'hypothèse de départ, dans le sens où, même si les pigments biomarqueur des diatomées (*e.g.* fucoxanthine et diadinoxanthine) sont retrouvés dans les intestins de *C. bioculata* et *C. viridis*, les résultats indiquent que le remplissage de l'intestin des nématodes en équivalents chlorophylle *a* (Chl *a*-eq) reste proportionnel à la concentration de Chl *a* dans le milieu. Ce qui implique que *C. bioculata* et *C. viridis* consomment non-sélectivement le MPB du biofilm (Gasparini *et al.*, 1999; Tackx *et al.*, 2003). Ce comportement opportuniste a déjà été observé en laboratoire (Schiemer, 1983; Montagna *et al.*, 1995; Moens & Vincx, 2000). Un tel opportunisme est compréhensible : compte-tenu du caractère fluctuant et hétérogène de la répartition des micro-organismes dans les biofilms (Murga *et al.*, 1995; Lyautey *et al.*, 2005; Leflaive *et al.*, 2008), les nématodes ont tout intêret à pouvoir adapter leur régime alimentaire en fonction de la disponibilité des ressources, comme c'est le cas en milieu estuarien (Moens & Vincx, 1997).

Dans un contexte bibliographique où la caractérisation des processus digestifs des nématodes est encore largement restreinte aux nématodes bactérivores (Moens *et al.*, 1999b, 2006), il est délicat de pouvoir utiliser des temps de passage intestinaux appropriés pour estimer précisément une pression de broutage d'après la quantification des contenus digestifs. Quoi qu'il en soit, la pression de broutage exercée par *C. bioculata* et *C. viridis* sur la biomasse phototrophe est plutôt réduite : ils ingèrent journalièrement en moyenne 0.03–0.67% de la Chl *a* du biofilm (en prenant en compte un large intervalle de temps de passage intestinaux potentiels). Des pressions de broutage comparables sont observées pour les nématodes de milieux marins et saumâtres (Epstein & Shiaris, 1992; Nozais *et al.*, 2001; Moens *et al.*, 2002; Rzeznik-Orignac *et al.*, 2003; Pascal *et al.*, 2008b). Le rôle principal des nématodes dans le fonctionnement du biofilm se situe donc probablement autour d'une régulation secondaire (*e.g.* par bioturbation) des micro-organismes et/ou des propriétés diffusives du biofilm—par des modifications physicochimiques liées aux déplacements à travers la matrice épilithique—comme suggéré par Mathieu *et al.* (2007) et Pinckney *et al.* (2003).

En comparant les taux de broutage avec les besoins énergétiques de *C. bioculata* et *C. viridis*, il est estimé que la consommation des contenus cellulaires du MPB ne satisfait en moyenne qu'entre 1 et 27% de leurs besoins énergétiques. Ces résultats suggèrent l'emploi d'autres sources trophiques par ces nématodes, et notamment, de leur possible utilisation des exo-

polymères et du carbone organique du biofilm avec l'aide de l'activité enzymatique de bactéries commensales (Riemann & Schrage, 1978; Riemann & Helmke, 2002).

De par sa première application aux nématodes, l'analyse par HPLC des contenus pigmentaires intestinaux s'est révélée particulièrement utile, dans la mesure où les informations collectées sont les premières qui détaillent le comportement trophique des nématodes dans des biofilms naturels. Cependant, quelques limitations quand à l'utilisation de cette technique sont soulignées :

(1) Afin d'obtenir des concentrations pigmentaires suffisantes à la discrimination des pigments minoritaires, et compte-tenu de la taille réduite des nématodes et des difficultés inhérentes à leur tri dans le biofilm, il serait recommandable de n'appliquer cette technique qu'à des nématodes algivores de grande taille (*e.g.* Dorylaimidae) et/ou habitant des milieux où leur tri est plus aisé.

(2) L'analyse est appliquée à la communauté entière de nématodes car un tri à l'espèce serait trop fastidieux. Ainsi le comportement alimentaire obtenu concerne un assemblage d'espèces. Dans cette étude, la dominance écrasante de deux espèces du même genre limite un possible biais, d'autant que les rares autres espèces sont principalement bactérivores. Cependant, l'emploi de cette technique devrait être restreint à de telles communautés ou ciblé sur une espèce particulière pour donner des informations satisfaisantes.

(3) Seul le broutage (i.e. la consommation des contenus cellulaires du MPB) est considéré. Ainsi, cette méthode n'apporte qu'une information limitée si les nématodes consomment d'autres ressources. Par contre, il est envisageable d'améliorer la définition de cette méthode—en modulant la méthodologie utilisée—pour analyser des pigments et/ou des métabolites biomarqueurs d'autres micro-organismes (voir *e.g.* Buffan-Dubau *et al.*, 1996).

Feeding of biofilm-dwelling nematodes examined using HPLC-analysis of gut pigment content

N. Majdi[1,2], M. Tackx[1,2], W. Traunspurger[3] and E. Buffan-Dubau[1,2]

[1] Université de Toulouse-UPS-INP, EcoLab UMR 5245, 118 route de Narbonne, 31062 Toulouse, France

[2] CNRS, EcoLab UMR 5245, 31062 Toulouse, France

[3] University of Bielefeld, Animal Ecology, Morgenbreede 45, 33615 Bielefeld, Germany

III.2. Abstract

The natural feeding behaviour of the nematodes *Chromadorina bioculata* (Schultze *in* Carus 1857) and *Chromadorina viridis* (Linstow 1876) was studied *in situ*, within epilithic biofilms of the Garonne River (France). Based on their feeding-type characteristics and population dynamics, it was hypothesized that these species feed selectively on microphytobenthos (MPB) within the biofilm, and that among MPB groups, diatoms are preferred. High-performance liquid chromatography (HPLC) was used for separation, identification and quantification of pigments both in nematode guts and in the biofilm. This is the first time that nematode gut pigment contents were examined under natural conditions. Diatoms dominated the MPB which also comprised cyanobacteria and green microalgae. The comparison between chlorophyll *a* content in nematode guts versus in the biofilm showed that *C. bioculata* and *C. viridis* fed opportunistically (non-selectively) on MPB within the biofilm. Only diatom biomarker pigments were found in nematode guts suggesting that they could preferentially fed on diatoms. However, the non-detection of biomarker pigments for other microphyte groups could be also linked to HPLC detection limits. It was estimated that *Chromadorina* nematodes daily ingested on average 0.03–0.67% of the MPB standing stock. This grazing covered only a small part of their energetic requirements, suggesting that besides MPB they probably also fed on other biofilm food sources. Some considerations on the applicability of the HPLC gut pigment analysis technique for the examination of nematode feeding are also presented.

Keywords: selectivity, grazing, diatoms, periphyton, meiofauna, *Chromadorina*

III.3. Introduction

Meiofauna is extremely species rich and abundant in freshwater benthos, contributing substantially to secondary production, acting as food web intermediates and informing general ecological theories such as the metabolic theory of ecology (Schmid-Araya & Schmid, 2000; Schmid-Araya *et al.*, 2002; Stead *et al.*, 2005; Reiss *et al.*, 2010; Reiss & Schmid-Araya, 2010). Free-living nematodes are among the most important contributors to meiofauna (Traunspurger, 2002). Nematodes feed on a variety of microorganisms including microphytes (Moens & Vincx, 1997; Höckelmann *et al.*, 2004), protozoans (Hamels *et al.*, 2001), fungi (Ruess *et al.*, 2002) and bacteria (Traunspurger *et al.*, 1997) and probably also on organic matter through enzyme-sharing interactions with bacteria (Riemann & Helmke, 2002).

In freshwater epilithic biofilms, microphytes, protozoans, fungi and bacteria are embedded in close connection within a three-dimensional mucous matrix of self-produced exo-polymeric substances (Flemming & Wingender, 2010). These biofilms offer a shelter and a rich variety of potential food items for nematodes (Höckelmann *et al.*, 2004; Peters & Traunspurger, 2005). In return, nematode activity might influence key biofilm processes such as detachment, oxygen turnover and secondary metabolites release (Sabater *et al.*, 2003; Gaudes *et al.*, 2006; Mathieu *et al.*, 2007). Biofilm biomass dynamics can be, to a considerable extent, modelled as a function of hydrodynamics and self-detachment (*e.g.* Boulêtreau *et al.*, 2006). However, functional field studies assessing nematode feeding habits within these biofilms are lacking (Moens & Vincx, 1997), hampering an appropriate assessment of their trophic role within the mat and their potential feeding impact on biofilm biomass. This lack of *in situ* data is mostly due to the difficulty of measuring nematode feeding in such complex habitats: not only are epilithic biofilms composed of a complex organic matrix containing a variety of potential food sources for nematodes, but the mucous nature of the biofilm itself poses practical experimental problems.

The quantification of the chlorophyll *a*-equivalent (Chl *a*-eq, *i.e.* Chl *a* + phaeopigments) contained in guts allows to obtain *in situ* data on the grazing activity of post-mortem isolated taxa of animals. To date, this technique is routinely used with *e.g.* planktonic copepods: the quantitative measurement of their gut Chl *a*-eq content with regards to Chl *a* concentration in the surrounding habitat has allowed to investigate their selective grazing on phytoplankton,

with a disproportion between gut Chl *a*-eq content and Chl *a* concentration indicating a selective grazing (*e.g.* Price, 1988; Gasparini *et al.*, 1999; Irigoien *et al.*, 2000; Tackx *et al.*, 2003).

Gut pigment analyses using high-performance liquid chromatography (HPLC) can inform on feeding selectivity among various microphytic taxa by identifying and quantifying their biomarker pigments. This technique was applied with some meiobenthic groups: harpacticoid copepods (Buffan-Dubau *et al.*, 1996; Buffan-Dubau & Carman, 2000a) and chironomids (Goldfinch & Carman, 2000) in muddy salt marshes, but not with nematodes (Moens *et al.*, 2006). Although both selective and non-selective feeding strategies were observed for free-living marine bacterial feeding or predaceous nematodes under laboratory conditions, nematode selectivity on microphytobenthos (MPB) *in situ* and in freshwater habitats is poorly documented (Moens & Vincx, 1997; Moens *et al.*, 2006).

In order to determine ingestion rates from gut pigment contents, these have to be reported to gut passage times (GPT). However, information on nematode GPT and their dependence on environmental factors remain scarce and mainly restricted to bacterial-feeding nematodes (Moens *et al.*, 1999b; Moens *et al.*, 2006). Thus, a careful approach is needed for determining ingestion rates from measurements of gut pigment contents by using literature GPT. Nonetheless, given our generally limited knowledge about the grazing rates of freshwater nematodes (Borchardt & Bott, 1995), even such estimations represent, at present, a significant advancement in the evaluation of their grazing pressure on MPB.

In a recent study conducted in the Garonne River, Majdi *et al.* (2011) found a coupling pattern between epilithic diatom biomass and the density of the two dominant biofilm-dwelling nematode species: *Chromadorina bioculata* (Schultze *in* Carus 1857) and *Chromadorina viridis* (Linstow 1876). According to their buccal morphology, both these species were classified as epistrate-feeders after Traunspurger (1997), and hence are expected to feed predominantly on microphytes (Traunspurger, 2000). In marine environments, a diatom-feeding behaviour is well-documented for Chromadoridae (*i.e.* the family including *Chromadorina* spp. nematodes), which puncture or crack diatom frustules to suck inner cellular contents (Tietjen & Lee, 1977; Jensen, 1982; Romeyn & Bouwman, 1983; Moens & Vincx, 1997). Examining the digestive physiology of C*hromadorina germanica* Bütschli 1874, Deutsch (Deutsch, 1978) also suggested that it must have a fairly narrow diet primarily

composed of diatoms. As stated above, river epilithic biofilms offer a vast variety of potential food items to the nematode community. Within this offer, MPB seem a likely preferred food source considering the above mentioned knowledge on the feeding behaviour of the dominant nematode species (*Chromadorina* spp.). It can also be expected that epilithic diatoms are selected among the other microphyte groups available in the biofilm.

In this context, this study aims:
(1) to test the hypothesis that biofilm-dwelling *Chromadorina bioculata* and *Chromadorina viridis* nematodes feed selectively on biofilm MPB under natural conditions and that diatoms are preferred among microphyte groups,
(2) to estimate their grazing pressure on MPB biomass.

III.4. Methods

III.4.1. Study site and sampling

With a total length of 647 km and a drainage basin of 57 000 km², the Garonne is the largest river of south-western France. The Garonne is characterised by strong hydrodynamics (Chauvet & Décamps, 1989) displaying a pluvio-nival flow regime with relatively short flash-floods caused by heavy rainfall and a long and intense spring flood period due to snow-melt. The river bed consists mainly of cobbles and gravels, and between floods, a thick epilithic phototrophic biofilm typically coats the upper surfaces of cobbles. Sampling was undertaken at a cobble bar of the Garonne river situated 36 km upstream the city of Toulouse (01°17’53”E, 43°23’45”N). At this site, the residence time is too low for important phytoplankton development and it is assumed that benthic biofilms provide most of the riverine primary production (Ameziane *et al.*, 2003).

Epilithic biofilm samples were weekly collected on September and October 2008, January, March and September to November 2009. On each sampling occasion (N = 23), water temperature (T), dissolved oxygen concentration (O_2), conductivity, pH and flow velocity were recorded at 5 cm above the streambed using an automated YSI 6000 multi-parameter probe (YSI inc., Yellow springs, OH, USA) and a Flow-meter Flo-Mate 2000 (Flow-Tronic, Welkenraedt, Belgium). Twelve submerged cobbles covered by epilithic biofilm (diameter

~10 cm) were collected and processed: (1) to determine nematode species assemblages, density and individual biomass, (2) to measure total epilithic dry mass (DM) and ash-free dry mass (AFDM), (3) to measure biofilm MPB pigment concentrations using HPLC-analysis, and (4) to estimate the relative contribution of the different MPB groups to total MPB biomass in terms of chlorophyll *a* (Chl *a*) using CHEMTAX version 1.95 software (Mackey *et al.*, 1996). These procedures are detailed in Majdi *et al.* (2011).

For nematode gut pigment analysis, four more cobbles were collected on each sampling occasion. The biofilm covering cobbles was collected in the field by scraping-off the upper cobble surface with a scalpel and immediately immerged into liquid N_2. This instant freezing minimizes nematode gut content egestion (Moens *et al.*, 1999b). Frozen biofilm samples were then stored at –80°C until nematode sorting for gut pigment analyses.

III.4.2. Nematode sorting for gut pigment analysis

A biofilm sample was allowed to thaw in a 5 L-bucket with 100 mL tap water. Once defrosted, aggregates were crumbled with scissors. Then, a water jet was used to mix the biofilm suspension, in order to facilitate the separation of nematodes from heavier particles by decantation after Hodda & Abebe (2006). After 2 min of decantation, the supernatant containing nematodes and other light particles was poured through a 40 μm sieve to retain nematodes. The decantation operation was repeated four times. Then, undamaged nematodes were sorted from the bulk of gathered filtrate and isolated in small groups of 50 individuals under a stereomicroscope (9×–90×) while avoiding rare large suction-feeding nematodes. Each group was transferred with a 10 μL pipette to a petri dish containing a cold milliQ water rinsing bath. The operation was repeated until at least 400 nematodes lay in the rinsing bath. There, nematodes were thoroughly cleaned from any adherent particles, isolated by groups of 20 individuals, photographed and carefully pipetted in an eppendorf tube. All sorting operations were conducted under minimum light exposure and above a thin ice block to limit pigment photo- and/or thermo-degradation. At least a 400 nematode sample was prepared on each sampling occasion.

III.4.3. Extraction and HPLC-analysis of nematode gut pigment contents

Each sample of sorted nematodes was centrifuged (500 g, 5 min) to allow the settlement of a "nematode pellet". Excess water was removed by freeze-drying and pigments were extracted from nematode samples in 200 µL of 98% cold-buffered methanol (with 2% of 1 M ammonium acetate) by sonicating for 90 seconds in an ultrasonic bath (Elmasonic S-10 series, IMLAB, Lille, France). Extraction was then allowed overnight at –20°C in the dark. The pigment extract so obtained was then filtered on a 0.2 µm PTFE syringe filter with very low dead volume <10 µL (ReZist series Ø13 mm, Whatman inc., Florham Park, NJ, USA) and analyzed using the method described for biofilm pigment analyses in Majdi *et al.* (2011). The high-performance liquid chromatograph (HPLC) consisted of a 100 µl loop auto-sampler 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 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 phaeopigments (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 authentic standards (DHI LAB products, Hørsholm, Denmark), except for peridinin and diatoxanthin, which were obtained from the dinoflagellate species *Amphidinium carterae* Hubert 1967, CCAP strain 1102/3 (Culture Collection of Algae and Protozoa, Oban, UK). For pigment quantification, a response factor was calculated for each standard from the linear relationship between the concentration and the corresponding peak area on HPLC chromatograms. Pigments that were spectrally similar to, but did not have the same retention time as standards were designated 'like'-pigments. They were quantified using the response factor obtained from corresponding standards and summed to the value of the corresponding original pigment, *e.g.* Chlorophyll *a* (Chl *a*) quantification = Chl *a*-like$_1$ + Chl *a*-like$_2$ + Chl *a* (see Table III.1).

The nematode community was strongly dominated by *Chromadorina bioculata* and *Chromadorina viridis* (see results). The few other species isolated concomitantly were all deposit-feeders which have a minute unarmed buccal cavity allowing them only to swallow small preys such as bacteria (Moens *et al.*, 2006). Therefore, the presence of potential MPB

pigments in their guts was presumed to be minor. Hence, pigment concentrations measured from nematode extracts were reported to the expected proportion (number) of *C. bioculata* and *C. viridis* individuals extracted. To correct for possible nematode pigment which did not stem from the gut content, 415 nematodes were starved for 48 h in filtered (0.2 µm) river water to represent a nematode control sample analysed using the same HPLC protocol described above.

