Leaf litter recycling in benthic and hyporheic layers in agricultural streams with different types of land use.

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Abstract

Changes in land use and intensification of agriculture pressure have greatly accelerated the alteration of the landscape in most developed countries. These changes may highly disturb the adjacent ecosystems particularly in streams, where pollutions are concentrated. In this study, we used the leaf litter breakdown rate to assess the functional integrity of stream ecosystems and river sediments along either traditional extensive farming or a gradient of vineyard area. At the benthic layer, the total litter breakdown process integrates the temporal variability of the anthropogenic disturbances and is strongly influenced by land use changes in the catchment even though a low concentration of toxics was measured during the study period. This study also confirmed the essential role played by amphipods in the litter breakdown process. In contrast, microbial processes clearly integrated the variations in available nutrients and dissolved oxygen concentrations, but failed to integrate the disturbances induced by wine production during the study period. The response of microbes may not be reliable enough for assessing the global effect of seasonal agricultural practices. Finally, the leaf litter breakdown measured in the hyporheic zone seemed mainly driven by microbial activities and was hence more affected by vertical exchanges with surface water than by land use practices. However, the breakdown rate of leaf litter in the hyporheic zone may constitute a relevant way to evaluate the impact on river functioning of any human activities which induce massive soil erosion and sediment clogging.

Key words: livestock farming, land use, particulate organic matter dynamic, river sediment, aquatic macroinvertebrates, and aquatic hyphomycetes.

Capsule: This study highlights the consequences of land uses on benthic and interstitial leaf litter recycling in streams.

1 Introduction

2 In most developed countries, changes in land use associated to the intensification of
3 agriculture pressure have greatly accelerated the alteration of the landscape (removal of
4 hedgerows, increase in field size, and reduction of habitat diversity) in the last 20-30 years.
5 The contribution of agriculture to the nutrient enrichment in rivers (Meybeck, 1982; Vitousek
6 et al., 1997) or the rising concentration of pollutants (herbicides, pesticides, fungicides)
7 (IFEN, 2007) have been widely considered. Several studies have pointed out changes in
8 assemblage structure and composition for microbial, invertebrate and fish communities
9 following landscape modifications (see a review in Harding et al., 1998; Allan, 2004; Dolédec
10 et al., 2006; Hagen et al., 2006; Pesce et al., 2010), but changes in their role in ecosystem
11 processes were rarely considered (Piscart et al., 2009).

12 In recent years, there has been a growing interest in the use of leaf litter breakdown to
13 assess the functional integrity of stream ecosystems (Gessner and Chauvet, 2002; Lecerf et
14 al., 2006; Piscart et al., 2009). This Particulate Organic Matter (POM) represents the main
15 source of organic carbon and nutrients in low-order streams (Cummins, 1974; Webster and
16 Meyer, 1997). In such streams, leaf litter breakdown is partially controlled by physical factors
17 (e.g. abrasion and fragmentation) but the major part of leaf mass loss is due to the
18 complementary activities of a wide variety of organisms (aquatic fungi, bacteria and shredder
19 invertebrates). Anthropogenic disturbances of river watersheds can severely affect the
20 production and recycling rates of POM, through decreases in quantity and quality (Lecerf and
21 Chauvet, 2008b), changes in microbial activities (Lecerf and Chauvet, 2008a,b; Piscart et al.,
22 2009) and in the density and composition of microbial and invertebrate assemblages (Hagen
23 et al. 2006; Lecerf and Chauvet, 2008a,b; Piscart et al., 2009). In western France, Piscart et al.
24 (2009) showed that agricultural practices can strongly affect the activity of shredders and
25 reduce by 75% litter breakdown rates in farming areas. Physical disturbances of the bed

sediment also have a strong influence on the litter breakdown (Tillman et al., 2003; Marmonier et al., 2010). For example, up to 50% of autumnal allochthonous leaf input were reported by Herbst (1980) to be buried in sediment, and the breakdown rate of this leaf litter is known to be highly reduced due to burial in sediments (Crenshaw and Valett, 2002; Marmonier et al. 2010; Cornut et al., 2010). However, the effects of land use changes on sediment quality are still poorly understood and nothing is known about the consequences of sediment disturbance on the litter breakdown rates in the hyporheic zone.

The originality of our work lies in the combined study of leaf litter breakdown rates in the benthic and hyporheic layers along gradients of agricultural activities in the same watershed: a traditional hedged farming area used as a control gradient (livestock production with low use of pesticides and fertilizers) and a gradient of vineyard area considered as highly disturbed in its downstream part (with regular use of pesticides and heavy metals during the growing period, Pesce et al. 2008). Six sites were selected in two rivers along an up- to downstream gradient of either traditional hedged farming or vineyard areas. We hypothesized that traditional farming areas (1) should have a limited effect on invertebrates, and consequently on the breakdown activity of shredder invertebrates (Piscart et al. 2009, Hladysz et al. 2010). However, the increase in available nutrients along the river should increase the microbial breakdown activity in the benthic layer (Suberkropp and Chauvet, 1995, Gulis et al. 2004, Paul et al. 2006, Hladysz et al. 2010). For the vineyard area, we hypothesized (2) a deleterious effect of pesticides and heavy metals on invertebrates (Schäfer et al. 2007) and fungal assemblages (Solé et al. 2008) and a resulting decrease in breakdown rates in the benthic layer along the gradient of vineyard density (Piscart et al. 2009, Medeiros et al. 2010). For the hyporheic layer, we predicted (3) that the increase in available nutrients along the river in the control area should increase the microbial breakdown activity (Claret et al. 2001). However, in the vineyard area a decrease in the vertical connectivity between the river and the

hyporheic zone, due to tillage and soil erosion in vineyards, river sediment clogging, and accumulation of toxics inside the sediment, may result in a decrease in breakdown rates in the hyporheic zone. Indeed, vertical connectivity controls the movements and the activities of benthic shredders inside sediments (Marmonier et al. 2010; Navel et al. 2010) while pesticides and heavy metals have a toxic effect on fungi and biofilm (Morin et al. 2010).

Material and Methods

Study sites

The study takes/took place in the upstream first-order part of the Ardières River and its first-order tributary, the Morcille River, in the well-known wine area of Beaujolais (France, Fig. 1). The upstream part of the Ardières River consists in a patchy landscape with forests, pastures, and traditional extensive livestock farming (Montuelle et al., unpublished data). The Morcille River flows in a region of intense wine growing (more than 70% of the catchment area is occupied by vineyards). For each river, three different sites were selected with similar riparian vegetation (with at least one forested bank) and instream characteristics (e.g. riffles with similar local water velocity, discharge and geomorphology), but differing in the surrounding land-use. The three sites of the Ardières River (A1, A2, and A3 in Fig. 1) were all located in the upstream part of the stream with, along extensive farming with a weak up- to downstream pattern of increasing livestock production. The three sites of the Morcille River (M1, M2, and M3 in Fig. 1) were subject to an increasing vineyard pressure from M1 with less than 5% of the catchment covered by vineyard and 90% by forest (considered as a reference site), to M2 where vineyard represents 30%, and M3 with more than 50% of the catchment devoted to wine production (Dorigo et al. 2007). Bottom sediments are similar in all stations (coarse gravel and sand) with high hydraulic conductivity (from $7.13 \cdot 10^{-3}$ to $2.10 \cdot 10^{-2}$

m.sec⁻¹ at -20 cm).

