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Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services

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ABSTRACT

Effects of anthropogenic and environmental stressors on freshwater communities can propagate to ecosystem functions and may in turn impede ecosystem services. We investigated potential shifts in ecosystem functions that provide energy for freshwater ecosystems due to pesticides and salinity in 24 sites in streams of southeast Australia. First, effects on allochthonous organic matter (AOM) breakdown using three different substrates (leaves, cotton strips, wood sticks) in coarse and fine bags were investigated. Second, we examined effects on stream metabolism that delivers information on the ecosystem functions of gross primary production and ecosystem respiration. We found up to a fourfold reduction in AOM breakdown due to exposure to pesticides and salinity, where both stressors contributed approximately equally to the reduction. The effect was additive as, no interaction or correlation between the two stressors was found. Leaf breakdown responded strongly and exclusively to exposure to pesticides and salinity, whereas cotton strip breakdown was less sensitive and responded also to other stressors such as nutrients. No functional redundancy for the effects of pesticides and salinity on leaf breakdown was observed. For wood stick breakdown, no relationship to environmental gradients was found, however, the sample size was lower. We did not detect effects of pesticides or salinity on gross primary production or ecosystem respiration. A reduction in AOM breakdown by pesticides and salinity may impair the ecosystem services of food provision and possibly water purification. Hence, future studies should examine the spatial extent of these effects.

1. Introduction

Freshwater ecosystems deliver various goods and services for human societies such as clean water, food (e.g. fish), purification of wastes, recreation and spiritual values. However, freshwater biota are severely threatened as outlined in the Millennium Ecosystem Assessment (MEA) which identified among others organic pollution, heavy metals and pesticides as anthropogenic stressors of major importance (MEA, 2005). For most anthropogenic stressors it is unclear to which extent effects on freshwater biota (structural changes) propagate to effects on ecosystem functions and potentially ecosystem services (Covich et al., 2004; MEA, 2005).

Organic matter represents the basic energy for ecosystems and is mainly provided by the ecosystem functions of organic matter breakdown and primary production via photosynthesis. In freshwater ecosystems, these functions deliver organic matter resulting from (1) the breakdown of allochthonous organic matter (AOM) and (2) the photosynthesis or breakdown of aquatic biota (autochthonous organic matter) (Tank et al., 2010; Webster, 2007). Since a proportion of the organic matter in lotic ecosystems is exported downstream, both ecosystem functions deliver energy for local as well as downstream food webs (Allan and Castillo, 2007; Webster, 2007). Hence, any alteration in one or both of these functions may also propagate downstream (Delong and Brusven, 1994; Wallace et al., 1997).

Macroinvertebrates (especially shredders) and microorganisms (bacteria and fungi) are the main decomposers of AOM (Graca et al., 2001; Hieber and Gessner, 2002). To determine the ecosystem function of AOM breakdown, breakdown of leaves, cotton strips or wood sticks were suggested as measures (Young and Collier, 2009). For these three measures, with a few exceptions, only leaf breakdown has been investigated with respect to anthropogenic stressors (Young et al., 2008). The leaf breakdown is especially inhibited by toxicants, whereas other stressors such as excess nutrients may increase breakdown (Gessner and Chauvet, 2002; Young et al., 2008).

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While the AOM breakdown is mainly relevant for first- to fifth-order streams, the relevance of photosynthesis as energy source increases along the stream network (Vannote et al., 1980). For example, a study of Webster (2007) assigned approximately 81% of available organic carbon of the whole stream network of a medium-sized US river to gross primary production (GPP), primarily in the lower reaches. The contribution of GPP to the local energy budget can be estimated by dividing GPP by the ecosystem respiration (ER) (Tank et al., 2010). GPP and ER can be calculated by measuring the stream metabolism, for which several methods are available (Tank et al., 2010). Despite the major importance of GPP for freshwater ecosystems, only few studies have examined potential adverse effects of anthropogenic stressors on this ecosystem function (Gücker et al., 2009; Tank et al., 2010), except for several studies on the effects of agricultural land-use on GPP (Bernot et al., 2010; Gücker et al., 2009; McTammany et al., 2007; Young and Huryn, 1999).

Pesticides represent an important stressor for freshwater ecosystems and can impact all groups of organisms (Liess et al., 2008; Schäfer et al., 2011c). Nevertheless, to date only one field study examined the relationship between leaf breakdown and estimated site specific pesticide toxicity as derived from measured pesticide concentrations (Schäfer et al., 2007). This study found a reduction in leaf breakdown by invertebrates with increased pesticide toxicity (Schäfer et al., 2007). We are not aware of a field study on the effects of pesticides on the breakdown of cotton or wood on stream metabolism.

Beside the input of pesticides, agriculture in arid and semi-arid regions such as the Middle East, central Asia and southeast Australia is also a leading cause for anthropogenic salinisation that can result in a rise of electrical conductivity (EC) from below 500 µS/cm to several thousand µS/cm in freshwater ecosystems (Williams, 1987). Additionally salinity can be elevated by saline effluent from industry or mining (Piscart et al., 2005). Although changes in conductivity to several thousand µS/cm affect all major groups of freshwater biota (Hart et al., 1990), the consequences for ecosystem functions such as AOM breakdown or GPP are largely unknown (Gutierrez-Canovas et al., 2009).

In the present study, we investigated whether these two stressor pesticides and salinisation affect the ecosystem functions of AOM breakdown, GPP and ER. Furthermore, we compared different methods for the determination of the breakdown of AOM with respect to their sensitivity for both stressors. Finally, we assessed the relevance of observed effects on ecosystem functions for associated ecosystem services.

2. Methods

2.1. Study design and sampling schedule

The study was conducted in 24 sites in streams in an agriculturally dominated region of southern Victoria in southeast Australia (Fig. 1). The streams were selected to exhibit a gradient in the exposure to pesticides and salinity. The sampling was scheduled for the expected main time of pesticide application in spring and summer of 2008/2009 and encompassed six pesticide samplings, two times monitoring of leaf and cotton strip breakdown and one monitoring of wood breakdown and stream metabolism (Fig. 2). Due to weather extremes during the summer 2008/2009 in Victoria, Australia (0 mm precipitation and highest temperatures on record in 120 years across several regions between 1/1/2009 and 28/2/2009) and due to catastrophic forest fires (Schäfer et al., 2010), some stream sites fell dry or were not accessible so that the samplings in February and March comprised only 16 of the 24 sites (BOM, 2009a,b,c). This lead to a reduced sample size for the monitoring methods employed in this period (Fig. 2, Appendix Table A.1). The study presented here was complemented by a study on the effects of pesticides on macroinvertebrates and densities of microorganisms, and these results as well as further details on the sampling sites and region are given in Schäfer et al. (2011b).