III.4.4. Nematode ingestion rates, production and energy requirements

Data on nematode gut passage times (GPT) are rare, but since their gut is completely emptied with each defecation (Duncan *et al.*, 1974), and defecation intervals are very short (Avery & Thomas, 1997), GPT are likely to last only few minutes for most nematode species (Moens *et al.*, 2006). GPT shorter than 2 min were reported for the bacterial-feeding *Caenorhabditis elegans* Maupas 1900 (Ghafouri & McGhee, 2007). Defecation intervals of <4–43 min were observed in the marine Monhysterida *Daptonema* sp., and defecation intervals of 14–23 min were observed for the marine Chromadoridae *Spilophorella* sp. while feeding on diatoms (Moens *et al.*, 1999b, see discussion). Consequently, and knowing that *C. bioculata* was reported to be very active (Croll & Zullini, 1972), we assumed an average GPT of 14 min for all sampling occasions to estimate daily ingestion rates based on gut pigment content data. However, due to our uncertainty about the GPT of *Chromadorina* in field conditions, ingestion rates were calculated with an error interval using GPT 5-fold shorter or longer than 14 min (*i.e.* 2.8–70 min).

Nematode wet weights were calculated from their body dimensions (length and width) after Andrássy (1956) and converted into carbon content assuming a dry/wet weight ratio of 0.25 (Warwick & Gee, 1984) and a carbon/dry weight ratio of 0.45 (Peters, 1983). Nematode production was calculated for each sampling after Plante & Downing (1989):

$$\text{Log}\,(P) = 0.06 + 0.79 \times \text{Log}\,(B) - 0.16 \times \text{Log}\,(M_{max}) + 0.05 \times T$$

With mean nematode biomass (B, mgC m^{-2}), maximum individual biomass ($M_{max,}$ µgC ind^{-1}) and average surface water temperature (T). Nematode production was then expressed per day by dividing P by 365. This method was recently recognized to give the most reliable estimates of invertebrate production over other regressions available in the literature, partly because it

take into account the effect of temperature on invertebrate metabolism (Butkas *et al.*, 2011). Further, nematode energetic requirements (in terms of carbon) were estimated from production assuming a 20% factor for energy conversion efficiency (Heip *et al.*, 1990). Assuming an assimilation/ingestion efficiency of 25% (Herman & Vranken, 1988), assimilation rates of MPB were compared to energetic requirements, to infer the contribution of MPB to the diet of nematodes.

III.4.5. Statistical analyses

All data fulfilled normality assumptions (Kolmogorov–Smirnov test) and homogeneity of variances (Levene test). Hence they were not further transformed. Correlations were examined by Pearson correlation coefficient. To disentangle the potential co-influence of correlated predictors, *e.g.* biofilm biomass, pigment concentrations, temperature and O_2 on gut Chl *a*-equivalents (Chl *a*-eq, *i.e.* Chl *a* + phaeopigments), a multiple regression was performed using stepwise forward selection. *F* to enter was set at 1 with a *P*-value < 0.001. By comparing the statistical significance of predictors in a stepwise design, this procedure allowed selecting the most relevant predictor(s) which explained gut Chl*a*-eq variations. All tests were performed using Statistica software (version 8.0, Statsoft inc., Tulsa, OK, USA).

III.5. Results

III.5.1. Biofilm microphytobenthos (MPB)

Along the study period, the biofilm DM averaged 328 g m^{-2} (ranging from 91–679 g m^{-2}), AFDM averaged 26.1 g m^{-2} (8.8–58 g m^{-2}) and Chl *a* averaged 235 mg m^{-2} (46–803 mg m^{-2}). The identified pigments from biofilm extracts are listed in Table III.1 and examples of biofilm HPLC-chromatograms are shown in Fig. III.1a,b. Among biomarker pigments, fucoxanthin and chlorophyll *c* (Chl *c*) were present in substantial concentrations (>50 µg gDM^{-1}). They may originate from diatoms and other groups of chromophyte microalgae, *e.g.* prymnesiophytes and chrysophytes (Stauber & Jeffrey, 1988). However, typical biomarkers for prymnesiophytes and chrysophytes such as 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin (Jeffrey *et al.*, 1997) were not detected in the biofilm, indicating that fucoxanthin and Chl *c* mainly originated from diatoms. Likewise, diadinoxanthin which may

be produced by diatoms, euglenophytes and dinoflagellates, was detected. However neoxanthin which is a typical biomarker pigment for euglenophytes (Schagerl *et al.*, 2003) as well as peridinin and diatoxanthin which are biomarker pigments for dinoflagellates (Johansen *et al.*, 1974) were not detected, implying that diadinoxanthin also mainly originated from diatoms. Zeaxanthin was detected in the biofilm, and although it may be found as a minor pigment in green algae, it is primarily a product of cyanobacteria (Brotas & Plante-Cuny, 1998). Lastly, biomarker pigments chlorophyll *b* (Chl *b*) and lutein accounting for green algae and vascular plants were also detected. However, field and microscopic observations did not reveal the presence of macrophytes within the biofilm community. Furthermore, the biofilm Chl *a*/phaeopigments ratio averaged 36.5, indicating that the epilithic phototrophic community was in a viable state (Buffan-Dubau *et al.*, 1996) and that the potential contribution of fine particulate plant and/or macrophyte-derived detritus to the biofilm matrix was minute. Hence, green microalgae were likely the main source of lutein and Chl *b*.

Table III.1. Microphytobenthic pigments in biofilm and nematode extracts. Biofilm pigment concentrations are reported to corresponding biofilm dry mass (DM). Gut pigment contents are expressed per individual *Chromadorina* spp. Pigments are listed following their elution order. Probable pigment sources were compiled after Johansen *et al.* (1974), Jeffrey *et al.* (1997) and Majdi *et al.* (2011)

Peak #	Pigment	Biofilm (µg gDM^{-1})		Gut (pg ind^{-1})		Probable pigment source
		Mean	Range	Mean	Range	
1	Chlorophyll *c*[a]	67	6–158	0.25	0–1.21	Diatoms
2	Pheophorbide *a*	8	2–23	0.29	0.03–1.56	Chlorophyll *a* degradation
3	Pheophorbide *a*-like					
4	Fucoxanthin-like	290	26–704	1.16	0.05–2.74	Diatoms
5	Fucoxanthin					
6	Violaxanthin	7	2–20	*not detected*		Green microalgae
7	Diadinoxanthin-like	41	3–128	0.03	0–0.18	Diatoms
8	Diadinoxanthin					
9	Zeaxanthin	5	1–16	*not detected*		Cyanobacteria
10	Lutein	8	2–19	*not detected*		Green microalgae
11	Chlorophyll *b*	11	2–23	*not detected*		Green microalgae
12	Chlorophyll *a*-like$_1$[b]					
13	Chlorophyll *a*	709	72–1740	0.90	0.06–4.51	All microphytes
14	Chlorophyll *a*-like$_2$					
15	Pheophytin *a*	13	2–24	4.61	1.46–7.56	Chlorophyll *a* degradation
16	Carotenes (α+β)	23	3–58	0.21	0–0.96	All microphytes

[a]chlorophyll *c* = chlorophylls $c_1 + c_2$.

[b]chlorophyll *a*-like$_1$ = three chlorophyll *a* allomer compounds.

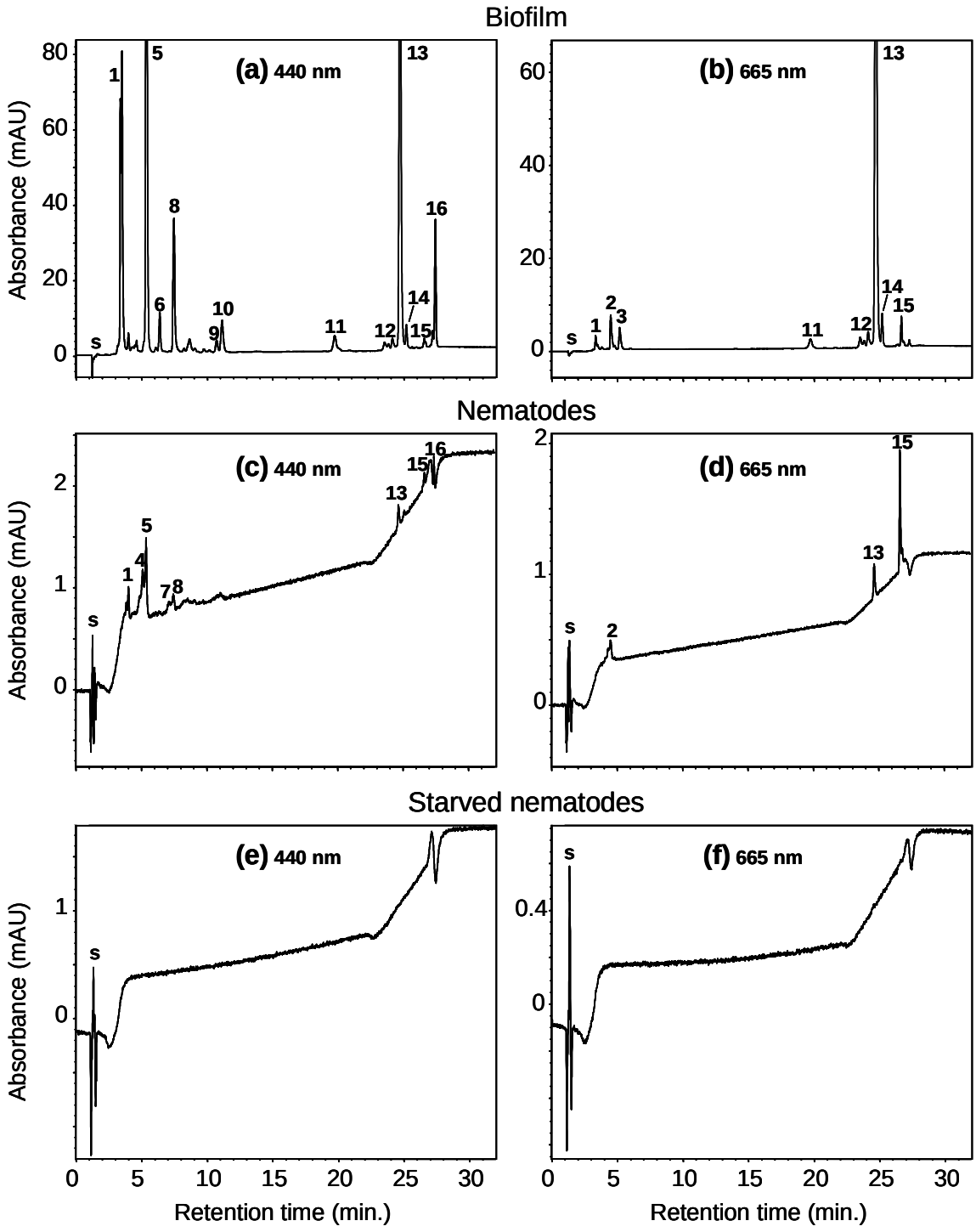

Figure III.1. Examples of absorbance HPLC-chromatograms measured at 440 and 665 nm of **(a)**,**(b)** biofilm extract, **(c)**,**(d)** extract of nematodes collected in the biofilm (comprising 400 individuals), **(e)**,**(f)** extract of nematodes starved for 48h (comprising 415 individuals). For peak identification see Table III.1. S: solvent-front peak. Absorbance is expressed in milli Arbitrary Units (mAU).

Considering all sampling occasions, it was estimated that the total biofilm MPB biomass consisted in average of 82% diatoms, 17% green microalgae and 1% cyanobacteria. This dominance of diatoms was also underlined by significant positive correlations found between Chl *a* and diatom biomarker pigment concentrations in the biofilm (Pearson correlation, N = 23; Chl *a* & Chl *c*: R = 0.98, P < 0.001; Chl *a* & fucoxanthin: R = 0.97, P < 0.001; Chl *a* & diadinoxanthin: R = 0.94, P < 0.001). Biofilm Chl *a* concentration correlated also positively with AFDM and O_2 (Pearson correlation, N = 23; Chl *a* & AFDM: R = 0.61, P < 0.001; Chl *a* & O_2: R = 0.47, P < 0.01), whereas negatively with water temperature (Pearson correlation, N = 23, R = –0.6, P < 0.001, see Fig. III.2a).

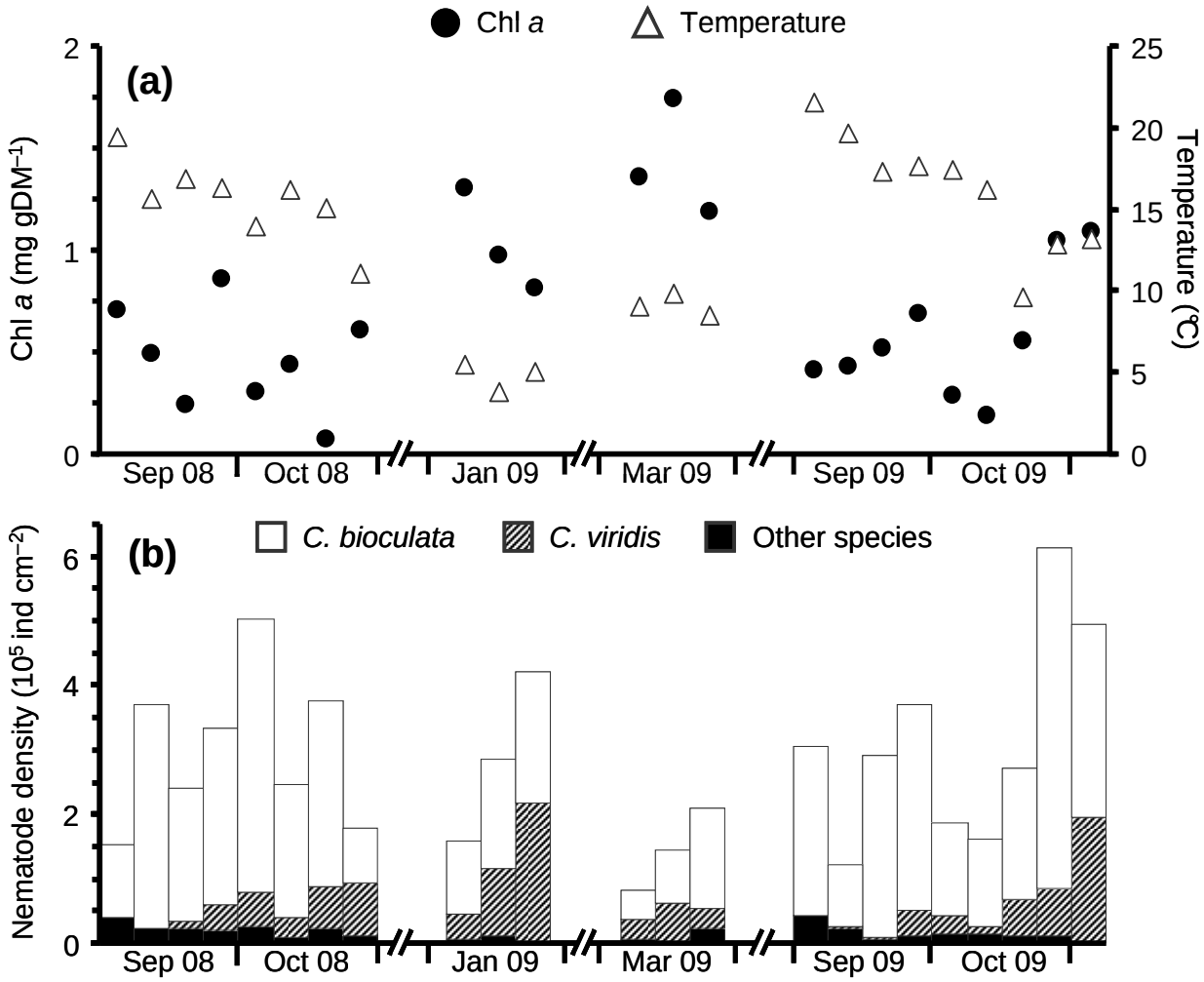

Figure III.2. Temporal dynamics (N = 23) of **(a)** water temperature and biofilm chlorophyll *a* concentration (Chl *a*), **(b)** nematode density in the biofilm with the relative proportion of *Chromadorina bioculata*, *Chromadorina viridis* and other nematode species.

III.5.2. Nematode community

Over the study period, nematode density averaged (±SD) 2.8 $\times 10^5$ ±0.3 $\times 10^5$ ind m^{-2} (ranging from 0.8–6.1 $\times 10^5$ ind m^{-2}). Nematode individual biomass averaged 0.11 µgC ind^{-1} (0.08–0.14 µgC ind^{-1}). The total biomass of nematodes in the biofilm averaged 32.4 ±4 mgC m^{-2} (9.4–78 mgC m^{-2}). The epistrate-feeding species *Chromadorina bioculata* and *Chromadorina viridis* dominated strongly, averaging 94.2% (75–100%) of nematode species inhabiting the biofilm (Fig. III.2b). The other species contributing to nematode community were all deposit-feeders: *Eumonhystera dispar* (Bastian 1865), *Eumonhystera vulgaris* (de Man 1880), *Eumonhystera barbata* Andrássy 1981, *Monhystrella paramacrura* (Meyl 1954), *Plectus opisthocirculus* Andrássy 1952 and *Plectus aquatilis* Andrássy 1985. Large suction-feeding *Dorylaimus* cf *subtiliformis* (Andrássy 1959) were rarely encountered.

