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Préambule

Cette thèse correspond à la synthèse d'une partie de mes travaux de recherche effectués au sein du laboratoire EcoLab depuis trois ans. Ce travail s'inscrit dans deux programmes de recherche :

- ANR Biodiversité "InBioProcess" (ANR-06-BDIV-007) coordonné par Pierre Marmonier (Université Lyon 1, UMR-CNRS 5023, Laboratoire d'Ecologie des Hydrosystèmes Fluviaux) dont l'objectif principal est d'estimer le rôle de la biodiversité sur les processus écologiques aux interfaces eaux souterraines/eaux de surface, dans la perspective de développer une politique de gestion durable de leur fonctionnement. Ce programme de recherche vise à intégrer le continuum de décomposition de la matière organique sous une approche fonctionnelle, dans un contexte d'anthropisation des milieux et sur différents types d'organismes (invertébrés, champignons et bactéries).

- Programme bilatéral franco-portugais dans le cadre du Partenariat Hubert Curien, (Pessoa : 228 08 TB), soutenu par EGIDE et coordonné par Cristina Canhoto (Imar-CMA, Dept.of Zoology, University of Coimbra), dont l'objectif est d'évaluer les effets des changemenst globaux sur la biodiversité et les processus écosystémiques dans les cours d'eau forestiers.

La décomposition de la matière organique allochtone dans les cours d'eau forestier

Le compartiment hyporhéique des cours d'eau forestier

Chapitre 1: Organic matter storage in the hyporheic zone of headwater streams

Chapitre 2: Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams

Chapitre 3: Multiple screening of aquatic hyphomycetes in the hyporheic zone of woodland streams

Chapitre 4: Effects of burial on leaf physico-chemical traits, microbial conditioning and palatability to three invertebrate shredders

4.5. Results

Chapitre 5: Effects of anthropogenic acidification on leaf litter processing in the hyporheic zone of headwater streams

Discussion générale et perspectives

Annexe: Bottom-up effects mediate top-down impacts in a detritus-based food web

Résumé

Dans les cours d'eau forestiers, la décomposition des litières végétales constitue un processus écologique fondamental. Cette matière organique allochtone est rapidement colonisée par les microorganismes, notamment des champignons qui augmentent la palatabilité des litières pour les invertébrés détritivores et contribuent à l'incorporation du carbone allochtone dans ces écosystèmes. Bien qu'une grande quantité de la litière soit décomposée en surface du sédiment, une part substantielle, suite aux crues automnales ou à des mouvements de sédiment, peut intégrer le compartiment hyporhéique sous-jacent et ainsi être soumise à des conditions physico-chimiques et biologiques différentes. Ma thèse s'inscrit dans ce contexte et a pour objectif, la caractérisation du processus de décomposition des litières sous l'effet des contraintes abiotiques de la zone hyporhéique de cours d'eau forestier, et la quantification de la dynamique des invertébrés et des champignons décomposeurs associés, le tout inséré dans une vision intégrative à l'échelle de l'écosystème. Cette étude s'articule en cinq parties.

L'estimation des stocks de litières réalisée dans des cours d'eau du sud-ouest de la France a tout d'abord permis d'évaluer l'importance du processus d'enfouissement dans les sédiments.

La comparaison des processus de décomposition des litières au sein des compartiments benthique et hyporhéique a ensuite révélé que, dans ce dernier, le processus était presque exclusivement contrôlé par des champignons : les hyphomycètes aquatiques. De plus, le processus de décomposition des litières établi sous la forme d'un bilan de carbone a permis de montrer des différences entre ces compartiments en termes de patrons d'allocations des ressources entre le développement mycélien, la production de CO_2 et la libération de spores.

La zone hyporhéique constitue par ailleurs une barrière physique et chimique, qui exerce un criblage sur les communautés de décomposeurs. Ainsi, des manipulations *in situ* et au laboratoire ont permis d'évaluer ces mécanismes sur les communautés fongiques.

Les caractéristiques granulométriques des sédiments exercent un criblage physique des conidies d'hyphomycètes aquatiques en fonction de leurs traits morphologiques : la sélection des espèces par le milieu hyporhéique est l'un des résultats originaux de cette thèse.

Le gradient vertical en O_2 dissous dans la zone hyporhéique entraîne par ailleurs un stress hypoxique.

L'interaction de ces deux mécanismes conduit à des changements marqués dans la composition et la structure des communautés d'hyphomycètes aquatiques de l'habitat hyporhéique, ayant pour conséquence un ralentissement de la décomposition en zone hyporhéique.

Nous avons ensuite pu montrer que l'histoire d'enfouissement de la litière dans les sédiments affecte nettement la qualité du matériel foliaire (composition élémentaire et dureté de la feuille) et sa palatabilité pour les invertébrés détritivores, en raison de changements de la communauté fongique et de la biomasse mycélienne. Cette diminution de l'attrait des invertébrés pour les feuilles issues du milieu hyporhéique a entraîné une baisse des taux de croissance des invertébrés détritivores, traduisant ainsi une efficacité moindre des transferts trophiques dans l'écosystème.

Enfin, une expérience conduite dans un contexte de forte altération anthropique (acidification du massif forestier des Vosges) a apporté la preuve que la diminution du processus de décomposition des litières, observée à la fois dans les zones benthiques et hyporhéiques des cours d'eau acidifiés, ne dépendait pas de modifications du développement fongique. En dépit de changements prononcés dans la structure et la composition des assemblages de décomposeurs le long du gradient de pH, la décomposition des litières était moins affectée par l'acidification dans le secteur hyporhéique que dans le secteur benthique des cours d'eau. Ce phénomène résulte de l'absence d'invertébrés décomposeurs en milieu hyporhéique et d'une sensibilité relativement faible de l'activité des décomposeurs fongiques à l'acidification.

Les résultats acquis au cours de cette thèse suggèrent de réexaminer le fonctionnement écologique des cours d'eau de tête de bassin, jusque là uniquement basé sur la compréhension des relations trophiques et la dynamique du carbone liées au processus de décomposition des litières situé à la surface du sédiment, en y intégrant la dimension verticale conceptualisée par les "hyporhéologistes". Ces résultats conduisent également à repenser la dynamique et le rôle des hyphomycètes aquatiques à une échelle plus large que celle de la colonne d'eau uniquement, et mettent en évidence l'importance de la zone hyporhéique qui à cet égard restait jusqu'à présent en grande partie négligée.

Summary

In headwater streams, leaf litter decomposition constitutes a key ecosystem-level process. Once entered into the stream, leaf litter is quickly colonized by microorganisms, particularly by fungi (aquatic hyphomycetes), which enhance leaf palatability to invertebrates and contribute to the incorporation of allochthonous carbon into the stream food web. A substantial part of leaf litter entering running waters may however be buried into the streambed as a consequence of sediment movements during autumnal spates, and thus be subjected to the constraining conditions prevailing in the hyporheic zone. The objective of this thesis was to characterize leaf decomposition in the hyporheic habitat of streams, quantify the associated dynamics of fungal and invertebrate decomposers together with the effect of abiotic factors, and incorporate this process into a stream ecosystem perspective.

Budgets of coarse particulate organic matter stored in the sediment of three headwater streams (South-Western France) have allowed to evaluate the extent of burial process on the fate of leaf detritus. Leaf decomposition experiments conducted on the streambed sediment and in the hyporheic zone have revealed that this process is mostly mediated by aquatic fungi in the latter environment. A comparison of carbon budgets also showed contrasted patterns of fungal allocation to the production of mycelium, reproductive structures (conidia) and CO_2 between both habitats.

I hypothesized that the hyporheic zone performed as a physical and chemical barrier to leaf decomposers. In field and laboratory experimentations, I evaluated such mechanisms on fungal communities. A major finding was the physical screening effect of sediment grain size on the aquatic hyphomycete conidia circulating in stream water, in relation with their specific morphological traits (size and shape). This species selection from the potential fungal inoculum expectedly resulted in strongly modified fungal community structure in the hyporheic zone compared to the bentic one. Moreover, the vertical gradient of dissolved O_2 in the hyporheic zone led to an hypoxic stress that could affect fungal species differently. The interaction of both factors induced pronounced changes in the aquatic hyphomycete community together with a slower leaf litter decomposition in the hyporheic zone.

In an additional experiment, the time sequence of leaf litter burial in the sediment markedly affected the elemental composition and toughness of leaf material together with its palatability to leaf shredding invertebrates, due to changes in microbial conditioning. The observed decrease in leaf litter quality in the hyporheic zone led to lower growth rates of shredding invertebrates and was suggested to result in lower trophic transfer.

Finally, an experiment conducted in the Vosges Mountains, *i.e.* an area highly disturbed by anthropogenic acidification, provided evidence that the observed reduced decomposition of alder leaves in relation with acidification of both benthic and hyporheic zones of streams was not primarily due to changes in fungal biomass. If both structures of aquatic hyphomycete and macroinvertebrate communities were strongly modified in acidic conditions, leaf decomposition was much less affected by acidification in the hyporheic zone than in its benthic counterpart. This could partly be explained by a drastic reduction in shredder abundance in the hyporheic zone and a lower sensitivity of fungal decomposers to acidification.

Taken as a whole, these findings suggest that the functioning of woodland stream ecosystems, largely based on trophic relationships and carbon dynamics related to leaf decomposition occurring at the sediment surface, should be reconsidered with the incorporation of the vertical dimension conceptualized by "hyporheologists". These results lead to think out the dynamics and role of aquatic hyphomycetes at a broader scale than the stream water column alone, and highlight the crucial function of the hyporheic zone, which up to now remained mostly neglected to this regard.

Introduction générale

La décomposition de la matière organique allochtone dans les cours d'eau forestier

Hétérotrophie des cours d'eau forestier

Les cours d'eau de tête de bassin représentent l'ensemble des écosystèmes aquatiques lotiques [*i.e.*, sources, cours d'eau intermittents, cours d'eau de premiers ordres (Strahler 1952)] présents à la périphérie des réseaux hydrographiques. Bien qu'étant des éléments abondants et particuliers au sein des réseaux hydrographiques, la valeur et le rôle écologique de ces cours d'eau sont souvent sous-estimés et, par conséquent sujets à controverse quant à leurs gestions en vue d'assurer leurs conservations dans le cadre de pratiques forestières durables. Ils représentent près de 80% de la longueur totale de chenal au sein des réseaux hydrographiques (Sidle *et al.* 2000 ; Gomi *et al.* 2002 ; Meyer *et al.* 2003 ; 2007 ; Naiman *et al.* 2005 ; Lowe & Likens 2005). Ces cours d'eau sont si petits et répandus dans le paysage qu'ils sont souvent négligés. De plus, le manque de considération de ces systèmes écologiques réside dans le fait qu'un manque de compréhension évident persiste vis-à-vis de ce que ces cours d'eau de tête de bassin offrent en termes d'habitat, de services écologiques, et de qualité de l'eau pour l'ensemble des paysages naturels et aménagés.

Du fait d'importantes variations de leurs caractéristiques physico-chimiques, géomorphologiques et biotiques, les cours d'eau de tête de bassin, sont d'étonnants pourvoyeurs d'habitats pour une multitude d'espèces remarquables. Parce que la surface de leurs bassins versants est de dimensions réduites et qu'ils peuvent ainsi être facilement influencés par des différences à petite échelle, les ruisseaux de tête de bassin sont sans doute les plus variés de tous les écosystèmes d'eau courante. Ils offrent un étonnant éventail d'habitats pour les compartiments végétal, animal et microbien, cependant leurs petites tailles les rend particulièrement sensibles aux perturbations. En dépit de leurs abondances dans le paysage, de leurs importances en termes d'habitat et qu'ils soient à l'origine de la ressource en eau, le fonctionnement des cours d'eau de tête de bassin n'a longtemps suscité que peu d'intérêts à l'égard des gestionnaires et des chercheurs (Meyer & Wallace 2001).

Malgré leurs contributions uniques et leurs importances dans le maintien de la diversité et de l'intégrité fonctionnelle des réseaux hydrographiques dans leurs ensembles, ils sont en permanence sous la menace d'activités humaines (Meyer & Wallace 2001). La littérature décrivant les assemblages biologiques et le fonctionnement écologique des ces systèmes regorge malheureusement d'exemples d'espèces menacées par des activités anthropiques. Les menaces comprennent l'exploitation des eaux souterraines (Hubbs 1995), les activités modifiant le paysage telles que l'agriculture, l'exploitation forestière, l'exploitation minière, et l'urbanisation qui dégradent et sont à l'origine de la disparition des ces écosystèmes d'eau courantes (Meyer & Wallace 2001). L'ensemble de ces cours d'eau est donc extrêmement vulnérable aux effets des activités humaines. L'impact cumulé de l'ensemble des dégradations au niveau des cours d'eau de tête de bassin contribue à la perte de l'intégrité écologique des écosystèmes situés en aval. La dégradation des habitats de ces cours d'eau et la perte de connectivités avec ceux d'ordres supérieurs ont des conséquences dramatiques non seulement pour les espèces inféodées à ces petits systèmes écologiques, mais aussi pour la diversité des écosystèmes situés en aval et riverains. À bien des égards, l'intégrité biologique des réseaux hydrographiques entiers peut être largement tributaire de l'impact individuel et cumulatif se produisant dans l'ensemble de ces petits cours d'eau constituant leurs sources.

Une différence évidente qui distingue les cours d'eau de tête de bassin des cours d'eau d'ordres supérieurs est le rapport particulièrement élevé entre le linéaire de ces systèmes et la surface des écosystèmes adjacents (*e.g.*, Vannote *et al.* 1980 ; Gomi *et al.* 2002). Les cours d'eau de tête de bassin interagissent très fortement avec les écosystèmes terrestres environnants. Leur forte dépendance vis-à-vis des apports de matière organique allochtone (Richardson *et al.* 2005), réside dans le fait que le couvert forestier réduit considérablement la qualité et la quantité des rayonnements solaires disponibles pour les producteurs primaires aquatiques dans ces écosystèmes (Hill *et al.* 1995 ; Kiffney *et al.* 2003 ; 2004). Les cours d'eau forestiers reçoivent de grandes quantités de matières organiques sous forme de feuilles mortes, de bois de petites et grandes tailles, de graines, de fleurs, provenant du couvert végétal environnant. Ainsi, ces écosystèmes d'eau courante sont connus pour être largement tributaires des apports énergétiques allochtones provenant des communautés riveraines (Bilby & Bisson 1992).

Aussi, il a été démontré que les cours d'eau forestiers pouvaient recevoir jusqu'à 99% de leurs sources de carbone organique par le biais des apports de la végétation riveraine (Fisher & Likens 1972 ; Webster & Meyer 1997). Plus généralement, la contribution relative de la matière organique à la base énergétique des réseaux trophiques des cours d'eau de tête de bassin est proportionnel au degré de fermeture de la canopée, ce qui dans le cas des petits ruisseaux forestiers est souvent complète.

La plupart des apports annuels de litière se font à l'automne, en particulier ceux provenant des arbres caducifoliés, mais certaines espèces d'arbres à feuillage persistant renouvèlent constamment leurs feuilles. Ainsi, sous des latitudes moyennes (35°-60°), les cours d'eau forestiers reçoivent en moyenne 570 g m^{-2} an^{-1} (d'après Benfield 1997). Ces quantités relativement importantes de matières organiques induisent une prépondérance des consommateurs (déchiqueteurs et collecteurs) de matière organique associée à ces écosystèmes, et l'abondance de ces consommateurs diminue à mesure que l'on se dirige vers l'aval (Haggerty *et al.* 2002).

Les taux de transformation de la litière peuvent varier énormément entre les espèces, ce qui s'explique en partie par de grandes différences qualitatives dans la chimie et la structure physique des litières. Ces différences qualitatives affectent considérablement leurs valeurs en tant que ressource alimentaire pour les invertébrés déchiqueteurs. Ainsi, la teneur en nutriments et la teneur en substances réfractaires telles que lignine et tannins sont deux éléments essentiels permettant d'estimer la qualité intrinsèque de la litière (Webster & Benfield 1986 ; Gessner & Chauvet 1994 ; Ostrofsky 1997 ; Driebe & Whitam 2000 ; Lecerf & Chauvet 2008).

Les organismes impliqués dans le processus de décomposition

Les hyphomycètes aquatiques

A ce jour, près de 2000 espèces de champignons ont été signalés, associés à des substrats en décomposition immergés, sous forme de spores dans l'eau, de parasites de plantes ou d'animaux aquatiques, ou encore associé à des macrophytes en décomposition dans les zones humides.

Dans l'ensemble, les champignons sont considérés comme l'un des plus grands groupes d'eucaryotes sur la terre, en second lieu après les insectes, avec à ce jour seulement environ 5% des espèces décrites. Par conséquent la diversité des champignons dans les eaux douces, est susceptible d'être sensiblement supérieure aux 2000 espèces mentionnées ci-dessus. Des représentants de tous les grands groupes taxonomiques de champignons (Chytridiomycètes, Zygomycètes, Ascomycètes, et Basidiomycètes) ont été observés, isolés, et /ou décrit dans les écosystèmes dulçaquicoles.

Les champignons les plus importants écologiquement, et étant par ailleurs les mieux étudiés dans les cours d'eau sont les hyphomycètes aquatiques, qui sont des anamorphes (formes asexuées) des Ascomycètes et Basidiomycètes.

Ces champignons sont capables de réaliser la totalité de leur cycle de vie asexué dans l'eau, à partir de la colonisation d'un substrat approprié suivi ensuite de l'invasion de la matrice foliaire par la croissance du mycélium et d'une abondante libération de conidies (*i.e.*, spores asexuées). Les conidies d'hyphomycètes aquatiques sont souvent tétraradiées, diversement ramifiées ou filiformes. La forme inhabituelle de leurs conidies est une adaptation à la dispersion dans l'eau courante, et cela renforce également la probabilité d'attachement à leurs substrats (*i.e.*, feuilles mortes et débris ligneux provenant de la végétation riveraine) (Webster 1987 ; Webster & Descals 1981 ; Webster & Davey 1984). Une fois que les conidies sont entrées en contact avec le substrat, la sécrétion de mucilage, le développement des appressoriums, et la germination rapide (*i.e.*, en quelques heures) permettent de sécuriser leurs attachements et d'assurer la colonisation au nouveau substrat. Le mycélium se développe alors essentiellement dans la matrice foliaire, et enfin, donne lieu à des conidiophores (structure portant les conidies). Une fois libérées, les conidies sont transportées par le courant afin de coloniser de nouveaux substrats. Les conidies peuvent également être emprisonnées et concentrées dans l'écume à l'interface eau-atmosphère (neuston) où ils peuvent survivre pendant des semaines. En automne, lorsque des feuilles ou des branches tombent dans les cours d'eau, ils sont immédiatement colonisés par les conidies du neuston ou par des spores en suspension dans la colonne d'eau. Jusqu'à 80% de la production de champignons peut être investie dans la production de conidies (Suberkropp 1991).

De ce fait, ces champignons aquatiques sont les principaux colonisateurs de la litière d'origine végétale dans les cours d'eau forestier. Les maxima de la biomasse mycélienne et des taux de sporulation des hyphomycètes aquatiques sont assez bien corrélés avec les taux de décomposition de la litière (Gessner & Chauvet 1994 ; Niyogi *et al.* 2003), suggérant que ce groupe de champignons aquatiques est aussi un des principaux décomposeurs de cette matière organique allochtone. Ces derniers possèdent des capacités enzymatiques pour dégrader les constituants majeurs de la litière végétale. Les exo-enzymes produites par ces champignons sont des enzymes qui hydrolysent la cellulose (endo-et exoglucanases, exoglucosidase) et les hémicelluloses, pectine (polygalacturonase et pectine-lyase), les protéines et les lipides. La biomasse fongique représente de 95 à plus de 99% du total de la biomasse microbienne (bactéries et champignons) associées à la litière en décomposition dans les cours d'eau (Baldy & Gessner 1997 ; Gulis & Suberkropp 2003, Hieber & Gessner 2002). Par ailleurs, la production des champignons aquatiques est généralement supérieure à celle des bactéries (Baldy *et al.* 2002 ; Weyers & Suberkropp 1996). La production annuelle de ces derniers sur la base de bilans réalisés à l'échelle de petits cours d'eau peut être comparable ou supérieure à la production secondaire des invertébrés (Methvin & Suberkropp 2003 ; Suberkropp 1997). Les champignons aquatiques transforment la litière végétale en carbone organique, convertissant ce dernier en leur propre biomasse, ce qui peut représenter jusqu'à 18-23% de la masse totale détritique (Methvin & Suberkropp 2003 ; Suberkropp 1995).

Ainsi, au cours de la décomposition de la litière végétale, les hyphomycètes aquatiques sont impliqués dans une variété de processus qui aboutissent à la conversion du carbone organique végétal en biomasse fongique, et en CO_2 en raison de leurs activités respiratoires (Gessner *et al.* 1997). En outre, la décomposition fongique de la litière végétale, ainsi que les activités d'alimentation des consommateurs détritivores sur cette litière végétale "conditionnée" facilite l'exportation de carbone végétal sous forme de matière organique particulaire fine (incluant les conidies) ou de matière organique dissoute disponibles pour d'autres organismes des cours d'eau. Le "conditionnement" de la litière végétale du fait de la colonisation fongique augmente sa valeur nutritive et sa palatabilité pour les invertébrés déchiqueteurs.

En effet, les invertébrés déchiqueteurs aquatiques préfèrent se nourrir sur des litières préalablement colonisées par des hyphomycètes aquatiques comparé à des feuilles non colonisées (Bärlocher 1985 ; Suberkropp 2003). De plus, il a été démontré que la fitness de ces mêmes invertébrés étaient significativement améliorés (Chung & Suberkropp 2009). Par conséquent, en dehors de leur rôle direct dans la décomposition de la matière organique, les hyphomycètes aquatiques jouent également un rôle déterminant en tant que médiateur dans les transferts d'énergie et de nutriments vers les niveaux trophiques supérieurs (Bärlocher & Kendrick 1976). Malgré leur rôle clé dans le fonctionnement des écosystèmes lotiques, les champignons aquatiques, ont historiquement reçu beaucoup moins d'attention que les macroinvertébrés, les algues, les poissons, ou encore les bactéries (Gulis *et al.* 2009). Ces champignons n'ont été découverts en tant que groupe écologique distinct que dans le milieu du siècle dernier (Ingold 1942). Les chercheurs étudiant le fonctionnement écologique des cours d'eau forestier ont reconnu l'importance de ces champignons il y a une quarantaine d'années (Bärlocher & Kendrick 1974 ; Kaushik & Hynes 1968 ; Suberkropp & Klug 1976). Plusieurs articles résumant les avancées dans le domaine ont depuis été publiés (Bärlocher 1992 ; Gessner *et al.* 2003 ; Gessner & Newell 2002 ; Gessner *et al.* 1997 ; Suberkropp 1992).

Les activités fongiques associées à la litière végétale sont contrôlées par les caractéristiques du substrat, les caractéristiques environnementales, et les interactions biotiques. Le type de litière, et plus précisément, les concentrations en lignine, azote et phosphore peuvent exercer un fort contrôle sur la croissance et la reproduction des hyphomycètes aquatiques. La lignine est difficile à dégrader par voie enzymatique en raison de sa structure réfractaire. Ainsi, la litière végétale constituée de carbone de mauvaise qualité (*i.e.*, importante concentration en lignine) induit une faible activité fongique et se décompose lentement.

Au contraire, d'importantes concentrations en azote et phosphore dans la litière stimulent souvent l'activité des hyphomycètes aquatiques. Ainsi, pour un substrat donné, leurs activités dépendent en grande partie de l'interaction de ces facteurs intrinsèques (*e.g.*, les rapport entre la concentration en lignine et celles des nutriments). Par ailleurs, l'activité fongique est également affectée par les concentrations en azote et phosphore inorganique dissous dans les cours d'eau, car ces champignons sont capables d'assimiler l'azote et le phosphore provenant à la fois du substrat et de la colonne d'eau.

Des d'études sur le terrain et en laboratoire ont montré que dans des conditions d'approvisionnement limitées en azote et en phosphore des champignons via le substrat ou la colonne d'eau (*e.g.*, dans des cours d'eau quasiment dépourvus en nutriments), de petites augmentations en nutriments inorganiques dissous induisent une augmentation considérable de la biomasse fongique, de la production et la reproduction, et par conséquent, une accélération de la décomposition des litières. Plus généralement, l'eutrophisation des écosystèmes lotiques conduit souvent à la stimulation de l'activité des champignons, à une décomposition plus rapide des litières, et par conséquent, à une réduction des ressources disponibles (*i.e.*, en quantité et dans le temps) pour les niveaux trophiques supérieurs (*e.g.*, les invertébrés déchiqueteurs). Outre les éléments nutritifs dissous, d'autres paramètres chimiques, tels que le pH, l'alcalinité, l'oxygène dissous, les polluants influencent également l'activité fongique dans les cours d'eau. La pollution organique peut nuire à la diversité des champignons et à leurs activités en limitant la concentration en oxygène dissous dans l'eau (Medeiros *et al.* 2009).

Les effluents de mines et les concentrations élevées en métaux lourds dans l'eau réduisent généralement la diversité des hyphomycètes aquatiques. Cependant, certaines espèces d'hyphomycètes aquatiques semblent pouvoir résister à de fortes concentrations en métaux lourds durant les épisodes de pollutions chroniques et semblent plus tolérants que les invertébrés aquatiques.

La décomposition des litières dans ces cours d'eau est généralement lente, et est probablement le reflet de la diminution en abondance et en diversité des invertébrés déchiqueteurs. Par ailleurs, l'élévation de la température des cours d'eau affecte positivement la croissance fongique et l'activité de décomposition de la litière (Ferreira & Chauvet 2010). Par conséquent, l'augmentation des températures à l'échelle planétaire, comme prévu à partir de scénarios de changement climatique pourrait mener à une activité fongique accrue résultant en une augmentation des taux de décomposition de la litière. Ainsi, ces réponses pourraient avoir des conséquences négatives à l'échelle des niveaux trophiques supérieurs comme mentionné précédemment pour les effets des nutriments (Ferreira & Chauvet 2010). Enfin, la diversité de la végétation riveraine a un effet positif, mais assez limité sur les communautés d'hyphomycètes aquatiques en affectant la diversité des ressources disponibles pour ces derniers.

Les interactions biotiques avec d'autres organismes des cours d'eau incluent principalement celles avec les bactéries et les invertébrés déchiqueteurs. Ces champignons sont évidemment en concurrence directe avec les bactéries pour les ressources détritiques. Certaines espèces d'hyphomycètes aquatiques produisent des antibiotiques qui limitent ou inhibent la croissance bactérienne (Romani *et al.* 2006). Cependant, les interactions entre les champignons aquatiques et les bactéries sont complexes et encore mal comprises (Romani *et al.* 2006). Les interactions avec les invertébrés déchiqueteurs inclut la concurrence directe pour les ressources, la consommation par les invertébrés, et secondairement l'ingestion directe de spores, qui peut passer intact dans le système digestif des invertébrés facilitant ainsi la dispersion des champignons.

Les invertébrés détritivores

Les invertébrés les plus représentatifs trouvés dans les cours d'eau forestier de tête de bassin sont généralement des insectes de type éphéméroptères, plécoptères, trichoptères, diptères et odonates (Tachet *et al.* 1987). Ces organismes sont généralement assignés à des groupes fonctionnels en fonction du mode d'acquisition de leurs nourritures plutôt que par ce qu'ils mangent (Wallace & Webster 1996 ; Graça 2001). En ce sens, les prédateurs se nourrissent des tissus des autres animaux. Les brouteur-racleurs se nourrissent du biofilm qui recouvre la surface d'éléments et de structures immergées, telles que par exemple des pierres, du bois, de la litière, des tiges et des feuilles de macrophytes. D'autres connus sous le nom de collecteurs s'alimentent à partir de particules fines de matière organique, filtrées à partir de la colonne d'eau ou collectées directement à partir du substrat.

Enfin, un dernier groupe d'organismes, les déchiqueteurs se nourrissent à partir de particules grossières de matière organique (Graça 2001). Ces derniers sont intimement liés à la nature des apports de matière organique provenant des communautés riveraines et à la période où ceux-ci surviennent. Ces invertébrés déchiqueteurs consomment la litière végétale piégée dans le cours d'eau (Cummins 1973 ; 1974 ; Cummins & Wilzbach 1985 ; Merritt & Cummins 1984). Cette dernière s'accumule sur l'ensemble des éléments (*e.g.*, pierres, bois, racines) constituant des obstacles dans les courants, et se dépose dans les mouilles et autres zones de sédimentation (Cummins *et al.* 1980 ; Kaushik & Hynes 1971).

La dépendance des populations d'invertébrés déchiqueteurs des cours d'eau de tête de bassin vis-à-vis des apports de matière organique sous forme de litière végétale est un élément important du River Continuum Concept (Minshall *et al.* 1983 ; Vannote *et al.* 1980).

Comme nous l'avons abordé précédemment, la plupart du temps, les invertébrés déchiqueteurs vont préférentiellement consommer la litière végétale qui a été au préalable conditionnée. Le processus de conditionnement commence généralement dès que la litière intègre le cours d'eau. Ce processus implique le lessivage de matières organiques solubles (polyphénols et polysaccharides) (Nykvist 1962 ; Suberkropp *et al.* 1976 ; Chauvet 1987), qui peut induire jusqu'à 25-30% de perte de masse des litières (Nykvist 1962 ; Petersen & Cummins 1974 ; Gessner & Schwoerbel 1989), suivi par la colonisation des micro-organismes du cours d'eau. Certaines litières peuvent être pré-conditionnées à des degrés divers à la surface du sol avant d'être emportées par le cours d'eau (Merritt & Lawson, 1979). Les invertébrés déchiqueteurs ne sont pas spécialisés sur les litières d'une espèce végétale donnée, mais plutôt sur une litière ayant subi un niveau de conditionnement suffisant indépendamment de l'espèce. Ainsi, les invertébrés déchiqueteurs commencent à se nourrir de la litière lorsque l'activité du compartiment microbien a induit suffisamment de changements au niveau structurel et biochimique des tissus foliaires pour les convertir dans un état qui améliorent considérablement sa digestibilité et sa valeur nutritionnelle. Par ailleurs, des travaux ont également démontré qu'au-delà de l'augmentation de la digestibilité et de la valeur nutritionnelle de la litière induit par les hydrolyses partielles des tissus foliaires et de la destruction de la cuticule par les exo-enzymes fongiques, ce phénomène de facilitation résultait également d'une translocation des enzymes fongiques à l'intérieur de l'appareil digestif des invertébrés (Bärlocher 1982 ; Sinsabaugh *et al.* 1985). Ce temps nécessaire de conditionnement peut varier de quelques semaines à quelques mois selon les espèces végétales considérées et le contexte environnemental. Les invertébrés déchiqueteurs changent continuellement de substrats pour se nourrir sur ceux qui apparaissent les mieux colonisés (Cummins & Klug 1979).

Le compartiment hyporhéique des cours d'eau forestier

La zone hyporhéique: une definition

White (1993): "Despite considerable use of the term, there still is no single conceptual definition or framework that would convey a common and concise meaning to biologists, hydrologists, geomorphologists, and aquatic chemists alike".

La zone hyporhéique peut être conceptuellement définie comme l'espace interstitiel saturé en eau, situé sous le lit du cours d'eau et adjacent à ce dernier, et contenant une certaine proportion d'eau de surface provenant du chenal (White 1993). Cette définition sépare la zone hyporhéique de la zone des eaux souterraines dans le sens où les eaux souterraines représentent l'eau sous la nappe phréatique qui n'a pas encore été influencée par les processus biologique et physico-chimique se produisant dans l'eau de surface.

La définition de la zone hyporhéique telle qu'évoquée par White (1993) est quelque peu différente de celles proposées par Schwoerbel (1961) et Triska *et al.* (1989). Schwoerbel (1961) considère la zone hyporhéique comme étant une zone intermédiaire située entre les eaux de surface provenant du chenal au-dessus et les eaux souterraines dessous. Des précisons à ce concept ont été apportées, au travers notamment des patrons de distribution des invertébrés hypogés et épigés, appelés "hyporheos" par Williams & Hynes (1974). Cette approche idéale fondée sur des considérations écologiques a rapidement montré ses limites. En effet, les patrons de distribution de la faune hypogée et épigée sont souvent amenés à se chevaucher du fait de la complexité des variations spatiale et temporelle des masses d'eau. De plus, la diversité des types de zone hyporhéique (Malard *et al.* 2002) et sa concomitance avec beaucoup d'autres facteurs régissant la distribution des invertébrés hyporhéiques rendent particulièrement peu aisée une définition uniquement basée sur la distribution des organismes mobiles (Danielopol 1989 ; Ward 1989 ; Bretschko 1991 ; Marmonier 1991 ; White 1993 ; Boulton *et al.* 2010). Ce problème a suscité des réactions, et des définitions alternatives ou complémentaires ont été proposées, notamment basées sur des variables physico-chimiques.

Triska *et al.* (1989) ont réalisé des suivis pour tenter de délimiter la zone hyporhéique en se basant sur des différences ayant trait à des variables chimiques entre les eaux de surface et les eaux souterraines. Ainsi, ils définissaient la zone de surface comme étant la région située immédiatement sous le cours d'eau, chimiquement indiscernable de l'eau de surface provenant du chenal et contenant plus de 98% de cette dernière. Sous la zone de surface, ils délimitaient une zone interactive, contenant entre 10 et 98% d'eau de surface, qui fixait la profondeur de la frontière hydrologique du cours d'eau.

Bien qu'ayant une nouvelle fois suscité des critiques (Vervier *et al.* 1992) en raison de critères de catégorisation jugés arbitraires et statiques, cette dernière définition a tout de même permis des applications intéressantes notamment en offrant un meilleur cadre de travail dans lequel des perspectives longitudinales intégrant les caractéristiques hydrauliques du cours d'eau (*e.g.*, pente, porosité du lit du cours d'eau, résistance hydraulique) ont pu être envisagées.

Le concept d'écotone dynamique

Gibert *et al.* (1990) en conceptualisant le rôle de la zone hyporhéique comme un écotone dynamique ont apporté une vision et une dimension nouvelles à la définition de ce compartiment qui faisait jusqu'alors l'objet de débats énergiques (*e.g.*, hyporheic workshop du congrès de la North American Benthological Society de 1991) (Palmer 1993). Le terme "écotone" fait naturellement référence à la dimension spatiale de la zone hyporhéique insérée entre le cours d'eau situé juste au-dessus, et les eaux souterraines en-dessous, et "dynamique" dans le sens où ces mêmes dimensions spatiales sont amenées à fluctuer en réponse aux caractéristiques du sédiment et aux échanges hydrologiques. En associant ces deux termes dans ce nouveau concept, Gibert *et al.* (1990) soulignaient les propriétés d'élasticité, de perméabilité, de biodiversité et de connectivité de la zone hyporhéique, facteurs cruciaux régissant le rôle fonctionnel de cette dernière dans le fonctionnement global des cours d'eau (Fig. 1) (Boulton *et al.* 2010).

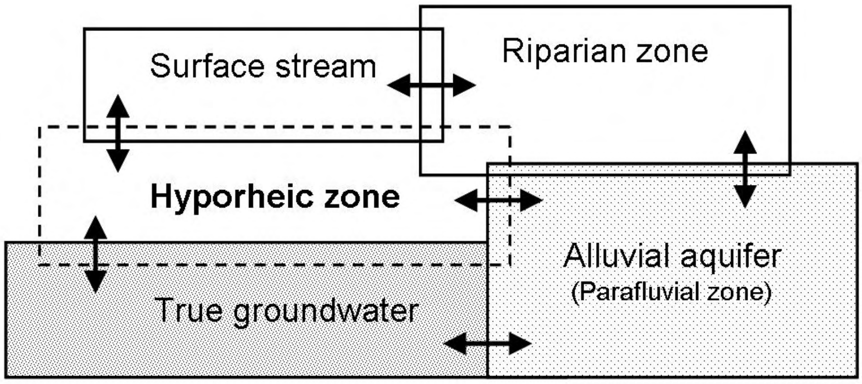

Fig. 1 The central location of the hyporheic zone exchanging water with the surface stream above, true groundwater below, and the riparian zone and alluvial aquifers laterally, (adapted from Boulton 2000).

Le rôle fonctionnel de la zone hyporhéique

Grimm & Fisher (1984) ont été les premiers à faire la démonstration de l'importance du rôle potentiel de la zone hyporhéique dans le métabolisme global des cours d'eau. Leur étude a révélé que la zone hyporhéique contribuait à hauteur de 40 à 50% de la respiration totale de l'écosystème. La portée de ces résultats a incité Grimm et Fisher à suggérer l'extension du traditionnel modèle conceptuel des écosystèmes lotiques à deux dimensions vers un nouveau modèle incluant la contribution de la zone hyporhéique.

La zone hyporhéique agit comme un filtre physique gouverné par les caractéristiques granulométriques du sédiment et la circulation de l'eau, comme un filtre biochimique contrôlé par les processus biologique et chimique, et enfin comme un filtre photique (Boulton *et al.* 2010). Ces caractéristiques influencent des processus fonctionnels clés, tels que la productivité primaire et le recyclage des nutriments dans ces systèmes lotiques. En dépit de l'importance des processus qui ont lieu dans ce compartiment, et bien qu'assez tôt des perspectives de plusieurs auteurs aient attiré l'attention sur la zone hyporéique en suggéreraient son rôle potentiel dans le fonctionnement écologique des cours d'eau (Orghidan 1959 ; Schwoerbel 1964 ; Williams & Hynes 1974), la prise en considération explicite de la connectivité hydrologique verticale dans les modèles conceptuels d'écosystèmes lotiques a tardé à être mise en place par rapport à l'intégration des liens longitudinaux (Vannote *et al.* 1980) et latéraux (Junk *et al.* 1989) plus évidents (*e.g.*, River Continuum Concept, Flood Pulse Concept).

Le compartiment hyporhéique: une zone refuge

Pour certains organismes, l'habitat interstitiel peut être occupé pendant une partie de leurs vies et/ou sous des formes de résistance. Par exemple, les œufs de certains poissons salmonidés se développent à l'intérieur du sédiment. Après l'éclosion, ces poissons juvéniles peuvent aussi trouver refuge dans la zone superficielle du sédiment pendant des périodes de basses eaux, pour regagner les zones de radiers plus tard lorsque le niveau d'eau sera revenu à la normale (Debowski & Beall, 1995). Pour certains insectes (Ephéméroptères, Trichoptères, Plécoptères, Coléoptères ou Diptères), les œufs peuvent éclore et les larves résider dans la zone hyporhéique pendant les premiers stades de développement, tandis que la majeure partie de leurs cycles de vie prédominent dans la zone benthique, ou occasionnellement en hyporhéique (Gray & Fisher ; 1981; Williams 1984 ; Pugsley & Hynes 1985 ; 1986).

Le compartiment hyporhéique peut être une zone refuge pendant des périodes de sécheresse à la surface du sédiment. Cette hypothèse a notamment été discutée par Gagneur & Chaoui-Boudghane (1991) et Boulton & Stanley (1995), et ces derniers ont montré que des organismes survivaient en grand nombre dans les sédiments humides ou saturés du lit du cours d'eau. Alors que le compartiment hyporhéique pour la majorité des insectes est temporairement utilisé comme une zone refuge pour se protéger contre les variations des conditions environnementales des eaux de surface (*e.g.*, augmentation brutale du débit, températures extrêmes, dessiccation, prédateur), pour d'autres la vie dans le milieu interstitiel est prédominante, avec une étape épigée restreinte à la phase d'émergence puis le stade aérien de l'adulte (Berthelemy 1968 ; Stanford & Ward 1988).

Facteurs limitants de la zone hyporhéique

La structure du sédiment

La nature de l'habitat dans le sédiment est déterminée par un ensemble de facteurs hiérarchisés (Fig. 2). Les sédiments détermine l'environnement du cours d'eau et correspond précisément à l'habitat des communautés interstitielles. La porosité est la relation entre le volume d'eau contenu dans un échantillon de sédiment et le volume total de l'échantillon.

La taille des interstices et leurs connexions sont des facteurs écologiques déterminants. Généralement, à mesure que le diamètre diminue, le grain de sédiment se rapproche de plus en plus d'une forme globuleuse (Zingg 1935), les quartzites font cependant exception à la règle et présentant des formes irrégulières. Par conséquent et pour des raisons géométriques, bien que la taille des pores soit petite dans les sédiments fins, la quantité d'eau y est élevée. La majorité de l'eau contenue dans le sédiment est liée à la grande surface offerte par les petits grains de sédiment (Leichtfried 1985), ainsi la consistance de la matrice devient de plus en plus fluide (Schlieper 1972), au moins dans ses couches superficielles. Les organismes benthiques peuvent ainsi s'y mouvoir en déplaçant les sédiments et en creusant à travers eux.

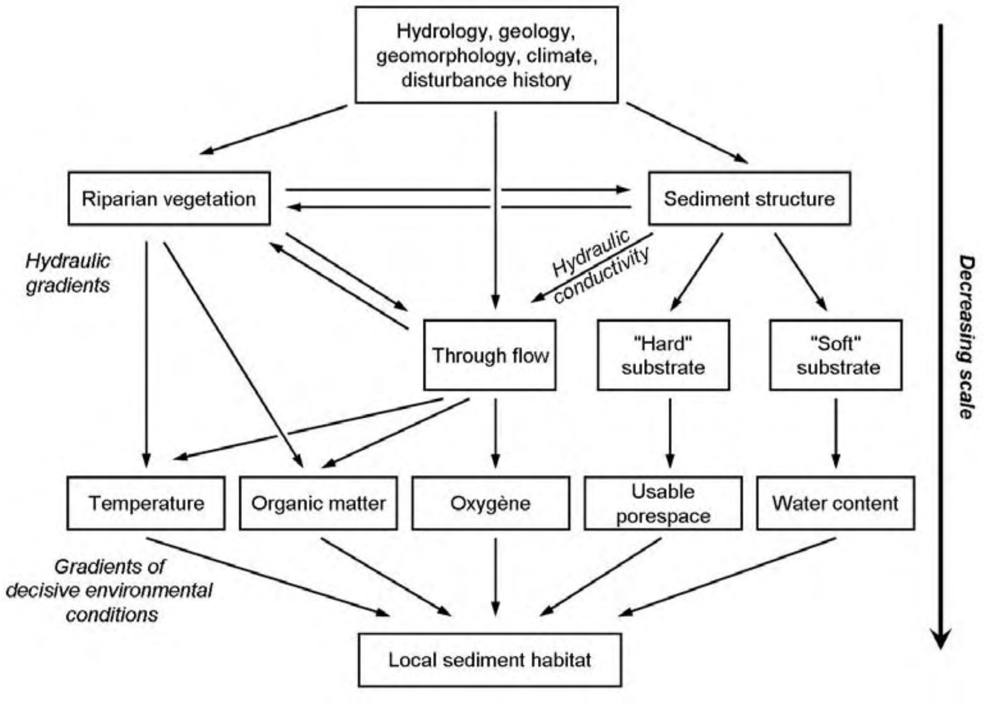

Fig. 2. Hierarchical conceptualisation of factors controlling local sediment habitat conditions (adapted from Ward *et al* 1998).

Par ailleurs, les pierres, cailloux et graviers tendent à être arrondis et globuleux (Bretschko 1994). Du fait de la géométrie de ces matériaux, les espaces interstitiels sont relativement larges, cependant la quantité d'eau y est inférieure à celle contenue dans les sédiments fins (Bretschko & Klemens 1986).

La majorité de l'eau transite librement à travers l'espace interstitiel et adhère très peu aux surfaces sédimentaires, du fait de l'importante diminution de la surface de contact avec l'augmentation du diamètre des matériaux. La perméabilité y est élevée et les organismes interstitiels ne peuvent ni modifier la structure du sédiment ni la taille de ses pores. Toutefois, ces derniers peuvent nager à travers les interstices de grandes dimensions, ou s'accrocher et ramper à la surface des matériaux de la même manière qu'ils le font à la surface du lit du cours d'eau.

L'oxygène dissous

L'oxygène dissous est un paramètre environnemental clé dans la zone hyporhéique. Il est utilisé pour la respiration de la plupart des organismes et de nombreuses bactéries, ainsi sa raréfaction dans l'environnement produit des changements qualitatifs et quantitatifs notables au niveau de l'assemblage de ces organismes. Les concentrations en oxygène dissous dans le compartiment hyporhéique dépendent de la perméabilité, de la porosité des sédiments, de la saturation des espaces interstitiels par l'eau et de l'intensité de la respiration des microorganismes du sédiment. Cette dernière est liée à l'activité bactérienne, qui dépend à son tour de la disponibilité en carbone assimilable présent dans la matrice sédimentaire. Les sédiments à faible porosité et perméabilité présentent généralement de faibles concentrations en oxygène dissous. En revanche, les concentrations en oxygène dissous sont souvent plus élevées dans des sédiments grossiers et homogènes, telles que rapportées par Bretschko (1991) et Stanford *et al.* (1994) qui trouvaient des concentrations en oxygène dissous relativement élevées dans la zone hyporhéique de cours d'eau de montagne. Ainsi, ils suggéraient que ce sont les forts écoulements en subsurface qui approvisionnent en eau relativement bien oxygénée le compartiment hyporhéique.

Un tel contexte est relativement favorable à l'installation d'assemblages biologiques diversifiés dans la zone interstitielle, il peut même permettre à des invertébrés épigés d'évoluer en profondeur dans la zone hyporhéique. Cependant, les concentrations en oxygène dissous dans les sédiments ne sont souvent pas uniformes.

Ainsi, les zones proches des eaux de surface sont généralement bien oxygénées, alors que les concentrations diminuent à mesure que l'eau descend dans la matrice sédimentaire (Marmonier & Dole 1986 ; Findlay *et al.* 1993), et cela, principalement en raison de la respiration au sein des sédiments (Chapelle 1993).

En conséquence, les patrons de distribution de la faune se composent essentiellement d'espèces oxyphiles dans les sédiments superficiels tandis que les niveaux de sédiments inférieurs présenteront plutôt des espèces tolérantes à de faibles concentrations en oxygène dissous (Danielopol *et al.* 1994 ; Plénet *et al.* 1995). Enfin, il est à noter que ces patrons généraux de distribution des organismes au sein de la zone hyporhéique sont soumis à des fluctuations spatio-temporelles liées entre autres, aux gradients longitudinaux (*i.e.*, zones d'upwelling et de downwelling) et latéraux des cours d'eau, aux variations hydrologiques saisonnières, à des évènements météorologiques extrêmes.

Flux de la matière organique dans les cours d'eau forestier

Dans les cours d'eau hétérotrophes, la matière organique fournit l'énergie qui régit les cycles des nutriments dans les écosystèmes. Selon les taux de minéralisation des différents composés organiques, et des divers mécanismes de transport, les sites de production et de minéralisation peuvent différer dans l'espace et dans le temps. Cette séparation est particulièrement visible dans les cours d'eau de tête de bassin, dans lesquels la production autotrophe est généralement inférieure à la demande d'énergie de la communauté de consommateurs (Fisher & Likens 1973 ; Minshall *et al.* 1985). Le River Continuum Concept est en grande partie basé sur cette dépendance des apports allochtones de matière organique (Vannote *et al.* 1980).

La matière organique est transportée au sein de la zone hyporhéique à la fois sous ses formes dissoute (Findlay *et al.* 1993 ; Jones *et al.* 1995) et particulaire, cependant très peu de travaux se sont attachées à étudier cette dernière. Moser (1991) a mesuré les apports de matière organique particulaire dans le sédiment d'un cours d'eau de montagne (Autriche). Il a estimé qu'en moyenne presque 2g TOC j^{-1} m^{-2} était importé, ce qui représente environ 6 fois les apports dans le chenal via les flux verticaux et latéraux.

Ces valeurs qui peuvent paraître erronées tiennent au fait que la matière organique particulaire est souvent piégée puis libérée de la zone hyporhéique lorsque celle-ci est constituée de sédiments fins (*i.e.*, sable et gravier), phénomène qui d'ailleurs est transposable à la matière organique dissoute. Cette suggestion est par ailleurs étayée par les rapports C:N de la matière organique piégée, qui étaient significativement plus faibles que ceux de la matière organique provenant des apports par le chenal. Le transport de la matière organique s'accentue avec l'augmentation des débits, et les quantités de matière organique perdues par dérive en aval sont étroitement liées à la capacité de rétention du cours d'eau.

Par ailleurs, gardons à l'esprit qu'une proportion de la matière organique dérivant en aval, et donc perdue pour le site en question, peut par la suite subir une redistribution latérale et/ou verticale. A l'heure actuelle, la dynamique des apports, du stockage et de la décomposition de la matière organique particulaire dans les sédiments des cours d'eau reste très peu connue (Maridet *et al.* 1996). Une importante proportion de la matière organique particulaire entrant dans les cours d'eau au moment de l'abscision des feuilles, peut être enfouie et ainsi immobilisée dans la zone hyporhéique, suite à des mouvements de sédiments ou lors de crues ou de tempêtes (Herbst 1980 ; Metzler & Smock 1990 ; Naegeli *et al.* 1995). Ainsi, ce compartiment peut potentiellement être un site important de stockage de matière organique. Des comparaisons entre les deux compartiments ont révélé que la zone hyporhéique pouvait immobiliser de 25 à 82% de la totalité de la matière organique stockée dans le cours d'eau (Cummins *et al.* 1983 ; Metzler & Smock 1990 ; Smock 1990 ; Jones 1997 ; Jones *et al.* 1997).

Les champignons de la zone hyporhéique

A ce jour, quelques études seulement se sont intéressées à la présence, l'abondance et la diversité des champignons dans le compartiment hyporhéique des écosystèmes d'eau courantes (Bärlocher & Murdoch 1989 ; Smith & Lake 1993 ; Naamane *et al.* 1999 ; Bärlocher *et al.* 2006 ; 2008). Cependant, un manque évident persiste quant à la compréhension de leurs rôles, leurs activités, leurs dynamiques dans la zone hyporhéique.

Toutefois, il est suggéré que ces derniers jouent un rôle important dans la décomposition de la matière organique particulaire immobilisée dans le sédiment des cours d'eau, de la même manière qu'ils le font à la surface du sédiment.

