Leaf litter breakdown budgets in streams of various trophic status: effects of dissolved inorganic nutrients on microorganisms and invertebrates

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SUMMARY

1. We investigated the effect of trophic status on the organic matter budget in freshwater ecosystems. During leaf litter breakdown, the relative contribution of the functional groups and the quantity/quality of organic matter available to higher trophic levels are expected to be modified by the anthropogenic release of nutrients.

2. Carbon budgets were established during the breakdown of alder leaves enclosed in coarse mesh bags and submerged in six streams: two oligotrophic, one mesotrophic, two eutrophic and one hypertrophic streams. Nitrate concentrations were 4.5–6.7 mg L^{-1} and the trophic status of each stream was defined by the soluble reactive phosphorus concentration ranging from 3.4 (oligotrophic) to 89 μg L^{-1} (hypertrophic). An ammonium gradient paralleled the phosphate gradient with mean concentrations ranging from 1.4 to 560 μg L^{-1} NH_4-N. The corresponding unionised ammonia concentrations ranged from 0.08 to 19 μg L^{-1} NH_3-N over the six streams.

3. The dominant shredder taxa were different in the oligo-, meso- and eutrophic streams. No shredders were observed in the hypertrophic stream. These changes may be accounted for by the gradual increase in the concentration of ammonia over the six streams. The shredder biomass dramatically decreased in eu- and hypertrophic streams compared with oligo- and mesotrophic.

4. Fungal biomass increased threefold from the most oligotrophic to the less eutrophic stream and decreased in the most eutrophic and the hypertrophic. Bacterial biomass increased twofold from the most oligotrophic to the hypertrophic stream. Along the trophic gradient, the microbial CO_2 production followed that of microbial biomass whereas the microbial fine particulate organic matter and net dissolved organic carbon (DOC) did not consistently vary. These results indicate that the microorganisms utilised the substrate and the DOC differently in streams of various trophic statuses.

5. In streams receiving various anthropogenic inputs, the relative contribution of the functional groups to leaf mass loss varied extensively as a result of stimulation and the deleterious effects of dissolved inorganic compounds. The quality/quantity of the organic matter produced by microorganisms slightly varied, as they use DOC from

stream water instead of the substrate they decompose in streams of higher trophic status.

Keywords: budget, invertebrates, microorganisms, organic matter, trophic status

Introduction

Leaf litter represents an important amount of the allochthonous inputs entering heterotrophic streams running through mountain forests or covered by riparian woods (Webster & Benfield, 1986; Webster, Wallace & Benfield, 1995). Both aquatic hyphomycetes and bacteria colonise leaves, and fungal contribution to leaf mass loss may be as high as 50% (Baldy, Gessner & Chauvet, 1995; Weyers & Suberkropp, 1996; Baldy *et al*., 2002; Findlay *et al*., 2002; Gulis & Suberkropp, 2003a,b). At the end of the decomposition, the bacteria balance fungi by increasing their number, while fungal biomass decreases (Baldy *et al*., 1995, 2002). These microorganisms process coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) and dissolved organic carbon (DOC) through exoenzyme activity. The accumulation of microbial biomass of high nutritive value enhances the palatability of leaf detritus for shredders, which in turn convert leaf litter into FPOM (Petersen & Cummins, 1974; Suberkropp, Arsuffi & Anderson, 1983; Covich, Palmer & Crowl, 1999). Other invertebrates (e.g. filtering and gathering collectors) feed on the organic matter transformed in this way and are prey to numerous invertebrate and vertebrate predators (Merritt & Cummins, 1996). One way to describe leaf litter processing is to construct carbon budgets at various breakdown stages (Gessner, Suberkropp & Chauvet, 1997) so as to follow the changes in the quality and quantity of organic matter. Such a quantitative and dynamic approach contributes to a better understanding of how lotic ecosystems function by linking biodiversity and function.

Anthropogenic release of nutrients into aquatic systems changes their natural trophic status. In lowland streams, runoff from agricultural fields and urban lawns causes an increase in nitrate and phosphate concentrations which vary over a broad range. As a consequence of increased dissolved nutrient concentration, microbial respiration and biomass increase (Gulis & Suberkropp, 2003a; Stelzer, Heffernan & Likens, 2003; Pascoal & Cassio, 2004). Conversely, the effluents of sewage treatment plants, which contain toxic substances such as unionised ammonia and nitrite, represent an important point-source of pollution. Both compounds have shown deleterious effects on macroinvertebrates upon short (Williams, Green & Pascoe, 1986; Maltby, 1995; Alonso & Camargo, 2006) and prolonged (Berenzen, Schultz & Liess, 2001) exposure, depending on their concentrations. Overall, changes in trophic status because of human activities could enhance or slow down leaf litter breakdown in streams, depending on the balance between nutrient-dependence and the effect of toxic substances.

Carbon budgets have been constructed during leaf litter breakdown in (sub) natural low-order streams (Baldy & Gessner, 1997; Hieber & Gessner, 2002). However, no organic matter budget involving fungi, bacteria and invertebrates has so far been established to track the effect of anthropogenic nutrient release in lotic ecosystems. In coastal regions, it has been shown that human-induced terrestrial inputs can have an impact on the annual global carbon budget (Smith & Hollibaugh, 1993). In freshwaters, it can be hypothesised that, during leaf litter breakdown, the quantity and quality of organic matter available to higher trophic status should be modified by the anthropogenic release of nutrients.

The objective of our study is to determine the effects of trophic status on the organic matter budget during leaf litter breakdown in nitrogen-enriched streams along a gradient of soluble reactive phosphorus (SRP) concentration. We specifically attempted to answer two questions: (i) what is the relative contribution of the functional groups involved in the flow of organic matter? and (ii) what are the quality and quantity of organic matter available for the aquatic food web during the breakdown process?

Methods

Study sites

The six second to fourth order streams (Lemboulas, Lère, Lupte, Seye, Tauge and Tescou) are located in

south-western France between Cahors, Montauban and Gaillac. The yearly rainfall ranges from 700 to 800 mm. The physical characteristics of the streams were previously described in detail (Lecerf *et al.*, 2006). Briefly, elevation and discharge range from 100 to 300 m.a.s.l. and 47–820 L s^{-1}, respectively. The dominant substrates are boulders and pebbles in river Seye or gravel and sand in the other five rivers. The bedrock is mainly composed of limestone and clay and the streams flow through an intensively farmed landscape. Dense riparian vegetation consisting primarily of alder (*Alnus glutinosa* (L.) Gaertn.), oak (*Quercus robur* L.) and ash (*Fraxinus excelsior* L.) covers the stream bed.