III.5.3. Gut pigment contents and feeding-behaviour of nematodes

The identified pigments from nematode extracts are listed in Table III.1 and examples of HPLC-chromatograms are shown in Fig. III.1c,d. Neither MPB pigments nor nematode body constituent pigments were detected from the control sample conducted with starved nematodes (Fig. III.1e,f). Thus, it was assumed that pigments detected in field nematode extracts stem from their gut contents. Fucoxanthin was the major biomarker pigment observed in nematode extracts, indicating that nematodes fed on diatoms. This was corroborated by the presence of diadinoxanthin and Chl *c* in nematode extracts (Fig. III.1c and Table III.1). Biomarker pigments of cyanobacteria and green microalgae (*e.g.* zeaxanthin and Chl *b*) were not detected in nematode extracts (Table III.1). The Chl *a*/phaeopigments ratio averaged 0.18 in nematode extracts. This value, which is very low compared to that found in biofilm extracts, reflects the Chl *a* breakdown during digestive processes of nematodes. Hence, to account for this degradation, the Chl *a*-equivalent (Chl *a*-eq) was quantified by summing Chl *a*, pheophorbide *a* and pheophytin *a*. Chl *a*-eq was considered as a proxy for total microalgal biomass in nematode guts. It averaged (±SD) 5.8 ±0.3 pg ind^{-1} (range: 2.6–9.1 pg ind^{-1}).

Gut Chl *a*-eq and gut pheophytin *a* correlated positively with biofilm Chl *c*, fucoxanthin, diadinoxanthin, Chl *a*, AFDM and dissolved oxygen (O_2) (Table III.2), whereas negatively with water temperature (T). Gut Chl *a* correlated positively with biofilm Chl c, fucoxanthin, AFDM and O_2, whereas negatively with T. Gut pheoporbide *a* correlated positively with O_2,

whereas negatively with T. Gut diadinoxanthin correlated positively with O_2. Lastly, gut Chl *c* correlated negatively with T. Conductivity, pH, streambed flow velocity, biofilm DM, pheophytin *a*, pheophorbide *a* and α,β-carotenes concentrations were not presented in Table III.2, since they did not show any significant correlation with gut pigment contents.

Table III.2. Pearson correlations (N = 31) between nematode gut pigment concentration and biofilm habitat characteristics. Abbreviations: chlorophyll *c* (Chl *c*), fucoxanthin (Fuco), diadinoxanthin (Diad), α,β-carotenes (Car), chlorophyll *a* (Chl *a*), pheophorbide *a* (Pheob *a*), pheophytin *a* (Pheo *a*), chlorophyll *a*-equivalents (Chl *a*-eq), ash-free dry mass (AFDM), water temperature (T) and dissolved oxygen (O_2). Pearson correlation abbreviations: not significant (*ns*), significantly negative at $P < 0.05$ (–), $P < 0.01$ (– –) and $P < 0.001$ (– – –); significantly positive at $P < 0.05$ (+), $P < 0.01$ (+ +) and $P < 0.001$ (+ + +)

Gut pigments (pg ind^{-1})	Biofilm pigments (µg gDM^{-1})				AFDM (g m^{-2})	T (°C)	O_2 (mg l^{-1})
	Chl *c*	Fuco	Diad	Chl *a*			
Chl *c*	*ns*	*ns*	*ns*	*ns*	*ns*	–	*ns*
Fuco	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Diad	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	+
Car	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Chl *a*	+	+	*ns*	*ns*	+ +	– –	+ +
Pheob *a*	*ns*	*ns*	*ns*	*ns*	*ns*	–	+ +
Pheot *a*	+ + +	+	+ + +	+ + +	+ +	– – –	+ + +
Chl *a*-eq	+ + +	+ + +	+ + +	+ + +	+ +	– – –	+ +

Results from the stepwise multiple regression analysis indicated that among the predictors which were correlated with gut Chl *a*-eq variations (Table III.2), only Chl *a* concentration in the biofilm was significantly selected (F = 34, $P < 0.001$). This was expected since all of these predictors were also correlated with biofilm Chl *a* concentration (see above). The relationship between nematode gut Chl *a*-eq and biofilm Chl *a* concentration (Fig. III.3) was rectilinear (N = 31, R^2 = 0.54, $P < 0.001$), showing that nematodes ingested MPB (in terms of Chl *a*-eq) proportionally to MPB availability in the biofilm (in terms of Chl *a* concentration).

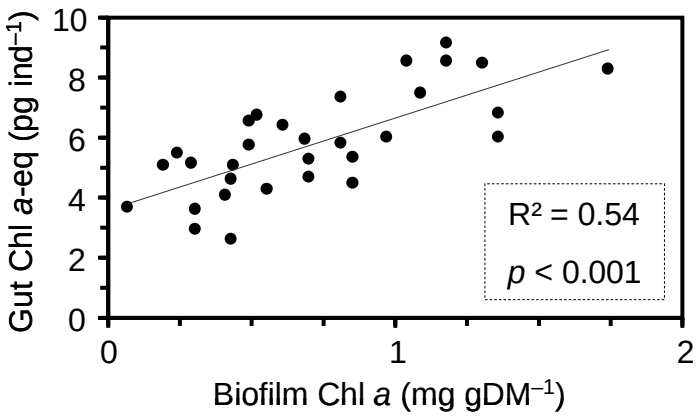

Figure III.3. Linear correlation (N = 31) between individual gut content of *Chromadorina bioculata* and *Chromadorina viridis* in chlorophyll *a*-equivalent (Chl *a*-eq) and the biofilm chlorophyll a concentration (Chl *a*).

III.5.4. Grazing pressure and energy requirements covered by MPB ingestion

Assuming GPT of 2.8, 14 and 70 min (see methods), the *C. bioculata* and *C. viridis* population grazed a mean (min–max) of 875 (271–3023), 175 (54–605) and 35 (11–120) µgChl*a*-eq m^{-2} d^{-1}, respectively. Compared to biofilm Chl *a* standing stocks, this means that they daily ingested 0.67 (0.04–1.87), 0.13 (0.01–0.37) and 0.03 (0.002–0.07) % of biofilm MPB biomass (in terms of Chl *a*), respectively. Assuming a carbon (C)/Chl *a* ratio of 17.2, estimated from biofilm-microphyte biovolume measurements at the study site (Leflaive *et al.*, 2008), the MPB C ingested yearly averaged 5.5, 1.1 and 0.2 gC m^{-2} y^{-1}, respectively.

Yearly production of *C. bioculata* and *C. viridis* was 1.4 gC m^{-2} y^{-1}. However, daily production fluctuated substantially: 1–9 mgC m^{-2} d^{-1} (Fig. III.4). When production was expressed in terms of carbon requirements, *C. bioculata* and *C. viridis* needed to assimilate yearly 7.2 gC m^{-2} y^{-1} to fulfil 100% of their requirements. Always assuming GPT of 2.8, 14 and 70 min, the MPB C assimilated (25% of ingestion, see methods) yearly covered on average 1, 5 and 27% of their requirements, respectively. But this fluctuated from 0.1 to 100% depending on the sampling date and on the GPT assumed (Fig. III.4).

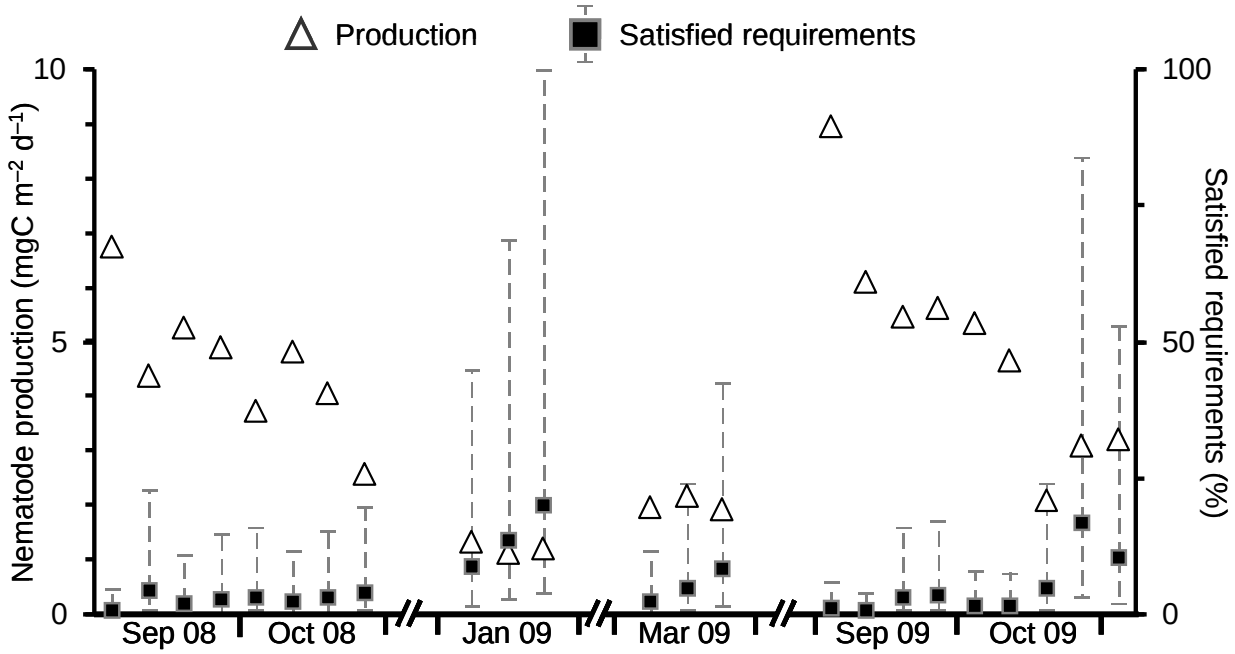

Figure III.4. Temporal dynamics (N = 23) of the daily production of *Chromadorina bioculata* and *Chromadorina viridis*, and the proportion of their energetic requirements satisfied by MPB consumption. The use of gut passage times (GPT) of 2.8, 14 and 70 min is depicted by upper interval, black square and lower interval, respectively.

III.6. Discussion

The nematodes *Chromadorina bioculata* and *Chromadorina viridis* strongly dominated the biofilm-dwelling nematode community at the study site. Widespread in European freshwater periphytic habitats (Decraemer & Smol, 2006), these two species show a typical epilithic lifestyle with their ability to attach themselves to hard substrates with sticky silks produced by their caudal glands (Meschkat, 1934; Croll & Zullini, 1972; Decraemer & Smol, 2006). Both species are described as epistrate-feeders expected to feed predominantly on MPB, although feeding on bacteria or on unicellular heterotrophic eukaryotes is not excluded (Traunspurger, 2000).

III.6.1. Gut Chl *a*-eq content and non-selective feeding on MPB

Our study confirms that biofilm-dwelling *C. bioculata* and *C. viridis* fed on MPB under natural conditions, as Chl *a*-eq was found in their guts. However, our results also show that

their gut Chl *a*-eq content was rectilinearly correlated with biofilm Chl *a* concentration, implying that their grazing on MPB was proportional to MPB availability in the biofilm. Some previous laboratory studies highlighted such proportional feeding responses to prey density with bacterial-feeding nematodes (*e.g.* Nicholas *et al.*, 1973; Schiemer, 1983; Moens & Vincx, 2000), predaceous nematodes (*e.g.* Bilgrami *et al.*, 1984; Bilgrami & Gaugler, 2005) and marine algal-feeding nematodes (Montagna *et al.*, 1995; Pascal *et al.*, 2008b). Nevertheless, to the best of our knowledge, this is the first time that such proportionality was observed for nematodes feeding under natural conditions. A linear relationship between ingestion and prey concentration reveals that either preys are taken up non-selectively, proportionally to their abundance in the medium (*e.g.* Gasparini *et al.*, 1999), or that the prey abundance is below the critical concentration at which ingestion is saturated (*i.e.* type II and III functional responses: Holling, 1959). Only a minor fraction of the biofilm MPB biomass (in terms of Chl *a*) was consumed by nematodes. Hence, it seems unlikely that biofilm-dwelling *Chromadorina* nematodes were capable of selecting MPB, but did not arrive at their ingestion saturation given the high MPB availability encountered. While a strong competition with other biofilm inhabitants (*e.g.* rotifers, insect larvae) could perhaps explain such a situation, we find rather likely that the linear relationship between nematode gut Chl *a*-eq content and biofilm Chl *a* concentration reflected a non-selective feeding on biofilm MPB.

River epilithic biofilms are structurally complex assemblages where distribution of organisms can be very patchy, constrained by environmental biotic and abiotic disturbances (*e.g.* Murga *et al.*, 1995; Leflaive *et al.*, 2008; Majdi *et al.*, 2011; 2012a). For instance, in the Garonne River, the observed negative correlation between temperature and biofilm MPB biomass is likely linked to a temperature-dependent bacterial degradation of the biofilm inducing its self-detachment from the cobbles occurring during summer–autumn low-flow periods (Lyautey *et al.*, 2005; Boulêtreau *et al.*, 2006). Hence, to overcome biofilm biotic composition fluctuations, biofilm-dwelling nematodes likely have an interest to adopt a non-selective, opportunistic feeding behaviour in response to available food, as observed for many estuarine nematodes (Moens & Vincx, 1997).

III.6.2. Gut biomarker pigments and nematode feeding on diatoms

A non-selective nematode feeding on MPB in general does not necessarily exclude that a potential selectivity occurred for (a) specific group(s) of microphytes among MPB. Only

diatom biomarker pigments were found in nematode gut extracts. This could perhaps suggest that they mainly ingested diatoms. This result would not be surprising, since diatoms strongly dominated the biofilm MPB community throughout the sampling occasions. It is also well known that diatoms are a high-quality food resource often used by benthic invertebrates—including marine nematodes—probably because of their high content of polyunsaturated fatty acids (*e.g.* Phillips, 1984; Goedkoop & Johnson, 1996; Buffan-Dubau & Carman, 2000a). Besides, marine nematodes can also feed on green algae and cyanobacteria (Tietjen & Lee, 1973; Evrard *et al.*, 2010). In our study, no biomarker pigments for green algae (*e.g.* lutein) or cyanobacteria (zeaxanthin) were detected in nematode extracts.

However, this non-detection of green algal and cyanobacterial biomarker pigments could be due to the detection limit of the HPLC device. Indeed, in biofilm chromatograms, the average ratio of fucoxanthin/lutein peak areas was 49, and fucoxanthin/zeaxanthin was 106. In nematode chromatograms, the peak area of fucoxanthin averaged 3 millivolt-seconds (m*V*sec). Hence, assuming a grazing over MPB groups proportional to their availability in the biofilm, the peak area of lutein and zeaxanthin would have been 0.06 and 0.03 m*V*sec, respectively, which is below the detection limit (0.1 m*V*sec) of the HPLC device used.

III.6.3. Grazing pressure

Even using the shortest GPT considered (*i.e.* 2.8 min), it was estimated that *C. bioculata* and *C. viridis* nematodes exerted a rather small grazing pressure on biofilm MPB standing stocks (0.67%). Comparable low nematode grazing pressures are reported from various marine and brackish habitats (Epstein & Shiaris, 1992; Nozais *et al.*, 2001; Moens *et al.*, 2002; Rzeznik-Orignac *et al.*, 2003; Pascal *et al.*, 2008b). In superficial sediments of a third order stream, Borchardt & Bott (1995) find a negligible algivory of nematodes using fluorescently labelled diatoms. However, only swallowed whole diatoms are detected with this technique, so that the grazing of nematodes such as Chromadoridae, which suck out inner frustule contents, was probably underestimated by these authors. Our estimates also emphasized that nematode grazing pressure fluctuated with temporal constraints, as observed from other meiobenthic organisms (Buffan-Dubau & Carman, 2000a; Goldfinch & Carman, 2000). River epilithic biofilms show high turnover rates, especially under grazing pressure (Lamberti & Resh, 1983). Hence, the low estimated nematode grazing pressure suggests that, although rotifers and Chironomidae larvae are also abundant in the biofilm at the study site (Majdi *et al.*, 2012a),

the MPB biomass was probably more than sufficient to supply all biofilm-dwelling meiobenthic consumers. This also supports the hypothesis that direct top-down control of MPB biomass by meiofaunal grazing is not a primary regulating mechanism. Indeed, biofilm-dwelling meiofauna likely play a secondary role by modifying the potential bottom-up controls of MPB through *e.g.* bioturbation, which leads to alterations in the light environment and the enhancement of solute transport rates within the mat (Pinckney *et al.*, 2003; Mathieu *et al.*, 2007).

III.6.4. Contribution of MPB to *Chromadorina*'s diet

Although it can highly fluctuate depending on GPT, on production efficiency and on MPB availability, the energetic requirements of *C. bioculata* and *C. viridis* satisfied by grazing on MPB remained globally rather low (5% assuming a GPT of 14 min) compared to values reported in literature for marine nematodes (50%, Van Oevelen *et al.*, 2006; 15%, Pascal *et al.*, 2008b). Hence, to fulfil 100% of their food requirements, *C. bioculata* and *C. viridis* probably depended on other food sources than MPB cell contents. Meschkat (1934) observed that freshwater Chromadoridae can collect and agglutinate detritus using their sticky silks to form a kind of pellet around their tail. This behaviour was also observed during our study with living specimens. Riemann & Schrage (1978) suggested that these detritus agglutinations, being crowded by bacteria, may contribute to nematode diet. In a more recent study, Riemann & Helmke (2002) pointed out that within these agglutinations, bacterial external enzymatic activity can contribute to cleave refractory polysaccharides, so that resulting sugars can easily be assimilated by nematodes. Considering the large proportion of exo-polymeric substances (EPS) exuded by MPB and bacteria within the biofilm matrix (Nielsen *et al.*, 1997), and the typical detritus-agglutinating behaviour of Chromadoridae nematodes described above, it can be speculated that organic matter uptake through "gardening" interactions with bacteria might contribute substantially to the diet of biofilm-dwelling *C. bioculata* and *C. viridis*.