Dans ce contexte, la décomposition de la matière organique par les champignons au sein du compartiment hyporhéique peut être une voie significative dans le processus de minéralisation dans les cours d'eau. Bien que ce processus ait été très largement étudié à la surface du sédiment (Kaushik & Hynes 1971 ; Webster & Benfield 1986 ; Suberkropp 1998), il demeure virtuellement inconnu dans la zone hyporhéique. Ainsi, des études comparatives relativement simples quant à la production et l'activité des champignons au-dessus et en dessous des sédiments pourraient être très informatives et permettraient une meilleure compréhension du rôle et de la contribution des champignons du compartiment hyporhéique à l'échelle globale du fonctionnement écologique des cours d'eau.

Les invertébrés de la zone hyporhéique

Les espaces interstitiels entre les particules de sédiments dans la zone hyporhéique de nombreux ruisseaux et rivières sont occupés par une grande diversité d'invertébrés aquatiques, appelée "hyporheos" (Orghidan 1959 ; Williams & Hynes 1974). L'hyporheos comprend de nombreux types d'organismes tels que des crustacés, rotifères, acariens aquatiques, et les stades juvéniles la plupart des insectes aquatiques (Williams 1984 ; Stanford & Ward 1988 ; Danielopol 1989 ; Hakenkamp & Palmer 1999 ; Boulton 1999). Le biofilm constitue une source importante de l'alimentation de la faune interstitielle (Bärlocher & Murdoch 1989 ; Williams 1993 ; Brunke & Gonser 1997 ; Boulton 1999). Par conséquent, les facteurs influençant le développement, la croissance, la composition du biofilm sont précisément les facteurs qui pèseront sur les patrons de répartition des invertébrés brouteur/racleur (Dole-Olivier & Marmonier 1992 ; Boulton 1993 ; Gibert *et al.*1994 ; Strayer 1994 ; Boulton 1999). Des études ont démontré que leurs activités d'alimentation pouvaient stimuler la production du biofilm (Boulton 1999) et qu'ils pouvaient également être indirectement impliqués dans la décomposition des particules de matière organique immobilisées dans le sédiment en régulant la productivité microbienne.

La densité des invertébrés dans la zone interstitielle peut être très importante [>3000 individus par litre (Strayer *et al.* 1997)]. La plupart d'entre eux appartiennent à la méiofaune, c'est-à-dire qu'ils mesurent moins de 1 mm au stade adulte (Hakenkamp & Palmer 1999 ; Palmer & Strayer 1996). Leur petite taille et leur taux de reproduction élevés suggèrent qu'ils occupent un rôle important à l'échelle des sédiments. Cette méiofaune peut constituer une ressource alimentaire pour les invertébrés prédateurs de la zone hyporhéique, ceux à la surface du sédiment mais également pour les poissons (Palmer & Strayer 1996).

Objetcifs et organisation de la thèse

Le compartiment hyporhéique, en dépit de son indéniable contribution dans le fonctionnement écologique des écosystèmes d'eau courante à longtemps été négligé. Toutefois, la persévérance d'une poigné de chercheurs a permis son intégration dans le modèle conceptuel des écosystèmes lotiques. L'appréhension du fonctionnement écologique des cours d'eau dans trois dimensions a conduit à une vision intégrative de la complexité de ces systèmes. Cependant, peu d'études à l'heure actuelle se sont attachées à intégrer ce compartiment dans la compréhension du fonctionnement des cours d'eau forestier de tête de bassin.

Ainsi, au cours de cette thèse, je me suis principalement attaché à tester expérimentalement diverses hypothèses quant au processus de décomposition des litières dans le compartiment hyporhéique et à la contribution et les interactions des principaux agents impliqués dans ce processus, et cela, mené à différents degrés de complexité et en considérant différents niveaux trophiques. J'ai en particulier cherché à étudier les mécanismes biotiques et abiotiques impliqués dans le processus de décomposition des litières dans la zone hyporhéique, en l'insérant dans une vision intégrée à l'échelle du cours d'eau. Cette démarche m'a amené à apporter des éléments de réponses aux questions suivantes :

1. Comment les contraintes de la zone hyporhéique peuvent-elles influencer la composition et la structure des communautés d'hyphomycètes aquatiques et d'invertébrés déchiqueteurs au sein de ce compartiment, et en retour le processus de décomposition des litières ?

2. Comment les contraintes abiotiques du compartiment hyporhéique, et les modifications du compartiment microbien qui en résultent, peuvent-elles affecter la qualité de la matière organique particulaire, et par conséquent l'efficacité des transferts trophiques au sein des cours d'eau forestiers ?

3. Dans quelle mesure et par quels mécanismes, une perturbation d'origine anthropique affecte-t-elle le processus de décomposition des litières dans la zone hyporhéique de cours d'eau forestiers ? Ce compartiment ne constitue-t-il pas une zone refuge pour les communautés de décomposeurs face à ce type de phénomène ?

Ces questions sont très vastes, et aucune d'entre-elles n'a donc pu être traitée de façon exhaustive au cours de ces travaux. Toutefois, un nombre significatif d'éléments de réponse ont pu être apportés, permettant de formuler plusieurs hypothèses quant aux mécanismes biotiques et abiotiques régissant en partie le processus de décomposition des litières dans la zone hyporhéique de cours d'eau forestiers et, plus généralement quant à la contribution de ce compartiment dans le fonctionnement écologique de ces écosystèmes d'eau courante. Ces hypothèses, leur fondement ainsi que les principaux résultats vont être présentés dans la suite de ce document, et s'articulent en cinq chapitres présentés dans le corps de ma thèse, et d'une partie située en annexe.

La première partie de mon travail de thèse, qui est le point de départ de l'ensemble des réflexions qui ont alimenté mes recherches, a été consacrée à l'estimation des stocks de matière organique particulaire grossière (CPOM) situés à la surface du sédiment et au sein du compartiment hyporhéique de trois cours d'eau forestier de tête de bassin de la Montagne noire (Sud-Ouest de la France), volontairement sélectionnés sur la base de profils granulométriques contrastés. Les estimations des CPOM ont été réalisées à deux dates d'échantillonnages, la première à l'automne, juste après l'abscission des feuilles et la seconde au début du printemps. Cette étude préliminaire a permis d'évaluer la contribution relative du compartiment hyporhéique dans le stockage de la matière organique d'origine végétale constituant la base énergétique des réseaux trophiques des cours d'eau de tête de bassin.

Dans la seconde partie, j'ai mesuré l'effet de l'enfouissement de la litière dans le compartiment hyporhéique au travers de l'étude de son processus de décomposition et de l'activité, la composition et la structure des assemblages de décomposeurs associés à cette litière.

Une approche sous forme de bilan de carbone a notamment permis d'identifier la contribution relative des deux principaux agents impliqués dans le processus de décomposition de la litière, à savoir les hyphomycètes aquatiques et les invertébrés déchiqueteurs.

La troisième partie a permis de tester à partir d'approches expérimentales conduites sur le terrain puis au laboratoire, deux des principaux mécanismes identifiés comme régissant la composition et la structure des communautés d'hyphomycètes aquatiques de la zone hyporhéique :

- le criblage physique des conidies par la matrice sédimentaire,
- le criblage physiologique en raison du stress hypoxique dans les sédiments,

et par conséquent leurs implications potentielles directes dans le fonctionnement écologique de ces derniers.

La quatrième partie présente une expérience de terrain conduite en collaboration avec Michael Danger, alors post-doctorant dans notre laboratoire. L'objectif de cette étude visait à déterminer dans quelle mesure la localisation de la litière dans le cours d'eau (*i.e.*, à la surface ou enfouie dans le sédiment) et l'historique d'enfouissement affectent l'assemblage microbien associé à la litière et, par conséquent, la décomposition de cette dernière. Nous avons testé les hypothèses selon lesquelles (1) l'enfouissement dans la zone hyporhéique influence la diversité de l'assemblage microbien associé aux feuilles en décomposition et (2) diminue le taux de décomposition de la litière du fait de la diminution de la palatabilité et de la qualité du matériel foliaire, et (3) le devenir de la litière dans le cours d'eau dépend de l'historique de colonisation microbienne. Nous avons également testé l'hypothèse selon laquelle une colonisation microbienne plus importante, en augmentant la qualité de la litière végétale, pourraient améliorer la fitness des consommateurs et par conséquent l'efficacité des transferts trophiques aux niveaux supérieurs.

Dans ce contexte, les ratios stœchiométriques peuvent être importants, en particulier le ratio C:P dans des systèmes écologiques dépourvus en phosphore.

Enfin, dans la cinquième partie, mes investigations ont porté sur les effets de l'acidification anthropique vis-à-vis du processus de décomposition des litières dans la zone hyporhéique de cours d'eau forestier. Cette expérience de terrain a été menée en collaboration avec Pierre Gierlinski et Hugues Clivot, tous deux doctorants dans le laboratoire LIEBE de l'Université Paul Verlaine de Metz. Cette étude, basée sur un ensemble de cinq cours d'eau forestiers situés le long d'un gradient d'acidité, a permis de tester l'hypothèse selon laquelle la décomposition des litières serait relativement moins affectée par le processus d'acidification dans la zone hyporhéique qu'à la surface du sédiment.

Finalement, l'annexe 1 est une étude consacrée à l'évaluation des effets d'interactions trophiques et non-trophiques impliquant des prédateurs (top-down effect) sur le processus de décomposition des litières, eux-mêmes contrôlés à la base par la qualité de la ressource (bottom-up effect). L'ensemble des études précédemment présentées m'a permis de constater que les interactions biotiques dans leur ensemble étaient particulièrement limitées dans le compartiment hyporhéique, du fait de contraintes physiques évidentes. Ainsi, seule une partie des consommateurs peuvent avoir accès à la ressource immobilisée dans la zone hyporhéique, et les prédateurs sont pour l'essentiel naturellement exclus de cet habitat. Toutefois, les différents signaux chimiques libérés par les prédateurs peuvent quant à eux parvenir jusqu'aux consommateurs de litière situés quelques centimètres sous la surface du sédiment, et ainsi modifier l'activité de ces derniers. De telles interactions sont susceptibles d'altérer les fonctions de l'écosystème assurées par les consommateurs primaires (à la fois invertébrés déchiqueteurs et hyphomycètes aquatiques). Cette annexe présente une partie des résultats d'une étude menée en collaboration avec Jérémy Jabiol également doctorant dans notre laboratoire.

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Chapitre 1:

Organic matter storage in the hyporheic zone of headwater streams

1. Organic matter storage in the hyporheic zone of headwater streams

1.1. Résumé

La matière organique particulaire allochtone à la surface du sédiment est une composante essentielle du fonctionnement des cours d'eau forestiers de tête de bassin. A la base du réseau trophique de ces écosystèmes, les litières végétales (i.e., feuilles mortes, brindilles et bois) provenant des communautés végétales riveraines constituent la principale source de carbone et d'énergie disponible pour les communautés microbiennes et de macroinvertébrés. Les travaux traitant de la dynamique de la matière organique dans les cours d'eau se sont principalement attachés à décrire ces processus à la surface du sédiment, réduisant ces écosystèmes lotiques à un compartiment unique.

L'intégration de la zone riveraine dans le modèle conceptuel du fonctionnement des écosystèmes d'eau courante comme un second compartiment, et non plus comme une simple source de matière organique, a permis une vision plus intégrative de la dynamique de la matière organique dans les cours d'eau. Enfin, bien que l'importance générale d'un troisième compartiment, la zone hyporhéique, ait été notée assez tôt, l'importance des processus associés à ce compartiment en lien avec le fonctionnement du cours d'eau a longtemps été négligée. Des études suggèrent que le stockage dans la zone hyporhéique peut être supérieur à celui à la surface du sédiment, du fait de taux de décomposition de la matière organique enfouie relativement faible, et d'une plus faible probabilité d'être transportée vers l'aval en période de crue. Ainsi, la compréhension de la dynamique de la matière organique allochtone est nécessaire afin de mieux appréhender les mécanismes qui régissent la structure et le fonctionnement de ces écosystèmes. Cependant, très peu d'étude ce sont attachées à quantifier ce processus à l'intérieur du sédiment.

La première étape de ma thèse a donc consisté à évaluer les quantités de matière organique particulaire grossière (CPOM > 1 mm) stockées dans trois cours d'eau de tête de bassin de la Montagne noire (Sud-Ouest de la France) présentant des caractéristiques granulométriques différentes. Les mesures ont été effectuées à deux dates différentes (25 novembre 2009 et 21 mars 2010), à la surface du sédiment et dans la zone hyporhéique.

J'ai testé les hypothèses selon lesquelles, (1) une part substantielle de matière organique particulaire grossière intégrant les cours d'eau après l'abscission des feuilles pourrait être immobilisée dans le compartiment hyporhéique et ainsi être soumise à des conditions physico-chimiques et biologiques différentes de celles à la surface du sédiment, (2) les quantités de matière organique allochtone stockées dans le compartiment hyporhéique pourraient fortement varier entre cours d'eau du fait des caractéristiques granulométriques de leurs sédiments.

Les résultats indiquent que des quantités de matière organique allochtone importantes peuvent effectivement être immobilisées par le compartiment hyporhéique, et que des différences marquées apparaissent à l'intérieur des cours d'eau, entre cours d'eau, et entre les deux dates d'échantillonnages. L'enfouissement des litières est donc un processus dynamique dans l'espace et dans le temps, et semble être influencé par les caractéristiques granulométriques du lit des cours d'eau. Par ailleurs, les résultats des analyses physico-chimiques ont entre autres révélé une forte diminution de l'approvisionnement en O_2 dissous dans l'eau interstitielle des trois cours d'eau, pouvant potentiellement induire une importante diminution de l'activité des communautés de décomposeurs. Ainsi, l'immobilisation de la matière organique dans la zone hyporhéique peut affecter le recyclage du carbone et des nutriments à l'échelle de l'écosystème.

1.2. Abstract

Headwater woodland streams are primarily heterotrophic since they receive substantial inputs of organic matter from the riparian vegetation while primary production is generally low. A substantial part of leaf litter entering running waters may be buried in the streambed as a consequence of flooding and sediment movement. Although the general significance of the hyporheic zone for stream metabolism has been reported early, organic matter storage within the sediment of streams has received less attention, most studies only quantifying storage on streambed surface and ignoring other compartments of the stream.

Storage of coarse particulate organic matter divided into three categories (CPOM : > 16 mm, 4-16 mm and 1-4 mm) was measured in three low-order headwater streams of the Montagne Noire, in South-Western France, at two different times of the year. Our findings clearly demonstrated that total CPOM storage in the hyporheic zone can be higher than on the sediment surface. Indeed, storage was up to one order of magnitude higher in the hyporheic zone than in the benthic one.

A sandy bottomed stream stored a higher quantity of CPOM compared with others streams, and whatever the CPOM size categories. Thus, the present study suggests that sediment particle size of streambed may be an important determinant which control leaf litter storage both in benthic and hyporheic zones of streams. Given the large quantity of organic matter stored in the hyporheic zone, this latter compartment may play an important role in the stream’s carbon turn-over, energetics, and trophic dynamics.

1.3. Introduction

Low-order forested streams, where light limitation restricts primary production, rely upon the input of organic matter from the riparian zone to fuel in-stream processes (Vannote *et al.*, 1980). Thus, benthic coarse particulate organic matter (*e.g.* leaves, wood and twigs) is an integral component of the functioning of these streams. Indeed, it often forms the basis of the trophic structure of streams, being the major source of organic matter and energy in woodland stream ecosystems (Cummins *et al.* 1989). The decomposition of this organic matter is a key ecosystem-level process integrating the activities of both microbial decomposers and aquatic invertebrates (Gessner & Chauvet; 1994; Suberkropp 1998 ; Graça 2001 ; Hieber & Gessner 2002). These litter accumulations also provide physical structure, affecting geomorphology (Keller & Swanson 1979) and serving as habitat for organisms (Malmqvist *et al.* 1978 ; Short & Ward 1981).

During the last four decades, studies on the dynamic of organic matter in streams have mostly been limited to benthic habitats, *i.e.* with processes occurring above the streambed surfaces (Kaushik & Hynes 1971 ; Webster & Benfield 1986), denoting the reductionist view of researchers at this time, considering these lotic ecosystems only as "pipes" conducting water, solutes and organic matter from the catchment downstream (Boulton 2000). Although the general significance of a third compartment, the hyporheic zone, has been reported early (Orghidan 1959 ; Schwoerbel 1961 ; 1964 ; Williams & Hynes 1974 ; Gibert *et al.* 1990 ; Vervier *et al.* 1992 ; White 1993 ; Triska *et al.* 1989), the specific significance of associated processes linked with stream functioning remains often neglected. The major contribution of the hyporheic zone to stream metabolism has been reported by Grimm & Fisher (1984).

Moreover, this zone may also play a crucial role in the organic matter dynamics within streams. Some studies suggest that the organic matter storage within the hyporheic zone may be considerably higher compared to the one at the sediment surface, partly because buried organic matter has a slower decomposition rate (Herbst 1980 ; Rounick & Winterbourn 1983, Cornut *et al. in press*) and a lower probability of downstream transport during storms and floods.

Thus, understanding of spatial and temporal dynamic storage is needed to better understand the mechanisms that control the structure and functioning of these ecosystems. However, very few studies have attempted to evaluate the importance of organic matter storage within streambed sediment.

The present study was performed to measure coarse particulate organic matter (CPOM > 1 mm) storage in three low-order headwater streams of the Montagne Noire (South-Western France) varying in granulometric characteristics. Attention has been focused on the spatial variability of storage at two different sampling dates (25 november 2009 and 21 march 2010), measurements being made above and within stream sediments. The following hypotheses have been tested : (1) a substantial part of CPOM entering streams should be buried into streambed sediment, during storms or as a consequence of sediment movements, and therefore be submitted to physico-chemical and biological conditions strongly contrasting with those at the sediment surface; (2) allochthonous organic matter stored in the hyporheic habitat should differ between streams due to changes in granulometric characteristics of their streambed, potentially differing in frequency and magnitude of disturbances affecting the hyporheic zone.

1.4. Methods

The experiment was carried out in the Montagne Noire, South-Western France, a 1,450-km^2 region covered by a mixed broadleaf forest with an altitudinal range of 250–1211 m a.s.l. Climatic conditions are marked by high rainfall (average 1,500 mm year^{-1}). Three 1st- or 2nd-order permanent streams with similar physical and chemical characteristics were selected in forested areas, situated between 02°09′25′′E and 02°19′51′′E longitude and 43°21′15′′N and 43°27′03′′N latitude. Broadleaf forests dominated by oak (*Quercus petraea* (Mattus.) Liebl.), hazel (*Corylus avellana* L.), alder (*Alnus glutinosa* (L.) Gaertn.), ash (Fraxinus excelsior L.), sweet chestnut (*Castanea sativa* Mill.) and beech (*Fagus silvatica* L.) form the riparian zones.

Water chemistry was determined at two sampling dates in three representative riffles per stream. Temperature, pH, conductivity and dissolved oxygen concentration were measured in the field using portable instruments (pH-meter 320i and Oxi 330i, WTW, Weilheim, Germany; Conductimeter Dist5, HANNA, Woonsocket, Rhode Island, USA). Water samples were filtered in the field with 0.7 µm glass fiber filters (Glass fibre GF/F, Whatman, Clifton, New Jersey, USA), stored in pre-rinsed polyethylene bottles, and placed in an icebox until they were returned to the laboratory. Concentrations of P-PO_4^{3-}, measured as soluble reactive phosphorous (SRP), N-NO_3^-, N-NO_2^-, and N-NH_4^+, were measured using standard colorimetric methods by flow injection analysis with an Alpkem Flow Solution IV system (OI Analytical, College station, Texas, USA). Alkalinity was determined using potentiometric titration.

CPOM on the channel surface and subsurface was quantified using a Surber net (30 cm × 30 cm; 500 µm mesh size) and a benthometer (15 cm × 16 cm inner diameter), respectively. During CPOM sampling, the number of samples has been doubled compared to those made at the surface of the sediment to minimise bias due to contrasting sampling efforts in terms of area considered in both compartments. Thus, three samples on the sediment surface, and six in the hyporheic zone have been performed on each of the three selected riffles in the three streams. Each riffle has been virtually divided into nine zones (*i.e.* 3 sections in the length × 3 zones in the width) (Fig. 1). One zone of each of the three sections was randomly selected and sampled. Samples and sediment cores from the benthic and hyporheic zones respectively, were stored individually in plastic zip-lock bags and transported to the laboratory in an icebox. At the laboratory, all samples were wet-sieved under tap water and CPOM was sorted and divided into three categories as proposed by Minshall *et al.* (1983) : > 16 mm, 4-16 mm and 1-4 mm. Once the organic matter had been removed from each grain size fraction, each core layer was meticulously washed and wet-sieved through a series of mesh sizes (20 mm, 10 mm, 5 mm, 2 mm, 1 mm, 0.5 mm and 0.25 mm) for grain size analysis. The CPOM of each category and the remaining sediment from each grain size were placed into an oven at 105°C until constant mass and weighed to the nearest 0.01 g.

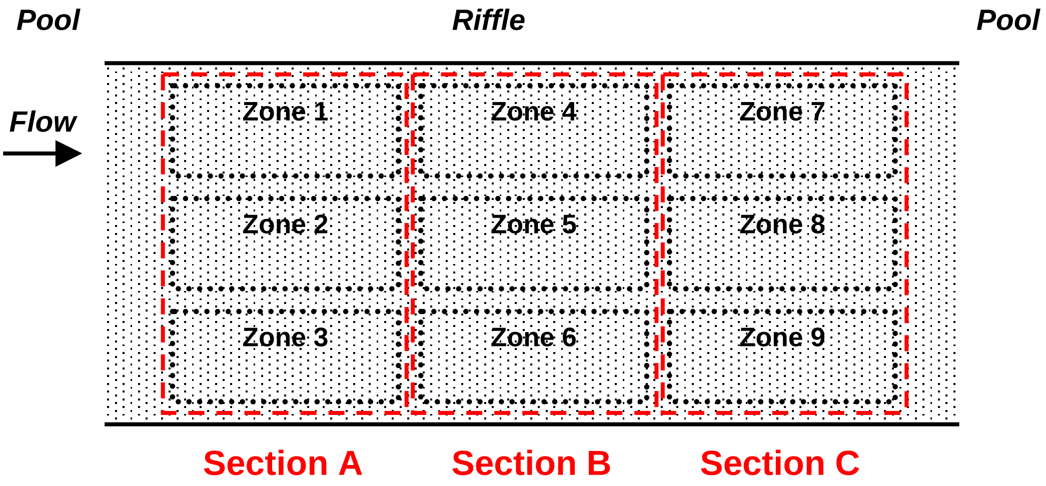

Fig. 1: Sampling design used for the measurement of CPOM storage.

A three-way factorial ANOVA was used to assess differences in CPOM storage among streams with treatment (*i.e.* benthic or hyporheic zones) and sampling date as the main effects. When significant differences were detected, Tukey HSD tests were then carried out for *post hoc* pairwise comparisons. Total masses of CPOM for each fraction were log-transformed to meet the assumptions of ANOVA. STATISTICA 6.0 (StatSoft Inc., 2001) was used for all statistical analyses. Differences were considered significant when $P < 0.05$.

1.5. Results

Mean water temperature patterns in the three streams were similar (Benthic: Béal = 9.1°C; Bergnassonne = 7.6°C; Orbiel = 6.2°C; Hyporheic: Béal = 9.5°C; Bergnassonne = 8.2°C; Orbiel = 7.6°C). The streams had very similar geomorphologic characteristics, especially in terms of water depth (≤ 0.40 m) and width (≤ 2.30 m). They had an average discharge ranging between 81.5 and 137 L s^{-1}. The stream-bed substrata were unconsolidated and mostly made of coarse sediments as determined from fractionation of the grain size of sediment cores for the Bergnassonne and the Orbiel, while the Béal had a stream-bed substratum comprising a mixture of gravel and coarse sand (Fig. 2).

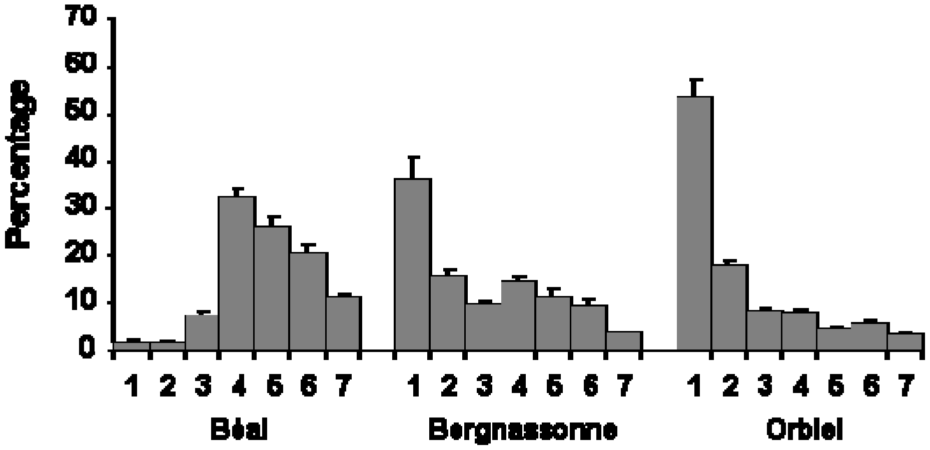

Fig. 2: Frequency distributions of average masses (+ SE, n = 9 per steam) of seven size-classes of sediments (1 = >20 mm; 2 = 10-20 mm; 3 = 5-10 mm; 4 = 2-5 mm; 5 = 1-2 mm; 6 = 0.5-1 mm; 7 = 0.25-0.5 mm) in the three streams.

The three stream waters were slightly acidic (pH 6.0-7.0) with low conductivity (28-58 μS cm^{-1}) and relatively low buffering capacity (3.1-9.6 mg CaCO3 L^{-1}). Concentrations of SRP (2.0-4.2 μg L^{-1}) were low, while concentrations of N- NO_3^- (0.338-1.749 mg L^{-1}) were relatively high. The streams were always well oxygenated (97-102 % of the saturation). Physical and chemical conditions in the hyporheic zone (*i.e.* at 15 cm below the sediment surface; interstitial water pumped from a specific sampling device using a hand-held vacuum pump) were comparable to those at the surface level, except for dissolved oxygen (51-79 %) and SRP (2.0-8.5 μg L^{-1}) that were usually lower and higher, respectively, in the hyporheic zone (Table.1).

Table 1: Minimum and maximum values of physico-chemical parameters of water of the three streams, measured at each date (25 november 2009 and 21 march 2010) (n = 6 per stream and per compartment).

	Béal		**Bergnassonne**		**Orbiel**	
Altitude a.s.l (m)	551		700		780	
Discharge (L s^{-1})	75-88		79-122		108-166	
	Benthic	Hyporheic	Benthic	Hyporheic	Benthic	Hyporheic
Temperature (°C)	8.5-9.8	8.8-10.1	6.8-7.5	8.1-8.2	5.6-6.8	7.2-8.0
pH	6.3-7.0	6.2-6.6	6.0-6.6	6.0-6.4	6.1-6.7	5.7-5.9
D.O (% of the saturation)	98-100	56	97-99	59-79	97-102	51-71
Conductivity (μS cm^{-1})	56-58	58-59	35	55-59	28-30	31-34
Alkalinity (mg CaCO3 L^{-1})	9.2-9.6	10.3-10.6	3.3-4.3	14.1-15.2	3.1-3.4	4.7-5.9
P-PO$_4$ (μg L^{-1})	3.2-4.2	2.0-3.6	2.0	7.7-8.3	2.2-3.6	3.2-8.5
N-NO$_3$ (mg L^{-1})	0.756-1.749	0.555-1.355	0.744-1.479	0.503-1.421	0.338-1.073	0.216-0.900

Our findings clearly demonstrated that total CPOM storage in the hyporheic zone can be higher than on the sediment surface (ANOVA, $F_{1,96} = 7.07$, $P < 10^{-3}$) (Fig.3). Indeed, storage was up to one order of magnitude higher in the hyporheic zone than in the benthic one. Our results also showed that total organic matter storage markedly differed between streams (ANOVA, $F_{2,96} = 6.85$, $P < 10^{-3}$ et HSD test) (Fig.3). Storage of organic matter strongly varied according to CPOM size categories between the three streams. Indeed, storage ability of the three streams for the large CPOM (*i.e.* >16 mm) was different (ANOVA, $F_{2,96} = 4.26$, $P < 10^{-3}$ and HSD test) (Fig.4). Large CPOM storage was significantly higher in the Béal than in the Bergnassonne. However, it was not different when compared with Orbiel. Moreover, these patterns were similar whatever the compartment and the sampling date. Regarding the CPOM between 4 and 16 mm, our results showed differences in terms of stream, compartment and sampling date. Thus, once again, the Béal had a higher storage ability for this type of particles compared with the other streams (ANOVA, $F_{2,96} = 4.27$, $P < 10^{-3}$ et HSD test). Storage of CPOM between 4 and 16 mm was markedly higher in the hyporheic zone than on the surface of sediment (ANOVA, $F_{1,96} = 28.44$, $P < 10^{-7}$), with this pattern being more pronounced at the first sampling date, as indicated by the significant interaction date × compartment (ANOVA, $F_{1,96} = 4.10$, $P < 10^{-3}$).

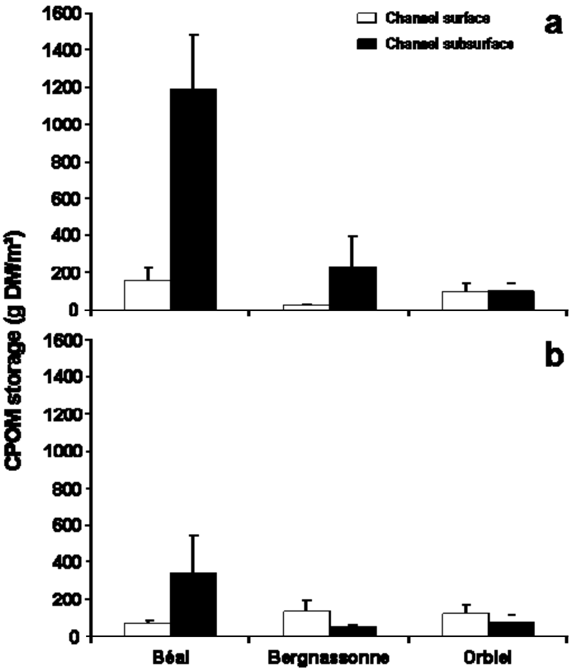

Fig. 3. Total CPOM storage (+ SE, n = 9 per stream) on the streambed surface and in the hyporheic zone of the three streams, a: 25 november 2009, b: 21 march 2010.

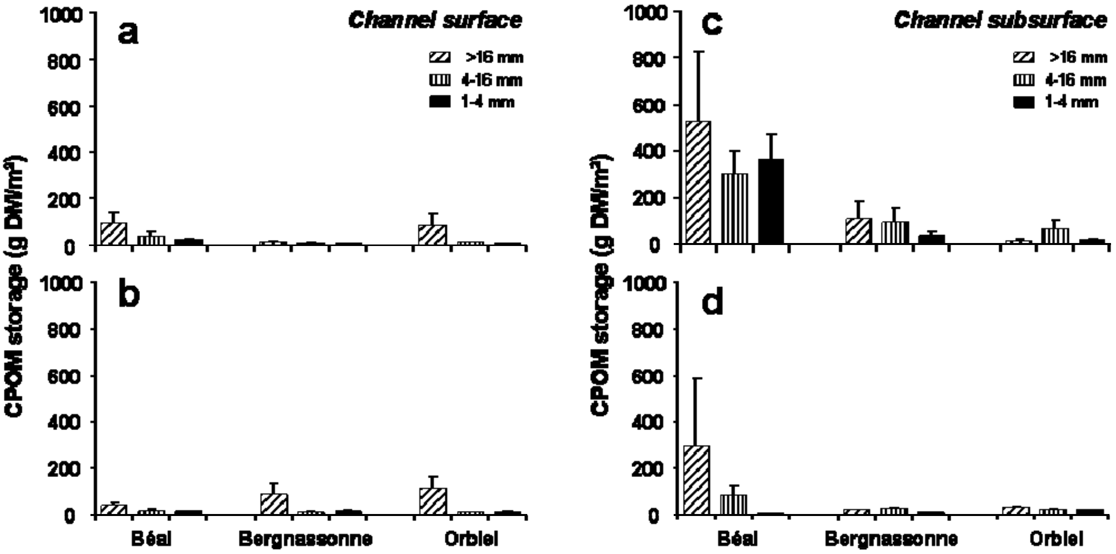

Fig. 4. Mean storage of the three CPOM size-classes (+ SE, n = 9 per stream) on the streambed surface and in the hyporheic zone of the three streams, a and c: 25 november 2009, b and d: 21 march 2010.

Finally, the same although more contrasted patterns were observed with finest CPOM (1-4 mm). As previously, results showed markedly differences between streams (ANOVA, $F_{2,96} = 6.87$, $P < 10^{-4}$), compartments (ANOVA, $F_{1,96} = 5.98$, $P < 10^{-3}$) and sample dates (ANOVA, $F_{1,96} = 15.88$, $P < 10^{-5}$). It is also interesting to note that CPOM storage within the sediment of the three streams was either higher or similar than at the sediment surface.

1.6. Discussion

Despite a high variability between streams, the present study demonstrated that concentrations of CPOM buried in the sediment were not negligible. Generally, CPOM contents in the hyporheic zone of the three streams were higher or equivalent to those at the surface of the sediment (Fig. 3). At the first sampling date, CPOM storages in the Béal and the Bergnassonne were up to one order of magnitude higher in the hyporheic zone than in the benthic one. The highest CPOM storage was found in the Béal, a sandy bottomed stream, where almost 1.2 kg DM/m² was retained in subsurface. This value is lower that those reported by Metzler & Smock (1990) and Smock (1990), 3.5 and almost 3 kg AFDM/m², respectively, but represents a snapshot assessment of CPOM storage, in contrast with these authors who reported CPOM storage in subsurface cumulated through one year.

It is interesting to note that CPOM storage in subsurface at the second sampling date markedly decreased when compared to the previous one. This result suggests that changes in the pool of hyporheic detritus can be explained both through their processing and their transport after they have been released from subsurface storage. Thus, and in line with the few studies that have measured CPOM storage in the hyporheic habitat, our findings demonstrate that burial can be an important storage mechanism, particularly in streams with loose sediment and potentially high sediment disturbance frequency (Herbst 1979 ; Godbout & Hynes 1982 ; Cummins *et al.* 1983 ; Metzler & Smock 1990).

Sediment particle size of streambed is indeed an important determinant which controls the storage ability of streams both in benthic and hyporheic zones. Thus, the storage ability of the hyporheic zone of the Béal, unconsolidated substratum mostly made of fine sediments, was significantly higher compared with the two others streams. An examination of Fig. 3 reveals that this process was dynamic. Indeed, the differences between the two sampling dates were evident, even though these were not statistically significant, probably because high variability in the Béal. This high variability in the Béal (sandy-bottomed stream) is not surprising and may be partly explained by sediment instability which is function of particle size. The hyporheic zone of sandy-bottomed streams is generally characterised by a high frequency of disturbance, increasing its ability to store large quantities of organic matter. This potentially high disturbance frequency of the hyporheic zone increases the spatial variability of organic matter stored. In other respects, the size of CPOM was also a crucial factor which influenced its storage by streams.

It is important to keep in mind, however, the limits of the approach used in this experiment, grounding our conclusion only on two sampling dates. A budget-based approach during one year would have been preferable to overcome potential effects related to species phenology or meteorological conditions that could advance or delay the fall of leaves, thus leading to a lag in the input periods of allochthonous organic matter in the three streams.

Organic matter storage within the hyporheic zone of streams leads to significantly reduced downstream carbon transfers. This process seems to be particularly significant in sandy-bottomed streams, where organic matter storage on the streambed surface is relatively inefficient, specifically during floods (Smock *et al.* 1989). Thus, hyporheic storage affects carbon *spiralling lengths* (Newbold *et al.* 1982), both the transport and biological utilization components of spiralling being decreased relative to those for surface organic matter. The lower microbial and invertebrate utilization of buried organic matter, compared to the surface, is potentially important for the overall stream metabolism (Grimm & fisher 1984 ; Cornut *et al. in press*) and for the release of dissolved organic carbon and nutrients (Croker & Meyer 1987). Finally, release from hyporheic storage will make available a large quantity of new sources of carbon to the stream surface, potentially at times when surface supplies may be scarce (Metzler & Smock 1990). Spatial and temporal patterns of organic matter storage are undoubtedly significant factors affecting stream energy dynamics, and may result from the interaction of litter fall phenology, processing rates of CPOM, storage abilities of different geomorphologic structures, and exchanges between stream compartments.

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Chapitre 2:

Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams

2. Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams

2.1. Résumé

Les cours d'eau forestier ont un fonctionnement écologique original basé sur l'hétérotrophie. Ainsi, la litière d'origine végétale provenant des communautés riveraines constitue la principale source de carbone et d'énergie dans ces écosystèmes aquatiques. L'étude dans le chapitre 1 a permis de révéler qu'une partie importante de cette matière organique allochtone intégrant les cours d'eau forestiers après l'abscission des feuilles, pouvait subir un enfouissement partiel ou total suite à des crues ou à des mouvements de sédiments, et ainsi être piégée et immobilisée dans la zone hyporhéique sous-jacente. Bien que la décomposition des litières soit relativement bien documentée lorsqu'elle a lieu à la surface du sédiment, son devenir quand elle est incorporée à la matrice sédimentaire des cours d'eau, mais également les organismes impliqués dans ce processus restent très peu étudiés.

Par une expérience de terrain, nous avons testé l'hypothèse selon laquelle, la taille réduite des interstices du sédiment restreint l'accès des plus gros macroinvertébrés déchiqueteurs à la matière organique d'origine végétale enfouie dans le sédiment, sans compromettre celui des micro-organismes, notamment les hyphomycètes aquatiques. Ainsi, il est attendu que les décomposeurs fongiques dans la zone hyporhéique compensent au moins partiellement le rôle des déchiqueteurs à la surface du sédiment. Dans ce but, nous avons introduit des feuilles d'aulne glutineux dans l'Alzeau, un cours d'eau de deuxième ordre de la Montagne Noire (Sud-Ouest de la France). Les sachets à litières contenant le matériel foliaire ont été exposés selon trois conditions d'incubation : (1) enfouis dans les sédiments (hyporhéique), (2) enfouis après 2 semaines d'exposition à la surface des sédiments (benthique-hyporhéique), ou exposés à la surface des sédiments pendant la totalité de l'expérience (benthique).

Nos résultats ont révélé que la décomposition des feuilles d'aulne glutineux est nettement plus rapide à la surface du sédiment que dans les deux autres traitements (i.e. 2,1 et 2,8 fois plus rapide que dans les traitements benthiques-hyporhéique et hyporhéique, respectivement). Les assemblages fongiques sont généralement moins diversifiés dans l'habitat hyporhéique, avec quelques espèces qui tendent toutefois à être relativement favorisées par de telles conditions. La biomasse fongique et le taux de sporulation sont réduits dans le traitement hyporhéique, avec les litières associées au traitement benthiques-hyporhéique présentant un patron intermédiaire. L'abondance et la biomasse des invertébrés déchiqueteurs dans la zone hyporhéique sont considérablement diminuées, à l'exception des Leuctra *spp.*, qui augmentent quant à eux pour être de loin les taxons les plus souvent associés à la litière dans la zone hyporhéique. Par ailleurs, les Leuctra *spp.* sont les rares invertébrés déchiqueteurs à présenter des caractéristiques morphologiques susceptibles d'augmenter leurs performances dans les espaces limités de la zone interstitielle.

La réalisation d'un bilan de carbone a révélé que la contribution relative des deux principaux décomposeurs, à savoir les invertébrés détritivores et les hyphomycètes aquatiques, varie considérablement selon la localisation des litières dans le cours d'eau. Bien que la biomasse des invertébrés représente près de 50% du carbone initial transformé après 80 jours dans le traitement benthique, leurs contributions est inférieure à 0,3% dans la zone hyporhéique et à 2,0% dans le traitement combiné benthique-hyporhéique. En revanche, la production de mycélium et de conidies dans le traitement hyporhéique représentent 12% de la perte de masse foliaire, soit 2 à 3 fois plus que dans les deux autres conditions. Ces résultats suggèrent que le rôle des champignons est particulièrement important dans la zone hyporhéique. Nos résultats confirment donc que l'enfouissement de la litière dans le lit des cours d'eau réduit son taux de décomposition en limitant l'accès des décomposeurs. Ainsi, le compartiment hyporhéique peut être une zone importante de stockage de la matière organique dans les cours d'eau forestier et servir de réservoir d'inoculum fongique. Du fait, de la rétention temporaire de la matière organique allochtone par l'enfouissement, la zone hyporhéique joue un rôle important dans le métabolisme du carbone et le fonctionnement global des écosystèmes d'eau courante.

SUMMARY

1. Leaf litter constitutes the major source of organic matter and energy in woodland stream ecosystems. A substantial part of leaf litter entering running waters may be buried in the streambed as a consequence of flooding and sediment movement. While decomposition of leaf litter in surface waters is relatively well understood, its fate when incorporated into river sediments, as well as the involvement of invertebrate and fungal decomposers in such conditions, remain poorly documented.
2. We tested experimentally the hypotheses that the small interstices of the sediment restrict the access of the largest shredders to buried organic matter without compromising that of aquatic hyphomycetes, and that fungal decomposers in the hyporheic zone, at least partly, compensate for the role of invertebrate detritivores in the benthic zone.
3. Alder leaves were introduced in a stream either buried in the sediment (*hyporheic*), buried after 2-weeks exposure at the sediment surface (*benthic-hyporheic*), or exposed at the sediment surface for the entire experiment (*benthic*). Leaf decomposition was markedly faster on the streambed surface than in the two other treatments (2.1-fold and 2.8-fold faster than in the benthic-hyporheic and hyporheic treatments, respectively).
4. Fungal assemblages were generally less diverse in the hyporheic habitat with a few species tending to be relatively favoured by such conditions. Both fungal biomass and sporulation rates were reduced in the hyporheic treatment, with the leaves subject to the benthic-hyporheic treatment exhibiting an intermediate pattern. The initial 2-week stage in the benthic habitat shaped the fungal assemblages, even for leaves later subjected to the hyporheic conditions.
5. The abundance and biomass of shredders drastically decreased with burial, except for *Leuctra spp.* which increased and was by far the most common leaf-associated taxon in the hyporheic zone. *Leuctra spp.* was one of the rare shredder taxa displaying morphological characteristics that increased performance within the limited space of sediment interstices.

6. The carbon budgets indicated that the relative contributions of the two main decomposers, shredders and fungi, varied considerably depending on the location within the streambed. While the shredder biomass represented almost 50% of the initial carbon transformed after 80d in the benthic treatment, its contribution was less than 0.3% in the hyporheic one and 2.0% in the combined benthic-hyporheic treatment. In contrast, mycelial and conidial production in the permanently hyporheic environment accounted for 12% of leaf mass loss, i.e. 2-3 times more than in the two other conditions. These results suggest that the role of fungi is particularly important in the hyporheic zone.
7. Our findings indicate that burial within the substrate reduces the litter breakdown rate by limiting the access of both invertebrate and fungal decomposers to leaves. As a consequence, the hyporheic zone may be an important region of organic matter storage in woodland streams and serve as a fungal inoculum reservoir contributing to further dispersal. Through the temporary retention of litter by burial, the hyporheic zone must play a significant role in the carbon metabolism and overall functioning of headwater stream ecosystems.

Introduction

Leaf litter constitutes the major source of organic matter and energy in woodland stream ecosystems (Cummins *et al*., 1989). A substantial part of leaf litter entering running waters may be buried into the streambed during storms (Herbst, 1980) or as a consequence of flooding and sediment movement (Metzler & Smock, 1990; Naegeli *et al*., 1995). Thus, the hyporheic zone of streams is potentially a major site of organic matter storage. Comparisons between benthic and hyporheic zones have shown that the latter may account for 25-82% of the total stored organic matter (Cummins *et al*., 1983; Metzler & Smock, 1990; Smock, 1990; Jones, 1997; Jones *et al*., 1997). During the last four decades, studies on the decomposition of leaf litter have mostly been limited to benthic habitats, i.e. with processes occurring above the streambed surfaces (Kaushik & Hynes, 1971; Webster & Benfield, 1986; Suberkropp, 1998). Although a few authors have attempted to determine the fate of leaf litter when incorporated into stream sediments, its ecological significance for this compartment of headwater streams remains poorly documented (but see Herbst, 1980; Rounick & Winterbourn, 1983; Metzler & Smock, 1990; Smith & Lake, 1993; Naamane, Chergui & Pattee, 1999). From these studies however, burial has been generally shown to reduce the rate of leaf litter decomposition (Reice, 1974; Herbst, 1980; Rounick & Winterbourn, 1983; Metzler & Smock, 1990; Naamane *et al*., 1999), with a few other studies reporting ambiguous or less well defined patterns (Mayack, Thorp & Cothran, 1989; Smith & Lake, 1993). In contrast, Nichols & Keeney (1973) observed an enhancement of leaf litter decomposition rate by the presence of sediment in the lentic environment.

Aquatic hyphomycetes, or Ingoldian fungi, are a phylogenetically heterogenous group of fungi that are involved in leaf litter colonization and decomposition in streams, i.e. environments where aerobic conditions generally prevail (Bärlocher, 1992; Storey, Fulthorpe & Williams, 1999; Gulis, Kuehn & Suberkropp, 2009). Despite their key importance in the functioning of stream ecosystems, aquatic fungi have historically received much less attention than macroinvertebrates, algae, fish, or even bacteria (Gulis & Suberkropp, 2006).

These fungi play an essential role in the decomposition of leaf litter in aquatic environments (Bärlocher, 1992; Gessner & Chauvet, 1994; Gessner, Chauvet & Dobson, 1999) by producing extracellular enzymes able to degrade the structural constituents of vascular plants such as cellulose, hemicelluloses, pectin and, to a lesser degree, lignin, and in transforming leaf material into a more suitable food source for invertebrates in streams (Bärlocher, 1992). Therefore aquatic hyphomycetes are important mediators in the energy and nutrient transfer to higher trophic levels (Gulis *et al*., 2009), and more generally in the ecological functioning of headwater streams. Fungal abundance, distribution and population dynamics are strongly linked to the seasonal timing and extent of terrestrial plant matter inputs into the surface zone of running waters (Suberkropp, 1997). Hyporheic fungi might thus play a fundamental role in processing allochthonous organic matter and the incorporation into the trophic webs like fungi do in surface waters (Storey *et al*., 1999). Surprisingly, and despite the considerable amount of plant matter buried in sediment, little is known about the role of microorganisms – and aquatic hyphomycetes in particular – on the decomposition of leaf litter in the hyporheic zone of headwater streams. From the very few studies available, the conclusions concerning the role of aquatic hyphomycetes remain unclear.

Essafi *et al*. (1994) and Metzler & Smock (1990) suggested that due to constraints prevailing in the hyporheic zone, fungal conditioning was more limited than in surface waters and thereby led to a decrease in the attractiveness of leaves to invertebrates. In more extensive studies, Rounick & Winterbourn (1983) recorded a lower protein content and microbial activity in buried litter, while Herbst (1980) found no consistent difference in the protein content of leaves incubated above and below the surface of sediment, and Naamane *et al*. (1999) did not find aquatic hyphomycete spores on poplar leaves buried in the sediments of a Moroccan stream. Finally, Smith & Lake (1993) observed microbial colonization, particularly by fungal hyphae, on leaf litter incubated either above or below the surface of sediment. Bärlocher *et al.* (2006, 2008) found that the occurrence of aquatic hyphomycetes in streambed sediment was closely linked to that of deciduous leaves, and suggested that aquatic hyphomycetes readily disperse within the hyporheic zone. Clearly, additional data are needed to fully understand the abundance, the role, the activities and the dynamics of aquatic fungi colonizing leaves in the hyporheic zone of headwater streams.

The aim of this study was to determine how the location of leaf litter within the streambed, i.e. at the surface or buried, may affect the leaf-associated decomposer communities and therefore leaf litter decomposition. Hyporheic sediment may act as a physical barrier, allowing only shredders with thin and elongated bodies to penetrate the substratum layers. Similarly, the specific conditions prevailing in the hyporheic zone are likely to considerably influence the presence, dynamics and activity of aquatic hyphomycetes. As a result of such constraints, our first hypothesis was that the density and biomass of both detritivorous macroinvertebrates and aquatic hyphomycetes would be lower in the hyporheic zone than in the benthic habitat. We expected leaf litter burial to lead to an impoverished community and lowered activity of aquatic hyphomycetes, not only due to the physical barrier but also due to the decreased oxygen concentration in the hyporheic water. Our second hypothesis was that leaf litter decomposition depends on initial microbial colonisation. Specifically, we predicted that leaves exposed for a short period at the sediment surface before burial, due to higher microbial inoculation, would be decomposed more rapidly than those buried from the beginning, but at a rate lower than leaves decomposing entirely at the sediment surface. We tested these hypotheses by conducting an experimental study in a 2nd-order stream located in southern France where leaf-associated microbial and macroinvertebrate communities, as well as decomposition rates, were compared across controlled treatments differing by their location within the streambed (buried in the sediment, buried after 2-weeks exposure at the sediment surface, or exposed at the sediment surface throughout the experiment).

Methods

Study site

The experimental site was located in the Alzeau, a 2nd-order stream of the Montagne Noire in South-Western France (02°13'23" E, 43°25'51" N; elevation 743 m a.s.l). The surrounding forest consisted of mixed broadleaf species including alder (*Alnus glutinosa* (L.) Gaertn.), oak (*Quercus petraea* (Mattus.) Liebl.), hazel (*Corylus avellana* L.) and aspen (*Populus tremula* L.). The area was subject to a mountain climate with oceanic influences marked by high rainfall in the autumn and winter (1,300 mm year^{-1}). The mean monthly temperatures ranged from -0.7 to 21.4°C.

