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### The landscape drives the stream

*Unraveling ecological mechanisms to improve restoration*

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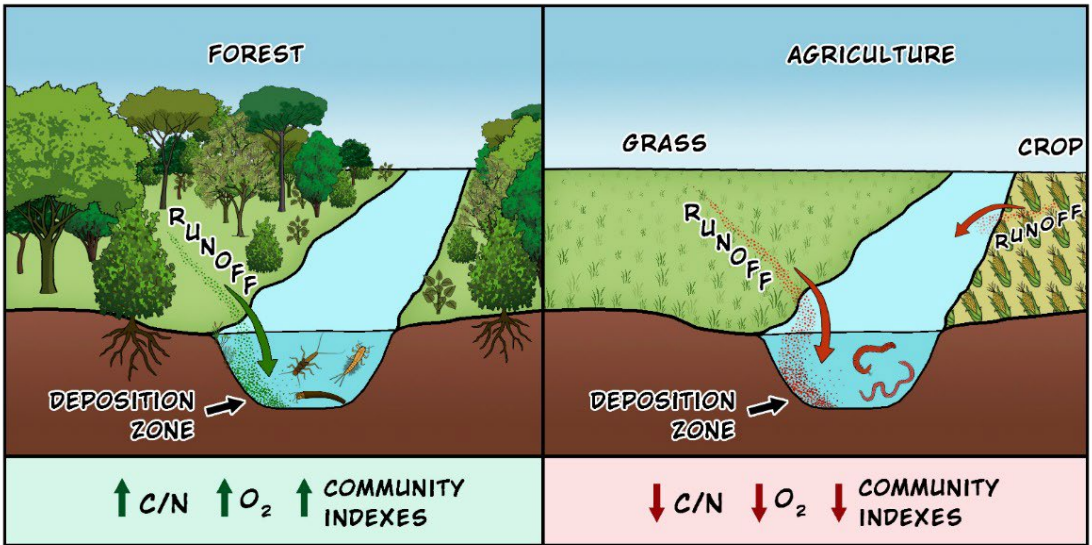
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# Chapter 3

## Sediment composition mediated land use effects on lowland streams ecosystems

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Author contributions: PCRO, PFMV and MK designed the experiment. PCRO and SN conducted the experiment. PCRO analyzed most of the data, and wrote most of the manuscript together with SN, PFMV and MK. PFMV, MK and HG advised on practical issues during the course of the experiment and data processing and contributed to editing and revising draft versions of the manuscript.

### Highlights

- Instream deposition zone sediment composition is land use specific.
- Agricultural land use affects streams at the species, community and ecosystem level.
- Agricultural land use effects are linked to lower C/N ratios and higher SOD levels.
- Stream deposition zone sediment C/N ratio reflects runoff sediment C/N ratio.
- Agriculture affects stream via altered food quality and sediment oxygen demand.

### Abstract

Despite the widely acknowledged connection between terrestrial and aquatic ecosystems, the contribution of runoff to the sediment composition in lowland stream deposition zones and the subsequent effects on benthic invertebrates remain poorly understood. The aim of this study was therefore to investigate the mechanisms by which runoff affects sediment composition and macroinvertebrates in deposition zones of lowland stream ecosystems. To this end, sediment from runoff and adjacent instream deposition zones from streams with different land use was chemically characterized and the biological effects were assessed at the species, community and ecosystem level. Runoff and deposition zone sediment composition as well as biological responses differed clearly between forest and agricultural streams. The stream deposition zone sediment C/N ratio reflected the respective runoff sediment composition. Deposition zones in the forest stream had a higher C/N ratio in comparison to the agricultural streams. Growth of *Hyalella azteca* and reproduction of *Asellus aquaticus* were higher on forest stream sediment, whereas chironomids and worms suffered less mortality on the agricultural sediments containing only natural food. The forest stream deposition zones showed higher values for indices indicative of biological integrity and had a lower sediment oxygen demand. We concluded that agricultural land use affects lowland stream ecosystem deposition zones at the species, community and ecosystem level via altered food quality (C/N ratio) and higher oxygen demand of the sediment.

**Key words:** deposition zone, runoff, C/N ratio, macroinvertebrates, sediment respiration, food quality.

## 1. Introduction

The strong connection between terrestrial and aquatic ecosystems is a central issue in understanding ecological processes in freshwater environments (Allan, 2004; Palmer, 2010; Ward, 1998; Wiens, 2002). Stream ecosystems receive allochthonous detritus, wood debris and sediment-bound chemicals from the adjacent terrestrial environment (Cummins and Klug, 1979; Jordan et al., 1997; Schriever and Liess, 2007). These allochthonous materials represent a key source of resources for stream food webs, supporting biodiversity and ecosystem services (Dodds, 2002; Bunn et al., 1999; Rosi-Marshall et al., 2016; Tank et al., 2010; Vannote et al., 1980; Webster et al., 1999). Yet, intense agricultural activities may lead to a lower input of coarse particulate organic matter and to increased concentrations of nutrients in the receiving streams (Bernhardt et al., 2017; Collins and Anthony, 2008). This may cause changes in instream detritus and sediment quality (Allan, 2004; Ekholm and Krogerus, 2003; MacDonald et al., 2001; Rabení et al., 2005), which is posing pressure on freshwater ecosystems worldwide (Leal et al., 2016; Wood et al., 2005).

Once allochthonous material reaches a stream, it may partly accumulate in deposition zones (Callisto and Graça, 2013; Pusch et al., 1998; Haan et al., 1994; Golladay, 1987). Previous studies argued that alterations in the input of fine particles in stream deposition zones often lead to changes in the amount and type of organic matter, nutrient dynamics (Jordan et al., 1997; Stelzer et al., 2014), oxygen concentration (Jones et al. a, 2012) and the presence of potentially toxic substances (Jones et al. a, 2012; Larsen and Ormerod, 2010). Due to these changes, organisms inhabiting sediment deposition zone, such as benthic invertebrates, are confronted with altered food quality (Graham, 1990; Jones et al. a, 2012; Lenat and Crawford, 1994; Parkhill and Gulliver, 2002; Rowe and Dean, 1998), and physico-chemical conditions, such as oxygen concentration (Larsen and Ormerod, 2010; McDonald et al., 1991; Zweig and Rabeni, 2001; Von Bertrab et al., 2013).