III.6.5. Methodological considerations

Through its first application to nematodes, the HPLC-analysis of gut pigment contents proved useful to examine their grazing behaviour and pressure on the MPB community as a whole and on diatoms in particular. The main advantage of this technique is that it gives ingestion data under natural conditions without utilization of artificial markers and that it is applicable

to organisms—in our case nematodes—embedded in complex matrices such as epilithic biofilms. However, three shortcomings have to be acknowledged concerning this HPLC-approach:

(1) Based on our experience, the HPLC detection of non-dominant microphyte biomarker pigments in guts of *Chromadorina*-sized nematodes (dry weight ~0.2 µg ind^{-1}) would require sorting at least 1300 individuals. Besides the fact that this would be extremely time consuming, isolating such a large number of nematodes would increase the risk of contamination and pigment degradation. As a comparison, Buffan-Dubau *et al.* (1996) recommend a minimum of 400 individuals of the meiobenthic harpacticoid *Canuella perplexa* Scott 1893 (dry weight 2–10 µg ind^{-1}), to analyse gut pigments in detail. Hence, the detection of biomarker carotenoids for non-dominant microphyte groups may be practically restricted to larger algal-feeding nematode taxa (*e.g.* Dorylaimidae), if one wants to sort a reasonable number of nematodes.

(2) The analysis was applied to the entire natural nematode community and therefore the relevance of drawing conclusions from gut content data depends mainly on the complexity of the species assemblage occurring at the time of sampling. Hence, to overcome possible bias due to species specific diet, we recommend that this technique should either be restricted to the examination of nematode communities strongly dominated by a few species—as was the case in our study—or be applied to nematodes sorted to the best taxonomic level.

(3) With this technique only feeding on MPB cells is considered. Hence, potential feeding on heterotrophic preys (*e.g.* bacteria) and/or on EPS was not detected, while these latter resources likely contributed considerably to the diet of *C. bioculata* and *C. viridis* inhabiting epilithic biofilms of the Garonne River.

III.7. Conclusion

Our results showed that biofilm-dwelling *Chromadorina* spp. nematodes fed on MPB within epilithic biofilms of the Garonne River, and that this feeding was non-selective. Only diatom biomarker pigments were found in their guts, however a potential additional feeding on green algae and cyanobacteria can not be completely excluded. Our estimates of their ingestion rates emphasized a low grazing pressure on biofilm MPB cells and suggested that these nematodes used additional food sources (*e.g.* bacteria, EPS), which were not detected by means of HPLC gut pigment analysis. Thus, this aspect should be investigated in future studies.

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Chapitre IV

Positionnement trophique de la méiofaune et incorporation du carbone microphytobenthique dans un biofilm de rivière tempérée

Article soumis dans
Freshwater Biology

IV.1. Résumé de l'article

IV.1.1. Contexte et objectifs

Pouvoir apprécier les transferts de carbone (C) entre les organismes autotrophes et les niveaux trophiques supérieurs est essentiel pour comprendre le fonctionnement des écosystèmes (*e.g.* Minshall, 1978). Dans les biofilms épilithiques de rivière, micro-, méio- et macro-organismes vivent rassemblés dans une matrice de substances exo-polymériques (EPS) qui piège les apports allochtones de matière organique et inorganique (*e.g.* Romaní *et al.*, 2004). L'implication des plus petits invertébrés (la méiofaune) dans le broutage de la biomasse phototrophe du biofilm reste peu explorée (**chapitre III**). Plus généralement, la consommation *in situ* du C microphytobenthique (CMPB) par la méiofaune dans les milieux d'eau douce est peu décrite (Borchardt & Bott, 1995; Moens *et al.*, 2006), bien que le rôle d'intermédiaire joué par la méiofaune dans les réseaux trophiques soit reconnu (Schmid-Araya *et al.*, 2002; Schmid & Schmid-Araya, 2002; Woodward *et al.*, 2005a). Par ailleurs, de nombreux travaux documentent les interactions trophiques entre la macrofaune et le biofilm épilithique (*e.g.* Sabater *et al.*, 2002; Hillebrand, 2009), mettant même en évidence une influence positive (Hillebrand *et al.*, 2002) ou négative (Peters & Traunspurger, *in press*) des macro-brouteurs sur l'abondance de la méiofaune dans les biofilms. L'analyse des rapports isotopiques naturels du C ($\delta^{13}C$) et de l'azote ($\delta^{15}N$) couplée à l'utilisation de ces isotopes comme marqueurs trophiques (*e.g.* ^{13}C) est une approche qui s'est avérée pertinente pour caractériser et quantifier *in situ* les interactions trophiques incluant la méiofaune en milieu marin et saumâtre (*e.g.* Middelburg *et al.*, 2000; Moens *et al.*, 2002; Galvan *et al.*, 2008; Evrard *et al.*, 2010). En utilisant une telle approche isotopique, cette étude a pour objectifs :

(1) de préciser le positionnement de la méiofaune au sein du réseau trophique du biofilm en comparant les signatures naturelles $\delta^{13}C$ et $\delta^{15}N$ des invertébrés et des ressources basales potentielles (*i.e.* fractions de taille 1.2–25 et 25–40 µm du biofilm et litière végétale).

(2) de spécifier et de quantifier l'utilisation du CMPB par les invertébrés du biofilm en utilisant un design expérimental *in situ* incluant une étape de marquage du CMPB par une solution de $NaHCO_3$ enrichie en ^{13}C puis une période de suivi de l'incorporation de ce marqueur par les populations d'invertébrés.

Enfin, cette approche devrait permettre de compléter les informations obtenues à partir de l'analyse des contenus pigmentaires intestinaux des nématodes par HPLC (**chapitre III**), et devrait donner un aperçu du devenir du CMPB dans le biofilm épilithique.

IV.1.2. Principaux résultats et discussion

La comparaison des signatures isotopiques naturelles $\delta^{13}C$ et $\delta^{15}N$ indique que la méiofaune utilise clairement les ressources trophiques basales. Les nématodes *Chromadorina* spp. se nourrissent principalement de la fraction du biofilm comprise entre 1.2 et 25 µm (la plus riche en diatomées). Les oligochaetes Naididae et les larves méio- et macrobenthiques de Chironomidae (Insecta, Diptera) vivant dans le biofilm semblent avoir un régime alimentaire plus diversifié incluant des ressources allochtones (*i.e.* litière végétale) en plus des fractions 1.2–25 et 25–40 µm du biofilm. Ces résultats renforcent l'hypothèse d'un biofilm utilisé comme ressource trophique par la méiofaune (Höckelmann *et al.*, 2004; Peters & Traunspurger, 2005; Gaudes *et al.*, 2006). La prédation accidentelle de la méiofaune par les macro-brouteurs (ici les Chironomidae) n'est pas observée. Par contre, les larves macrobenthiques de Rhycophilidae (Insecta, Trichoptera) sont des prédateurs, se nourrissant vraisemblablement des oligochaetes Naididae.

Les résultats de l'expérience *pulse-chase* montrent que le C marqué est fixé par le microphytobenthos, puis généralement rapidement incorporé par les invertébrés. Cependant, différentes dynamiques d'enrichissement soulignent différentes stratégies alimentaires : herbivorie manifeste pour les nématodes *Chromadorina* et les larves meio- et macrobenthiques de Chironomidae, détritivorie pour les oligochaetes Naididae et prédation pour les larves de Rhyacophilidae.

La pression de broutage et les besoins énergétiques comblés par la consommation du CMPB complètent les résultats obtenus par HPLC pour les nématodes *Chromadorina* : en effet, leurs besoins énergétiques sont comblés (104%) par l'assimilation du CMPB (*i.e.* contenus cellulaires + EPS), tandis qu'à la même date leur seule ingestion des contenus cellulaires du MPB ne couvre qu'entre 1 et 23% de leurs besoins (**chapitre III**). Ainsi dans les biofilms épilithiques, les nématodes *Chromadorina* semblent se nourrir principalement des EPS exsudés par le MPB, et dans une moindre mesure, du contenu cellulaire des microphytes.

Les macro-brouteurs Chironomidae incorporent davantage de CMPB que la méiofaune (36 mgC m^{-2} j^{-1} contre 8 mgC m^{-2} j^{-1}). Compte-tenu de la baisse significative de leur rétention du CMPB trois jours après le marquage, les Chironomidae semblent donc pouvoir mobiliser et exporter davantage ce C que les nématodes.

Cependant, le broutage des invertébrés considérés dans cette étude ne semble pas être le vecteur principal du transfert du C fixé par photosynthèse dans le biofilm épilithique. En effet, 81% du ^{13}C initialement fixé ne se retrouve ni chez les invertébrés ni dans les fractions de biofilm considérées après trois jours.

Trophic positioning and microphytobenthic carbon uptake of biofilm-dwelling meiofauna in a temperate river

N. Majdi, M. Tackx and E. Buffan-Dubau

Université de Toulouse-UPS-INP, EcoLab UMR 5245, 118 route de Narbonne, 31062 Toulouse, France
CNRS, EcoLab UMR 5245, 31062 Toulouse, France

IV.2. Abstract

1. $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures combined with an *in situ* microphytobenthic ^{13}C labelling experiment were performed in epilithic biofilms of a large temperate river (the Garonne, France) to infer the trophic positioning and the microphytobenthic carbon (MPBC) uptake of biofilm-dwelling meio- and macrofauna.

2. Chironomidae larvae and *Chromadorina* spp. nematodes rapidly incorporated freshly produced MPBC contrary to Rhyacophilidae larvae and Naididae oligochaetes. Quantitatively, macrofaunal Chironomidae daily incorporated more MPBC than did meiofauna. Moreover, Chironomidae seemed more involved in MPBC spatial export than nematodes.

3. Rhyacophilidae larvae were predators feeding on large meiofauna (Naididae and Chironomidae) but not on nematodes. Naididae oligochaetes primarily gained their carbon from allochthonous and/or microbial-loop recycled sources.

4. A rapid and important loss of labelled MPBC was observed. Feeding activity of biofilm-dwelling invertebrates seemed not primarily involved in this loss.

Keywords: meiofauna, periphyton, food web, stable isotopes, carbon flows

IV.3. Introduction

The epilithic biofilm is a complex assemblage comprising microphytes, bacteria, meiofauna and macrofauna embedded in a mucous matrix of exopolymeric substances (EPS) together with entrapped allochthonous imports (*e.g.* Romaní *et al.*, 2004). This biofilm coats any hard submerged substrate, and when enough light is available, microphytobenthos (and their EPS exudates) contribute copiously to the biofilm organic content (Azim & Asaeda, 2005). Epilithic biofilms contribute significantly to biogeochemical processes and sustain secondary production (*e.g.* Lock *et al*., 1984; Pusch *et al.*, 1998; Battin *et al.*, 2003; Cardinale, 2011).

Meiofauna are small invertebrates which pass through a 500 µm mesh and are retained on a 40 µm mesh (Fenchel, 1978). Understanding their trophic role is a key issue to disentangle energy flows in freshwater food webs (Hildrew, 1992; Ward *et al.*, 1998; Reiss & Schmid-Araya, 2010). In freshwater sediments, meiofauna can specifically ingest microphytobenthos and smaller heterotrophic organisms (Borchardt & Bott, 1995; Traunspurger *et al.*, 1997; Bott & Borchardt, 1999; Reiss & Schmid-Araya, 2011). From *in situ* studies conducted in marine intertidal habitats, it is now well-established that meiofauna can rapidly take up freshly photosynthetically-fixed microphytobenthic carbon (MPBC), as such improving its transfer rate to higher trophic levels (*e.g.* Montagna, 1984; Middelburg *et al.*, 2000; Moens *et al.*, 2002; Pinckney *et al.*, 2003). Generally, the quantitative *in situ* uptake of MPBC by meiofauna has received little attention in freshwater systems (Borchardt & Bott, 1995; Moens *et al.*, 2006), though the role of meiofauna as stream food web intermediates is increasingly advocated (*e.g.* Schmid-Araya *et al.*, 2002; Schmid & Schmid-Araya, 2002; Woodward *et al.*, 2005a; Dineen & Robertson 2010; Spieth *et al.*, 2011).

Concerning epilithic biofilms, there are some indications that meiofauna can influence key processes such as oxygen turnover, secondary metabolites release and detachment (Sabater *et al.*, 2003; Gaudes *et al.*, 2006; Mathieu *et al.*, 2007). Some recent studies address biofilm-dwelling meiofauna feeding habits: Kathol *et al.* (2011) budget the importance of pelagic-benthic import through rotifer and ciliate filtration activity. Majdi *et al.* (2012b) show that *Chromadorina* spp. nematodes feed on biofilm diatoms non-selectively. However, most studies addressing grazing within epilithic biofilms remain focused on macrofauna (Hillebrand, 2009).

Stable isotope analysis (SIA) multi-approaches (*i.e.* trophic tracers in addition to natural isotopic signatures) are relevant to unravel trophic processes in ecosystems (Boschker & Middelburg, 2002). SIA multi-approaches have been abundantly and successfully applied to examine *in situ* trophic linkages and carbon flows involving meio- and macrofauna in marine and brackish benthic systems (Herman *et al.*, 2000; Middelburg *et al.*, 2000; Galvan *et al.*, 2008; Pascal *et al.*, 2008a; Evrard *et al.*, 2010). SIA multi-approaches using addition of dissolved ^{13}C and/or ^{15}N as trophic tracers are also commonly used in freshwater benthic systems to disentangle a variety of trophic processes (*e.g.* Hall, 1995; Parkyn *et al.*, 2005; Cardinale, 2011). However, so far no freshwater studies have applied SIA multi-approaches to examine trophic positioning and *in situ* grazing of meiofauna.

Using an *in situ* SIA multi-approach, our objectives are: (1) to specify the organization of the biofilm food-web by including meiofauna, (2) to quantify the importance and rate of carbon transfer from benthic photosynthesis to both meio- and macrofauna inhabiting the biofilm. These objectives should contribute to elucidate the fate of biofilm MPBC.

IV.4. Methods

IV.4.1. Site description

With a total length of 647 km and a drainage basin of 57 000 km², the Garonne is the largest river of south-western France, displaying alternate cobble bars even in channels up to the seventh order. The study site is on one of these cobble bars at 36 km upstream the city of Toulouse, where the Garonne is of sixth order (lat 01°17’53”E, long 43°23’45”N; elevation: 175 m asl, Fig. IV.1a). The dynamics of epilithic phototrophic biofilm with its bacterial, microphytobenthic, and meiofaunal components is detailed at this site (Lyautey *et al.*, 2005; Boulêtreau *et al.*, 2006; Leflaive *et al.*, 2008; Majdi *et al.*, 2011; 2012a)**.** In this stretch of the Garonne, the residence time of the water is too low to allow substantial phytoplankton development, and it is assumed that benthic biofilms provide most of the riverine primary production (Ameziane *et al.*, 2003). On the basis of these data, the study site was located along a transect at 45 m from the riverside, so that depth lied within 40–50 cm, *i.e.* the depth where the phototrophic biofilm typically develops (Ameziane *et al.*, 2002).

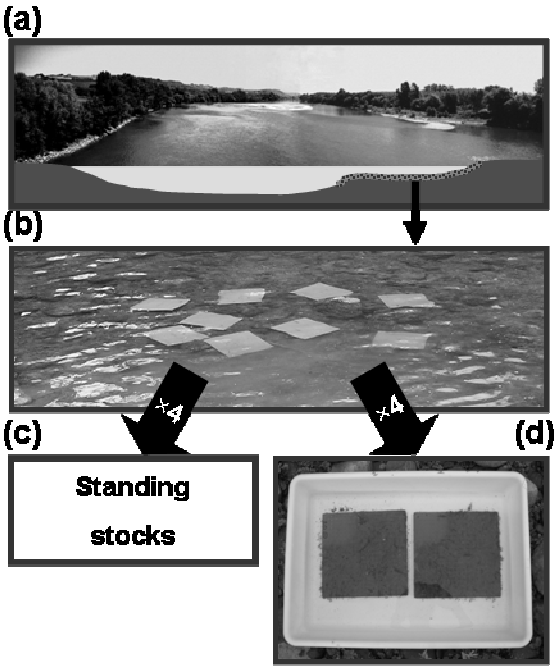

Figure IV.1 Experimental design: **(a)** Cross-section view of the Garonne River at the study site. **(b)** Ceramic tiles lying on the streambed before colonization by biofilm (20th of July 2009). After two months of colonization: **(c)** biofilm was gathered from four tiles to estimate initial standing stocks, and **(d)** biofilm from four other tiles was labelled with $NaH^{13}CO_3$ solution for 3 h.

IV.4.2. Growth of the epilithic biofilm and natural $\delta^{13}C$ and $\delta^{15}N$ signatures

On the 20th of July 2009, eight 400 cm² ceramic tiles were settled on the river bed at the chosen site (Fig. IV.1b). Biofilm was allowed to colonize tiles during two months, since this exposure period is sufficient for the establishment of mature biofilm communities in temperate rivers (Pusch *et al.*, 1998; Norf *et al.*, 2009).

On the 20th of September 2009, four 50 cm² biofilm samples were collected (by scraping with a scalpel and a toothbrush) from four of the eight biofilm-colonized tiles to measure the natural $\delta^{13}C$ and $\delta^{15}N$ signatures of the biofilm and its associated invertebrates.