2.2. Pesticide monitoring and recording of environmental variables

A total of 97 insecticides, herbicides and fungicides were monitored in the sampling period from September 2008 to March 2009 (Fig. 2) using grab water sampling, sediment sampling and passive sampling with trimethylpentane passive samples (TRIMPS) (Leonard et al., 2002). A detailed overview of the substances, sampling methods, chemical analysis and pesticide detections is described elsewhere (Schäfer et al., 2011b). The pesticide concentrations were

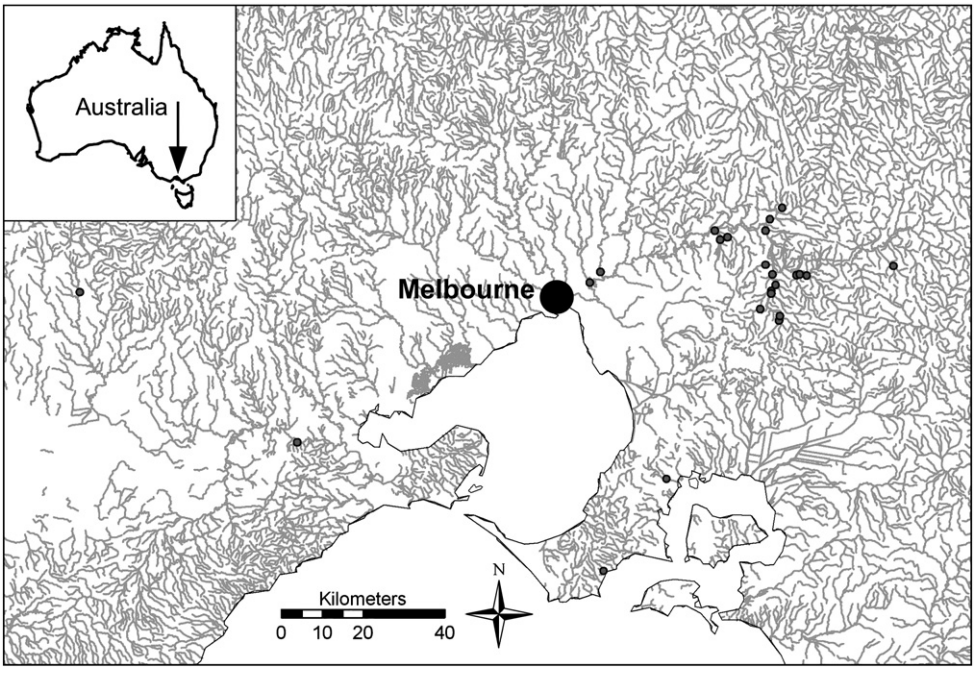

Fig. 1. Location of the sampling sites (small dots) in the stream network in the region around Melbourne (large dot) in Victoria, Australia.

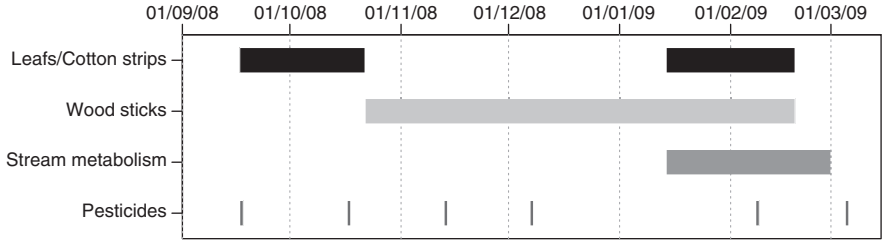

Fig. 2. Schedule for the monitoring of leaf and cotton strip breakdown, wood stick breakdown and stream metabolism as well as pesticide sampling. Bars indicate the period of substrate or logger deployment in the 24 sites. For pesticide sampling, the bars refer to the time points of sediment and water sampling or retrieval of continuous TRIMPS passive samplers (see Schäfer et al., 2011b for details on pesticide sampling).

used to estimate the toxicity in each site in terms of the maximum toxic unit (mTU):

$$\mathrm{mTU} = max_{i=1}^{n}\left(\frac{c_i}{\mathrm{EC50}_i}\right) \quad (1)$$

where c_i is the concentration of pesticide i, $\mathrm{EC50}_i$ is the corresponding 48-h to 96-h median effect concentration for a given standard test species and n is the number of pesticide detections in the site. The standard test species were selected with respect to the organism groups that are involved in the ecosystem functions investigated in this study, which were macroinvertebrates (AOM breakdown), microorganisms (AOM breakdown) and primary producers (GPP). Therefore, *Daphnia magna* and *Selenastrum capricornutum* were selected as standard test species for macroinvertebrates and primary producers, respectively. In addition, the predictions of both species were used as measure for microorganisms, as for the study compounds no sufficient toxicity data for microbial (e.g. *Vibrio fischeri*)

Table 1
Maximum (Max.), minimum (Min.), median, mean, % standard deviation (SD), potential transformations (Trans.) and category for the biotic and environmental variables.