Despite the well documented qualitative effects of land use on stream ecosystem structure and functioning (Jones et al. a, 2012; Rabení et al., 2005; Schriever and Liess, 2007), the contribution of altered runoff to sediment composition in deposition zones and the subsequent effects on benthic invertebrates still remains poorly understood (Bernhardt et al., 2017; Larsen et al., 2009; (Kefford et al., 2010); Larsen and Ormerod, 2010; Von Bertrab et al., 2013; Zweig and Rabeni, 2001; Allan et al., 1997). This knowledge gap is even greater in lowland streams, where the runoff

from the surrounding land may be less frequent, but more intense due to the accumulation of high amounts of nutrients on the upper layer of the soil in flat areas (Stieglitz et al., 2003), leading to accumulation of contaminated material in stream deposition zones. The aim of this study was therefore to investigate the mechanisms by which runoff affects sediment composition and macroinvertebrates in deposition zones of lowland stream ecosystems. We hypothesized that land use specific runoff substantially affects deposition zone sediment composition and benthic ecosystem structure and functioning by changing food quality and oxygen availability. To test this hypothesis, sediment from runoff and adjacent instream deposition zones from streams with different land use was characterized chemically and the biological effects were assessed at the species (whole sediment bioassay), community (macroinvertebrate community composition) and ecosystem level (sediment oxygen demand).

## 2. Materials and methods

### *2.1 Study area*

This study was conducted in three tributaries of the Hierden stream (52° 23' N, 5° 41' O) in the Netherlands, (Fig. 1). The Hierdense stream catchment is characterized by sandy soils and a slope of 1.3 m/km (Klein and Koelmans, 2011). The headwater is surrounded by agricultural areas followed by a forested area downstream. The three selected stream are no more than four kilometers apart from each other, maintaining similar climatic and geological conditions. However, the intense human occupation in this relatively small catchment created a patchy landscape formed by diverse land uses.

For each stream, the cover percentages of land use types were estimated using the topographic map from the Kadaster (<https://www.kadaster.nl>) and confirmed in the field (Table 1). The catchment of the forest stream was dominated by deciduous and coniferous forest (98%); the grass stream was surrounded mainly by fertilized grasslands used for animal grazing (50%) and urban areas (31%); and the crop stream was surrounded by non-perennial fertilized crop fields (36%) and urban areas (31%) (Table 1).

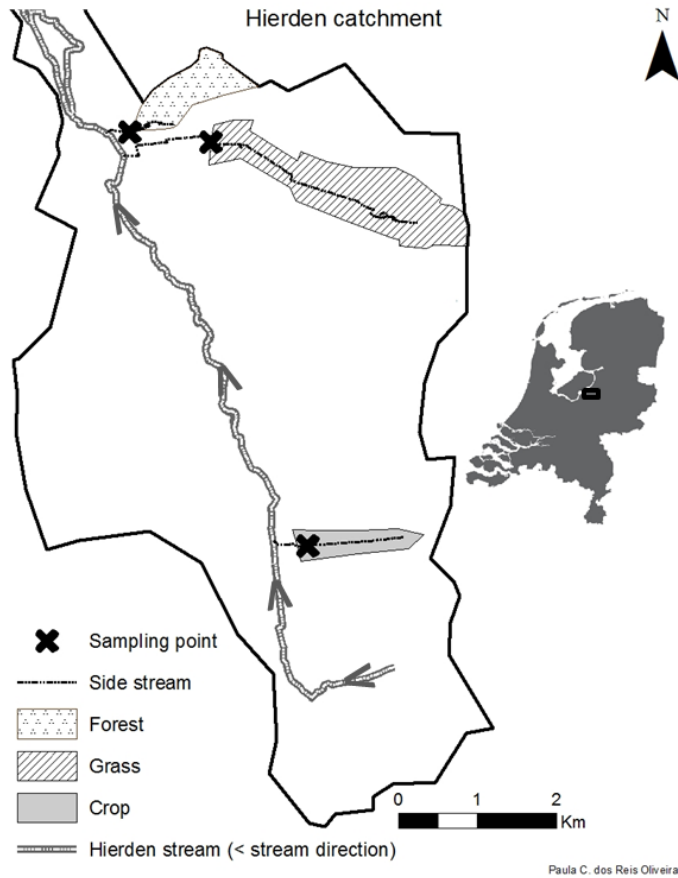


Figure 1: The Hierden stream catchment and the sampling sites, based on the topographic map of the Kadaster [1:25.000 scale] at RD new projection, created using ArcMap<sup>®</sup>. Forest, grass and crop are the tree studied streams catchments.

Table 1: Surface cover (Km<sup>2</sup>) and percentage of the major land use types in each of the three catchments.

Stream	Land use				Total (Km <sup>2</sup> )
	Forest (Km <sup>2</sup> )	Grass (Km <sup>2</sup> )	Crop (Km <sup>2</sup> )	Urban (Km <sup>2</sup> )	
Forest	0.69 (98%)	0.00	0.00	0.01 (2%)	0.70
Grass	0.09 (4%)	1.01 (50%)	0.30 (15%)	0.64 (31%)	2.03
Crop	0.01 (4%)	0.12 (30%)	0.14 (36%)	0.12 (31%)	0.39

In each of the three lowland streams (flow velocity and depth, respectively in: forest 0.097 m/s  $\pm$  0.08 m/s, 11cm  $\pm$ 3; grass 0.111 m/s  $\pm$ 0.005 m/s, 24  $\pm$ 3; and crop 0.091 m/s  $\pm$  0.003 m/s, 12 cm  $\pm$ 1) a downstream sampling site was selected (Fig. 1) to collect sediment from runoff and instream deposition zones. The runoff was sampled adjacent to the stream in the forest, the grasslands and the crop fields respectively, representing the dominant land use surrounding the sample site (Table 1). In each stream, a 15-meter-long stretch was selected in order to estimate substrate cover percentages according to Hering et al. (2003) (Table 2). Additionally, deposition zones were identified, defined as deeper areas where current velocity was low and where fine particulate organic matter (FPOM) accumulated, quantified based on Hering et al. (2003).

Table 2: Substrate cover percentage estimates per sampling site over a 15-meters long stretch.

Sample site	Akal (gravel)	Psammal (sand, mud)	Algae	Submerged macrophytes	Emergent macrophytes	Xylal (wood)	CPOM	FPOM
Forest	9	25	0	0	1	15	10	50
Grass	1	3	40	45	0	0	1	10
Crop	4	10	33	5	30	0	3	15

### 2.2 Runoff and deposition zone sediment composition

Sediment from runoff was collected simulating soil erosion by wash (Bryan, 1974), flushing the soil with demineralized water (5 to 6 L) over an area of 283 cm<sup>2</sup> by pouring water from a container vertically on the soil of the river bank. Per site, five replicate runoff sediment samples were taken. Water and sediment were collected in 3L glass bottles and stored in a refrigerator at 4°C for about 15 hours, decanted and the remaining particles were analyzed.