Water chemistry

Water temperatures were measured at each site in surface water and in the hyporheic zone (at 20 cm deep) every hour throughout the study period, using miniature data logger MINILOG8-TDR (WEMCO division, Amirix Systems Inc, Halifax, Canada). Electrical conductivity, pH, (LF92, WTWTM, Weilheim, Germany), dissolved oxygen concentration (HQ20, HACHTM, Dusseldorf, Germany) and vertical hydraulic gradient were measured in the field at each sampling date. Water samples were collected in surface and hyporheic waters every month from January to March 2009 and analyzed the same day. In the laboratory, filtered-water samples (GF/C, 1.2 mm pore size, WhatmanTM, Maidstone, UK) were analyzed for NH₄⁺, NO₃⁻, and NO₂⁻ using an automatic analyzer Easychem Plus (Systea, Italia) based on standard colorimetric methods (Grashoff et al., 1983). Water samples for DOC were filtered through 0.22 µm pore size Whatman GSWP filters (Millipore), acidified with three drops of HCl (35%) and stored at 4°C for further analysis. DOC concentration was measured with a total carbon analyzer (multi N/C 3100; Analytik Jena, Jena, Germany) based on combustion at 850°C after removing dissolved inorganic C with HCl and CO₂ stripping under O₂ flow. The 8 most frequently found pesticides (Morin et al. 2010) were analyzed in the surface water samples using standardized protocols and a ESI-LC-MS/MS (API 4000, Applied Biosystems) and the 7 most frequently found metals were analyzed by ICP-MS (Thermo Electron X7) following standard procedures (NF EN ISO 17294-2). To evaluate toxic storage in the hyporheic zone, bottom sediment was sampled in January for heavy metal analysis. After samples acidification with HNO₃ SUPRAPUR 0.5%, the analyses were performed by ICP-MS (THERMO ELECTRON X7 Series 2) to meet the standard NF EN ISO 17294-2. The quantification threshold was 0.05 µg/L.

Leaf litter decomposition

The litter-bag method (Chauvet, 1987; Boulton and Boon, 1991) was used to assess the leaf litter breakdown rate along the gradient of agricultural impact. Freshly fallen leaves of *Alnus glutinosa* were collected in December 2008 from forests adjacent to the study sites.

To study the breakdown rate in the surface water, about 2 (± 0.1) g of air-dried leaves were enclosed in coarse (3 mm mesh, 15 x 15 cm plastic bags) and in fine (0.5 mm mesh, 12 x 8 cm nylon bags) mesh bags closed in a quadratic shape (Piscart et al. 2009). The coarse mesh allowed large shredders (such as Gammaridae and Limnephilidae) to enter the bag and feed on leaves, whereas the fine mesh excluded most of the invertebrates without interfering with microbial colonization (Boulton and Boon, 1991, Boulton, 1993). Twelve bags of each type were firmly tethered to steel pegs placed within shallow riffles with similar current velocities (range from 0.2 m.s⁻¹ in M1 to 0.3 m.s⁻¹ in A1) at each site, on the 21st of January 2009. Three bags were collected at each site, each every week for coarse mesh bags and every two weeks for fine mesh bags, because the leaf litter breakdown rate without invertebrates was much slower.

Small size bags were used for the measure of breakdown rates in the hyporheic zone to avoid modifications of sediment characteristics linked to the burial process (Marmonier et al. 2010; Navel et al., submitted). About 0.5 (± 0.1) g of air-dried leaves were enclosed in coarse mesh bags (3 mm mesh, 5 x 4 cm plastic bags). In order to reduce the disturbance of the river bed, bags were carefully introduced inside the sediment using a mini-piezometer (1 m long, 3 cm diameter) pushed to a 20 cm depth into sediment using an internal metallic rod (Navel et al., submitted). Twenty-four hyporheic bags per site were buried at the upstream end of shallow riffles (i.e. downwelling zone with negative hydraulic gradient). The upstream end of riffles was chosen because it represents a downwelling area (infiltration of surface water into the hyporheic zone, Hendricks and White, 1991) fed with oxygen and nutrients, resulting in a

high breakdown rate (Tillman et al., 2003). In order to have enough remaining material for analyses, six bags were removed every two weeks (same dates as the fine mesh benthic bags).

Upon retrieval, the leaves of each type of bags were washed individually to remove sand, exogenous organic matter and invertebrates. The remaining leaf material was dried at 105°C for 24 h and weighed to the nearest mg. For each type of bag, three control litter bags were used to estimate the initial dry mass of leaf litter after a short immersion time (leaching) and drying at 105°C for 24 h.

Exponential litter breakdown rate (k) was calculated using the relationship,

$$W_t = W_i * e^{-k*t}$$

where W_t is the leaf dry mass remaining at the time t and W_i the leaf dry mass at the initial time (Petersen and Cummins, 1974). Because of the temperature changes between sites, we used the coefficients based on thermal sums ($k_{\text{degree day}}$) (Cummins et al, 1989). For degree day calculations, we substituted for time (t) the thermal sum in degree days above 0°C on the collection day (i.e. the sum of each daily mean water temperature over the period that the bags spent in the water). Calculations were performed separately for interstitial (k_i), fine (k_f), and coarse mesh (k_c) bags.

Invertebrates sampling

At mid-term litter exposure (February 2009), both benthic and interstitial invertebrate assemblages were characterized. Three samples of invertebrates present in the benthic layer were taken in each site using a Surber net sampler (0.375 m², 0.1 mm mesh size). Three samples of interstitial invertebrates were collected using the Bou-Rouch sampler (Bou and Rouch, 1967; Bou, 1974) through which 10 L of water and sediment were pumped and filtered (0.1 mm mesh size). The samples were fixed with 96% ethanol in the field. In the laboratory, organisms were sorted using a stereomicroscope and identified to species or genus

(most Crustaceans and Insect larvae, Tachet et al., 2000) except for Nematoda and Hydracarina (not identified further), and most Diptera (identified to the family level), and counted.

Microbial biomass

After 42 days, the remaining leaf litter from both benthic fine mesh and hyporheic bags were used to study the fungal and the bacterial biomasses. The fungal biomass on leaves was assessed through the content in ergosterol (Gessner & Chauvet, 1993). Leaf material was lyophilized and weighed to the nearest 0.1 mg, and then lipids were extracted with alkaline methanol heated at 80°C for 30 min. The extracts were purified using solid-phase extraction cartridges (Oasis HLB, 60 mg, 3 cc, Waters, Milford, Massachusetts, USA) and the ergosterol was quantified by high-performance liquid chromatography (procedure slightly modified from Gessner, 2005). The extraction efficiency (89-94%) was determined for each series using controls to which known amounts of ergosterol were added, and was applied to calculate the ergosterol content in leaf litter.