A total of twenty leaves (mainly poplar and alder) were hand-collected underwater from natural accumulations in small depositional zones between the tiles to determine the leaf litter isotopic signature. Four replicates of five leaves each were carefully rinsed with milliQ water

to remove epibionts and stored (–20 °C) for further measurement of their natural $\delta^{13}C$ and $\delta^{15}N$ signatures.

IV.4.3. Labelling experiment

Just after the collection of biofilm samples to measure natural $\delta^{13}C$ and $\delta^{15}N$ signatures (see above), the same four sampled tiles were placed into two rectangular basins (Fig. IV.1d) filled with 1.5 L of low mineralized water (<25 mg L^{-1} dry residue, Mont-Roucous, Lacaune-les-bains, France), at ambient river water pH (7.1), containing 160 mg L^{-1} $NaH^{13}CO_3$ (>99% ^{13}C, Sigma-Aldrich, Buchs, Switzerland) for ^{13}C-labelling of biofilm microphytobenthos (MPB). Photo-incorporation of ^{13}C by MPB was favoured by leaving the tiles well-exposed to sunlight from 11 h a.m. to 2 h p.m. at ambient river water temperature (~17 °C). At the end of this labelling period (t = 3 h), four 50 cm² biofilm samples (one from each tile) were collected by scraping with a scalpel and a toothbrush. The tiles were then resettled in the river at the colonization site. Four additional 50 cm² biofilm samples (one from each tile) were collected on each of the following three days (at t = 24, 48 and 72 h). Attention was paid (1) to minimize any detachment of biofilm during the removal and the resettlement of the tiles by gentle handling, and (2) to always gather biofilm surfaces from non-previously scrubbed locations of the tile. All biofilm samples were preserved in 100 mL formaldehyde solution (4%) immediately after collection. No corrections were applied for any carbon added through the formaldehyde preservation.

IV.4.4. Sample processing for stable isotope analysis (SIA)

All biofilm samples were thoroughly homogenized and poured through stacked 500, 40 and 25 µm mesh sieves. The resulting filtrate was then filtered on 1.2 µm glass fibre filter (GF/C, Whatman, Clifton, NJ, USA). Macro- and meiobenthic metazoans were isolated from their corresponding size fractions, *i.e.* >500 and 40–500 µm respectively, and sorted to the best practical taxonomic level under a stereomicroscope (9–90×). From each biofilm sample, two 80 µL aliquots of biofilm filtration residues were collected: one from the 25 µm sieve fraction (representing the 25–40 µm biofilm fraction) and the other from the GF/C filter (representing the 1.2–25 µm biofilm fraction). These aliquots were transferred into tin cups and prepared for SIA as described below.

Nematodes were not identified to the species level, however at the time and site of the study, most biofilm-dwelling nematodes (>95%) belonged to two species from the genus *Chromadorina* (Majdi *et al.*, 2011). Hence, we assumed that nematode SIA results should depict mainly the feeding habits of *Chromadorina* spp. Oligochaetes were strongly dominated by Naididae (>85%). Naididae were selectively isolated for SIA since they were easily recognizable from Lumbricidae and Tubificidae which contributed the rest of the oligochaete assemblage. Chironomidae larvae were pooled without further taxonomic distinction. However, a size distinction was made between meio- and macrofaunal Chironomidae. Psychomyiidae and Rhyacophilidae larvae (Trichoptera) contributed the rest of biofilm-dwelling macrofauna. Psychomyiidae were not found in sufficient abundance in each sample, so only Rhyacophilidae were isolated for SIA. From each biofilm sample, 500 *Chromadorina* nematodes, 30 Naididae oligochaetes, 30 meio-, 10 macrofaunal Chironomidae and one Ryacophilidae were isolated, checked for body integrity, thoroughly washed in two successive milliQ water baths to remove any adherent particles, transferred to cleaned pre-weighted tin cups (one cup for each taxa), dried in a oven at 55 °C overnight, pinched closed, weighted and stored (–20 °C) until SIA.

Leaf litter samples were freeze-dried and ground to a homogeneous powder prior to encapsulation into tin cups for SIA.

IV.4.5. Isotopic analyses

Samples were analysed for organic carbon (C) and nitrogen (N) content and isotopic composition using a FLASH EA-1112 elementary analyser coupled to a DELTA V Advantage mass spectrometer (both Thermo Fisher Scientific Inc., Waltham, MA, USA). C and N percentages to organic dry weight were measured for each sample. $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios were expressed with the standard ‰ unit notation: $\delta X(‰) = ([R_{sample}/R_{standard}] - 1) \times 1000$. Where R is either the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ standardised according to the Vienna Pee Dee Belemnite (VPDB) for C, and to atmospheric N_2 for N. Measurement reproducibility was <0.15 ‰ for both C and N. The incorporation of ^{13}C label was defined as excess ^{13}C.
Specific uptake was calculated as $\Delta\delta^{13}C = \delta^{13}C_{sample} - \delta^{13}C_{control}$, with $\delta^{13}C$ expressed relatively to VPDB. Total uptake (I) was quantified in $mg\,^{13}C\,m^{-2}$ with I = excess ^{13}C (E) × organic C content, according to standing stocks. E is the difference between the ^{13}C fraction of the control ($F_{control}$) and the sample (F_{sample}), where $F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R + 1)$. The

carbon isotope ratio (R) was derived from the measured $\delta^{13}C$ values as $R = (\delta^{13}C/1000 + 1) \times R_{VPDB}$, with $R_{VPDB} = 0.0112372$. For invertebrates, daily total MPBC assimilated was calculated as the product of I with the initial (t = 3h) proportion of ^{13}C to MPBC stock of the pooled 1.2–25 µm and 25–40 µm biofilm fractions, assuming equal uptake of labelled versus non-labelled carbon.

IV.4.6. Standing stocks

On the 20th of September 2009, concomitantly to the beginning of the labelling experiment, the biofilm from four additional replicate tiles (Fig. IV.1c) was entirely scraped and each sample was thoroughly homogenized and suspended in 200 mL formaldehyde solution (4%) for the following analyses:

Four 20 mL subsamples obtained from the four homogenized biofilm suspensions were used for biofilm biomass determination. Each subsample was dried overnight at 55 °C, weighted for its dry mass (DM), then combusted during 8 h at 450 °C to weigh its ash-free dry mass (AFDM).

Four 500 µL subsamples from the four homogenized biofilm suspensions were gently sonicated for 15 min at 35 kHz in an ultrasonic bath (Transsonic T460, Elma, South Orange, NJ, USA), and vortexed for 15 min to crumble bacterial aggregates (Garabétian *et al.*, 1999). Then, the density of bacteria was determined following a DAPI-staining method (Porter & Feig, 1980). Bacterial counting was carried out using a Leitz Dialux microscope (1250×) fitted for epifluorescence: HBO 100 W mercury light source (Osram, Winterthur, Switzerland), with an excitation filter for 270 and 450 nm, a barrier filter of 410 nm and a 515 nm cut-off filter. Bacterial biomass was assumed to be 20 fgC $cell^{-1}$ after Lee & Fuhrman (1987).

The remaining four replicate 179.5 mL biofilm suspensions were size-fractionated by sieving as described above. Since microphytes were rarely encountered in >40 µm fractions, we focused on 1.2–25 and 25–40 µm biofilm fractions to determine biofilm MPB density and biomass. Microphytes were enumerated using a Malassez counting chamber under a Nikon Optiphot-2 microscope (50–600×). Diatoms were identified to genus level. For each replicate, 50 cells of each MPB group (*i.e.* diatoms, green algae and cyanobacteria) were measured and

converted into biovolume after Hillebrand *et al.* (1999). Then, cell carbon content was calculated from its biovolume after Menden-Deuer & Lessard (2000).

The density of meio- and macrobenthic invertebrates was determined by counting four replicates of the 40–500 and >500 µm fractions, respectively. Their biomasses were measured from DM and Carbon (C)/DM values obtained after SIA.

IV.4.7. Estimation of invertebrate carbon budgets

Daily production (*P*, mgC m^{-2} d^{-1}) was calculated from invertebrate taxa biomasses using Plante & Downing's regression (1989), considering an average surface water temperature of 17 °C. We assumed net production efficiencies (NPE = P/Assimilation) of 0.6 for nematodes, 0.55 for predators and 0.4 for other taxa (Smock & Roeding, 1986; Herman & Vranken, 1988). Hence, the daily assimilation demand (DAD, in terms of C) of each invertebrate taxa was estimated from P and NPE. DAD was compared to the total MPBC assimilated daily, to budget the contribution of MPBC to consumer's DAD. For predators, we estimated the number of potential preys needed daily to fulfil their DAD.

IV.4.8. Data analyses

The organization of the biofilm food-web was assessed by plotting natural $\delta^{13}C$ versus natural $\delta^{15}N$ isotopic signatures, so that trophic levels were identified using $\delta^{15}N$, and food sources were identified using $\delta^{13}C$ (Peterson & Fry, 1987). Natural $\delta^{13}C$ and $\delta^{15}N$ signatures of food sources were compared using one-way ANOVA, after assessing variance homogeneity using Levene's test. The expected trophic enrichment factor (TEF) during food assimilation was assumed to be +0.5‰ for $\delta^{13}C$ and +2.2‰ for $\delta^{15}N$ (McCutchan *et al.*, 2003). Therefore, the isotopic signature of the probable food was estimated by removing 0.5‰ for C and 2.2‰ for N from the mean isotopic values of the consumers. Differences between specific label uptake dynamics were analysed by two-way ANOVA with meiofaunal taxa and post-labelling times as factors, after assessing variance homogeneity using Levene's test. Tukey's HSD test was performed for *a posteriori* pairwise comparisons. All statistical analyses, as well as total ^{13}C uptake dynamics fitting were performed with STATISTICA software (version 8.0, Statsoft inc., Tulsa, OK, USA).

IV.5. Results

IV.5.1. Standing stocks

On the 20th of September 2009, the biofilm averaged (±SD) 129 ±6 gDM m^{-2} and 16.1 ±1.5 gAFDM m^{-2}. Thus, organic content contributed 12% of the total biofilm DM. Biofilm C/AFDM ratio was assumed to 0.45 (Whittaker & Likens, 1973), so that the whole biofilm organic C stock could be estimated at 7.2 gC m^{-2}. Bacterial density in the biofilm averaged 9.6 ±1.1 $\times 10^{12}$ cells m^{-2}, or 193 mgC m^{-2}, *i.e.* 2.7% of the biofilm organic C stock.

Green algae and diatoms contributed equally to biomass in the 25–40 µm biofilm fraction (Table IV.1): encountered diatom genera were mostly *Diatoma* spp., *Melosira* spp., *Cymbella* spp., *Amphora* spp. and *Gyrosigma* spp. In the 1.2–25 µm biofilm fraction, cyanobacteria were numerically dominant, but diatoms represented most of the MPB biomass (Table IV.1): most abundant diatom genera were *Achnantidium* spp. and *Cyclotella* spp. Taken together, MPBC in both biofilm fractions (1165 mgC m^{-2}) contributed 16.2% of the estimated biofilm organic C stock.

Table IV.1. Invertebrates and microphytobenthos (MPB) mean density (± SD, *n* = 4), carbon (C) to dry mass (DM) content, biomass, contribution to biofilm organic C stock and natural $\delta^{13}C$ and $\delta^{15}N$ signatures (± SD, *n* = 4) in the epilithic biofilm on the 20th of September 2009

Biofilm invertebrates and MPB	Density (ind m^{-2}) or (cell m^{-2})	C/DM (%)	Biomass (mgC m^{-2})	C stock (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Macrofauna (>500 µm)						
Chironomidae larvae	3257 ±98	42.9	125.1	1.74	-23.39 ±0.5	7.80 ±0.5
Rhyacophilidae larvae	230 ±13	43.1	14.9	0.21	-21.65 ±0.2	9.25 ±0.1
Meiofauna (40–500 µm)						
Chromadorina nematodes	369954 ±32790	45.3	14.2	0.20	-21.22 ±0.7	8.56 ±0.3
Chironomidae larvae	11642 ±1834	38.4	7.2	0.10	-23.36 ±0.4	7.77 ±0.1
Naididae oligochaetes	3631 ±436	38.5	3.5	0.05	-23.18 ±1.0	6.99 ±0.4
Biofilm fraction (25–40 µm)						
Diatoms	0.28×10^{9} $\pm46\times10^{6}$	–	138.7	1.93	-19.22 ±1.3	5.96 ±0.4
Green algae	0.15×10^{9} $\pm38\times10^{5}$	–	123.7	1.72		
Biofilm fraction (1.2–25 µm)						
Diatoms	11.18×10^{9} $\pm1.6\times10^{9}$	–	804.2	11.17		
Green algae	2.24×10^{9} $\pm0.5\times10^{9}$	–	68.0	0.94	-21.73 ±0.7	5.60 ±0.3
Cyanobacteria	44.72×10^{9} $\pm3.3\times10^{9}$	–	30.3	0.42		

Meiofauna and especially nematodes dominated the invertebrate community in terms of density (Table IV.1). Rotifers were also abundant but had a very small individual biomass (~15 ngC ind^{-1}). Hence, for practical reasons (>1000 individuals per sample needed for SIA measurements), they were not considered further. Macrofauna and especially Chironomidae larvae dominated in terms of biomass (Table IV.1). Taken together, invertebrate C (164.6 mgC m^{-2}) contributed 2.3% of the biofilm organic C stock.

IV.5.2. Natural $\delta^{13}C$ and $\delta^{15}N$ signatures

The $\delta^{13}C$ versus $\delta^{15}N$ signatures of leaves, biofilm fractions and invertebrates are shown in Fig. IV.2. From their low $\delta^{15}N$ values, leaves and both biofilm fractions can be considered as basal food sources (Peterson & Fry, 1987). Their $\delta^{13}C$ signatures differed significantly (ANOVA, $F_{2,11} = 66.6$, $P < 0.001$), leaves having the most negative $\delta^{13}C$ values.

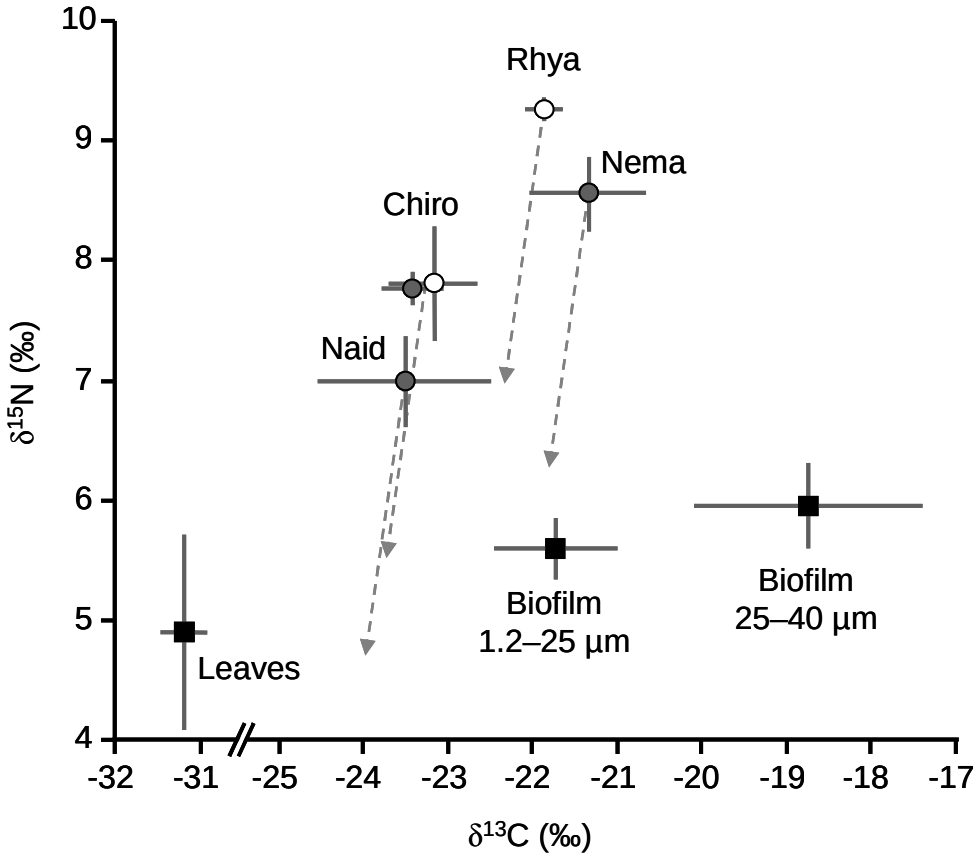

Figure IV. 2. Mean (n = 4, ±SD) $\delta^{13}C$ and $\delta^{15}N$ natural signatures of basal resources (black squares), meiofauna (grey circles) and macrofauna (white circles) gathered from the epilithic biofilm of the Garonne River. The expected trophic enrichment factors between consumers and their probable diet are shown by dashed arrows. (Nema) *Chromadorina* nematodes, (Chiro) Chironomidae larvae, (Naid) Naididae oligochaetes, (Rhya) Rhyacophilidae larvae.

Observed TEF (Fig. IV.2) suggested that: (1) *Chromadorina* nematodes, Naididae oligochaetes and Chironomidae larvae depended on basal food sources: *Chromadorina* fed on the 1.2–25 µm biofilm fraction. No clear food source was identified under the TEF of Naididae and Chironomidae. (2) Rhyacophilidae trichopters were predators, feeding at least on Naididae.