Forestry was the only anthropogenic disturbance, although limited within the study area. The study was conducted in a pool-riffle-pool sequence, extending over ca. 100 m. At baseflow, the stream was 3.5-4.7 m wide and 0.25-0.35 m deep in riffles. The mean water temperature during the leaf decomposition study was 7.7°C both in the benthic and hyporheic zones, and the average discharge was *ca*. 500 L s^{-1}. Vertical hydraulic gradients (VHGs) through the streambed were calculated as follows: VHG (m m^{-1}) = $\Delta h/\Delta l$, where $\Delta h = h_{\text{stream}} - h_{\text{piezometer}}$ and Δl is the distance between the streambed and the top of the plexiglas minipiezometer screen (Baxter, Hauer & Woessner, 2003). Vertical hydraulic gradients were measured to identify downwelling (positive VHG) and upwelling (negative VHG) areas in the hyporheic zone, and the sediment characteristics (particle size and hydraulic conductivity) were studied in four representative riffles of the study site. The substratum was unconsolidated and mostly made of coarse sediments as determined from fractionation of the grain size of sediment cores (20 cm length x 30 cm diameter) sampled from downwelling zones in the middle of four representative riffles of the study site: > 20 mm: 8.6%; 20-10 mm: 25.9%; 10-5 mm: 23.4%; 5-2 mm: 19.3%; 2-1 mm: 9.5%; 1-0.5 mm: 9.3%; 0.5-0.25 mm: 3.9%. The average flow rate through the sediment of the study site was relatively fast (22 cm h^{-1}). The stream water temperature was monitored every two hours with calibrated data loggers (SmartButton, ACR System Inc., Surrey, Canada). Water chemistry was determined at the six dates when leaf bags were introduced into or retrieved from the stream. pH, conductivity, and dissolved oxygen concentration were measured in the field using portable instruments (pH-meter 320i and Oxi 330i, WTW, Weilheim, Germany; Conductimeter Dist5, HANNA, Woonsocket, Rhode Island, USA). Water samples were filtered in the field with 0.7 μm glass fiber filters (Glass fibre GF/F, Whatman, Clifton, New Jersey, USA), stored in pre-rinsed polyethylene bottles, and placed in an icebox until they were returned to the laboratory. Concentrations of P-PO_4^{3-}, measured as soluble reactive phosphorous (SRP), N-NO_3^-, N-NO_2^- and N-NH_4^+, and alkalinity were measured using standard colorimetric methods by flow injection analysis with an Alpkem Flow Solution IV system (OI Analytical, College station, Texas, USA) and potentiometric titration, respectively. The stream water was slightly acidic (pH 6.0-6.6) with low conductivity (24-34 μS cm^{-1}) and low buffering capacity (1-5 mg $CaCO_3$ L^{-1}).

Concentration of SRP (0.7-2.9 μg L^{-1}) was low, while concentration of N-NO_3^- (0.802-0.980 mg L^{-1}) was relatively high. The stream was always well-oxygenated (88-111 % of the saturation). Interstitial water was pumped from plexiglas minipiezometers using a hand-held vacuum pump. Physical and chemical conditions in the hyporheic zone (i.e. at 15 cm below the sediment surface) were comparable to those at the surface level, except for dissolved oxygen (45-86 %) and SRP (8.3-12.4 μg L^{-1}) which were usually lower and higher, respectively, in the hyporheic zone.

Leaf decomposition

We selected alder as the test decomposing leaf species because it was by far the most common deciduous riparian tree species in the study area and provided a large part of leaf litter input to the stream. Alder leaves were collected from trees at abscission using nets in the autumn of 2007. Leaf bags consisted of 3.00 g (mean air-dry mass ± 0.03 g) of leaves enclosed in plastic net bags (15×15 cm, 5 mm mesh) to simulate natural accumulations of leaf detritus in the stream. Before incorporated into bags, the leaves were moistened with distilled water from a vaporizer to prevent breakage during handling and transport.

A total of 60 leaf bags were introduced in the stream at the head of riffles (downwelling zones). Leaf bags were subjected to three treatments: benthic, hyporheic (i.e. buried in the sediment), or benthic-hyporheic (i.e., buried in the sediment after 17 days of benthic exposure). Benthic leaf bags were placed at the sediment surface and anchored to an iron bar driven into the streambed. Hyporheic leaf bags were positioned approximately 15-20 cm below the sediment surface using a small shovel, with a coloured plastic wire attached to facilitate localization and retrieval. At the time of the burial of the benthic-hyporheic leaf bags, the latter were disassembled and all macroinvertebrates associated with leaves were meticulously removed. Four replicate bags per treatment were randomly retrieved after 17, 31, 45, 59 and 80 days.

In parallel, artificial leaves were used to test whether invertebrates utilized leaves as refuge rather than food. Artificial leaves were made of polyethylene sheets with the shape and thickness similar to that of real leaves. Before use, these 'leaves' were pre-soaked for 2 weeks with several changes of distilled water to extract any soluble artificial chemicals (Boulton & Foster, 1998).

A total of 24 bags with artificial leaves were introduced at the head of riffles as previously, with two treatments (i.e. benthic artificial and hyporheic artificial) applied to four replicate leaf bags and three sampling times (17, 45 and 80 days).

During leaf bag sampling, a Surber net (500 µm mesh size) was used to minimize invertebrate loss due to passive or active drift. Leaf bags were stored individually in plastic zip-lock bags and transported to the laboratory in an icebox. Leaves were washed individually to remove sediments, exogenous organic matter and macroinvertebrates, which were collected in a 500 µm screen sieve and then preserved in 70% ethanol until processing. Two sets of five 12 mm diameter discs and another set of ten were cut from leaves, avoiding the central vein. One set of five leaf discs was frozen at – 18°C until processing for ergosterol extraction, the second one was used to determine microbial respiration and the last set of ten to characterize fungal sporulation. The remaining leaf litter was dried at 65°C to constant mass and weighed to the nearest 0.01 g. The leaf material was ground using a micro hammer mill (Culatti, Zurich, Switzerland) with a 0.5 mm mesh. Portions of leaf material of about 250 mg were ashed at 550°C for 4 h and weighed to determine the organic matter content. The leaf mass remaining in bags was expressed as the ratio of the ash-free dry mass (AFDM) between the final and initial leaf litter. Four unexposed batches of leaf litter were used to determine the leaf initial oven-dried mass by unit of air-dried mass and the initial AFDM according to the procedures above. Ground aliquots of leaf material were used to determine the nitrogen content with a CHN analyzer (NA 2100 Protein, CE Instruments, Milan, Italy).

Microbial respiration on leaves

Respiration rates associated with decomposing leaf material were inferred from measurements of oxygen consumption from five leaf discs. Once cut, leaf discs were quickly placed in a measuring chamber containing 3 mL of membrane-filtered (0.45 µm pore size, diameter 47 mm; Millipore Corporation, Bedford, Massachusetts, USA) stream water, and incubated in a water bath at stream temperature. Oxygen concentrations were monitored with oxygen electrodes connected to a data acquisition module (SI 130 Microcathode and 928 6-Channel Oxygen System, Strathkelvin Instruments, North Lanarkshire, UK) and a computer serving as a control and storage unit. Oxygen concentration was recorded every second for l.5 h.

Determinations were carried out simultaneously with six electrodes, i.e. in five chambers containing samples plus one control chamber containing stream water but no leaf material. Respiration rates (mg O_2 g^{-1} leaf AFDM $hour^{-1}$) were calculated as the slope of regression lines during periods of linear decreases in oxygen concentrations, and were corrected for oxygen loss in the control chamber.

Spore production and fungal diversity

Once cut, the ten fresh leaf discs were quickly placed in glass Petri dishes filled with 20 ml filtered (Glass fibre GF/F, Whatman) stream water. The production of fungal asexual spores was stimulated by gentle shaking (60 rpm, 25.4-mm orbital path) at 10°C for 48 h. Thereafter, spore suspensions were transferred into 50 mL polyethylene centrifuge tubes, the Petri dish and discs were rinsed with distilled water to collect spores no longer in suspension, and the volume was adjusted to 35 ml with 2 mL of 37% formalin and distilled water. The discs were then lyophilized and weighed to the nearest 0.1 mg. 0.5 mL of Triton X-100 (0.5% solution) was added to the spore suspensions and they were stirred gently to ensure uniform distribution of spores. 5 mL aliquots were then filtered through a membrane filter (5 μm pore size, diameter 25 mm; Millipore Corporation), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid (Iqbal & Webster, 1973). Spores were counted and identified under the microscope (× 200). Spore production was calculated as the number of spores released per g leaf AFDM per day. The Shannon & Weaver index of diversity (H’) and the Shannon & Weaver index of evenness (E) were computed for fungal communities associated with each of the three treatments.

Fungal biomass

Mycelial biomass in leaves was assessed through the content of ergosterol (Gessner & Chauvet, 1993). Leaf material was lyophilised and weighed to the nearest 0.1 mg, and then lipids were extracted with alkaline methanol heated at 80°C for 30 min. Extracts were purified using solid-phase extraction cartridges (Oasis HLB, 60 mg, 3 cc, Waters, Milford, Massachusetts, USA) and ergosterol quantified by high-performance liquid chromatography (procedure slightly modified from Gessner, 2005).

The extraction efficiency (85-92 %) was determined for each series from controls to which known amounts of ergosterol were added and was applied to calculate the ergosterol content in leaf litter.

Macroinvertebrates

Macroinvertebrates retained over a 500 µm mesh sieve were counted and identified to the lowest practicable level. Identification was to the genus/species level whenever possible, except for Oligochaeta and Diptera (family and sub-family or tribe, respectively), and individuals were classified as shredders, grazers and others (Tachet *et al.*, 2000). Macroinvertebrates assigned to the shredder group were measured to the nearest 0.5 mm and individual body mass was calculated using body length–dry mass relationships from the literature (Meyer, 1989; Burgherr & Meyer, 1997).

Carbon budget

Mycelial biomass was estimated from the leaf content of ergosterol by using 182 as conversion factor (Gessner & Chauvet, 1993; Charcosset & Chauvet, 2001). The biomass of released conidia was calculated from individual conidial mass for species listed in Bärlocher and Schweizer (1983) and Chauvet & Suberkropp (1998), or the average value of 200 pg for the other species (Gessner, 1997). The daily consumption rate of leaf litter by shredders was assumed to be 10% of the animals' body mass (Hieber & Gessner, 2002). Cumulative production of CO_2, conidial biomass and shredder biomass were calculated by interpolating production rates (CO_2, conidia) and consumption rates (shredders) between sampling dates and summing over the decomposition period. A factor of 0.5 was used to convert leaf AFDM, shredder biomass, mycelial biomass and conidial biomass into carbon (Baldy et al., 2007). Partial decomposition budgets were built, either based on initial leaf carbon or leaf carbon loss.

Statistics

Leaf decomposition rates (*k*) for each treatment were calculated using a nonlinear regression of the proportion of leaf mass remaining versus time, following an exponential decay model, $M_t = M_0 \cdot e^{-kt}$, where M_0 is the initial AFDM, M_t is the AFDM remaining at time t, and t is the time in days (Petersen & Cummins, 1974). Leaf decomposition rates of the three treatments were compared using generalised linear models based on the log-link function and by a likelihood type I test (Lecerf & Chauvet, 2008). We used a two-way factorial ANOVA to assess differences in microbial and macroinvertebrate parameters (leaf-litter associated respiration, spore production, and fungal biomass and abundance) and nitrogen content with treatment (i.e. benthic, hyporheic, or benthic-hyporheic) and exposure time as the main effects. When significant differences were detected between treatments, Tukey HSD tests were then carried out for *post hoc* pairwise comparisons. The ergosterol content and abundance of shredders were log-transformed to meet the assumptions of ANOVA. In cases of persistent heterogeneity of variances, a non-parametric test was applied. The Kruskal-Wallis test was used to investigate for differences in total shredder biomass across the three treatments. Mann-Whitney U tests (with Bonferroni corrected *P* values) were then carried out for pairwise comparisons. Differences in total shredder abundance and biomass associated with artificial leaves were tested using two-way ANOVA with treatments and exposure time as the main effects, and Mann-Whitney U test, respectively. STATISTICA 6.0 (StatSoft Inc., 2001) was used for all statistical analyses. Differences were considered significant when $P < 0.05$. The similarity between aquatic hyphomycete communities at each sampling date under the various treatments was measured with the Steinhaus index (Legendre & Legendre, 1998) using R software version 2.6.0 (R Development Core Team, 2007). The differences among the indices were estimated by Monte-Carlo procedure. For this purpose, these indices were compared to 9999 pseudo-values of the Steinhaus index obtained from comparisons of virtual aquatic hyphomycete samples randomly generated (using a normal distribution for each species, with its observed mean and standard deviation in the original data set).

Results

Litter decomposition and nitrogen dynamics

The leaf decomposition rate was 2.8 and 2.1 fold higher at the sediment surface than for samples buried in the sediment and those buried after 17 d-exposure at the sediment surface, respectively (Fig. 1a and Table 1). Consequently, the decomposition rates differed significantly among these three conditions (GLM test, $\chi^2 = 12.5$, $P = 0.002$). The loss in leaf mass after 17 days did not differ between treatments: not only were the benthic (67.5 ± 1.9%, mean ± SE of leaf AFDM remaining) and benthic-hyporheic (69.1 ± 3.0%) samples identical during this initial period, but also the hyporheic one was the same (69.8 ± 0.5%; Kruskal-Wallis test; $n = 12$; $P = 0.584$). Clearly, divergences between treatments occurred between d17 and d31, with the differences remaining constant thereafter (Fig. 1a). After 80 days, only 3% of initial leaf AFDM remained for the samples at the sediment surface, which contrasted with the 49% remaining for leaves exposed in the hyporheic habitat. The combined benthic-hyporheic treatment gave an intermediate value (37%). Extrapolation from the decomposition rates indicated that an incubation period of 255 and 341 days would be required for the benthic-hyporheic and hyporheic treatments, respectively, to reach the same decomposition stage as the benthic treatment after 80 days.

Total nitrogen concentrations during the course of leaf decomposition ranged from 2.09 to 3.47%, which corresponded to the initial litter and the last sampling date in the hyporheic treatment respectively (Fig. 1b). Nitrogen concentrations for all treatments showed constant increases, which however seemed to level off at the end of the decomposition. The concentrations were very similar across the treatments (ANOVA, $F_{2,40} = 1.26$, $P = 0.295$).

Leaf-litter associated microbial respiration

At day 0, microbial consumption of O_2 was 0.063 mg g^{-1} AFDM h^{-1}. Subsequently, respiration rates increased from 0.084 (hyporheic, d17) to 0.462 mg g^{-1} AFDM $hour^{-1}$ (benthic, d31; Fig. 2a), reached a maximum and then generally decreased. At any date, oxygen consumption from leaf material in the benthic habitat exceeded that from the hyporheic habitat with the combined benthic-hyporheic treatment showing intermediate values (ANOVA, $F_{2,34} = 9.123$, $P < 10^{-3}$ and HSD test).

Table 1 Decomposition rates of alder leaves in the three treatments: benthic (exposure at the sediment surface throughout the experiment), benthic-hyporheic (buried after 2-weeks exposure at the sediment surface), and hyporheic (buried in the sediment). The 95 % confidence limits and the regression coefficient are indicated.

Treatment	*k* (d^{-1})	95 % CL	R^2
Hyporheic	0.0103	0.0090-0.0114	0.83
Benthic-hyporheic	0.0138	0.0120-0.0156	0.83
Benthic	0.0292	0.0240-0.0345	0.88

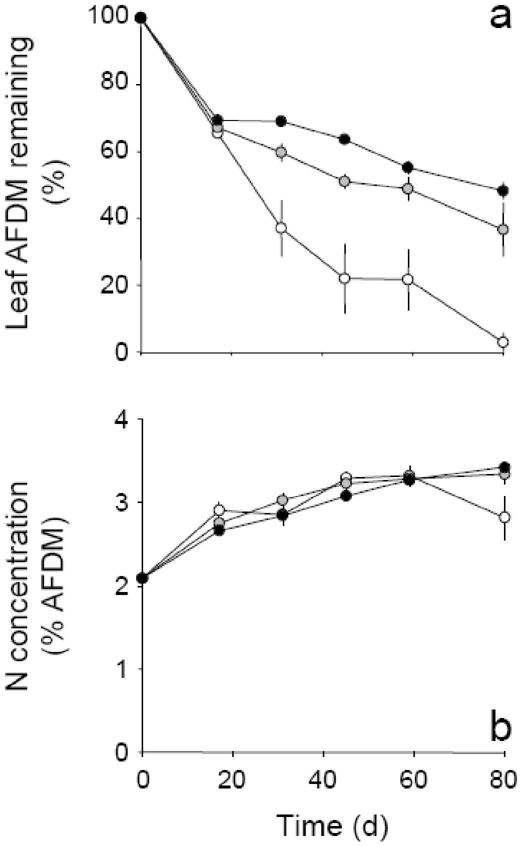

Fig. 1 Remaining ash free dry mass (**a**) and nitrogen concentration (**b**) of alder leaves in the three treatments, hyporheic: buried in the sediment (●), benthic-hyporheic: buried after 2-weeks exposure at the sediment surface (◎), and benthic: exposure at the sediment surface throughout the experiment (○). Vertical bars indicate ± 1 SE

Microbial O_2 consumption was 1.4 and 1.6 fold higher in the treatment at the sediment surface for the whole experiment compared with those samples buried after 2-weeks exposure at the sediment surface and those buried in sediment throughout experiment, respectively.

Spore production and fungal diversity

The pattern of leaf-associated sporulation rate of aquatic hyphomycetes was comparable across the treatments, however with very different dynamics (Fig. 2b). In the former, the sporulation rate showed a sharp increase to a peak of 1.9 conidia μg^{-1} AFDM d^{-1} at d31 followed by a rapid and pronounced decline, whereas the high rates also reached at d31 in the latter treatments were maintained up to the fourth sampling date before declining. The maximum rates were however lower in the hyporheic and benthic-hyporheic treatments with values of 1.2 and 0.7 conidia μg^{-1} AFDM d^{-1}, respectively. As a consequence, sporulation rates in the hyporheic habitat were intermediate between those of the two other treatments, which differed from patterns of both microbial respiration and fungal biomass (see below). Due to the large variations between dates and in spite of the differences in maximum values, sporulation rates were found not to differ significantly across treatments (ANOVA, $F_{2,34}$ = 1.048, P = 0.631). Finally, because of the sustained sporulation rates over a longer period in the hyporheic treatment, the cumulative conidial production in relation to the initial leaf mass was much higher than in the other two treatments (data not shown, but see *Carbon budgets* below).

A total of 23 sporulating species of aquatic hyphomycetes were identified from decomposing leaves with a similar number, i.e. 19 or 20, found in the three exposure treatments (Table 2). However, species richness at any time was generally much lower, i.e. by about 50%, in the permanently hyporheic treatment than in the two other treatments (data not shown). Only very slight differences in species abundance were observed between benthic and benthic-hyporheic treatments (Table 2). Similarly, the species composition of fungal assemblages on the leaves was much more comparable between benthic and benthic-hyporheic treatments than between any of these treatments and the permanently hyporheic one.

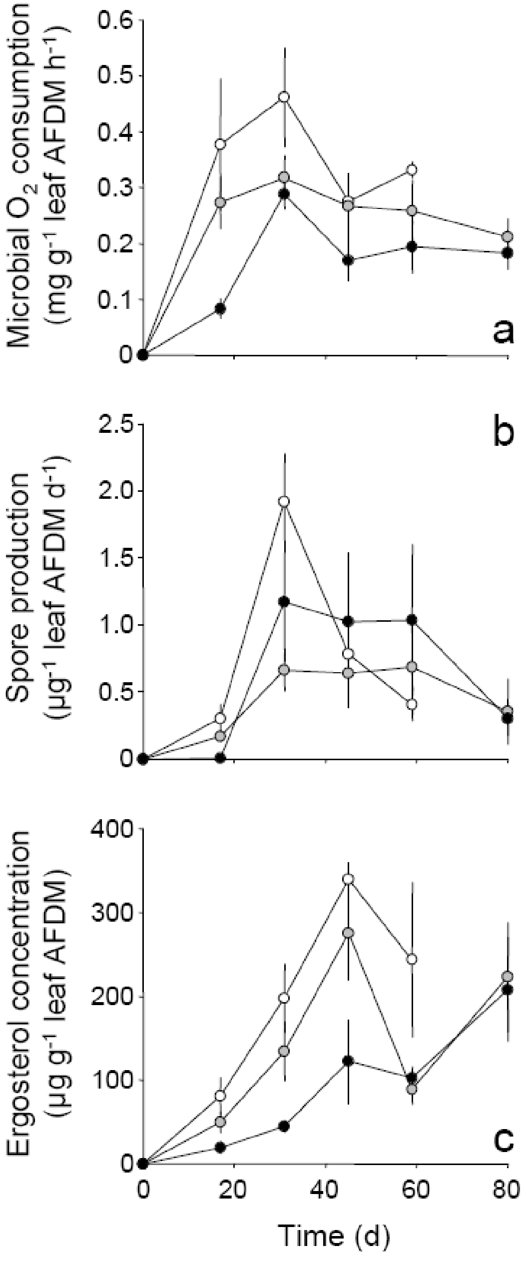

Fig. 2 Microbial respiration (a), sporulation rate of leaf-associated aquatic hyphomycetes (b), and ergosterol concentration in alder leaves (c) in the three treatments, hyporheic: buried in the sediment (●), benthic-hyporheic: buried after 2-weeks exposure at the sediment surface (◎), and benthic: exposure at the sediment surface throughout the experiment (○). Vertical bars indicate ± 1 SE

At the first sampling date (i.e. before burial of leaf material in the benthic-hyporheic treatment), aquatic hyphomycete communities were very similar between benthic and benthic-hyporheic treatments (Steinhaus similarity index = 0.839, $P = 0.003$). In contrast, these two treatments differed markedly from the hyporheic one (Steinhaus similarity index amounting to 0.219 and 0.270 with P values of 0.962 and 0.930, respectively). The similarity between benthic and benthic-hyporheic treatments slowly decreased thereafter, but remained significant until the third sampling date, and was still high at the fourth sampling date although no longer significant (Steinhaus similarity index = 0.70, $P = 0.101$). At this date, the similarity of the hyporheic treatment with the benthic-hyporheic and benthic ones was only 0.48 and 0.37, respectively. The Shannon-Weaver diversity index observed for benthic and benthic-hyporheic treatments was much higher than that for the hyporheic one denoting lower evenness in the latter community (Table 2). The relative abundance of *Flagellospora curvula* conidia was higher in the benthic treatments, where it almost reached half of the overall conidial production (42-46%). Similarly, the abundance of *Tricladium chaetocladium* was one order of magnitude higher in the benthic and benthic-hyporheic treatments than in the hyporheic one (16-18% *vs* <2%, respectively). The same pattern, although less marked, applied to *Tetrachaetum elegans*. In contrast, *Articulospora tetracladia* was dominant (33%) in the hyporheic treatment, and had a lower abundance (8-10%) in the other two. The greatest difference was found in *Heliscus lugdunensis*, which contributed up to 20% of the overall conidial production in the hyporheic treatment compared to < 1% in the benthic one, and an intermediate 6% for the combined treatment.

Fungal biomass

Freshly collected leaves of alder in the present study contained minute amounts of ergosterol, indicating that fungal colonization was negligible at the beginning of the experiment (Fig. 2c). The ergosterol content in the leaf material in the three treatments was significantly different (ANOVA, $F_{2,34} = 19.43$, $P < 10^{-4}$): the benthic treatment had constantly higher values than the combined treatment, which itself was higher than the hyporheic one at the first three sampling dates. However, only the two extreme treatments differed significantly (benthic *vs* hyporheic, HSD test, $P < 10^{-4}$).

Table 2 Relative abundance (%) of the leaf-associated aquatic hyphomycete species and diversity indices for the three exposure treatments, determined from the cumulative conidial production over the first 4 sampling dates (4 replicate leaf bags combined).

Species	Hyporheic	Benthic-hyporheic	Benthic
Alatospora acuminata Ingold	0.35	0.27	0.37
Alatospora flagellata (Gönczöl) Marvanová	0.09	0.60	0.37
Anguillospora filiformis Greathead	3.78	5.03	4.30
Anguillospora longissima (Sacc. & Syd.) Ingold	0.44	3.16	3.18
Articulospora tetracladia Ingold	33.21	10.35	8.08
Casaresia sphagnorum Gonz. Fragoso	1.62	0.03	0.03
Clavariopsis aquatica De Wild.	0.33	0.54	1.79
Clavatospora longibrachiata (Ingold) Marvanová & Nilsson	1.22	2.50	0.43
Crucella subtilis Marvanová	0.28	0.06	0.30
Culicidospora aquatica Petersen	0.05	1.78	1.69
Flagellospora curvula Ingold	29.79	42.09	46.34
Heliscella stellata (Ingold & Cox) Marvanová & Nilsson	0.46	0.12	0.03
Heliscus lugdunensis Sacc. & Thérry	20.47	5.91	0.65
Lemonniera aquatica De Wild.	1.71	2.93	4.12
Lemonniera cornuta Ranzoni	-	0.04	-
Lemonniera terrestris Tubaki	0.12	0.13	0.23
Mycofalcella calcarata Marvanová, Om-Kalth. & Webster	-	-	0.03
Taeniospora gracilis Marvanová	-	0.03	0.03
Tetrachaetum elegans Ingold	4.12	8.55	9.97
Tricladium chaetocladium Ingold	1.73	15.85	17.99
Tricladium splendens Ingold	-	0.03	0.03
Varicosporium elodeae Kegel	0.18	-	-
Unknown tetraradiate	0.06	-	-
Total number of species	19	20	20
Shannon-Weaver Diversity Index	0.91	1.54	1.53
Shannon-Weaver Evenness Index	0.52	0.67	0.64

The ergosterol content at the sediment surface reached a maximum of 340 µg g^{-1} (i.e. corresponding to a mycelial biomass of 6.2% leaf detrital mass) after 45 days, whereas maxima of 276 and 208 µg g^{-1} (5% and 3.8%) were attained in benthic-hyporheic and hyporheic treatments, respectively. The treatments did not differ, however, with regards to these maximum ergosterol values (ANOVA, $F_{7,40}$ = 1.387, P = 0.238 and HSD test). The maximum ergosterol content for the hyporheic treatment was delayed by 35 days in comparison with the benthic and benthic-hyporheic treatments, even though the latter also showed a strong increase at the final sampling date (Fig. 2c).

Shredders

The shredder abundance differed significantly between treatments (four last sampling dates; ANOVA, $F_{2,33} = 12.37$, $P < 10^{-4}$) and the HSD test indicated that the differences were significant for all comparisons except between the two treatments subject to burial. The highest abundance was associated with benthic leaves and the lowest with hyporheic leaves (Fig. 3a, Table 3). A considerable reduction in the abundance on the benthic-hyporheic leaves was observed following burial (d17), with the densities thereafter resembling those on the hyporheic leaves (Fig. 3a). Apart from this, shredder numbers tended to increase gradually over time, except at the last sampling date (d80) for both benthic and benthic-hyporheic treatments, which showed large increases to 200 and 50 individuals g^{-1} leaf AFDM, respectively.

Table 3 Relative occurrence (%) and biomass (%) of shredder genera associated with alder leaves in the three treatments, hyporheic: buried in the sediment, benthic-hyporheic: buried after 2-weeks exposure at the sediment surface, and benthic: exposure at the sediment surface throughout the experiment (all sampling dates and replicates combined)

	Abundance			Biomass		
	Hyporheic	Benthic-hyporheic	Benthic	Hyporheic	Benthic-hyporheic	Benthic
Average per bag per date	8.8	18.5	21.7	1.1	12.0	59.5
Plecoptera						
Leuctra	91.4	84.1	32.6	83.9	25.5	3.9
Amphinemura	2.9	5.9	29.8	1.9	1.5	3.0
Nemoura	1.1	3.0	2.3	0.2	0.7	0.2
Protonemura	1.7	1.9	16.6	1.4	1.0	1.1
Trichoptera						
Potamophylax	-	0.3	2.8	-	18.8	25.2
Pseudopsilopteryx	-	0.3	-	-	7.0	-
Limnephilinae	-	0.8	0.9	-	0.2	1.4
Micrasema	0.6	-	-	0.1	-	-
Sericostoma	2.3	3.5	14.1	12.5	45.1	60.0
Others						
Tipula	-	-	0.2	-	-	4.8
Gammarus	-	0.3	0.7	-	0.2	0.4

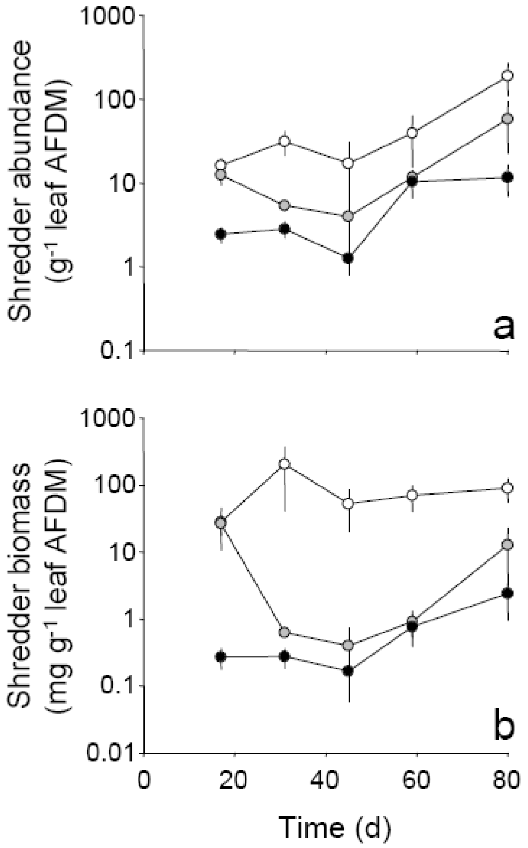

Fig. 3 Total shredder abundance (a) and biomass (b) associated with alder leaves in the three treatments, hyporheic: buried in the sediment (●), benthic-hyporheic: buried after 2-weeks exposure at the sediment surface (◉), and benthic: exposure at the sediment surface throughout the experiment (○). Note the logarithmic scale. Vertical bars indicate ± 1 SE

The same although more contrasted patterns were observed with the total shredder biomass (Fig. 3b, Table 3). The benthic-hyporheic leaves showed similarly intermediate values between benthic and hyporheic ones, being lower and higher by factors of 5 and 10, respectively (Table 3). The peak in shredder biomass observed on benthic leaves at d31 was only found in two replicate bags and was mostly attributable to movements of individuals of *Sericostoma* and *Potamophylax*, i.e. the largest dominant shredder taxa in the stream (Table 3). In contrast, *Leuctra spp.* was a strongly dominating taxa associated with hyporheic leaves, both in terms of biomass and numbers. The structure of the shredder community for the benthic-hyporheic treatment was intermediate between the two others, with the three latter taxa together representing 90% of the total shredder biomass.

The synthetic leaves exhibited the same differences between benthic and hyporheic treatments as natural leaves, although the absolute values were lower by *c.* one order of magnitude (maximum numbers of 13 and 11 individuals, and 29 and 1 mg per leaf bag, respectively). The same patterns were also observed in the community structure.

Carbon budget

The relative contribution of carbon products of leaf decomposition differed substantially among treatments, especially benthic (Fig. 4a) *vs* the other two (Fig. 4b, c). While shredder biomass represented almost 50% of the initial carbon transformed after 80d in the benthic treatment, its contribution was less than 0.3% in the hyporheic one and 2.0% in the combined benthic-hyporheic treatment. In contrast, the cumulative release of microbial C-CO_2 was comparable, with values ranging from 14.3% (hyporheic) and 18.6% (benthic-hyporheic). The maximum mycelial biomass ranged from 2.0 and 2.8% for the hyporheic and combined treatments, respectively. The cumulative conidial production was twice as high in the hyporheic (4.1%) as in the benthic and combined treatments (1.9 and 2.2%, respectively). Overall, the four carbon products represented a total of 21% to 36% of initial leaf carbon transformed, depending on the treatment (Fig. 4). Table 5 shows that these four carbon products accounted for a total of up to 40% of leaf carbon loss at the time of their maximum contribution (i.e. around the end of the decomposition period). The same, although generally more pronounced, patterns as for the budgets of initial leaf carbon were observed. Microbial CO_2 accounted for as much as 28-30% in the two hyporheic treatments while the contribution of the shredders consumption was almost as high as that of microbial respiration in the benthic treatment. Mycelial and conidial production in the permanently hyporheic environment accounted for 12% of leaf mass loss, i.e. 2-3 fold more than in the two other conditions.

Discussion

Benthic versus hyporheic litter breakdown rates

Our results indicate that the decomposition of alder leaves was strongly affected by burial in the stream sediment, with rates being 2.8 and 2.1 higher in the benthic habitat than when buried either for the whole period or after 2-weeks initial exposure at the sediment surface, respectively. The few studies that have compared leaf litter decomposition rates in benthic and hyporheic habitats have provided contradictory results, although most of them have also shown a reduced decomposition in the hyporheic zone. In line with the present study, Metzler & Smock (1990) reported very low decomposition rates from leaves of black gum, red maple and water ash buried in the substratum of a first-order Coastal Plain stream with a sand bottom. Likewise, leaves of silver maple and cottonwood were found to decompose 2.7 to 5.1 faster at the sediment surface than when buried in a headwater stream (Herbst, 1980). In contrast, Smith & Lack (1993) did not observe significant differences in decomposition rates for *Eucalyptus viminalis* leaves in an Australian stream, and Mayack *et al.* (1989) in South Carolina reported a reduction in decomposition rates of sweetgum in winter but not in spring where decomposition was, on the contrary, increased by burial. Such discrepancies from the literature are probably linked to the substratum and hydrological conditions together with the biological characteristics of the stream biota, like in the latter study where the activity of tipulid shredders was responsible for the increased decomposition in the spring. Overall, the results support the conclusion that leaf decomposition is generally depressed in the hyporheic zone, which leads to speculation about the causes and mechanisms of such an effect.

Physical constraints

An examination of Fig. 1 reveals lower coefficients of variation in loss of leaf mass, indicating that the decomposition process was more stable over time within rather than above the sediment, and this also holds for leaves buried after a short exposure at the sediment surface. We suspect that the reduced mechanical abrasion in the "protected" hyporheic habitat is at least partly responsible for the slower decomposition of buried leaves and also explains the reduced variability of leaf mass loss.

This effect of current velocity on the breakdown rate of leaf litter has been reported in many situations (e.g. Chergui & Pattee, 1988; Canton & Martinson, 1990; Heard *et al*., 1999), with the study by Ferreira *et al.* (2006) being an exception.

Burial within the substratum can also reduce the access of decomposers to leaves with the small interstices of the gravelly sediment acting as a constraint, especially for the largest shredders. Within a few centimetres, the sediment of the hyporheic zone constitutes a physical barrier, allowing only the taxa with appropriate morphological characteristics (i.e., smaller, narrower, more flexible) to penetrate to the deeper layers (Omesová, Horsák & Helešic, 2008). Indeed, shredders were particularly rare both in numbers and biomass in the hyporheic zone although they were abundant on leaf detritus at the surface, and the only taxa with the appropriate morphology to penetrate the substratum in the present study were also the less efficient decomposers (e.g. *Leuctra spp.*).

Oxygen limitation and biotic responses

Not only physical parameters but also the related chemical characteristics such as dissolved oxygen concentration may explain the observed patterns. Indeed, Strommer & Smock (1989) observed a significant correlation between dissolved oxygen concentration and invertebrate abundance, and Strayer *et al*. (1997) found a significant relationship between levels of dissolved oxygen, sediment grain size, organic matter concentration and the density of hyporheic animals. Shredders probably were limited in the subsurface sediments by low oxygen concentrations, unsuitable substratum and a lower palatability of buried *vs* surface leaves.

Like the shredder biomass, leaf-associated fungal biomass was strongly affected by burial in our study. Crenshaw, Valett & Tank (2002) found a significantly reduced fungal biomass on woody debris decomposing in the hyporheic zone compared to the benthic zone of a headwater mountain stream. The lower dissolved oxygen concentrations in the hyporheic zone probably explained this reduction in fungal biomass. Medeiros, Pascoal & Graça, (2009) recently reported a decrease in fungal biomass associated with decomposing alder leaves from 99 to < 30 mg g^{-1} leaf AFDM together with a comparable reduction in fungal sporulation rate, at exposures of 94% and 76% of O_2 saturation, respectively.

Dissolved oxygen is a limiting factor for biological colonization and activity in the interstitial habitats, with its depletion leading to qualitative and quantitative changes in macroinvertebrate and microbial assemblages (Ward *et al*., 1998).

The composition and structure of fungal assemblages were also strongly affected by burial in sediment, which suggests a better adaptation of some aquatic hyphomycete species to the physical and chemical conditions prevailing in the hyporheic zone. *H. lugdunensis* and *A. tetracladia*, for example, seem to be relatively favoured in such conditions, in contrast to *F. curvula* and *T. chaetocladium*. Whether these species respond differently to depletion of oxygen concentrations is currently unknown. However, the initial stage of fungal inoculation was clearly of major importance depending on whether it occurs in the stream flow, i.e. with abundant and highly diverse spores, or in the quantitatively and qualitatively impoverished hyporheic habitat. This led to diverging communities, potentially differing in enzymatic capacities and performance in litter breakdown.

Relative contribution of benthic and hyporheic decomposers

Decomposition rates of leaf litter buried in sediment whether or not initially exposed for two weeks at the sediment surface were similar and markedly low compared with the benthic habitat. This observation shows that microbial decomposers did not fully compensate for the lack of shredders, and underlines the fundamental role of macroinvertebrates as mediators of leaf litter processing. The carbon budgets drawn from our study however indicated that the relative contributions of shredders and fungi varied considerably with the location within the streambed. While this variation was obviously related to the unconstrained abundance of shredders in the benthic zone, trophic interactions in both habitats were also modified. Shredders are known to act both as competitors and predators of fungi, due to their preferential feeding on leaf patches colonized by fungi, thus reducing microbial activity (Bärlocher, 1980; Suberkropp 1992, 1998). The lack of shredders in the hyporheic zone virtually suppressed the competition between the two decomposers and allowed fungi to grow and decompose the leaf litter to a higher extent than in the benthic habitat. Over the whole decomposition period a much higher fungal contribution than shown in Fig. 4 and Table 4 is even expected in the hyporheic habitat.

This is discernible when extrapolating data beyond the final sampling date, i.e. at a time where the 50% leaf matter remaining can sustain further fungal growth in contrast to the benthic habitat where the decomposition budget is nearly completed. From Fig. 4 it is thus expected that fungal decomposers in the hyporheic zone compensate, if not fully, at least partly for the action of invertebrate detritivores in the benthic zone. At late decomposition stages, *i.e.* in spring and summer at a period of high biological activity and limitation of resource on the streambed, the buried leaf litter may also become essential in supporting a portion of the invertebrate community (Mayack *et al.*, 1989). In such conditions, we hypothesize the leaves buried after exposure at the sediment surface to be preferentially consumed by shredders in comparison to constantly buried leaves, due to their higher fungal colonization and palatability. This highlights the fundamental role of the history of leaf litter, i.e. the sequence of events responsible for multiple microbial assemblage scenarios, which in turn can alter leaf litter processing.

Table 4. Relative contribution (%) of the leaf decomposition carbon products to carbon mass loss, derived from microbial activity and shredders consumption for the three treatments after 59 or 80 days.

	Hyporheic	Benthic-hyporheic	Benthic
Time (d)	80	80	59
Fungal mycelia	3.8	2.4	1.9
Fungal conidia	8.0	3.5	2.2
Microbial CO_2	27.7	29.5	19.2
Shredders	0.5	3.3	17.1

Links between benthic and hyporheic food webs

In natural environments, the community structure is in part driven by requirements for the resource, which is distributed heterogeneously in space and time (Bulling *et al.*, 2008). However, Strayer *et al*. (1997) suggest that the influence of organic matter depends on, and is sometimes outweighed by, other mechanisms and processes determining the number and composition of co-occurring species in a local environment. For instance, the variability of pore sizes in the three dimensions of the hyporheic zone result in complex fluid distribution patterns (Zilliox, 1994).

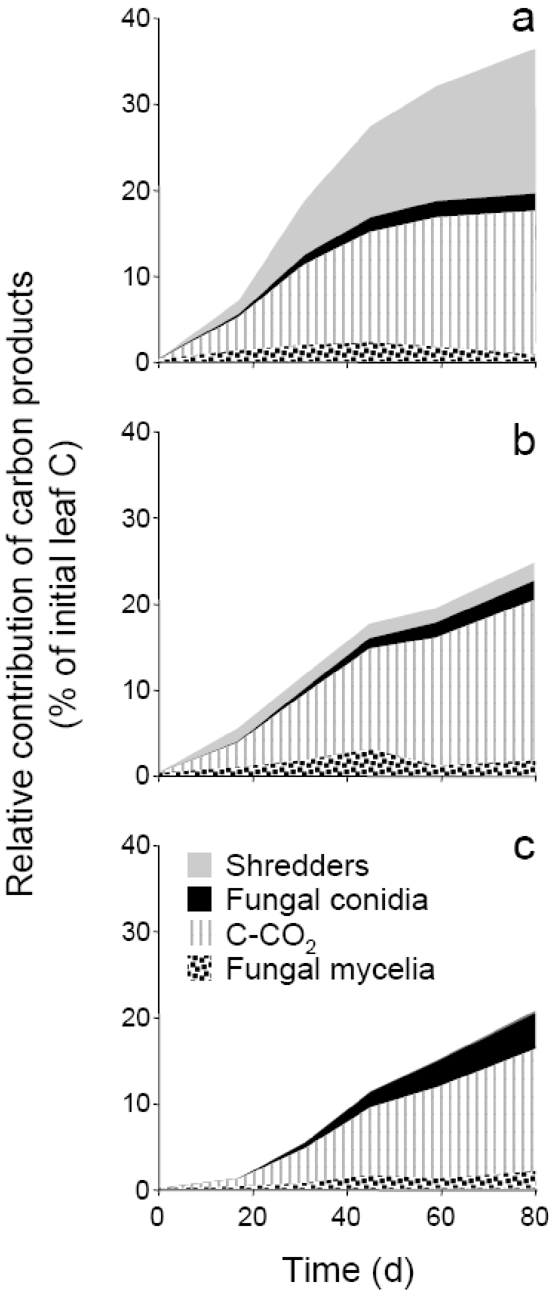

Fig. 4 Changes in the relative contributions of shredders consumption and microbial products to leaf decomposition during decomposition for the three treatments: (a) benthic, (b) benthic-hyporheic, and (c) hyporheic. Values are expressed as percentage of initial leaf carbon.

These spatial complexity and heterogeneity are reflected in the dispersion patterns of invertebrates. Our results lead us to conclude that at this site the food sources, whether leaf litter or fungal biomass, are not as determinant for controlling hyporheic invertebrate distribution as other site-specific factors such as the characteristics of sediment and water physicochemical parameters. Clearly, the functioning of surface and hyporheic sub-systems is interdependent, being linked through hydrologic exchange as a transport vector for materials and nutrients (Jones, Fisher & Grimm, 1995). In hyporheic zones with abiotic characteristics of the sediment matrix less limiting to invertebrates and biotic interactions (e.g. large pore), a greater involvement of macroinvertebrates in leaf litter processing is expected as suggested by the findings of Brunke & Fischer (1999) and Navel *et al.* (2010). Physically, the higher interstitial water flow rates at such sites may allow greater access for invertebrates to migrate to deeper layers. Due to a higher channel water infiltration and a shorter retention time of hyporheic water, chemical conditions may be less constraining as well. Extrapolation from decomposition rates indicated a required incubation period of 255 and 341 days for the benthic-hyporheic and hyporheic treatments, respectively, to reach the same decomposition stage as in the benthic treatment after 80 days (i.e. 3% of mass remaining). This suggests that the hyporheic zone of woodland streams is an important compartment for organic matter storage with the release of decomposition products being extended for up to one year for fast-decomposing leaf species. Depending on the structure and porosity of the sedimentary matrix together with the amount, quality and seasonality of litter input, this temporary reservoir may differently affect the overall stream metabolism. Compositional changes in species assemblages, as well as their relative abundance and biomass resulting from limiting dispersal conditions are likely to affect leaf litter decomposition. In addition to providing organic sources for the stream food web, plant matter decomposing in the hyporheic zone sustains a substantial fungal biomass that can serve as a complementary food source and as a source of fungal inoculum. Fungal conidial production in the hyporheic zone was surprisingly high, but whether this is linked to changes in the metabolic strategy of fungi in adverse conditions is questionable. Nevertheless, it remains likely that the high release of spores by fungal species partly differing from those dominating the benthic habitat constitutes a major trait of hyporheic decomposition of leaf litter, with potential relevance for the overall stream functioning.

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Chapitre 3:

Multiple screening of aquatic hyphomycetes in the hyporheic zone of woodland streams

3. Multiple screening of aquatic hyphomycetes in the hyporheic zone of woodland streams

3.1. Résumé

Les résultats issus du chapitre 2 ont permis de mettre en évidence le rôle fondamental des champignons en tant que médiateur principal du processus de décomposition de la matière organique d'origine végétale dans la zone hyporhéique. Par ailleurs, nous avons également vu que le compartiment hyporhéique, du fait de ses caractéristiques physico-chimique et biologique particulières, constituait localement un site important de stockage de la matière organique dans les cours d'eau forestiers et pouvait servir de réservoir d'inoculum fongique. Il est suggéré que les spores ou autres propagules fongiques pourraient se déplacent assez facilement dans les sédiments constituant le lit des cours d'eau, et qu'elles pourraient ainsi établir des colonies sur les substrats qu'elles rencontrent. A ce jour, cette hypothèse demeure inexplorée et un déficit de connaissance important persiste dans notre compréhension des voies de dispersion verticale et de colonisation de nouveaux substrats par les hyphomycètes aquatiques dans les conditions contraignantes de la zone hyporhéique.

Nous avons expérimentalement testé l'hypothèse selon laquelle l'habitat hyporhéique pourrait agir comme une barrière physique en filtrant sélectivement les spores de champignons provenant des eaux de surface en fonction de leur taille et de leur morphologie. En outre, la diminution de la taille des interstices dans le sédiment pourrait influencer spécifiquement l'efficacité de dispersion des conidies d'hyphomycètes aquatiques dans la zone hyporhéique. Enfin, les conditions physico-chimiques de l'habitat hyporhéique, et en particulier le faible approvisionnement en dioxygène dissous, devraient conduire à d'importants changements dans la composition et la structure des communautés fongiques et/ou dans la capacité de sporulation des différentes espèces.

Dans ce contexte, la présence d'hyphomycètes aquatiques à travers l'identification des conidies présentes dans l'eau interstitielle de la zone hyporhéique de différents cours d'eau a été déterminée.

En parallèle, des feuilles d'aulne glutineux ont été inoculées pendant 17 jours dans un cours d'eau de premier ordre, pour ensuite être mises à incuber pendant 57 jours, dans deux types de microcosmes : microcosmes aérés (simulant les conditions environnementales de la zone benthique de petits cours d'eau) et colonnes sédimentaires (mimant la zone hyporhéique).Ceci a permis de déterminer dans chaque cas la contribution relative des différentes espèces d'hyphomycètes à la production conidienne. Enfin, une autre série d'expériences en microcosmes a permis la manipulation de la granulométrie des sédiments et l'évaluation de l'influence des traits morphologiques des conidies sur leur efficacité de dispersion au sein de la matrice sédimentaire.

L'abondance et la diversité des hyphomycètes aquatiques provenant de l'eau interstitielle des zones hyporhéiques des trois cours d'eau étudiés se sont révélées très inférieures à celles observées dans les eaux de surface, avec quelques espèces qui, relativement aux autres, tendent à être favorisées par de telles conditions. Les expériences au laboratoire ont montré que la production conidienne dans les colonnes sédimentaires était majoritairement due à deux espèces, tandis que cinq espèces étaient co-dominantes dans les microcosmes aérés. La différence la plus marquée concerne l'espèce *Flagellospora curvula* qui a contribué jusqu'à 68% de la production globale de conidies dans les colonnes sédimentaires contre moins de 17% dans les microcosmes aérés. Dans l'expérience de criblage par le sédiment, le nombre de conidies avait été réduit de près de 85% entre le sédiment le plus grossier (4-8 mm) et l'intermédiaire (2-4 mm), et d'environ 95% entre le plus grossier et le plus fin (1-2 mm). En outre, des différences très significatives existent entre les espèces d'hyphomycètes aquatiques, avec une efficacité de dispersion systématiquement plus élevée pour les espèces présentant des spores filiformes/sigmoïdes, en comparaison avec d'autres spores de forme compacte ou ramifiée/tétraradiée.

Cette étude révèle donc un certain nombre de mécanismes potentiellement impliqués dans la structuration des communautés de champignons de l'habitat hyporhéique des cours d'eau forestiers. Elle montre que la contribution relative des différentes espèces à la production conidienne peut être modifiée en milieu hyporhéique.

Elle souligne également l’importance de la porosité du sédiment, et des caractéristiques intrinsèques des conidies, dans les processus de dissémination des hyphomycètes aquatiques au sein de la zone hyporhéique.

3.2. Abstract

1. In headwater woodland stream ecosystems, the input of allochthonous organic matter from the riparian vegetation is their major source of energy and nutrients. During autumnal storms, abundant coarse particulate organic matter accumulates on the stream bottom at that time may be buried deeper, thus forming a substantial storage pool that may be released gradually over the year. Consequently, the hyporheic zone is a major site of organic matter processing within the riverine ecosystem.

2. Fungal decomposers are important mediators in the energy and nutrient transfer to higher trophic levels, and more generally in the ecological functioning of headwater streams. It is suggested that spores or other fungal propagules move relatively easily through the streambed sediment, and may establish colonies on the substrates they encounter. However, this hypothesis remains unexplored and a significant knowledge gap persists in our understanding of vertical dispersal pathways and colonization of new substrates by aquatic hyphomycetes in the constrained conditions of the hyporheic habitat.