Leaf discs were collected and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 h. The fixed samples were subsequently washed twice in PBS and were stored in ethanol and PBS (50 : 50) at 20°C. After storage (2 weeks), the leaf discs were homogenized in 20 mL of 0.1% pyrophosphate in PBS using a sonicator with a 2-mm-diameter probe at 50 W for two periods of 60s. All homogenized samples were finally supplemented with the detergent NP-40 (Fluka, Buchs, Switzerland) to a final concentration of 0.01%. Aliquots (10 µL) of homogenized samples were stained with the DNA intercalating dye DAPI (200 ng µL⁻¹, Sigma, Buchs, Switzerland) according to Navel et al. (2010). Slides were mounted with Citifluor solution (Citifluor Ltd, London, U.K.) and the preparations were examined at 1000 × magnification with a microscope fitted for epifluorescence (Nikon

Labophot-2, Nikon, Japan). Bacteria from the samples were counted in 20 microscope fields per sample with up to 30 cells per field.. Results were expressed as numbers of bacteria g⁻¹ of sediment dry mass (DM). Conversion into C biomass was then calculated using a coefficient of 4.65×10^{-12} µg C/cell (Sevais & Garnier, 1993).

Statistical analysis

For each river, between-site differences were compared statistically for each physico-chemical parameter using the Friedman nonparametric ANOVA method to take the variability between the sampling dates into account.

Benthic and hyporheic taxonomic richness and abundances were calculated for all feeding groups, for the sensitive Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT), for the shredders that control the POM degradation, and for organisms associated to fine sediment habitats (i.e. the Oligochaeta). We used the Spearman's Rank correlation to link the gradient of land use and the invertebrate richness and abundances, microbial biomass, and leaf litter breakdown rates (k_c , k_f and k_i). To evaluate the local vertical connectivity between benthic and hyporheic zones, we calculated a difference in oxygen concentrations between the surface and -20 cm (Marmonier et al., 2010).

Leaf breakdown rates were determined by nonlinear regression (Petersen and Cummins, 1974). For each type of bag, the mean remaining leaf mass (Ln(x+1) transformed) in sites was analyzed using a mixed-model ANOVA with 'river' and 'site' (repeated measure) as fixed factors and the number of degree days as covariable, followed by Tukey's HSD tests for multiple comparisons. All analyses were performed using procedures from Statistica 7.1 (StatSoft, 2004).

Results

Water chemistry

The surface water of both the Ardières and the Morcille rivers had low electrical conductivities ($\leq 170 \mu\text{S.cm}^{-1}$), low-to-intermediate nutrient concentrations (e.g. $\square 10.8 \text{ mg NO}_3\text{.L}^{-1}$), and dissolved organic carbon concentrations ($\leq 6 \text{ mg DOC.L}^{-1}$, Table 1). Chemical characteristics changed little along the Ardières River, with only a significant decrease ($\chi^2 = 6.0$, $p = 0.05$) in DOC concentrations from A1 ($6.0 \pm 3.3 \text{ mg.L}^{-1}$) to A3 ($3.0 \pm 1.2 \text{ mg.L}^{-1}$) (Table 1). Similarly, in the Morcille River we only observed a significant increase ($\chi^2 = 6.0$, $p = 0.05$) in the electrical conductivity from M1 ($144 \pm 16 \mu\text{S.cm}^{-1}$) to M3 ($170 \pm 10 \mu\text{S.cm}^{-1}$).

Hyporheic water was much more variable between sites (Table 1). We observed a significant increase in temperature, dissolved oxygen and pH from upstream to downstream in the Ardières River (all $p < 0.016$) and a decrease in nitrite concentrations at A2 ($\chi^2 = 6.9$, $p = 0.032$). In the Morcille River, we observed a significant increase in the dissolved oxygen, the pH and the electrical conductivity from M1 to M3 (all $p < 0.05$), but a significant decrease in the temperature and the ammonium concentration ($p = 0.001$ and $p = 0.008$, respectively).

Pesticides were only found in February (14 days after the beginning of the study) at M3 and A3. Four different pesticides were found at relatively low concentrations at M3: Diuron ($0.2 \mu\text{g.L}^{-1}$), Dimethomorphe ($0.2 \mu\text{g.L}^{-1}$), Tebuconazole ($0.1 \mu\text{g.L}^{-1}$), and Procymidone ($0.1 \mu\text{g.L}^{-1}$). At A3, only Procymidone was measured at low concentrations ($0.1 \mu\text{g.L}^{-1}$).

Heavy metals were found in all sampling sites, dissolved in water and associated to the sediments (Table 2). According to Water Quality standards in France (Water Agency, 2003), some of them indicate a low water quality: Cu, Cr, Cd in both rivers; Zn was medium for the Ardières; Pb, Zn (in the Morcille River), Al and Fe indicated a high water quality. No specific trend was observed from upstream to downstream and between rivers: Cu, Zn, Pb and Cd

were higher in the Ardières and Fe, Al, Cr were higher in the Morcille.

Invertebrate communities and microbial biomass

In the benthic layer (Table 3), the invertebrate richness significantly decreased from upstream to downstream in both the Ardières ($r = -0.73$, $p = 0.03$) and the Morcille ($r = -0.66$, $p = 0.05$) rivers. For sensitive organisms, the EPT richness decreased with the proportion of vineyard along the Morcille River ($r = -0.93$, $p < 0.001$) whereas no significant change in EPT richness was measured between the three stations of the Ardières River ($p = 0.72$). Similarly, shredders richness, abundances, and amphipod abundances slightly varied along the Ardières River whereas they significantly decreased with vineyard land-cover in the Morcille River ($r = -0.75$, $p = 0.02$, for amphipod abundances). Finally, the richness of organisms linked to fine sediment (the Oligochaeta) increased along the Ardières River ($r = -0.69$, $p = 0.04$) but remained similar in the Morcille River ($p = 0.94$). In the M2 and M3 sites surrounded by vineyard, most deposit feeders Oligochaeta disappeared (Tubificinae and Lombriculidae), while algivorous species increased (Naidinae)

In the hyporheic zone (Table 3), strong changes were observed with the increasing proportion of vineyard around the Morcille River, with a significant decrease in the invertebrate richness ($r = -0.87$, $p = 0.002$), abundances ($r = -0.74$, $p = 0.02$), Oligochaeta richness ($r = -0.75$, $p = 0.02$) and Oligochaeta abundances ($r = -0.74$, $p = 0.02$). Algivorous Oligochaeta species that developed abundant populations in the benthic layer were not collected inside the sediment. In the Ardières River, we observed slight changes in macroinvertebrate assemblages from upstream to downstream, with only a significant slight increase in the Oligochaeta abundances ($r = -0.69$, $p = 0.04$).