From each stream deposition zone, the 2-cm top layer of the sediment was sampled using an acrylic core and a scaled core-cutter (Uwitec). Five replicate



sediment samples were collected per site for chemical analysis, freeze-dried, sieved over a 2 mm sieve and ball-milled for five minutes at 400 RPM. For the bioassays, five replicate sediment samples per site were first frozen at -20°C for two days and thawed at 4°C for a period of three to four days.

### 2.2.1 Chemical analyses

Carbon (C) and nitrogen (N) concentrations were determined using an elemental analyzer (Elementar Vario EL, Hanau, Germany). Phosphorus was determined by first igniting one to two grams of sediment at 500°C for 16h, after which the remaining sediment was extracted with 0.5M sulfuric acid and finally, total orthophosphate content was determined by the colorimetric molybdenum blue method (Murphy and Riley, 1962). The inorganic phosphorus (IP) corresponds to the orthophosphate fraction determined from unburned samples, according to the method described by Murphy and Riley (1962). Organic phosphorus (OP) was calculated by subtracting inorganic from total orthophosphate. The organic matter (OM) content of the sediment was measured by loss-on-ignition. After overnight drying at 105 °C, the sediment was weighted using a precision scale (0.1 mg) before and after burning at 550°C for 16 hours.

## 2.3 Biological analyses

### 2.3.1 Sediment bioassays

To measure the chronic (sub)lethal biological effects of the sediment samples at the species level, five benthic invertebrate species, *Asellus aquaticus*, *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus* and *Sericostoma personatum* were tested in a series of whole sediment bioassays. There were five replicates per treatment, each replicate consisting of a 150 ml jar containing a ratio of 4:1 local stream water to sediment. The experiments were started by introducing ten (five for *S. personatum*) specimens of a single test species per replicate. The experiments were started with juvenile *A. aquaticus* (<2.5 mm), first instar (aged ≤24h) *C. riparius* larvae and juvenile *H. azteca* (<3.3 mm). *Lumbriculus variegatus* (0.3 mg ± 0.07 mg dry weight) consisted of juvenile individuals of similar size, and *S. personatum* larvae (12.6 mg ± 3.6 mg dry weight) were similar sized individuals of unknown instar. The test species originated from the University of Amsterdam's in-house laboratory cultures, except for *S. personatum* that was collected from a reference site (Springendal stream) and acclimatized for one week to laboratory conditions. The test jars were kept at 20°C, continuously aerated and maintained under a 16:8 light:dark regime for

a period of 28 days in a climate room. The controls contained artificial sediment made according to OECD guideline 218 (OECD, 2004), modified by Marinković et al. (2011). The bioassay with *C. riparius* was fed according to Marinković et al. (2011); for *S. personatum*, approximately 1.5 g incubated wet oak leaves (12 days incubation in stream water) and 0.5 mg fish food/larva/day for a period of 28 days (mixture of Trouvit - Trouw, Fontaine-les-Vervins, France and Tetraphyll - Tetrawerke, Melle, Germany in a ratio of 20:1) were added at the onset of the experiment. Seven days after the start of the test 1.5 g incubated oak leaves were added and after 14 days 0.5 mg fish food/larva/day for a period of 14 days. *A. aquaticus* were fed by administrating approximately 1.5 g incubated oak leaves at the onset of the experiment. For *H. azteca*, 5% of sediment dry weight ground *Urtica dioica* was added to each jar at the onset of the experiment. To evaluate the carrying capacity of the natural sediments, the bioassay with *L. variegatus* and an additional test with *C. riparius* did not receive food. Conductivity, dissolved oxygen, pH and temperature were checked prior to the experiment, and measurements were repeated weekly. Ammonia levels were checked at the beginning and at the end of the experiment. After 28 days, the bioassays were terminated and the sediment was sieved (mesh size 500 µm). The end-points were survival and emergence for *C. riparius*; survival, growth and reproduction for *A. aquaticus* and *H. azteca*; survival for *L. variegatus*; and survival and growth for *S. personatum*. Survival was defined as the percentage of alive individuals per jar; emergence was the percentage of adult midges per jar; reproduction was the mean number of juveniles per jar; and, finally, growth was the individual final length or weight subtracted from the initial average length or weight per jar.

### 2.3.2 Community analysis

Macroinvertebrates were sampled from stream deposition zones by a Surber sampler (625 cm<sup>2</sup>; mesh size: 0.25 mm). The collected organisms were sorted within 48 hours and preserved in 70% ethanol for later identification. Species richness (number of taxa), Shannon–Wiener diversity index and the percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT) individuals and the SPEAR index (Liess and Von Der Ohe, 2005) were calculated. Additionally, the saprobity and the relative abundance of functional feeding groups (Moog, 1995) were derived from the autecological database for freshwater organisms, version 7.0, accessed on 28.03.2017 ([www.freshwaterecology.info](http://www.freshwaterecology.info)).

### 2.3.3 Ecosystem level

At the ecosystem level, the sediment oxygen demand (SOD) was measured. SOD of five replicate sediment cores per site was determined as described by Belanger (1980), with slight modifications: the undisturbed sediment cores were kept at 20°C in dark and saturated with air immediately after sampling. Next, the dissolved oxygen concentrations were measured after 1, 2, 9 and 24 hours with a portable meter (HQ440d HACH). Finally, SOD was calculated according to Rong et al. (2016).

### 2.4 Statistics

Deposition zone sediment composition, biological effects and runoff sediment composition non-transformed data were tested separately using one-way analysis of variance (ANOVA), followed by a Tukey post hoc test (R-package stats). In the cases where the conditions of data normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test) were violated, differences between means were calculated using the non-parametric Kruskal–Wallis test, followed by a Mann–Whitney pairwise comparisons (Bonferroni corrected: 0.05/2,  $\alpha = 0.025$ ) to compare the 3 streams (R-package multcompView).

The effect of environmental variables on macroinvertebrate communities was analyzed by using a Canonical Correspondence Analyses (CCA), CANOCO for Windows version 4.55 (ter Braak and Smilauer, 2002). All nine sediment chemical parameters measured in both the deposition zone and the runoff were included in the analysis. The significance of the relation between the macroinvertebrates and the environmental parameters was evaluated using a Monte Carlo permutation test (999 permutations,  $p < 0.05$ ). In the bioassays, the laboratory control treatments performed for test validation (OECD, 2004) were not included in the statistical test.