Stream water analyses

During the study, temperature was recorded using two data loggers (SmartButton, ACR Systems, Pelham, AL, U.S.A.) in each stream. On each sampling date, the pH (pH 320, WTW GmbH, Weilheim, Germany), oxygen saturation (Oxi 330i, WTW GmbH) and conductivity (HI 98311, Hanna instruments, Woonsocket, RI, U.S.A.) were measured. Water samples were collected for alkalinity determination by titration at pH 4.5 ± 0.05 (precision: 2%). An aliquot of water was filtered in the field on a GF/F glass fibre filter (Whatman International, Florham Park, NJ, U.S.A.; retention 0.7 μm) for SRP determination (Motomizu, Wakimoto & Tôei, 1983; precision: 5%). Nitrate concentration was measured by UV spectral deconvolution (Thomas *et al.*, 1993) using an Anthelie 70MI (Secomam, Ales, France; precision: 2%). Ammonium determination was carried out by the indophenol blue method as already described (APHA, 1998; precision: 5%). At each sampling date, the fraction of unionised ammonia was determined according to the water pH and temperature from data previously reported by Emerson *et al.* (1975). The values varied from 0.0245 (Tauge at T28: pH 8.29, temperature 3.9 °C) to 0.0603 (Seye at T0: pH 8.50, temperature 11.1 °C). This fraction was then multiplied by the NH_4-N concentration to obtain the corresponding NH_3-N concentration. An aliquot of 0.45-μm filtered water was acidified and stored at 4 °C before DOC determination on a TOC-VCSH analyser (Shimazu, Osaka, Japan; precision: 2%).

Experimental field protocol

Organic matter budgets were calculated from alder leaf breakdown experiments conducted in December 2003. Freshly fallen leaves were collected in the riparian zone and air-dried for 2 weeks at 19–22 °C. One hundred and sixty-eight leaf packs of 5 ± 0.05 g were weighed and then sprayed with deionised water to prevent break-up when they were introduced into 10-mm mesh bags. Four leaf bags were anchored to an iron bar driven into the stream bed. After 7, 14, 21 and 28 days, four replicate leaf bags were retrieved at random from the seven iron bars installed in each stream and transferred into zip-lock plastic bags with about 100 mL of stream water. At T0 (before submersion), four leaf litter samples were humidified with stream water, and leaf discs were cut for processing as described below.

Leaf bag processing

In the laboratory, leaves were gently rinsed with demineralised water over a 350-μm mesh sieve to remove sediment and exogenous organic matter. The macroinvertebrates collected on the sieve were preserved in 70% ethanol until identified and counted. From each replicate bag, leaf discs were rapidly cut with a 1-cm diameter cork borer in at least five leaves: (i) five discs were stored in 10 mL of a 2% formaldehyde solution at 4 °C for bacteria counts; (ii) five discs were stored at −20 °C in a zip-lock plastic bag until ergosterol determination and (iii) ten discs were used for incubation in microcosms and five to measure oxygen consumption. The rest of the leaves were then dried at 105 °C for 3 days and weighed to the nearest 0.01 g. Portions of about 500 mg of ground sample were ashed at 550 °C for 4 h and weighed to determine the organic matter content (ash-free dry mass, AFDM).

The logarithm of leaf litter AFDM was plotted versus cumulated temperature in degrees Celsius-day (Petersen & Cummins, 1974). The exponential breakdown rate, k, was determined from linear least-squares fitting.

Macroinvertebrate biomass

Taxa were identified to the lowest practicable taxonomic level (mostly species or genus). Invertebrates

were assigned to the shredder functional feeding group following Merritt & Cummins (1996) and after gut content analyses of sampled individuals. The biomass of the shredders was determined by weighing dried individuals (105 °C, 24 h) to the nearest 0.01 mg.

Bacterial biomass

The bacterial cells were detached from the leaf discs by a 12.7-mm flat tip ultrasonic probe connected to a Digital Sonifier 250 (Branson Ultrasonics, Danbury, CT, U.S.A.) operated continuously for 1 min at a 50% amplitude (Buesing & Gessner, 2002 and preliminary assays to set the parameters in our experimental conditions). The bacterial suspension was re-suspended by vortexing and, after the homogenate had been allowed to settle for 10 s, a 0.5-mL sub-sample was taken about 5 mm below the surface and mixed with sterile water to a final volume of 5 mL. The bacterial suspension was stained by adding 0.72 μL of a 40 mg L^{-1} 4′,6-diamidino-2-phenylindole, 2 HCl (cat # D-9542, Sigma, St Louis, MI, U.S.A.) for 5 min. Bacterial numbers were determined by epifluorescence microscopy at ×1000 magnification.

Mycelial biomass

Ergosterol was extracted from leaf discs and determined as previously described (Gessner & Schmitt, 1996). Briefly, the leaf discs were lyophilised, weighed and heated in alkaline methanol for 30 min at 80 °C. The extract was purified by solid-phase extraction on an Oasis HLB 30 μm extraction cartridge (cat. # WAT094226, Waters, Milford, MA, U.S.A.). Ergosterol was separated by reversed phase HPLC on C_{18} column and quantified by measuring absorbance at 282 nm.

Fungal reproductive biomass

At each time of sampling, 10 leaf discs were incubated at 10 °C in aerated microcosms (Suberkropp, 1991) with 40 mL of stream water filtered on 0.45 μm pore size cellulose nitrate membrane first washed with 50-mL GF/F filtered stream water to eliminate soluble carbon. A 5 mL aliquot of stream water was taken for DOC determination (see below). After 48 h, a 5 mL aliquot was transferred from the microcosm into a polypropylene vial containing 125 μL 0.5% (w/v) Triton X-100 and immediately mixed before 0.5 mL 35–37% formaldehyde solution was added. Before conidia identification and counting, the samples were filtered on 5-μm pore size cellulose nitrate membrane which was stained with 0.3% Trypan blue in 50% lactic acid solution. Conidial biovolumes were determined as described in Bärlocher & Schweizer, 1983 and conidial masses were calculated from the conidial biovolumes as described in Baldy *et al.* (2002).