3. We tested experimentally the hypotheses that the hyporheic habitat acting as a physical barrier can filter out unsuccessful strategists regarding conidial size and shape from the potential pool of species, and that a decreased pore size of the interstices selectively influences the dispersal efficiency of aquatic hyphomycetes conidia, both processes lowering the density and diversity of aquatic hyphomycete conidia in the interstitial water. Finally, the specific conditions prevailing in the hyporheic habitat, particularly the low dissolved oxygen supply is expected to lead to strong changes in the structure of fungal community.

4. The occurrence of aquatic hyphomycetes by identifying conidia present in interstitial water collected at different streams within the hyporheic zone has been determined. Moreover, experimental approaches in microcosms have allowed manipulation of sediment structure characteristics and investigation of the influence of conidia life-history traits on their dispersal efficiency within sediment columns. In parallel, senescent leaves of *Alnus glutinosa* immersed for 17 days in a first order stream, for fungal colonization, were then incubated for 57 days in both slow filtration columns and stream-simulating microcosms and fungal diversity was determined.

5. The abundance and diversity of aquatic hyphomycetes drastically decreased in the interstitial water form the hyporheic zone of the three study streams, with a few species tending to be relatively favoured by such conditions.

Average proportion of conidia able to disperse within the sediment was found to dramatically decrease with decreasing availability of the sediment interstice size, the number of conidia was reduced by almost 85% between the coarsest sediment (*i.e.* S1: 4-8 mm) and the intermediate one (S2: 2-4 mm), and almost 95% between the coarsest and the finest one (S3: 1-2 mm). The order of magnitude of these values was corroborated and relatively closed to data from the field. However, highly significant differences existed among aquatic hyphomycete species, with dispersal efficiency of species exhibiting filiform/sigmoid conidia being always significantly higher in comparison with others displaying compact and branched/tetraradiate conidia. In addition, this pattern was consistent across the minimal obstruction size range of tested conidia.

6. Generally, fungal communities associated with alder leaf discs from the slow filtration columns were dominated by only two species whereas five species were co-dominant on leaf discs from the stream-simulating microcosms. The greatest difference was found in *Flagellospora curvula* which contributed up to 68% of the overall conidial production in the slow-filtration column treatments compared to < 17% in the stream-simulating microcosms.

7. This study highlights the importance of the interstices availability in streambed sediment, as well conidial shape and size and additional properties of aquatic hyphomycete conidia in determining dispersal efficiency into the sediment layers and they point to the potential role of these factors in structuring fungal communities in the hyporheic habitat of streams.

3.3. Introduction

Shade by the riparian vegetation considerably depresses the primary productivity of small headwater woodland streams. Therefore, the important input of allochthonous organic matter from the riparian vegetation is the major source of carbon and energy for these aquatic ecosystems (Fisher & Likens 1973 ; Kaushik & Hynes 1968 ; Webster & Meyer 1997). A substantial part of leaf litter entering running waters may be buried into the streambed during storms (Herbst 1980) or as a consequence of flooding and sediment movement (Metzler & Smock 1990 ; Naegeli *et al.*, 1995). Thus, the hyporheic zone of streams is potentially a major site of organic matter storage. Aquatic heterotrophic microorganisms, especially aquatic hyphomycetes – a phylogenetically heterogeneous group of fungi, are crucial for the mineralization of leaf litter and also for the conditioning of leaf litter to improve its nutritional quality for invertebrate detritivores (Kaushik & Hynes 1971 ; Bärlocher & Kendrick 1976 ; Bärlocher 1985 ; Suberkropp 1992). Consequently, aquatic hyphomycetes are important mediators in the energy and nutrient transfer to higher trophic levels (Gulis *et al.* 2009), and more generally in the ecological functioning of headwater streams.

During plant litter decomposition, fungal biomass on decaying leaves may reach 17% of total detrital mass (Gessner 1997). Several studies estimated that *ca.* 50% of the total fungal production is allocated to the formation of conidia (Findlay & Arsuffi 1989 ; Suberkropp 1991 ; Sridhar & Bärlocher 2000), that are asexual reproductive spores. This can result in conidial concentrations in headwater streams that can reach several thousands per litre during autumn-shedding of leaves (Iqbal & Webster 1973 ; Webster & Descals 1981 ; Bärlocher 1992). Gessner (1997) estimated that 20 g of conidial mass pass daily through a cross-section of a small stream (discharge of 60 L s^{-1}). Similarly, on an annual basis, Suberkropp (1997) calculated that the total amount of fungal biomass as spores exported from a low-nutrient stream with a base flow of 4-5 L s^{-1} was 375 g dry mass. Undoubtedly, conidia are the dominant propagules responsible for the rapid expansion of aquatic fungi after the annual leaf fall in temperate streams (Bärlocher 2009) and their release appears as being the predominant mechanism involved in the colonization of distant leaf patches (Sridhar & Bärlocher 1997).

This huge part of hyphomycete production allocated to the formation of asexual reproductive spores suggests that the colonisation of new substrata via conidia is crucial for their continued existence in a stream (Kearns & Bärlocher 2008).

Unlike most terrestrial fungi, aquatic hyphomycetes show a variety of characteristic conidial shapes, that can be compact, filiform or branched (Dang 2007). The most obvious adaptation of aquatic hyphomycetes to their habitat is the size and morphology of their conidia (Webster 1987). Many of them are either tetraradiate, branched or filiform and exceed 100 µm in length (although small ovoid conidia also exist). The unusual features of aquatic hyphomycete conidia are therefore interpreted as an adaptation to the turbulent stream environment (Webster 1987 ; Webster & Davey 1984), to minimise downstream displacement (Bärlocher 1992). Moreover, conidia of aquatic hyphomycetes are not designed as a resting stage (Dang 2007), and are relatively fragile. Thereby, they can quickly lose their ability to germinate, even under benign conditions (Sridhar & Bärlocher 1994).

Although there can be intense water transfers in the hyporheic zone of headwaters (Boulton, 2000), sediment properties will largely determine the displacement ability of any suspended matter, including hyphomycete conidia. In addition, the specific conditions prevailing in the hyporheic zone, particularly low dissolved oxygen supply, are likely to considerably influence the development and sporulation of aquatic hyphomycetes. Bärlocher *et al.* (2006) suggest that spores or other fungal propagules move relatively easily through the streambed sediment, and may establish colonies on the organic substrates they encounter. However, this hypothesis remains unexplored, and a significant knowledge gap persists in our understanding of vertical dispersal pathways and colonization of new substrates by aquatic hyphomycetes in the constrained conditions of the hyporheic habitat.

The aim of the present study was to determine how the hyporheic habitat can filter out unsuccessful strategists from the pool of benthic aquatic hyphomycete species, and thereby control fungal community composition within sediment. For this purpose, the abundance and identity of aquatic hyphomycete conidia circulating in interstitial water was examined in different streams within the hyporheic zone, and compared to the conidial pool in surface water. In parallel, laboratory experiments were conducted to try to explain the field patterns observed.

Because the circulation of conidia in the hyporheic zone may depend both on their intrinsic dispersal ability (i.e. ability to cope with physical filtering) and on the intensity of endogenous spore production (i.e. ability to cope with physiological filtering), we addressed these two potential filtering mechanisms in dedicated microcosms. The following hypotheses have been tested: (1) the density and diversity of aquatic hyphomycete conidia should be lower in the hyporheic zone than in the surface water; (2) the ability of hyphomycetes to develop and sporulate within the hyporheic zone should be reduced compared to benthic habitats, and should differ across species; (3) the dispersal ability of conidia through the sedimentary matrix should be influenced both by sediment grain size and by conidial traits.

3.4. Materials and methods

Aquatic hyphomycete spores circulating in the hyporheic zone

Study sites

The experiment was carried out in the Montagne Noire, South-Western France, a 1,450-km^2 region covered by a mixed broadleaf forest with an altitudinal range of 250–1211 m a.s.l. Climatic conditions are marked by high rainfall (average 1,500 mm year^{-1}). Three 1st- or 2nd-order permanent streams with similar physical and chemical characteristics were selected in forested areas, situated between 02°09′25′′E and 02°19′51′′E longitude and 43°21′15′′N and 43°27′03′′N latitude. In all catchments, forestry was the only anthropogenic disturbance, although limited within the study area (Laitung *et al.* 2002). The Bergnassonne and the Béal, on the South-Western side of the study area, flow through a well-shading riparian vegetation composed predominantly of oak (*Quercus petraea* (Mattus.) Liebl.), hazel (*Corylus avellana* L.), alder (*Alnus glutinosa* (L.) Gaertn.), ash (Fraxinus excelsior L.), sweet chestnut (*Castanea sativa* Mill.) and beech (*Fagus silvatica* L.). The Orbiel, on the North-Eastern side, flows through a riparian vegetation composed predominantly of oak, alder, hazel and beech.

Stream characterisation

Water chemistry was determined at two sampling dates in three representative riffles per stream. Temperature, pH, conductivity and dissolved oxygen concentration were measured in the field using portable instruments (pH-meter 320i and Oxi 330i, WTW, Weilheim, Germany; Conductimeter Dist5, HANNA, Woonsocket, Rhode Island, USA).

Water samples were filtered in the field with 0.7 µm glass fiber filters (Glass fibre GF/F, Whatman, Clifton, New Jersey, USA), stored in pre-rinsed polyethylene bottles, and placed in an icebox until they were returned to the laboratory. Concentrations of P-PO_4^{3-}, measured as soluble reactive phosphorous (SRP), N-NO_3^-, N-NO_2^-, and N-NH_4^+, were measured using standard colorimetric methods by flow injection analysis with an Alpkem Flow Solution IV system (OI Analytical, College station, Texas, USA). Alkalinity was determined using potentiometric titration.

The sediment characteristics were studied from the grain-size fractionation of three sediment cores (20 cm length x 16 cm diameter) sampled from downwelling zones in the middle of three representative riffles of each stream. At the laboratory, each core layer was meticulously washed and wet-sieved under tap water through a series of mesh sizes (20 mm, 10 mm, 5 mm, 2 mm, 1 mm, 0.5 mm and 0.25 mm) for grain size analysis. Once the organic matter had been removed from each grain size fraction, the remaining sediment was placed into a oven at 60°C until constant dry weight was reached for each fraction. Mean water temperature patterns in the three streams were similar (Benthic: Béal = 9.1°C; Bergnassonne = 7.6°C; Orbiel = 6.2°C; Hyporheic: Béal = 9.5°C; Bergnassonne = 8.2°C; Orbiel = 7.6°C). The streams had very similar geomorphologic characteristics, especially in terms of water depth (≤ 0.40 m) and width (≤ 2.30 m). They had an average discharge ranging between 81.5 and 137 L s^{-1}. The stream-bed substrata were unconsolidated and mostly made of coarse sediments as determined from fractionation of the grain size of sediment cores for the Bergnassonne and the Orbiel, while the Béal had a stream-bed substratum comprising a mixture of gravel and coarse sand (Fig. 1).

The three stream waters were slightly acidic (pH 6.0-7.0) with low conductivity (28-58 µS cm^{-1}) and relatively low buffering capacity (3.1-9.6 mg CaCO3 L^{-1}). Concentrations of SRP (2.0-4.2 µg L^{-1}) were low, while concentrations of N-NO_3^- (0.338-1.749 mg L^{-1}) were relatively high. The stream was always well oxygenated (97-102 % of the saturation). Physical and chemical conditions in the hyporheic zone (*i.e.* at 15 cm below the sediment surface; interstitial water pumped from a specific sampling device using a hand-held vacuum pump – see below) were comparable to those at the surface level, except for dissolved oxygen (51-79 %) and SRP (2.0-8.5 µg L^{-1}) that were usually lower and higher, respectively, in the hyporheic zone.

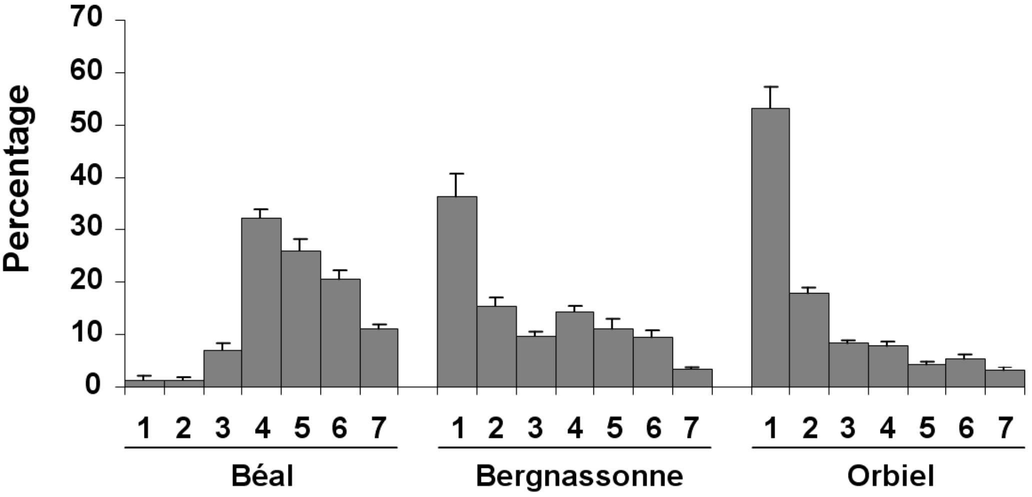


Fig. 1 Frequency distributions of mean masses (+ SE bars) of seven size-classes of sediments (1 = >20 mm; 2 = 10-20 mm; 3 = 5-10 mm; 4 = 2-5 mm; 5 = 1-2 mm; 6 = 0.5-1 mm; 7 = 0.25-0.5 mm) in the three riffles (six samples per riffle) in the Béal, Bergnassonne and Orbiel.

Construction and placement of sampling devices

Fifty-four cylinders of PVC (10 cm length, 12 mm inner diameter) were cut and pierced (2.5 mm diameter hole × 50), and capped at bottom with a plastic stopper. A length of plastic tubing (Tygon, 5 mm inner diameter) was hermetically sealed at the top of each cylinder to allow sampling of interstitial water for spores and water chemistry, and kept isolated from surface water with a clamp.

Six cylinders were carefully introduced in the stream sediment at the head of three representative riffles of each stream (downwelling zone), by using a piezometer pushed to 20 cm depth into streambed using an internal metallic rod, as described in Baxter et al. (2003). The middle of each cylinder was approximately 15 cm below the streambed surface, and were left several months to allow the initial hydraulic properties to stabilise, thereby avoiding any bias due to exchanges between surface and interstitial waters. At each sampling dates, four from six cylinders per riffle were randomly chosen. One of them was used for water chemistry, and the three others to characterise fungal communities. 250 mL of interstitial water was slowly collected from cylinders with a hand vacuum pump placed at the end of the Tygon tubing (5 mm inner diameter).

Spore sampling

Aquatic hyphomycete spores were collected from the benthic (water column) and hyporheic (interstitial water) zones of the riffles studied. Three replicate samples per riffle and per zone were taken on each occasion. Samples of spores were collected by filtering on site 250 mL of stream water through a membrane filter (5 μm pore size, diameter 47 mm; Millipore Corporation, Bedford, Massachusetts, USA). The filters were fixed and stained with a 60% lactic acid and 0.1% Trypan blue solution (Iqbal & Webster 1973). All filters were examined microscopically at 200× magnification and spores present were counted and identified to species. Conidial density (number of spores per litre of stream water) and species richness (number of species per zone and per stream riffle) were determined. The Simpson's dominance index and the Simpson's diversity index were computed for fungal communities associated with each of the two zones for the three streams.

Effects of hyporheic constraints on spore production by aquatic hyphomycete species

Stream inoculation

The stream inoculation was performed in the Montauds, an oligotrophic 1st-order stream of the Montagne noire in South-Western France (43°28′26′′ N, 2°15′46′′ E; elevation 580 m a.s.l), running through a mixed broadleaf forest of beech (*Fagus silvatica* L.), sweet chestnut (*Castanea sativa* Mill.), hazel (*Corylus avellana* L.), oak (*Quercus petraea* (Mattus.) Liebl.), alder (*Alnus glutinosa* (L.) Gaertn.), wild cherry (*Prunus avium* (L.) L.), and holly (*Ilex aquifolium* L.).

At baseflow, the stream was 1-3 m wide and 0.1-0.2 m deep in riffles. During a previous 12-month period, stream water was slightly acidic (pH 6.5-6.9) with low conductivity (21-29 µS cm-1) and low buffering capacity (3-5.4 mg CaCO3 L-1). Stream displayed a relatively elevated concentration of N- NO_3^- (0.85-1.29 mg L-1) while concentration of SRP (0-2.9 µg L-1) was low. Stream water had a temperature ranging between 3.1-18.1°C and the average discharge was ca. 29 L s-l (Laitung & Chauvet 2005). The bed substratum was unconsolidated and mostly made of a mixture of coarse and fine sand, with sporadic boulders.

The area was subject to a temperate climate with oceanic influences marked by high rainfall in the autumn and winter (1,200 mm year^{-1}). The mean monthly temperatures ranged from 3.0 to 20.2°C.

Alder leaves were collected from trees at abscission using nets in the autumn 2007, and dried at room temperature. Alder leaves were individually placed in litterbags (12 x 12 cm, 1-mm mesh size), which were attached to an iron bar driven into the streambed of the Montauds. The field study was initiated on 22 February 2008 by introducing 8 rows, each with four bags. After 17 days of stream exposure, leaf bags were retrieved for the laboratory study.

Microcosms, medium and experimental set up

Leaves retrieved after 17-days stream exposure were individually rinsed with stream water and cut into discs (15 mm diameter) avoiding the central vein, with a cork borer. Batches of 15 alder leaf discs were prepared and placed under a layer of filtered (Whatman glass fibre GF/F) stream water to avoid desiccation. Batches of 15 alder leaf discs chosen at random were enclosed in circular fine mesh bags (9.6 cm diameter, 1-mm mesh size) and were placed either at 10 or 30 cm below the sediment surface in slow filtration columns (50 cm height, 10 cm diameter). Each column was filled to a height of 40 cm with gravel (4-10 mm) from Garonne River following similar procedure as previously (see above, *Spore dispersal efficiency in the hyporheic zone*). Openings at different depths in each column allowed water sampling (Mermillod-Blondin *et al.*, 2000).

After setting-up sediment and leaf discs, columns were supplied from above with mineral salt solution using a peristaltic pump. Interstitial water velocity was fixed to 3.48 cm per hour in columns, in accordance with values reported in the hyporheic zone of streams (Triska *et al.* 1993; Morrice *et al.* 2000). Supplied water was aerated to maintain concentrations of dissolved oxygen (O_2) at 9 mg L^{-1} ± 0.5 at the inlet of the columns throughout the experiment. For each column, infiltration flow rate was determined to obtain a similar retention time of water across all experimental devices. Three other batches of 15 alder leaf discs from leaves collected after 17-days stream exposure were placed in stream-simulating microcosms (see above, *Preliminary production of spores*) and mineral salt solution (40 mL) was added to microcosms through the open top which was otherwise closed with a glass lid. The experiments were performed at a constant temperature of 15 ± 0.5°C in the dark. We kept both types of microcosms in the dark to suppress photoautotrophic processes.

Relative spore production of aquatic hyphomycete species

Every two days, when renewing the mineral salt solution of the stream-simulating microcosms, suspensions of conidia released from leaf discs were transferred and stored into 50 mL centrifuge tube, the sample volume was adjusted to 45 mL with 2 mL of 37% formalin and distilled water.

Every four days, water circulation in the slow filtration columns was shunted at each treatment depth (*i.e.* 10 and 30 cm), just below the leaf litter fine mesh bags to collect 45 mL of water into 50 mL centrifuge tube and preserved with 2 mL of 37% formalin. During conidial suspension sampling, a tubing system installed in the slow filtration columns just below the leaf litter fine mesh bags was used to minimize conidia loss. 0.5 mL of Triton X-100 (0.5% solution) was added to the spore suspensions and they were stirred gently to ensure uniform distribution of spores.

The relative spore production of the different hyphomycete species was assessed from the total spore output. The spore suspensions of each microcosm resulting from multiple changes of the medium (stream-simulating microcosms) or spore suspension samples (slow filtration column) were combined. 5 mL aliquots were then filtered through a membrane filter (5 µm pore size, diameter 25 mm; Millipore Corporation), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid (Iqbal & Webster 1973). Spores were counted and identified under the microscope (× 200).

Dissolved oxygen measurement

Dissolved oxygen (O_2) concentrations were measured every four days in the slow-filtration columns, with a time lag of one day with spore sampling. Water circulation in the slow filtration columns was shunted at each treatment depth (*i.e.* 10 and 30 cm below the sediment surface) by openings situated just below and above the leaf litter fine mesh bags and at the input and output of the devices.

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

Spore dispersal efficiency across the sediment

Preparation of spore suspensions

To encompass the different conidial sizes and shapes (*e.g.* compact, filiform, branched) exhibited by aquatic hyphomycetes, seven species were used. Strains were isolated from single conidia trapped in naturally occurring foam, or released from leaf accumulations, collected from neighbouring lowland streams of the Montagne noire and the Pyrenees (south-western France): *Heliscus lugdunensis* Saccardo & Théry (HELU), *Lemonniera terrestris* Tubaki (LETE) and *Tricladium chaetocladium* Ingold (TRCH) from the Orival (02°05′25′′E, 43°26′18′′N, elevation 460 m a.s.l); *Tetracladium marchalianum* de Wildeman (TEMA) from the Malcoustat (02°05′11′′E, 43°26′05′′N, elevation 470 m a.s.l); *Articulospora tetracladia* Ingold (ARTE) from the Lampy (02°11′15′′E, 43°25′06′′N, elevation 709 m a.s.l); *Tetrachetum elegans* Ingold (TEEL) from the Montauds (02°15′42′′E, 43°29′14′′N, elevation 560 m a.s.l); *Flagellospora curvula* Ingold (FLCU) from the Remillassé (01°05′26′′E, 42°56′35′′N, elevation 530 m a.s.l).

Growing colonies were kept at 15 °C, in Petri dishes with 10mL of 2% malt agar, until they were used for conidial production. Sporulation of aquatic hyphomycetes was induced by incubation of agar plugs of 7- to 14-days-old colonies in stream-simulating microcosms designed for studying fungal leaf decomposition, as described by Suberkropp (1991). Each microcosm consisted of a 50 mL glass chamber connected from the bottom to an aeration tube, which provides a continuous cotton-filtered air flow (100 mL min^{-1}), and creating turbulence, keeping the leaf discs in permanent agitation. A tap at the bottom allowed for the aseptic drainage of the chamber and recovery of the spore suspension. Fresh mineral salt solution (40 mL) consisting of 100 mg $CaC1_2{\cdot}2H_2O$, 10 mg $MgSO_4{\cdot}7H_2O$, 10 mg KNO_3, 0.055 mg K_2HPO_4, and 0.5 g MOPS (3-morpholinopropanesulfonic acid) buffer in 1 L of water (pH adjusted to 7.0), was added to microcosms through the open top which was otherwise closed with a glass lid. Microcosms and mineral salt solution were autoclaved before inoculation.

All manipulations of stream-simulating microcosms were carried out in a horizontal laminar flow cabinet. The period of incubation was performed in a constant temperature room at 15 ± 0.5 °C in the dark. Conidia produced after 24-48 h were used in experiments.

An aliquot of each specific conidial suspension, based on conidial numbers, was used to make conidial suspensions of a multiple-species or a single species to inoculate each slow filtration column (see below).

Microcosms for the study of spore dispersal through sediment

Experiments were carried out in slow filtration columns designed to simulate the physical and chemical conditions occurring in water-saturated sediments of the hyporheic zone of gravel-dominated stream, similar to those used by Torreiter *et al.* (1994), Griebler (1996), Mermillod-Blondin *et al.* (2005) and Navel *et al.* (2010) at constant temperature (15 ± 0.5 °C). Each column was 40 cm high and 10 cm in diameter. To test the influence of sediment physical characteristics on the dispersal efficiency of aquatic hyphomycete spores, the grain size of gravel and sand (1-8 mm) inserted in columns was manipulated. We tested three ranges of grain size, 4-8 mm (S1), 2-4 mm (S2) and 1-2 mm (S3). These grain sizes were in the range of streambed sediments of the three studied streams (Fig. 1). For each range of grain size, experimental column was filled to a height of 30 cm with sand or gravel depending on the treatment. For the three treatments S1, S2 and S3, each experimental column was filled exclusively with 4-8 mm gravel, 2-4 mm gravel or 1-2 mm sand, respectively. Before introduction into the columns, sand and gravel were thoroughly washed with tap water, sieved and shuffled in the laboratory. A water column (10 cm) was left at the surface of the sediment to simulate the overlying water of the stream. After installation of sediment, columns were supplied from above with the same mineral salt solution used to induce conidial production (see above, *Preparation of spore suspensions*) using a peristaltic pump. Interstitial water velocity was fixed to 0.5×10^{-3} m s^{-1} in columns, in accordance with values reported in the hyporheic zone of streams (Claret *et al.* 1998; Cornut *et al. in press*). Supplied mineral salt solution was aerated to maintain concentrations of dissolved oxygen at 9 mg L^{-1} ± 0.5 at the inlet of the columns throughout the experiment. For each column, infiltration flow rate was determined to obtain a similar retention time of water across all experimental devices. The experiment was started three days after the beginning of water flow to allow the initial hydraulic properties to stabilise.

Experiments on single- and multispecies spore suspensions

In a first experiment, to assess the dispersal efficiency of aquatic hyphomycete conidia within the interstitial pores of sediment, slow filtration columns were inoculated simultaneously with conidia of seven aquatic hyphomycetes species. Each of three replicate microcosms received *ca.* 39 000 conidia divided as follows: 25 000 conidia for *Flagellospora curvula*, 1 500 for *Articulospora tetracladia*, 3 500 for *Heliscus lugdunensis*, 2 500 for *Tetrachaetum elegans,* 2 000 for *Lemonniera terrestris,* 1 000 for *Tetracladium marchalianum* and 3 500 for *Tricladium chaetocladium*.

In a second experiment, to assess the dispersal efficiency of the extreme conidial shapes and sizes, and to suppress possible inter-specifics interactions, the same protocol as previously was followed, except that slow filtration columns were inoculated with single-species suspensions of conidia of four aquatic hyphomycete species. For this purpose, ca. 8 000 conidia for *Flagellospora curvula,* 11 000 for *Articulospora tetracladia,* 1500 for *Heliscus lugdunensis* or 6500 for *Tetrachaetum elegans* were used. Numbers of conidia in the inocula were verified by staining and counting the conidia of three additional inocula on membrane filters (Gessner *et al.* 2003).

These conidial suspensions were introduced as a single pulse from the top of each of nine slow filtration columns. Aquatic hyphomycete spores were counted in samples collected at the column outlets over 5 h (each 10 minutes after the first 35 minutes following the inoculation of slow filtration columns for the first hour, and at 110, 125, 180 and 300 min. thereafter). For this purpose, water circulation in the sediment columns was shunted to collect 40 mL of water. The conidial suspensions from three slow filtration columns from each treatment were stored into 50mL centrifuge tubes, and preserved with 2mL of 37% formalin. 0.5 mL of Triton X-100 (0.5% solution) was added to the spore suspensions and they were stirred gently with a magnetic stirring bar to ensure uniform distribution of spores. 10 mL aliquots were then filtered through a membrane filter (5 µm pore size, diameter 25 mm; Millipore Corporation), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid (Iqbal & Webster 1973). Spores were counted and identified under the microscope (× 200).

Aquatic hyphomycete spore dispersal efficiency was calculated as the cumulative number of spores (% of inoculum) sampled at the outlet of the slow filtration column depending on grain size ranges.

Statistical analyses

Aquatic hyphomycete spores circulating in the hyporheic zone

Three-way nested ANOVA was used to test whether conidial density, hyphomycete species richness, and Simpson dominance and diversity indices were related to the zone (*i.e.* benthic *vs.* hyporheic), the riffle and the stream. The zone effect (fixed) was crossed with the riffle effect (random; nested within streams), and the stream effect (random).

As the total number of spores varied largely across samples, and particularly between hyporheic and benthic ones, it was necessary to correct the species richness observed in the hyporheic and benthic zones of the different streams to account for a possible "sampling effect". For this purpose, the data corresponding to all the samples from a same zone (*i.e.* hyporheic or benthic) and from a same stream have been pooled. From each of these pools (containing between 277 and 6806 spores), 200 spores have been randomly selected, and the number of species within these subgroups determined. This procedure has been repeated 999 times to be able to calculate an average ± SD species richness for each zone in each stream. The significance of the differences in fungal species richness between the benthic and hyporheic zones was tested using a Student's *t*-test on paired samples.

Effects of hyporheic constraints on spore production by hyphomycete species

The similarities in the species composition of hyphomycete spore outputs under the various exposition treatments was measured with the Steinhaus index (Legendre & Legendre 1998). The modification of O_2 concentrations with depth was tested using one-way ANOVA with depth as the main effect. When significant differences were detected between treatments, Tukey HSD tests were then carried out for *post hoc* pairwise comparisons.

Non-homogeneity of variance precluded the use of parametric statistical methods when studying the dispersal efficiency of conidia with the multiple-species inoculum.

The non-parametric Kruskal-Wallis test was used to test the effect of sediment grain sizes (S1, S2 and S3) on dispersal efficiency of aquatic hyphomycetes conidia. In addition, the assumption of independence among treatments (Aquatic hyphomycete species) was violated. Therefore, the non-parametric Friedman test was performed to compare the dispersal efficiency among species.

In the single-species test, the relation between dispersal efficiency of aquatic hyphomycetes conidia and sediment grain sizes was tested using a two-way ANOVA with aquatic hyphomycete species and sediment grain sizes (S1, S2 and S3) as the main effects. When significant differences were detected between treatments, Tukey HSD tests were then used for *post hoc* pairwise comparisons.

Possible relationships between the dispersal ability of aquatic hyphomycetes and their conidial traits (*i.e.* biovolume and minimal obstruction size of conidia) were investigated using Spearman correlation tests. Biovolume and minimal obstruction size of conidia were calculated for each species from conidia descriptions obtained from the literature (Chauvet, 1990).

STATISTICA 6.0 (StatSoft Inc., 2001) was used for all statistical analyses. Differences were considered significant when $P < 0.05$.

3.5. Results

Aquatic hyphomycete spores circulating in the hyporheic zone

Conidial densities were similar among riffles within streams (Nested-ANOVA, $F_{6,44} = 0.55$, $P = 0.77$), but differed between streams, with the Bergnassonne and the Béal exhibiting a similar total conidial density, contrasting with higher values of the Orbiel (Nested-ANOVA, $F_{2,44} = 18.71$, $P < 0.01$). There was a marked decrease in conidial densities in interstitial water compared to the surface (Nested-ANOVA, $F_{1,44} = 183.05$, $P < 0.001$).

Conidial densities averaged 2794 L^{-1} (range 2222-3680) in the benthic zone and 404 L^{-1} (range 185-617) in the hyporheic zone. The average conidial density in the hyporheic zone was thus almost 7-fold lower than in the surface water, with a particularly strong decrease in the Béal (*i.e.* 12-fold), contrasting with the Bergnassonne and the Orbiel exhibiting similar ratios close to 6-fold (Fig. 2a).

A total of 39 aquatic hyphomycete species was recorded from the three streams and the two zones (Table 1). There was no difference in species richness between streams and between riffles within streams (Nested-ANOVA, $F_{2,44} = 2.32$, $P = 0.19$ and Nested-ANOVA, $F_{6,44} = 0.53$, $P = 0.78$, respectively). The number of aquatic hyphomycete species from the benthic and hyporheic zones of each stream ranged from 14 to 24 and from 1 to 14, respectively. Species richness was markedly higher in the benthic zone than in the hyporheic one (Nested-ANOVA, $F_{1,44} = 256.65$, $P < 0.001$) (Fig. 2b). Moreover, even after the correction of the species richness observed in the hyporheic and benthic zones of the different streams, to account for a possible "sampling effect" due to highly contrasting conidial densities between zones, the difference remained significant (Student's *t*-test on paired samples, $t = 7.82$, $P = 0.016$). The Simpson dominance slightly differed among streams (Nested-ANOVA, $F_{2,44} = 6.87$, $P = 0.03$), but not between riffles within streams (Nested-ANOVA, $F_{2,44} = 1.53$, $P = 0.19$). The Simpson dominance index observed in the benthic zone was significantly lower than in the hyporheic one (Nested-ANOVA, $F_{1,44} = 8.70$, $P < 0.01$), denoting lower evenness in the spores circulating within sediment (Fig. 2c).

The fungal community was dominated by two species (*Taeniospora gracilis* and *Flagellospora curvula*), which accounted for 59-83 % of the total conidial production in the benthic zone, and 74-80 % in the hyporheic zone. Although the other species accounted for low contributions, often less than 5 % of the total conidial densities, some differences in relative abundances of aquatic hyphomycete species between the two zones were evident. Conidia of *Alatospora acuminata, Alatospora constricta, Articulospora tetracladia, Clavatospora longibrachiata, Heliscus lugdunensis, Lunulospora curvula, Myccentrospora sp1* and *Tricladium chaetocladium* (either tetraradiate / branched or arched filiform / sigmoid) exhibited higher relative abundance in the surface water compared to the interstitial one.

Table 1 Relative abundance (%) of the aquatic hyphomycete species for the three streams in the benthic and hyporheic zones (Berg. = Bergnassonne; Béal; Orb. = Orbiel).

	Benthic zone			**Hyporheic zone**		
Species	Berg.	Béal	Orb.	Berg.	Béal	Orb.
Alatospora acuminata Ingold	5.58	2.33	3.16	1.45	2.45	1.90
Alatospora constricta Dyko	3.57	2.00	2.37	0.61	0.07	0.54
Alatospora flagellata (Gönczöl) Marvanová	0.04	-	0.02	-	-	-
Anguillospora crassa Ingold	0.25	0.03	0.31	1.66	-	5.62
Anguillospora filiformis Greathead	0.39	11.26	0.80	4.15	6.65	1.62
Anguillospora furtiva Webster & Descals	-	0.04	-	-	-	-
Anguillospora longissima (Sacc. & Syd.) Ingold	0.86	1.48	1.06	2.70	6.57	1.24
Articulospora tetracladia Ingold	3.27	1.76	0.78	0.39	-	0.19
Casaresia sphagnorum Gonz. Fragoso	-	-	-	-	-	0.03
Clavariopsis aquatica De Wild.	0.49	0.60	0.12	0.07	-	-
Clavatospora longibrachiata (Ingold) Marvanová & Nilsson	5.41	3.00	1.65	0.52	0.26	1.73
Crucella subtilis Marvanová	2.82	0.96	2.63	2.58	-	2.09
Culicidospora aquatica Petersen	0.10	0.06	0.10	-	-	-
Dendrospora erecta Ingold	0.02	0.04	-	0.11	-	-
Flagellospora curvula Ingold	22.99	36.49	9.16	47.04	43.73	21.78
Fontanospora alternibrachiata Dyko	-	0.37	0.19	-	0.07	-
Goniopila monticola (Dyko) Marvanová & Descals	0.02	0.29	0.07	-	0.43	-
Heliscella stellata (Ingold & Cox) Marvanová & Nilsson	0.61	0.20	0.11	-	-	-
Heliscina campanulata Marvanova	-	-	-	-	-	0.18
Heliscus lugdunensis Sacc. & Théry	2.73	0.93	1.43	0.29	-	0.57
Lateriramulosa uni-inflata Matsushima	0.15	-	-	-	-	-
Lemonniera aquatica De Wild.	-	0.72	-	-	-	-
Lemonniera cornuta Ranzoni	-	0.06	-	-	-	-
Lemonniera terrestris Tubaki	0.04	3.48	-	-	1.05	-
Lunulospora curvula Ingold	5.96	0.81	-	4.09	0.33	-
Mycocentrospora sp.*1* cf. *angulatum* R.H. Petersen	4.46	1.03	1.55	0.82	0.63	0.06
Mycocentrospora sp.*2* ?	0.10	0.28	0.03	-	0.07	-
Mycofalcella calcarata Marvanová, Om-Kalth. & Webster	0.02	0.15	0.10	-	-	-
Stenocladiella neglecta (Marvanová & Descals) Marvanová & Descals	-	0.14	-	-	-	-
Taeniospora gracilis Marvanová	35.91	29.97	74.12	33.21	34.99	52.07
Tetrachaetum elegans Ingold	2.39	0.48	0.06	0.29	2.51	-
Tetracladium marchalianum De Wild.	-	0.18	-	-	-	-
Tricelophorus monosporus Ingold	-	0.20	-	-	-	-
Tricladium chaetocladium Ingold	1.66	0.60	0.17	-	0.20	0.12
Tricladium splendens Ingold	-	0.05	0.01	-	-	-
Tumularia tuberculata (Gönczöl) Descals & Marvanová	0.13	-	0.02	-	-	-
Varicosporium elodeae Kegel	0.02	0.04	-	-	-	-
Unknown filiform ≤ 60 µm	-	-	-	-	-	9.03
Unknown tetraradiate	-	-	-	-	-	1.23
Total number of species	27	32	24	16	15	17

In contrast, the relative abundance of scolecoid or filiform conidia from species such as *Anguillospora crassa, Anguillospora filiformis, Flagellospora curvula,* and an undetermined filiform conidia (with a length ≤ 60 μm) increased in the interstitial water compared to the surface water.

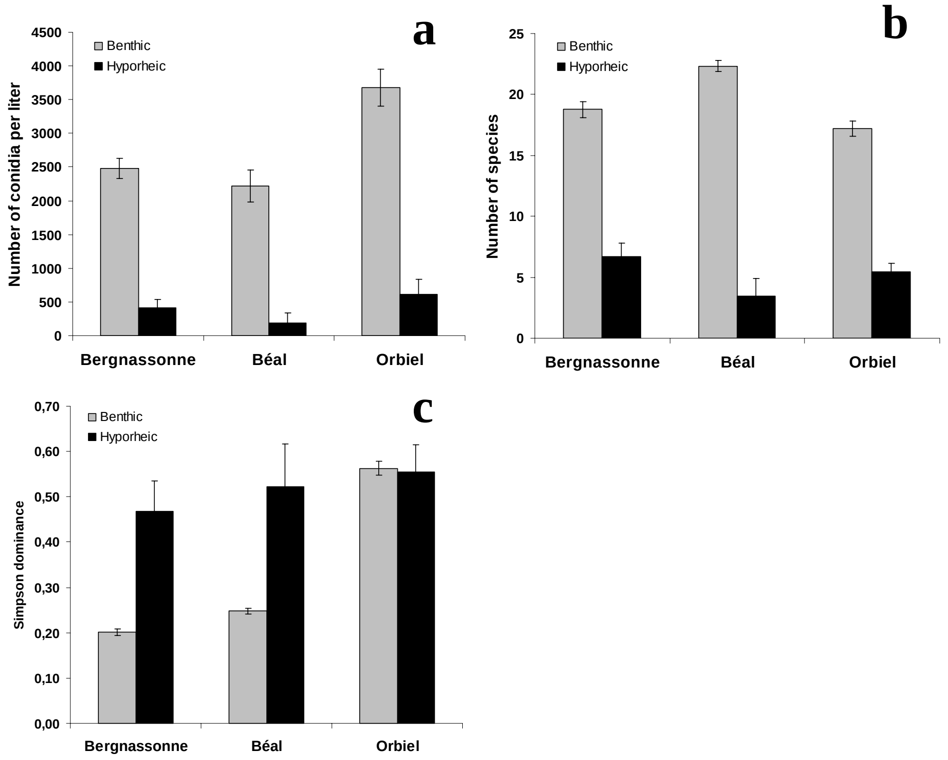


Fig. 2 Mean conidial density (a), species richness (b) and Simpson dominance index (mean ± SE) of the fungal communities in the benthic and hyporheic zones of the three streams (Bergnassonne, Béal and Orbiel).

Effects of hyporheic constraints on spore production by aquatic hyphomycete species

In the stream-simulating microcosms, the constant water agitation kept O_2 concentrations at saturation throughout the experiment. In contrast, in the slow-filtration columns used to mimic the hyporheic habitat, O_2 concentrations presented a sharp drop with depth (Fig. 5), from 8.8 ± 0.1 mg L^{-1} at the sediment surface to 5.1 ± 0.1 mg L^{-1} in the deepest sediment layer (ANOVA, $F_{4,10} = 7.19$, $P < 10^{-6}$ and HSD tests, comparison of O_2 concentrations between surface layer and other layers, $P < 10^{-3}$) (Fig. 3).

After 57 days of incubation, a total of 15 and 11 sporulating species of aquatic hyphomycetes were identified on alder leaf discs from stream-simulating microcosms and slow-filtration columns, respectively. The mean number of fungal species associated with alder leaf discs from stream-simulating microcosms was markedly higher than that from the slow filtration columns (10 and 30 cm below the sediment surface), with 15, 9 and 10 species found in the three exposure treatments, respectively.

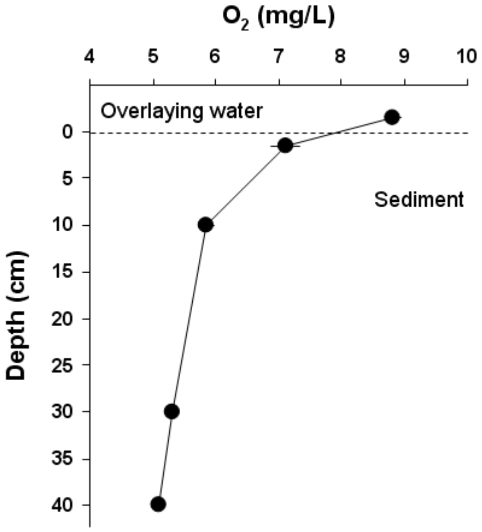


Fig. 3 Vertical distribution of O_2 in the slow filtration columns (mean ± SE).

There were clear differences in the relative abundance of dominant species between exposure treatments. The relative contribution of *Flagellospora curvula*, *Articulospra tetracladia* and *Lemonniera terrestris* to the conidial production were lower in the stream-simulating microcosms than in the slow filtration columns. But the most striking difference was the disappearance of *Alatospora acuminata*, *Clavariopsis aquatica*, *Clavatospora longibrachiata*, *Culicidospora aquatica* and *Tricladium chaetocladium*, and the decrease of *Anguillospora longissima's* contribution, from the conidial pool in slow-filtration columns. The conidial output from slow filtration columns was dominated by only two species (*Flagellospora curvula* and *Articulospra tetracladia*), whereas five species were co-dominant in the conidial output from stream-simulating microcosms (Fig. 4).

The greatest difference was found in *Flagellospora curvula* which contributed up to 75% of the overall conidial production in the slow-filtration column treatments compared to < 17% in the stream-simulating microcosms. This strong dominance markedly increased with depth, averaging 69% and 81% for exposure treatments at 10 and 30 cm below the sediment surface, respectively.

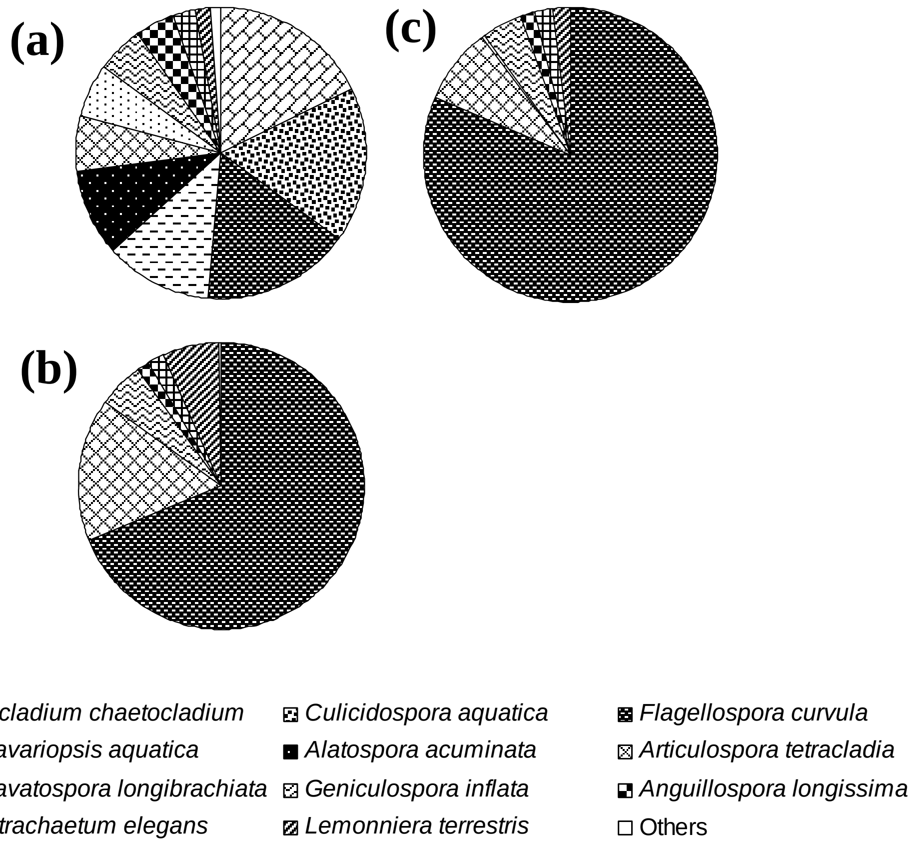


Fig. 4 Relative abundance (%) of the leaf-associated aquatic hyphomycete species for the three exposure treatments, determined from the cumulative conidial production over the whole period of the experiment (three replicate microcosms combined). (a) Aerated microcosms, (b) slow filtration columns: at 10 cm below the sediment surface, (c) slow filtration columns: at 30 cm below the sediment surface.

The species composition of conidial outputs was much more comparable between the two exposure treatments in slow-filtration columns (Steinhaus similarity index = 0.736) than between any of these treatments and the stream-simulating microcosm treatment (Steinhaus similarity index ranging between 0.307 and 0.341).

Spore dispersal efficiency across the sediment

In the multiple-species test, the average dispersal efficiency measured during the experiment was clearly influenced by sediment grain size (Kruskal-Wallis test, $H = 7.2$, d.f. = 2, $P < 0.05$). The proportion of conidia able to disperse within the sediment dramatically decreased with decreasing grain size, thereby reducing the availability of interstices. As a consequence of this screening, the number of conidia was reduced by almost 85% between the intermediate and the coarsest grain size (S1 *vs.* S2), and almost 95% between the coarsest and the finest one (S1 *vs.* S3). In addition, highly significant differences existed among aquatic hyphomycete species (Friedman tests, $Q = 33.19$, d.f. = 6, $P < 10^{-4}$), demonstrating that conidial features greatly influenced dispersal efficiency within the interstices of stream sediment (Fig. 5a).

In addition, each aquatic hyphomycete species from the seven species tested showed differences in their ability to disperse along the gradient of sediment grain size (*Flagellospora curvula*: Kruskal-Wallis test, $H = 7.2$, d.f. = 2, $P < 0.05$; *Articulospora tetracladia*: Kruskal-Wallis test, $H = 6.7$, d.f. = 2, $P < 0.05$; *Tetrachaetum elegans*: Kruskal-Wallis test, $H = 7.6$, d.f. = 2, $P < 0.05$; *Tetracladium marchalianum*: Kruskal-Wallis test, $H = 4.5$, d.f. = 2, $P = 0.10$; *Lemonniera terrestris*: Kruskal-Wallis test, $H = 5.7$, d.f. = 2, $P = 0.06$; *Tricladium chaetocladium*: Kruskal-Wallis test, $H = 4.5$, d.f. = 2, $P = 0.10$; *Heliscus lugdunensis*: Kruskal-Wallis test, $H = 3.3$, d.f. = 2, $P = 0.20$), denoting that the potential importance of dispersal was strongly related to the conidial traits (*i.e.* shape and size).

In the single-species test, dispersal efficiency measured during the experiment was also influenced by sediment grain sizes (ANOVA, $F_{2,24} = 156.44$, $P < 10^{-6}$). Species identity also had a highly significant effect (ANOVA, $F_{3,24} = 118.06$, $P < 10^{-6}$) (Fig. 5b). These results were consistent with those from the multiple-species experiment, showing once more that large differences in dispersal efficiency exist among aquatic hyphomycete species.

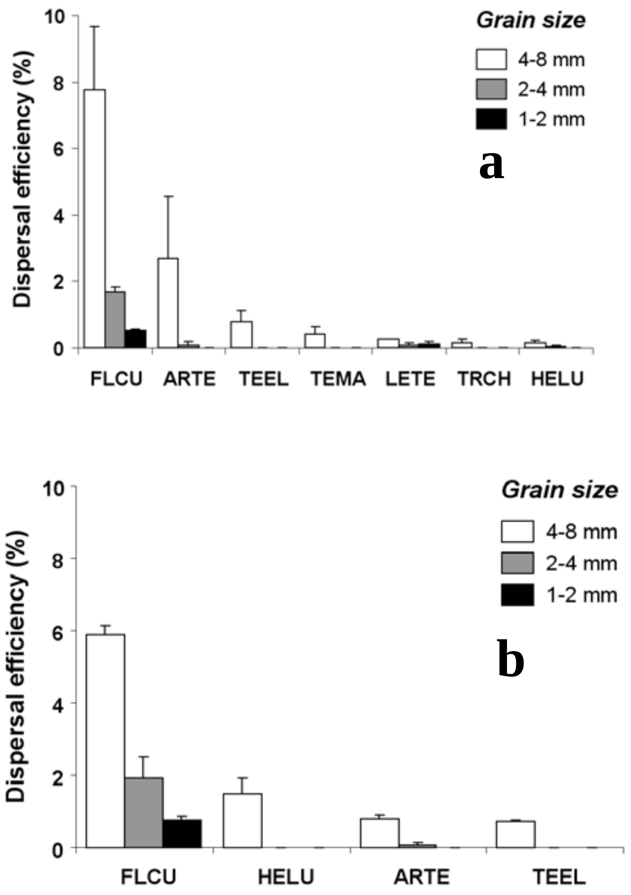


Fig. 5 Cumulated number of spores for the (a) 7 different aquatic hyphomycete species, (b) 4 different aquatic hyphomycete species (mean + SE) for the three different sediment grain sizes at the outlet of slow filtration columns, as a function of time.