Variable[a]	Min.	Max.	Median	Mean	%SD	Trans./category[b]
$k_{leaves\ invertebrates}$ ($dday^{-1}$)	0.0002	0.0016	0.0008	0.0008	48	n/Ef (AOM)
$k_{leaves\ microorganisms}$ ($dday^{-1}$)	0.001	0.002	0.0016	0.0015	19	n/Ef (AOM)
$k_{cotton\ invertebrates}$ ($dday^{-1}$)	0.00001	0.00026	0.00009	0.0001	82	n/Ef (AOM)
$k_{cotton\ microorganisms}$ ($dday^{-1}$)	0.00007	0.0003	0.00016	0.00017	36	n/Ef (AOM)
$k_{wood\ sticks}$ ($dday^{-1}$)	0.00004	0.00033	0.00017	0.00019	48	n/Ef (AOM)
Ergosterol (μg g^{-1})	0.01	123	3	29	133	log/Ef (Ass.)
Functional microbial richness (after 72 h)	13	26	20	21	18	n/Ef (Ass.)
GPP (mg O_2 L^{-1} d^{-1})	1	44	5	7	147	n/Ef (GPP)
ER (mg O_2 L^{-1} d^{-1})	3	101	19	28	93	n/Ef (ER)
T (°C)	11.9	19.3	15.2	15.4	10	n/PC
pH	6.7	8	7.2	7.3	5	n/PC
EC at 25 °C (μS cm^{-1})	47	3563	181	798	139	log/PC
Dissolved oxygen (% sat.)	22	87	73	72	21	n/PC
NH4[c] (mg L^{-1})	0.2	4.4	0.4	0.7	132	log/PC
NO2[c] (mg L^{-1})	0.001	0.9	0.005	0.098	232	log/PC
NO3[c] (mg L^{-1})	0.1	4	0.48	0.62	131	log/PC
PO4[c] (mg L^{-1})	0.15	21	9	8.6	66	log/PC
Alkalinity (mmol)	0.9	13.4	2	3.7	91	n/PC
Turbidity (NTU)	3.7	18.9	8	9.1	49	n/PC
Depth (m)	0.08	0.87	0.33	0.38	58	n/PC
Current velocity (m s^{-1})	0.01	0.35	0.15	0.16	73	n/PC
PAR (mol m^{-2} d^{-1})	2.71	24.8	8.09	9.12	61	n/PC
Width left bank (m)	3	50	10	15	81	log/Geo
Width right bank (m)	3	35	15	16	65	log/Geo
Pool sections (%)	20	100	96	88	24	n/Habitat
Bedrock (%)	0	23	0	1	345	n/Habitat
Boulder (%)	0	20	0	4	164	n/Habitat
Cobble (%)	0	20	4	7	107	n/Habitat
Pebble (%)	0	25	1	7	133	n/Habitat
Gravel (%)	0	30	6	9	104	n/Habitat
Sand (%)	5	50	15	20	65	n/Habitat
Clay (%)	10	95	44	52	63	n/Habitat
$mTU_{D.\ magna}$	0.0003	0.57	0.008	0.049	242	log/PC
$mTU_{S.\ capricornutum}$	0.00002	1.76	0.0078	0.18	244	log/PC
Recovery section[d]	0	1	0	n	n	n/Geo
Left bank cover[d]	1	5	4	n	n	n/Geo
Right bank cover[d]	1	5	4	n	n	n/Geo
Shading[d]	1	5	3	n	n	n/Habitat
Filamentous algae[d]	0	3	1	n	n	n/Habitat
Total macrophytes[d]	1	4	2	n	n	n/Habitat
Coarse particular organic matter[d]	1	3	2	n	n	n/Habitat

[a] See EPA (2003) for details on the measurement of habitat, geographical and physicochemical variables.
[b] Transformation: n = no; log = log-transformed. Category: Ef = ecosystem function; AOM = allochthonous organic matter; GPP = gross primary production; ER = ecosystem respiration; Ass. = associated with ecosystem function AOM breakdown; PC = physicochemical variable; habitat = habitat variable; Geo = geographical variables.
[c] NH4 = ammonium; NO2 = nitrite; NO3 = nitrate; PO4 = phosphate.
[d] Ordinal variables classifying the prevalence/coverage from 0 (absent) to 5 (very high), except for Recovery section where 1 refers to the presence of undisturbed upstream sections, else 0. See Schäfer et al. (2011b) for details.

standard test species were available. For further details on the compilation of toxicity data and site-specific results for toxic units see Schäfer et al. (2011b).

Environmental parameters were recorded in concert with the pesticide sampling in September, November and February (Fig. 2) and included physicochemical, landscape and habitat variables (Table 1). Temperature, pH, salinity as EC and dissolved oxygen were measured in the field with a TPS FL90 (Brisbane, Australia) water quality metre. Ammonium, nitrite, nitrate and phosphate concentrations were determined on site using a HI 83200 photometer (Hanna Instruments, Melbourne, Australia) with the respective reagents. We used a Hach 2100P turbidimeter (Loveland, USA) to measure turbidity in the field and an Aquamerck (Merck, Melbourne, Australia) test kit to measure alkalinity. Further habitat and landscape variables (Table 1) were recorded with a ruler, visual inspection or maps according to protocols of the Environment Protection Authority (EPA) Victoria (EPA, 2003). Summary statistics for all environmental variables are given in Table 1, site-specific information are reported in Schäfer et al. (2011b).

2.3. Determination of AOM breakdown rates

We determined the breakdown of leaves, cotton strips and wood sticks in order to compare different methods that have been suggested for the assessment of AOM breakdown (Tiegs et al., 2007; Young and Collier, 2009). For leaf breakdown, *Eucalyptus camaldulensis* leaves from a locally common riparian tree that were prior to abscission were collected in spring and oven-dried (48 h at 60 °C). Approximately 2.5 g of dried leaves was placed into coarse polyethylene mesh bags (mesh size: app. 6 mm; bag size: 20×20 cm) and into fine cylindrical nylon bags (mesh size: 50 μm; cylinder length: 15 cm). Leaves in the coarse bags were accessible to invertebrates and microorganisms, whereas leaves in the fine bags were accessible for microorganisms only and served as control for microbial degradation and leaching (Gessner and Chauvet, 2002). The fine bags consisted of two separate sections, where one section contained the 2.5 g of leaves and the second section contained leaves that were used to estimate the fungal biomass and functional groups of microorganisms according to the carbon sources metabolised by them (see below). Triplicate coarse and fine bags were deployed in each site approximately 10 cm above the stream bed so that they touched the bottom. The bags were retrieved after approximately 5 weeks (Fig. 2). The remaining litter was carefully taken out, washed to remove deposits, oven-dried at 60 °C (48 h), reweighed and averaged for each type of bag for every site. To correct for handling losses three coarse and fine bags were treated the same way as the others but returned immediately to the laboratory after a brief immersion in the stream. Physical abrasion may also contribute to leaf breakdown but was not measured. However, a study on streams in the same region reported only minor influence (3–7%) of physical abrasion on the leaf weight mass loss (Imberger et al., 2008).

For cotton strip breakdown, unbleached standardised cotton was obtained from EMPA (St. Gallen, Switzerland), cut into 5×10 cm strips and autoclaved for 1 h at 120 °C. Subsequently, one cotton strip was placed in the coarse bags and in each section of the fine bags. After retrieval the cotton strips were cleaned, soaked in 70% ethanol for a few minutes to inhibit microbial decay during storage, air dried and stored at −18 °C. Three strips were treated the same way as the others but returned immediately to the laboratory after a brief immersion in the stream to serve as control for handling losses. Tensile strength was measured using an Instron Series IX Tensiometer (Instron, Melbourne, Australia) after cutting 1×5 cm strips from the centre of the cotton strips. The instrument parameters were: 3 mm s^{-1} crosshead speed, 23 °C temperature and 50% relative humidity.