IV.5.3. Labelling experiment

IV.5.3.1. *Invertebrate specific uptake ($\Delta\delta^{13}C$)*

Invertebrate specific uptake showed taxa and time effects (two-way ANOVA taxa and time as factors, $F_{12,77} = 2.1$, $P < 0.05$). Significant $\Delta\delta^{13}C$ differences were detected at 24 h and at 48 h, between a group comprising *Chromadorina* and Chironomidae, versus a group comprising Naididae and Rhyacophilidae (Fig. IV.3, ANOVA taxa as factor, $F_{4,15} = 17.3$, $P < 0.001$ and HSD test).

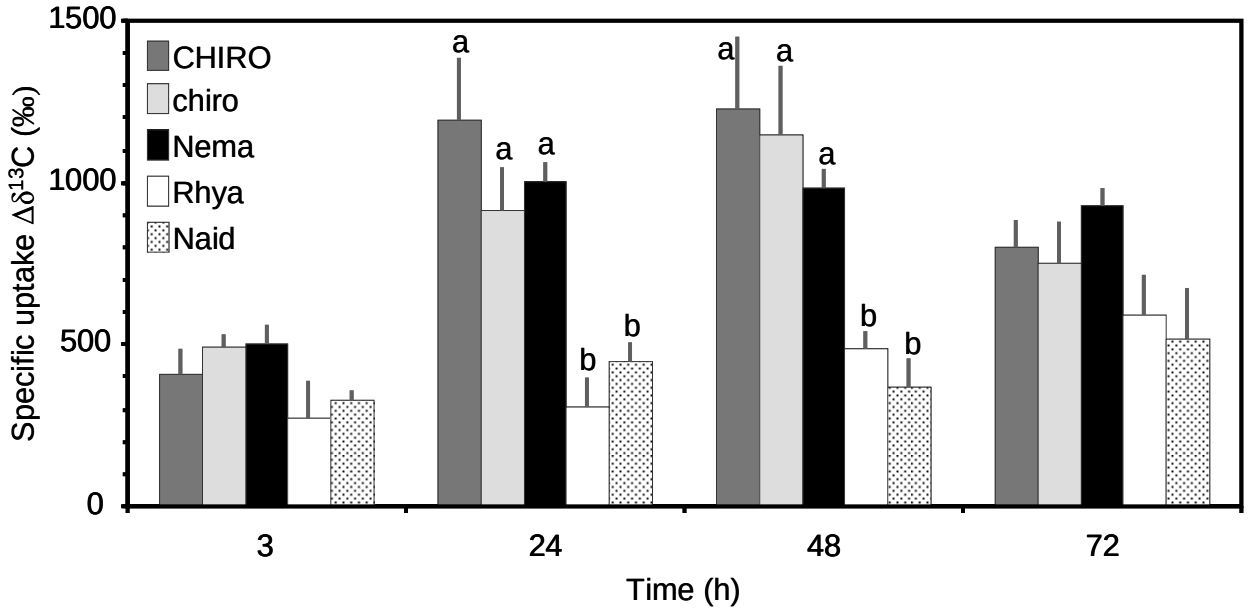

Figure IV.3. Specific ^{13}C uptake ($\Delta\delta^{13}C$) by biofilm-dwelling invertebrates during the post-labelling period (t = 3–72 h). Values are means (n = 4, +SD). (CHIRO) macrofaunal-sized Chironomidae larvae, (chiro) meiofaunal-sized Chironomidae larvae, (Nema) *Chromadorina* nematodes, (Rhya) Rhyacophilidae larvae, (Naid) Naididae oligochaetes. Different letters above bars show significant $\Delta\delta^{13}C$ differences between invertebrate taxa at a given time.

On the one hand, the $\Delta\delta^{13}C$ of *Chromadorina* increased significantly between 3 and 24 h. The $\Delta\delta^{13}C$ temporal dynamics of meio- and macrofaunal Chironomidae was similar to that of *Chromadorina*, excepted that it decreased significantly at 72 h. On the other hand, the $\Delta\delta^{13}C$ temporal dynamics of Naididae and Rhyacophilidae did not show significant changes after labelling (ANOVA time as factor, $F_{3,19} = 13.3$, $P < 0.001$ and HSD test).

IV.5.3.2. *Total ^{13}C uptake (I)*

I evolution in biofilm fractions is shown in Fig. IV.4. After labelling (t = 3 h), MPB from the 1.2–25 µm biofilm fraction have incorporated on average (±SD) 41.1 ±5.3 $mg^{13}C\ m^{-2}$. MPB from the 25–40 µm biofilm fraction have incorporated on average 5.8 ±1 $mg^{13}C\ m^{-2}$. Thus, at the end of the labelling period (t = 3 h), 4% of the MPBC stock was ^{13}C-labelled in pooled biofilm fractions. Then, *I* decreased exponentially in both biofilm fractions (n = 16; 1.2–25 µm: $R = -0.89$, $P < 0.001$; 25–40 µm: $R = -0.85$, $P < 0.001$) with similar attenuation coefficients ($-0.026\ h^{-1}$; Fig. IV.4), corresponding to a ^{13}C half-life period of 1.1 day (loss of the half initial excess ^{13}C = ln2/attenuation coefficient).

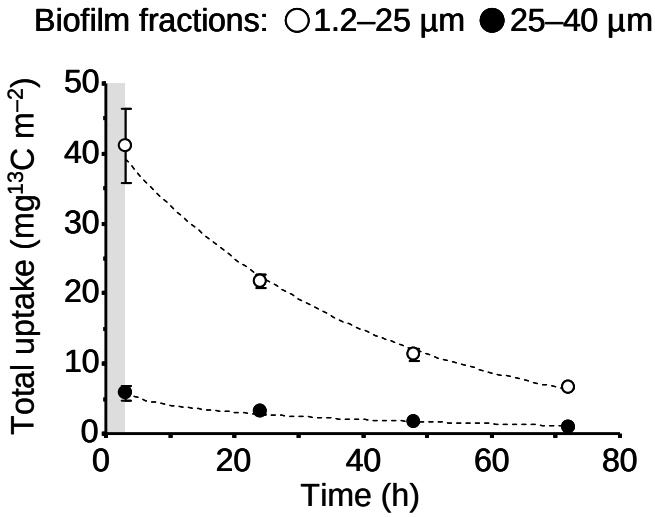

Figure IV.4. Evolution of total ^{13}C uptake (*I*) in the 1.2–25 µm and 25–40 µm biofilm fractions during the post-labelling period (t = 3–72 h). Values are means (n = 4, ±SD). Exponential fits: $y = 42.47^{-0.026x}$ (1.2–25 µm fraction) and $y = 6.14^{-0.026x}$ (25–40 µm fraction). The shaded area shows the period of microphytobenthos labelling using $NaH^{13}CO3$ (t = 0–3 h).

I evolution in invertebrates is shown in Fig. IV.5. Macrofaunal Chironomidae incorporated the highest amount of label: with *I* peaking up to 1.36 ±0.23 $mg^{13}C\ m^{-2}$ at t = 48 h, whereas Naididae were minor contributors to total invertebrate *I*. Second order polynomial regressions (bell-shaped curves) best fitted *I* evolution after labelling for *Chromadorina* (n = 16, R = 0.69, P < 0.001), meiofaunal Chironomidae (n = 16, R = 0.54, P < 0.05) and macrofaunal Chironomidae (n = 16, R = 0.52, P < 0.05). Linear regressions best fitted *I* evolution for Naididae (n = 16, R^2 = 0.31, P < 0.05) and Rhyacophilidae (n = 16, R^2 = 0.54, P < 0.001).

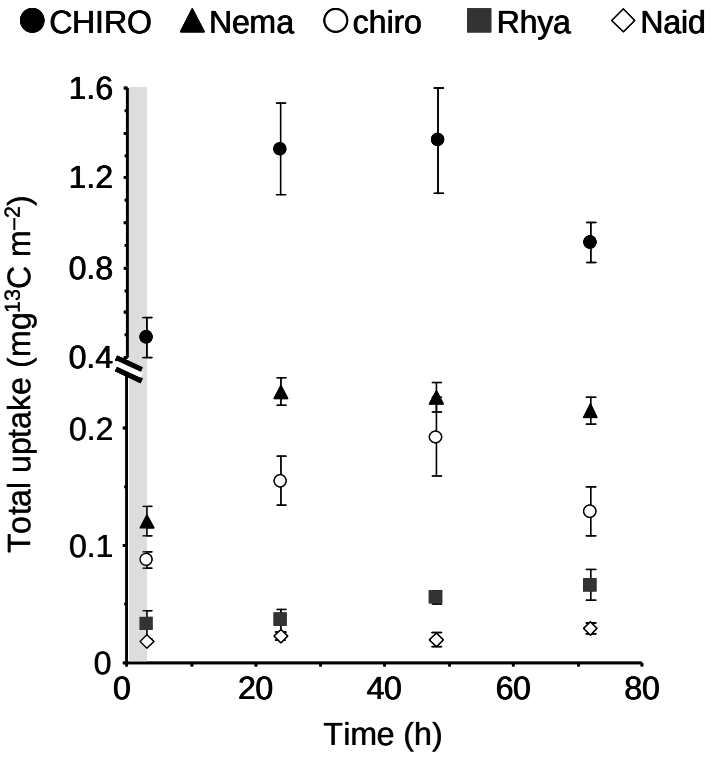

Figure IV.5. Evolution of total ^{13}C uptake (*I*) by biofilm-dwelling invertebrates during the post-labelling period (t = 3–72 h). Values are means (n = 4, ±SD). (CHIRO) macrofaunal-sized chironomidae larvae, (Nema) *Chromadorina* nematodes, (chiro) meiofaunal-sized chironomidae larvae, (Rhya) Rhyacophilidae larvae, (Oligo) Naididae oligochaetes. Shaded area show the period of microphytobenthos labelling using $NaH^{13}CO3$ (t = 0–3 h).

Because MPB became progressively less labelled, the temporal attenuation of available ^{13}C was implemented for the following calculation: between t = 3–24 h, daily invertebrate ^{13}C uptake rates were estimated at 1460, 117, 193, 8 and 5 µg^{13}C m^{-2} d^{-1} for macro- and meiofaunal Chironomidae, *Chromadorina*, Naididae and Rhyacophilidae, respectively. Daily total MPBC uptake rates were calculated from these ^{13}C uptake rates (Table IV.2). It was estimated that the MPBC from the pooled 1.2–25 and 25–40 µm biofilm fractions contributed daily 309, 99, 104 and 13% to the DAD of macro- and meiofaunal-sized Chironomidae, *Chromadorina* and Naididae, respectively (Table IV.2). As predators, Rhyacophilidae daily needed to assimilate 6.7 µgC ind^{-1}, corresponding to the assimilation of e.g., 6 Naididae individuals.

Table IV.2. Biofilm-dwelling invertebrate daily production (P), daily assimilation demand (DAD), daily microphytobenthic carbon (MPBC) assimilation rates and contribution of assimilated MPBC to DAD in the epilithic biofilm of the Garonne River on the 20th of September 2009

Biofilm-dwelling invertebrates	P (mgC m^{-2} d^{-1})	DAD (mgC m^{-2} d^{-1})	MPBC assimilation (mgC m^{-2} d^{-1})	DAD fulfilled (%)
Macrofauna (>500 µm)				
Chironomidae larvae	4.73	11.82	36.48	309
Rhyacophilidae larvae	0.85	1.54	–	–
Meiofauna (40–500 µm)				
Chromadorina nematodes	2.79	4.65	4.82	104
Chironomidae larvae	1.18	2.95	2.93	99
Naididae oligochaetes	0.59	1.48	0.19	13

After labelling (t = 3 h), 98% of the label was stocked in the pooled 1.2–25 and 25–40 µm biofilm fractions and 2% in invertebrates (Fig. IV.6). At t = 72 h, 16% of the initial amount of label was still stocked in the pooled biofilm fractions and 3% in invertebrates. However, 81% of the label was found neither in biofilm fractions nor in invertebrates (Fig. IV.6).

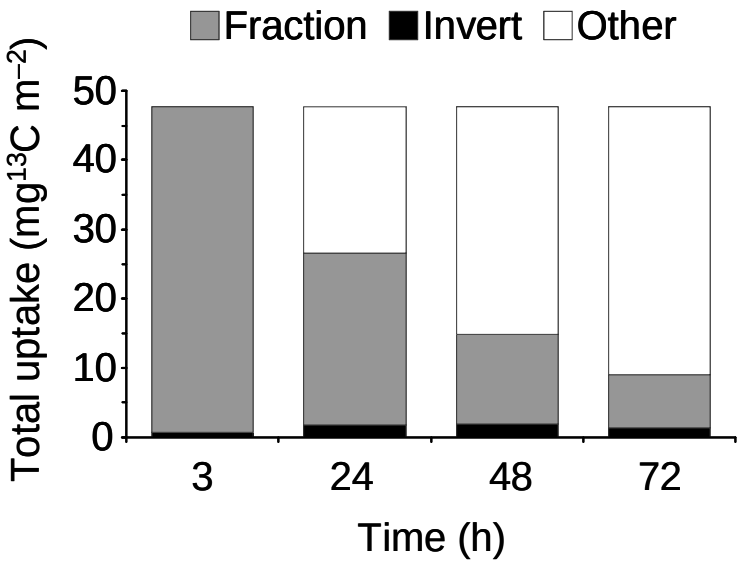

Figure IV.6. Evolution of ^{13}C stocks among main biofilm compartments during the post-labelling period (t = 3–72 h). (Fraction) ^{13}C incorporated in pooled 1.2–25 and 25–40 µm biofilm fractions (mean, *n* = 8), (Invert) ^{13}C incorporated by biofilm-dwelling invertebrates (mean, *n* = 20), (Other) missing ^{13}C relative to post-labelling (t = 3 h) ^{13}C stocks.

IV.6. Discussion

In our study, MPB represented 16% of biofilm organic content, which is in agreement with values commonly found in these habitats (Azim & Asaeda, 2005). Most of the MPB biomass consisted of diatoms, which can exude high amounts of EPS for adhesion, nutrition and protection functions (Winsborough, 2000). Our study did not disentangle the part of MPBC incorporated by direct grazing on MPB cells from that incorporated indirectly by MPB-EPS consumption. However, on the basis of contrasting label uptake dynamics, our results allowed to discriminate *Chromadorina* nematodes and Chironomidae larvae, which seemed to use this freshly produced MPBC, from Naididae oligochaetes and Rhyacophilidae larvae, which used MPBC through its recycling by microbial-loop or through predation, respectively.

From natural $\delta^{13}C$ and $\delta^{15}N$ signatures, we deduced that biofilm-dwelling *Chromadorina* spp. nematodes fed on the 1.2–25 µm biofilm fraction which contained high amounts of small diatoms. This result makes sense, since biomarker pigments of diatoms were found in guts of *Chromadorina* inhabiting epilithic biofilms of the Garonne River (Majdi *et al.*, 2012b). Moreover, marine Chromadoridae nematodes (comprising *Chromadorina*) feed commonly on diatoms by piercing and/or cracking frustules to suck out cellular contents (*e.g.* Tietjen & Lee,

1973; Romeyn & Bouwman, 1983; Moens & Vincx, 1997). In the present study, *Chromadorina* nematodes rapidly incorporated MPBC, as such realising a rapid transfer of freshly photosynthesized C through the food-web by feeding on MPB cells and possibly on their EPS exudates. A similar process is also reported for nematodes inhabiting intertidal sediments (Montagna, 1984; Middelburg *et al.*, 2000; Moens *et al.*, 2002). Considering only direct grazing on MPB cells, it would lead *Chromadorina* to assimilate daily 0.4% of biofilm MPB cell C stocks. This would correspond to a daily ingestion of ~1.6% of biofilm MPB cell C stocks, since nematode ingestion approximates four times assimilation (Herman & Vranken, 1988). This estimation fits well with grazing pressure commonly reported for marine nematodes (*e.g.* Moens *et al.*, 2002; Pascal *et al.*, 2008b). In superficial sediments of a third order stream, Borchardt & Bott (1995) found a negligible algivory of nematodes using fluorescently labelled diatoms (FLD). However, these authors specify that, with FLD, only diatoms ingested whole are detected. In our study, the assimilation demand of *Chromadorina* was fully met (104%) by MPBC sources. Majdi *et al.* (2012b) estimate at the same date and site, that MPB cell-content ingestion contributes only 1–23 % to *Chromadorina*'s demand. *Chromadorina* nematodes can agglutinate surrounding detritus using mucus silks exuded by their caudal glands (Meschkat, 1934). Bacterial colonisation and growth is generally promoted on these mucus silks (Moens *et al.*, 2005). Riemann & Helmke (2002) propose that bacterial external enzymatic activity initiates the decomposition of complex molecules associated to these agglutinations, so that resulting simple molecules can in return be easily ingested and incorporated by nematodes. In this context, we suggest that within epilithic biofilms of the Garonne River, *Chromadorina* nematodes feed to a considerable extent on EPS exuded by MPB through "gardening" interactions with bacteria, and in a lesser extent can graze directly on MPB cells.

Like nematodes, Chironomidae larvae rapidly incorporated freshly produced MPBC. Contrary to what was observed for nematodes, the ^{13}C incorporated by Chironomidae decreased significantly three days after labelling. This could depict rapid population turnover processes (*e.g.* migration, emergence and removal by predation). We therefore suggest that Chironomidae could represent more important vectors for biofilm MPBC spatial export than nematodes (on a scale of 400 cm^2). Quantitatively, the MPBC daily incorporated by pooled meio- and macrofaunal Chironomidae represented 3.3% of biofilm MPB cell C stocks (considering only direct grazing on cells). However, their ingestion of MPBC must be substantially higher than this assimilation value (Rasmussen, 1984). From our estimates, the

assimilation of MPBC largely exceeded (309%) the demand of macrofaunal Chironomidae, while it fulfilled (99%) the demand of meiofaunal Chironomidae. The feeding activity and energetic needs of Chironomidae larvae can strongly increase in latest larval instars (Berg, 1995), and we probably underestimated this extensive energetic demand increase for macrofaunal Chironomidae. Besides, it was somewhat surprising that both meio- and macrofaunal Chironomidae showed similar $\delta^{13}C$ and $\delta^{15}N$ signatures, which were slightly stretched towards allochthonous signatures. A possible explanation is that Chironomidae larvae have a rather broad and flexible diet including MPB, as well as fine particulate organic matter (FPOM)—their diet also fluctuates with species and larval development (Berg, 1995; Schmid & Schmid-Araya, 2002). In this context, and since our study concerned pooled Chironomidae species, extrapolation of our findings concerning Chironomidae larvae must be done with caution and considered as a preliminary estimation.