## 3. Results

### 3.1 Runoff sediment composition

In runoff sediment, organic matter percentage decreased significantly ( $p < 0.05$ ) from forest ( $62\% \pm 11\%$ ) to grasslands ( $44\% \pm 2\%$ ) to crop fields ( $25\% \pm 8\%$ ) (Fig. 2A). C/N ratio was significantly ( $p < 0.05$ ) higher in forest runoff sediment ( $17 \pm 1$ ) than in the runoff sediment from the two agricultural sites ( $14 \pm 0.5$  and  $14 \pm 1$ , grass and crop respectively) (Fig. 2B). Inorganic phosphorus (IP) was significantly ( $p < 0.05$ ) higher in crop runoff ( $19 \text{ mmol/Kg} \pm 5 \text{ mmol/Kg}$ ) than in grass ( $10 \text{ mmol/Kg} \pm 6 \text{ mmol/Kg}$ ) and forest runoff ( $3 \text{ mmol/Kg} \pm 0.5 \text{ mmol/Kg}$ ) (Fig. 2C). Organic phosphorus (OP) was not significantly ( $p < 0.05$ ) different between sampling sites (Fig. 2D).

The OP concentration ( $15 \text{ mmol/Kg} \pm 9 \text{ mmol/Kg}$ ) was significantly ( $p < 0.05$ ) higher than the IP concentration ( $3 \text{ mmol/Kg} \pm 0.5 \text{ mmol/Kg}$ ) in forest runoff, but the opposite was observed in crop runoff, where the OP concentration was significantly ( $p < 0.05$ ) lower ( $8 \text{ mmol/Kg} \pm 2 \text{ mmol/Kg}$ ) than the IP concentration ( $19 \text{ mmol/Kg} \pm 5 \text{ mmol/Kg}$ ).

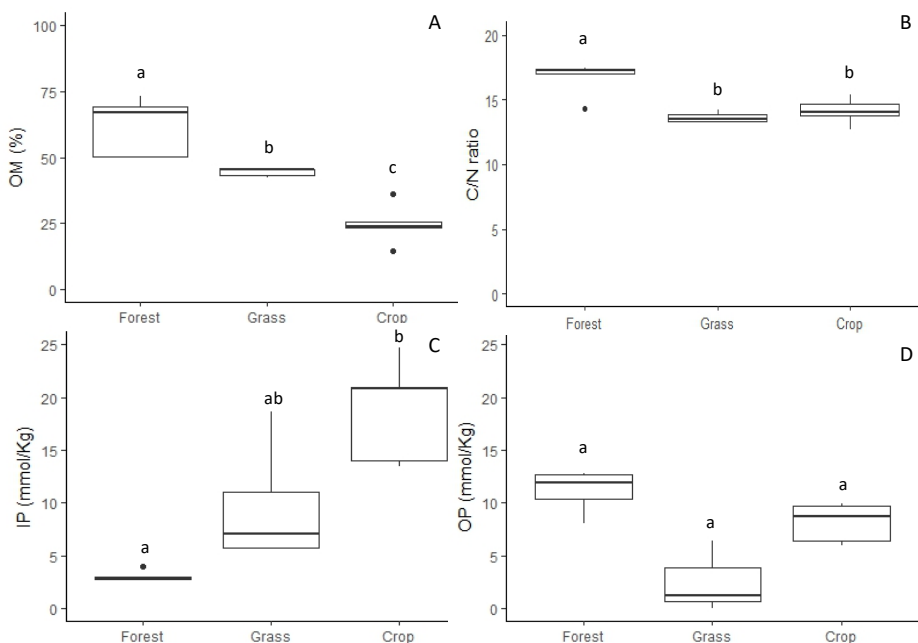


Figure 2: Runoff sediment composition: particulate organic matter percentage (OM %) (A), carbon/ nitrogen ratio (C/N ratio) (B); Inorganic phosphorus (IP) (C) and organic phosphorus (OP) (D). The boxes indicate the first to third quartile. The bottom, middle and top line indicate the minimum, median and maximum values. Treatments labelled with different letters indicate a significant difference between the means ( $p < 0.05$ , analyses of variance followed by multiple comparison test).

### 3.2 Deposition zone sediment composition

Percentage of organic matter in sediment deposition zones did not differ significantly ( $p > 0.05$ ) between forest and agricultural streams. The C/N ratio was significantly ( $p < 0.05$ ) higher in forest stream sediment ( $17.5 \pm 0.4$ ) compared to both agricultural sites ( $15.2 \pm 0.3$  and  $14.6 \pm 1$ , respectively) (Fig. 3B), fully reflecting the runoff sediment composition. Organic and inorganic phosphorus did not differ significantly ( $p > 0.05$ ) between sampling sites (Fig. 3C, D). Finally, the IP concentration was higher in runoff ( $4.1 \pm 3.9$ ;  $5.9 \pm 1.3$ ;  $2.1 \pm 2.1$ , forest, grass and crop respectively)

than in deposition zones ( $3.1 \pm 0.5$ ;  $12.6 \pm 8.6$ ;  $17.4 \pm 3.6$ , forest, grass and crop respectively), except for forest.

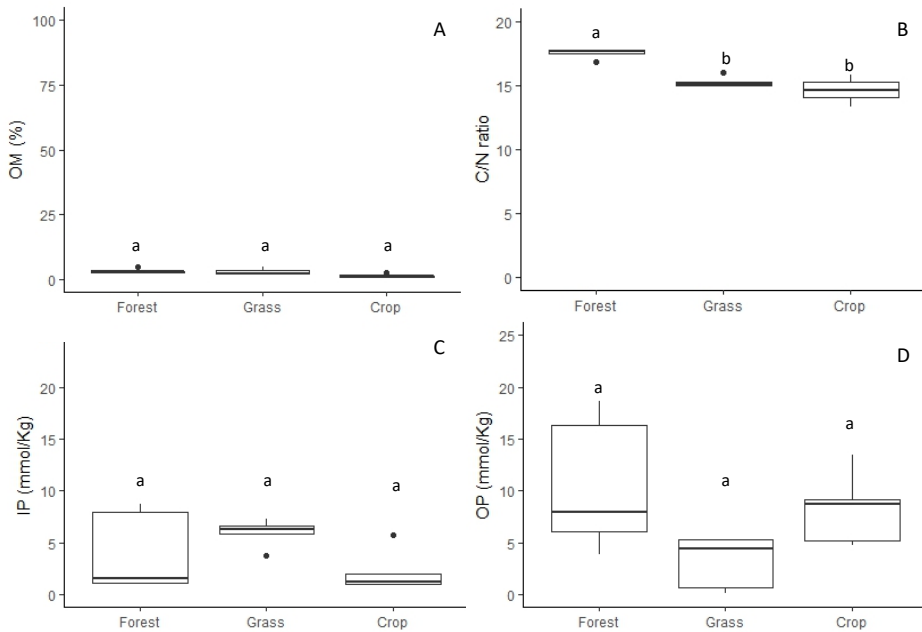


Figure 3: Deposition zone sediment composition: particulate organic matter percentage (OM %) (A), carbon/nitrogen ratio (C/N ratio) (B), Inorganic phosphorus (IP) (C) and organic phosphorus (OP) (D). The boxes indicate the first to third quartile. The bottom, middle and top line indicate the minimum, median and maximum values. Treatments labelled with different letters indicate a significant difference between the means ( $p < 0.05$ , analyses of variance followed by multiple comparison test).