The fungal biomass in both benthic and hyporheic layers (Table 3) significantly increased from upstream to downstream in both the Ardières (all $r > 0.79$ and $p < 0.01$) and

the Morcille (all $r = 0.68$ and $p < 0.05$) rivers. For a similar position along the up- to down-stream gradient (i.e. site 1, 2 or 3), the fungal biomass was much higher in the Ardières River than in the Morcille River, whereas the bacterial biomass did not change either along the up- to downstream gradient or between rivers (Table 3).

Litter breakdown rates

In the benthic layer (Fig. 1), breakdown rates in coarse mesh bags (k_c) significantly varied between rivers ($F_{1,65} = 13.69$, $p < 0.001$) and sites ($F_{4,65} = 8.21$, $p < 0.001$). In the Ardières River, k_c was not significantly different between sites (Fig. 1a, all $p > 0.241$), whereas it significantly decreased with the increasing proportion of vineyard in the Morcille River (Fig. 1b, all $p < 0.004$). When invertebrates were excluded, breakdown rates in fine mesh bags (k_f) did not differ between rivers ($F_{1,61} = 2.58$, $p < 0.113$), but significantly changed between sites ($F_{4,61} = 4.08$, $p = 0.005$). In the Ardières River, k_f increased from upstream to downstream (Fig. 1a, $p = 0.033$), while it remained similar at all sites in the Morcille River (Fig. 1b, all $p > 0.97$).

No correlation was found between the breakdown rates in coarse mesh bags (k_c) and in fine mesh bags (k_f), neither with chemical characteristics (all $p > 0.266$) nor with all invertebrate and microbial characteristics (all $p > 0.208$) with the exception of the amphipod abundances. Indeed, k_c was significantly and positively correlated with the amphipod abundances (Fig. 2a, $r = 0.88$, $p = 0.02$), which were negatively correlated with the percentage of vineyard in the catchment (Fig. 2b, $r = -0.82$, $p = 0.046$).

The breakdown rates measured in the hyporheic zone (k_i , Fig. 3) were very low compared to the benthic rates (Fig. 1): k_i was divided by 7 compared to the breakdown rates that included shredders activity (the mean ratios between k_c and k_i were 6.8 ± 2.3 and 7 ± 5 in the Ardières and the Morcille Rivers, respectively) and was reduced 2-fold compared to the

benthic breakdown rates without invertebrates (the mean ratios between k_f and k_i were 2.4 ± 1.2 and 2.2 ± 1.2 for the Ardières and the Morcille Rivers, respectively).

The hyporheic breakdown rates were significantly higher in the Ardières River, surrounded by traditional agriculture, than in the vineyard-dominated Morcille River ($F_{1,93} = 5.4$, $p = 0.023$). In contrast to the benthic layer, k_i did not change between sites in both rivers ($F_{4,93} = 2.1$, $p = 0.083$). k_i was positively correlated with DOC ($r = 0.82$, $p = 0.05$), dissolved oxygen concentrations ($r = 0.94$, $p < 0.01$), but negatively correlated with the water temperature ($r = -0.82$, $p = 0.05$). When the vertical connectivity between surface and hyporheic waters was considered (i.e. the decrease in dissolved oxygen concentration between the surface and -20 cm), the hyporheic breakdown rates decreased with the decreasing vertical exchanges ($r = -0.94$, $p = 0.005$; Fig. 4).

Discussion

In the control area (i.e. the traditional hedged farming area), we did not observe a strong change in the nutrient concentrations from upstream to downstream in the surface water. Nitrate concentrations ($\leq 9.1 \text{ mg NO}_3\text{.L}^{-1}$) were generally lower than concentrations found in streams surrounded by similar land uses (e.g. $13.6 \text{ mg NO}_3\text{.L}^{-1}$ in Brittany, Piscart et al., 2009). Hence, we did not observe a strong impact of traditional agricultural practices on the surface water chemistry, at least during the study period (i.e. winter with high water levels). In the vineyard area, we did not observe a strong up- to downstream gradient, either in the water chemistry or in the toxic or heavy metal concentrations, at least during the study period (winter high flow period). In the same way, pesticides were only observed at the M3 site in very low concentrations. The lack of vineyard treatment by herbicides during the cold season and the high river discharge probably explain the low concentrations of Diuron ($0.2 \text{ }\mu\text{g.L}^{-1}$) and Dimethomorphe ($0.2 \text{ }\mu\text{g.L}^{-1}$) measured in the Morcille River. Previous studies

1 have highlighted significant amounts of pesticides at the M3 site, with maximum
2 concentrations of 1 $\mu\text{g.L}^{-1}$ of Diuron in spring 2008 (noted “Les Versauds” in Morin et al.
3 2010) and 0.3 $\mu\text{g.L}^{-1}$ in summer 2008 (noted “Intermediate site” in Pesce et al. 2010).

4 Traditional farming did not lead to a significant change in the invertebrate
5 communities, with no changes in the total abundances and sensitive EPT taxa, but a slight
6 change in the composition of the benthic assemblages (with a decrease in taxonomic richness
7 and an increase in Oligochaeta abundances). This result supports our first hypothesis of a
8 limited effect of extensive farming on invertebrates. When the riparian zone is protected and
9 direct access of the cattle to the river is avoided, traditional agriculture has little effect on the
10 benthic and interstitial invertebrate assemblages (Sarriquet et al. 2006, Piscart et al. 2009,
11 Marmonier et al. 2010). These changes, which probably did not affect the shredder
12 invertebrates, explain the low impact observed on the organic matter processing in coarse
13 mesh bags. In contrast, the increase in fungal biomass observed downstream in the Ardères
14 River probably explains the increase in breakdown rates measured in the fine mesh bags
15 (three times higher at A3 than at A1 or A2). The role played by aquatic fungi in the
16 breakdown process is crucial (Gessner and Chauvet, 1994) for two reasons: they increase the
17 microbial breakdown activity *per se*, but they also stimulate the feeding activity of shredders
18 (Graça et al., 1993). This result supports our first prediction of a positive effect of traditional
19 agricultural practices on microbial activities associated with an increase in nitrate
20 concentrations (Table 3).

21 In the vineyard area, even if toxic concentrations were low during the study period,
22 we observed a strong decrease in the total invertebrate richness, in the sensitive EPT taxa
23 richness, in the abundances and richness of organisms linked to fine sediment (i.e. the
24 Oligochaeta), and a complete disappearance of amphipods at the M3 site. Schäfer et al. (2007)
25 observed a negative impact of Linuron (a urea herbicide of the Diuron family) on the

Percentage of Species at Risk (%SPEAR_{pesticide}) for a concentration of 0.097 $\mu\text{g.L}^{-1}$, concentrations much lower than those observed in the Morcille River during our study (i.e. 0.2 $\mu\text{g Diuron.L}^{-1}$). The Diuron may also have an indirect impact on invertebrates through a toxic effect on microbial communities. For example, Morin et al. (2010) observed a 70% decrease in biofilms biomass at M3 in spring 2008. Such a decrease may alter the trophic resources for several invertebrates. We also measured significant concentrations in Dimethomorphe (i.e. 0.2 $\mu\text{g.L}^{-1}$). This fungicide of the morpholine family is considered as less toxic than urea herbicides for invertebrates (Schäfer et al. 2007) and we did not observe any significant decrease in fungal biomass (the biological target of this fungicide) at the M3 site. Its effect on the stream functioning may thus have been limited during the study period. Breakdown rates in coarse mesh bags (k_c) strongly decreased at M2 and M3 whereas k_f was not affected by the increasing proportion of vineyard in the watershed. The strong decrease in k_c may be explained by the decrease (in M2) and disappearance (in M3) of the amphipods, highlighted by the positive correlation between k_c and amphipod abundances (Figure 2). These results demonstrate the crucial role of amphipods in the breakdown process and the lack of compensation by other shredders or by aquatic fungi when the amphipods disappeared (Piscart et al., 2009). The sensitivity of amphipods to pesticides and other toxics is hence crucial for several ecological processes in streams (organic matter recycling, fish productivity).