Naididae oligochaetes feed on various food items, but particularly on FPOM (including bacteria) and diatoms (Learner *et al.*, 1978). Naididae can heavily colonize leaf packs settled in the Garonne River, which are pools of FPOM through the abundance of litter-processing microorganisms and/or the entrapment of fine suspended particles (Chauvet *et al.*, 1993). In our experiment, $\delta^{13}C$ and $\delta^{15}N$ signatures suggested that Naididae could use a mixture of biofilm fractions and leaf litter. Although we observed entire diatom frustules in Naididae guts, they only gained slight ^{13}C enrichment during the post-labelling period. This strengthened that allochthonous C and/or microbial-loop recycled MPBC contributed predominantly to Naididae's diet.

The $\delta^{13}C$ and $\delta^{15}N$ signature of Rhyacophilidae larvae suggested that they were mainly predators feeding at least on Naididae oligochaetes and probably also on Chironomidae. This result corresponds to the well-known predatory habits of Rhyacophilidae larvae (Wiggins, 2004). We found no evidence of any predation on nematodes, probably because they were too small to be successfully handled. Rhyacophilidae showed a similar slight labelling than Naididae, strengthening their predatory behaviour on Naididae. A previous monitoring study report that there is only one Rhyacophilidae species living downstream sixth order reaches of the Garonne River basin: *Rhyacophila dorsalis* Curtis 1834 (Cayrou *et al.*, 2000). It is therefore likely that our results mainly concerned *R. dorsalis*. We estimated that, on average, each Rhyacophilidae individual needed to feed daily on 6 Naididae to satisfy its C demand. This agrees with results of enclosure experiments, in which individual *R. dorsalis* consumes

2–19 blackfly larvae per day (Wotton *et al.*, 1993). Our predation estimation based on a 21 h period should overcome potential biases caused by diel feeding rhythms of *R. dorsalis* (Elliott, 2005). However, we can not exclude a potential bias due to predator mobility, although Otto (1993) reports that *Rhyacophila nubila* Zetterstedt 1840 larvae are sedentary, ambushing their preys from "favourite" sheltered positions.

After three days, on average 81% of the label initially fixed by MPB photosynthesis was detected neither in invertebrates nor in biofilm fractions (Fig. IV.6). The ^{13}C half-life loss period ($\tau_{½}$) was 1.1 day in biofilm fractions. In comparison, from intertidal top 5 mm sediments of the Scheldt estuary, Middelburg *et al.* (2000) report slower label losses for diatoms ($\tau_{½}$ = 1.9 d) and for the total carbon pool ($\tau_{½}$ = 2.5 d). To explain their observed MPBC loss, the latter authors point out processes such as resuspension, respiration and mixing to deeper sediment layers. For instance, respiration contributes 40% of the MPB ^{13}C loss in intertidal flats after three days. While respiration is indeed an important C loss pathway occurring also in the biofilm, river epilithic biofilm habitats deeply differ from intertidal sediments, and some specific hypotheses can be proposed to explain the rapid label loss observed:

(1) A major part of the C initially fixed by photosynthesis is rapidly exuded by diatoms as EPS, which are mostly low-molecular-weight compounds being preferentially and quickly assimilated by bacteria (*e.g.* Romaní & Sabater, 1999). This C pathway could substantially contribute to the observed label loss, since it was not accounted for in our experimental setup (the <1.2 µm biofilm fraction comprising bacteria and EPS was not analysed).

(2) The biofilm can be detached from its substrate by flow constraints (*e.g.* Biggs & Close, 1989). However, this is not likely, since during our three day experimental time-window, the streambed flow velocity at the study site remained low (~10 cm s^{-1}), with discharge of the Garonne River ranging between 30–37 m^3 s^{-1} (Majdi *et al.*, 2011). Boulêtreau *et al.* (2006) show—at the same site of the Garonne River—that a self-detachment of the biofilm from its substrate occurs during extended low-water periods with high temperatures (typically in summer), presumably due to bacterial growth destabilizing senescent algal layers. This self-detachment of free-floating biofilm fractions could be partly involved in the observed label loss.

(3) Lastly, it is plausible that during our experiment, highly mobile grazers (*e.g.* fishes, Van Dam *et al.*, 2002) might have grazed and exported some label away from the experimental biofilms.

IV.7. Conclusion

To conclude, our results showed that, in the epilithic biofilm of the Garonne River, macrofaunal Chironomidae took up quantitatively more MPBC than did meiofauna. While macrofaunal Chironomidae incorporated MPBC as rapidly as *Chromadorina* nematodes, they seemed more involved in MPBC spatial export. Rhyacophilidae fed on large meiofauna (*e.g.* Naididae) but not on nematodes. We observed an important and rapid loss of MPBC. Invertebrate assimilation *per se* seemed not primarily involved in the fate of biofilm MPBC.

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Chapitre V

Discussion générale et conclusion

Discussion générale et conclusion

V.1. Résilience et résistance à l'hydrodynamique

Les perturbations hydrodynamiques ont une influence primordiale sur le développement et l'organisation fonctionnelle des biofilms épilithiques de rivière (*e.g.* Biggs & Close, 1989; Peterson & Stevenson, 1992; Boulêtreau, 2007). Dans la Garonne, les épisodes de crues ont clairement provoqué une baisse dramatique de la densité de la méiofaune associée à l'arrachage du biofilm. Il s'en est suivi une phase de recolonisation induisant une modification et une adaptation progressive de l'assemblage d'organismes du biofilm suivant le continuum de la succession écologique du biofilm (*e.g.* Korte & Blinn, 1983; Peterson & Stevenson, 1992). Habituellement cantonnée au milieu microbien (*e.g.* Jackson *et al.*, 2001; Lyautey *et al.*, 2005), la succession d'organismes durant le processus de recolonisation d'un biofilm épilithique en milieu lotique a, pour la première fois, été mise en évidence pour des métazoaires (**chapitre I**). Les résultats de cette étude ont montré que parmi la méiofaune, les rotifères étaient les colonisateurs les plus rapides (relativement dominants dès 7 jours après une crue), tandis que les nématodes étaient plus avantagés par des biofilms épais et matures—les nématodes devenaient relativement plus abondants que les rotifères de façon durable ~140 jours après une crue (Fig. I.2b). Ces résultats corroborent les observations de Peters *et al.* (2007b) lors de l'étude de la colonisation de substrats artificiels (plaques d'aluminium) immergés dans un lac : les rotifères sont majoritaires en début de succession, détrônés par les nématodes après 57 jours de colonisation. Cette dynamique de colonisation, aussi soulignée par Smith & Brown (2006) dans le sédiment, pourrait correspondre à une tendance assez générale en milieu aquatique. Peters *et al.* (2007b) montrent également que le processus de recolonisation du biofilm par la méiofaune dépend plus du transport actif et/ou passif dans la colonne d'eau plutôt que d'une recolonisation par le sédiment. Les rotifères étant de meilleurs nageurs que les nématodes, leur propension à être les premiers métazoaires de la succession écologique du biofilm épilithique est logique. De plus, contrairement aux nématodes, les rotifères ne sont pas dépendants des seules ressources alimentaires benthiques : en effet Kathol *et al.* (2011) montrent que les rotifères des biofilms épilithiques du Rhin sont d'efficaces filtreurs actifs de plancton. Enfin, les rotifères possèdent des caractéristiques physiologiques (parthénogénèse, formes de résistance) et

morphologiques (glandes de fixations) les rendant très concurrentiels pour la colonisation précoce de milieux fraîchement perturbés par des crues (Ricci & Balsamo, 2000).

Le suivi détaillé de la communauté de nématodes (**chapitre II**) a montré que les espèces *Chromadorina bioculata* et *C. viridis* dominent très largement un assemblage d'espèces, par ailleurs peu diversifié en comparaison avec la diversité communément observée dans les habitats interstitiels (*e.g.* Traunspurger, 2000). Or, ces deux espèces sont capables de s'attacher au substrat grâce à leurs sécrétions muqueuses, et sont ainsi communément retrouvées dans des habitats épilithiques et épiphytiques (*e.g.* Croll & Zullini, 1972; Jensen, 1984; Traunspurger, 1992; Peters & Traunspurger, 2005). En plus de l'influence de la situation trophique du biofilm, il est possible que la faible diversité de l'assemblage de nématodes et la nette dominance de *C. bioculata* et *C. viridis* soit aussi liée aux fortes contraintes hydrologiques rencontrées dans les biofilms épilithiques de rivière.

V.2. Interrelations méiofaune–biofilm

La densité des principaux groupes de la méiofaune permanente (*i.e.* nématodes et rotifères) était corrélée à la biomasse (*i.e.* l'épaisseur : voir Peters *et al.*, 2007b) du biofilm et à son contenu en microphytes. Ainsi, c'est paradoxalement en fin d'étiage d'hiver, lorsque les températures sont basses et les vitesses du courant relativement élevées, que la densité de la méiofaune permanente—suivant la biomasse du biofilm—a atteint son apogée annuelle (**chapitre I**). Cette interrelation entre la biomasse du biofilm épilithique et la densité de la méiofaune permanente corrobore les observations de Peters & Traunspurger (2005) en milieu lentique, et semble transcender les dynamiques saisonnières observées communément pour la méiofaune du sédiment qui montre des pics de densité en fin de printemps ou en été (Beier & Traunspurger, 2003a; Stead *et al.*, 2003; Abebe *et al.*, 2006b). L'épaisseur du biofilm (donc la place disponible), et parallèlement son contenu en microphytes (nourriture potentielle) ont donc conditionné le développement de la méiofaune. D'un autre côté, Pinckney *et al.* (2003) suggèrent que l'activité de bioturbation et de broutage de la méiofaune dans les vasières intertidales favorise la pénétration de la lumière et la circulation des nutriments, et donc paradoxalement la production primaire du microphytobenthos. Plus récemment, Mathieu *et al.* (2007) montrent que de fortes densités de nématodes (>50 ind cm^{-2}) augmentent la production et la distribution verticale de l'O_2

dans des biofilms expérimentaux de diatomées. L'activité de la méiofaune pourrait donc tout à fait favoriser plutôt qu'impacter la croissance du biofilm en décloisonnant son architecture au bénéfice des processus de production primaire. D'autant que la pression de broutage de la méiofaune sur le compartiment microphytobenthique du biofilm est assez faible (**chapitres III et IV**). En revanche, Gaudes *et al.* (2006) suggèrent que de très fortes densités de nématodes favoriseraient le détachement des biofilms cyanobactériens dans la rivière Llobregat (Catalogne). Les fractions flottantes de ces biofilms abritent en effet des densités considérables de nématodes (jusqu'à 752 ind cm^{-2}), bien supérieures aux densités observées dans le biofilm fixé. De plus, les fractions flottantes contiennent une proportion importante de juvéniles et de femelles gravides, tant et si bien que ces auteurs avancent l'hypothèse que ces « radeaux » de biofilm favoriseraient la reproduction et la dispersion des nématodes. La fragmentation des biofilms cyanobactériens de rivière en fractions flottantes stimule l'émission de géosmine, affectant sérieusement la qualité de l'eau (Sabater *et al.*, 2003). Or, l'activité de la méiofaune au sein de ces fractions flottantes de biofilm pourrait stimuler davantage encore les rejets de géosmine en endommageant et en dispersant les agrégats de cellules cyanobactériennes (Sabater *et al.*, 2003; Gaudes *et al.*, 2006). Ce travail de thèse n'a pas clairement mis en évidence de tels liens entre la méiofaune et le détachement du biofilm épilithique. Cependant, le suivi à long-terme de la méiofaune associée au biofilm (**chapitre I**) a précisé un seuil de vitesse de courant (30 cm/s), au-dessus duquel un détachement important du biofilm et de sa méiofaune associée (en particulier les nématodes) est constaté. Cette valeur est en accord avec les valeurs seuils de résistance des assemblages de microphytes du biofilm (Poff *et al.*, 1990; Biggs *et al.*, 1998). Mais surtout est supérieure au seuil critique d'érosion de la méiofaune habitant les sédiments fins (12 cm/s) déterminé par Palmer (1992), laissant penser que les biofilms pourraient servir de refuge et de réserve pour la recolonisation du sédiment en cas d'augmentation modérée de l'hydrodynamique—observée pour 54% des dates d'échantillonage, et notamment pendant l'étiage d'hiver. Ce rôle de refuge pour la méiofaune joué par le biofilm (tout comme l'hyporheos) doit-être d'autant plus crucial dans les cours d'eau de tête de bassin généralement soumis à de fortes contraintes hydrodynamiques, et qui constituent une importante source d'organismes méiobenthiques dérivants pour la colonisation des zones avals (Gaudes *et al.*, 2010). Compte-tenu de l'importance des biofilms épilithiques pour la connectivité écologique et le fonctionnement biogéochimique des rivières (Pusch *et al.*, 1998; Battin *et al.*, 2003)—notamment par la rétention des nutriments et du carbone organique dissout (Romaní *et al.*, 2004; Teissier *et*

al., 2007)—il serait avisé de préciser *e.g.* dans des milieux à l'hydrodynamique contrôlée, si des densités croissantes de méiofaune pourraient modifier les valeurs de seuil de vitesse de courant entraînant le détachement du biofilm.

V.3. Interactions trophiques

V.3.1. Consommation de la biomasse épilithique par la méiofaune

Concernant la méiofaune permanente, les rotifères étaient très abondants dans le biofilm de la Garonne. Ils peuvent contribuer significativement au transfert de la biomasse planctonique vers le biofilm par filtration (Kathol *et al.*, 2011). Le comportement trophique des rotifères n'a pas été examiné dans le détail dans cette thèse, ceci principalement en raison du très grand nombre d'individus requis pour l'analyse isotopique. Cependant, une étude préliminaire des contenus pigmentaires intestinaux des rotifères Bdelloidea du biofilm de la Garonne, suggère que ces derniers pourraient se nourrir sélectivement des cyanobactéries (Mialet, 2010). Il reste cependant à déterminer si ce sont des cyanobactéries d'origine benthique ou pélagique. L'impact du broutage de la méiofaune (permanente et temporaire) s'est avéré moindre que celui des larves de Chironomidae macrobenthiques (**chapitre IV**). Ceci était probablement lié au fait que, bien qu'étant abondants, les organismes de la méiofaune ne représentaient qu'une faible biomasse cumulée. Cependant leur nombre, leur rapport production/biomasse élevé, leur cycle de vie très court ainsi que leur activité au sein du biofilm suggèrent une régulation subtile des processus du biofilm de par leur bioturbation et leur interaction fine avec le compartiment microbien (*e.g.* Borchardt & Bott, 1995; Bott & Borchardt, 1999; Moens *et al.*, 1999a; Schroeder *et al.*, 2010). Ce travail de thèse a mis en évidence des comportements trophiques variés (algivorie, détritivorie, non-sélectivité) au sein de la méiofaune du biofilm, mais il ne fait aucun doute que la palette des comportements alimentaires possibles dans des milieux tels que les biofilms est très diverse (*e.g.* chimiotactisme voir Höckelmann *et al.*, 2004), et mériterait une attention particulière.

V.3.2. Comportement trophique des nématodes

Dans l'étude détaillée de la dynamique de l'assemblage d'espèces de nématodes (**chapitre II**), un couplage a été mis en évidence entre le contenu en diatomées du biofilm et l'abondance des deux espèces dominantes *Chromadorina bioculata* et *Chromadorina viridis*. Ces deux espèces appartiennent au type trophique *epistrate-feeders*, impliquant une certaine affinité alimentaire pour les microphytes (Traunspurger, 1997), bien que la consommation de bactéries et d'eucaryotes unicellulaires hétérotrophes par ces nématodes ne soit pas exclue (Traunspurger, 2000). Des études menées en milieu marin soulignent une spécialisation comportementale, morphologique et même digestive des nématodes Chromadoridae (famille comprenant le genre *Chromadorina*) envers la consommation de diatomées (Tietjen & Lee, 1973; Deutsch, 1978; Jensen, 1982; Romeyn & Bouwman, 1983; Moens & Vincx, 1997). Le couplage observé entre nématodes *Chromadorina* et diatomées pouvait donc être de nature trophique. Aussi, l'hypothèse d'une consommation sélective des diatomées du biofilm a été énoncée et testée en dosant pour la première fois par chromatographie liquide à haute-performance (HPLC) les pigments contenus dans les intestins des nématodes (**chapitre III)**. Cette hypothèse a été invalidée, car ces nématodes consomment les microphytes en proportion avec leur disponibilité dans le biofilm (*i.e.* non-sélectivement). De plus, la consommation des contenus cellulaires des microphytes ne comblait que peu (1–27%) leurs besoins énergétiques. Cependant, l'éventualité d'une consommation sélective des diatomées vis-à-vis des autres groupes de microphytes n'est pas à exclure. L'approche isotopique a montré une incorporation rapide du carbone microphytobenthique (CMPB), et a complété les résultats des analyses HPLC en montrant que la demande énergétique des nématodes *Chromadorina* était entièrement satisfaite (104%) par l'assimilation du CMPB (**chapitre IV**).