Fine particulate organic matter and dissolved organic carbon

After removal of the 5 mL aliquot for fungal reproductive biomass determination (see above), the contents of the microcosm were filtered on a 1 mm-mesh screen to separate the CPOM. Twenty to 25 mL were then filtered on a 0.45-μm pore size 25-mm diameter nitrate cellulose membrane first washed three times with 10-mL pure water, dried at 80 °C and preweighed to the nearest μg. After filtration, the membrane was dried at 80 °C and weighed to determine the FPOM. A 5 mL aliquot of 0.45-μm filtered microcosm water was taken, acidified and stored at 4 °C for DOC determination on a TOC-VCSH analyser (Shimazu).

Leaf-litter associated microbial respiration

Respiration rates associated with decomposing leaf material were inferred from measurements of oxygen consumption by leaf discs. Five leaf discs were incubated at 10 °C in stream water filtered on GF/F saturated with air in 3-mL chambers closed by a 1302 oxygen electrode connected to a microcomputer via a 928 oxygen interface (Strathkelvin Instruments, Motherwell, U.K.). The oxygen consumption of stream water filtered on 0.2-μm pore-size nitrate cellulose membrane was measured as a control that also included electrode consumption. The slope of oxygen consumption was calculated on a 15–20 min period of monotonous decrease and the control value was subtracted from each sample. A respiratory ratio of 1.0 was used to convert the oxygen consumption rate into a carbon dioxide production rate.

Carbon budget

Mycelial biomass was estimated from the ergosterol content by multiplying by 182 (Gessner & Chauvet,

1993; Charcosset & Chauvet, 2001). A conversion factor of 0.5 was used to convert leaf AFDM, shredder biomass, mycelial biomass and FPOM into carbon (FPOC). The conversion to carbon of conidial volumes was obtained using a density of 250 fg carbon μm^{-3}. A mean value of 20 fg carbon per bacterial cell was used to estimate the bacterial biomass (Norland, 1993). The relative organism biomass and microbial product proportions were calculated at each date and displayed in Fig. 8.

The relative contribution of the organisms to leaf mass loss was calculated to establish the budget of carbon allocation in each stream. The contribution of shredders to leaf mass loss was assumed to be 10% of the animal body mass consumed per day (Hieber & Gessner, 2002). The contribution of fungi and bacteria was obtained by dividing the biomass by the average growth efficiency (0.35 for fungi: Suberkropp, 1991; 0.3 for bacteria: (del Giorgio & Cole, 1998). At each date, shredder contribution, FPOC, DOC and CO_2 production were integrated and these components were cumulated at T28 while fungal and bacterial contribution to the leaf mass loss was estimated from the respective carbon mass at this date and reported in Table 4.

Data analysis

Differences between exponential breakdown rates in the six streams were assessed by analysis of covariance (ANCOVA) followed by Tukey's test for *post hoc* pairwise comparisons, as the overall differences were significant. Two-way (stream × time) analysis of variance (ANOVA) was used to test for differences in organisms or microbial products between streams and for interaction between variables. Tukey's tests were carried out after one-way ANOVA (stream) for *post hoc* pairwise comparisons between streams. The difference between the two trophic status groups in each compartment was tested by one-way ANOVA. For these analyses, values at T0 (before submersion) were excluded. Pearson's correlation analysis was used to relate the different biotic and abiotic parameters studied. Test assumptions were verified on either raw data or natural log-transformed data before analysis.

The ammonium and SRP concentrations as well as the ammonia and ammonium concentrations were highly correlated [regression equations: $(NH_4\text{-N}) = 6.62\ (SRP) - 49.4$, $R = 0.975$, $P = 0.0009$ and $(NH_3\text{-N}) = 0.0349\ (NH_4\text{-N}) - 0.498$, $R = 0.988$, $P = 0.0006$, respectively). The difference between the two Pearson's coefficients was not significant ($P = 0.66$) and the dissociation of the effects of these independent variables on dependent variables was not possible in further general comparisons.

ANCOVA and ANOVA were performed using STATISTICA 6.0 software (Statistica for Windows, Edition 98, StatSoft, Maisons-Alfort, France) and Pearson's correlation analyses were carried out using Minitab (release 1.3 for Windows 2000, Minitab SARL, Paris, France). Differences were considered significant when $P < 0.05$.

Results

Physical and chemical characteristics of stream water

Because of the similar environmental conditions prevailing in the area of the study, mean stream water temperatures were similar among streams during the breakdown experiment (6.9–8.9 °C, Table 1). The oxygen saturation measured during the day was close to 100% in all streams as expected from the shade effects of the riparian forest which precluded the growth of aquatic macrophytes and limited that of phytoplankton (chlorophyll-*a* concentration varied from 6.8 $\mu g\ L^{-1}$ in the MsTES to 22 $\mu g\ L^{-1}$ in the HsTAU during 2002; Lecerf *et al.*, 2006). Conductivity was high and the high level of alkalinity resulted in a pH around 8.4. All sites showed high NO_3-N concentrations (4.5–6.7 $mg\ L^{-1}$) which were not limiting for growth of microorganisms (Ferreira, Gulis & Graça, 2006) and aquatic vegetation productivity (Newbold & Palmer, 1979). The phosphorus concentration determined the trophic status with reference to total *P*-value thresholds published by Newbold & Palmer (1979) and OECD (Vollenweider & Kerekes, 1980). Considering that the SRP represented 50–60% of the total phosphorus (unpublished data), the six streams were assigned to four trophic statuses along the SRP gradient, from oligotrophic (OsSEY) to hypertrophic (HsTAU) (Table 1). Ammonium and ammonia gradients paralleled the SRP gradient with mean NH_4-N concentrations ranging from 1.4 (OsSEY) to 560 $\mu g\ L^{-1}$ (HsTAU) and mean NH_3-N concentrations ranging from 0.08 (OsSEY) to 19 $\mu g\ L^{-1}$ (HsTAU). Finally, the carbon budgets were compared between two groups of

Table 1 Physical and chemical characteristics of stream water. Streams are classified according to their trophic status based on the mean soluble reactive phosphorus (SRP) concentration (cf. 'Methods' section). NH_3-N concentration is calculated from the fraction of unionised ammonia determined as a function of water pH and temperature (cf. 'Methods' section). The acronym includes the information on the trophic status (e.g. 'OsSEY' means 'oligotrophic stream Seye'). Mean (SD), $n = 4$.