In the hyporheic area, the expected increase in breakdown rates in the traditional farming area (our third hypothesis) was not measured, despite increases in nitrate concentration and fungal biomass along the river. In the hyporheic layer, the suggested positive effect of nutrient concentrations on k_i at A3 was not significant, indicating a non-linear response of k_i to the range of available nutrients. In the same way, the expected decrease in breakdown rates with the increasing proportion of vineyard was not supported by the results. Heavy metals could

1 impact microbial processes, with very different results depending on the way these metals act
2 and their bioavailability, as well as on the biological targets. The concentrations found in both
3 rivers were sometimes relatively important with values above the level of French high
4 ecological quality standards (Agence de l'Eau, 2003). However, a study on the heterotrophic
5 biofilm in the same river (Tlili et al., 2011) indicated that the respiration of the heterotrophic
6 community has a low sensitivity to metals. The results indicate that the concentrations of
7 heavy metals (Fe, Al, Cu, Cr, Pb, Cd) found during this survey were quite below the CE50.
8 These concentrations were not associated to changes in macroinvertebrate communities along
9 the two rivers. We also observed that concentrations of the most toxic heavy metals found in
10 the Ardières River were higher (for Cu et Cd) or comparable (for Cr) to concentrations
11 measured in the Morcille River. This observation was probably due to the geological nature of
12 the Morcille and Ardières catchments, mainly constituted of granitoid rocks rich in biotite,
13 orthoclase and amphibole (Arène et al., 1982), acting as natural sources of heavy metals for
14 rivers in the study area. Therefore, the heavy metals concentrations found in rivers were not
15 tightly linked to human activities and could not explain the distributions of
16 macroinvertebrates observed in our study. However, the decrease of the hyporheic taxonomic
17 richness, total abundances, and Oligochaeta abundances at the M3 site suggested local
18 toxicity of the sediment, but not strong enough to reduce fungal and bacterial biomasses and
19 activities. The lack of decrease in breakdown rates in the hyporheic zone of the M3 site may
20 be explained by a high vertical connectivity between the surface and interstitial waters at this
21 site, where the river was surrounded by a wide strip of trees preventing fine particle inputs
22 and bottom sediment clogging (Gibert et al., 1995; Pichtel et al., 1997). The vertical
23 connectivity could allow the colonization of buried leaf litter by shredder organisms
24 originated from the surface (Cornut et al., 2010; Navel et al., 2010) and favour aerobic
25 conditions in sediments which permit an optimal breakdown rate of buried leaf litter by

micro-organisms (Navel et al., in press).

Conclusion

Our study demonstrates that the total litter breakdown process (measured in coarse mesh bags, *kc*) was strongly influenced by land use changes in the catchment. The breakdown rate *kc* thus represents an efficient indicator of the level of disturbance linked to agriculture, even during periods when agricultural practices are reduced. It is still not clear how litter breakdown integrates the temporal variability of the anthropogenic disturbance: through changes in the composition and activity of invertebrate and fungal communities or the direct effect of toxics stored inside bottom sediments. In any case, this measure of a global biological process may be of greater use than punctual water chemical analyses *per se*. Our study confirms the results of Piscart et al. (2009) in a completely different biogeographical context: a single group of shredders, the Gammarid amphipods, may be considered as the key organisms controlling litter breakdown. In contrast, microbial processes (measured in our study by *kf*) clearly integrated the variations in available nutrients and dissolved oxygen concentrations, but failed to integrate disturbances induced by wine production during the growing seasons. The response of microbes, which is mainly driven by local factors, may not be reliable enough for assessing the global effect of seasonal agricultural practices. Finally, the leaf litter breakdown measured in the hyporheic zone seems mainly driven by microbial activities. The response to land use of the breakdown rates in the hyporheic zone was hence mainly affected by vertical exchanges with surface water. The analysis of the breakdown rate of leaf litter in the hyporheic zone may thus constitute a relevant way to evaluate the impact on river functioning of any human activities that induce massive soil erosion and sediment clogging.

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References

- Allan JD. Landscape and riverscape: the influence of land use on river ecosystems. *Annu Rev Ecol Evol Syst* 2004; 35: 257–284.
- Arène J, Bambier A, Carroué J-P, Debrand-Passard S, Monjuvent G, Putallaz J. Carte géologique de la France au 1/50000 - Feuille de BEAUJEU, 1982, BRGM, Orléans, France.
- Bou C, Rouch R. Un nouveau champ de recherches sur la faune aquatique souterraine. *C R Acad Sci* 1967; 265: 369-370.
- Bou C. Recherches sur les eaux souterraines -25- Les méthodes de récolte dans les eaux souterraines interstitielles. *Ann Spéléol* 1974; 29: 611-619.
- Boulton AJ, Boon PI. A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? *Aust J Mar Fresh Res* 1991; 42: 1-43.
- Boulton AJ. Stream ecology and surface-hyporheic hydrologic exchange: implications, techniques and limitations. *Aust J Mar Fresh Res* 1993; 44: 553-564.
- Chauvet E. Changes in the chemical composition of alder, poplar and willow leaves during decomposition in a river. *Hydrobiologia* 1987; 148: 35-44.
- Claret C, Boulton AJ, Dole-Olivier MJ, Marmonier P. Functional processes versus state variables: interstitial organic matter pathways in floodplain habitats. *Can J Fish Aquat Sci* 2001; 58: 1594-1602.
- Cornut J, Elger A, Lambrigtot D, Marmonier P, Chauvet E. Early stages of leaf decomposition

are mediated by aquatic fungi in the hyporheic zone of woodland streams. *Freshwat Biol* 2010; 55: 2541-2556.

Crenshaw CL, Valett HM. The effect of coarse particulate organic matter on fungal biomass and invertebrate density in the subsurface of a headwater stream. *J N Am Benthol Soc* 2002; 1: 28-42.

Cummins KW. Structure and Function of Stream Ecosystems. *BioScience* 1974; 24: 631-641.

Cummins KW, Wilzbach MA, Gates DM, Perry JA, Taliaferro, WB. Shredders and riparian vegetation. *Bioscience* 1989; 39: 24-30.