HSD test indicated that there were significant differences between conidial shapes, with one filiform/sigmoid *vs.* compact and branched/tetraradiate, exhibiting the highest dispersal efficiency. The influence of conidial traits on the dispersal efficiency depended on sediment characteristics as indicated by the significant interaction “species identity” × “sediment grain size” (ANOVA, $F_{6,24} = 4.18$, $P < 10^{-2}$). The dispersal efficiency of *Flagellospora curvula*, an aquatic hyphomycete species exhibiting filiform/sigmoid conidia, was always significantly higher in comparison with the three other species with compact and branched/tetraradiate conidia, whatever the sediment grain size (HSD test, $P < 10^{-3}$).

In the coarsest sediment (S1), dispersal efficiency varied greatly among the four fungal species with a total dispersal efficiency of 5.88 ± 0.47 % (mean ± SD) for the small filiform/sigmoid conidia of *Flagellospora curvula*, 1.48 ± 0.80 % for the compact conidia of *Heliscus lugdunensis*, 0.81 ± 0.21% for the small tetraradiate conidia of *Articulospora tetracladia* and 0.71 ± 0.11 for the large tetraradiate conidia of *Tetrachaetum elegans*. As a consequence, the dispersal efficiency of *Flagellospora curvula* was higher by 4, 7 and 8-fold in comparison with the three others species, *Heliscus lugdunensis*, *Articulospora tetracladia* and *Tetrachaetum elegans*, respectively. In contrast to the other species, *Flagellospora curvula* was able to disperse within sediment whatever its grain size (Fig. 6). *Heliscus lugdunensis* and *Tetrachaetum elegans* did not disperse at all within the two finest sediments (S2 and S3), while *Articulospora tetracladia* dispersed through the interstices of the sediment S2, however almost one order of magnitude less in comparison with the coarsest sediment (S1).

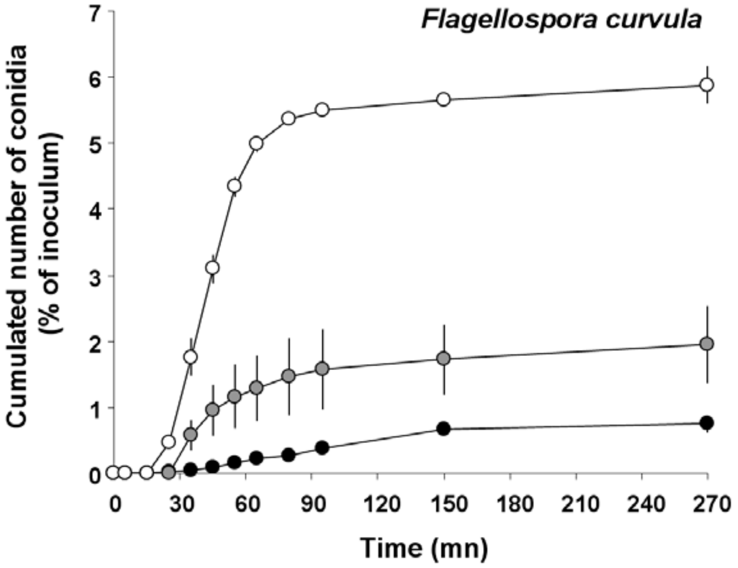


Fig. 6 Cumulated number of spores of *Flagellospora curvula* (mean ± SE) for the three different sediment grain sizes at the outlet of slow filtration columns, as a function of time.

In the multi-species experiment, Spearman correlation analyses between the two morphological traits of conidia and their dispersal efficiency revealed a significant relationship for minimal obstruction size of conidia (r = -0.44; $P < 0.05$) but not for conidia biovolume (r = -0.17; P = 0.46).

The relationships between dispersal efficiency and conidial traits obtained through the series of experiments with the single-species inocula were consistent with the results from the multiple-species inoculum, and were even more pronounced (dispersal efficiency *vs.* minimal obstruction size of conidia: r = -0.78; P < 0.01; dispersal efficiency *vs.* conidia biovolume: r = -0.50; P = 0.10).

3.6. Discussion

Abundance and diversity of hyphomycetes in the hyporheic zone of woodland streams

Several authors have previously suggested that aquatic hyphomycetes and other fungi readily disperse within the hyporheic zone (Bärlocher & Murdoch 1989 ; Bärlocher *et al.* 2006 ; 2008). Smith & Lake (1993) observed fungal hyphae on leaf litter incubated either above or below the surface of sediment, while Krauss *et al.* (2003) reported that groundwater wells can support a surprisingly high fungal diversity. In this latter study, submerged alder leaves were colonised by aquatic hyphomycetes. However, as far as we know, our study is the first to estimate the regular occurrence of aquatic hyphomycete conidia in the hyporheic zone of woodland streams.

Surprisingly, and despite the considerable amount of plant matter buried in sediment, little is known about the role of aquatic hyphomycetes on the decomposition of leaf litter in the hyporheic zone of headwater streams. Some insights have been brought by Cornut *et al.* (*in press*) who have recently examined the role of hyphomycetes in processing allochthonous organic matter in streambed sediments. However, a significant gap persists in our knowledge concerning pathways of vertical dispersal and colonization of new substrates by aquatic hyphomycetes into the hyporheic habitat. Colonisation of leaves and other substrates by aquatic hyphomycetes may occur through various mechanisms: by direct contact as a result of hyphal outgrowth from a colonised substrate touching another one, or at a distance by either detached hyphal fragments (Park 1974 ; Knudsen & Stack 1991) or conidia (Read *et al.* 1992) landing on a leaf surface. This last mechanism is predominant to colonize distant leaf patches (Sridhar & Bärlocher 1997).

Bärlocher *et al.* 2006 support the hypothesis suggesting that spores or other fungal propagules move relatively easily through the streambed sediment, and may establish colonies on the substrates they encounter. However, this hypothesis remained to be tested. Our results indicate that the conidial assemblage found in the interstitial water from the hyporheic zone differed markedly of the one from the benthic zone. Compared to streambed surfaces, the hyporheic habitat is clearly impoverished, with an average decrease in conidial density and species richness in the three streams ranging from 83 to 92% and from 65 to 85%, respectively. Higher values of Simpson dominance index observed in the hyporheic zone in comparison with those from the benthic zone suggest that some aquatic hyphomycetes species are favoured, relatively to other species, by the physical and chemical conditions prevailing in the hyporheic zone.

Bärlocher *et al.* (2006) underline the crucial role played by the presence of suitable substrates within stream sediments, provided however that external nutrients and, above all, oxygen are available (Field & Webster 1983). Differences in the composition of conidial assemblages observed in our field study between the benthic and the hyporheic zones may therefore be due both to (1) the physiological constraints of the hyporheic habitat, particularly the low dissolved oxygen supply, modulating the establishment of the different hyphomycete species and the endogenous spore production, and (2) the dispersal efficiency of conidia, likely to vary among aquatic hyphomycete species, as a result of contrasting conidial traits.

Differential spore production among hyphomycete species in the hyporheic zone

The spores released by fungal assemblages were strongly affected by the exposure treatment, and also by depth in the slow filtration columns. This suggests a better ability of some aquatic hyphomycete species to develop, relatively to others, under the physical and chemical conditions prevailing in the hyporheic zone of headwater streams. Our microcosm system used to mimic hyporheic conditions reproduced a clear vertical gradient leading to a rapid decrease of dissolved oxygen concentrations with depth in the sediment, which was comparable with downwelling zones in streams (Cornut *et al. in press*).

The lower dissolved oxygen concentrations in the slow filtration columns probably explained these changes in fungal assemblages. Dissolved oxygen is indeed a limiting factor for biological colonization and activity in the interstitial habitats, with its depletion leading to qualitative and quantitative changes in macroinvertebrate and microbial assemblages (Ward *et al.* 1998).

Although it is difficult to assess the effects of dissolved oxygen concentrations on the structure of fungal communities in streams, separately from other confounding environmental factors, several studies underline the likely effect of this element. For instance, Chergui & Pattee (1988) reported a lower number of aquatic hyphomycete species in a side arm of the Rhone River than in the main channel, and this difference was attributed to lower dissolved O_2. Many studies conducted in rivers have also shown that low dissolved oxygen concentrations may selectively eliminate some species of aquatic hyphomycetes, conducting to impoverished communities in hypoxic conditions (Rajashekhar & Kaveriappa 2003 ; Pascoal *et al.* 2003 ; Pascoal & Cássio 2004). All these results confirm the theory of Field & Webster (1983) who consider that aquatic fungi potentially tolerate hypoxic conditions in streams thanks to some species being able to grow under such conditions.

Medeiros *et al.* (2009) recently reported some clear changes in the structure of the fungal community associated with decomposing alder leaves exposed in the laboratory at low concentrations of dissolved oxygen. They found a strong decrease of the community evenness under hypoxic stress (*i.e.* 54% of O_2 saturation, equivalent to *ca.* 5 mg L^{-1}), compared to a reference stream. In their study, *Flagellospora curvula* was the dominant species under hypoxic conditions. In line with these results, we also observed a clear decrease of fungal community evenness in our study, both from alder leaf-discs incubated in the slow filtration columns and in the interstitial water from the hyporheic habitat of the three streams sampled. *Flagellospora curvula* was also one of the two dominant species associated with alder leaves incubated under low dissolved oxygen concentrations (*i.e.* 5.30 ± 0.12 mg L^{-1}) in our slow filtration columns (*i.e.* 81%), and in the hyporheic habitat of the three streams sampled (*i.e.* 38%; average abundance for the 3 streams). These findings demonstrate that some species respond differently to oxygen depletion, confirming those of Field & Webster (1983).

A common point of all these studies is the fact to quantify spore production and to use it as a proxy of fungal community structure. However, at this stage, it is not possible to know whether hypoxic conditions modulate the relative development of different hyphomycete species, or only have an impact on their sporulation ability. Indeed, active mycelium of some species can be present and not release any spores (Sridhar & Bärlocher 1997). On the basis of molecular data, Bärlocher *et al.* (2006) even reported that a substantial proportion of the fungal biomass does not belong to species that dominate spore production.

Thus, the actual fungal diversity associated with alder leaves incubated under low dissolved oxygen concentrations in our slow filtration columns may be higher than what is suggested by the conidial output observed. However, for understanding the structure of conidial assemblages circulating within the sedimentary matrix in field conditions, the important point raised by our results is the differential ultimate spore production of various hyphomycete species in benthic and hyporheic zones, even with a similar inoculation of leaf litters at early stages of the decomposition process.

Conidial traits and dispersal efficiency in the hyporheic zone

In our microcosm experiment, the sedimentary matrix had a strong filtering effect on hyphomycete conidia. The dispersal ability of conidia was positively related to sediment grain size. However, only *ca.* 15% of the inoculum passed through the column with the coarsest sediment. The results also demonstrated significant differences among aquatic hyphomycete species, showing the great influence played by conidial traits in the dispersal efficiency within stream sediment. Among the seven aquatic hyphomycetes tested, *Flagellospora curvula,* a species with sigmoid conidial shape, had the highest dispersal efficiency along the studied gradient of sediment grain size.

Through our first laboratory experiment, it has been shown that this species outperformed species with other conidial shapes from the multiple-species inoculum in which large tetraradiate (*Tetrachaetum elegans* and *Tricladium chaetocladium*), small tetraradiate (*Articulospora tetracladia, Tetracladium marchalianum* and *Lemonniera terrestris*) and compact (*Heliscus lugdunensis*) forms occurred.

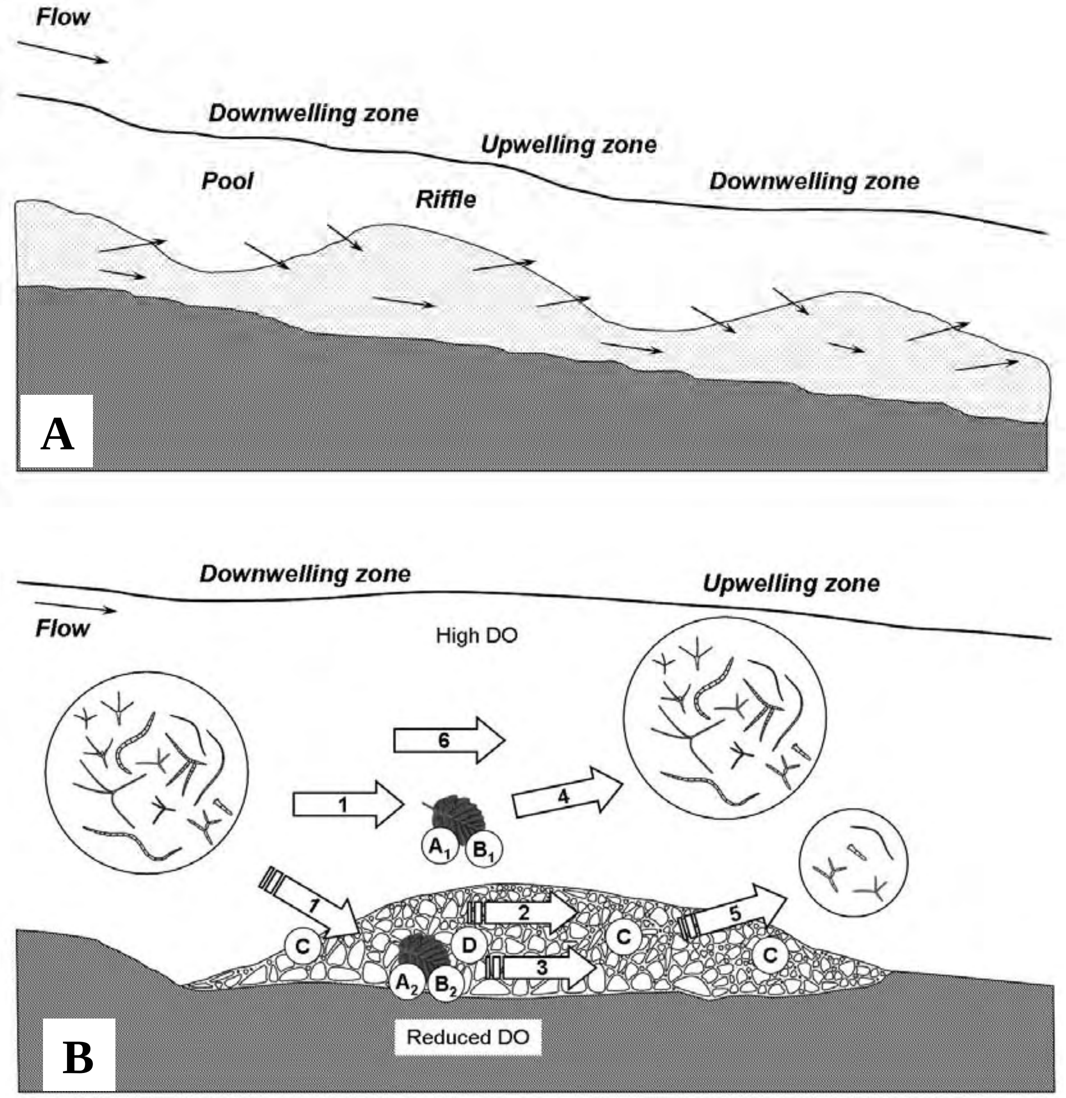
These results were partly corroborated by those from the second series of experiment using single-species inocula, which confirmed the higher dispersal efficiency of *Flagellospora curvula* compared to two species with tetraradiate and one with compact conidial shapes. Not surprisingly, one of the decisive factors for dispersal ability was the conidial size, expressed in terms of minimal obstruction size, while there was no relationship between conidial biovolume and dispersal efficiency. The conidial minimal obstruction sizes of *Flagellospora curvula* and *Heliscus lugdunensis* were by far the smallest compared with the others species. We cannot exclude that other characteristics of conidia also play a decisive role in their dispersal ability, singly or in combination with conidial minimal obstruction size.

We have actually only considered two conidial morphological traits, while other factors such as the quantity and type of mucilage at the tip of conidial arms (Au *et al.* 1996 ; Read *et al.* 1992), the speed for attached conidia to form appressoria (Read *et al.* 1992), or the alteration of conidial shape by germination, might improve attachment to various substrates and thereby compromise dispersal efficiency (Cox 1983). Interspecific interactions among conidia may be also influential for dispersal, as indicated by the results obtained with single- and multi-species inocula, showing a different hierarchy across species in terms of dispersal ability. Such interactions may contribute to explain the weaker relationship between conidial minimal obstruction size and dispersal efficiency in multi-species than in single-species inocula. It may also be the reason for the large differences in dispersal patterns of *Heliscus lugdunensis* depending whether it was singly or in mixture with other species.

These results highlight the importance of interstice availability in streambed sediment, as well as conidial shape and size and additional properties of aquatic hyphomycete conidia, in determining their dispersal efficiency into the sediment layers. The current study suggests that decreasing the interstice availability in the hyporheic habitat, due to physical and/or biological clogging for instance, will likely result in lower density and diversity of aquatic hyphomycetes.

3.7. Overview

Adverse physicochemical conditions in the hyporheic zone, notably the lowered dissolved oxygen concentration, are likely to select some hyphomycete species from among the benthic pool. As observed in our study, this physiological screening may cause important shift in the structure of fungal decomposer communities, potentially differing in their enzymatic capacities and performance in litter breakdown. Colonisation by conidia seems ideal to quickly respond to the regular cycle of superabundance and scarcity of discrete, ephemeral substrates common in many temperate streams (Bärlocher 2009). However, due to the physical screening of conidia by the streambed sediment, and even when the abiotic conditions could otherwise be favourable to fungi development, communities from hyporheic habitats will probably differ to what could be expected from the benthic hyphomycete species pool. Our results therefore suggest a multiple screening of aquatic hyphomycetes in the hyporheic zone of woodland streams (Fig. 7). However, molecular techniques may provide powerful new tools to deepen our knowledge of fungal diversity in streams (Bärlocher 2010), and would notably allow us to unequivocally determine the respective influences of hyporheic conditions on hyphomycete colony development and sporulation activity.



A1, **A2**, fungal colonization; **B1**, **B2**, spore production; **C**, physical screening; **D**, physiological stress (hypoxic stress); **1**, benthic conidia pool; **2**, spore dispersal from benthic zone into the hyporheic habitat; **3**, dispersal spore autogenic production; **4**, release of spore; **5**, release of spore from hyporheic zone; **6**, spore dispersal from benthic zone

Fig. 7 (A) Reach-scale surface subsurface exchange flows, (B) conceptual scheme of how hyporheic habitat affects conidial dispersal and identity. Hyporheic zone exerts two types of screening on aquatic hyphomycetes community: a physical screening of benthic condia pool; a physiological stress inducing changes in the structure and composition of aquatic hyphomycete assemblage.

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Chapitre 4:

Effects of burial on leaf physico-chemical traits, microbial conditioning and palatability to three invertebrate shredders

4. Effects of burial on leaf physico-chemical traits, microbial conditioning and palatability to three invertebrate shredders

4.1. Résumé

Le chapitre précédent a révélé que la zone hyporhéique constitue pour les communautés fongiques une barrière physique et physiologique. Les caractéristiques granulométriques des sédiments exercent un criblage physique des conidies d'hyphomycètes aquatiques en fonction de leurs traits morphologiques. Le gradient vertical en dioxygène dissous dans la zone hyporhéique entraîne par ailleurs un stress hypoxique susceptible de moduler l'abondance et/ou la capacité à sporuler des différentes espèces d'hyphomycètes. L'interaction de ces deux mécanismes conduit à des changements marqués dans la composition et la structure des communautés d'hyphomycètes aquatiques de l'habitat hyporhéique, ayant pour conséquence un ralentissement de la décomposition en zone hyporhéique.

Le devenir de la matière organique enfouie dans cette zone hyporhéique, et en particulier le rôle du conditionnement microbien dans de telles conditions, reste largement inexploré. Nous verrons dans cette quatrième partie de ma thèse comment la localisation de la litière dans les cours d'eau (*i.e.* à la surface ou enfouie dans les sédiments), mais aussi l'histoire de l'enfouissement de la litière, peuvent affecter les communautés microbiennes associées à cette dernière et, par conséquent, le processus de décomposition.

Nous avons testé les hypothèses selon lesquelles (1) l'enfouissement de la litière conduirait à un taux de décomposition plus faible, associé à des changements dans les assemblages microbiens par rapport à la litière exposée à la surface du sédiment, (2) les changements dans le conditionnement microbien de la litière, du fait de l'enfouissement dans les sédiments, induirait une diminution de la qualité de la litière (composition élémentaire et dureté de la feuille) et de sa palatabilité pour les consommateurs, et (3) le devenir de la litière serait fortement lié à l'histoire de son inoculation microbienne.

Par ailleurs, nous nous attendons à ce qu'une meilleure inoculation microbienne, en améliorant la qualité de la litière –(en particulier son rapport C:P) ait une incidence directe sur les taux de croissance des invertébrés détritivores.

Dans cette optique, une expérience a été conduite dans un cours d'eau forestier de deuxième ordre, situé dans le sud-ouest de la France. Les communautés microbiennes, les taux de décomposition, la composition élémentaire et la dureté de la litière ont été comparés entre quatre traitements différant par l'emplacement de la litière dans le cours d'eau : (1) enfouie dans les sédiments, (2) enfouie après 3 semaines d'exposition à la surface des sédiments, (3) enfouie dans les sédiments pendant 3 semaines avant d'être exposée à la surface des sédiments ou (4) exposée à la surface des sédiments pendant toute la durée de l'expérience. En parallèle, nous avons examiné l'effet de ces quatre traitements sur la palatabilité des litières vis-à-vis de trois taxons d'invertébrés détritivores, par le biais de tests de consommation, et sur le taux de croissance de l'Amphipode *Gammarus fossarum*.

Les résultats ont montré que l'histoire de l'enfouissement de la litière dans les sédiments a nettement affecté la qualité du matériel foliaire et sa palatabilité pour les invertébrés détritivores, en raison de changements dans le conditionnement microbien. La diminution de la qualité de la litière a également conduit à des taux de croissance plus lents de *Gammarus fossarum*, ces derniers étant négativement corrélés aux rapports C:P du matériel foliaire. Ces résultats soulignent le rôle clé du conditionnement par les champignons de la litière présente dans la zone hyporhéique des cours d'eau de tête de bassin. Ce conditionnement affecte directement les consommateurs contrôlant le processus de décomposition, et joue donc un rôle crucial dans l'efficacité des transferts de matière et d'énergie dans les écosystèmes aquatiques de tête de bassin.

4.2. Abstract

1. In headwater streams, leaf litter decomposition constitutes a key ecosystem-level process. Aquatic heterotrophic microorganisms (fungi and bacteria) are crucial for the mineralization of leaf litter and also render it more palatable for leaf shredding invertebrates. Although this ecological process largely occurs at the sediment surface of streams, a substantial part of leaf litter entering running waters may be buried in the streambed as a consequence of flooding and sediment movement, and thus be subject to constraining conditions prevailing in the hyporheic zone. The fate of organic matter buried in this hyporheic zone, and particularly the role of microbial conditioning in such conditions, remain largely unexplored.

2. The aim of this present study was to determine how the location of leaf litter within the streambed, *i.e.* at the surface or buried, but also leaf litter burial history, may affect the leaf-associated microbial decomposer communities and therefore leaf litter consumption by invertebrate detritivores. We tested the hypotheses that (1) burial of leaf litter would lead to lower decomposition rates associated with changes in the microbial assemblages compared to leaf litter exposed at the surface of the sediment, (2) changes in the microbial conditioning of leaf litter after burial would lead to decreased palatability to consumers and quality (elemental composition and leaf toughness) of decaying leaves, and (3) the fate of leaf litter would be strongly linked to microbial inoculation history. In addition, we expected that higher microbial colonization, by enhancing leaf litter quality – particularly through decrease of leaf C:P ratio – would directly affect shredding invertebrates' growth rates.

3. We experimentally tested these hypotheses by conducting an experimental study in a second-order stream located in southern France, where leaf-associated microbial community, as well as decomposition rates, elemental stoichiometry, and leaf litter toughness were compared across controlled treatments differing by their location within the streambed: (1) buried in the sediment, (2) buried after 3 weeks of exposure at the sediment surface, (3) buried in the sediment for 3 weeks before being exposed at the sediment surface or (4) exposed at the sediment surface throughout the experiment.

In parallel, we examined the effects of the four conditioning treatments on decaying leaves palatability to consumers through feeding trials on three taxa of shredding invertebrates, and the effects of leaf litter burial on growth rates of a freshwater amphipod model, *Gammarus fossarum*.

4. Our results showed that burial history within the sediment of leaf litter markedly affected the quality of leaf material and its palatability to leaf shredding invertebrates, due to changes in microbial conditioning. The decrease of the quality of leaf litter led to slower growth rates of shredding invertebrates, growth rates being negatively correlated to leaf litter C/P ratios.

5. These results underline the key role of aquatic fungi conditioning in the hyporheic zone of headwater streams. This conditioning directly affects consumer-mediated processes, and therefore plays a crucial role in nutrient and energy transfer efficiencies in headwater stream ecosystems.

4.3. Introduction

Headwaters (*i.e.* springs and intermittent, first and second-order streams) are abundant and unique components of a river network (Meyer *et al.* 2007). It is now unanimously recognized that headwater streams are crucial for sustaining the structure, function, productivity, and biocomplexity of downstream ecosystems (Haigh *et al.* 1998). Headwater streams provide downstream habitats with a multitude of ecosystem services, including clean water, nutrients (*e.g.*, nitrogen and phosphorus), food [*e.g.*, organic matter (OM) and invertebrate prey for fishes, insectivorous birds], woody debris (Wipfli & Gregovich 2002 ; Compton *et al.* 2003 ; Gregory *et al.* 2003), and habitats for some species at some points in their life cycles (Wipfli *et al.* 2007). Headwater streams also serve as refugia and source areas for biodiversity (Meyer *et al.* 2007). Improvement in the understanding of their ecological functioning is clearly needed to propose rational means of conservation. Low-order forested streams, where light limitation restricts primary production, rely upon the input of organic matter from the riparian zone to fuel in-stream processes (Vannote *et al.* 1980). Therefore, their metabolism is primarily heterotrophic (detritus-based). Carbon and energy flow from submerged coarse particulate organic matter (CPOM, basically leaf litter and woody debris) to higher trophic levels is mediated by microorganisms (Gessner & Chauvet 1994 ; Suberkropp 1998b ; Graça, 2001 ; Hieber & Gessner 2002).

After entering running waters, a substantial part of leaf litter and other organic detritus may be buried into the streambed during storms (Herbst 1980) or as a consequence of flooding and sediment movement (Metzler & Smock 1990 ; Naegeli *et al.* 1995). Thus, the hyporheic zone of streams often represents a major site of organic matter storage. Indeed, comparisons between benthic and hyporheic zones have shown that the latter may account for 25–82% of the total stored organic matter (Cummins *et al.* 1983 ; Metzler & Smock 1990 ; Smock 1990 ; Jones 1997 ; Jones *et al.* 1997). Despite hyporheic zones of streams have long been recognized as sustaining many physico-chemical and biological processes (Orghidan 1959 ; Schwoerbel 1961 ; 1964 ; Williams & Hynes 1974 ; Gibert *et al.* 1990 ; Vervier *et al.* 1992 ; White 1993 ; Triska *et al.* 1989), the specific significance of such processes in whole stream functioning has long been neglected.

Most of studies on the dynamic of organic matter in streams have mostly been limited to benthic habitats, *i.e.* with processes occurring above the streambed surfaces (Kaushik & Hynes 1971 ; Webster & Benfield 1986). As underlined by Boulton (2000), these lotic ecosystems were at the time only considered as "pipes" conducting water, solutes and organic matter from the catchment downstream, without considering other flowpaths above or below the ground where constant exchanges of water and materials occurs. The few authors who have attempted to determine the fate of leaf litter when incorporated into stream sediments have reported that burial led generally to reduced rates of leaf litter decomposition (Reice 1974 ; Herbst 1980 ; Rounick & Winterbourn 1983 ; Metzler & Smock 1990 ; Naamane *et al.* 1999), or sometimes reported ambiguous or less well-defined patterns (Mayack *et al.* 1989 ; Smith & Lake 1993). On the whole, ecological significance of leaf litter decomposition in the hyporheic zone for headwater stream metabolism remains poorly documented (but see Herbst 1980 ; Rounick & Winterbourn 1983 ; Metzler & Smock 1990 ; Smith & Lake 1993 ; Naamane *et al.* 1999 , Cornut *et al. in press*).

Aquatic hyphomycetes are responsible for the initial decomposition of leaves and, thanks to this microbial conditioning, for enhancing the palatability of leaf detritus to invertebrates. Detritic organic matter thus provides a more suitable food source for shredders which further contribute to the conversion of leaf litter into fine particles and other decomposition products (Arsuffi & Suberkropp 1985 ; Arsuffi & Suberkropp 1989 ; Suberkropp 1998 ; Covich *et al.* 1999 ; Gessner *et al.* 1999 ; Bärlocher 2005).

Cornut *et al.* (*in press*) have recently examined how the location of leaf litter within the streambed, *i.e.* at the surface or buried, may affect the leaf-associated decomposer communities and therefore leaf litter decomposition. Their findings indicate that burial within the substratum reduces the litter breakdown rates by limiting the access of invertebrate decomposers to leaves, and therefore suggest that the role of fungi is particularly important in the hyporheic zone of streams. Fungal colonization results in increasing leaf litter nutritional value and palatability to shredding invertebrates through the presence of their biomass and associated changes in leaf tissue due to their decomposing activity (Rounick & Winterbourn 1983 ; Bärlocher 1985 ; Graça 1993).

Some studies revealed that shredders prefer feeding on leaves with certain characteristics, *e.g.* high nitrogen content, low levels of structural or secondary leaf compounds, and a high degree of fungal colonization (Schlief & Mutz 2006). Moreover, it is well documented that beyond the enhancement of leaf detritus nutritional quality by fungi, feeding on leaf litter first colonized by fungi also affects some life-history traits of shredders such as growth, survivorship, and reproduction (Chung & Suberkropp 2009).

Therefore, in addition to their role in the decomposition of organic matter, aquatic hyphomycetes also mediate energy and nutrient transfers to higher trophic levels (Bärlocher & Kendrick 1976). Herbst (1980) first reported that burial within the sediment affected negatively consumption rates of leaves by shredders compared with those incubated on streambed surface. It was suggested that this response pattern may be related to the greater percent crude protein per ash-free dry weight found in surface incubated leaves. Other hypotheses have been proposed such as differences in leaf softness, or the intensity of microbial colonization. Considering the scarcity of the literature on the subject, and particularly of microbial focused studies, the conclusions concerning the role of aquatic hyphomycetes in the conditioning of leaves within the hyporheic zone, and their consequences in terms of palatability to shredders and leaf litter decomposition, remain largely unknown.

Through aquatic fungi activity, increases in the food quality of leaf detritus buried in the hyporheic zone of streams could enhance some life-history traits of shredders such as growth, survivorship, and reproduction. In particular, improvements in the elemental composition of resources, and particularly phosphorus content, is now recognized as a main driver of nutrient transfer efficiency in food-webs, affecting in return a wide range of ecological processes (Cebrian 1999 ; Elser & Urabe 1999 ; Sterner & Elser 2002). Phosphorus is often the most limiting element for growth and activity of microbial and invertebrate decomposers in streams (Cross *et al.* 2003 ; Grattan & Suberkropp 2001 ; Rosemond *et al.* 2002 ; Stallcup *et al.* 2006). In particular, P requirements of organisms directly reflect their organismal requirements of P-rich ribosomal RNA, molecules closely related to organisms growth rates.

This observation, called the "growth rate hypothesis" (Elser *et al.* 2000), has now been tested on several types of organisms in diverse ecosystems (Sterner & Elser 2002). However, relationship between leaf litter C:P and growth rates of shredding invertebrates in these aquatic ecosystems has never been questioned.

The aim of this study was to determine how the location of leaf litter within the streambed, *i.e.* at the surface or buried, may affect the leaf litter-associated microbial decomposer communities and therefore leaf litter decomposition. Specific conditions prevailing in the hyporheic zone are likely to considerably influence the presence, dynamics and activity of aquatic hyphomycetes. Our first hypothesis was that the biomass of aquatic hyphomycetes would be lower in the hyporheic zone than in the benthic habitat. We expected leaf litter burial to lead to an impoverished community and lowered activity of aquatic hyphomycetes, not only because of the physical barrier but also because of the decreased oxygen concentration in the interstitial water.

As a consequence, our second hypothesis was that leaf litter palatability depending on initial microbial colonisation should be altered by leaf litter burial. In addition, the history of burial, *e.g.* leaf exposed at the surface of the sediment for a short period before being buried, by permitting leaf litter colonization in better conditions, could reduce the negative effect of burial on leaf litter decomposition and on palatability to decomposers.

We tested experimentally these hypotheses in a second-order stream located in southern France, where leaf-associated microbial community, as well as decomposition rates, elemental composition, shredding invertebrate consumption rates and growth rates, were compared across controlled treatments differing by their location within the streambed (buried in the sediment, buried after 3 weeks of exposure at the sediment surface, buried in the sediment for 3 weeks before being exposed at the sediment surface or exposed at the sediment surface throughout the experiment).

4.4. Material and Methods

Study site

The experimental site was located in a 2nd-order stream, called Alzeau, in the Montagne Noire - South-Western France (02°13′23" E, 43°25′51" N; elevation 743 m a.s.l). The area is subject to a mountain climate with oceanic influences marked by high rainfall in the autumn and winter (1,300 mm year^{-1}). The surrounding forest consisted of mixed broadleaf species dominated by alder *Alnus glutinosa* (L.) Gaertn and oak *Quercus petraea* (Mattus.) Liebl. The study was conducted in a pool-riffle-pool sequence, extending over ca. 100 m, similar to those described by Cornut *et al.* (*in press*). Four representative riffles were selected and used as replicates sites for decomposition study. The stream was 3.5-4.7 m wide and 0.25-0.35 m deep in riffles, and the average discharge was ca. 500 L s^{-1}. Vertical hydraulic gradients (VHGs) through the streambed were calculated as follows: VHG (m m^{-1}) = $\Delta h/\Delta l$, where $\Delta h = h_{\text{stream}} - h_{\text{piezometer}}$ and Δl is the distance between the streambed and the top of the Plexiglas minipiezometer screen (Baxter *et al.* 2003). Vertical hydraulic gradients were measured to identify downwelling (positive VHG) and upwelling (negative VHG) areas in the hyporheic zone. Sediment characteristics (particle size and hydraulic conductivity) were studied in the four selected riffles of the study site.

The substratum was unconsolidated and mostly made of coarse sediments as determined from fractionation of the grain size of sediment cores (20 cm length x 30 cm diameter) Sediments were on average dominated by particles of 10-20mm, and small size particles were rare (particles > 20 mm: 8.6%; 20-10 mm: 25.9%; 10-5 mm: 23.4%; 5-2 mm: 19.3%; 2-1 mm: 9.5%; 1-0.5 mm: 9.3%; 0.5-0.25 mm: 3.9%). The average flow rate through the sediment of the study site reached 22 cm h^{-1}. To monitor stream water temperature throughout the experiment, calibrated data loggers (SmartButton, ACR System Inc., Surrey, Canada) recording temperatures every 2 hours were used. Water chemistry was determined on seven dates: at the beginning of the experiment then on every sampling date. Water sampling was carried out on the four selected riffles, both at the surface of the sediment, and in the hyporheic zone (*i.e.* at 15 cm below the sediment surface) using a plexiglas minipiezometers and a hand-held vacuum pump.

Conductivity, pH, and dissolved oxygen concentration were measured in the field using portable instruments (pH-meter 320i and Oxi 330i, WTW, Weilheim, Germany; Conductimeter Dist, HANNA, Woonsocket, Rhode Island, USA). Water samples were filtered in the field using GF/F filters (Glass fibre filters, Whatman, Clifton, New Jersey, USA - nominal cut-off 0.7 μm) and stored at 4°C in pre-rinsed polyethylene bottles until analyses in the laboratory. Concentrations of P-PO_4^{3-}, measured as soluble reactive phosphorous (SRP), and N-NO_3^- were measured using standard colorimetric methods.

Leaf litter conditioning and mass loss

Litter conditioning was carried out using the classical decomposition method of litter bags (Graça *et al.* 2005). Oak (*Quercus robur* L.) leaves were selected as a slow decomposing material and as a species with a particularly low palatability without microbial colonization. Oak leaves were collected at abscission using a suspended net in autumn 2008, in the riparian zone of the Alzeau river. About 2.5 g (±0.02) of air-dried leaves were enclosed in fine (0.5-mm nylon) mesh bags, 10cm x 10cm sized. The fine mesh excluded most of the invertebrates without interfering with microbial colonization (Boulton & Boon 1991). A total of 144 litter bags were introduced into the stream, following 4 conditions that simulates 4 different scenarios after leaf introduction into a stream: 1) Strictly benthic conditioning (B), *i.e.* leaf exposed at the surface of the sediment throughout the experiment, 2) Benthic-Hyporheic (BH), *i.e.* leaf exposed at the surface of the sediment for 19 days, allowing benthic microorganisms conditioning, then buried into the sediment until the end of the experiment, 3) Hyporheic-Benthic (HB), *i.e.* leaf directly buried into the sediment, then returned to the surface of the sediment, and 4) strictly hyporheic, *i.e.* leaf exposed in the experiment throughout the experiment. Hyporheic leaf bags were buried at 15-20 cm depth into the sediment using a small shovel. The experiment lasted 117 days, and one litter bag of each treatment was randomly sampled on each of the four replicate riffles on six dates, after 7, 19, 35, 56, 82, and 117 days to follow leaf conditioning and decomposition. In addition, after 35, 56, and 82 days, one supplementary litter bag was randomly sampled for each treatment on each of the four riffles to measure leaf palatability to diverse macroinvertebrate species.

On sampling dates, each litter bag was stored individually in plastic zip-lock bag, and brought back to the laboratory at 4°C.

Then, leaves were quickly washed individually to remove small sediments, until processing for diverse analyses and consumption measurements. A set of 5 discs of 12 mm diameter were stored at -18°C until ergosterol content measurements, and 7 discs of the same size were used to quantify conidia production by aquatic hyphomycetes. Remaining organic matter was stored at -18°C until being freeze dried and reweighted. Leaves were then grinded using a bead-grinder (Culatti, Zurich, Switzerland) and aliquots of 300 to 400 mg were then combusted at 450°C for 5h to estimate organic matter Ash-Free Dry Mass (AFDM). To estimate leaf litter decomposition rate, the leaf mass remaining on each date was expressed as the ratio of the AFDM between the remaining litter and the litter introduced at the beginning of the experiment.

Microbial colonization of leaves and litter quality

On each sampling date, several parameters were followed to estimate leaf litter microbial colonization and quality for consumers. To estimate fungal diversity in leaves, seven fresh leaf discs were quickly placed in glass Petri dishes filled with 20mL of pre-filtered (GF/F, Whatman, nominal cut off: 0.7µm) stream water. Dishes were gently agitated in the dark on an orbital shaker (60 rpm) at 10°C for 48h. Thereafter, spore suspensions were transferred into 50 mL polyethylene centrifuge tubes, the Petri dish and discs were rinsed with distilled water to collect spores no longer in suspension, and the volume was adjusted to 35 ml with 2 mL of 37% formalin and distilled water.

The discs were then lyophilized and weighed to the nearest 0.1 mg. 0.5 mL of Triton X-100 (0.5% solution) was added to the spore suspensions and they were stirred gently to ensure uniform distribution of spores. 5 mL aliquots were then filtered through a membrane filter (5 µm pore size, diameter 25 mm; Millipore Corporation), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid (Iqbal & Webster 1973). Spores have not all been analyzed yet, and this part of the results will be included in the manuscript later.

Fungal biomass in leaf litter was quantified through measurements of ergosterol content, a proxy for mycelium biomass (Gessner & Chauvet 1993). Five freeze dried leaf discs were weighted to the nearest 0.1mg then heated at 80°C for 30min with alkaline methanol to extract lipids. Extracts were purified usingsolid-phase extraction cartdridges (Oasis HLB, 60mg, 3cc, Waters, Milford, Massachusetts, USA), then quantified by high performance liquid chromatography (Kontron). Ergosterol content were then converted to mycelium biomass (mg g^{-1} of litter) using a conversion factor (Gessner & Chauvet 1993).

To describe leaf litter quality to invertebrates, we chose two important parameters relative to the species chosen, oak: leaf litter toughness and elemental composition. Leaf toughness is an important parameter involved in leaf litter consumption by invertebrates (Arsuffi & Suberkropp 1984), and particularly in low palatability species such as oak leaves. This parameter was estimated by measuring the force needed to penetrate leaf samples (*e.g.* Arsuffi & Suberkropp 1984) using a penetrometer. This penetrometer is similar to those described in Graça *et al.* (2005). The punch (diameter: 1.55 mm) was placed in the center of an area bounded by the leaf veins. Water was loaded on a piston until the punch broke through the leaf. The mass (mg) of water necessary to break through the leaf was taken as a measure of leaf toughness. For each treatment, leaf toughness corresponds to the mean of 15 replicate measurements.

Finally, leaf litter elemental ratios were chosen as good estimators of detritic organic matter decomposability (Enriquez *et al.* 1993), and essential parameters affecting consumers' life history traits (Sterner & Elser 2002). To estimate leaf litter elemental quality for consumers, carbon (C), nitrogen (N) and phosphorus (P) content were measured on each sampling date. Analyses were carried out on previously grinded material. The percentage of carbon and nitrogen contained in leaf litter organic matter was quantified using a CHN elemental analyser (NA 1500 Series 2; Fisons, Manchester, UK). Organic P content was determined using the Ormaza-Gonzales & Statham (1996) method after oxidation by sodium persulphate. All elemental ratios are expressed as molar ratios.

Palatability test

To investigate the effect of leaf litter burial history in the hyporheic zone of streams on its palatability to invertebrates, we chose 3 distinct representative taxa of west European headwater streams: *Gammarus fossarum*, *Leuctra spp*. and *Sericostoma personatum.* The crustacean species, *Gammarus fossarum,* is widespread and common in headwater streams, often playing a major role in the leaf litter breakdown process (Felten *et al.*, 2008). *S. personatum* is the most common Trichoptera present in the studied stream, and is also known to strongly impact leaf litter breakdown. Finally, the plecoptera *Leuctra spp.* are among the most efficient taxa that are able to penetrate the hyporheic zone thanks to their morphology, and that can access to buried organic matter (Cornut *et al. in press*).

Leaf litter palatability to *Gammarus fossarum*, *Leuctra sp*. and *Sericostoma personatum* was determined through non-choice feeding assays (see Elger *et al.* 2005), on three distinct dates: after 35, 56, and 82 days of leaf litter conditioning in the stream. Individuals of the three species were acclimatized in the laboratory for at least 7 days at 10°C before the experiments, and supplied *ad libitum* with detritus from the stream. Organisms were then sorted out by size and put in clear water without food 48h before starting the experiments. The experiments were carried out in plastic containers (125 mL) filled with 60 mL of filtered stream water (GF/C filters, Whatman, nominal cut off 1.2 μm). Experiments were carried out at 10°C. For *G. fossarum* and *S. personatum,* tests were performed individually, whereas for *Leuctra sp*., a relatively small species compared to the two other species, 5 individuals were used for each measurement to limit the duration of the test and consequently, avoid any problem of organism maintenance. For each species, individuals were sorted out by size class to avoid any bias linked to differences in initial size (Dry mass: *G. fossarum:* 6.8 ± 0.7 mg, *Leuctra spp*.: 0.59 ± 0.07 mg, and *S. personatum:* 13.4 ± 2.3 mg). Three pairs of 10-mm discs from the same leaf were obtained with a cork borer, one pair for each of the three species tested. All discs were weighted to the nearest 0.01 mg after being quickly dried on a clean absorbent paper. For each species, one disc was available as food on the bottom of the container, and the second disc was placed inside a 250 μm mesh on the side of the container (no choice experiment). This second disc, inaccessible for the consumers, served as a control for potential leaf mass losses during the tests.

The length of the experiment depended on the species tested, and was adjusted to reach at least c.a. 50% of mass loss for one of the treatment discs.

Thus, palatability measurements lasted 11h for *S. personatum*, from 45 to 48h for *Leuctra spp.* and from 68 to 72h for *G. fossarum*. Then, all litter fragments and control discs were recovered, as well as macroinvertebrates, dried at 65 °C to constant weight, and weighed to the nearest 0.01 mg. Dry mass (initial mass minus losses due to non-consumption processes) of the disc available as food for consumers (DM_0) was estimated from the initial fresh weight of the disc multiplied by the ratio between control disc dry weight and control disc fresh weight. Relative consumption rates were calculated as the differences in the dry mass between estimated available leaf (DM_0) and leaf remaining after consumption (DM_f) and divided by the dry mass of consumers and by the time of the consumption experiment (in days). Relative consumption rates are expressed as mg $mg_{consumer}^{-1}$ day^{-1}. To limit variability, for each species of consumer tested, consumption rates were measured independently 4 times per treatment per replicate riffle (*i.e.* 16 measurements per treatment per date). To avoid pseudo-replication, the four measurements made with litter coming from the same riffle were averaged, and final analyses were carried out on the means obtained for each riffle (4 data per treatment per date).

Individual consumer growth rates

To test for the effects of leaf litter conditioning in the different zones of the stream on consumer life history traits, we carried out individual growth measurements of *Gammarus fossarum* fed with leaf litter from the 4 distinct treatments. This crustacean species was preferred to the two other insect species, due to its simplicity to be manipulated on relatively long term experiments. As for palatability measurements, *G. fossarum* were collected in the field and acclimatized in the laboratory for at least 7 days at 10°C, then sorted out by size and put in clear water without food 48h before starting the experiments. Organisms did not differ significantly between treatments and dates at the beginning of the experiment (15.6 ± 1.8 mg wet weight).

To test for the effect of the duration of leaf litter conditioning in the different compartments of the stream, the experiment was carried out on two sampling dates: after 35 and 82 days of conditioning. On these sampling dates, 12 mm leaf discs were cut. To avoid any alteration of leaf litter quality during the 3-weeks experiment, discs were immediately frozen at -18°C before their distribution to consumers. *G. fossarum* were put individually in 125 mL containers filled with 60mL of filtered stream water. Organisms were fed *ad libitum* with 2 discs. Every second day, leaf discs were recovered and replaced by new discs, and water was changed and replaced by filtered aerated stream water.

Experiments were carried out at 10°C. All organisms were weighted at the beginning and at the end of the experiment to the nearest 0.1 mg after being quickly dried on a clean absorbent paper. As for palatability tests, each measurement was replicated four times, then averaged to obtain one mean value for each treatment and replicate site. One individual died on the first day of the experiment with 35-days litter, and was replaced immediately. *G. fossarum* growth was calculated as the difference between initial mass and final mass divided by initial mass. Values have been multiplied by 100 to be converted in % of initial mass.

Statistical analyses

Leaf litter decomposition rates (k) were calculated using a classical exponential decay model as follows: $M_t = M_o.e^{-kt}$, where M_o represents the initial AFDM, M_t the remaining AFDM at time t, and t the time spent since the beginning of the experiment, in days. Analysis of covariance (ANCOVA) made on logarithmically transformed AFDM data was used to test for the effects of burial history on leaf litter decomposition. A test of comparison of mean (t-test) was used to test for differences between physico-chemical conditions in hyporheic and benthic zone of the stream. The effects of burial history and time and their potential interactions were investigated using two-way ANOVAs. Data were log transformed to improve homoscedasticity when necessary. Due to systematic differences in physico-chemical conditions on one of the four replicate riffles, and to correct for this potential bias, this parameter was introduced in all analyses as a block effect.

This parameter was eliminated of analyses when non-significant, and was only shown as significantly affecting phosphorus content of buried leaf litter. For all analyses, post-hoc tests used Tukey HSD procedure. For all statistical analyses, the significance $P = 0.05$ threshold was chosen.

4.5. Results

Physico-chemical conditions in benthic and hyporheic zones

All physico-chemical parameters measured throughout the experiment differed significantly between the hyporheic and the benthic zone of the river (Table 1). Oxygen was significantly lower in hyporheic than in benthic zone, showing a reduction by about 40%.

Conductivity and nutrient content of interstitial water, and particularly phosphorus, were systematically higher than stream water. Temperature was in average 0.5 °C higher in the hyporheic compartment. This result can be related to the fact that one of the four riffles showed higher temperature of interstitial water during the 80 first days of the experiment, and showed less variation in temperature throughout the experiment than other riffles. This riffle was characterized by a positive vertical hydraulic gradient (VHG), *i.e.* an upwelling-type functioning (discharge from the interstitial zone into surface water), whereas the 3 others riffles showed negative VHG throughout the experiment, *i.e.* were downwelling zones.

Leaf litter decomposition and conditioning

There was a marked variation in decomposition of oak leaves as a function of burial history (Table 2). In all treatments, leaves lost up to 10% of their mass during the first 7 days of the experiment, through leaching processes. Then, decomposition followed a classical exponential leaf mass loss, that differed between burial history treatments (ANCOVA, $F_{3,104} = 3.0$; $P < 0.034$). Leaf litter decomposition was significantly faster when exposed in the benthic zone of the river, intermediate in the Hyporheic-Benthic treatment, and was the lowest in the Hyporheic and Benthic-Hyporheic treatments (Figure 1).

Mycelium development of aquatic fungi, as measured by ergosterol content, was significantly affected by burial history ($F_{15,72}$ = 1.87, P = 0.04). Fungal biomass first increased quickly to a first plateau during the first 20 days of the experiment (Figure 2), this plateau being independent of burial treatment. Then, after 60 days, fungal biomass reached a second plateau, this one being significantly higher (by more than 30 %) in the benthic treatment than in the 3 other treatments.

Table 1 Physical and chemical characteristics of the two compartments, benthic zone and hyporheic zone. Values are means ± SE of data collected during the experiment (from January to May 2010). Values in parentheses represent minimal and maximal recorded values.