Stream	Trophic status	Acronym	Latitude E	Longitude N	Temperature (°C)	O_2 saturation (%)	Conductivity at 25 °C (μS cm^{-1})
Seye	Oligo	OsSEY	44°15′04″	1°52′01″	8.9 (1.3)	102 (5)	639 (11)
Lemboulas	Oligo	OsLEM	44°16′17″	1°28′02″	7.9 (1.8)	98 (3)	633 (17)
Tescou	Meso	MsTES	44°54′41″	1°45′45″	7.5 (2.3)	97 (2)	616 (37)
Lère	Eu	EsLER	44°09′15″	1°31′29″	8.8 (1.5)	102 (2)	620 (7)
Lupte	Eu	EsLUP	44°15′34″	1°21′48″	7.6 (1.4)	95 (3)	692 (1)
Tauge	Hyper	HsTAU	44°03′07″	1°26′43″	6.9 (2.2)	83 (5)	734 (90)

Acronym	pH	Alkalinity (mg $CaCO_3$ L^{-1})	NO_3-N (mg L^{-1})	SRP (μg L^{-1})	NH_4-N (μg L^{-1})	NH_3-N (μg L^{-1})
OsSEY	8.49 (0.04)	214 (6)	6.7 (1.0)	3.4 (0.9)	1.4 (1.1)	0.08 (0.07)
OsLEM	8.39 (0.02)	227 (28)	6.4 (0.4)	4.7 (2.2)	9.2 (4.6)	0.41 (0.24)
MsTES	8.39 (0.02)	389 (49)	5.3 (0.1)	7.4 (2.6)	14 (8)	0.64 (0.40)
EsLER	8.33 (0.05)	148 (4)	5.0 (0.4)	26 (4)	22 (10)	0.92 (0.47)
EsLUP	8.45 (0.02)	275 (14)	6.3 (0.8)	40 (18)	198 (80)	9.8 (4.1)
HsTAU	8.31 (0.03)	318 (2)	4.5 (1.2)	89 (38)	560 (53)	19 (3)

trophic status according to the SRP concentration: the OsSEY, OsLEM and MsTES were grouped as 'low trophic status' (LTS) and the EsLER, EsLUP and HsTAU were grouped as 'high trophic status' (HTS).

Litter breakdown

Leaf mass loss in OsSEY was 2.7- to 5.9-fold faster in the OsSEY compared with the five other streams (Fig. 1 and Table 2). Breakdown rates differed significantly between streams (ANCOVA, $F_{5,113} = 35.01$, $P < 0.0001$), but *post hoc* pairwise comparisons indicated that only the breakdown rate in the OsSEY differed from that in the other streams (Table 2). No significant relationship between the breakdown rate and each of the chemical parameters measured was found.

Shredder invertebrates

In the OsSEY, shredder density peaked at T7 and declined until T28 as leaf-limb progressively disappeared, whereas an unexpectedly low density of shredders was found in the other oligotrophic stream LEM (Fig. 2). The pattern in the MsTES was similar to that observed in the OsSEY with lower densities, except at T28. In the EsLER and HsTAU, almost no shredders were found in the litter bags, while the leaves displayed large areas of leaf-limb throughout the study. A shift of the dominant shredder taxa was

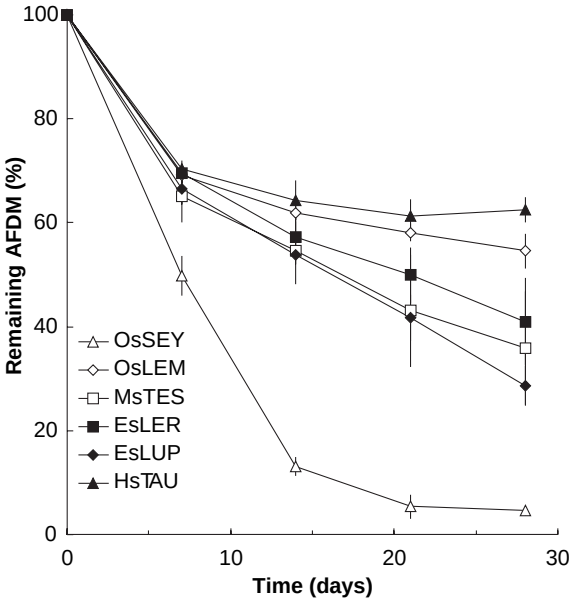

Fig. 1 Time course of the mean percentage of ash-free dry mass (AFDM) remaining in streams of various trophic statuses. Streams are ordered along the soluble reactive phosphorus (SRP) gradient from oligotrophic (OsSEY) to hypertrophic (HsTAU). Symbol meaning in the figure. Bar: SD ($n = 4$).

observed from *Gammarus* (77% of the shredder invertebrates collected over the 4 weeks) in the OsSEY to *Echinogammarus* in the OsLEM and MsTES (86% and 98%, respectively), and sF Limnephilinae in the EsLER (100%). In the EsLUP, only two individuals of

Table 2 Breakdown rates k of alder leaf litter in streams of various trophic status. Different letters mean significant difference in a pair of streams (*ancova* and Tukey's test).

	k (degree day^{-1})	R^2	P-value
OsSEY	0.0124 a	0.956	0.004
OsLEM	0.0025 b	0.876	0.020
MsTES	0.0043 b	0.988	0.001
EsLER	0.0028 b	0.977	0.002
EsLUP	0.0046 b	0.986	0.001
HsTAU	0.0021 b	0.767	0.051

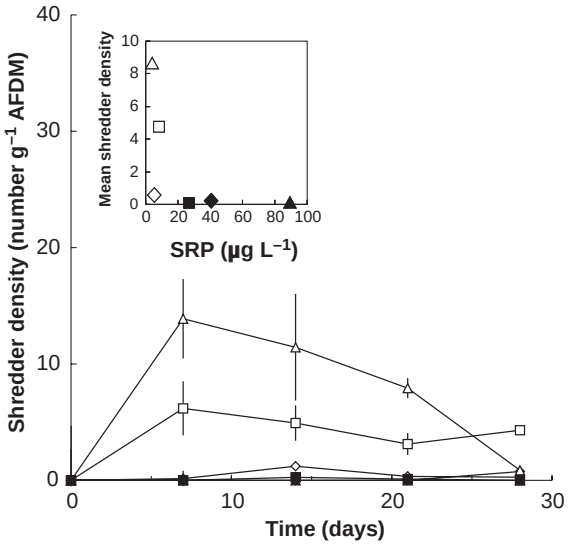

Fig. 2 Shredders density in leaf litter packs submerged in streams of various trophic statuses (same symbols as in Fig. 1; bar: SD, $n = 4$). Insert: mean shredder density (mean of the four sampling dates in each stream) versus soluble reactive phosphorus (SRP) concentration.