Birch wood ice cream sticks (length: 12 cm, width: 1 cm, depth: 0.2 cm) were used to determine wood breakdown. They were weighed after drilling a hole in the stick that allowed for securing with wire. In each sampling site four sticks were tied to the stream bottom using a metal peg in the same spot where the coarse and fine bags were deployed. The sticks were retrieved after approximately 3.5 months, cleaned, oven-dried at 60 °C (24 h) and re-weighed. Triplicate wood sticks were treated as outlined but returned immediately to the laboratory after a brief immersion in stream water to correct for handling losses. A disadvantage of wood sticks is their long deployment period (Fig. 2) that is owed to the much lower breakdown rate in comparison with leaves or cotton (Webster et al., 1999). In our study this lead to major losses of samples resulting in the recovery of only 9 samples after the deployment period (Table 2), thus decreasing the statistical power to detect relationships with environmental gradients.

Temperature loggers (Hobo Pendant, Onset, Pocasset, USA) with hourly temperature recording were deployed in concert with the bags and wood sticks. The temperature data was used for the calculation of the sum of degree days (dday) for the deployment period of leaves, cotton strips and wood sticks in order to standardise the breakdown rates for temperature. The breakdown rate k for each of the three substrates in a site i was calculated based on the exponential mass loss or tensile strength loss per dday:

$$k_i = \frac{-\ln\left(\frac{S_i(t)}{S_i(0)}\right)}{\sum_{j=1}^{t} \overline{T_i}(j)} \quad (2)$$

where S is the mass or tensile strength as a function of the deployment time, t being the total number of deployment days and T is the mean temperature for a day j. $S_i(t)$ was corrected for handling losses. In

Table 2
Environmental variables (see Table 1 for full variable names) with highest explanatory power for biotic response variables with % contribution in hierarchical partitioning, r^2 and Bayesian Information Criterion (BIC) for the best-fit model and sample size n.

Response variable	$mTU_{D.\ magna}$[a]	$mTU_{S.\ capri.}$[a,b]	EC (μS cm^{-1})[a]	Sand (%)	PO4[d] (mg/L)[a]	T (°C)	r^2	BIC	n
$k_{leaves\ invertebrates}$	40		60				0.67	−378	23
$k_{leaves\ microorganisms}$[c]		32 (35)	68 (65)				0.74 (0.56)	−380 (−384)	22 (23)
$k_{cotton\ invertebrates}$[c]	41 (52)				59 (48)		0.63 (0.38)	−433 (−436)	22 (23)
$k_{cotton\ microorganisms}$		47		53			0.44	−450	23
$k_{wood\ sticks}$									9
Functional microbial richness[c]	100						0.44 (0.30)	28 (38)	14 (15)
Ergosterol concentration[a,c]			100				0.74 (0.61)	1 (6)	14 (15)
GPP[c]						100	0.59 (0.41)	23 (68)	14 (16)
ER									16

[a] Variable log-transformed in linear model.
[b] *capri.* = *capricornutum.*
[c] Values in brackets give the result for inclusion of observations that exhibited unduly influence according to Cook's distance.
[d] PO4 = phosphate.

addition, $S_i(t)$ of leaves and cotton strips in coarse bags was corrected for losses due to microbial degradation and leaching in site i to determine the contribution of invertebrates to breakdown (for details see Benfield, 2007).

2.4. Estimation of fungal biomass and microbial carbon source use

We determined the fungal biomass and the richness of carbon source use by microorganisms (in the following called functional microbial richness) to allow for an attribution of potential effects on AOM breakdown to changes in the microbial community (Hieber and Gessner, 2002; Stefanowicz, 2006). Leaf-associated fungal biomass was estimated by measuring ergosterol, which is a component of the fungal cell membrane. This was done according to a method developed by Gessner and Schmitt (1996) using leaves from the second section of the fine bags. Briefly, ergosterol was extracted from the leaves in 10 mL alkaline methanol at 80 °C for 0.5 h and then purified by solid-phase extraction using 500 mg Sep-Pac® Vac RC tC18 cartridges (Waters, Eschborn, Germany). Separation of ergosterol was done on an Agilent 1200 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) equipped with a LiChrospher 100 RP18 column (CS-Chromatographie Service, Langerwehe, Germany). Subsequently, ergosterol was measured at a wavelength of 282 nm with an ultraviolet detector and quantified using a standard curve prepared with the respective chemical standard (Fluka, purity 97.8%).

The carbon source use of the microbial community was assessed using 96-well Biolog EcoPlates™ (Biolog, CA, USA) that consisted of triplicated 31 different carbon sources and water blanks (Appendix Table A.2) (Garland, 1996; Stefanowicz, 2006). For each site, 10 g of wet leaves, the cotton strip from the second section of the fine bags and one randomly selected wood stick were placed in a sterile 250 mL glass bottle containing 90 mL of the maximum recovery diluent CM0733 (Oxoid, Adelaide, Australia) and 10 g of glass beads. The bottle was placed on a rotary shaker and mixed at 400 rpm for 4 min. 100 µL samples of the supernatant were inoculated into each well of the Ecoplate™. Subsequently the plates were incubated at 20 °C for 72 h. Absorption was measured at 595 nm in a plate reader. For each site, the absorption was corrected by subtraction of the absorption from (1) the water blank and (2) the respective carbon source for control leaves, cotton strips and wood sticks that were not deployed. Carbon sources with a statistically significant higher absorption than the water blank of the respective plate in Dunnett's test were considered as being used by microorganisms. The p-values in Dunnet's test were adjusted for multiple testing according to a method developed by Benjamini and Hochberg (1995). The number of carbon sources used by microorganisms were summed per site and used as variable in data analysis.