Variable	Benthic	Hyporheic	*P*-value
O_2 (% saturation)	104.9 ± 0.2 (101-107)	61.4 ± 17.7 (39-95)	<0.0001
pH	6.2 ± 0.1 (5.6-6.6)	5.9 ± 0.1 (5.0-6.6)	<0.0001
Conductivity (µS cm^{-1})	27.4 ± 0.9 (14-35)	35.3 ± 2.5 (22-46)	<0.0001
Temperature (°C)	6.2 ± 0.2 (4.0-10.4)	6.6 ± 0.5 (4.1-10.4)	0.01
$N\text{-}NO_3^-$ (mg L^{-1})	0.91 ± 0.02 (0.71-1.09)	0.83 ± 0.09 (0.52-1.01)	0.005
$P\text{-}PO_4^{3-}$ (µg L^{-1})	1.55 ± 0.23 (0.45-5.07)	9.61 ± 1.89 (3.98-19.62)	<0.0001

Table 2 Daily exponential decay rates (k) of oak leaves as a function of burial history in the hyporheic zone: benthic (leaf exposed at the surface of the sediment throughout the experiment), benthic-hyporheic (leaf exposed at the surface of the sediment for 19 days, then buried in the hyporheic zone), hyporheic-benthic (leaf buried in the hyporheic zone for 19 days, then exposed at the surface of sediment), and hyporheic (leaf exposed in the hyporheic zone throughout the experiment). The 95% confidence limits, regression coefficients, and time to reach 50% of leaf mass loss are also indicated.

Treatment	k (day^{-1})	95% CL	R^2	T50
Benthic	0.0036	0.0033-0.0043	0.96	192
Benthic-Hyporheic	0.0029	0.0020-0.0031	0.93	239
Hyporheic-Benthic	0.0032	0.0027-0.0035	0.94	216
Hyporheic	0.0029	0.0020-0.0031	0.93	239

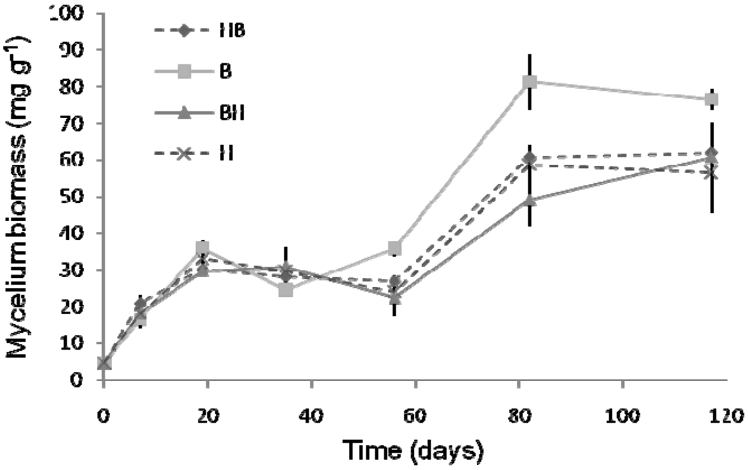

Figure 1 Dynamic of fungal mycelium biomass (derived from ergosterol contents) on oak litter as a function of burial history: B: leaf litter exposed at the surface of the sediment throughout the experiment, BH: leaf litter exposed for 3 weeks at the surface of the sediment before being buried in the hyporheic zone of stream, HB: leaf litter exposed for 3 weeks in the hyporheic zone before being exposed at the surface of the sediment, H: leaf litter exposed in the hyporheic zone of stream throughout the experiment. Vertical bars correspond to standard errors.

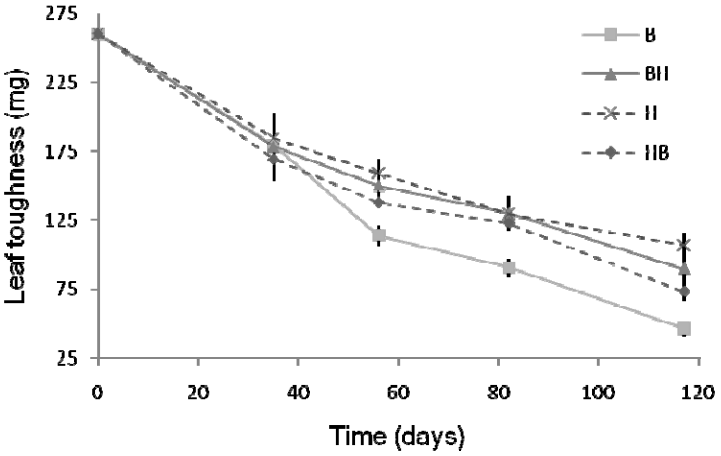

Figure 2 Leaf litter toughness as a function of burial history in the hyporheic zone of the stream. Legends of treatments are the same as in Figure 1. Vertical bars correspond to standard errors.

Leaf litter quality to consumers

Oak leaves litter quality was assessed by two distinct parameters: leaf toughness and leaf litter elemental ratios. Leaf litter toughness (Figure 2) decreased continuously during the experiment ($F_{3,48}$ = 44.4; $P < 0.0001$), but was in average 20% lower in the benthic exposed leaves than in the three other treatments ($F_{3,48}$ = 7.6; $P < 0.0003$). Both C/P and C/N ratios (Figure 3) of leaf litter exposed in the stream decreased significantly throughout the experiment ($F_{5,69}$ = 19.4; $P < 0,0001$ and $F_{5,72}$ = 9.76; $P < 0.0001$, respectively). On the contrary, leaf litter N/P ratio was maintained constant during the experiment ($F_{5,72}$ = 0.82; $P < 0.53$). Leaf litter C/N and N/P ratios were not significantly affected by the burial treatment ($F_{3,72}$ = 0.52; $P = 0.66$ and $F_{3,72}$ = 0.42; $P = 0.53$, respectively)., whereas leaf litter C/P ratio was significantly lower in the benthic treatment than in the three other buried treatments ($F_{3,69}$ = 2.76; $P < 0.04$). Leaf litter C/N and C/P ratios were negatively correlated to the mycelial content of the leaf ($R^2 = 0.34$; $P < 0.0001$ and $R^2 = 0.49$, $P < 0.0001$, respectively). In addition, leaf litter toughness was also negatively correlated to the mycelial content of the leaf ($R^2 = 0.54$, $P < 0.0001$).

Leaf litter palatability to consumers

Leaf litter palatability, as measured by instantaneous consumption rates of a leaf disc, differed as a function of the duration of the conditioning and the type of consumer tested (Figure 4). Leaf litter consumption rate by *Gammarus fossarum* significantly increased with the length of conditioning in the stream ($F_{2,36}$ = 17.7; $P < 0.0001$). Consumption rates of leaf litter by *Sericostoma personatum* were slightly higher after 56 days of exposure in the stream than on the two other conditioning length tested ($F_{2,36}$ = 5.06; $P < 0.01$). On the contrary, consumption rates of leaf litter by the Plecoptera, *Leuctra spp.*, was unaffected by the length of leaf litter conditioning ($F_{2,36}$ = 0.78; $P = 0.46$).

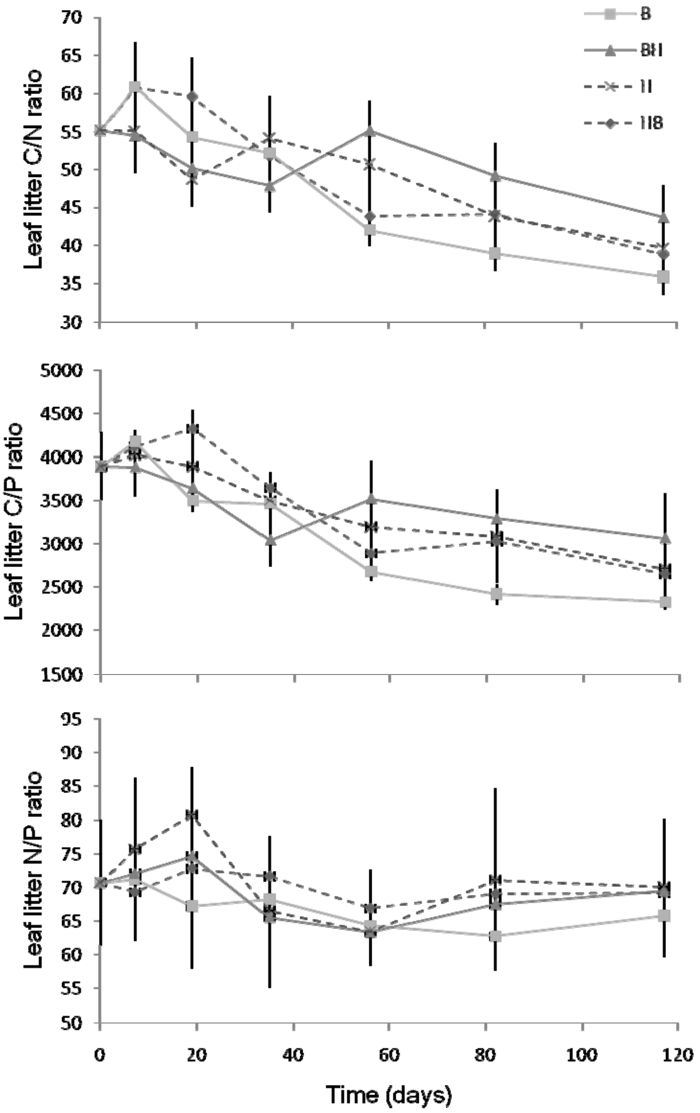

Figure 3 Leaf litter elemental quality, *i.e.* C/N, C/P, and N/P ratios as a function of burial history in the hyporheic zone of the stream. Elemental ratios are molar ratios. Legends of treatments are the same as in Figure 1. Vertical bars correspond to standard errors.

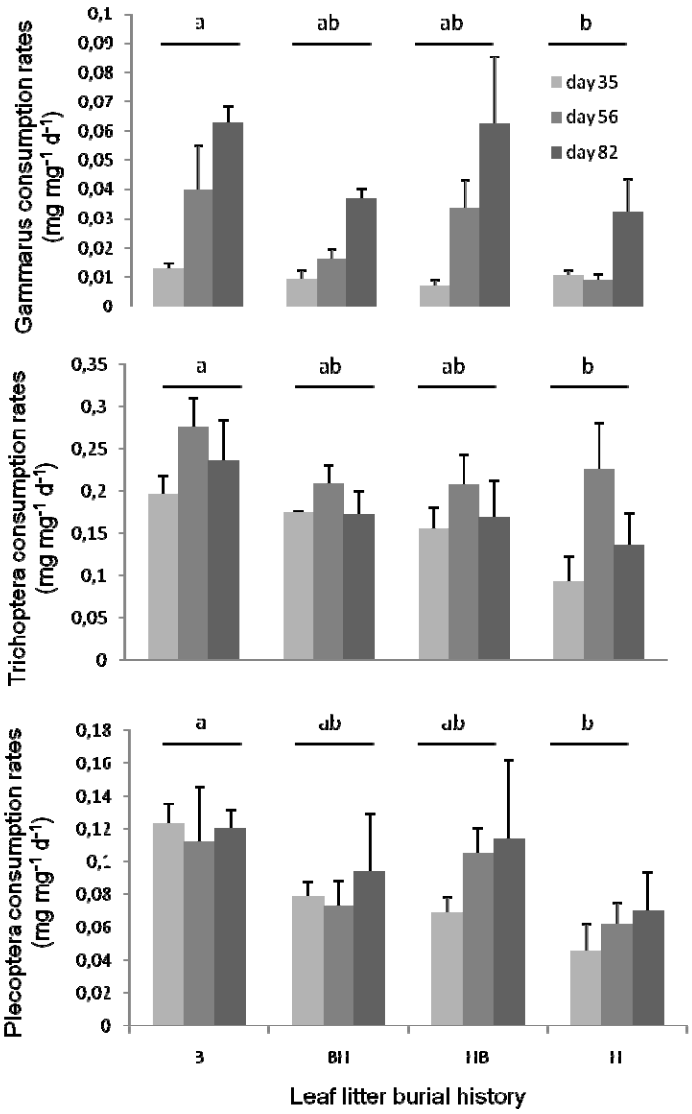

Figure 4 Leaf litter palatability to three taxa of invertebrate benthic consumers: *Gammarus fossarum* (Crustacea), *Sericostoma personatum* (Trichoptera), and *Leuctra spp.* (Plecoptera), as a function of leaf litter burial history in the hyporheic zone of the stream. Leaf litter palatability measurements were carried out with leaf litter exposed for 35, 56, and 82 days in the different treatment conditions. Legends of treatments are the same as in Figure 1. Vertical bars correspond to standard errors.

For the three invertebrates tested, *Gammarus fossarum*, *Sericostoma personatum*, and *Leuctra spp.*, leaf litter exposed in the benthic zone of the stream was on average more consumed than leaf litter exposed in the hyporheic zone throughout the experiment, litter coming from the two other treatments (Benthic-Hyporheic and Hyporheic-Benthic) showing intermediate consumption rates ($F_{3,36} = 3.69$; $P < 0.02$, $F_{3,36} = 3.20$; $P < 0.04$, and $F_{3,36} = 3.44$; $P < 0.03$, for *G. fossarum*, *S. personatum*, and *Leuctra spp.*, respectively).

Leaf litter palatability to *S. personatum* was both negatively related to leaf litter toughness ($R^2 = 0.07$; $P < 0.0001$) and negatively to the C/P ratio ($R^2 = 0.14$, $P < 0.0001$). In the same way, leaf litter palatability to *Leuctra spp.* was slightly negatively related to leaf litter toughness ($R^2 = 0.09$; $P < 0.0001$) and C/P ratio ($R^2 = 0.09$, $P < 0.0001$) and positively to the mycelium content ($R^2 = 0.06$; $p < 0.0001$). Finally, leaf litter palatability to *G. fossarum* was more strongly negatively related to leaf litter toughness ($R^2 = 0.31$; $P < 0.0001$) and C/P ratio ($R^2 = 0.13$, $P < 0.0001$). and strongly positively related to the mycelium content ($R^2 = 0.31$; $P < 0.0001$).

Effect of leaf litter burial on consumer growth rates

Individual growth rate measurements of *Gammarus fossarum* showed a significant effect of leaf litter burial history (Figure 5).

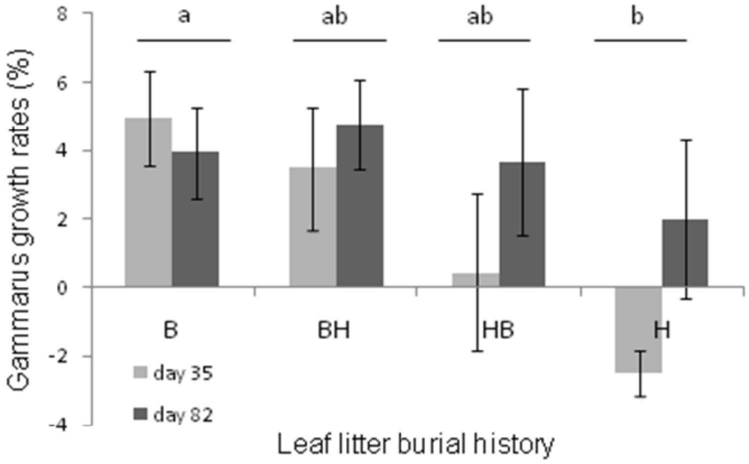

Figure 5 Effects of leaf litter burial history in the hyporheic zone of the stream on Gammarus fossarum growth rates. Values are expressed in percentage of initial mass. Growth rates measurements were carried out after two conditioning durations in the different treatment conditions: 35 and 82 days. Legends of treatments are the same as in Figure 1. Vertical bars correspond to standard errors.

G. fossarum growth rate did not differ significantly when fed with leaf litter conditioned for 35 and 82 days in the stream ($F_{1,24} = 2.62$; $P = 0.10$). However, their growth rates were significantly higher when fed with leaves coming from the benthic treatment than with leaves buried throughout the experiment, leaves from the two other treatments (Benthic-Hyporheic and Hyporheic-Benthic) leading to intermediate growth rate values. *G. fossarum* growth rate was neither correlated to leaf litter toughness nor to leaf litter palatability. However, *G. fossarum* growth rate was negatively related to leaf litter C:P ratio ($R^2 = 0.13$; $P < 0.0001$).

4.6. Discussion

Effect of burial of leaf litter on its conditioning and quality

Independently of the burial treatment, oak leaves mass loss was moderate (between 31 and 37%) during the 117 days of the experiment. However, this result is quite logical considering that in such fine mesh bags, only microbial processes can occur, and that oak leaves are considered as quite recalcitrant compared to other leaf types (Petersen & Cummins 1974 ; Suberkropp *et al.* 1976 ; Butler & Suberkropp 1986).

Even if the decomposition proceses of leaf litter in headwater streams is now quite well understood in surface waters, its fate when incorporated in the hyporheic zone, and particularly the role of microbial decomposers, are still poorly documented. Our results first indicate that leaf litter decomposition by microorganisms is significantly reduced in the hyporheic zone compared to the benthic zone of headwater streams, even if differences are small. We found only one study in the literature comparing leaf litter decomposition rates in fine-mesh bag between hyporheic and benthic zones of streams (Rounick & Winterbourn 1983). In their study, decomposition patterns did not differ between the two types of treatments.

Even if aquatic hyphomycetes play a relatively higher role than invertebrates in the decomposition of buried organic matter (Cornut *et al. in press*), our results show that in absence of shredding invertebrates, microbial mediated decomposition is more important in the benthic treatment than in the hyporheic treatment.

Interestingly, there were neither differences in mycelial biomass nor differences in decomposition rates between leaves that have been buried throughout the experiment (Hyporheic treatment) and leaves that have either been exposed for two weeks at the surface of the sediment (Benthic-Hyporheic treatment) or those that have been buried for two weeks and then brought back to the surface (Hyporheic-Benthic treatment). After 115 days of leaf conditioning, mycelial biomass measured in leaf litter exposed at the surface of the sediment remained nearly 20% lower than mycelial biomass measured in the Hyporheic-Benthic treatment. Consequently, the initial burial history of detritic organic matter entering these ecosystems seems to play a great role in microbial colonization and leaf litter decomposition. Further analyses of fungal communities through spore identification could permit to determine if oak leaves burial history, in addition to the observed effects on microbial biomass, also affects fungal communities, as shown by Cornut *et al.* (*in press*) on alder (*Alnus glutinosa*) leaves.

Observed effects of burial on fungal biomass and putative effects on species present on leaf litter could be related to differences in physico-chemical conditions between the hyporheic and the benthic zone. The most pronounced difference concerns oxygen concentrations, these ones being on average 45% lower in the hyporheic than in the benthic zone of the stream. This observation could at least partly explain negative impacts on microbial processes, this parameter being essential for aquatic fungi activity. Medeiros *et al.* (2009) showed that from 76% of oxygen saturation to lower concentrations, fungal biomass and decomposing activities are two-fold reduced compared to 94% of oxygen saturated water.

Finally, even if we observed higher phosphorus concentrations in interstitial water compared to surface water, leaf litter elemental composition remained always lower in the benthic zone of the stream.

This result could be explained by the lower development of decomposers observed in the hyporheic zone of the stream, these decomposers leading to lower nutrient immobilization.

Effect of leaf litter burial on leaf litter palatability and consumer growth rates

After colonization by microbial decomposers, microorganisms condition the leaves for shredders through the presence of their own organic matter and numerous changes brought about in leaf tissue chemistry by their enzyme activity and nutrient immobilization (Rounick & Winterbourn 1983 ; Bärlocher 1985 ; Graça 1993). In particular, aquatic hyphomycetes are essential to increase palatability of leaf litter to shredders through increases in nitrogen content and reductions in the concentration of structural or secondary leaf compounds. Despite the numerous studies focused on the palatability to shredding invertebrates of organic matter conditioned in surface water of streams, data on the effect of organic matter conditioning in the hyporheic zone of streams remains very scarce. In this study, leaf litter palatability greatly differed between the three species of invertebrate consumers tested. Leaf litter consumption rates increased with time only for *Gammarus fossarum*. In addition, for the three species of consumers tested, leaf litter palatability was related to leaf litter toughness. However, the relationship was far much stronger for *Gammarus fossarum* than for the two other taxa.

These results could be explained by differences in the feeding behavior and anatomical differences in the mouth parts. Several previous studies showed that leaf toughness can influence food selection by building a physical barrier for invertebrate feeding, harder leaves being more difficult to tear than soft ones (Pennings *et al.* 1998 ; Motomoro *et al.* 2001). The small Plecoptera, *Leuctra spp.*, only feed by scraping the surface of the leaf discs. Toughness measurements integrate all the thickness of the leaf, and this parameter is certainly not pertinent for this type of consumer. The Trichoptera, *S. personatum*, seems to be only weakly dependent on leaf toughness, probably thanks to their abilities to eat low-conditioned and hard material. In contrast, *G. fossarum* seems to be really dependent on this parameter.

This type of shredder eats the whole leaf disc, *i.e.* fungi and the leaf matrix, and could be limited by its feeding apparatus when leaf remains too hard (Graça *et al.* 1993). Thus, this organism appears as a particularly interesting biological model that greatly depends on the conditioning of detritus made by decomposers.

With the measured biological and physico-chemical parameters, only a small part of the observed patterns of leaf palatability to consumers can be explained. Several studies showed that more than total fungal biomass in the leaf litter, fungal species present in organic matter can shape the response of invertebrate consumers (*e.g.* Butler & Superkropp 1986). Physical screening of spores by sediment and local physico-chemical conditions in the hyporheic zone of streams playing a great role in the selection of fungal species colonizing buried organic matter (Cornut *et al. in press* ; Cornut *et al. in prep*), burial could indirectly affect leaf litter palatability by selecting more or less palatable fungal species. Analyses of fungal communities that are currently in progress will perhaps permit to relate the structure of these communities to the observed palatability of leaf litters to macroinvertebrates.

Considering the advantages described above, we selected *G. fossarum* as a pertinent model to test the effect of leaf litter burial on invertebrate consumer growth rates. Our results clearly show that leaf litter burial in the hyporheic zone negatively impacted *G. fossarum* growth rates. Maximal growth rates were observed when organisms were fed with leaf litter exposed at the surface of sediment throughout the experiment. We found no effects of leaf litter toughness or palatability on *G. fossarum* growth rate. However, *G. fossarum* growth rate was negatively correlated to leaf litter C/P ratio. This result is in total accordance with the "Growth Rate Hypothesis" (Elser *et al.* 2000), phosphorus content in resources being often the main factor limiting consumer growth. Low C/P resources allow increased allocation to P-rich ribosomal RNA that permits protein synthesis by ribosomes and support organisms' growth. Even if this kind of relationship has been showed in many ecosystems and on very diverse taxa (Elser *et al.* 2003), there are very few data available on headwater stream organisms. Frost & Elser (2002) showed that benthic mayflies (*Caenis sp.* and *Ephemerella sp.*) growth increased when fed with P-poor biofilm artificially enriched with inorganic P. To our knowledge, it is the first time that such relationship is shown on detritivorous invertebrates.

Nevertheless, the relationship is weak, and several other factors may interact with phosphorus content on invertebrate growth. Oak leaves being particularly rich in structural and other secondary compounds, the impact of elemental composition of resources is certainly reduced compared to other physical or chemical constraints. We could expect stronger stoichiometric effects on consumer growth rates with higher quality detrital material, such as *Alnus* or *Aspen* leaves.

4.7. Overview

Our study shows that burial of leaf litter in the hyporheic zone of headwater streams, even for a short period, is able to alter microbial development and leaf litter conditioning. These modifications can in turn negatively impact leaf litter palatability to consumers as well as some detritivorous consumer life history traits, such as growth rates. These results underline the key role of microbial decomposers, and particularly aquatic fungi in the fate of organic matter buried in the hyporheic zone of headwater streams. Further analyses of aquatic hyphomycetes species present in leaf litter could help explain observed results on leaf litter palatability to consumers. The low decomposition rates observed as well as the delay in quality improvement compared to benthic exposed leaves also confirm that the hyporheic zone of woodland streams is an important compartment for organic matter storage that could provide food sources to detritivorous organisms after sediment movements, when new detrital material incomes in the stream are scarce. This temporary reservoir of food resources may thus affect the overall stream metabolism, and should be taken into account in further studies considering the functioning, the conservation or the restoration of headwater stream ecosystems.

4.8. References

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Chapitre 5:

Effects of anthropogenic acidification on leaf litter processing in the hyporheic zone of headwater streams

5. Effects of anthropogenic acidification on leaf litter processing in the hyporheic zone of headwater streams

5.1. Résumé

L'ensemble des chapitres précédents ont permis de mettre en évidence le rôle déterminant joué par la zone hyporhéique dans le fonctionnement écologique des cours d'eau forestiers. Ils ont également mis en lumière la contribution des différents agents impliqués dans le processus de décomposition, en particulier celle des champignons. Enfin, ils ont révélé le contrôle important qu'exerce le compartiment hyporhéique sur la composition des communautés de décomposeurs, et par conséquent son implication dans l'efficacité des transferts trophiques. Il est important de noter que l'ensemble des ces expériences a été réalisé dans des systèmes peu ou pas altérés par les impacts anthropiques. Dans ce contexte, il semble pertinent d'évaluer l'effet d'un stress d'origine anthropique sur le fonctionnement écologique du compartiment hyporhéique, et d'identifier les mécanismes impliqués dans les dysfonctionnements qui pourraient être observés. A ce titre, l'acidification anthropique est un processus connu pour induire des effets délétères sur la structure et le fonctionnement des écosystèmes d'eau de surface.

La cinquième partie de ma thèse a donc consisté àexaminer comment ce processus pouvait affecter le taux de décomposition des litières, ainsi que la structure et l'activité de la communauté de décomposeurs dans la zone hyporhéique de cinq cours d'eau de tête de bassin, le long d'un gradient d'acidification , se répartissant de très acide (pH 4.6) à neutre (pH 7,4). Nous avons testé l'hypothèse selon laquelle la décomposition des litières serait moins affectée par l'acidification dans la zone hyporhéique en raison de (i) la sensibilité moindre des décomposeurs fongiques à ce facteur de stress, et (ii) l'absence de gros invertébrés détritivores dans l'habitat hyporhéique des cours d'eau de tête de bassin.

Les résultats ont montré que la décomposition des litières est beaucoup plus rapide tant dans les zones hyporhéiques et benthiques du cours d'eau neutre (k = 0,0068 et 0,0534 j^{-1}, respectivement) que dans le plus acide (k = 0,0016 et 0,0055 j^{-1}, respectivement), et que les taux de décomposition sont bien corrélés au gradient d'acidité dans les deux habitats.

Les invertébrés détritivores sont beaucoup moins abondants et efficaces dans leurs contributions au processus de décomposition des litières dans les cours d'eau acides. En revanche, la biomasse fongique ne montre aucune tendance particulière, excepté une valeur de pic supérieure dans le cours d’eau le plus acide, résultant probablement d’une diminution de la compétition et de la prédation par les invertébrés détritivores. Dans l’ensemble, les réponses à l'acidification dans les zones hyporhéiques sont moins prononcées, bien que suivant le même patron que leurs homologues benthiques.

Cette étude n’a pas permis de discriminer sans équivoque l'importance relative du pH, de l'aluminium, des nutriments et d'autres variables liées à la toxicité des eaux acidifiées. Le fait que le ralentissement de la décomposition des litières dans les cours d'eau acidifiés, à la fois dans les zones benthiques et hyporhéiques, soit ou non directement relié à l’érosion de la diversité des hyphomycètes aquatiques reste à déterminer. Quoi qu’il en soit, la subsistance de décomposeurs fongiques actifs dans un environnement inoccupé par les invertébrés détritivores est l’assurance pour les cours d’eau dégradés par l’acidification du maintien d’un flux de carbone, avec le compartiment hyporhéique agissant comme une source importante de matière organique et de propagules pour les zones avales. Ces résultats plaident donc en faveur de la conservation des habitats hyporhéiques des cours d'eau affectés par l'acidification.

5.2. Abstract

Anthropogenic acidification has been shown to induce deleterious effects on both structure and functioning of surface water ecosystems. This study examined how it may affect the leaf decomposition rate and the community structure and activity of decomposers in the hyporheic zone of five headwater streams along an acidification gradient from highly acidic (pH 4.6) to circumneutral (pH 7.4). We hypothesized leaf decomposition to be less affected by acidification in the hyporheic zone due to (i) the expectedly lower sensitivity of fungal decomposers to this stressor, which are supposedly maintained in this habitat, and (ii) the lack of large macroinvertebrate decomposers in the hyporheic habitat of streams. Leaf decomposition was much faster both in the hyporheic and benthic zones of the circumneutral stream (k = 0.0068 and 0.0534 d^{-1}, respectively) than in the most acidic one (k = 0.0016 and 0.0055 d^{-1}, respectively), and leaf decomposition rate correlated well with the acidic gradient in both habitats. Shredder taxa were much less abundant and performing in leaf fragmentation in acidic streams. In contrast, fungal biomass showed no particular trend except that maximum was higher in the most acidic stream, probably resulting from the depressed competition and predation by shredders. Overall, responses to acidification in the hyporheic zones were generally less pronounced, although following the same pattern than in their benthic counterparts. The reduced leaf decomposition in the hyporheic zone of acidified streams was likely due to a depressed fungal activity, in relation with the adverse chemical conditions and the lower fungal diversity. These results argue for a conservation of the hyporheic habitats in streams impaired by acidification as they can maintain fluxes of matter and species that are essential to the ecosystem.

5.3. Introduction

Acid deposition has degraded terrestrial and freshwater systems, and despite reductions in sulfate and nitrogen oxides emissions, acidification remains a widespread problem (Schindler 1988, Driscoll *et al.* 2001). Anthropogenic acidification has been shown to induce deleterious effects on both structure and functioning of surface water ecosystems (Muhlolland *et al.* 1992 ; Dangles & Guérold 2001 ; Dangles *et al.* 2004 ; Baudoin *et al.* 2008), with low-order streams being especially impacted. The ecosystem health implies preservation of the natural components, processes, and functions of all parts of the ecosystem, even internal ones that are not evident from a surperficial perspective (Boulton 2000). Therefore, we need to assess the well-being of both surface and hyporheic zones if we intend to measure health of the whole stream ecosystem.

At present, only the surface appearance is being measured, overlooking some fundamental attributes below the streambed surface (Boulton 2000). In the last decades, hydrologists and rivers ecologists have been aware of the significance of the vertical dimension of rivers and have concerted efforts to integrate subsurface hydrology and ecology into conceptual models of river ecosystem function (Boulton 2000). Indeed, one of the major outputs of this conceptual advance was the recognition that rivers do not function simply as channel conducting water and solutes from the catchment downstream, but involve multiple and complex water and material exchanges above and below the ground (Williams & Hogg 1988 ; Bencala 1993 ; Findlay 1995 ; Boulton 2000). As water flows through the hyporheic zone, microbial and chemical processes alter the water chemistry by transforming nutrients, consuming oxygen, decomposing organic matter, and others activities (Boulton 2000). Consequently, such exchange flows can play a substantial role in the surface and subsurface energetics (Grimm & Fisher 1984 ; Valett *et al.* 1994 ; Jones *et al.* 1995 ; Mulholland *et al.* 1997 ; Holmes *et al.* 1998). Moreover, studies on biologic communities in hyporheic zones have shown that the latter are important sites of refuge, dwelling and development for the freshwater fauna (Nagorski and Moore 1999).

Leaf litter decomposition is a pivotal process in woodland stream ecosystems (Cummins *et al.* 1989). Several studies have shown the extent to which this process is depressed by anthropogenic disturbances like atmospheric acidification (Burton *et al.* 1985 ; Chamier 1987 ; Groom & Hildrew 1989 ; Mulholland *et al.* 1992 ; Griffith & Perry 1993 ; Dangles & Guérold 1998 ; Dangles *et al.* 2004 ; Baudoin *et al.* 2008).

However, a substantial portion of leaf litter entering running waters is subject to burial into the stream bed as a consequence of flood events (Herbst 1980) and sediment movement (Metzler & Smock 1990 ; Naegeli *et al.* 1995). Under the control of abiotic processes and decomposers' activity, the hyporheic zone may act as a sink or a source of detrital organic matter to surface waters. Compared to the benthic habitat, leaf litter decomposition in the hyporheic zone tends to be slowed down with the decomposers community being dominated by aquatic fungi (Cornut *et al. in press*). During plant litter decomposition in benthic environments, fungal biomass on decaying leaves may reach 17% of total detrital mass (Gessner *et al.* 1997) and may account for up to 98% of microbial decomposer (*i.e.* fungi + bacteria) biomass on leaf litter (Gulis & Suberkropp 2003). Reduced leaf decomposition rates following stream acidification are often attributed to microbial impairment (Mulholland *et al.* 1987 ; Baudoin *et al.* 2008 ; Simon *et al.* 2009). Several studies have clearly shown that aquatic hyphomycetes respond negatively to stream acidification (Chamier 1987 ; Chamier & Tipping 1997 ; Baudoin *et al.* 2008), with pectin lyases activity being higher in neutral or alkaline waters than in acid or soft waters and pectinases production being affected by monomeric aluminium (Chamier 1992 ; Griffith *et al.* 1995 ; Jenkins & Superkropp 1995 ; Chamier & Tipping 1997).

On one hand, burial within the substratum can reduce the access of decomposers to leaves with the small interstices of the gravelly sediment acting as a constraint, especially for the largest shredders. Within a few centimetres, the sediment of the hyporheic zone constitutes a physical barrier, allowing only the taxa with appropriate morphological characteristics (*i.e.*, smaller, narrower, more flexible) to penetrate to the deeper layers (Omesová *et al.* 2008).

On the other hand, the eradication of the acid-sensitive species, which are often among the largest ones, *i.e. Gammarus fossarum* Köch (Amphipoda, Gammaridae) and trichopterans, from acidic streams is at least partly responsible for the reduction of leaf litter decomposition under acidic conditions (Dangles & Guérold 1998). Even if these shredders are replaced by plecopteran shredders in acidic streams – sometimes in greater abundance as observed by Sutcliffe & Hildrew *et al.* (1989) and Dangles & Guérold (1999) – the latter were suspected to be less efficient shredders and consequently unable to fulfill the functional role of *G. fossarum* and trichopterans in neutral streams.

The aim of this study was thus to determine how the location of leaf litter within the streambed, *i.e.* at the surface or buried, along an acidification gradient may affect the leaf-associated decomposer communities and therefore leaf litter decomposition.

The hyporheic zone was susceptible to constitute an important site of refuge for freshwater biota, preventing or buffering the effects of acidification from the surface water. Specifically, we hypothesized leaf decomposition to be much less affected by acidification in the hyporheic zone, because large macroinvertebrate detritivores like *Gammarus fossarum* and Trichopterans are supposedly absent from the hyporheic zone and more sensitive to acidification than fungal decomposers, which were expected to disappear more gradually along the acidification gradient and be less constrained by the hyporheic conditions. We tested this hypothesis by conducting an experimental study where leaf-associated microbial and macroinvertebrate communities, as well as leaf decomposition rates and the activity of microbial decomposers were compared in the benthic and hyporheic zones of five low-order streams along a pronounced acidification gradient.

5.4. Materials and methods

Site

The experiment was conducted in the Vosges Mountains (North-eastern France) where anthropogenic acidification has adversely affected surface waters. Forestry was the only other anthropogenic disturbance, although limited within the study area. Five first and second-order streams along an acidification gradient, differing in pH, and total Al concentration, but with similar hydrological and morphological characteristics were selected. Courbeline (CL) was the most acidified stream. Ravines (RV) and Gravelle (GV) were two moderately acidic streams, differing by their concentrations of total Al, NO_3^- and acid neutralising capacities (ANC). Two non-acidified streams, Menombru (MB) and La Maix (LM), showed a circumneutral pH. They exhibited rather similar physical and chemical characteristics, but strongly differed regarding their ANC and total Al concentrations (448 vs. 204 µEq/L and 26 vs. 93 µg/L, respectively) which contrasted with those in LM (Table 1). Vegetation in the selected catchments was dominated by silver fir *Abies alba* Mill., Norway spruce *Picea abies* L. and beech *Fagus sylvatica* L. Beech and alder (*Alnus glutinosa* (L.) Gaertn.) were by far the most common deciduous tree species adjacent to streams and provided almost all leaf litter inputs.

Water chemistry was determined at the seven dates when leaf bags were introduced into or retrieved from the streams. Samples of surface and interstitial water were collected from each stream, stored in pre-rinsed polyethylene bottles, and placed in an icebox until they were returned to the laboratory to be analysed within 48 h. Interstitial water was pumped from plexiglas minipiezometers using a hand-held vacuum pump. Stream pH was measured in the laboratory using a microprocessor pH meter (pH 3000, WTW), and acid-neutralizing capacity (ANC) was determined by Gran's titration. Conductivity was measured with a Metrohm Herisau Conductometer E518 (Herisau, Switzerland) at 25°C. Concentrations of Ca^{2+}, Mg^{2+}, Na^+, K^+ and total Al (after acidification with HNO_3) were determined by atomic absorption spectrophotometry (Analyst 100; Perkin Elmer and Varian SpectrAA-300) and concentrations of Cl^-, SO_4^{2-} and NO_3^- by ion chromatography (Dionex 1500i with a AS 4 A SC column; Sunnyvale, USA).

Table 1. Means values of the physico-chemical parameters of water of the five streams from November 2008 to January 2009 (*n* = 7). Minimum and maximum values are given in brackets. ANC = Acid neutralizing capacity.

	Streams	**LM**	**MB**	**GV**	**RV**	**CL**
pH	Benthic	7.4 (7.3/7.4)	7.0 (6.8/7.2)	6.4 (6.2/6.5)	6.1 (5.9/6.3)	4.6 (4.5/4.6)
	Hyporheic	7.4 (7.3/7.6)	6.9 (6.6/7.1)	6.4 (6.3/6.5)	6.3 (6.2/6.7)	4.7 (4.5/5.1)
Temp. (°C)	Benthic	6.0 (4.7/7.0)	5.8 (4.3/7.3)	6.6 (5.3/7.6)	5.6 (3.8/6.8)	4.2 (2.0/5.7)
	Hyporheic	5.9 (4.7/6.9)	5.8 (4.5/7.1)	6.6 (5.3/7.8)	5.5 (3.8/6.7)	4.2 (2.4/5.9)
DO (mg/L)	Benthic	11.8 (11.3/12.4)	11.8 (10.1/12.5)	11.3 (10.8/11.8)	12.1 (11.7/12.8)	12.0 (10.7/12.9)
	Hyporheic	10.3 (9.4/10.9)	10.1 (9.2/11.1)	9.9 (9.4/10.4)	10.2 (9.9/10.6)	11.0 (10.1/12.4)
Cond. (µS/cm)	Benthic	71.3 (60.0/76.0)	52.7 (41.5/60.5)	35.5 (34.9/36.0)	37.0 (36.5/37.8)	33.8 (30.9/35.5)
	Hyporheic	75.9 (62.5/83.0)	61.7 (48.5/75.5)	36.2 (35.0/37.0)	39.0 (37.5/40.0)	33.4 (30.0/35.5)
ANC (µEq/L)	Benthic	448 (348/512)	204 (113/286)	53 (44/63)	25 (15/33)	-27 (-32/-13)
	Hyporheic	486 (372/548)	302 (198/509)	60 (48/72)	43 (31/76)	-19 (-33/-3)
Total Al (µg/L)	Benthic	26 (11/67)	93 (72/110)	32 (23/53)	105 (44/310)	697 (630/844)
	Hyporheic	140 (29/237)	249 (145/427)	67 (32/119)	360 (70/868)	682 (144/1962)
Ca^{2+} (mg/L)	Benthic	6.7 (5.8/8.4)	4.0 (3.0/4.7)	2.4 (2.3/2.5)	2.2 (2.2/2.3)	1.2 (1.0/1.5)
	Hyporheic	7.4 (5.8/8.4)	5.4 (4.0/7.4)	2.4 (2.3/2.6)	2.4 (2.2/2.6)	1.4 (1.2/1.6)
NO_3^- **(mg/L)**	Benthic	3.2 (3.0/3.4)	2.8 (2.5/2.9)	1.6 (1.6/1.7)	2.6 (2.5/2.8)	4.4 (4.1/4.7)
	Hyporheic	3.2 (3.0/3.4)	2.3 (1.0/2.8)	1.6 (1.5/1.6)	2.6 (2.5/2.7)	4.4 (4.2/4.7)

During the leaf litter decomposition study, water temperatures of surface and interstitial were recorded every 4 h (SmartButton, ACR System Inc.). The five streams had relatively similar substratum, which was unconsolidated and mostly made of gravel and coarse/fine sand sediments. The grain size fractionation was determined from one sediment core (20 cm length x 10 cm diameter) sampled in the middle of a representative riffle of each stream. At the laboratory, each sediment sample was meticulously washed and wet-sieved under tap water through a collection of mesh sizes (32, 18, 10, 5, 2, 1, 0.5 and 0.2 mm) for grain size analysis. Once the organic matter had been removed from each grain size fraction, the remaining sediment was placed into a 105 °C oven until a constant dry mass was reached for each fraction (Table 2).

Table 2 Frequency distributions (%) of sediment size classes (1: > 32 mm; 2: 18-32 mm; 3: 10-18 mm; 4: 5-10 mm; 5: 2-5 mm; 6: 1-2 mm; 7: 0.5-1 mm; 8: 0.2-0.5 mm) in the downwelling zone of the five streams.

	1	2	3	4	5	6	7	8
LM	4.2	8.1	11.3	18.1	13.4	8.1	21.3	15.4
MB	-	9.7	15.0	21.9	14.9	9.6	22.5	6.4
GV	4.3	21.7	23.0	15.8	8.7	4.7	18.8	3.0
RV	-	9.9	10.5	4.5	2.8	1.5	46.8	23.9
CL	2.9	15.4	11.2	7.1	4.8	5.2	30.2	23.1

Leaf decomposition

We selected alder as one of the two most common riparian tree species in the study sites. In October 2008, alder leaves were collected from trees at abscission and air-dried for 15 days at 20-22 °C. Leaf bags consisted of 3.00 g (mean air-dry mass ± 0.03 g) of leaves enclosed in plastic net bags (15×10 cm, 5 mm mesh) to simulate natural accumulations of leaf detritus in the stream. Before incorporated into bags, the leaves were moistened with distilled water from a vaporizer to prevent breakage during handling and transport. A total of 48 leaf bags per stream were introduced at the head of riffles (downwelling zones) the 18 November 2008. Leaf bags were subjected to two treatments: benthic and hyporheic (*i.e.* buried in the sediment).

Benthic leaf bags were placed at the sediment surface and secured to the bank with plastic-coated wire, which was anchored to the stream bottom with large boulders. Hyporheic leaf bags were located approximately 15-20 cm below the sediment surface using a small shovel, with a coloured plastic wire attached to facilitate localization and retrieval (Cornut *et al. in press*). Four replicate bags were randomly retrieved from the five streams after 7, 14, 21, 28, 49, and 70 days of exposure, immediately placed and stored individually in plastic zip-lock bags with stream water and transported to the laboratory in a cool box. During leaf bag sampling, a Surber net (500 μm mesh size) was used to minimize invertebrate loss due to passive or active drift.

Leaves were washed individually with water from the respective stream to remove sediments, exogenous organic matter and macroinvertebrates, which were collected in a 500 μm screen sieve and then preserved in 70% ethanol until processing. One set of five 12 mm diameter discs and another of ten were cut from leaves, avoiding the central vein. The set of five leaf discs was promptly frozen at - 20 °C until processing for ergosterol extraction, and the second one of ten was immediately used for incubation in aerated microcosms (see below). Then, the leaf toughness was determined as described below. The remaining leaf litter was dried at 105 °C to constant mass and weighed to the nearest 0.01 g. The leaf material was ground using a micro hammer mill (Culatti, Zurich, Switzerland) with a 0.5 mm mesh. Portions of leaf material of about 500 mg were ashed at 550°C for 4 h in a muffle furnace and weighed to determine the organic matter content. The leaf mass remaining in bags was expressed as the ratio of the ash-free dry mass (AFDM) between the final and initial leaf litter. Four unexposed batches of leaf litter were used to determine the initial AFDM and oven-dried mass to air-dried mass ratio of leaves according to the procedures above.

Fungal diversity

Once cut, the ten fresh leaf discs were quickly placed in aerated microcosms (Suberkropp, 1991) filled with 40 ml filtered (Glass fibre GF/F, Whatman) water from the respective stream and incubated at 10 °C. After 48 h, a 20 mL aliquot of spore suspension was transferred into a 30 mL glass tube and preserved with 1.5 mL of 37% formalin. The discs were then lyophilized and weighed to the nearest 0.1 mg. 0.5 mL of Triton X-100 (0.5%

solution) was added to the spore suspensions and was stirred gently to ensure uniform distribution of spores. 5 mL aliquots were then filtered through a membrane filter (5 µm pore size, diameter 25 mm; Millipore Corporation), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid (Iqbal & Webster 1973). Spores were counted and identified under the microscope (× 200). Spore production was calculated as the number of spores released per g leaf AFDM per day.

Fungal biomass

Mycelial biomass in leaves was assessed through the content of ergosterol (Gessner & Chauvet 1993). Leaf material was lyophilised and weighed to the nearest 0.1 mg, and then lipids were extracted with alkaline methanol heated at 80°C for 30 min. Extracts were purified using solid-phase extraction cartridges (Oasis HLB, 60 mg, 3 cc, Waters, Milford, Massachusetts, USA) and ergosterol quantified by high-performance liquid chromatography (procedure slightly modified from Gessner, 2005). The extraction efficiency (87-100 %) was monitored for each series using control samples and used to correct the determination of ergosterol content in leaves.

Macroinvertebrates

Macroinvertebrates retained over a 500 µm mesh sieve were counted and identified to the lowest practicable level. Identification was to the genus/species level whenever possible, except for Oligochaeta and Diptera (family and sub-family or tribe, respectively), and individuals were classified as shredders, grazers and others (Tachet *et al.* 2000). For each leaf sample, the biomass of macroinvertebrates assigned to the shredder group was determined to the nearest 0.01 mg after drying to constant weight at 60 °C.

Fine particulate organic matter production

After removal of the 20 mL aliquot for spore production and fungal diversity determinations (see above), the contents of the microcosms were filtered through a 1 mm-mesh screen to retain coarse particules. The 20 ml remaining were then filtered through a membrane filter (0.45 µm pore size, diameter 25 mm; Millipore Corporation) prior being washed three times with 10 mL pure water, dried at 80 °C and preweighed to the nearest µg.

The fine particulate organic matter (FPOM) release due to microbial activity was determined to the nearest μg after drying to constant weight at 80 °C.

Statistical analysis

Leaf decomposition rates (*k*) for each treatment were estimating by using the exponential decay model, $M_t = M_o \cdot e^{-kt}$, where Mo is the initial AFDM, Mt is the AFDM remaining at time t, and t is the time in days (Petersen & Cummins, 1974). The *k* values were determined by using linear regression in the log-transformed relationship. An analysis of covariance (ANCOVA) was used to compare *k* values among the five streams and the two exposure treatments, followed by a multiple-comparison (Tukey HSD test). A three-way factorial ANOVA was used to assess differences in microbial and macroinvertebrate parameters (leaf-litter-associated respiration, spore production, and fungal biomass and abundance) with stream, treatment (*i.e.* benthic or hyporheic) and exposure time as the main effects. When significant differences were detected between treatments, Tukey HSD tests were then carried out for post hoc pairwise comparisons.

The Simpson's dominance index and the Simpson's diversity index were computed for fungal communities associated with decaying leaves from each of the two zones for the five streams. Non-metric multidimensional scaling (NMDS) analyses of sporulation data and abundance of shredder were used to assess differences among sites in aquatic hyphomycete and shredder assemblages, respectively. This ordination method is a robust procedure for analysing ecological data (Minchin 1987). The Bray-Curtis coefficient was used to quantify the dissimilarity among sites based on joint occurrence and abundance of taxa. NMDS attempts to maximise the fit between measured dissimilarities and distance between resulting data points within a predefined number of spatial dimensions (Legendre & Legendre 1998 ; Legendre & Marti 1999). Stress function for each NMDS plot indicates the goodness of representation of differences among sites. Stress values range from 0 to 1, with values close to zero indicating a good fit. Axes from the NMDS analysis were correlated (Spearman rank correlation) with physical and chemical data to identify variables most strongly corresponding to among-site differences in aquatic hyphomycete and shredder assemblages (Hawkins *et al.* 1997 ; Baudoin *et al.* 2008).

Non-metric multidimensional scaling (NMDS) analysis was performed with PRIMER 6 (Clarke & Gorley 2001). Otherwise STATISTICA 6.0 (StatSoft Inc., 2001) was used for all statistical analyses. Differences were considered significant when $P < 0.05$.

5.5. Results

Litter decomposition

In both benthic and hyporheic zones, a significant decrease in leaf decomposition rates was observed under acidic conditions. After 10 weeks, the percentage of remaining AFDM in the benthic zone was less than 2% in the circumneutral LM and MB, while it was more than 30% in the GV and RV and 60% in the most acidic stream CL. Leaf decomposition rates at the sediment surface in the two streams closest to neutrality LM and MB were significantly faster than in the others streams ($F_{9,229} = 74.08$, $P < 0.001$).

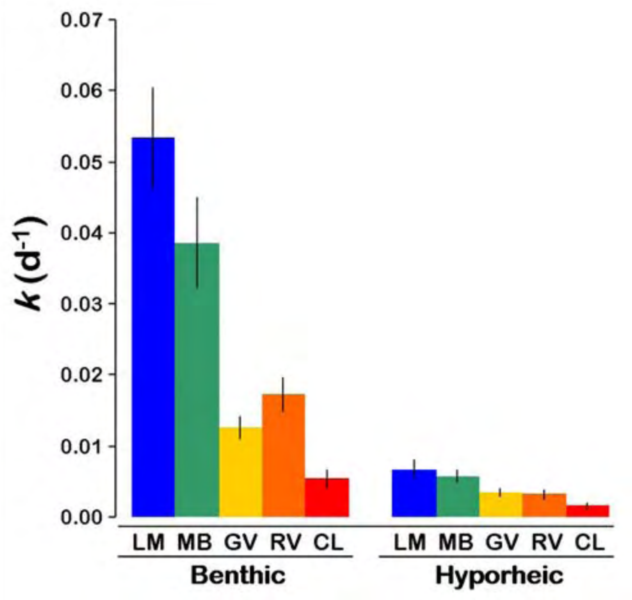

Fig. 1 Leaf decomposition rate in benthic and hyporheic zones of five streams along an acidification gradient (mean ± 95% confidence interval, n = 24). LM: La Maix, MB: Ménombru, GV: Gravelle, RV: Ravines, CL: Courbeline.