Echinogammarus berilloni and two others belonging to the sub-family Limnephilinae were identified at T28 and counted as shredders whereas 10s of *Asellus aquaticus* L. individuals were counted per gram of litter at each sampling date. The gut contents of the *A. aquaticus* that were examined were mainly composed of FPOM and mineral material but no coarse particulate matter was found and this species was not considered as a shredder in this stream. Overall, in the six streams, the leaf mass loss was positively correlated with the shredder density ($R = 0.294$, $P = 0.004$).

Mycelial biomass

The ergosterol content in the leaf litter increased almost linearly and significantly with time in the six streams (Fig. 3; ANOVA, $F_{3,72} = 68.48$, $P < 0.0001$). The mean ergosterol content at the four dates of submersion (T7–T28) in each stream increased along the SRP gradient (insert to Fig. 3) from the OsSEY (76.8 µg g^{-1}) to the EsLER (258 µg g^{-1}) and then decreased to the HsTAU (158 µg g^{-1}). The mean value was significantly different between streams (ANOVA, $F_{5,72} = 25.19$, $P < 0.0001$), the one of the OsSEY being significant from the other five streams and that of the EsLER being significant from that of the HsTAU (ANOVA, $F_{5,90} = 6.94$, $P < 0.0001$ and Tukey's tests). The ergosterol dynamics differed between streams as the effect of each stream depended on the time (ANOVA, stream × time, $F_{15,72} = 3.30$, $P = 0.0003$) but no clear pattern could be observed.

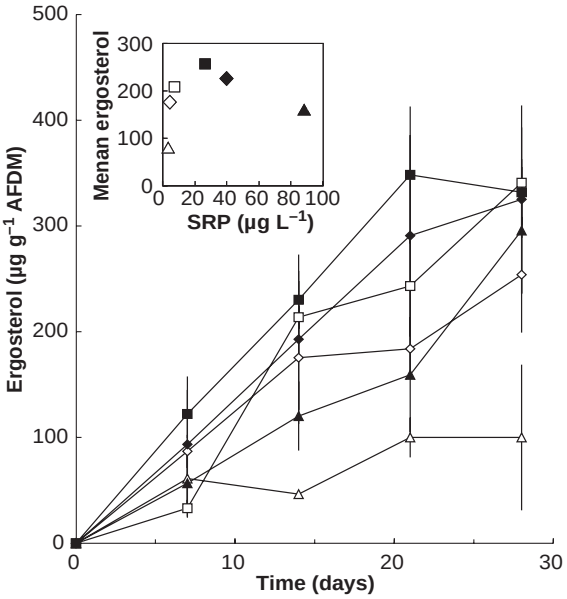

Fig. 3 Ergosterol content in leaf litter from streams of various trophic statuses (same symbols as in Fig. 1; bar: SD, $n = 4$). Insert: mean ergosterol content (mean of the four sampling dates in each stream) versus soluble reactive phosphorus (SRP) concentration.

Bacterial abundance

The numbers of bacterial cells dislodged from the leaf litters increased with time in the six streams (Fig. 4). The mean bacterial cell number increased along the trophic gradient to reach a plateau in the EsLUP and the HsTAU (insert to Fig. 4; ANOVA, $F_{5,72} = 8.4$, $P < 0.0001$).

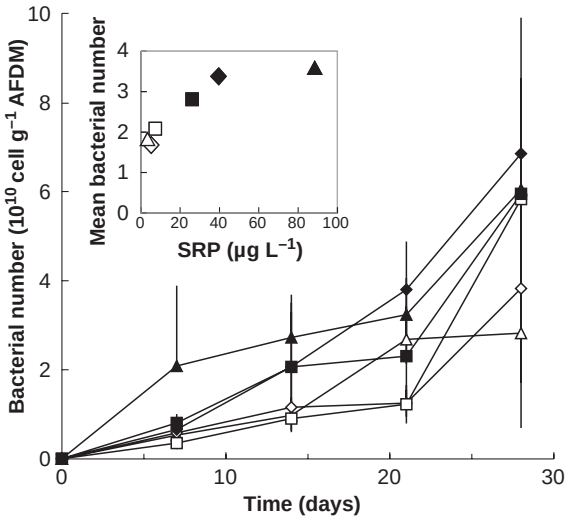

Fig. 4 Bacterial numbers in leaf litter from streams of various trophic statuses (same symbols as in Fig. 1; bar: SD, $n = 4$). Insert: mean bacterial number (mean of the four sampling dates in each stream) versus soluble reactive phosphorus (SRP) concentration.

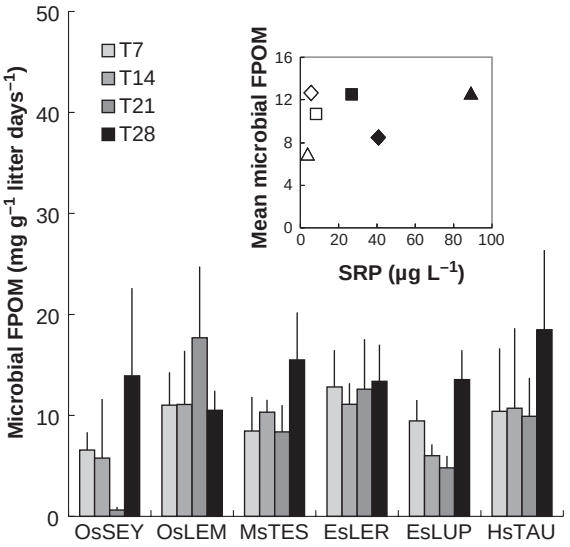

Fig. 5 Microbial fine particulate organic matter (FPOM) produced from leaf litter discs incubated in microcosms with water from streams of various trophic statuses in four sequential 7-day intervals (same symbols as in Fig. 1; bar: SD, $n = 4$). Insert: mean FPOM (mean of the four sampling dates in each stream) versus soluble reactive phosphorus (SRP) concentration.