### 3.3 Biological effects

#### 3.3.1 Bioassays

Control survival for *A. aquaticus*, *H. azteca*, *S. personatum* and *C. riparius* was higher than 80%, meeting the validity criteria of the tests (OECD, 2004) (Table 3). Moreover, there was no treatment-related effect on survival. Control survival of *C. riparius* and *L. variegatus* without additional food was obviously very low due to starvation. These experiments did show, however, that both the chironomid and the worm suffered significantly ( $p < 0.05$ ) less mortality on the agricultural sediments than on the forest sediment.

Considering the chronic sublethal end points, growth of *H. Azteca* and reproduction of *A. aquaticus* was significantly ( $p < 0.05$ ) higher on the forest stream

sediment than on the two agricultural sediments. In contrast, emergence of *C. riparius* in the treatment without additional food was significantly ( $p < 0.05$ ) higher on the two agricultural sediments than on the forest sediment.

Table 3: Survival (%), growth (mm or mg), reproduction (number of offspring) and emergence (number of individuals) of benthic invertebrate species. Different letters indicate a significant difference between the means in landscape types ( $p < 0.05$ , analyses of variance followed by multiple comparison test).

	Forest	Grass	Crop	Laboratory control
<b>Survival (%)</b>				
<i>Asellus aquaticus</i>	76 ( $\pm 11$ )	84 ( $\pm 13$ )	80 ( $\pm 7$ )	82 ( $\pm 24$ )
<i>Hyalella azteca</i>	94 ( $\pm 9$ )	86 ( $\pm 15$ )	84 ( $\pm 11$ )	90 ( $\pm 10$ )
<i>Sericostoma personatum</i>	96 ( $\pm 9$ )	100 ( $\pm 0$ )	96 ( $\pm 9$ )	100 ( $\pm 0$ )
<i>Chironomus riparius</i>	90.9 ( $\pm 13$ )	69 ( $\pm 40$ )	82 ( $\pm 29$ )	92.7 ( $\pm 16$ )
<i>Chironomus riparius</i> _no food	54 ( $\pm 11$ ) <sup>a</sup>	67 ( $\pm 15$ ) <sup>b</sup>	74 ( $\pm 34$ ) <sup>b</sup>	2 ( $\pm 4$ )
<i>Lumbriculus variegatus</i> _no food	24 ( $\pm 32$ ) <sup>a</sup>	100 ( $\pm 58$ ) <sup>b</sup>	114 ( $\pm 21$ ) <sup>b</sup>	24 ( $\pm 32$ )
<b>Growth</b>				
<i>Asellus aquaticus</i> (mm)	6.4 ( $\pm 0.7$ )	6.4 ( $\pm 1.2$ )	6.1 ( $\pm 0.8$ )	4.8 ( $\pm 0.5$ )
<i>Hyalella azteca</i> (mm)	4.6 ( $\pm 1.7$ ) <sup>a</sup>	2.2 ( $\pm 0.4$ ) <sup>b</sup>	2.4 ( $\pm 0.4$ ) <sup>b</sup>	3.8 ( $\pm 0.4$ )
<i>Sericostoma personatum</i> (mg)	20 ( $\pm 3.4$ )	19.4 ( $\pm 3$ )	17 ( $\pm 1.6$ )	16 ( $\pm 2.4$ )
<b>Reproduction (number of offspring)</b>				
<i>Asellus aquaticus</i>	17.4 ( $\pm 18$ ) <sup>a</sup>	2.2 ( $\pm 5$ ) <sup>b</sup>	0 ( $\pm 0$ ) <sup>b</sup>	0 ( $\pm 0$ )
<i>Hyalella azteca</i>	1 ( $\pm 1$ )	0.5 ( $\pm 0.5$ )	0 ( $\pm 0$ )	1.2 ( $\pm 0.4$ )
<b>Emergence (number of individuals)</b>				
<i>Chironomus riparius</i>	91 ( $\pm 13$ )	69 ( $\pm 40$ )	82 ( $\pm 29$ )	93 ( $\pm 16$ )
<i>Chironomus riparius</i> _no food	0 ( $\pm 0$ ) <sup>a</sup>	24 ( $\pm 15$ ) <sup>b</sup>	16 ( $\pm 12$ ) <sup>a</sup>	0 ( $\pm 0$ )

### 3.3.2 Macroinvertebrate community composition

Macroinvertebrate community composition differed strongly between land use type (Fig. 4A) in terms of richness, functional feeding groups (FFG) and saprobity categories.

All indices of good ecological quality scored higher for forest (Table 4). Richness and diversity were significantly ( $p < 0.05$ ) higher in the forest stream community than in the agricultural streams communities. EPT individuals as well as sensitive species according to the SPEAR classification were present only in the forest stream.

Table 4: Mean (n=5)  $\pm$  standard deviation (sd) of macroinvertebrate community indices. EPT is the relative abundance of Ephemeroptera, Plecoptera and Trichoptera individuals; SPEAR is the number of species that are classified as “at risk” (Lies and Von Der Ohe, 2005). Different letters indicate a significant difference between the means (n=5  $\pm$  sd) ( $p < 0.05$ , analyses of variance followed by multiple comparison test).