The differences among streams in the hyporheic zone were less pronounced (Fig. 1), with percentages of remaining AFDM varying between 55.0 and 79.0% for the circumneutral LM and the most acidic CL streams, respectively.

As a consequence, the ratio of circumneutral-to-acidic decomposition rates (LM:CL) for the hyporheic habitat was <4.5 while it was 9.8 in the benthic counterpart.

Fungal diversity and biomass

A total of 34 sporulating species of aquatic hyphomycetes was detected during leaf decomposition (Table 3). The highest fungal diversity (cumulated number species from benthic and hyporheic zones) was found in the circumneutral stream LM (24 species) and the lowest (15 species) in the two most acidic ones, RV and CL. Whatever the stream, the fungal diversity associated with decaying leaf-litter was always lower in the hyporheic zone than in the benthic counterpart (Table 3).

Throughout the experiment, the most striking difference between aquatic hyphomycete assemblages from decaying leaves was the dominance of *Flagellospora curvula* in the most acidic stream CL, both in the benthic and hyporheic zones (*i.e.* 97.7% and 98.3% of the pool of conidia produced, respectively). In contrast, four to six species (*i.e. Flagellospora curvula, Heliscus lugdunensis, Tetrachaetum elegans, Lemonniera cornuta, Alatospora pulchella, Alatospora acuminata*) were codominant on leaf-litter from the circumneutral stream LM in both the hyporheic and benthic zones. The NMDS ordination emphasized the overall differences in aquatic hyphomycete assemblages between the five streams and the two zones (*i.e.* benthic and hyporheic) (Fig. 2). It revealed marked differences in aquatic hyphomycete assemblages along the acidification gradient: Axis 2 strongly correlated with pH ($r = 0.83$, $P = 0.001$). The position along this axis reflected the acidification status of the streams and strongly separated the acidic streams with lower richness and evenness in fungal communities from circumneutral streams (Table 3). The NMDS ordination also separated well aquatic hyphomycete assemblages from the benthic and hyporheic zones, with Axis 1 correlating with the concentration in dissolved oxygen ($r = -0.80$, $P = 0.003$).

Freshly collected leaves of alder contained minute amounts of ergosterol, indicating that fungal colonization was negligible at the beginning of the experiment. Fig. 3 shows that fungal biomass associated with decaying leaves of alder at the sediment surface increased rapidly within the first 7 weeks, except for the circumneutral stream LM, which reached a maxima of 26 mg g^{-1} after 3 weeks, then rapidly declined.

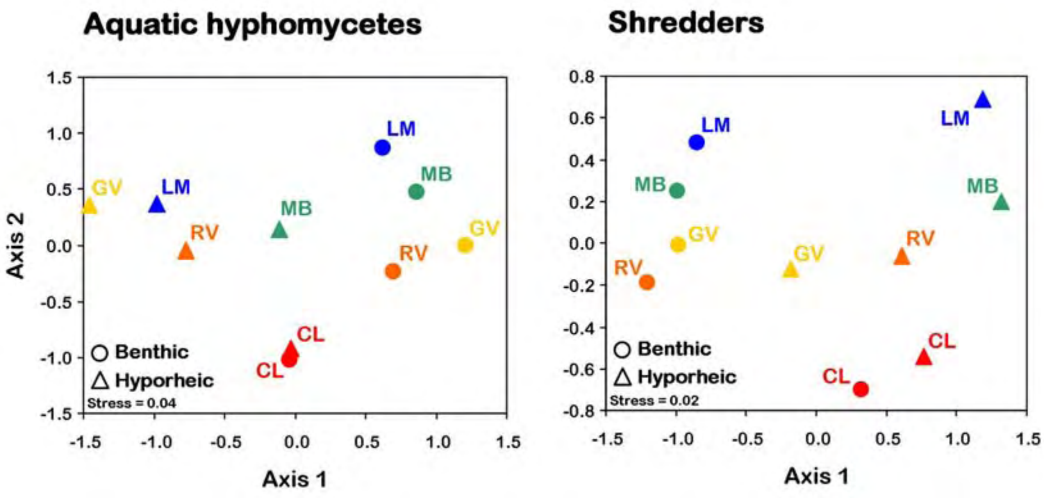

Fig. 2 NMDS plot of sites based on (a) aquatic hyphomycete and (b) shredder assemblages associated with leaves decomposing in benthic and hyporheic zones of five streams along an acidification gradient. LM: La Maix, MB: Ménombru, GV: Gravelle, RV: Ravines, CL: Courbeline.

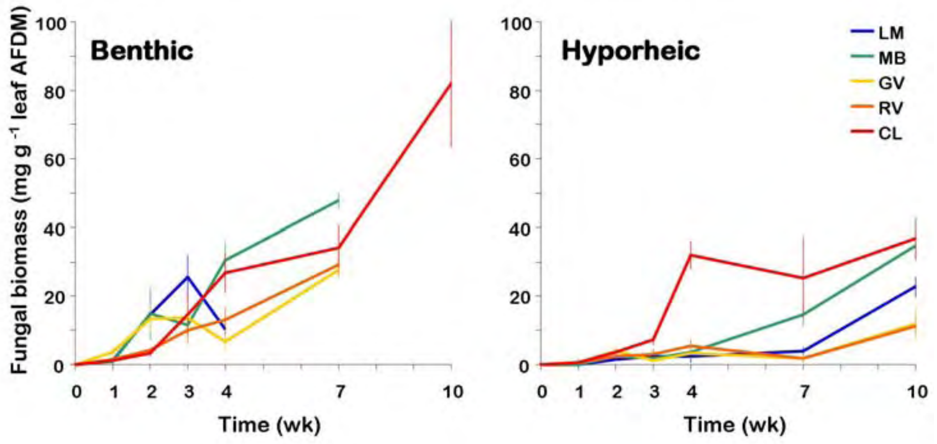

Fig. 3 Changes in fungal biomass associated with leaves decomposing in benthic and hyporheic zones of five streams along an acidification gradient (mean ± SE, n = 4). LM: La Maix, MB: Ménombru, GV: Gravelle, RV: Ravines, CL: Courbeline.

Alder leaves exposed at the sediment surface in the most acidic stream CL differed from this general pattern in that mycelial biomass continuously increased to reach a maximum of 82 mg g^{-1} after 10 weeks, whereas alder leaves in the four others streams had been decomposed almost entirely before the last sampling date. Consequently, biomass associated with decaying alder leaves in the latter streams was determined from the first five sampling dates only. The mycelial biomass showed no particular trend regarding the acidification gradient, except that maxima were higher in the most acidic stream, both in benthic and hyporheic zones.

The mycelial biomass from the first four sampling dates differed significantly between habitats ($F_{1,149} = 25.12$, $P < 0.001$) and streams ($F_{4,149} = 2.60$, $P < 0.001$). The maximum biomass in the hyporheic zone, except for the most acidic stream CL, was delayed by 3-7 weeks in comparison with the benthic habitat. The maximum differed significantly between streams in the benthic ($F_{4,12} = 5.38$, $P < 0.001$) and hyporheic zones ($F_{4,15} = 7.99$, $P < 0.001$). The most acidic stream CL showed values similar to or higher than the others streams suggesting that fungal biomass associated with decaying alder leaves was not affected by acidification.

Shredders

The shredder biomass differed significantly between streams ($F_{4,240} = 5.58$, $P < 0.001$) and location within the stream ($F_{1,240} = 34.74$, $P < 0.001$). The highest biomass on benthic leaves, averaged over the sampling period, was found in the two circumneutral streams LM (20.2 mg g^{-1} AFDM) and MB (15.0 mg g^{-1} AFDM), where they differed significantly from their hyporheic counterpart of these streams (0.004 and 0.007 mg g^{-1}, respectively). A considerable reduction in shredder biomass on the benthic leaves was observed along the acidification gradient, with values from the two circumneutral streams LM and MB being significantly higher than in the three acidic streams (CL: 0.145, RV: 7.060 and GV: 2.297 mg g^{-1} AFDM). In the hyporheic habitat, shredder biomass was similar among streams and mostly dominated by *Leuctra spp.* and *Amphinemura sulcicollis* (data not shown). The peaks in shredder biomass observed on benthic leaves at d49 were only found in the two circumneutral streams LM and MB and was mostly attributable to individuals of *G. fossarum* and Trichoptera (*Sericostoma personatum*, *Potamophylax sp.* and unidentified Limnephilinae), *i.e.* the largest dominant shredder taxa in the stream (data not shown). However, the latter taxa were generally found at low densities. In acidic streams GV and RV, *G. fossarum* and Trichoptera shredders were replaced by stonefly shredders, mainly *Leuctra spp.*, *Amphinemura sulcicollis*, *Protonemura sp.* and *Nemoura sp.*, which were frequently abundant. In the most acidic stream CL, shredder assemblages in both benthic and hyporheic habitats were dominated, in biomass and numbers, by *Leuctra spp.* Shredder assemblages among the five streams were well discriminated on Axis 2 of the NMDS analysis (Fig. 2), which was correlated with pH ($r = 0.75$, $P = 0.007$).

Shredder assemblages associated with leaves from the benthic and hyporheic zones were distinguished along Axis 1, which was well correlated with the concentration in dissolved oxygen (r = -0.79, P = 0.003). The discrimination between macroinvertebrate communities along the acidification gradient held for both benthic and hyporheic zones of the streams, which tended to exhibit symmetrical responses. This overall pattern was remarkably similar to that of fungal assemblages (Fig. 2).

Microbial FPOM

The pattern of microbial FPOM production associated to alder leaves was comparable across the streams and treatments, however, with contrasted dynamics (Fig. 4). Overall, the release of FPOM by microbial activity increased significantly with time ($F_{4,150}$ = 315.42, P < 0.001) and decreased in a consistent trend along the acidification gradient ($F_{4,150}$ = 116.56, P < 0.001) in both habitats ($F_{1,150}$ = 71.42, P < 0.001). At any date, the release of microbial from leaf material in the benthic habitat of the five streams exceeded that from the hyporheic habitat. The mean FPOM production at the streambed surface varied from 50.2 to 11.0 mg g^{-1} litter d^{-1} for the circumnenutral stream LM and the most acidic one CL, respectively and 42.3 to 8.5 mg g^{-1} litter d^{-1} for the hyporheic habitat of the same streams. At day 49, the release of FPOM by microbial activity showed a sharp increase to a maximum, except for the most acidic stream CL where the pattern was much less pronounced.

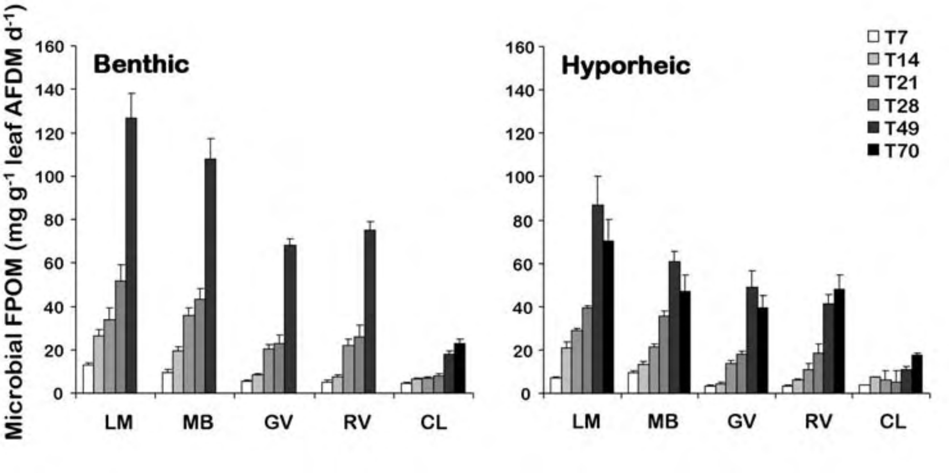

Fig. 4 Rate of release of fine particulate organic matter from leaves decomposing in benthic and hyporheic zones of five streams along an acidification gradient at six incubation dates (mean + SE, n = 4). LM: La Maix, MB: Ménombru, GV: Gravelle, RV: Ravines, CL: Courbeline.

Table 3 Occurrence of the leaf-associated aquatic hyphomycete species for the two exposure treatments, determined from the cumulative conidial production over the 6 sampling dates.

	Benthic					**Hyporheic**				
Species	**LM**	**MB**	**GV**	**RV**	**CL**	**LM**	**MB**	**GV**	**RV**	**CL**
Alatospora acuminata Ingold	+	+			+	+			+	
Alatospora flagellata (Gönczöl) Marvanová					+	+				
Alatospora pulchella Marvanová	+	+				+				
Anguillospora crassa Ingold			+							
Anguillospora filiformis Greathead		+	+	+	+	+		+	+	+
Anguillospora furtiva Descals & Marvanová			+							+
Anguillospora longissima (Sacc. & Syd.) Ingold	+				+				+	+
Anguillospora rosea Descals & Marvanová	+	+	+	+	+		+			
Articulospora tetracladia Ingold	+	+	+	+	+	+	+	+	+	+
Clavariopsis aquatica De Wild.	+	+	+							
Clavatospora longibrachiata (Ingold) Marvanová & Nilsson	+	+	+	+	+	+	+	+	+	
Culicidospora aquatica Petersen	+		+					+		
Flagellospora curvula Ingold	+	+	+	+	+	+	+	+	+	+
Fontanospora eccentrica (Petersen) Dyko	+	+	+		+					
Fusarium like	+			+		+			+	
Heliscella stellata (Ingold & Cox) Marvanová & Nilsson		+					+			
Heliscus lugdunensis Sacc. & Théry	+	+	+	+	+	+	+	+	+	
Lemonniera aquatica De Wild.	+	+	+	+	+		+	+	+	
Lemonniera centrosphaera Marvanová		+	+							
Lemonniera cornuta Ranzoni	+	+	+			+	+			
Lemonniera terrestris Tubaki	+	+	+	+						
Mycocentrospora sp. (cf. *angulata* (Petersen) Iqbal)	+		+				+			
Stenocladiella neglecta Marv. & Descals	+		+			+				
Tetrachaetum elegans Ingold	+	+	+	+		+	+	+	+	+
Tetracladium marchalianum De Wild.						+				
Tetracladium furcatum Descals					+			+		
Tricellula aquatica Webster				+	+					
Tricladium chaetocladium Ingold	+	+	+	+		+	+			
Tripospermum camelopardus Ingold, Dann & McDougall							+		+	
Tripospermum myrti (Lind.) Hughes								+		
Tumularia aquatica (Ingold) Marvanová & Descals						+				
Tumularia tuberculata (Gonczol) Descals & Marvanová		+					+			
Sigmoid (<60 µm)						+				
Sigmoid (60-120 µm)						+				
Total number of species	18	18	12	18	13	17	13	11	10	6
Simpson's diversity index	0.70	0.57	0.69	0.03	0.00	0.49	0.26	0.51	0.56	0.01
Simpson's dominance index	0.30	0.43	0.31	0.97	1.00	0.51	0.74	0.49	0.44	0.99

5.6. Discussion

This study provides evidence that the anthropogenic acidification reduced decomposition of alder leaves both in benthic and hyporheic zones of headwater streams. The overall, responses to acidification in the hyporheic zones were however generally less than in their benthic counterparts, which fully supports our initial hypothesis. Although a substantial decrease in decomposition rates of alder leaves was observed under acidic conditions, this effect was not primarily due to changes in fungal biomass. Indeed, fungal biomass accrual has generally been shown to increase to a maximum and then decline during leaf decomposition (Gessner & Chauvet 1994), the maximum being controlled by both internal and external variables such as the nutritional quality of the leaves, the concentration of refractory leaf constituents (*e.g.* lignin) and the nutrients availability or the presence of pollutants in the stream water (Gessner *et al.* 1997). Our findings thus corroborated the results from Dangles and Chauvet (2003) and Baudoin *et al.* (2008) showing that fungal biomass in the same or nearby streams in the Vosges mountains was not affected by acidification, but contrasted markedly with the substantial reduction in fungal biomass on oak leaves in an acidified West Virginia stream reported by Griffith & Perry (1994). Surprisingly, the leaves exposed in the stream with the lowest pH in the present study (CL; 4.6 and 4.7 in benthic and hyporheic zones, respectively) and highest total aluminium concentrations (697 and 682 μg/L, respectively) showed the highest fungal biomass accrual, both in benthic and hyporheic zones. As suggested by Dangles & Chauvet (2003), this pattern might be partly explained by the relatively high concentrations of atmospheric-derived nitrates in this stream (4.4 mg/L in the two zones), providing an inorganic N source to fungal production (Suberkropp 1998) and thus compensating for the unfavorable acidic conditions.

Our results show that the maximum mycelial biomass in the hyporheic habitat of the five streams was delayed by 7 weeks in comparison with the benthic habitat, even though the latter also showed a strong increase at the final sampling date. Moreover, the amount of leaf-associated mycelial biomass was depressed in burial conditions, like reported from alder leaves in a nearby stream (Cornut *et al. in press*) or woody debris in a headwater mountain stream (Crenshaw *et al.* 2002).

The lower dissolved oxygen concentrations in the hyporheic zone probably explained this reduction in fungal biomass, as supported by experimental data from hypoxic environments (Medeiros *et al.* 2009).

Dissolved oxygen acts as a limiting factor for biological colonisation and activity in the interstitial habitats, with its depletion leading to qualitative and quantitative changes in microbial assemblages (Ward *et al.* 1998). Dissolved oxygen concentrations within sediments are not uniform along the vertical gradient with close to surface running waters being usually well oxygenated, whereas concentrations decrease during the subsurface passage of water (Marmonier & Dole, 1986 ; Findlay *et al.* 1993), mainly because of sediment respiration (Chapelle 1993) and differential water renewal rates. Dissolved oxygen in subsurface sediments depends on the permeability and porosity of the sediments, the saturation of pore spaces with water and the intensity of sediment respiration (Ward *et al.* 1998). Surprisingly, our results showed relatively high concentrations in dissolved oxygen within sediments in the five streams (mean: 10.3 mg L^{-1}; range: 9.6-11.1 mg L^{-1}) despite our observations of markedly lower water renewal, this can only partly explained the reduced fungal biomass on alder leaves in the hyporheic habitat. The colonization of plant matter by aquatic hyphomycetes can theorically occur through three pathways: (i) by direct contact as a result of hyphal outgrowth from a colonized leaf neighboring another leaf, or at a distance, by either (ii) detached hyphal fragments (Park 1974 ; Knudsen & Stack 1991) or (iii) conidia (Read *et al.* 1992) that settle on a leaf surface. Based on what is known about aquatic hyphomycetes, the latter appears to be predominant for the colonization of leaves as discrete resource units in streams (Sridhar & Bärlocher 1997). Thus, we expect the number of conidia in water to drop from the surface to the deepest sediment layers, a process further increased by the presumably lower amount of leaf litter as a compensating source of conidia in the hyporheic habitat. Even though fungal spores may disperse and allow small, short-lived colonies to develop on the substrates they encounter in the hyporheic habitat (Bärlocher *et al.* 2006) , the lower amount of conidia circulating in the sediment may have also contributed to the observed delay to reach a substantial accrual of mycelial biomass.

The contribution of shredders to leaf decomposition in the hyporheic zone of the present streams was similarly low as reported in a recent study (Cornut *et al. in press*). Shredders were rare on alder leaves whatever the level of acidification, although they were relatively abundant on leaf detritus at the surface. In addition, the only taxa with the appropriate morphology to penetrate the substratum in this study (Omesová *et al.* 2008) were also the less effective decomposers, *i.e. Leuctra spp.* and *Amphinemura sulcicollis*. The burial of leaves in the sandy substratum thus reduced the access of invertebrate decomposers to leaves due to the small interstices of the sediment and the subsequent bottom instability acting as a constraining disturbance, especially for the largest shredders.

In line with the findings exposed above, a major result was the contrasting contributions of fungi *vs* shredders to leaf decomposition in the two habitat types of the present acidified streams. If only because the physical constraints acted differently on large invertebrate detritivores and microfungi, trophic interactions in the hyporheic habitat differed strongly from those occurring in the benthic one. The absence of detritivores, which actually feed on both leaf and fungi (Bärlocher 1992 ; Suberkropp 1998), results in a less restrained development of fungi and consequently higher implication in litter decomposition in the hyporheic environment a phenomenon that has been recently documented (Cornut *et al. in press*). Overall, the prolonged leaf decomposition could sustain further fungal growth in contrast to the benthic habitat where decomposition was nearly completed after 70 days. Thus, fungal decomposers in the hyporheic zone compensate, if not fully, at least partly for the action of invertebrate detritivores in the benthic zone. Otherwise, the diversity of aquatic hyphomycetes on leaves in hyporheic habitat, assessing through the release of conidia, were much lower than on plant litter on the streambed. The significant reduction of leaf litter decomposition rates in the hyporheic zone of woodland streams means that this latter is an important compartment for organic matter storage (Cornut *et al. in press*), and suggests that this habitat may serve as a long-term reservoir contributing to their rapid expansion and dispersal during the yearly leaf fall (Bärlocher *et al.* 2006).

The acidification of freshwater ecosystems resulting from atmospheric pollution is often accompanied by increased concentrations of aqueous aluminium, which is being considered a major environmental issue due to its high toxicity to aquatic organisms.

Indeed, aqueous aluminium has been recognized as a main toxicant for aquatic animals (Gensemer & Playle 1999), and several studies have also suggested a direct effect on microbial metabolism (Myrold & Nason 1992 ; Pina & Cervantes 1996 ; Chamier & Tipping 1997) and diversity (Baudoin *et al.* 2008). Several studies have pointed out deleterious effects on fungal communities due to various chemical alterations of aquatic ecosystems. As examples, declines in aquatic hyphomycetes diversity have been reported in streams contaminated with either heavy metals (Bermingham *et al.* 1996 ; Duarte *et al.* 2004) or organic compounds (Au *et al.* 1992 ; Raviraja *et al.* 1998).

The presence of aquatic hyphomycetes in our streams strongly contaminated by aqueous aluminium confirms that at least some are tolerant or adapted to aquatic systems contaminated with such a metal. Biodiversity has been found to be positively associated with rates of ecosystem functions, such as leaf detritus processing (Heneghan *et al.* 1999 ; Jonsson & Malmqvist 2000) and ecosystem stability and resilience (Naeem *et al.* 1996 ; Tilman *et al.* 1996 ; Hector *et al.* 1999). Such relationships have however been demonstrated for plants and animals, with patterns for microorganisms tending to be equivocal (*e.g.* Bärlocher & Corkum 2003 ; Dang *et al.* 2005). It has been suggested that aquatic hyphomycete communities respond to stressors according to the redundancy model, in which ecosystem functioning remains stable because increased biomass and activity of tolerant species compensate for the loss of sensitive species (Raviraja *et al.* 1998 ; Bärlocher & Graça 2002 ; Niyogi *et al.* 2002a ; b ; Pascoal *et al.* 2005). Thereby, the final outcome of the response of aquatic hyphomycetes may depend on the type and the severity of stress, with the impact on communities both in structural and functional terms remaining difficult to predict (Pascoal *et al.* 2005). The unequivocal discrimination of such effects is probably only possible by using experimental approaches.

It is important to note that, similarly to the study by Medeiros *et al.* (2009), our results relied on species richness determination from the conidia released during leaf decomposition. Nikolcheva & Bärlocher (2005) underlined the potential shortcoming of relying on conidial identification and counts due to seasonal and interspecific differences in sporulation rates, which may distort the actual community composition of aquatic hyphomycetes.

Moreover, non-sporulating fungal species will not be detected. As an illustration, Bärlocher *et al.* (2006) showed, based on molecular data, that a substantial portion of the fungal biomass does not belong to any species dominating the spore production. Thus, conidial abundances may not truly reflect the contribution of individual species to the fungal pool, and even more specifically to the degradation of plant litter. Molecular techniques applied to fungal communities on plant detritus in streams have circumvented such obstacles, even though are still subject to incertitudes and limitations (Nikolcheva *et al.* 2003 ; Nikolcheva & Bärlocher 2004 ; Nikolcheva *et al.* 2005 ; Nikolcheva & Bärlocher 2005; Bärlocher 2010).

Both structures of aquatic hyphomycete and macroinvertebrate communities were strongly modified in acidic conditions. Fungi and macroinvertebrates responded similarly to the acidification gradient across the benthic and hyporheic habitats, however with communities between these two habitats differing much more in circumneutral streams than in the most acidic one (Fig. 2a and b). For invertebrates, this pattern was evidently attributable to the converging effects of physical (hyporheic) and chemical (acidic) constraints which both led to drastic reductions in their diversity and density and, when combined, ultimately to their disappearence (Fig. 5). The same pattern observed in fungal assemblages means that fungi responded to a similar extent to both physical and chemical barriers, even though individual responses differed among species (cf. Tab. 2). Despite the lower fungal diversity in acidic hyporheic environments, leaf decomposition was relatively less affected by acidification in this environment compared with the benthic one, which was supported by maintenance of a substantial biomass (Fig. 3) and activity (Fig. 4) of fungal decomposers. This argues a lower sensitivity of aquatic fungi to the present physical and chemical stressors, occurring at the functional but not structural level. This study does not allow to unequivocally discriminate the relative importance of pH, aluminium, nutrients and other variables related to the toxicity of acidified waters from the harsh conditions prevailing in the hyporheic zone. Whether the slower leaf decomposition in acidified streams both in benthic and hyporheic zones is linked to the decreased aquatic hyphomycete diversity is also questionable. It remains however that the subsistence of active fungal decomposers in environments unoccupied by invertebrate detritivores is a strong insurance for impaired stream ecosystems to maintain carbon fluxes, with the hyporheic compartment acting as an important source of organic matter and propagules to downstream.

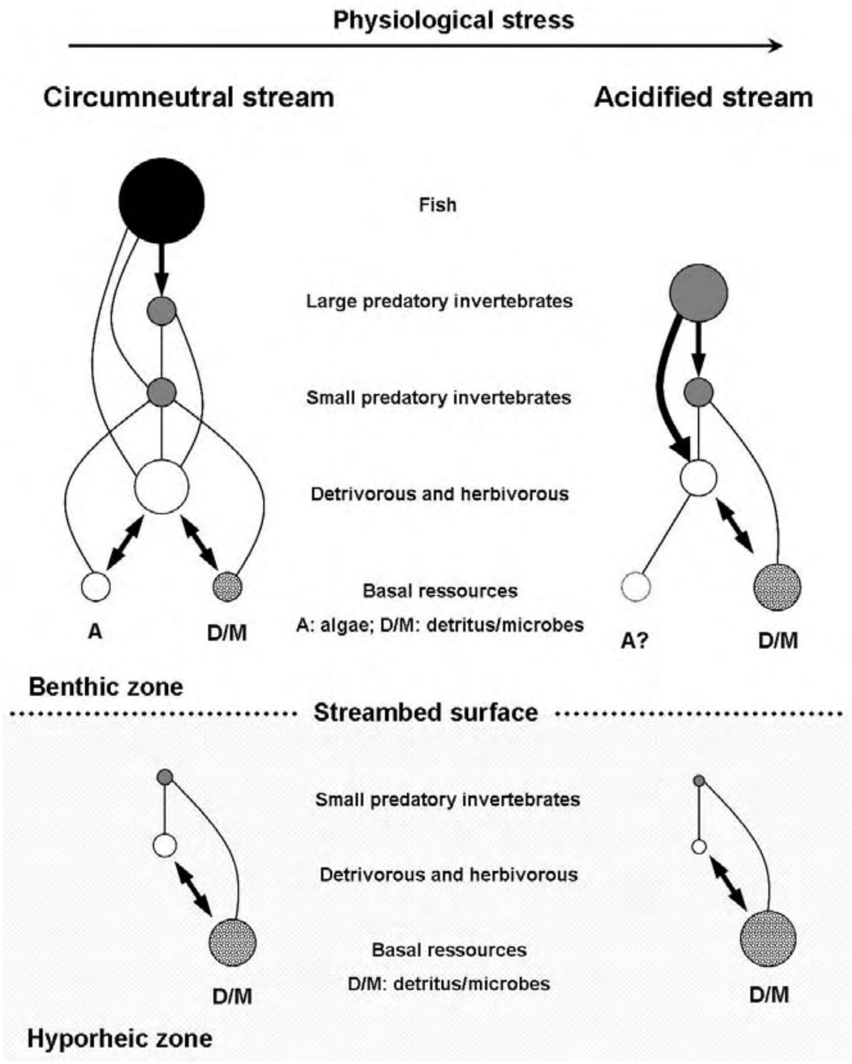

Fig. 5 Conceptual scheme of how anthropogenic acidification affects trophic and structural relationships in stream food web both in benthic and hyporheic zones. The figure is a simplified food web for the benthos and hyporheos, consisting of five elements (shown by circles): fish, large and small predatory invertebrates, herbivorous/detrivorous invertebrates and basal resources (algae, organic matter, fungi and bacteria). Trophic linkages within the web are indicated by lines and arrows passing between circles. The circle diameter is proportional to the abundance of each group and the thickness of lines and arrows to the intensity of interactions (adapted from Hildrew 1996).

5.7. References

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Discussion générale et perspectives

Discussion générale

L'objectif principal de ma thèse était d'étudier le processus de décomposition des litières dans le compartiment hyporhéique, mais également d'évaluer la contribution et les interactions des principaux agents impliqués dans ce processus à différents degrés de complexité et à différents niveaux trophiques. Cette thématique de recherche englobe un ensemble de mécanismes qu'il n'a bien évidement pas été possible d'aborder dans sa totalité au cours de ma thèse. Cependant, des expérimentations conduites à la fois au laboratoire et *in situ* ont permis d'apporter un certain nombre d'éléments de réponse concernant plusieurs hypothèses quant aux mécanismes biotiques et abiotiques régissant en partie le processus de décomposition des litières dans la zone hyporhéique de cours d'eau forestiers, et plus généralement dans la contribution de ce compartiment au fonctionnement écologique de ces écosystèmes d'eau courante.

De telles projections ont été possibles du fait de l'utilisation d'une approche comparative entre le compartiment benthique et hyporhéique pour la majorité des expérimentations réalisées. Ainsi, l'ensemble des résultats provenant des expériences menées dans la zone hyporhéique a été systématiquement comparé à ceux obtenus à la surface du sédiment afin d'avoir une vision intégrée des processus.

Stockage et décomposition de la matière organique particulaire dans la zone hyporhéique de cours d'eau forestiers

La première étape qui a été déterminante pour la suite des mes travaux de recherche a consisté en l'estimation du stockage de la matière organique particulaire grossière (CPOM) située au sein du compartiment hyporhéique et à la surface du sédiment de cours d'eau forestiers de tête de bassin de la Montagne Noire (Sud-Ouest de la France). En effet, en dépit de son indéniable contribution au fonctionnement écologique des écosystèmes d'eau courante, et cela à différents niveaux d'organisation et de processus, les études concernant l'évaluation des stocks de CPOM immobilisés dans le compartiment hyporhéique de cours d'eau de tête de bassin restent assez rares. Ainsi, un manque de connaissance flagrant persiste à ce niveau.

Il est bien évidemment impossible de pouvoir estimer et comprendre le rôle potentiel de la zone hyporhéique et de la contribution des agents impliqués dans le processus de décomposition de la CPOM sans avoir une estimation quantitative précise de cette dernière au sein de ce compartiment. L'estimation précise des flux de carbone à l'échelle d'une section d'un cours d'eau représente une charge de travail considérable, qui pourrait faire l'objet d'un travail de thèse à elle seule. J'ai donc décidé de réaliser des estimations ponctuelles, une première située à la fin du mois de novembre (*i.e.*, un mois environ après l'abscission des feuilles), puis la seconde à la fin du mois de mars de l'année suivante, et cela, sur trois cours d'eau présentant des profils granulométriques contrastés, afin d'intégrer ce paramètre dans l'estimation du processus de stockage. En effet, l'hydrologie et la géomorphologie des cours d'eau sont des composantes essentielles qui définissent l'aptitude de ces écosystèmes à stocker la matière organique. Par ailleurs, la taille des matériaux constituant le sédiment est un facteur déterminant qui gouverne l'ensemble des processus de la zone hyporhéique et le degré de couplage entre le compartiment de surface et celui de sub-surface. Ainsi, la diminution de la taille des particules constituant le sédiment implique une réduction de l'intensité des échanges chimique et de matériels (Beschta & Jackson 1979 ; Moring 1982), par exemple une diminution des échanges de matières organiques et d'oxygène dissous. Par ailleurs, les mouvements de sédiment et la profondeur d'érosion lors d'une crue peuvent être beaucoup plus importants dans un cours d'eau sableux que dans un cours d'eau constitué d'un autre type de sédiments, ainsi la probabilité que de la matière organique soit enfouie ou libérée dans un cours d'eau sableux paraît plus forte (Smock 1990 ; Metzler & Smock 1990 ; Naegeli *et al.* 1995).

Les résultats observés dans le chapitre 1 sont similaires à ceux des quelques études présentes dans la littérature, et démontrent que le compartiment sédimentaire de cours d'eau forestiers peut représenter d'importantes zones de stockage, dans lesquels une certaine quantité de CPOM peut rester immobilisée plusieurs mois (Cummins *et al.* 1983 ; Metzler & Smock 1990 ; Smock 1990 ; Jones 1997 ; Jones *et al.* 1997). Les différences qui ont été rapportées quant aux quantités de matière organique stockées dans les sédiments des trois cours d'eau étudiés, sont difficiles à interpréter sur l'unique base des deux campagnes d'échantillonnage.

Toutefois, les valeurs rapportées de la littérature montrent que la capacité de stockage de la zone hyporhéique de ce type de cours d'eau peut très différente [*i.e.*, 25-82% de la matière organique totale stockée (Cummins *et al.* 1983 ; Metzler & Smock 1990 ; Smock 1990 ; Jones 1997 ; Jones *et al.* 1997)], en fonction par exemple des contextes hydrologique, géomorphologique et climatique considérés.

Enfin, du fait du caractère exploratoire de notre étude et bien qu'il serait spéculatif de faire des projections en terme de bilan de carbone à l'échelle des trois cours d'eau étudiés, nos estimations soulignent cependant l'importance du mécanisme de stockage de la matière organique dans le compartiment hyporhéique des cours d'eau de tête de bassin, et son rôle potentiel dans le recyclage du carbone et dans la libération de nutriments à l'échelle de l'écosystème (Newbold *et al.* 1982 ; Croker & Meyer 1987).

L'ensemble des manipulations réalisées dans le cadre de ma thèse, visant à évaluer la décomposition des litières a fait apparaître que ce processus est fortement diminué lorsqu'il a lieu au sein du compartiment hyporhéique. Bien qu'à ce jour ce processus ait été très peu exploré, sur la base des quelques études existantes, le patron de réponse observé jusqu'à présent est assez cohérent avec nos observations (Reice 1974 ; Herbst 1980 ; Rounick & Winterbourn 1983 ; Metzler & Smock 1990 ; Naamane *et al.* 1999), même si certains auteurs ont rapporté des tendances beaucoup moins claires (Mayack *et al.* 1989 ; Smith & Lake 1993) voire opposées (Nichols & Keeney 1973). Peu d'études ont cherché à identifier les facteurs qui conduisaient à cette réduction importante du processus de décomposition des litières dans ce compartiment. La seule étude réellement aboutie à ce niveau, et offrant des éléments de réponses clairs est celle de Herbst (1980). Il est cependant important de souligner que certaines de ces études ont été réalisées à une époque, où le rôle de tous les agents impliqués dans le processus de décomposition n'était pas encore clairement identifié. De plus, les moyens techniques ne permettaient pas non plus d'atteindre les niveaux de développement et de précision identiques à ceux utilisés aujourd'hui.

Les chapitres 2 et 5, et particulièrement l'approche sous la forme d'un bilan de carbone (chapitre 2) m'ont permis d'évaluer la contribution relative des deux principaux agents décomposeurs des cours d'eau forestier, à savoir les hyphomycètes aquatiques et les invertébrés détritivores.

Il est indiscutable que le processus de décomposition dans la zone hyporhéique des écosystèmes aquatiques de tête de bassin est essentiellement contrôlé par le compartiment fongique. La contribution des invertébrés détritivores dans ce même compartiment est quant à elle quasiment imperceptible. Ces résultats soulignent une fois de plus, le rôle majeur joué par les champignons dans le processus de décomposition des litières, que ce soit à la surface du sédiment, par leur propre activité de transformation et par facilitation de l'activité des invertébrés détritivores du fait du conditionnement du matériel foliaire, ou pour leur contribution quasi exclusive dans l'habitat hyporhéique.

Enfin, la fragmentation par le courant et l'abrasion, composantes purement abiotique, sont des variables essentielles qui pourraient expliquer les différences importantes observées dans le processus de décomposition entre les compartiments benthique et hyporhéique. En effet, bien que ces composantes soient souvent écartées du processus, parce que très difficilement quantifiables, elles n'en sont pas pour autant négligeables. La variabilité observée au niveau des cinétiques de pertes de masse des litières exposées à la surface du sédiment, comparées à celles enfouies dans le sédiment, traduit assez bien ce phénomène (chapitre 2).

L'habitat hyporhéique constitue en quelque sorte une zone de protection dans laquelle les litières ne subissent pas la décomposition par ces processus abiotiques. A ce titre, il est probable que la décomposition des litières dans les cours d'eau de tête de bassin soit davantage régulée par l'action synergique de l'activité microbienne et des processus physiques, que par l'action des invertébrés détritivores. Même si la contribution de ces derniers dans le processus représente une part non négligeable, elle reste, tout autant que la contribution des agents physiques, difficilement quantifiable via la méthode des sachets de litières traditionnellement utilisée dans les études de décomposition. En effet, les invertébrés ne s'établissent pas à "long terme" dans un paquet de litière, mais au contraire sont en perpétuel déplacement. Par conséquent, l'échantillonnage des sachets de litières ne donne qu'une image très ponctuelle des communautés d'invertébrés associés aux litières. Au contraire, la contribution de la communauté d'hyphomycètes aquatiques associés à la litière, est très précisément quantifiable, puisque fixée au substrat.

Par ailleurs, la colonisation fongique des litières est un processus passif et systématique, qui est nettement moins soumis à l'influence de facteurs biotiques et abiotiques contrairement à la colonisation par les invertébrés détritivores.

Finalement, il est important de signaler que la présence d'un compartiment hyporhéique n'est pas une donnée systématique, en particulier dans les cours d'eau de tête de bassin. La présence de ce compartiment et son importance en termes de volume, et par conséquent sa contribution potentielle dans le fonctionnement général de l'écosystème, est intrinsèquement reliée à la nature du substratum drainé par le cours d'eau. A ce titre, il serait intéressant de comparer la productivité et la résilience face à des perturbations de différents types, de systèmes avec ou sans zone hyporhéique, afin d'évaluer concrètement le bénéfice que ce compartiment procure à l'écosystème.

Pratiquement, il s'agirait de sélectionner, trois cours d'eau au minimum de chaque type et d'y évaluer un certain nombre de métriques dénotant la productivité globale des écosystèmes, et cela sur l'ensemble des niveaux trophiques représentés afin d'avoir une vision la plus intégrée possible.

D'un point de vue méthodologique, l'utilisation de sachets à litières pourrait être critiquée à différents niveaux (Marmonier *et al.* 2010). En effet, afin de simuler les paquets de litières accumulés dans le cours d'eau, nous avons utilisé des sachets de litières d'une dizaine de centimètres de côté. L'enfouissement de ces dispositifs dans le sédiment requiert l'utilisation d'une petite pelle. Une des principales critiques réside dans le fait que cette technique déstructure la matrice sédimentaire à l'endroit où le sachet de litières est disposé. Cette déstructuration peut conduire à une fuite des invertébrés, ou au contraire faciliter leur migration verticale en direction de la litière, et par conséquent dans les deux cas conduire à une réponse biaisée du processus évalué. La recolonisation de sites perturbés après des mouvements de sédiments suite à des crues est relativement rapide, de l'ordre d'une semaine (Dole-Olivier *et al.* 1997), et ne doit pas être un frein à l'utilisation de cette technique. Par ailleurs, il est vraisemblable que dans un contexte où la surface de la zone hyporhéique se trouve colmatée, l'utilisation d'une telle méthode peut en effet profondément modifier la connectivité hydraulique verticale (Marmonier *et al.* 2010), et aboutir à une réponse faussée quant à l'évaluation du processus de décomposition.

Pour limiter ce biais, j'ai systématiquement expérimenté dans des écosystèmes exempts de toutes activités anthropiques, en particulier de celles qui auraient pu avoir un impact sur la géomorphologie des cours d'eau.

Par ailleurs, les cours d'eau de tête de bassin sont des systèmes particulièrement dynamiques, dans lesquels la géomorphologie du lit est souvent amenée à évoluer rapidement, à l'échelle de quelques semaines. Les crues hivernales et/ou printanières ont été jusqu'à modifier des séquences mouille-radier-mouille (obs. pers.) sur certains des cours d'eau étudiés. Ainsi, même s'il est important de garder à l'esprit l'impact que peut avoir l'installation de dispositifs sur les réponses mesurées, dans notre cas, la perturbation générée par nos expérimentations doit être relativisée vis-à-vis de la dynamique et de la résilience des systèmes et des organismes étudiés.

Un des enjeux de ma thèse était de pouvoir mesurer l'activité et caractériser la composition et la structure des communautés fongiques du compartiment hyporhéique *in situ*. Dans ce contexte, des dispositifs de terrain ont été développés et des microcosmes adaptés. Les dispositifs de terrain consistaient en des morceaux de tube PVC sur lesquels des systèmes de prélèvements ont été adaptés afin de pouvoir échantillonner les communautés fongiques présentes dans l'eau interstitielle via l'identification des spores circulantes. De même, les microcosmes étaient des colonnes sédimentaires (Torreiter *et al.* 1994 ; Griebler 1996 ; Mermillod-Blondin *et al.* 2005 ; Navel *et al.* 2010) sur lesquelles des dispositifs d'échantillonnage pour prélever les spores d'hyphomycètes aquatiques circulantes ont été adaptés. Ces deux approches complémentaires se sont avérées particulièrement efficaces pour apporter des éléments de réponses aux questionnements abordés durant ma thèse. Il est cependant impossible de garantir que nos dispositifs ont mimé à l'identique les conditions abiotiques de la zone hyporhéique.

Toutefois, une manipulation conduite au laboratoire (non présentée dans ce document), visant à évaluer le processus de décomposition de litière dans les colonnes sédimentaires a montré que les pertes de masses mesurées étaient relativement proches de celles enregistrées en parallèle dans la zone hyporhéique d'un cours d'eau de la Montagne Noire. Dans la même expérience, les pertes mesurées en microcosmes aérés (nettement plus importantes) étaient proches de celles observées en milieu benthique.

De même, la réduction du taux d'oxygène dissous dans l'eau interstitielle en fonction de la profondeur, mesurée dans les colonnes sédimentaires et *in situ*, suivait la même tendance. Ainsi, ces résultats suggèrent que ce microcosme est un outil adapté pour étudier la décomposition de la matière organique particulaire dans les conditions du compartiment hyporhéique et offre de nombreuses perspectives dans le développement d'axes de recherches fondamentaux et appliqués.

Structuration des communautés d'hyphomycètes aquatiques dans la zone hyporhéique

L'ensemble des manipulations visant à caractériser la composition et la structure des hyphomycètes aquatiques associés ou non à la litière dans la zone hyporhéique m'ont permis de constater qu'il existait un fort effet de sélection de cette dernière à la fois sur les spores circulantes dans l'eau interstitielle, mais également sur les assemblages fongiques établis sur le matériel foliaire. L'appauvrissement en oxygène dissous dans l'eau interstitielle et l'effet de filtre physique joué par le sédiment, sont apparus comme étant les deux mécanismes responsables de la structuration des communautés fongiques dans l'habitat hyporhéique.

Toutefois, il est important d'insister sur le fait que la caractérisation des communautés fongiques a été réalisée via l'identification de spores libérées. De ce fait, en ce qui concerne le stress des communautés par l'hypoxie, il est vraisemblable que les champignons sous un stress physiologique de ce type, modifie l'allocation de leurs ressources à la production de conidies, et que la reproduction ne soit plus une priorité, voire que celle-ci soit inhibée. Sridhar & Bärlocher (1997) ont observé que certains mycéliums actifs d'hyphomycètes aquatiques ne libéraient plus de spores sous l'effet de conditions environnementales contrôlées. Ainsi, une diminution de la turbulence réduit le taux de sporulation des champignons. L'écoulement dans la zone hyporhéique est profondément modifié du fait du passage dans le sédiment par rapport à l'écoulement turbulent des eaux de surface. Ce facteur pourrait être impliqué dans le fait qu'une densité inférieure de spores circulantes ait été systématiquement mesurée dans l'eau interstitielle par rapport aux eaux de surface (chapitre 3). Le sédiment de la zone hyporhéique est également un filtre photique très efficace.

Or, des études ont rapporté que la lumière avait un pouvoir stimulateur vis-à-vis de la sporulation des hyphomycètes aquatiques (Fisher & Webster 1978 ; Thomas *et al.* 1991 ; 1992 ; Rajashekhar & Kaveriappa 2000).

Les mécanismes physiologiques impliqués dans ce phénomène sont encore peu connus, mais l'absence de lumière dans l'habitat hyporhéique pourrait potentiellement entrer en jeu dans la régulation de la sporulation des champignons dans ce compartiment. De même, Thomas *et al.* (1992) ont démontré que la sporulation suivait une rythmicité calquée sur les cycles nycthéméraux, avec une diminution de spores libérées pendant la nuit. Enfin, l'ensemble des ces études ont souligné le fait que toutes les espèces n'étaient pas affectées de la même manière par ce phénomène, ce qui pourrait expliquer que les communautés fongiques dans le compartiment hyporhéique soient dominées par quelques espèces peu ou pas affectées par ce mécanisme (chapitres 2, 3 et 5). Toutefois, les résultats présentés dans le chapitre 2 et ceux d'une expérimentation non exposée dans ce document démontrent que la production conidienne associée à du matériel foliaire dans l'habitat hyporhéique reste relativement élevée. Finalement, notons également, que l'absence de lumière dans la zone hyporhéique élimine de fait les interactions potentielles des champignons avec des producteurs primaires tels que les diatomées par exemple. Ces interactions peuvent quant à elles avoir lieu à la surface du sédiment, et modifier l'activité des champignons, la qualité du matériel foliaire, l'efficacité des transferts trophiques et par conséquent le processus de décomposition des litières.

De nombreuses études ont démontré que l'activité fongique était affectée par les concentrations en azote et phosphore inorganique dissous dans les cours d'eau (*e.g.*, Suberkropp & Chauvet 1995 ; Niyogi *et al.* 2003 ; Pascoal *et al.* 2001 ; 2003 ; Ferreira *et al.* 2006). En effet, les hyphomycètes aquatiques sont capables d'assimiler l'azote et le phosphore provenant à la fois du substrat et de la colonne d'eau. Ainsi, de petites augmentations en nutriments inorganiques dissous induisent une augmentation considérable de la biomasse fongique, de la production et la reproduction, et par conséquent, une accélération de la décomposition des litières (*e.g.*, Elwood *et al.* 1981 ; Grattan & Suberkropp 2001 ; Gulis & Suberkropp 2003a). Les cours d'eau dans lesquels j'ai réalisé l'ensemble des manipulations sont des systèmes quasiment dépourvus de nutriments, en particulier de phosphore.

A ce propos, il est important de rappeler que les cours d'eau Vosgiens ont subi d'importants dépôts atmosphériques qui ont considérablement augmenté les concentrations en azote dans l'eau, par ailleurs ces derniers présentent des concentrations extrêmement faibles en phosphore (chapitre 5). Dans ce contexte, j'ai été surpris de constater que la concentration en phosphore, principal facteur limitant dans ces systèmes, était systématiquement plus élevée dans l'eau interstitielle (chapitres 2, 3 et 4), en raison vraisemblablement d'une activité biologique accrue dans ce compartiment, et que cela ne semblait en rien stimuler la croissance et l'activité fongique. Cela suggère que d'autres facteurs écologiques beaucoup plus structurants sont impliqués dans ces mécanismes, et inhibent l'effet stimulant de l'augmentation de la concentration en phosphore dans l'eau interstitielle.

La matrice sédimentaire représente une barrière physique directement impliquée dans la structuration des communautés de décomposeurs (*i.e.*, invertébrés et champignons) de l'habitat hyporhéique (chapitres 2, 3 et 5). Ainsi, j'ai démontré que le criblage physique des spores d'hyphomycètes aquatiques pouvait en partie expliquer l'appauvrissement de la richesse spécifique des communautés fongiques associées aux litières ou circulantes dans le sédiment (chapitres 2, 3 et 5). Les caractéristiques morphologiques des spores et réciproquement la taille des espaces interstitiels semblent être les deux facteurs explicatifs de ce criblage. Dans ce sens, les espèces présentant de petites spores peu ramifiées devraient dominer. Au contraire, les espèces d'hyphomycètes aquatiques libérant de grandes spores ramifiées devraient être systématiquement contre-sélectionnées. Or, même si j'ai constaté que l'on assistait souvent à une forte diminution de la contribution de ces espèces dans les communautés hyporhéiques, l'ensemble des patrons observés ne suivent pas exactement cette hypothèse.

Par ailleurs, même s'il est communément admis que la libération de spores est la voie principale de dissémination impliquée dans la colonisation de nouvelles ressources (Sridhar & Bärlocher 1997 ; Kearns & Bärlocher 2008 ; Bärlocher 2009), nous ne pouvons pas exclure, au vu de ces résultats, que d'autres mécanismes tels que la dissémination par des fragments de mycélium ou par des spores sexuées entrent en jeu dans cette étape cruciale du cycle biologique de ces champignons.