Microbial fine particulate organic matter

Release of FPOM by microbial activity varied from 4.8 to 18 mg g^{-1} leaf litter day^{-1} between streams and dates except in the OsSEY where the production was exceptionally low (0.63 mg g^{-1} litter day^{-1}) at T21 (Fig. 5). Although the mean FPOM production was relatively constant (around 11 mg g^{-1}litter day^{-1}) in the various streams, differences were significant (insert to Fig. 5; ANOVA, $F_{5,72} = 4.83$, $P = 0.0007$). This production depended on the time (stream × time, $F_{15,72} = 1.96$, $P = 0.03$) but varied in a non-consistent fashion with time in the streams.

The FPOM included the conidia produced by the aquatic hyphomycetes in the microcosms. The fungal reproductive biomass (conidial production) contributed from 0.03% (OsSEY, T7) to 25% (OsSEY, T21) of the FPOM but if we except the last extreme value, the mean percentage was 2.3 ± 2.0% (mean ± SD). The maximal production was observed at T21 in all streams except the OsLEM and EsLER, in which it was observed at T28 and T14, respectively.

Microbial dissolved organic carbon

The DOC concentration was quite stable over time in all streams except the OsSEY (Table 3). The values reported in Fig. 6 correspond to the net production/consumption of DOC in the water during the 48-h incubation in the microcosms, i.e. corrected by the initial DOC concentration in the stream water at the beginning of the experiment. The negative values observed at T0 in all streams and at T28 in the OsSEY mean that the consumption of DOC by the litter-associated microorganisms was higher than its production. During a breakdown, the mean net DOC differed between streams (ANOVA, $F_{5,72} = 11.59$, $P < 0.0001$) and decreased over the four dates (ANOVA, $F_{3,72} = 77.77$, $P < 0.0001$). The temporal dynamics was significantly different between streams (ANOVA, stream × time, $F_{15,72} = 3.23$, $P = 0.0004$) but most probably because of the DOC production in the OsSEY. Indeed, the low mean value in the OsSEY which represents 25–35% of that observed in the other streams (insert to Fig. 6) is mainly because of the negative value observed at T28. The mean net DOC

Table 3 Dissolved organic carbon concentration (mg L^{-1}) in the stream water ($n = 1$)

Stream	T0	T7	T14	T21	T28
OsSEY	3.7	1.5	2.3	3.0	4.3
OsLEM	2.8	2.7	2.9	2.6	3.0
MsTES	4.4	4.4	4.4	4.5	4.1
EsLER	2.1	2.0	2.0	2.0	2.5
EsLUP	2.7	2.6	2.8	2.7	2.8
HsTAU	3.9	3.6	3.4	4.6	3.7

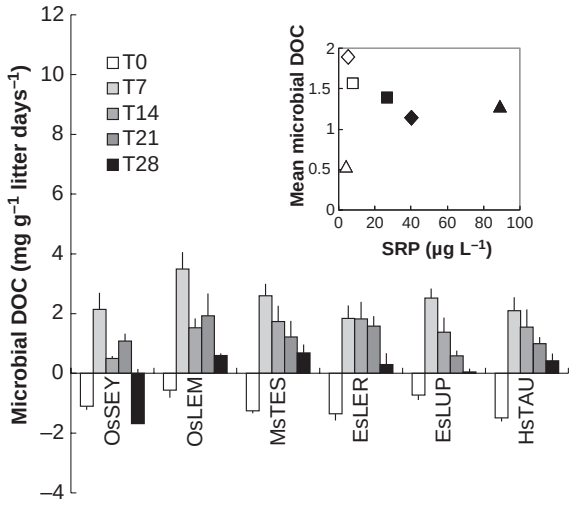

Fig. 6 Microbial dissolved organic carbon (DOC) produced from leaf litter discs incubated in microcosms with water from streams of various trophic statuses in four sequential 7-day intervals and on day 0 (same symbols as in Fig. 1; bar: SD, $n = 4$). Net DOC values (see text). Insert: mean DOC production (mean of four sampling dates, T7–T28, in each stream) versus soluble reactive phosphorus (SRP) concentration.

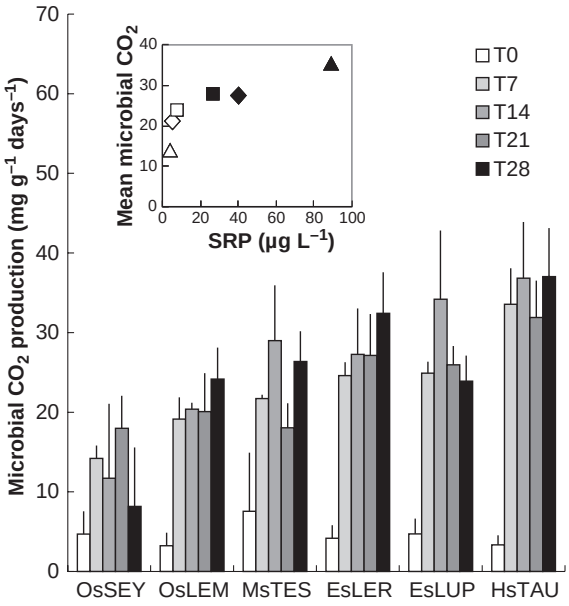

Fig. 7 Microbial carbon dioxide production from leaf litter discs incubated in closed chambers with water from streams of various trophic statuses in four sequential 7-day intervals and on day 0. Bar: SD ($n = 4$). Insert: mean carbon dioxide production (mean of four sampling dates, T7–T28, in each stream) versus soluble reactive phosphorus (SRP) concentration.

production in the other streams decreased from the OsLEM to the HsTAU (ANOVA, $F_{4,60} = 8.05$, $P < 0.0001$).

Microbial carbon dioxide

The carbon dioxide production of leaf litter submerged in streams inferred from oxygen uptake values varied from 9.5 (OsSEY, T28) to 37 mg g^{-1} leaf litter day^{-1} (HsTAU, T28) (Fig. 7). At T0, the production was low, varying from 4.5 (OsLEM) to 7.6 mg g^{-1} leaf litter day^{-1} (MsTES). From the OsSEY to the MsTES, mean carbon dioxide production almost doubled, from 13 to 24 mg g^{-1} leaf litter day^{-1}, whereas the values for EsLER, EsLUP and HsTAU were more similar, i.e. ranging from 27 to 35 mg g^{-1} leaf litter day^{-1} (insert to Fig. 7; sampling dates T7–T28, ANOVA, $F_{5,72} = 64.37$, $P < 0.0001$). The carbon dioxide production also depended on time (stream × time, $F_{15,72} = 35.06$, $P = 0.01$; no significant effect of time) in a non-consistent fashion.