Dolédec S, Philips N, Scarsbrook MR, Riley RH, Townsend CR. A comparison of structural and functional approaches to determining land-use effects on grassland stream invertebrate communities . *J N Am Benthol Soc* 2006; 25: 44–60.

Dorigo U, Leboulanger C, Berard A, Bouchez A, Humbert JF, Montuelle B. Lotic biofilm community structure and pesticide tolerance along a contamination gradient in a vineyard area. *Aquat Microb Ecol* 2007; 50: 91-102.

Gessner MO. Ergosterol as a measure of fungal biomass. In: Graça MAS, Bärlocher F & Gessner MO, editors. *Methods to Study Litter Decomposition: A Practical Guide*. Dordrecht: Springer; 2005. p. 189-195.

Gessner MO, Chauvet E. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Appl Environ Microb* 1993; 59: 502-507.

Gessner MO, Chauvet E. Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology* 1994; 75: 1807–1817.

Gessner MO, Chauvet E. A case for using litter breakdown to assess functional stream integrity. *Ecol Applic* 2002; 12: 498-510.

Gibert J, Marmonier P, Vanek V, Plénet S. Hydrological exchange and sediment characteristics in a riverbank: relationship between heavy metals and invertebrate

- community structure. Can J Fish Aquat Sci 1995; 52: 2084-2097.
- Graça MAS, Maltby L, Calow P. Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus*. Oecologia 1993; 96: 304-309.
- Gulis V, Rosemond A, Suberkropp K, Weyers HS, Benstead JP. Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. Freshwater Biology 2004 ; 49: 1437-1447.
- Hagen EM, Webster JR, Benfield EF. Are leaf breakdown rates a useful measure of stream integrity along an agricultural landuse gradient? J N Am Benthol Soc 2006; 25: 330–343.
- Harding JS, Benfield EF, Bolstad PV, Helfman GS, Jones EBD. Stream biodiversity: the ghost of land use past. Proc Natl Acad Sci USA 1998; 95: 14843–14847.
- Hendricks SP, White DS. Physicochemical patterns within a hyporheic zone of a Northern Michigan River, with comments on surface water patterns. Can J Fish Aquat Sci 1991; 48: 1645-1654.
- Herbst GN. Effects of burial on food value and consumption of leaf detritus by aquatic invertebrates in a lowland forest stream. Oikos 1980; 35: 411-424.
- Hladyz S, Tiegs SD, Gessner A, Giller PS, Risnoveanu G, Preda E et al. Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. Freshwat Biol 2010; 55: 1916-1929.
- IFEN, 2007, Les pesticides dans les eaux: données 2005, Institut Français de l'Environnement, 37p. www.ifen.fr.
- Iqbal SH, Webster J. The trapping of aquatic hyphomycete spores by air bubbles. Trans Brit Mycol Soc 1973; 60: 37-48.
- Lecerf A, Usseglio-Polatera P, Charcosset JY, Lambrigtot D, Bracht B, Chauvet E. Assessment of functional integrity of eutrophic streams using litter breakdown and benthic macroinvertebrates. Arch Hydrobiol 2006; 165: 105-126.

- 1 Lecerf A, Chauvet E. Diversity and functions of leaf-associated fungi in human-altered
2 streams. *Freshwat Biol* 2008a; 53:1658-1672.
- 3 Lecerf A, Chauvet E. Intra-specific variability in leaf traits strongly affects alder leaf
4 decomposition in a stream. *Bas Appl Ecol* 2008b; 9: 598-607.
- 5 Marmonier P, Piscart C, Sarriquet PE, Azam D, Chauvet E. Relevance of large litter bag
6 burial for the study of leaf breakdown in the hyporheic zone. *Hydrobiologia* 2010, 641: 203-
7 214.
- 8 Medeiros A, Duarte S, Pascoal C, Cassio F, Graca M. Effects of Zn, Fe and Mn on Leaf
9 Litter Breakdown by Aquatic Fungi: a Microcosm Study. *Int Rev Hydrobio* 2010; 95:12-26.
- 10 Meybeck M. Carbon, nitrogen, and phosphorus transport by world rivers. *Am J Sci* 1982;
11 282: 401–450.
- 12 Morin S, Pesce S, Tlili A, Coste M, Montuelle B. Recovery potential of periphytic
13 communities in a river impacted by a vineyard watershed. *Ecol indicat* 2010; 10: 419-426.
- 14 Navel S, Mermillod-Blondin F, Montuelle B, Chauvet E, Simon L, Piscart C et al.
15 Interactions between fauna and sediment control the breakdown of plant matter in river
16 sediments. *Freshwat Biol* 2010; 55: 753-766.
- 17 Navel S, Mermillod-Blondin F, Montuelle B, Chauvet E, Simon L, Marmonier P. Water-
18 sediment exchanges control microbial processes associated with leaf litter degradation in the
19 hyporheic zone: a microcosm study. *Microb Ecol* in press; DOI 10.1007/s00248-010-9774-
20 7.
- 21 Paul E, Johnson S, Skinner KM. Fish and invertebrate sensitivity to the aquatic herbicide
22 Aquakleen. *J Freshwat Ecol* 2006; 21: 163-168.
- 23 Petersen RC, Cummins KW. Leaf processing in a woodland stream. *Freshwat Biol* 1974; 4:
24 343–368.
- 25 Pesce S, Fajon C, Bardot C, Bonnemoy F, Portelli C, Bohatier J. Longitudinal changes in

1 microbial planktonic communities of a French river in relation to pesticide and nutrient
2 inputs. *Aquat Toxicol* 2008; 86: 352-360.

3 Pesce S, Margoum C, Montuelle B. In situ relationships between spatio-temporal variations in
4 diuron concentrations and phototrophic biofilm tolerance in a contaminated river. *Wat Res*
5 2010, 44: 1941-1949.

6 Pichtel J, Sawyerr HT, Czarnowska K. Spatial and temporal distribution of metals in soils in
7 Warsaw, Poland. *Environ Pollut* 1997; 98: 169-174.

8 Piscart C, Genoel R, Dolédec S, Chauvet E, Marmonier P. Effects of intense agricultural
9 practices on heterotrophic processes in streams. *Environ Pollut* 2009; 157: 1011-1018.

10 Sarriquet PE, Delettire Y, Marmonier P. Effects of catchment disturbance on stream
11 invertebrates: comparison of different habitats (vegetation, benthic and interstitial) using
12 bio-ecological groups. *Ann Limnol- Int J Limnol* 2006; 42: 205-219.

13 Schäfer RB, Caquet T, Siimes K, Mueller R, Lagadic L, Liess M. Effects of pesticides on
14 community structure and ecosystem functions in agricultural streams of three
15 biogeographical regions in Europe. *Sci Tot Environ* 2007; 382: 272-285.

16 Servais G., Garnier J. Contribution of heterotrophic bacterial production to the Carbon budget
17 of the River Seine (France). *Microb Ecol* 1993; 25: 19-33.