2.5. Estimation of stream metabolism

Stream metabolism was estimated with the single-station open-channel method as outlined in Grace and Imberger (2006) and Young and Collier (2009). This method relies on the continuous measurement of dissolved oxygen (DO) concentrations over a minimum period of 24 h at a site. In our study, we used three D-Opto (Zebratech, Nelson, New Zealand) DO loggers that were circulated between the sampling sites from middle of January to end of February 2009 (Fig. 2). In each site, DO loggers were installed in the middle of the water column and DO was measured in 10 minute intervals for a period of at least 72 h. An Odyssey photosynthetically active radiation (PAR) recording system 64 k (Dataflow Systems, Christchurch, New Zealand) was attached to each DO logger and recorded PAR every 10 min. Sites were monitored for at least one cloudless day to minimise variability due to differences in light exposure. Calculation of GPP and ER from the DO concentrations was based on the R software package StreamMetabolism (Sefick, 2009). This package computes temperature corrected GPP and DO from the diel oxygen curve for the open station method and requires the re-aeration coefficient K as well as temperature and oxygen as input data. In the current version of this software package (Sefick, 2009), K was estimated from the empirical O'Connor Dobbins surface renewal method that relies on hydromorphological parameters (O'Connor and Dobbins, 1958). Given the uncertainties related to methods based on hydromorphological parameters (Aristegi et al., 2009), we also implemented the nighttime regression method to estimate K (Grace and Imberger, 2006; Owens, 1974) (see Appendix B). The nighttime regression did not yield statistically significant regression estimates ($p>0.05$) of K for 9 of the 16 sites that were monitored (see Section 2.1), which is higher than in another study that did not find significant regression estimates for only 3 out of 18 sites (Aristegi et al., 2009). This may be explained by the fact that 6 of the 9 sites where the nighttime regression method failed were low productivity sites (GPP$\approx$1 mg O_2 L^{-1} d^{-1}), which presumably had insufficient variation in the oxygen deficit and change in oxygen concentration (Grace and Imberger, 2006). Indeed, the sites in our study with a GPP <2.4 mg O_2 L^{-1} d^{-1} did not yield a significant nighttime regression (Appendix Table A.1). However, since for sites with statistically significant nighttime regression the estimated reaeration coefficients of the nighttime regression method and the O'Connor Dobbins method were in reasonable agreement ($r^2=0.8$; Appendix Fig. A.1), the O'Connor Dobbins method was used to estimate K for all 16 sites.

2.6. Data analysis

Before analysis, the data for all variables was aggregated per site using the mean, as the sampling periods of the different methods differed (Fig. 2). Variables with a wide spread of values (maximum/minimum observation >100) or a very skewed distribution (checked visually) were log-transformed (Table 1). Linear models were used to examine the relationship between environmental variables and the response variables related to ecosystem functions, fungal biomass and functional microbial richness. We employed automatic model building to identify the environmental variables with the highest explanatory power for the respective response variable. Automatic model building started with the null model (no explanatory variable included) and used backward and forward entering variables with the Bayesian Information Criterion (BIC) as goodness-of-fit measure to identify the best-fit linear model. In addition, manual model building was used to check the results of the automatic modelling procedure (Sheather, 2009). Here, we started with models based on expert judgement and used the t-test for testing the significance of individual variables and the partial F-test for testing for significant differences during model simplification. However, automatic model building and manual model building lead to identical best-fit models.

Statistical models were checked for error assumptions (constant variance, non-correlation and normality of residuals) and unusual observations (leverage, outliers) (Sheather, 2009). A variable cluster analysis was conducted before modelling to identify pairs of abiotic variables with high intercorrelation (Pearson's $r>0.7$), where the variable with lower relevance for the respective response variable was omitted based on expert judgement. Hierarchical partitioning was used to determine the independent explanatory power of environmental variables in the best-fit models (Chevan and Sutherland, 1991; Grömping, 2006). To assess the relationship of the response variables with estimated pesticide toxicity and salinity, we built linear models for every response variable with each mTU and conductivity as explanatory variables. In addition, linear models with both explanatory variables and their interaction term were constructed to test for a potential interaction of pesticides and salinity.

Nonmetric Multidimensional Scaling (NMDS) (function metaMDS in the R package “vegan” (Oksanen et al., 2010)) was employed to

examine the similarity in microbial carbon source use between sites. The Sorensen index (Sorensen, 1948) was selected as similarity measure and a two-dimensional NMDS was started a maximum of 20 times with random configurations to find the global solution. The model with the lowest stress-value was regarded as the best-fit model. Since NMDS is an unconstrained ordination method, environmental variables were fitted afterwards (function envfit in the R package "vegan" (Oksanen et al., 2010)) in order to explore the relationship between gradients in microbial carbon source use and environmental variables. The fitted variables were selected based on the significant correlation ($p<0.05$) with the ordination that was assessed using 10,000 random permutations. All statistical computations and graphics were created with the open source software package R (www.r-project.org) using version 2.12.1 (for Mac OS X, 10.6.6) (R Development Core Team, 2011).

3. Results

3.1. Relationship between environmental variables and biotic endpoints

Of all the 32 environmental variables (Table 1), six mainly physicochemical variables such as the estimated pesticide toxicity ($mTU_{D.\ magna}$ and $mTU_{S.\ capricornutum}$), EC or phosphate concentrations were selected as best predictors for the different biotic endpoints (Appendix Table A.1), whereas habitat and geographical variables had minor explanatory power (see Table 2 for linear models, Fig. 3 for NMDS). Variation in leaf breakdown and the associated endpoint fungal biomass and functional microbial richness were best explained by the estimated pesticide toxicity and salinity in terms of EC (Table 2). For cotton strip breakdown, the % of sand in the habitat as well as nutrients in the form of phosphate concentrations exhibited strong explanatory power beside the estimated pesticide toxicity (Table 2). No reasonable linear model could be established for the breakdown of wood sticks in either manual or automatic model building. However, with only 9 sites due to losses of sticks at some sites, the statistical power was reduced (Appendix Table A.1). The physicochemical variables $mTU_{D.\ magna}$, EC, temperature and turbidity exhibited the closest correlation with the two-dimensional NMDS ordination for the carbon source use of microorganisms (Fig. 3). The ecosystem function of GPP displayed the highest correlation with temperature and no other variable was included in the best-fit model. For ER, no model was found with a good fit (Table 2).