	Forest	Grass	Crop
Richness	17 ( $\pm 6$ ) <sup>a</sup>	9 ( $\pm 5$ ) <sup>b</sup>	10 ( $\pm 2$ ) <sup>b</sup>
EPT (%)	12 ( $\pm 0.1$ ) <sup>a</sup>	0 <sup>b</sup>	0 <sup>b</sup>
Shannon-Winner diversity	2.03 ( $\pm 0.3$ ) <sup>a</sup>	1.4 ( $\pm 0.74$ ) <sup>ab</sup>	1.4 ( $\pm 0.4$ ) <sup>b</sup>
SPEAR	2.2 ( $\pm 1.6$ ) <sup>a</sup>	0 <sup>b</sup>	0.2 ( $\pm 0.4$ ) <sup>b</sup>
Alpha and polysaprobic (%)	23.1 ( $\pm 3.7$ ) <sup>a</sup>	34.02 ( $\pm 25.6$ ) <sup>a</sup>	38.8 ( $\pm 7.6$ ) <sup>a</sup>

For community composition, the similarity (Jaccard index) between grass and forest was 26%, but only 12% for crop and forest. Even between the two agricultural sites, similarity was low (22%). In forest, the evenness among different species was higher than grass, dominated by *Chironomus sp.*, and crop dominated by *Proasellus banyulensis* and *Stylaria lacustris*. All FFG and saprobity categories were present at the three locations, but in different proportions and therefore the Jaccard index was not applied. Macroinvertebrate species indicative of a high level of saprobity (alpha and polysaprobic) and a lower diversity and evenness of functional feeding groups occurred in the agricultural streams (Fig. 4B, C). Active filter feeders were more common and parasites occurred only in the forest stream.

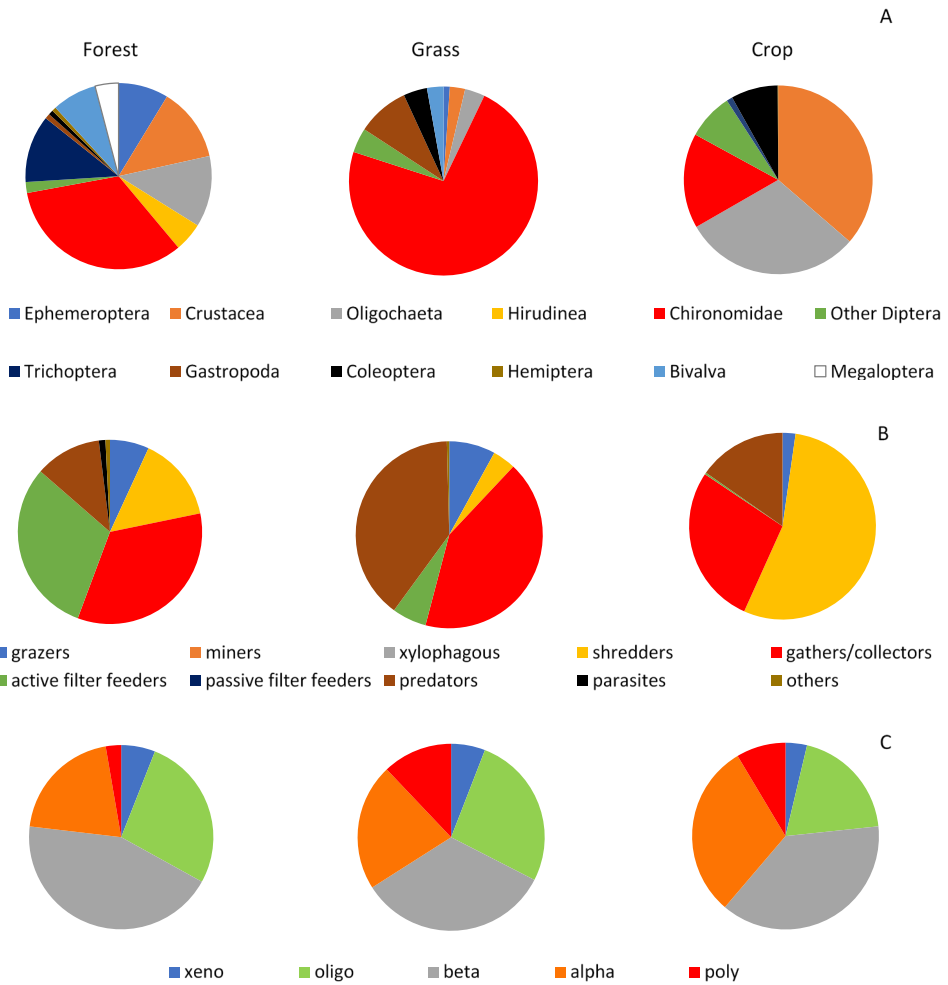


Figure 4: Relative abundance of macroinvertebrate taxa (A), functional feeding groups (B) and saprobity categories (C) at the three sampling sites.

### 3.3.3 Sediment oxygen demand

Sediment oxygen demand was significantly ( $p < 0.05$ ) lower in the deposition zones in the forest stream compared to both agricultural sites (Figure 5).