Budget of carbon allocation

Biomasses of organisms and microbial products increased during the first 2 weeks to peak at about 12% and 17% of the initial leaf carbon in the LTS and HTS groups, respectively (Fig. 8). As observed with biomass, the shredder contribution to leaf breakdown dramatically decreased in the HTS group (ANOVA, $F_{1,94} = 24.50$, $P = 0.0001$) (Table 4). It should be noted that the sum of the percentages (compartment carbon mass/litter carbon mass lost at 28 days × 100) did not reach 100% in the LTS group because the carbon dioxide produced by the invertebrates was not measured. The contribution of the microbial DOC remained unchanged in the HTS compared with the LTS group (ANOVA, $F_{1,94} = 0.063$, $P = 0.80$). The microbial FPOC 1.5-fold increase in the HTS group was not significant (ANOVA, $F_{1,94} = 0.95$, $P = 0.33$). The microbial carbon dioxide production in the HTS group was twice that in the LTS group and the contribution of the microorganisms increased to the same extent (Table 4; ANOVA: microbial CO_2, $F_{1,94} = 59.47$, $P < 0.0001$; mycelial biomass, $F_{1,94} = 7.81$, $P = 0.006$; bacterial biomass, $F_{1,94} = 13.68$, $P = 0.0004$). In both groups, the bacterial contribution represented only about 1.5% of the microbial contribution (Table 4).

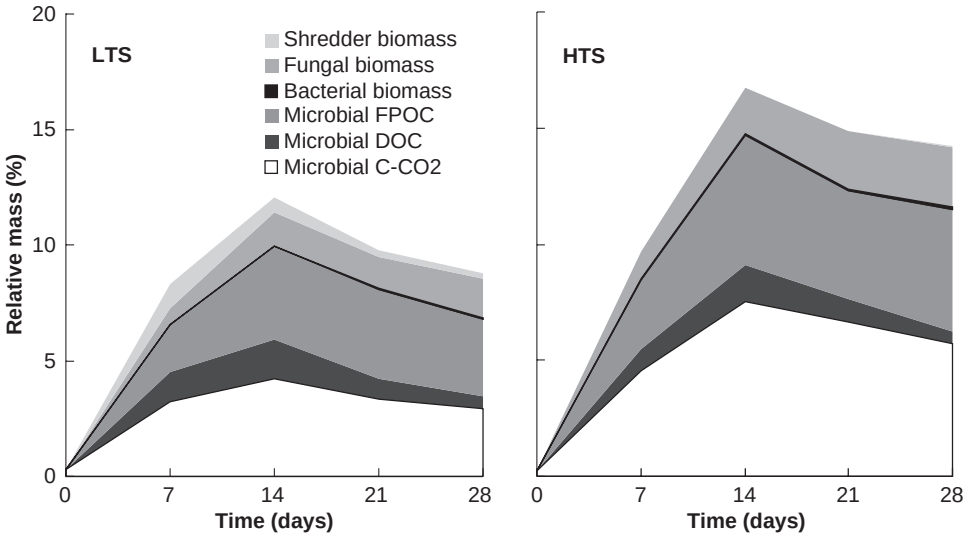

Fig. 8 Changes in the relative organism biomass and microbial product proportions during decomposition in the low trophic status (LTS) and high trophic status (HTS) groups. Areas corresponding to the compartments are labelled in the figure. Values are expressed as percentage of initial leaf carbon.

Table 4 Relative contribution of the different carbon products to leaf mass loss (percentage of initial leaf carbon) in the two trophic status groups after 28 days of alder leaf breakdown. The conidial mass is included in the microbial FPOC.

Product	LTS	HTS
Shredder	2.0	0.03
Fungi	7.8	15
Bacteria	0.11	0.24
Microbial FPOC	24	37
Microbial DOC	7.9	7.6
Microbial CO_2	25	50
Sum	67	110

FPOC, fine particulate organic carbon; DOC, dissolved organic carbon; LTS, low trophic status; HTS, high trophic status.

Discussion

Leaf litter breakdown rates and trophic status

The breakdown rate of alder litter was not related to the trophic status defined by SRP concentration in our streams whereas it could be fitted reasonably with linear or Michaelis–Menten models as a function of this parameter in the study of Gulis, Ferreira & Graça (2006). The comparison between our study and that of Lecerf *et al.* (2006) carried out 1 year before using identical experimental procedure shows that the breakdown rates in the OsSEY, EsLUP and HsTAU remain comparable, whereas the values in the OsLEM and MsTES are only 17% and 40%, respectively, of those reported previously. The concentration of ammonium and nitrite explained most of the overall variation in the breakdown rate across nine streams (Lecerf *et al.*, 2006), but the small differences in ammonia concentrations between years do not explain the large differences in breakdown rates in the OsLEM and MsTES. The diversity of shredders, although limited, was similar between years, although the densities cannot be compared because Lecerf *et al.* (2006) collected benthic macroinvertebrates whereas we collected those associated with litter bags. Nitrite also has a toxic effect on invertebrates (Alonso & Camargo, 2006) but, unfortunately, we did not determine nitrite concentrations in the present study. Nitrite and other substances may be responsible for the decreased density of shredders in the OsLEM and MsTES which is correlated with the low breakdown rates observed in the present study.

Relative contribution of the functional groups

The shredder contribution to the organic matter transformation differed dramatically between streams with low and high trophic status, as almost no shredders were associated with the leaf bags in the streams of the HTS group (Table 4). Beside the trophic status, other factors, such as differences in habitats, current velocity and predation in the various streams could also have an effect on the shredder community.

In the EsLUP, the presence of several individuals of *A. aquaticus* that were not feeding as shredders in the litter bags indicates that a change in the functional feeding groups occurred in this stream of high trophic status. In addition, *A. aquaticus* may have taken advantage of the increased litter quality linked to the growth of microorganisms during the 4 weeks (Graça, Maltby & Calow, 1993b).