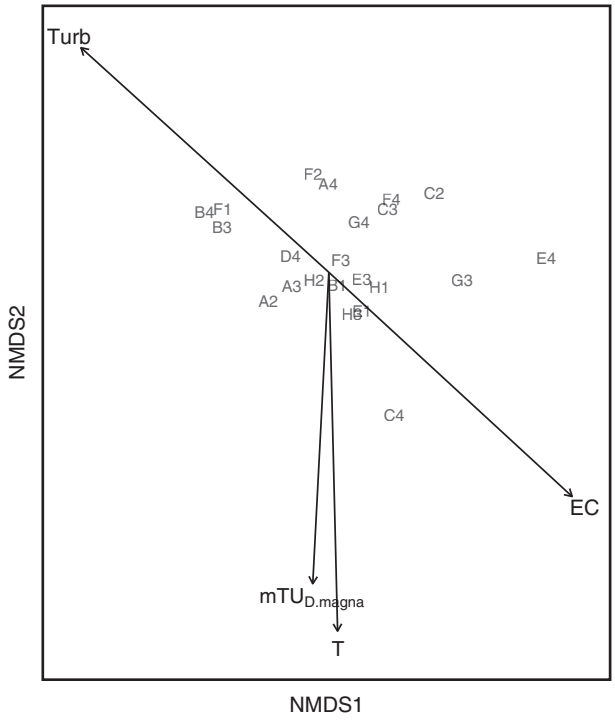

Fig. 3. Two-dimensional nonmetric multidimensional scaling for the carbon source use of microorganisms colonising AOM in the sampling sites with fitted environmental variables that exhibited a significant correlation with the gradients ($p<0.05$). The stress value was 9.5%. The r^2 after 10,000 permutations for log-transformed electrical conductivity (log EC), temperature (T), log-transformed maximum toxic units for *D. magna* ($mTU_{D.\ magna}$) and turbidity was 0.53 ($p=0.007$), 0.65 ($p=0.006$), 0.55 ($p=0.008$) and 0.49 ($p=0.02$), respectively. The substrates related to the carbon source codes are given in Appendix Table A.2. The following carbon sources are not displayed due to overlap (overlapping carbon source given in brackets): D2, D3 and H4 (B3); E2 and G1 (A3); G2 (A2); B2 (E3).

3.2. Influence of estimated pesticide toxicity and salinity on biological endpoints

The estimated pesticide toxicity and salinity as EC explained considerable parts (r^2 ranging from 0.13 to 0.48) of the variation in biotic variables related to the ecosystem function AOM breakdown except for wood stick breakdown (Appendix Table A.3). The leaf breakdown exhibited a stronger relationship (r^2 values between 0.04 and 0.44 greater for leaf breakdown than for cotton breakdown) with the $mTU_{D.\ magna}$, $mTU_{S.\ capricornutum}$ and EC than cotton strip breakdown (Fig. 4, Appendix Fig. A.2). Variables associated with AOM breakdown such as fungal biomass (ergosterol) and functional microbial richnessin most cases displayed a reasonable relationship (r^2 values between 0.08 and 0.74) with $mTU_{D.\ magna}$ and EC, respectively (Appendix Table A.3, Fig. A.3). Similarly, $mTU_{D.\ magna}$ and EC correlated significantly with the NMDS for the carbon source use of microorganisms colonising AOM (Fig. 3). Neither GPP nor ER displayed a linear or non-linear relationship with estimated pesticide toxicity or EC (Appendix Fig. A.4, Table A.3). For linear models that contained both mTU and EC, the inclusion of an interaction term for both variables was not statistically significant (all $p>0.35$).

4. Discussion

4.1. Predictors of AOM breakdown and associated biotic endpoints

The most important variables to explain the variation in the AOM breakdown and associated endpoints (fungal biomass, functional microbial richness and carbon source used by microorganisms) were estimated pesticide toxicity, salinity, percentage of sand in the habitat, phosphate, temperature and turbidity (Table 2, Fig. 3). The relevance of environmental factors such as temperature or nutrient concentrations for AOM breakdown rates is well established (Imberger et al., 2010; Tank et al., 2010; Webster and Benfield, 1986). Furthermore, sedimentation can affect the AOM breakdown (Blasius and Merritt, 2002; Imberger et al., 2010) and the variables' percentage of sand in the habitat and turbidity may represent a proxy for this stressor.

Much less is known on the importance of anthropogenic stressors in general and pesticides and salinity in particular on AOM processing. Although several field studies have investigated the general impact of agricultural land-use on leaf breakdown in streams (Hagen et al., 2006; Magbanua et al., 2010; Piscart et al., 2009; Schäfer et al., 2007), only one of these studies quantified pesticide exposure (Schäfer et al., 2007). Schäfer et al. (2007) reported a 2.5 fold reduction of leaf breakdown in streams subject to highest pesticide exposure and this effect size matches well with the reduction observed in the present study (Fig. 4). However, the three- to four-fold reduction in leaf breakdown in our study was equally attributed to estimated pesticide toxicity and salinity (Table 2, Fig. 4, Appendix Fig. A.2). We therefore suggest that the effects of salinity and pesticides were additive, as firstly, both stressors were not significantly correlated (Pearson's $r=0.23$, $p=0.28$, $n=23$) so that collinearity played no role, i.e. each stressor had an independent effect (Appendix Fig. A.6). Secondly, no