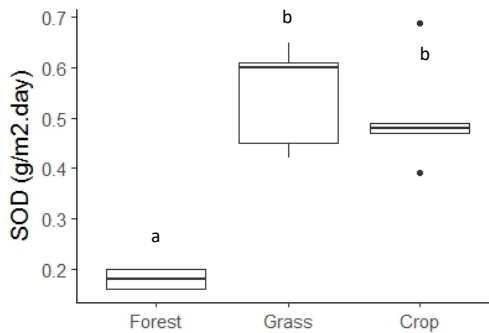


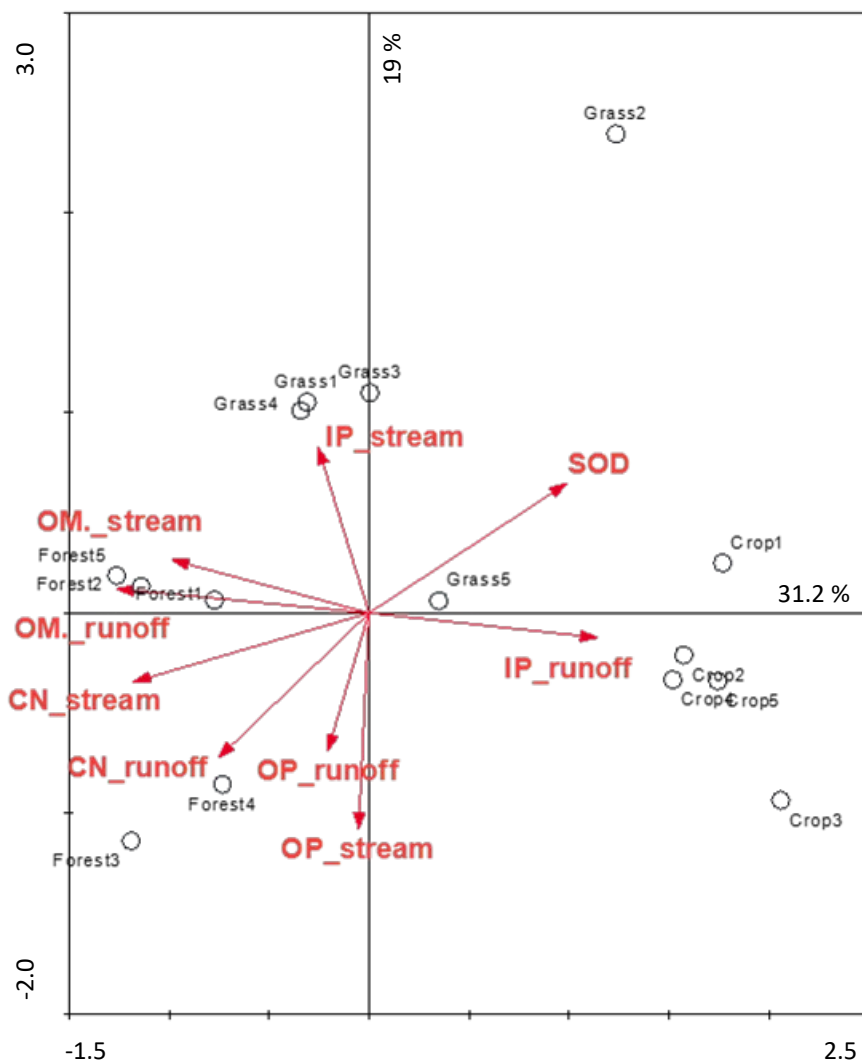
Figure 5: Sediment oxygen demand over 24 hours. The boxes indicate the first to third quartile. The bottom, middle and top line indicate the minimum, median and maximum values. Treatments labelled with different letters indicate a significant difference between the means ( $p < 0.05$ , analyses of variance followed by multiple comparison test).

#### 3.3.4 Canonical correspondence analysis

The CCA of macroinvertebrate community composition and environmental parameters shows that axis 1 accounted for 31.2% of the variation explained and axis 2 for another 19.1%, while the first four CCA axes jointly accounted for 74.6%. The CCA ordination plot shows that macroinvertebrate species in agricultural streams, were separated from those in forest streams (Fig. 6A). Both agricultural sites were positively related with a higher SOD, while streams in grassland also related to higher concentrations of inorganic phosphorus in deposition zones and streams in cropland to higher concentrations of inorganic phosphorus in runoff particles. Conversely, streams in forest related to higher C/N ratio's and percentage of organic matter in both runoff particles and deposited material.

The CCA of macroinvertebrate FFG and environmental parameters shows that axis 1 accounted for 47.9% of the variation explained, axis 2 for another 34.2% and the first four CCA axes jointly for 98.5%. The FFG CCA ordination plot (Fig 6B) is similar compared to the community composition plot (Fig. 6A). The major difference is that the sites in cropland are positioned in the diagram apart from those in grassland and forest. Here the streams in cropland are related to high SOD and concentrations of inorganic phosphorus in runoff particles.

The ordination analyses confirmed the results described above for C/N ratio, IP, SOD and macroinvertebrate community composition, where the forest stream was clearly distinct from the agricultural sites.



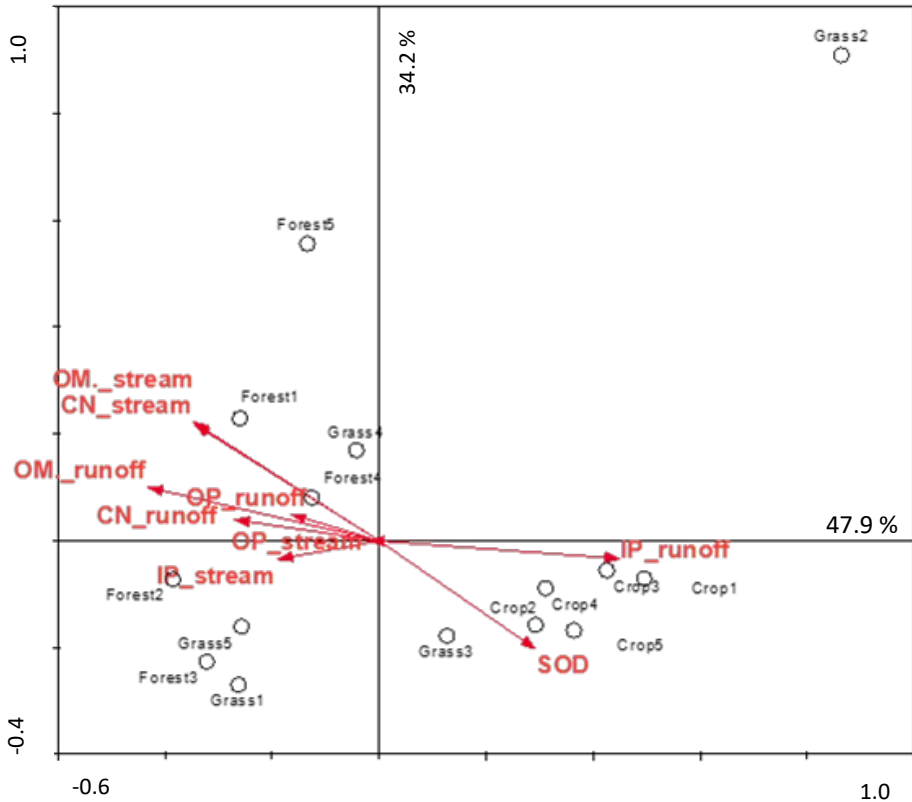


Figure 6: CCA biplots for data ordination of environmental variables with macroinvertebrates species (A) and functional feeding group (B) in forest, grass and crop streams.

#### 4. Discussion

Our results showed that the composition of runoff and instream deposition zone sediment is land use specific and that agricultural activities affect stream ecosystems at the species, community and ecosystem level. To evaluate the mechanisms by which runoff affects sediment composition and therewith macroinvertebrates in lowland streams, below we discuss deposition zone sediment characteristics and the corresponding biological effects.