Aquatic fungi represented about 98.5% of the microbial contribution to the leaf mass loss (Table 4). This group of organisms has been shown to be sensitive to nutrient enrichment (Pascoal & Cassio, 2004; Gulis *et al*., 2006; Ferreira *et al*., 2006). Meanwhile, the significant increase in bacterial numbers in the HTS group indicates that the growth of bacteria associated with leaf litter was also stimulated by increased nutrient concentration. Similarly, increased ammonium, nitrate and phosphorus in polluted sites enhanced bacterial production in a large river (Pascoal & Cassio, 2004). The microbial activity was measured in winter and can be expected to increase in spring and summer when temperature levels rise. During these seasons, increased decomposition in nutrient-enriched streams would contribute to litter disappearance in streams of the highest trophic status where the growth of microorganisms is stimulated. However, their efficiency to degrade plant polymers in these streams may be limited, as they can use DOC from sources other than decomposing litter (see below).

Quality and quantity of organic matter available

At T0 (before submersion in the streams), the net DOC consumption and the corresponding carbon dioxide production strongly suggest that the terrestrial microorganisms associated with leaf litter at the time of submersion (Nikolcheva, Bourque & Bärlocher, 2005) metabolised the stream water DOC that they consumed. The net DOC consumption suggests that these microorganisms assimilated stream water DOC rather than producing DOC by breaking down plant polymers into oligosaccharides and simple sugars. Therefore, their contribution to the leaf mass loss is expected to be very limited.

Upon submersion, carbon dioxide produced by the respiration of aquatic microorganisms was the major microbial product (Table 4). The values fit in with the 40% value determined from sycamore leaves decomposing in a small stream during summer (Findlay & Arsuffi, 1989). Determined in different conditions, these values indicate that a significant proportion of leaf mass loss is accounted for by mineralization of leaf material. The contribution of respiration even exceeded the values reported here, as shredder respiration was not measured. The twofold increase in microbial carbon dioxide production observed in the HTS group was directly associated with the metabolism of DOC by microorganisms.

In both trophic groups of streams, FPOM production (Table 4 and Fig. 5) indicates that the microorganisms, especially aquatic hyphomycetes, were actively degrading plant polymers into oligosaccharides (Suberkropp *et al*., 1983). Both FPOC and DOC account for 32–45% of leaf mass loss whatever the trophic status group. This is higher than the 10–20% estimated from calculations by Hieber & Gessner (2002) who did not measure these compartments in a woodland stream budget. In the OsSEY, whether or not the dramatic decrease in FPOM production at T21 and net DOC consumption at T28 are related to the refractory matter available to the microorganisms remains to be studied. Such limited modifications observed during one breakdown process should not have an effect on the food webs, as one can expect that litter breakdown would supply the stream with decomposition products over the leaf fall period. On the contrary, the variation in the mean DOC production (insert to Fig. 6) and the relatively constant FPOM production (insert to Fig. 5) linked to the increased carbon dioxide production (insert to Fig. 7) over the trophic gradient indicate that modification of water quality induced a modification in DOC production and/or utilization. In streams of higher trophic status, it seems that microorganisms, especially fungi, are using leaf litter as a support rather than a resource.

Impact of water quality on the organisms

The shift in the dominant taxa associated with the litter bags is similar to the data reported by Lecerf *et al*. (2006) in the benthos. The lethal effect of ammonia on invertebrates and its sub-lethal effect on feeding activity (Maltby *et al*., 2002) may have induced this shift. *Asellus aquaticus* which represented 98% of the invertebrates associated with litter bags in the EsLUP is more resistant to ammonia than *G. pulex* (Maltby, 1995). The ratio of mean NH_3-N concentrations in EsLUP and OsSEY was

123, whereas that of total N concentrations was 1. Hence, using total N in the determination of trophic state boundaries (Dodds, 2006) seems to be insufficient, because it does not discriminate between the four major chemical forms of inorganic nitrogen with respect to their effect on the activity and survival of various organisms.

Asellus aquaticus has been considered as a shredder in various natural conditions (Andersson, 1985; Cuppen *et al.*, 1995; Warren & Spencer, 1996). However, *A. aquaticus* feeds by scraping at the leaf surface and consumes only one-tenth the amount of conditioned leaf material compared with *G. pulex* that bites through the leaf material (Graça, Maltby & Calow, 1993a). Our results of the gut content examination of individuals collected in a stream impacted by anthropogenic inputs suggest to consider *A. aquaticus* as a scraper or a gathering-collector rather than a shredder in polluted streams.

The development of the microorganisms was influenced by the trophic status as indicated by the increased respiration rates from OsSEY to HsTAU (insert to Fig. 7). Such an increase of oxygen uptake by microorganisms has been described in a nutrient-enriched stream (Gulis & Suberkropp, 2003b) while increased bacterial production has been observed in polluted sites (Pascoal & Cassio, 2004). Over the trophic gradient of the six streams studied here, bacterial biomass reached a plateau in the EsLUP and HsTAU, whereas fungal biomass significantly decreased in the HsTAU, compared with the maximum in the EsLER. The stimulation of bacterial and fungal growth in streams of low trophic status can be related to the increase in phosphate concentration, the nitrate concentration being non-limiting (Suberkropp, 1998; Ferreira *et al.*, 2006). In streams of higher trophic status, the differential response of bacteria and fungi could be related to the effect of ammonia and other compounds that were not quantified in this study.

Budgets and water quality

Hieber & Gessner (2002) hypothesised that the growth of fungi and bacteria associated with litter could be sustained by carbon derived from sources outside the leaf compartment. Our results indicate that dissolved organic matter can be directly obtained from the stream water by microorganisms decomposing leaf litter. In streams of high trophic status, most of the increase in contribution to leaf mass loss was devoted to fungi and their respiratory activity. A change in these compartments has to be considered in eutrophication models. Besides, the importance of shredder contribution to leaf breakdown appeared to be highly variable among streams and mainly responsible for the differences in breakdown rates. In line with previous reports (Lecerf *et al.*, 2006; Gulis *et al.*, 2006), our study shows that scoring the stream integrity by measuring breakdown coefficients, as proposed by Gessner & Chauvet (2002), cannot be generalised to streams subjected to a mixture of anthropogenic stresses. This is particularly important in lotic ecosystems when modifications in the shredder assemblages integrate the toxic effect of various compounds of which the concentration can vary within wide ranges.

Carbon budgets established in streams receiving various anthropogenic inputs indicated that the relative contributions of the two main functional groups, shredders and microorganisms, largely varied. On the contrary, the quality and the quantity of organic matter produced by microorganisms and made available for higher trophic levels were not consistently modified between the two groups of low and high trophic status as they used DOC from water in streams of higher trophic status. Given the small sample sizes of streams of different trophic status retained here, more studies on lotic systems are needed to broaden these conclusions.

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