En outre, il est suggéré pour de nombreux organismes dépourvus de la capacité de déplacement actif dans leurs milieux, comme c'est le cas pour les champignons, qui sont contraints de dériver passivement le long du cours d'eau, que des formes de résistances existent leur permettant de compenser ce problème (Bärlocher 2010). Ces mécanismes de compensation pourrait entre autres comprendre la survie dans la zone hyporhéique, les eaux souterraines et le sol (Krauss *et al.* 2003 ; 2005 ; Bärlocher *et al.* 2006 ; 2008 ; Bärlocher 2010). Par ailleurs, plusieurs espèces d'hyphomycètes aquatiques sont connues pour être endophytes de racines de la végétation riveraine (Bärlocher 2009 ; 2010)

Enfin, la zone hyporhéique est reconnue comme étant caractérisé par une importante activité biologique, dénotant sa contribution dans les processus écologiques des cours d'eau. Ainsi, ce compartiment peut être un puits (Grimm & Fisher 1984) ou une source (Fiebig & Lock 1991) d'énergie pour l'écosystème. Grimm & Fisher (1984) et Push & Schwoerbel (1994) ont montré qu'une large part de cette activité biologique pouvait être attribuée au compartiment bactérien. Bien que les bactéries soient considérées comme jouant un rôle marginal dans la décomposition des litières, au moins dans les premiers stades (Newell 1993 ; Kominkova *et al.* 2000), des études ont rapporté d'autres tendances (*e.g.*, Gaur *et al.* 1992). Par ailleurs, des études ont démontré que la présence d'interactions entre champignons et bactéries pouvaient retarder la sporulation et affecter la production de conidies (Gulis & Suberkropp 2003b) ou encore affecter la croissance des champignons (Mille-Lindblom & Tranvik 2003). Toutefois, les résultats issus des chapitres 2 et 5 montrent clairement l'importance du compartiment fongique dans le processus de décomposition des litières au sein de la zone hyporhéique, et cela, même dans un contexte écologique très fortement perturbé. Cependant, les relations entre champignons et bactéries restent encore mal connues, et demandent à être étudiées plus en détails, particulièrement dans des conditions environnementales contrastées. Il est probable que ces interactions soient profondément modifiées dans la zone hyporhéique, du fait de conditions abiotiques particulièrement favorables aux bactéries.

Rôle des invertébrés détritivores

Les résultats provenant des chapitres 2 et 5 font apparaître l'existence de liens forts entre la structure et la composition des communautés d'invertébrés détritivores et la localisation de la litière dans les cours d'eau de tête de bassin. L'abondance, la biomasse et la diversité des invertébrés détritivores associées aux litières en décomposition sont significativement inférieures au sein de l'habitat hyporhéique qu'à la surface du sédiment. Ainsi, les communautés d'invertébrés détritivores associées à la litière dans les sédiments étaient principalement dominées par des Plécoptères de petites tailles, souvent dans leurs premiers stades de développement, et en particulier des espèces du genre *Leuctra*. Les sédiments constituant la zone hyporhéique représentent une barrière physique qui restreint l'accès à la litière enfouie pour les invertébrés les plus gros (*e.g.*, trichoptères). Plus généralement, il a été démontré qu'il existait des liens forts entre la structure des sédiments et les communautés d'invertébrés associées. Comme, cela a été évoqué précédemment, les communautés d'invertébrés dans la zone hyporhéique étaient exclusivement constituées d'individus de petites tailles appartenant à des taxons de type épigé. Ainsi, une ambigüité persiste quant aux mécanismes qui ont conduit ces invertébrés à migrer dans les litières disposés dans l'habitat hyporhéique. La recherche de conditions moins contraignantes (*e.g.*, stress hydraulique, compétition réduite) est un des facteurs pouvant expliquer la colonisation de la zone hyporhéique par les invertébrés benthiques (Creusé des Chatelliers & Marmonier 1990 ; Brunke & Gonser 1999).

Toutefois, la différence de colonisation observée au sein des feuilles naturelles et synthétiques (chapitre 2) ne paraît pas pouvoir être expliquée par ces deux éléments seuls. Ainsi, Brunke & Gonser (1999) suggèrent que la distribution des invertébrés est fortement influencée par la distribution des ressources trophiques. Cette hypothèse est cohérente avec la différence de patrons observés dans notre expérience (*i.e.*, feuilles naturelle versus synthétique), et pourrait de manière générale expliquer la faible abondance d'invertébrés associée aux litières enfouie dans le sédiment observée durant nos manipulations. En effet, nous avons conduit nos expériences de façon synchrone avec la période hivernale, époque à laquelle les quantités de litières à la surface des sédiments sont abondantes.

Par ailleurs, la porosité efficace des sédiments pourrait également influencer l'abondance, la distribution et la diversité des invertébrés (Navel *et al.* 2010).

En outre, la morphologie des invertébrés pourrait également être un élément explicatif de la composition et de la structure des communautés des invertébrés dans l'habitat hyporhéique. Ainsi, les taxons présentant des formes cylindriques de petite taille permettrait une meilleure pénétration et exploration des sédiments peu poreux. L'occupation de la matrice sédimentaire par les invertébrés peut varier au cours du cycle biologique des taxons (Williams 1984) mais également à la faveur de perturbations hydrauliques telles que les périodes d'étiage ou de crue (Boulton & Lake 1992 ; Dole-Olivier & Marmonier 1992). Dans ce contexte, l'utilisation de l'habitat hyporhéique a été vue comme un comportement de protection face aux contraintes liées de la vie à la surface du sédiment. Nos résultats montrent que la migration verticale de la macrofaune benthique n'est pas un comportement systématique dans les cours d'eau de tête de bassin, et que les dimensions spatiale et temporelle peuvent apporter une part de complexité supplémentaire aux patrons de distribution classiquement proposés.

Par ailleurs, cela permet également de souligner que le caractère aléatoire, bien que régi par un ensemble de facteurs précédemment abordés, de la colonisation des invertébrés, est en partie compensé par une colonisation systématique du compartiment fongique. Ainsi, les résultats acquis au cours de ma thèse pris dans leur ensemble suggèrent de repenser le fonctionnement écologique des cours d'eau de tête de bassin, uniquement basé sur la compréhension du processus de décomposition des litières situé à la surface du sédiment. L'intégration de la dimension verticale au modèle conceptuel du fonctionnement des écosystèmes d'eau courante, longtemps suggérée par les "hyporhéologistes", paraît maintenant justifié au regard du processus de décomposition. Ces résultats permettent également de repositionner les hyphomycètes aquatiques à l'échelle globale du fonctionnement des cours d'eau de tête de bassin, en soulignant leur fonction déterminante au sein d'un compartiment dans lequel jusqu'à ce jour, leur existence était à peine soupçonnée.

Perspectives de recherche

Généralisation des résultats obtenus

Dans le cadre de ma thèse, j'ai évalué le processus de décomposition des litières en me basant sur une espèce rapidement décomposable, l'aulne glutineux (*Alnus glutinosa*) afin de pouvoir limiter la durée des expérimentations sur le terrain mais en particulier au laboratoire, dans les colonnes sédimentaires. J'ai ainsi considéré que les patrons de réponses obtenus à partir de cette espèce végétale pouvaient être transposés à l'ensemble des espèces végétales composant les communautés riveraines des cours d'eau forestier. Cependant, les taux de transformation de la litière peuvent varier énormément entre les espèces, ce qui s'explique en partie par de grandes différences qualitatives dans la chimie et la structure physique des litières. Ainsi, la teneur en nutriments et la teneur en substances réfractaires telles que lignine et tannins sont deux éléments essentiels permettant d'estimer la qualité intrinsèque de la litière (Webster & Benfield 1986 ; Gessner & Chauvet 1994 ; Ostrofsky 1997 ; Driebe & Whitam 2000 ; Lecerf & Chauvet 2008a). Ainsi, l'utilisation de litière de chêne sessile dans le chapitre 3, même s'il n'est pas possible de faire une comparaison directe puisque nous avons utilisé des sachets de litière à maille fine (*i.e.*, 1 mm), montre que parmi les quatre modes d'exposition (*i.e.*, benthique, hyporhéique, benthique-hyporhéique ou hyporhéique-benthique), il n'y avait pas de différence dans les vitesses de décomposition. Ainsi, cette variable est peut être une des explications qui élucideraient les divergences de patrons de réponses rencontrées dans la littérature quant aux comparaisons des taux de décompositions des litières à la surface du sédiment versus dans la zone hyporhéique.

Par ailleurs, Smith & Lake (1993) dans leur étude rapportait des résultats intéressants quant à une divergence des patrons de réponse en fonction de la période à laquelle se déroule l'expérimentation. Ainsi, ces derniers trouvaient que la vitesse de décomposition de la litière enfouie dans la zone hyporhéique au printemps n'était pas différente de celle exposée à la surface du sédiment, contrairement à ce qu'ils avaient pu observer pendant la période hivernale. Ils suggéraient que les macroinvertébrés avaient été beaucoup plus actifs sur la litière enfouie dans le sédiment durant le printemps, du fait d'un manque de ressource dans la zone benthique.

Cette hypothèse bien que souvent suggérée, n'a jamais été testée expérimentalement. Il serait donc intéressant de vérifier si ce partitionnement de la ressource dans le temps et dans l'espace conduit à une migration active des invertébrés détritivores en direction des paquets de litières immobilisés dans le sédiment, et donc si ce compartiment jouerait un rôle de soutien d'une partie des communautés d'invertébrés détritivores des cours d'eau forestiers. Par ailleurs, il est également suggéré que la présence de prédateur pourrait être à l'origine de cette migration d'une partie des invertébrés dans la zone hyporhéique. Ce mécanisme serait d'autant plus important durant les périodes où les stocks de litière à la surface du sédiment sont rares. En effet, les structures de feuilles offrent des habitats idéaux pour les invertébrés, puisqu'elles permettent l'évitement d'une partie des prédateurs et sont source de nourritures. Toutefois, à la fin du printemps, lorsque les stocks de litière à la surface du sédiment ont pratiquement disparus, la prédation est susceptible d'avoir un impact sur la densité, la diversité et l'activité des proies (Malmqvist 1993 ; Usio 2000 ; Ruetz *et al.* 2002 ; Greig & McIntosh 2006 ; Nilsson *et al.* 2008). Il serait donc pertinent d'évaluer l'effet de la présence de prédateurs (*e.g.*, invertébrés ou poissons) sur l'activité de migration des invertébrés détritivores dans la zone hyporhéique, mais également sur le processus de décomposition de la litière enfouie. Nous pouvons imaginer qu'une partie des invertébrés détritivores (*e.g.*, plécoptères), sous la pression de prédation, se réfugie dans l'habitat hyporhéique et soit davantage focalisée sur la matière organique enfouie. Les résultats de l'expérimentation présentée en annexe renforcent cette hypothèse. En effet, ils soulignent le rôle déterminant de la structure de l'habitat pour les communautés d'invertébrés détritivores en présence d'un prédateur, et mettent l'accent sur le pouvoir structurant de ce dernier vis-à-vis de la communauté de proies. Enfin, ils mettent en évidence l'effet des interactions trophiques et non-trophiques sur le processus de décomposition des litières.

Les estimations faites à partir du modèle de décomposition de la litière dans la zone hyporhéique (chapitre 2) laissent penser que la litière d'une espèce à décomposition rapide, tel que l'aulne glutineux, pourrait résider près d'un an dans le sédiment avant d'être totalement décomposée. Ces estimations laissent imaginer quel pourrait être le temps de résidence de litières beaucoup plus réfractaires telles que les espèces de chêne (*Quercus sp.*) ou le hêtre commun (*Fagus sylvatica*).

Ainsi, de la même manière que le suggère Shearer (1992) pour le bois qui réside dans le lit des cours d'eau, ces litières immobilisées dans le sédiment pourraient constituer un réservoir fongique et contribuer au flux continu et minimal de spores durant le printemps et l'été jusqu'à l'abscision des feuilles en automne, et ainsi servir d'inoculum pour les feuilles nouvellement tombées dans le cours d'eau (Bärlocher *et al.* 2006). Par ailleurs, il est connu que plusieurs espèces d'hyphomycètes aquatiques sont des endophytes des racines terrestres et submergées (Bärlocher 2007), de plus une espèce a également été trouvée dans les aiguilles de *Picea mariana* (Sokolski *et al.* 2006). Ainsi, bien que ces études aient apporté un certain nombre d'éléments de réponse, l'origine des hyphomycètes aquatiques dans les cours d'eau reste partiellement inconnue (Bärlocher 1992).

Dans ce contexte, des études portant sur l'inventaire des espèces d'hyphomycètes aquatiques conjuguant les approches moléculaire et traditionnelle associées aux litières enfouies dans la zone hyporhéique pourraient apporter un éclairage supplémentaire sur la contribution de la zone hyporhéique quant à l'origine des hyphomycètes aquatiques dans les cours d'eau. Bärlocher *et al.* (2006 ; 2008) ont cependant utilisé les particules de matière organique présentes dans le sédiment, sans connaitre à priori l'histoire de ces dernières. Ce type d'étude pourrait être reproduit en introduisant de façon contrôlée le matériel foliaire sur lequel seraient réalisées les analyses. En parallèle, des études moléculaires pourraient être réalisées directement sur le sédiment de la zone hyporhéique, soit en y installant des substrats dans des dispositifs (tube PVC) soit en réalisant des carottages à l'aide de mini-piézomètres.

Approche moléculaire et étude de la diversité fongique

Un des principaux obstacles souligné par Bärlocher (2010) dans la recherche en écologie des champignons réside dans la difficulté d'identification du mycélium *in situ*. Cependant, ce dernier représente la phase métaboliquement active des champignons et est responsable des effets les plus directs sur les fonctions des écosystèmes (Bärlocher 2010). L'identification des espèces est généralement réalisée sur une phase de reproduction. En ce qui concerne les hyphomycètes aquatiques, la taxonomie est basée sur la taille et la morphologie des conidies (spores asexuées).

La majorité des études visant à évaluer les changements saisonniers dans la composition et la structure des communautés d'hyphomycètes aquatiques dans les cours d'eau (Iqbal et Webster 1973) ou la succession des espèces fongiques au cours de la décomposition des litières (Bärlocher 1982) est uniquement basée sur l'identification des spores filtrées dans la colonne d'eau ou de celles libérées par les champignons associés aux litières (Bärlocher 2010). Deux biais potentiellement importants apparaissent, le premier est que du fait d'une évolution convergente de la morphologie des spores, l'examen de ces dernières ne suffit pas toujours à elle seule pour obtenir une identification sans équivoque (Marvanová & Bärlocher 2001 ; Bärlocher 2010). Le second est que cette approche repose sur l'hypothèse que le nombre de spores libérées est directement relié à l'abondance relative et à l'activité des différentes espèces présentes sur les feuilles en décomposition. Toutefois, (Sridhar & Bärlocher 1997) ont fait remarquer que certains mycéliums actifs pouvaient ne pas libérer de spores, et que les espèces pouvaient réagir différemment, en termes de sporulation, aux conditions environnementales (*e.g.*, température, nutriments, turbulence modérée …).

Ainsi au cours de cette thèse (chapitres 2, 3 et 5), nous avons pu constater que les conditions physico-chimiques de la zone hyporhéique étaient un facteur déterminant de la composition et de la structure des assemblages fongiques. Cependant, une étude que j'ai réalisée au laboratoire, qui n'a pas été présentée dans ce document, montre que l'activité et le taux de sporulation d'espèces fongiques en culture pure et en mixture peuvent être différents, voire inhibés en ce qui concerne le taux de sporulation, selon les conditions d'incubation (*i.e.*, benthique versus hyporhéique). Il apparaît que toutes les espèces ne réagissent pas de la même manière aux conditions de l'habitat hyporhéique. L'allocation des ressources dans la biomasse mycélienne, dans la reproduction (*i.e.*, production de spores) et dans l'activité de décomposition est donc modifiée. Cela suggère que les interactions entre espèces peuvent être affectées, mais également que celles avec d'autres organismes (*e.g.*, bactéries et invertébrés) peuvent potentiellement être modifiées en fonction de la localisation des litières dans le cours d'eau, et par conséquent affecter les processus écologiques.

Dans ce contexte, l'utilisation de méthodes moléculaires pourrait être particulièrement intéressante et apporter des éclairages sur l'ensemble de ces mécanismes. L'immense avantage de l'approche moléculaire par rapport aux techniques traditionnelles, c'est qu'elle n'est pas dépendante de la présence de structures de reproduction pour identifier les espèces présentes sur le substrat, puisque les acides nucléiques sont présents dans chaque cellule indépendamment de sa position dans le cycle de vie (Bärlocher 2010). Ainsi, des études réalisées dans le cadre de ma thèse, non présentées dans ce document, ont révélé que le nombre d'OTU (operational taxonomic unit) associé à de la litière exposée à la surface du sédiment et dans la zone hyporhéique était presque similaire, alors que la richesse spécifique évaluée par l'identification des spores libérées montrait de nettes différences. De même, le nombre d'espèces d'hyphomycètes aquatiques associés à des litières incubées dans des cours d'eau le long d'un gradient d'acidité déterminé via la production de spores suivait le même patron que celui rapporté par Baudoin *et al.* (2008). Ainsi, la richesse spécifique était significativement corrélée au pH des cours d'eau. L'approche moléculaire a permis de montrer que le nombre d'OTU ne suivait pas cette tendance. Cela suggère que la vitesse de décomposition des litières dans ces cours d'eau est particulièrement bien corrélée avec la richesse taxonomique en espèces sporulantes mais pas avec le nombre d'OTU ("richesse spécifique rélle"). Ce constat soulève une nouvelle question d'autant que la biomasse mycélienne a été montrée comme n'étant pas affectée par le stress acide (chapitre 5, Baudoin *et al.* 2008 ; Dangles & Chauvet 2003).

Des études utilisant une approche enzymatique pourrait potentiellement fournir un certain nombre d'éléments de réponses quant au fait que la "richesse spécifique" déterminée via l'approche moléculaire n'apparait pas différente entre cours d'eau neutre et cours d'eau acidifié, alors que le processus de décomposition des litières est quant à lui affecté.

Par ailleurs, Bärlocher (2010) soulignait qu'en plus des hyphomycètes aquatiques, des analyses moléculaires avaient révélé la présence d'autres champignons associés aux litières submergées. A ce jour, assez peu de choses sont connus à propos de leurs fonctions écologiques. Dans ce sens, Bärlocher (2010) adresse une question intéressante, participent-ils à la décomposition ou sont-ils des agents pathogènes?

Ainsi, bien que les hyphomycètes aquatiques soient reconnus pour être le groupe de champignons dominant des communautés fongiques des milieux lotiques, d'autres groupes de champignons se développent sur les litières en décomposition, notamment dans les milieux moins oxygénés (Descals & Moralejo 2001), dans les eaux stagnantes, dans les fonds vaseux. La zone hyporhéique, du fait, de conditions abiotiques contrastées avec les eaux de surface pourrait potentiellement abriter des champignons appartenant à d'autres groupes taxonomiques, présentant des caractéristiques physiologiques leurs permettant d'être, dans ce contexte, beaucoup plus compétitifs que les hyphomycètes aquatiques. L'importance, le rôle de ces champignons et leur interaction avec les hyphomycètes aquatiques constituent des perspectives de recherches séduisantes.

Enfin, dans le chapitre 4 nous avons vu que la localisation de la litière dans le cours d'eau affecte considérablement l'efficacité des transferts trophiques vers les invertébrés détritivores. A l'échelle du cours d'eau, cela pourrait se traduire par une altération des flux de carbone dans l'écosystème dont dépendent la productivité et la stabilité des niveaux trophiques supérieurs. Ainsi, il serait intéressant d'identifier et comprendre les mécanismes sous-jacents qui régissent ce patron de réponse, d'étendre cette recherche à l'échelle de l'écosystème mais également de l'appliquer dans la compréhension des couplages entre écosystème terrestre et aquatique. Dans ce sens, l'exploration de ces mécanismes à l'aide d'une approche stœchiométrique pourrait être particulièrement fructueuse. La composition élémentaire d'organismes soumis à un facteur de stress est-elle affectée ? Quelles sont les conséquences de ces changements sur l'efficacité des transferts trophiques ?

La zone hyporhéique dans le contexte des changements globaux

Au cours de la seconde moitié du 20ème siècle, le niveau de dioxyde de carbone atmosphérique n'a cessé d'augmenter, et devrait encore augmenter pour passer d'une concentration actuelle d'environ 375 ppm à une valeur comprise entre 540 et 970 ppm à la fin du 21ème siècle (IPCC, 2001). Le CO_2 est l'une des ressources principales pour la croissance des plantes, son augmentation dans l'atmosphère telle que prévue par le précédent scénario est susceptible d'affecter profondément les interactions entre les espèces. De même, les patrons de réponse face au réchauffement climatique associé à l'augmentation de CO_2 devraient se manifester entre autres par des modifications phénologiques.

Ce mécanisme dans l'hémisphère nord est particulièrement bien documentée (Sparks & Menzel 2002 ; Walther *et al.* 2002 ; Parmesan & Yohe 2003 ; Root *et al.* 2003). Ainsi, une augmentation du dioxyde de carbone dans l'atmosphère implique des changements importants dans la composition chimique de la litière et par conséquent modifie sa vitesse de décomposition dans les cours d'eau (Rier *et al.* 2005). De plus, il a également été démontré que de tels changements climatiques induisent un retard dans la chute des feuilles et des changements dans la composition des communautés végétales riveraines (Menzel *et al.* 2006). Par ailleurs, d'autres effets directement liés à l'élévation de la température sont prévus, il s'agit de changements concomitants entre la température de l'eau et l'hydrologie, qui sont toutes deux des composantes déterminantes régissant l'activité biologique et la décomposition des litières.

Ainsi, il est prévu que l'augmentation de la température dans les cours d'eau devrait augmenter la respiration des bactéries de 26 à 63% (Sand-Jensen *et al.* 2007). De telles modifications du métabolisme bactérien pourraient profondément modifier les interactions avec les hyphomycètes aquatiques, et par conséquent celles avec les invertébrés détritivores et plus généralement l'efficacité des transferts trophiques.

A l'échelle des cours d'eau, cela pourrait se traduire par une altération des flux de carbone dans l'écosystème dont dépendent la productivité et la stabilité des niveaux trophiques supérieurs. Par ailleurs, dans un contexte de sécheresse particulièrement sévère comme prédit dans les différents scénarios, la zone hyporhéique ne pourrait-elle pas jouer un rôle majeur en tant que zone refuge et réservoir d'inoculum fongique? Des recherches visant à relier l'ensemble de ces processus et à comprendre comment les changements climatiques à l'échelle régionale influencent la dynamique du processus de décomposition des litières dans les cours d'eau sont primordiales.

Zone hyporhéique et intégrité fonctionnelle des cours d'eau forestier

Une autre direction qu'il serait intéressant d'explorer concerne le potentiel d'évaluation de l'intégrité fonctionnelle de la zone hyporhéique d'une manière équivalente à celle utilisée dans les eaux de surface.

Les écologues et les hydrologues sont depuis longtemps conscients de l'importance fonctionnelle de la zone hyporhéique des écosystèmes d'eau courante et de l'importance des échanges hydrologiques verticaux pour le maintien de l'intégrité biologique. Toutefois ces résultats ont tardé à être communiqués aux gestionnaires et par conséquent à être intégrés dans les politiques de gestion et de conservation (Boulton 2000 ; 2007 ; Woessner 2000). Malgré un intérêt certain du concept d'intégrité fonctionnelle des cours d'eau (Meyer 1997 ; Gessner & Chauvet 2002), l'application de ce concept à la zone hyporhéique a reçu assez peu d'attention (Boulton *et al.* 2008). Pourtant, de nombreuses activités humaines affectent la zone hyporhéique, soit par la perturbation des échanges hydrologiques ou par contamination directe (Mestrov & Lattinger-Penko 1981 ; Hancock 2002 ; Kasahara *et al.* 2009). Même la présence d'espèces exotiques dans les eaux de surface peut altérer les fonctions écologiques de la zone hyporhéique (Bickel & Closs 2008).

Des indicateurs efficaces, suffisamment sensibles, aisément mesurables et interprétables, évaluant le niveau d'intégrité fonctionnelle de la zone hyporhéique doivent être mis en place sans tarder (Boulton *et al.* 2008). Toutefois, le développement de tels indicateurs est actuellement limité par un manque évident de données de référence et de protocoles (Hahn 2006). Ainsi, un processus intégratif tel que le taux de décomposition des litières pourrait potentiellement être utilisé dans ce sens. Un dispositif contenant la litière, disposé dans la zone hyporhéique tel que proposé par Marmonier *et al.* (2010) pourrait être particulièrement adapté à cet usage. Par ailleurs, des métriques telles que la production de FPOM par le compartiment microbien (chapitre 5) semblent particulièrement pertinentes dans ce contexte.

Toutefois, bien que le processus de décomposition des litières soit un indicateur sensible de l'intégrité fonctionnelle des écosystèmes d'eau courante (Pascoal *et al.* 2003 ; Gulis *et al.* 2006 ; Lecerf *et al.* 2006 ; Mesquita *et al.* 2007 ; Castela *et al.* 2008 ; Lecerf & Chauvet 2008b), certaines études suggèrent que les taux de décomposition combinés à des mesures structurelles telles que la composition des communautés d'invertébrés et d'hyphomycètes aquatiques, ou encore la production de spores pourraient fournir des indicateurs encore plus pertinents (Lecerf *et al.* 2006, Castela *et al.* 2008, Lecerf & Chauvet, 2008b).

Ainsi, le développement de recherche intégrant ces deux composantes dans un contexte de "biomonitoring" de la zone hyporhéique pourrait être particulièrement utile dans l'élaboration de politiques de gestion, conservation et restauration des cours d'eau de tête de bassin.

A cet égard, la diversité des contextes écologiques et géomorphologiques peuvent rendre particulièrement peu aisée une démarche de normalisation d'un tel outil de « biomonitoring ». Il est cependant important de garder à l'esprit, que si la présence d'une zone hyporhéique fonctionnelle n'est pas systématique, le fonctionnement écologique d'une partie des cours d'eau de nos réseaux hydrographiques peut, à certaines époques de l'année, uniquement reposer sur cette dernière.

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Annexe

Bottom-up effects mediate top-down impacts in a detritus-based food web

Introduction

Les macroinvertébrés détritivores sont des organismes clé pour le fonctionnement et les flux de nutriments au sein de cours d'eau de tête de bassin hétérotrophes (Cuffney *et al.* 1990 ; Wallace & Webster 1996 ; Hieber & Gessner 2002). Or, si de nombreuses études ont porté sur les conséquences de divers facteurs abiotiques (qualité de l'eau, régime hydrique, température…) sur leur diversité et leur densité (Townsend *et al.* 1987 ; Reice & Wohlenberg 1993), un nombre plus restreint d'entre elles se sont intéressé aux influences biotiques responsables de la structuration de ces communautés, et les conséquences potentielles sur le fonctionnement de l'écosystème.

Ces communautés peuvent en effet être sous l'influence de facteurs top-down (prédation) et bottom-up (litière) (Rosemond *et al.* 2001 ; McIntosh *et al.* 2005). Ainsi, l'impact de prédateurs sur leurs proies a été détecté à des échelles spatiales variables, tels que micro-habitats (Kershner & Lodge 1995 ; Fairchild & Holomuzki 2005), macro-habitats (radiers, Peckarsky *et al.* (2002) et mouilles de cours d'eau, Power *et al.* (1985), Wiseman *et al.* (1993)), et également à l'échelle d'écosystèmes lenthiques (mares ou lacs, Carlisle & Hawkins (1998), Venturelli & Tonn (2005)).

La présence de prédateurs peut par divers mécanismes modifier les communautés de macroinvertébrés benthiques. Une action directe de ces derniers réduit la densité des communautés de proie (Greig & McIntosh 2006), et peut par le biais d'une prédation sélective modifier les proportions relatives de certaines populations (Nilsson *et al.* 2008).

En outre, par le biais d'interactions indirectes (non-trophique), la présence d'un prédateur peut entraîner une réduction de la densité ou de la diversité des macroinvertébrés benthiques, capables de détecter la présence du prédateur et adopter des comportements d'évitement et ainsi réduire leur activité (Åbjörnsson *et al.* 2000).

De ce fait, les proies potentielles peuvent éviter d'être consommées en vivant là où les prédateurs ne peuvent pas chasser efficacement, comme par exemple dans les interstices du sédiment (Suttle *et al.* 2004 ; Fairchild et Holomuzki 2005), dans des herbiers denses de macrophytes (Kelly & Hawes 2005), ou dans des courants rapides (Hart & Merz 1998 ; Hart & Finelli 1999).

Par conséquent, si différentes espèces ne présentent pas les mêmes comportements face à la prédation, la présence de prédateurs a le potentiel de générer des modifications de la structure des communautés de proies, par le biais d'interactions trophiques ou non-trophiques (Schmitz *et al.* 1997), et ainsi altérée les fonctions des écosystèmes assurées par ces dernières. Des études ont montré que la décomposition des litières dans des chenaux expérimentaux (Greig & McIntosh 2006) et dans des cours d'eau (Malmqvist 1993) diminuait en présence de truites et de plécoptères prédateurs, respectivement en raison d'effets létaux et non-léthaux des prédateurs sur les invertébrés détritivores. De plus, les litières peuvent être utilisées par les macroinvertébrés benthiques comme ressource nutritive et comme habitat (Richardson 1992). Ainsi il a été montré que la quantité, la qualité et la diversité des litières pouvaient influencer la structure des communautés de shredders (Wallace *et al.* 1997 ; Kominoski *et al.* 2008). En effet, les mélanges de litières labiles et réfractaires pouvaient présenter des assemblages de macroinvertébrés plus divers et/ou denses que les mêmes espèces isolées. De tels mélanges de litières constitueraient à la fois des ressources nutritives et un habitat favorable qui mènerait à un taux de décomposition accru des litières labiles au sein de tels mélanges.

Toutefois, peu d'études ont manipulé de manière concomitante la diversité des litières et la pression de prédation. Par une expérience de terrain, nous testons l'hypothèse selon laquelle les conséquences de la présence d'un prédateur sur les communautés de macroinvertébrés détritivores et sur la décomposition de paquets de litière dépendraient de la qualité et/ou de la diversité des litières. En effet l'effet de la prédation sur la structure des communautés de macroinvertébrés et les taux de décomposition pourrait être plus important au sein de paquets de litière labiles, conférant une faible complexité structurale et peu d'opportunités pour les macroinvertébrés d'échapper à la prédation. Au contraire, les paquets de litières réfractaires devraient être peu sujets à de telles altérations des communautés et des processus.

Enfin les paquets de litières présentant à la fois des litières labiles et réfractaires constitueraient une combinaison de ressources de qualité et un habitat favorable. Ainsi on s'attend à ce que l'effet de la prédation sur les communautés de macroinvertébrés et sur les taux de décomposition des litières labiles au sein de tels assemblages soient moins prononcés qu'au sein de monocultures d'espèces labiles.

Méthodes

L'expérience a été réalisée dans le Rieutort, un cours d'eau de deuxième ordre de la Montagne Noire (France). Des assemblages de 4g de litières d'aulne (*Alnus glutinosa*) et de chêne (*Quercus petraea*) en monocultures (4g d'une espèce) et en mélanges (2g de chaque espèce). Les sacs à litières consistaient en des tubes PVC (dimensions) séparés en leur milieu par une maille de 1mm constituant ainsi 2 compartiments distincts, les litières étant incluses dans le compartiment situé en aval. Afin de manipuler la présence de macroinvertébrés détritivores au sein des paquets de litière, nous avons clos les extrémités du tube par des mailles fines (0.30mm) ou grossières (5mm). Enfin un prédateur (*Cordulegaster boltonii,* Odonata Donovan) a été ajouté tantôt dans le compartiment amont afin d'évaluer l'effet indirect de la prédation, tantôt dans le compartiment aval pour les effets directs de la prédation. Nous avons croisé l'ensemble des traitements de litière (aulne, chêne et mélange) avec l'ensemble des traitements de macroinvertébrés (fine maille, prédation directe, prédation indirecte et absence de prédateur), et réalisé 10 répliques organisées en 5 blocks répartis le long du cours d'eau.

Après 33 jours d'incubation dans le cours d'eau nous avons déterminé les taux de décomposition associés à chaque espèce de litière pour chaque combinaison après nettoyage, lyophilisation et pesée au 0.01g près des litières restantes. Nous avons également déterminé la biomasse (0.01mg près après séchage) et l'identité (niveau générique) des macroinvertébrés détritivores associés à chaque traitement.

Nous avons traité les résultats de perte de masse de chaque espèce de litière par modèle mixte, incluant le block et le paquet de litière comme facteurs aléatoires, et l'espèce de litière, le traitement (fine maille, prédation directe, indirecte et absence de prédateur) et la diversité des litières comme facteurs fixes.

La biomasse des macroinvertébrés associés à chaque paquet de litière a été traitée de manière similaire, avec le bloc comme facteur aléatoire, le traitement litière (aulne, chêne ou mélange) et prédation en facteurs fixes.

Finalement, la composition des communautés de macroinvertébrés détritivores ont été représentées à l'aide d'une NMDS (non metric multidimensional scaling) en utilisant la distance de Bray-Curtis, et comparée entre traitements par une ANOSIM.

Masse restante de litière et biomasse de macroinvertébrés ont toutes deux été transformées (log (X+1)) afin de remplir les conditions d'application de l'ANOVA (normalité et homoscedasticité vérifiées graphiquement). Toutes les analyses ont été réalisées avec le logiciel R 2.6.0.

Résultats & discussion

Nos résultats suggèrent que quelque soit la variable considérée, le type de litière apparaît comme significatif (tables 1 et 2). En effet tant la biomasse de shredders et les taux de décomposition des litières sont moins importants pour le chêne que pour l'aulne, reflétant le fait que la qualité des litières est le principal facteur influençant le processus de décomposition (Melillo *et al.* 1982 ; Ostrofsky 1997) (figure 1).

Table 1 : Table ANOVA du modèle mixte sur le logarithme de la masse restante avec le bloc et le paquet de litière en facteurs aléatoires, et l'espèce la diversité des litières et le traitement de prédation comme facteurs fixes. Seuls les résultats concernant les facteurs fixes sont considérés.

	Df	SS	F	*P*
Espèce (Sp)	1	31.06	1542.31	<0.001
Diversité (D)	1	0.35	17.49	<0.001
Prédation (P)	3	3.41	56.42	<0.001
Sp x D	1	0.15	7.60	0.007
Sp x P	3	0.15	2.56	0.059
D x P	3	0.17	2.82	0.042
Résidus	107	2.155		

Table 2 : Table ANOVA du modèle mixte sur le logarithme de la biomasse de macroinvertébrés détritivores avec le bloc en facteur aléatoire, et l'espèce la diversité des litières et le traitement de prédation comme facteurs fixes. Seuls les résultats concernant les facteurs fixes sont considérés

	Df	SS	F	*P*
Litière (L)	2	28.63	21.85	<0.001
Predation (P)	2	6.30	4.81	0.011
L x P	4	3.18	1.21	0.312
Résidus	76	49.80		

Par contre, nous avons également mis en évidence un effet de la diversité des litières sur les taux de décomposition de l'espèce labile (l'aulne) (table 1), ce qui corrobore les résultats de plusieurs études antérieures (Swan & Palmer 2006 ; Sanpera-Calbet *et al.* 2009).

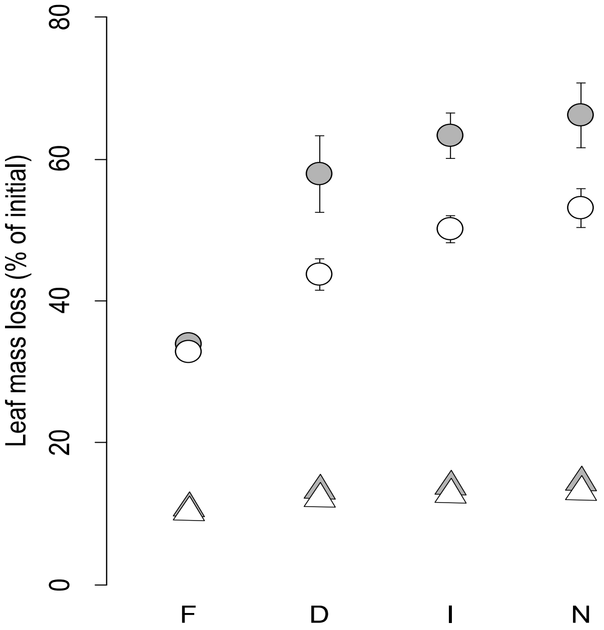

Figure 1 : Perte de masse moyenne (± *SE* , n = 10 par point) observée pour chaque espèce de litière en fonction du traitement (F : fine maille ; D : prédation directe ; I : prédation indirecte ; N : pas de prédateur), de l'espèce de litière considérée (ronds : aulne ; triangles : chêne) et de la composition du paquet de litière (gris : mélange ; blanc : monoculture). Les barres d'erreur représentent l'erreur standard.

En termes de communautés de détritivores (biomasse et composition), les paquets de litières comprenant à la fois de l'aulne et du chêne tendent à se rapprocher d'avantage de l'aulne en monoculture que du chêne (figures 2 et 3).

Ceci suggère que la présence d'une espèce hautement nutritive au sein d'un paquet de litière permet un recrutement d'une communauté dense et diverse de macroinvertébrés. Ainsi les préférences alimentaires des macroinvertébrés accédant aux paquets de litières divers, en focalisant leur consommation sur l'espèce la plus labile (présente en plus faible quantité que dans les monocultures) pourraient en second lieu expliquer l'accélération de la décomposition de l'aulne au sein des mélanges (Swan & Palmer 2006). Les taux de décomposition du chêne par contre ne sont pas altérés au sein des mélanges. Etant données les faibles pertes de masse observées sur ces litières, on peut suggérer que la contribution des macroinvertébrés à la décomposition initiale des litières de chêne a été mineure et qu'elle est plutôt due au lessivage des composés hydrosolubles, et une phase initiale d'activité fongique.

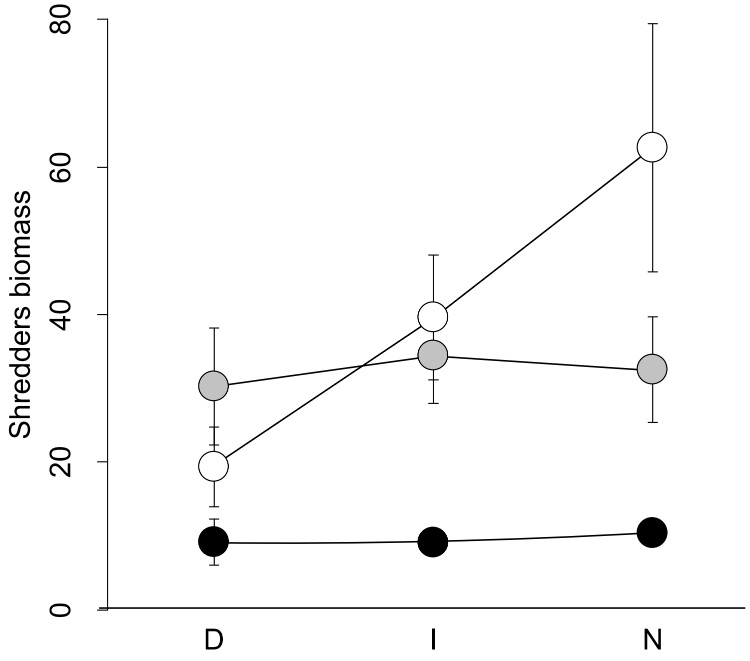

Figure 2 : Biomasse de macroinvertébrés détritivores moyenne (± *SE*, n= 10 par point) associés aux différents paquets de litières en fonction du traitement de prédation (D : prédation directe ; I : prédation indirecte ; N : pas de prédateur) et de la composition en espèces de litières du paquet (blanc : aulne ; noir : chêne ; gris : mélange). Les barres d'erreur représentent l'erreur standard.

On observe par ailleurs une augmentation de la décomposition et de la densité de détritivores le long d'un gradient de pression de prédation (prédation directe, prédation indirecte, et détritivores sans prédateurs) (figures 1 et 2). Toutefois les différences ne sont significatives qu'entre les extrémités de ce gradient (absence de prédation vs prédation directe).

Par contre, l'interaction significative entre le traitement de prédation et la diversité des litières (table 1) indique que l'effet de la prédation n'est pas identique entre monocultures et mélanges ou inversement. Cette interaction est essentiellement due au fait que les effets de diversité des litières, comme observé dans des études antérieures, n'apparaissent pas en l'absence de macroinvertébrés détritivores (fines mailles) et sont liés à leur activité de nutrition. Toutefois nous avons également observé que les effets directs du prédateur sur la décomposition de l'aulne sont moins importants lorsque des litières de chêne sont présentes au sein du paquet de litière que dans les monocultures d'aulne. De même si l'on observe une diminution de la biomasse de shredders avec la pression de prédation au sein des monocultures d'aulne, on n'observe aucune différence entre traitements pour les communautés issues de monocultures de chêne et de mélanges de litières. Enfin ces modifications corroborent avec les résultats de la NMDS qui montrent une altération de la composition des communautés due à une prédation directe au sein des monocultures d'aulne, mais pas au sein des autres combinaisons de litières.

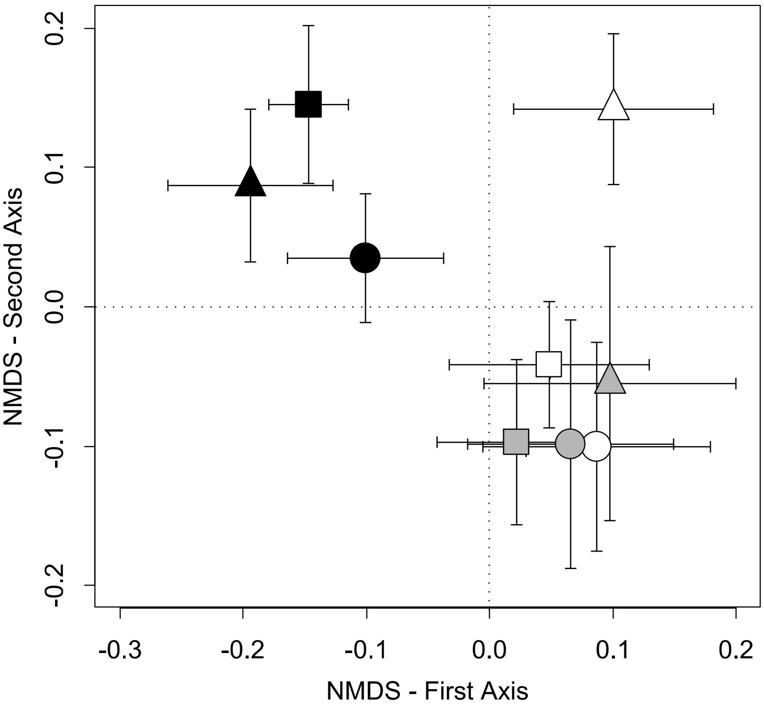

Figure 3 : Plan factoriel constitué des deux axes de la NMDS. Les moyennes des coordonnées sur les deux axes sont représentées par des cercles (pas de prédateurs), des carrés (prédation indirecte) et des triangles (prédation directe) de différentes couleurs correspondant à la composition en espèce de paquets de litière (blanc : aulne ; noir : chêne ; gris : mélange). Les barres d'erreur correspondent à l'erreur standard sur les axes 1 et 2.

Nous concluons que la qualité des litières et la pression de prédation (essentiellement au travers de consommation directe dans notre cas) ont le potentiel d'influencer à la fois la densité et la composition des communautés de macroinvertébrés détritivores par différents mécanismes (recrutement de différentes espèces en différentes proportions pour les litières, consommation préférentielle de la part du prédateur…) avec d'éventuels impacts sur les processus fonctionnels de l'écosystème. Les mélanges de litières n'influencerait pas directement ces paramètres mais constituerait une combinaison de ressource de qualité (aulne) permettant l'efficacité du processus de décomposition et d'un habitat structuré (chêne) permettant le maintien des communautés de détritivores et des processus face à la perturbation engendrée par la présence du prédateur.

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Communications scientifiques :

1. Publications

- Article accepté

Cornut J., Elger A., Lambrigot D., Marmonier P. & Chauvet E. (2010) Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams *Freshwater biology*, **55**: 2541-2556.

- Manuscrits en préparation

Danger M., Cornut J., Elger A. & Chauvet E. Effects of burial on leaf physico-chemical traits, microbial conditioning and palatability to three invertebrate shredders

Cornut J., Assémat F., Mermillod-Blondin F., Elger A., & Chauvet E. Multiple screening of aquatic hyphomycetes in the hyporheic zone of woodland streams

- **Reviewer** pour les revues internationals: **Water Research**, **Fungal Ecology** and **Fundamental and Applied Limnology**.

2. Communications

- Communications orales

Cornut J., Elger A. & Chauvet E. Effects of burial on leaf litter processing in a woodland stream. Symposium for European Freshwater Sciences. Sinaia (Roumania), 17th-21st August 2009.

Danger M., Cornut J., Elger A., Chauvet E., Chavez P., Ten-Hage L. & Lecerf A. Les algues benthiques accélèrent la décomposition de la matière organique en cours d'eau : un cas de « priming effect » aquatique? 1er Colloque National d'Ecologie Scientifique. Montpellier (France), 2-4 Septembre 2010.

Marmonier P., Navel S., Cornut J., Piscart C., Mermillod-Blondin F. Maazouzi C., Montuelle B. & Chauvet E. Décomposition de la matière organique particulaire dans le milieu interstitiel des cours d'eau. Proposition d'une méthode de terrain et premiers résultats. 10ème Journées Internationales de Limnologie. Thonon-les-Bains (France), du 5 au 8 Octobre 2010.

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- Communications affichées

Cornut J., Elger A. & Chauvet E. Effects of burial on leaf-associated fungi and litter decomposition: an experimental study in microcosms. 5th International Meeting on Plant Litter Processing in Freshwater. Coimbra (Portugal), 21st-26th July 2008.

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Cornut J., Danger M., Elger A. & Chauvet E. L'histoire de la litière dans les cours d'eau forestiers affecte l'efficacité des transferts trophiques. 1er Colloque National d'Ecologie Scientifique. Montpellier (France), 2-4 Septembre 2010. **3ème prix du meilleur poster**.

Activités pédagogiques :

1. Enseignements

Vacations en **Ecologie** et **Ecotoxicologie** à l'IUT Paul Sabatier d'Auch, Toulouse III en 2008-2009 (**33 heures**).

Vacations en **Ecologie** et **Ecotoxicologie** à l'IUT Paul Sabatier d'Auch, Toulouse III en 2009-2010 (**33 heures**).

2. Encadrement de stagiaire

Maëlys Bonnet, Stage de **Licence 1** « Sciences, Technologies, Santé mention Ecologie», Université Paul Sabatier de Toulouse III. Juillet-Août 2010. *Dynamique de la matière organique d'origine végétale en milieu hyporhéique.*

Marion Jouffroy, Stage de **DUT** « Génie biologique », IUT Paul Sabatier d'Auch, Toulouse III. Mars-Juin 2010. *Predator-prey relationships and leaf litter processing in woodland stream.*

Axel Greugny, Stage de **Master 1** « Eau Sol Environnement », Université Paul Sabatier de Toulouse III. Novembre 2009-Juin 2010. *Dynamique de la matière organique d'origine végétale et des peuplements fongiques associés en milieu hyporhéique.*

Fiona Assémat, Stage de **Master 2 Recherche** « Fonctionnement des écosystèmes et Anthropisation », Université Paul Sabatier de Toulouse III. Janvier-Juin 2009. *Rôle du sédiment sur la structuration des communautés d'hyphomycètes aquatiques dans le milieu hyporhéique.*

Caroline Bernadet, Stage de **Master 1** « Microbiologie et biotechnologies pour l'environnement », Université de Pau et des pays de l'Adour. Avril-Juin 2008. *Fonctionnement des cours d'eau de tête de bassin : étude de la décomposition des litières en zone hyporhéique par les hyphomycètes aquatiques.*

Title: Plant detritus breakdown in the hyporheic zone of headwater streams: importance of fungal decomposers

Abstract: In headwater streams, leaf litter decomposition constitutes a key ecosystem-level process. The objective of this thesis was to characterize leaf decomposition in the hyporheic habitat of streams, quantify the associated dynamics of fungal and invertebrate decomposers together with the effect of abiotic factors, and incorporate this process into a stream ecosystem perspective. Taken as a whole, these findings suggest that the functioning of woodland stream ecosystems, largely based on trophic relationships and carbon dynamics related to leaf decomposition occurring at the sediment surface, should be reconsidered with the incorporation of the vertical dimension conceptualized by “hyporheologists”. These results lead to think out the dynamics and role of aquatic hyphomycetes at a broader scale than the stream water column alone, and highlight the crucial function of the hyporheic zone, which up to now remained mostly neglected to this regard.

Keywords: headwater streams, litter breakdown, hyporheic zone, aquatic hyphomycetes, organic matter, shredders, abiotic constraints, river sediment

Auteur: Julien Cornut

Titre: Décomposition de détritus végétaux dans la zone hyporhéique de cours d'eau forestiers: implication du compartiment fongique

Directeurs de thèse: Eric Chauvet et Arnaud Elger

Lieu et date de soutenance: Toulouse, le 26 novembre 2010

Résumé : Dans les cours d’eau forestiers, la décomposition des litières végétales constitue un processus écologique fondamental. Ce processus sous l’effet des contraintes abiotiques de la zone hyporhéique (ZH) de ces cours d’eau, ainsi que la quantification de la dynamique des invertébrés et des champignons décomposeurs associés ont été étudiés, le tout inséré dans une vision intégrative à l’échelle de l’écosystème. Les résultats suggèrent de réexaminer le fonctionnement écologique de ces cours d’eau, uniquement basé sur la compréhension des relations trophiques et aux flux de carbone liés à la décomposition des litières situé à la surface du sédiment, en y intégrant la dimension verticale conceptualisée par les “hyporhéologistes”. Ces résultats conduisent à repenser la dynamique et le rôle des hyphomycètes aquatiques à une échelle plus large que celle de la colonne d’eau uniquement, et mettent en évidence l’importance de la ZH qui à cet égard restait jusqu’à présent en grande partie négligée.

Mots-clés: cours d’eau forestier, décomposition des litières, zone hyporhéique, hyphomycètes aquatiques, matière organique, invertébrés déchiqueteurs, contraintes abiotiques, sédiment

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Discipline : Ecologie fonctionnelle

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