18 Solé M, Fetzer I, Wennrich R, Sridhar KR, Harms H, Krauss G. Aquatic hyphomycete
19 communities as potential bioindicators for assessing anthropogenic stress. *Sci Tot Environ*
20 2008; 389: 557-565.

21 StatSoft. STATISTICA (data analysis software system).2004; Version 7.www.statsoft.com.

22 Suberkropp K, Chauvet E. Regulation of leaf breakdown by fungi in streams: influences of
23 water chemistry. *Ecology* 1995; 76: 1433-1445.

24 Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P. Invertébrés d'eau douce:
25 Systématique, biologie et écologie. Paris : CNRS edition ; 2000.

1 Tillman DC, Moerke AH, Ziehl CL, Lamberti GA. Subsurface hydrology and degree of burial
2 affect mass loss and invertebrate colonisation of leaves in a woodland stream. *Freshwat Biol*
3 2003; 48: 98-107.

4 Tlili A., Marechal M., Montuelle B., Volat B., Dorigo U., Berard A., Use of the MicroResp_
5 method to assess pollution-induced community tolerance to metals for lotic biofilms
6 *Environ Poll* 2011; 159:18-24.

7 Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW et al. Human
8 alteration of the global nitrogen cycle: Sources and consequences. *Ecol Applic* 1997; 7:
9 737–750.

10 Water Agency, 2003, Système d'évaluation de la qualité de l'eau des cours d'eau (SEQ-Eau),
11 MEED & Agence de l'Eau, 40p.

12 Webster JR, Meyer JL. Stream organic matter budgets. *J N Am Benthol Soc* 1997; 16: 3-13.

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Figure legends.

Fig. 1. Mean values ($\pm$ SE) of leaf litter breakdown rates in coarse (grey bars) and fine (open bars) mesh bags in the Ardières River (a) and the Morcille River (b). For each site, significant between-site differences (Tukey's HSD tests following the ANCOVAs) in breakdown rates are indicated by different letters.

Fig. 2. (a) Exponential relationship between the leaf litter breakdown rates in coarse mesh bags and the mean abundance of amphipods at the surface layer of the six study sites. (b) Linear relationship between the mean abundance ($\log x + 1$) of amphipods at the surface layer and the percentage of vineyard at the catchment scale of the six study sites.

Fig. 3. Mean values ($\pm$ SE) of the leaf litter breakdown rates measured within the sediment of the three sites in the Ardières River (a) and in the Morcille River (b).

Fig. 5. Linear relationship between the leaf litter breakdown rates measured within the sediment and the decrease in dissolved oxygen concentration between the surface water and the water within the sediment.

Table 1. Water chemical characteristics (mean $\pm$ SD) of sites of the Ardières River (A1, A2, A3) located in an extensive farming area and sites of the Morcille River (M1, M2, M3) located in the vineyard area.

Depth	Site	Temp. (°C)	O ₂ (mg.l ⁻¹)	pH	Cond. (µS.cm ⁻¹)	NO ₃ ⁻ (mg.l ⁻¹)	NO ₂ ⁻ (µg.l ⁻¹)	NH ₄ ⁺ (µg.l ⁻¹)	DOC (mg.l ⁻¹)
Surface	A1	5.6 $\pm$ 1.1	11.5 $\pm$ 0.8	7.9 $\pm$ 0.4	124 $\pm$ 27	7.5 $\pm$ 6.4	9.1 $\pm$ 10.0	105.5 $\pm$ 79.0	6.0 $\pm$ 3.3
	A2	5.8 $\pm$ 1.0	11.8 $\pm$ 0.6	8.4 $\pm$ 0.2	118 $\pm$ 18	6.8 $\pm$ 5.6	7.5 $\pm$ 7.4	38.3 $\pm$ 18.1	3.7 $\pm$ 2.0
	A3	5.9 $\pm$ 1.6	11.8 $\pm$ 0.5	8.0 $\pm$ 0.3	121 $\pm$ 10	10.8 $\pm$ 11.4	7.3 $\pm$ 7.0	19.2 $\pm$ 7.2	3.0 $\pm$ 1.2
Interstitial	A1	5.6 $\pm$ 1.6	8.1 $\pm$ 0.8	7.5 $\pm$ 0.2	114 $\pm$ 7	7.7 $\pm$ 6.1	12.0 $\pm$ 10.9	69.4 $\pm$ 33.0	5.4 $\pm$ 0.5
	A2	6.3 $\pm$ 1.6	8.7 $\pm$ 2.2	7.5 $\pm$ 0.2	120 $\pm$ 7	7.7 $\pm$ 7.7	10.4 $\pm$ 8.6	73.4 $\pm$ 27.5	5.7 $\pm$ 1.1
	A3	6.4 $\pm$ 1.9	10.5 $\pm$ 0.9	7.7 $\pm$ 0.2	122 $\pm$ 6	10.6 $\pm$ 10.7	11.2 $\pm$ 9.7	49.3 $\pm$ 18.6	5.4 $\pm$ 1.2
Surface	M1	5.9 $\pm$ 1.1	11.5 $\pm$ 0.8	8.0 $\pm$ 0.2	144 $\pm$ 16	3.3 $\pm$ 2.2	5.0 $\pm$ 4.4	8.9 $\pm$ 0.2	2.8 $\pm$ 1.0
	M2	5.4 $\pm$ 0.8	11.7 $\pm$ 0.6	7.9 $\pm$ 0.4	156 $\pm$ 11	3.3 $\pm$ 2.0	4.8 $\pm$ 4.4	12.8 $\pm$ 0.13	2.8 $\pm$ 1.3
	M3	4.5 $\pm$ 1.0	11.6 $\pm$ 0.7	7.8 $\pm$ 0.4	170 $\pm$ 10	5.0 $\pm$ 4.1	7.6 $\pm$ 7.7	22.9 $\pm$ 9.9	2.8 $\pm$ 0.
Interstitial	M1	6.1 $\pm$ 1.5	6.1 $\pm$ 2.0	7.2 $\pm$ 0.4	163 $\pm$ 22	5.0 $\pm$ 4.9	8.8 $\pm$ 8.0	44.7 $\pm$ 29.5	4.5 $\pm$ 0.6
	M2	6.1 $\pm$ 1.2	5.5 $\pm$ 1.2	7.1 $\pm$ 0.3	187 $\pm$ 17	5.1 $\pm$ 3.9	8.4 $\pm$ 8.0	43.0 $\pm$ 28.4	4.0 $\pm$ 0.9
	M3	4.7 $\pm$ 1.8	10.1 $\pm$ 1.5	7.6 $\pm$ 0.3	185 $\pm$ 8	6.4 $\pm$ 5.2	21.0 $\pm$ 23.1	43.3 $\pm$ 33.4	5.3 $\pm$ 0.5

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Table 2. Heavy metal concentrations (mean $\pm$ SD) in the water (in $\mu\text{g/L}$) and in sediment (in $\mu\text{g/g}$ of sediment at the first date only) of sites of the Ardières River (A1, A2, A3) located in an extensive farming area and sites of the Morcille River (M1, M2, M3) located in the vineyard area.