Ces résultats sont surprenants étant donné la spécialisation trophique mise en évidence pour des nématodes marins de la famille Chromadoridae. Une explication probable (soulignée dans le **chapitre III**) est que ces nématodes ont montré un comportement alimentaire non-sélectif—en ce qui concerne la consommation des contenus cellulaires des microphytes—qui pourrait les avantager étant donné la fluctuation et la répartition hétérogène des microphytes dans les biofilms (*e.g.* Cazaubon & Loudik, 1986). D'ailleurs les estimations de la contribution du microphytobenthos (MPB) au régime alimentaire des nématodes correspondraient bien avec la contribution du MPB à la biomasse organique totale du

biofilm (16.2%, **chapitre IV**). Pour ce qui est de l'essentiel de leur régime alimentaire, on sait que les nématodes *Chromadorina* secrètent des soies adhésives (Meschkat, 1934; Croll & Zullini, 1972), qui en plus d'aider à leur fixation sur des substrats durs, leur permettent d'accumuler des pelotes de détritus (Fig. V.1a). Ce comportement a été observé chez des *Chromadorina* vivants du biofilm de la Garonne (Fig. V.1b).

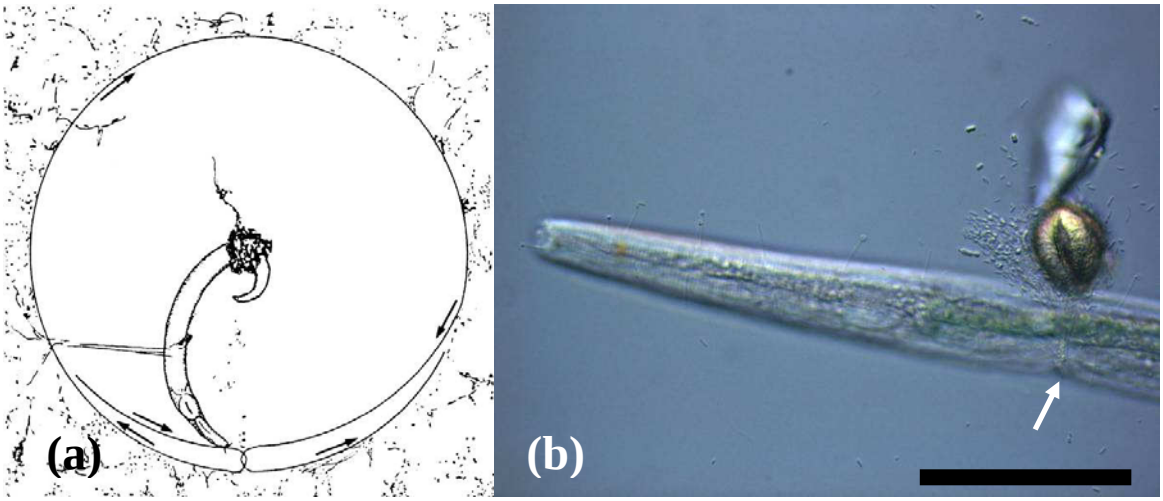

Figure V.1. (a) Schéma du processus de formation d'une pelote de détritus. Un nématode Chromadoridae fixé par le bout de sa queue à une surface plane collecte les particules détritiques en oscillant et en se balançant dans des directions opposées. Les particules adhèrent à ses soies et sont concentrées en position postérieure (d'après Meschkat, 1934; modifié dans Riemann & Helmke, 2002). **(b)** Microphotographie montrant la partie antérieure d'un *Chromadorina* spp. du biofilm de la Garonne et ses sécrétions de soies emprisonnant une diatomée. La *flèche blanche* désigne la constriction provoquée par l'attachement des soies. La densité bactérienne est importante autour de la pelote et de la diatomée emprisonnée. La *barre de légende* représente 50 µm.

Ces soies stimulent la croissance bactérienne (Moens *et al.*, 2005; Fig. V.1b), et Riemann & Helmke (2002) suggèrent que l'activité enzymatique externe des bactéries qui colonisent ces soies cliverait les molécules complexes (piégées dans les pelotes de détritus) en molécules simples plus facilement assimilables par les nématodes. Cet *enzyme–sharing concept* (*sensu* Rieman & Helmke 2002) permettrait donc à ces nématodes d'ingérer une « soupe » nutritive à partir des EPS principalement produits par le MPB et qui composent majoritairement la biomasse du biofilm (Azim & Asaeda, 2005). Cette hypothèse en plus de préciser l'essentiel du régime alimentaire des *Chromadorina*, expliquerait pourquoi ces

nématodes ont un comportement opportuniste dans leur consommation du MPB : ils consommeraient les microphytes en complément (*e.g.* les diatomées pour leurs acides gras essentiels), proportionnellement à leur densité dans le milieu et donc à leur emprisonnement dans les pelotes de détritus (**chapitre III** et Fig. V.1a,b). Cependant, cette explication, bien que séduisante, n'a pas été testée spécifiquement, et reste donc spéculative dans l'attente d'être plus précisément examinée *e.g.* par un traçage spécifique des EPS.

V.3.3. Impact du broutage et de la prédation de la macrofaune

Tandis que la plupart du temps *C. bioculata* et *C. viridis* ont dominé très largement la communauté de nématodes, la diversité de l'assemblage d'espèces a augmenté en été (**chapitre II**), avec notamment une proportion relative accrue d'espèces du type trophique *deposit-feeders* qui consomment principalement des bactéries (Traunspurger, 2000). Ces nématodes bactérivores, qui ont des cycles de vie courts et une reproduction parthénogénétique, semblaient bénéficier de conditions estivales pourtant très contraignantes : en effet, un bouleversement estival (Juillet 2005 et 2009, Fig. I.1) a visiblement impacté le biofilm et sa méiofaune associée en l'absence de perturbation hydrodynamique (**chapitre I**). Or, pendant ces périodes, les derniers stades larvaires de Psychomyiidae (Insecta, Trichoptera) pullulaient littéralement sur les galets. Les larves de Psychomyiidae sont sédentaires, mais construisent des abris constitués de petites particules organiques et/ou sédimentaires soudées par de la soie à la surface des galets (Fig. V.2). Elles broutent le biofilm tout en élargissant leurs abris pour atteindre de nouvelles « pâtures » sur la surface des galets (Wiggins, 2004). De par leur fortes densités, il est probable que leur pression de broutage ait pu réduire drastiquement la biomasse épilithique (Hillebrand, 2009), d'autant que leur activité de construction d'abris pourrait aussi déstabiliser la cohésion basale du biofilm et donc augmenter le phénomène de détachement autogène déjà important en été (Boulêtreau *et al.*, 2006). Ainsi ces larves de Psychomyiidae pourraient impacter le biofilm en général, ce qui induirait une concurrence globalement néfaste à la méiofaune permanente de par la réduction de leur habitat et l'épuisement de leurs ressources alimentaires. La combinaison de ce broutage avec un développement plus important des communautés bactériennes dans le biofilm (Lawrence *et al.*, 2002; Lyautey *et al.*, 2005; 2010) pourrait aussi expliquer pourquoi les espèces de nématodes bactérivores et opportunistes seraient plus « favorisées » que *C. bioculata* et *C. viridis* pendant l'été.

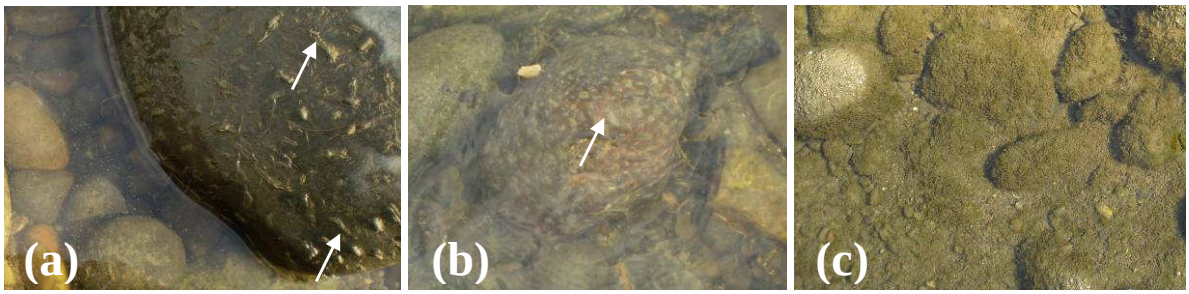

Figure V.2. Photographies de galets sur les bords de la Garonne, **(a)** et **(b)** 13 Juillet 2009 : biofilm réduit, présence de nombreux abris de larves de trichoptères Psychomyiidae. **(c)** 14 Septembre 2009 : biofilm développé, pas d'abris de Psychomyiidae. Les *flèches blanches* indiquent des abris de larves de trichoptères Psychomyiidae.

De plus, les macro-brouteurs pourraient également ingérer « par inadvertance » la méiofaune lors de leur broutage du biofilm (Peters *et al.*, 2007a; Peters & Traunspurger, *in press*). En tout cas, dans la Garonne, d'autres macro-brouteurs comme les larves de Chironomidae n'avaient aucun impact collatéral sur la méiofaune lors de leur broutage (**chapitre IV**). Cette différence pourrait provenir de la différence de taille des macro-brouteurs considérés : ainsi, les gastéropodes *Theodoxus fluviatilis* (>5 mm) considérés dans l'étude de Peters & Traunspurger (*in press*) sont beaucoup plus grands que les larves de Chironomidae (0.5–2 mm) considérées dans le **chapitre IV**. D'ailleurs, les macro-brouteurs de grande taille : *e.g.* les gastéropodes *Physa acuta* ou les larves d'Ephéméroptères *Ecdyonurus insignis* retrouvés dans le biofilm de la Garonne montrent un taux de broutage individuel important sur le biofilm, consommant respectivement ~1400 et 200 µgAFDM ind^{-1} $jour^{-1}$ (d'après les taux de broutage calculés pour les organismes de taille 5–8 mm : Nofdianto, 2005). En comparaison, les larves macrobenthiques de Chironomidae n'assimilaient que 11 µgC ind^{-1} $jour^{-1}$ soit ~25 µgAFDM ind^{-1} $jour^{-1}$ (en prenant un rapport C/AFDM = 0.45 : Whittaker & Likens, 1973). Bien que le taux de broutage et l'éventuelle consommation indirecte de méiofaune par les derniers stades larvaires de Psychomyiidae n'aient pas été examinés. Il peut être suggéré que ces Psychomyiidae de taille importante (~5 mm) puissent atténuer indirectement les densités de méiofaune associée au biofilm en impactant considérablement la biomasse épilithique. Cette hypothèse pourrait être explorée par une approche isotopique englobant les Psychomyiidae lors de leur pic d'abondance et de biomasse (*i.e.* au mois de Juillet).

La macrofaune peut aussi avoir un effet *top-down* direct (par prédation) sur la méiofaune dans le sédiment (*e.g.* Schmid-Araya & Schmid, 2000; Beier *et al.*, 2004). Les résultats issus des analyses isotopiques indiquent que dans le biofilm épilithique de la Garonne les larves de trichoptères Rhyacophilidae se nourrissaient des plus « gros » représentants de la méiofaune *i.e.* les oligochaetes Naididae et les larves de Chironomidae. En revanche, ils ne semblaient pas se nourrir de proies beaucoup plus petites qu'eux comme les nématodes (**chapitre IV**).

V.4. Considérations méthodologiques

Des estimations basées sur des données de la littérature ont été utilisées pour estimer *e.g.* les besoins énergétiques (en termes de carbone) et la pression de broutage des nématodes. Ces estimations ont pu conduire à une approximation de certains résultats, soulignant le besoin de disposer de données physiologiques propres aux nématodes *C. bioculata* et *C. viridis*. Par exemple, pour une date commune (21 Septembre 2009), la pression journalière de broutage de ces nématodes a été estimée à ~1.6% du stock microphytobenthique (en terme de carbone), à partir des analyses isotopiques (**chapitre IV)**, et à 0.07, 0.35 et 1.76% du stock microphytobenthique (en terme de chlorophylle *a*), à partir de l'analyse HPLC des contenus intestinaux et en prenant des temps de passage intestinaux de 70, 14 et 2.8 min, respectivement (**chapitre III**). Bien que ces résultats soient plutôt concordants, ces estimations pourraient être améliorées si l'on disposait de mesures physiologiques spécifiques pour ces nématodes (*e.g.* temps de passage intestinal du bol alimentaire et efficacité de digestion et d'assimilation de la cholorophylle *a*). Malheureusement *C. bioculata* est difficilement cultivable en laboratoire (Pieczynska, 1964), rendant des études physiologiques ciblées particulièrement délicates.

Plus globalement, bien que dépeignant plus vraisemblablement les processus écologiques en conditions naturelles, l'expérimentation *in situ* ne permet pas de s'affranchir des nombreux facteurs pouvant jouer, par exemple, sur l'étude d'un processus comme le broutage. D'autant que les biofilms sont des milieux caractérisés par une grande complexité structurelle et montrant toute une diversité de micro-habitats et de micro-gradients (Costerton, 2000; Romaní, 2010).

V.5. Conclusion

L'objectif général de cette thèse était de préciser la dynamique et les interactions trophiques de la méiofaune dans les biofilms de rivière. Comme souligné par Giere (2009), nos connaissances actuelles en la matière sont très limitées, tout particulièrement dans les fleuves. Ainsi cette étude s'est concentrée sur la Garonne moyenne comme site d'étude.

La question de la dynamique de la méiofaune a nécessité un suivi régulier sur le long-terme, afin de définir la réponse de la méiofaune du biofilm à l'instabilité de son habitat. Les scénarios hydrologiques (durée des périodes d'étiage), ainsi que la dynamique de la biomasse du biofilm se sont révélés comme les facteurs majeurs déterminant la dynamique de répartition des nématodes et des rotifères (**chapitre I**). De plus, le rôle du biofilm comme habitat-refuge assurant une protection contre l'érosion a notamment été montré, et un seuil de vitesse de courant limitant cette protection a été estimé à 30 cm s^{-1}.

Suite aux relations avérées entre la méiofaune (principalement les nématodes) et la composition microphytobenthique du biofilm (**chapitres I et II**), les interactions trophiques de la méiofaune du biofilm ont été étudiées en se focalisant sur l'importance des microphytes épilithiques comme source de nourriture. Différentes stratégies alimentaires ont été mises en évidence parmi les méio- et macro-invertébrés associés au biofilm. La pression de broutage de la méiofaune sur le compartiment microphytobenthique reste globalement modeste (**chapitres III et IV**). Ainsi, il est envisageable que la présence de méiofaune soit plus favorable que néfaste au développement de la biomasse épilithique. En insistant sur les nématodes, il a été montré que le genre dominant (*Chromadorina* spp.) utilise le carbone microphytobenthique : majoritairement sous forme d'EPS et dans une moindre mesure par la consommation directe des contenus cellulaires des diatomées.

En s'attachant à la caractérisation de processus *in situ*, ce travail de thèse doit être vu comme une première étape vers une meilleure compréhension de la dynamique et du comportement trophique de la méiofaune associée aux biofilms de rivière, qui incite à la poursuite de ces investigations (notamment par des études en milieu contrôlé) pour affiner les multiples influences constatées et explorer plus précisément les nouvelles questions soulevées.

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Abstract

The long-term dynamics of meiofauna with regards to environmental constraints was monitored in epilithic biofilms of the Garonne River, France. In addition, HPLC-analysis of nematode gut pigment content and a stable isotope multi-approach—including measurement of $\delta^{13}C$ and $\delta^{15}N$ natural signatures and an *in situ* ^{13}C-labelling experiment—were performed to disentangle trophic interactions involving biofilm-dwelling meiofauna. Hydrological scenarios and fluctuation of microphyte availability primarily shaped the structure of the meiobenthic community. Also, possible interferences with macro-invertebrate grazers were suspected during July. *Chromadorina* spp. nematodes grazed non-selectively on epilithic diatom contents, while most of their diet likely derived from extra-cellular polymeric substances produced by microphytes. Freshly photosynthesized carbon was rapidly incorporated by meiofauna, although quantitatively, macrofaunal Chironomidae were the most important grazers.

Résumé

La dynamique à long terme de la méiofaune a été examinée dans les biofilms épilithiques de la Garonne, en considérant les contraintes environnementales. En complément, les contenus pigmentaires intestinaux des nématodes ont été analysés par HPLC, et une multi-approche isotopique incluant une mesure des signatures naturelles $\delta^{13}C$ et $\delta^{15}N$ et une expérience de marquage au ^{13}C a été réalisée *in situ*, afin d'étudier les interactions trophiques impliquant la méiofaune habitant ces biofilms. Les résultats montrent que les scénarios hydrologiques (durée des étiages et fréquence des crues) et l'état du biofilm (dynamique de sa biomasse et de sa composition microphytique) ont façonné la structure de la communauté méiobenthique. De possibles interférences avec les macro-invertébrés brouteurs ont aussi été soupçonnées. Les nématodes *Chromadorina* spp. consommaient non-selectivement les diatomées épilithiques, tandis que l'essentiel de leur régime alimentaire était basé sur l'utilisation de substances polymériques extra-cellulaires sécrétées par les microphytes. Le carbone fraîchement photosynthétisé était rapidement incorporé par la méiofaune, bien que quantitativement, les larves macrobenthiques de Chironomidae broutaient davantage.

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Laboratoire écologie fonctionnelle et environnement, Ecolab UMR 5245 (CNRS-UPS-INPT),

Université Paul Sabatier – 118, route de Narbonne – 31062 TOULOUSE cedex 9 – FRANCE