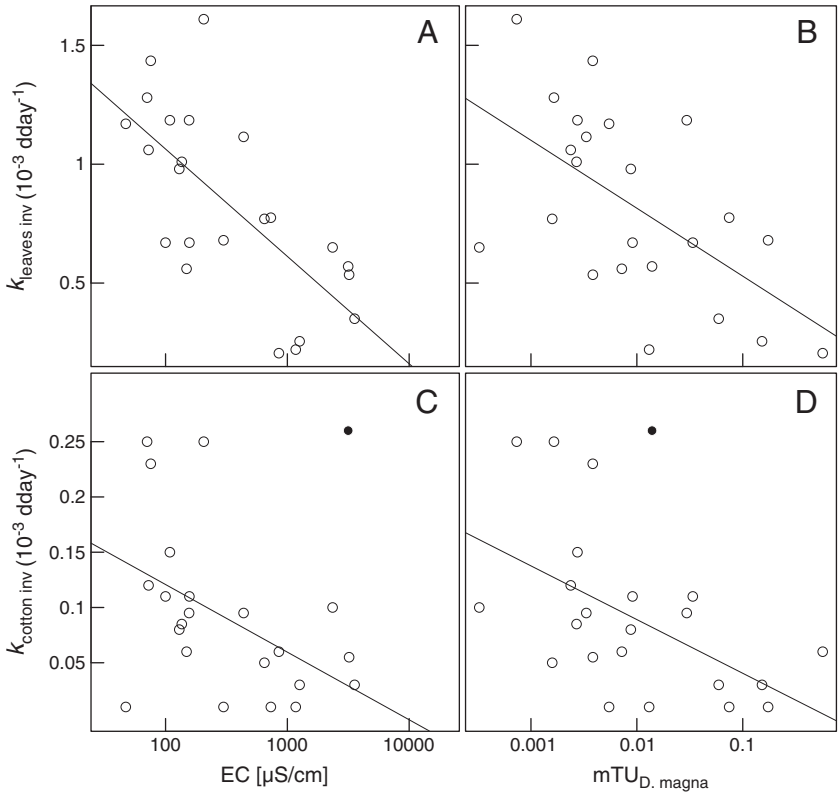

Fig. 4. Relationship of salinity (A, C) in terms of electrical conductivity (EC) and estimated pesticide toxicity (B, D) in terms of maximum toxic units for *D. magna* ($mTU_{D.\ magna}$) with the breakdown of leaves (k_{leaves})(A, B) and cotton strips (k_{cotton})(C, D) by invertebrates (inv) per degree day (dday). Log EC and log $mTU_{D.\ magna}$ explained 48% and 34% variation in $k_{leaves\ inv}$, respectively. For the variation in $k_{cotton\ inv}$, log EC and log $mTU_{D.\ magna}$ explained 23% and 30%, respectively (6% and 22% when including a point (filled dot) that unduly influenced the linear model according to Cook's distance).

statistically significant interaction ($p>0.35$) between the stressors was found, and this is in agreement with another study on joint effects of pesticides and salinity on macroinvertebrate communities in streams of southeast Australia (Schäfer et al., 2011a).

Both $mTU_{D.\ magna}$ and $mTU_{S.\ capricornutum}$ were included in linear models for AOM breakdown. Since the macroinvertebrate-related AOM breakdown showed a much stronger relationship to $mTU_{D.\ magna}$ than to $mTU_{S.\ capricornutum}$ (Appendix Table A.3, Fig. 4), this suggests direct effects of pesticides on macroinvertebrates that translated to effects on ecosystem functions. This hypothesis is also supported by a companion study that identified estimated pesticide toxicity as a dominant stressor for the structure of macroinvertebrate communities in the streams investigated here (Schäfer et al., 2011b). For microorganisms the situation is ambiguous. Firstly, there is no standard test species for microorganisms and hence no toxicity data was available to calculate microorganism-specific toxic units for all chemicals measured in the present study. Therefore, we used *D. magna* and *S. capricornutum* as surrogates, though their validity is uncertain. AOM breakdown by microorganisms showed a stronger relationship with $mTU_{S.\ capricornutum}$ than with $mTU_{D.\ magna}$ (Appendix Table A.3). This may either indicate direct effects on microorganisms, under the assumption that $mTU_{S.\ capricornutum}$ represents a valid surrogate, but could otherwise indicate indirect effects from alterations in primary producers that interact with the leaf-associated microbial communities (Franken et al., 2005). However, $mTU_{S.\ capricornutum}$ was not a major explanatory variable for the fungal biomass, functional microbial richness or the similarity in carbon source use of microorganisms (Appendix Table A.3, Fig. 3). By contrast, the functional microbial richness and the similarity in carbon source use responded to $mTU_{D.\ magna}$ (Fig. 3, Appendix Fig. A.3). In addition, the companion study did not find a link to potential effects on the density of different major groups of microorganisms (bacteria, flagellates, ciliates, amoebas, nematodes, and gastrotrichs) (Schäfer et al., 2011b), though this may not exclude changes in more sensitive microbial endpoints (Widenfalk et al., 2008). To sum up, it remains unclear, whether the reduction of the microbial breakdown is a direct effect from pesticide exposure or an indirect effect from the alteration of the community of primary producers (Franken et al., 2005).

We compared the performance of three different methods when used to determine the breakdown of AOM: leaf bags, cotton strips and wood sticks. Both leaf bags and cotton strips identified the explanatory variable estimated pesticide toxicity as of high importance, whereas wood sticks did not yield a reasonable relationship with any environmental variable (Appendix Fig. A.5). The wood stick breakdown rates were very similar to those reported in other studies, which also found no (Clapcott et al., 2010) or no clear (Young and Collier, 2009) relationship of wood stick breakdown with different stressor gradients.

Cotton strips have been employed as a substrate that is more standardised than leaves to determine AOM breakdown (Fritz et al., 2011; Tiegs et al., 2007). In agreement with the study of Tiegs et al. (2007) cotton strip and leaf breakdown in coarse bags were significantly correlated (invertebrates: Pearson's $r=0.58$, $p=0.004$, $n=23$; microorganisms: Pearson's $r=0.23$, $p=0.29$, $n=23$). In addition, they responded similarly to estimated pesticide toxicity (Fig. 4, Appendix Fig. A.2), albeit the response was weaker for cotton strips. However, while the variation in leaf breakdown and fungal biomass on leaves also correlated strongly with salinity in terms of EC, the cotton strip breakdown responded to percentage of sand in the habitat and phosphate concentrations (Table 2). In a study on cotton strip breakdown in 12 streams, cotton strips also responded strongest to sedimentation and phosphorus concentrations and not to specific land-use patterns (Imberger et al., 2010). Leaf breakdown may

represent the most sensitive indicator for effects of pesticides and salinity on the ecosystem function of AOM breakdown, while cotton strips may be more sensitive to gradients in nutrients or sedimentation.

The response of leaf breakdown to the logarithm of EC and mTU_{DM} was linear and therefore no obvious effect threshold was apparent (Fig. 4). Nevertheless, the leaf breakdown rate for sites with EC>1000 and mTU_{DM}>0.01 was reduced compared to sites with lower exposure (Fig. 4). Studies in southeast Australia on the response of the macroinvertebrate community structure to salinity and pesticides showed considerable community change when salinity levels exceed approximately 500–1000 μS/cm (Kefford et al., 2010a,b; Schäfer et al., 2011a). For pesticides, field and mesocosm studies in central European regions and southeast Australia reported adverse effects on the macroinvertebrate communities for mTU_{DM} exceeding between 0.001 and 0.01 (Beketov et al., 2008; Liess and von der Ohe, 2005; Schäfer et al., 2007, 2011b). These results indicate that salinity and pesticides trigger change in structural and functional endpoints at similar levels, hence suggesting that there is not much functional redundancy in the communities for these stressors (Rosenfeld, 2002). However, additional field studies are needed to confirm these results.