Depth	Site	Fe	Al	Cu	Zn	Cr	Pb	Cd
Water	A1	3.3 ± 0.2	0.2 ± 0.1	46.0 ± 30.8	6.4 ± 0.8	77.8 ± 9.2	0.1 ± 0.02	0.2 ± 0.1
	A2	3.3 ± 0.2	0.2 ± 0.1	53.7 ± 30.2	6.1 ± 0.7	82.2 ± 9.0	0.05 ± 0.01	0.4 ± 0.3
	A3	3.7 ± 0.2	0.2 ± 0.1	55.1 ± 31.3	5.5 ± 0.8	81.0 ± 7.0	0.04 ± 0.01	0.4 ± 0.2
	M1	4.3 ± 0.3	0.4 ± 0.0	31.0 ± 16.0	1.3 ± 0.1	74.1 ± 6.9	0.01 ± 0.01	0.1 ± 0.1
	M2	5.6 ± 0.1	0.3 ± 0.0	31.2 ± 21.6	1.4 ± 0.0	80.7 ± 8.7	0.01 ± 0.01	0.1 ± 0.1
	M3	11.8 ± 1.0	0.3 ± 0.0	33.1 ± 8.7	3.5 ± 0.5	100.6 ± 10.5	0.01 ± 0.01	0.1 ± 0.1
	A1	5800	9597	6.3	41.7	8.3	21	0.38
	A2	5484	8928	18.9	34	7.1	104	0.28
	A3	5484	8928	18.9	34	7.1	104	0.28
Sediment	M1	9078	11073	5.1	29	30	20	0.09
	M2	10618	13760	11.3	35.6	41	29.2	0.11
	M3	6030	11017	7.1	26.6	17.9	28.8	0.08

Table 3. Composition (mean $\pm$ SD for 0.05 m²) of macroinvertebrate assemblages (richness and abundance without Oligochaeta) and Oligochaeta assemblages (richness and abundance), and microbial biomasses at the surface layer and in the river sediment of sites of the Ardières River (A1, A2, A3) located in an extensive farming area and sites of the Morcille River (M1, M2, M3) located in the vineyard area.

Site	Assemblages at the surface layer						Interstitial assemblages					
	A1	A2	A3	M1	M2	M3	A1	A2	A3	M1	M2	M3
Invertebrate richness	22.7 $\pm$ 3.8	17 $\pm$ 3.6	15 $\pm$ 0	19.7 $\pm$ 2.5	20.7 $\pm$ 3.5	9 $\pm$ 2.6	10.3 $\pm$ 2.9	9.3 $\pm$ 1.5	7 $\pm$ 1	14.3 $\pm$ 3.5	8.3 $\pm$ 1.5	5.7 $\pm$ 2.5
Invertebrate abundance	414 $\pm$ 258	233 $\pm$ 90	284 $\pm$ 306	186 $\pm$ 173	240 $\pm$ 145	130 $\pm$ 69	85 $\pm$ 86	65 $\pm$ 49	14 $\pm$ 7	178 $\pm$ 56	32 $\pm$ 17	30 $\pm$ 6
EPT richness	6.3 $\pm$ 1.5	5.7 $\pm$ 0.6	5.3 $\pm$ 2.1	6 $\pm$ 0	5.3 $\pm$ 0.6	2 $\pm$ 0	1.7 $\pm$ 1.5	1.7 $\pm$ 1.5	2 $\pm$ 1	1.3 $\pm$ 1.2	1.3 $\pm$ 0.6	1 $\pm$ 1
EPT abundance	40 $\pm$ 28	76 $\pm$ 31	37 $\pm$ 44	48 $\pm$ 22	104 $\pm$ 56	37 $\pm$ 21	2 $\pm$ 2	2.3 $\pm$ 2.1	3.3 $\pm$ 2.5	2 $\pm$ 2	3 $\pm$ 2	1.7 $\pm$ 1.5
Shredder richness	7 $\pm$ 2.6	5.7 $\pm$ 2.1	5.7 $\pm$ 1.2	6.3 $\pm$ 1.2	7.3 $\pm$ 2.1	1.7 $\pm$ 0.6	1.3 $\pm$ 1.2	1.7 $\pm$ 0.6	2.3 $\pm$ 0.6	2 $\pm$ 1	2 $\pm$ 1	0.7 $\pm$ 0.6
Shredder abundance	218 $\pm$ 136	121 $\pm$ 72	214 $\pm$ 304	127 $\pm$ 143	124 $\pm$ 79	31 $\pm$ 16	1.3 $\pm$ 1.2	2 $\pm$ 1	3.7 $\pm$ 2.9	2.7 $\pm$ 1.5	3.7 $\pm$ 2.5	0.7 $\pm$ 0.6
Amphipod abundance	195 $\pm$ 121	70 $\pm$ 60	200 $\pm$ 312	90 $\pm$ 127	25 $\pm$ 20	0 $\pm$ 0	0.3 $\pm$ 0.6	0 $\pm$ 0	0.7 $\pm$ 0.6	0.3 $\pm$ 0.6	0 $\pm$ 0	0 $\pm$ 0
Oligochaeta richness	6 $\pm$ 2.6	11.7 $\pm$ 1.2	12 $\pm$ 3	3.3 $\pm$ 4.9	3.7 $\pm$ 4.7	2.7 $\pm$ 2.5	6 $\pm$ 1	7.3 $\pm$ 3.1	7.3 $\pm$ 2.1	4.3 $\pm$ 1.5	4 $\pm$ 1	0.3 $\pm$ 0.6
Oligochaeta abundance	129 $\pm$ 144	223 $\pm$ 158	81 $\pm$ 23	32 $\pm$ 55	10 $\pm$ 13	4 $\pm$ 4	27 $\pm$ 11.8	23 $\pm$ 13.9	37 $\pm$ 6	8 $\pm$ 4.4	7 $\pm$ 3.5	1.3 $\pm$ 2.3
Fungal biomass (μ g ergosterol. g of leaf ⁻¹)	457 $\pm$ 85	689 $\pm$ 160	2155 $\pm$ 318	178 $\pm$ 75	138 $\pm$ 48	1278 $\pm$ 168	20 $\pm$ 12	155 $\pm$ 66	182 $\pm$ 66	16 $\pm$ 4	19 $\pm$ 9	94 $\pm$ 57
Bacterial biomass (μ g Carbon. g of leaf ⁻¹)	1.5 $\pm$ 0.3	1.7 $\pm$ 0.2	1.6 $\pm$ 0.4	0.9 $\pm$ 0.2	0.9 $\pm$ 0.1	1.8 $\pm$ 0.1	1.4 $\pm$ 0.4	1.3 $\pm$ 0.3	1.8 $\pm$ 0.3	1.4 $\pm$ 0.2	2.2 $\pm$ 0.5	2.1 $\pm$ 0.1

Fig. 1. Piscart et al.

Fig. 2. Piscart et al.

Fig. 3. Piscart et al.

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2 Fig. 4. Piscart et al.