4.2. Relationship of stream metabolism with estimated pesticide toxicity and salinity

Stream metabolism comprises the ecosystem functions of GPP and ER and was determined in this study to elucidate potential effects of pesticides and salinity. More specifically, we hypothesised that an increase in estimated pesticide toxicity for primary producers in terms of $mTU_{S.\ capricornutum}$ would result in a decreasing GPP. The values for GPP of our streams ranged from 1 to 12 mg O_2 L^{-1} d^{-1}, except for one outlier with a value of 44 mg O_2 L^{-1} d^{-1} (Appendix Fig. A.4, Table A.1). These values are well within the range given for streams in Victoria, Australia (0.2–50 mg O_2 L^{-1} d^{-1}) (Grace and Imberger, 2006). Furthermore, the values for ER (3–101 mg O_2 L^{-1} d^{-1}) (Table 1) also corresponded well to those (8–100 mg O_2 L^{-1} d^{-1}) reported in the same publication. In the present study temperature was a main predictor for GPP (Table 2) and the same holds for a modelling study of Marcarelli et al. (2010) on ecosystem metabolism in a fifth-order river. However, the ecosystem functions of GPP and ER showed no relationship to estimated pesticide toxicity or to the salinity gradient (Appendix Fig. A.4).

To our knowledge, no other study has investigated the effects of pesticides and salinity on stream metabolism. Nevertheless, four studies have examined the effect of agricultural land-use on stream metabolism (Bernot et al., 2010; Gücker et al., 2009; McTammany et al., 2007; Young and Collier, 2009). Three of which reported an increase of GPP in agricultural streams that ranged from twofold to sixfold in comparison with reference streams (Bernot et al., 2010; Gücker et al., 2009; McTammany et al., 2007). By contrast, there was no statistically significant relationship between a land-use gradient that included agriculture and GPP in a study on 15 streams in New Zealand (Young and Collier, 2009). Regarding the general impact of stressors on GPP, a study on 213 sites proposed thresholds for non-impacted, lightly impacted and highly impacted sites at GPP<3.5, 3.5<GPP<7 and GPP>7, respectively (Young et al., 2008). When classifying the sites of our study according to these classes, the resulting groups were not statistically significantly different in their $mTU_{S.\ capricornutum}$ (ANOVA with F-test, $p=0.57$, $n=16$) or salinity in terms of EC (ANOVA with F-test, $p=0.19$, $n=16$). Overall, pesticides and salinity did not exhibit a major influence on GPP or ER in our study, despite measured levels of $mTU_{S.\ capricornutum}$ that would lead to acute mortality of the green algae *S. capricornutum* in the laboratory (Table 1). This may be explained firstly by a pollution-induced shift in the community of primary producers that did not compromise the ecosystem functions of GPP and ER due to functional redundancy (Rosenfeld, 2002). Future studies should examine this hypothesis by determining the community tolerance along a gradient of $mTU_{S.\ capricornutum}$, where a positive correlation between the community tolerance and $mTU_{S.\ capricornutum}$ would be expected according to the pollution-induced community tolerance concept (Blanck and Dahl, 1996; Blanck and Wangberg, 1988). Second, (1) the natural variability in GPP and ER due to groundwater inputs or hyporheic flows (Hall and Tank, 2005) and (2) uncertainty associated with the measurement (e.g. stream metabolism measured not simultaneously in all sites) and calculation (e.g. different methods available, see Aristegi et al. (2009)) of GPP and ER may have prevented identification of the effects of stressors. A third explanation would be that contrasting effects of different stressors in agricultural streams cancelled each other out (Clapcott et al., 2010). For example, the inhibition of GPP by pesticides may be compensated by stimulation of primary producers by nutrients. Further studies are required to examine the validity of these three explanations.

4.3. Relevance of the observed effects for ecosystem services

A first step for an ecological risk assessment based on ecosystem services is the identification of the relevant ecosystem services for a certain environmental compartment (e.g. freshwaters, soil), which is followed by the derivation of suitable and measurable endpoints in the second step (see Nienstedt et al., 2012). Based on the Millennium Ecosystem Assessment (MEA, 2005), Harrison et al. (2010) compiled an extended list of ecosystem services relevant for freshwater ecosystems. According to this list, freshwater ecosystems play a key role in the provision of food, energy, water and genetic resources and in the regulation of water flow and water purification. Finally, they deliver several cultural services such as education, recreation, aesthetic values and sense of place (Harrison et al., 2010). The ecosystem functions investigated in our study are central for the delivery of energy, in terms of food, to freshwater ecosystems. A reduction of energy processing, for example by impediment of AOM breakdown, will primarily translate to a lower carrying capacity i.e. less biomass in the system, and therefore affect the provision of food to humans e.g. fish (Wipfli, 2005; Wipfli and Baxter, 2010). In addition, the ecosystem service of water purification may also be affected if the biomass of species involved in this ecosystem service decreases, whereas effects on other of the abovementioned services are dependent on whether an alteration of the composition of the freshwater community occurs. However, effects of pesticides and salinity on ecosystem functions were only shown for AOM breakdown in the present study and this energy source is mainly important for first- to fifth-order streams (Vannote et al., 1980; Webster, 2007). Future studies should elucidate whether especially herbicides have any effect on the GPP or whether they are masked by stimulatory effects of nutrients. Furthermore, to quantify the effects on ecosystem services on the landscape or regional level (as for example Rutgers et al., 2012 for soil ecosystem services) data on the spatial extent of pesticides and salinity effects is required. For pesticides, the spatial and temporal dynamic of effects on ecosystem functions such as AOM breakdown in the field is largely unknown which is owed to the episodic (days) exposure (Schäfer et al., 2011c). By contrast, for salinity the exposure is relatively constant over time scales from days to months (McNeil and Cox, 2007) and the spatial extent can be delineated much easier. Moreover, the trophic linkages between macroinvertebrates and fish should be further explored to enable quantification of the dietary relevance of macroinvertebrate biomass and consequently the effects of their reduction (Wipfli and Baxter, 2010). Beside further field studies, ecological modelling may prove useful here, to extrapolate results to higher levels of ecological and spatial organisation and to guide the operationalisation of ecosystem services in research projects (see Galic et al., 2012). Finally, previous studies have demonstrated that landscape patterns such as undisturbed upstream sections can alleviate episodic disturbances on

macroinvertebrate communities (Hatakeyama and Yokoyama, 1997; Liess and von der Ohe, 2005; Schäfer et al., 2007; von der Ohe et al., 2009). Whether undisturbed upstream sections also prove beneficial for AOM breakdown in impacted stream reaches is therefore a question that may, among other measures, foster environmental risk assessment.

Supplementary materials related to this article can be found online at doi:10.1016/j.scitotenv.2011.05.063.

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