##### 4.1 The contribution of runoff to deposition zone sediment composition

Several authors attributed shifts in OM composition in streams to storm water runoff (e.g. Imberger et al., 2014; Wang et al., 2001; Withers and Jarvie, 2008). This is especially the case in agricultural catchments (Allan et al., 1997; Burcher and Benfield, 2006), because the nutrients and pollutants concentrated in the upper

horizons of the soils can be easily washed off from land to streams by runoff (Cai et al., 2015; Lewis and Grimm, 2007; Vidon et al., 2010). Our analyses of the terrestrial runoff contribution to the sediment composition in the deposition zones of the streams demonstrated that runoff sediment showed a land use specific signature in terms of OM percentage, C/N ratio and IP. Forest runoff sediment contained the highest OM percentage and C/N ratio and the lowest IP concentration, due to the input of forest tree leaves (Vidon et al., 2010; Withers and Jarvie, 2008). The importance of land use for dissolved and pore water phosphorus concentrations is well established (e.g. Meyer and Likens, 1979; Wetzel, 2001; Lijklema, 1993; Reddy et al., 1999), in contrast to phosphorus in the orthophosphate form, bound to runoff sediment. Focusing on orthophosphate, we showed that IP was indeed much higher in agricultural runoff, especially in the crop field, which was to be expected in agricultural systems with artificial fertilizer inputs (Ekholm and Krogerus, 2003; Oyewumi et al., 2017; Withers and Jarvie, 2008). Yet, for IP there was no deposition zone-runoff relationship, which may be explained by the rapid P uptake, turnover and regeneration times in streams (Mulholland et al., 1997, 1985), as a result of a combination of biotic and abiotic assimilation processes during downstream transport (Wetzel, 2001; Withers and Jarvie, 2008).

OM percentage was fifteen to nineteen times higher in runoff than in deposition zone sediments in all studied streams, demonstrating that the materials deposited in instream sediment are not representative of the substances washed in from the surrounding land (McCorkle et al., 2016) and because OM in deposition zones may be altered by temperature, light, upstream particle influx and decomposition (Imberger et al., 2014; McCorkle et al., 2016; Mulholland et al., 1985). Thus, although Golladay (1987) reported significant FPOM inputs during storms, our results showed no one to one correlation between deposition zones and runoff sediment composition, except for C/N ratio.

C/N ratio was higher in forest compared to agricultural runoff, indicating the higher carbon and lower nitrogen influx in forest streams. In all studied streams, the C/N ratio in deposition zones was very similar to that in runoff sediment. C/N ratio was therefore the only stream sediment parameter that fully reflected runoff sediment composition, in agreement with McCorkle et al. (2016), suggesting that runoff sediment might play an import role as a source of carbon and nitrogen in deposition zones.

Overall, the present study thus showed that the contribution of runoff to deposition zone sediment composition is parameter-specific (C, N, OM, IP). C/N ratio may represent a good finger print from terrestrial input in lowland streams (Bunn et al., 1999; Hamilton et al., 1992), which could be further improved by particle size determination.

#### *4.2 Deposition zone characteristics*

The sediment composition in the deposition zones of the studied streams differed specifically in OM content and C/N ratio. In the forest stream, OM percentage and C/N ratio were higher than in the agricultural streams. Several studies have suggested that lower C/N ratios indicate the influx of autochthonous material, whereas higher values are related to allochthonous-derived carbon (Wetzel, 2001; Hunt et al., 2012; Kendall et al., 2001; Leigh et al., 2010). This could well be the case in the present study, where the forest stream received more allochthonous carbon, such as wood and deciduous tree leaves (Bilby, 1981; Meyer et al., 1998), while the agricultural streams were characterized by the predominance of autochthonous carbon from instream sources such as algae and macrophytes. We concluded that instream deposition zone sediment composition is land use specific and that the C/N ratio showed the most pronounced differences between forest and agricultural sites.

#### *4.3 Biological effects*

Agricultural land use was not only reflected by a lower C/N ratio, but also by higher SOD levels. According to Imberger et al. (2014) and Pusch et al. (1998) alterations in C/N ratios and microbial detritus processing have the potential to impact ecosystem respiration, nutrient processing and water quality. The SOD in the agricultural streams was comparable to that in eutrophic water bodies (Sommaruga, 1991) and in water with low dissolved oxygen concentrations (Chau, 2002; Liu and Chen, 2012; Rong et al., 2016) and showed that the respiration rate in agricultural stream sediments was higher than in the forest sediment. Moreover, it has been documented that SOD strongly influences the dissolved oxygen budgets in the overlying water (e.g. Boynton and Kemp, 1985; Liu and Chen, 2012; Matlock et al., 2003), potentially affecting the benthic community (Larsen et al., 2011). The present study showed that land use affected benthic community composition and induced species-specific responses in bioassays. The forest stream showed higher values for all indices indicative of biological integrity than the agriculture sites, in agreement with e.g. Allan et al. (1997); Lammert and Allan (1999); Wang et al. (2001). The higher richness, biodiversity and EPT index in the forest stream deposition zones was related

to the higher C/N ratio and lower SOD. Von Bertrab et al. (2013) also reported a higher occurrence of EPT species at sites with a high C/N ratio, which, together with oxygen availability, explained benthic invertebrate community composition.

Since the substantial differences in C/N ratio hint at differences in food quality, we also analysed functional feeding group composition (FFG). We observed that the FFG diversity and evenness was higher in the forest stream, in agreement with Von Bertrab et al. (2013), who showed that a lower food quality affected active filter feeders and grazers most. Moreover, higher diversity of FFG allows differential energy flows through the benthic food web, which may lead to higher food web stability (Rooney and McCann, 2012).

Higher SOD may decrease the dissolved oxygen concentration in the water, especially during the night. Therefore, the differences in SOD between the forest and the agricultural sites suggest that community composition may also be explained by species specific sensitivities to oxygen demand in the sediment. Analysing the relative abundance of macroinvertebrates according to saprobity levels showed that more than one quarter of macroinvertebrate taxa present at the agricultural sites are resistant to low oxygen concentrations (alpha and polysaprobic), in line with the higher sediment oxygen demand at these sites. In contrast, the EPT species, generally sensitive to low oxygen concentrations (Collier et al., 1998; Von Bertrab et al., 2013) were only present in the forest stream with the lower SOD.

The differences in community composition between sites matched very well with the species specific responses in the whole sediment bioassays, showing that in the forest stream sediment growth of *H. azteca* and reproduction of *A. aquaticus* was higher, whereas *L. variegatus* and *C. riparius* survived better on the agricultural sediment containing only natural food with a low C/N ratio. This result can likely be explained by the variance in food intake and digestion efficiency between macroinvertebrate species (Cammen, 1980; Cummins and Klug, 1979). *H. azteca* and *A. aquaticus* are selective feeders with preferences toward a higher food quality (Cammen, 1980; Graça et al., 1993; Wang et al., 2004). In contrast, *L. variegatus* and *C. riparius* can ingest bacteria at high rate (Brinkhurst and Chua, 1969; Baker and Bradnam, 1976) and have the ability to compensate for reduced food quality by increasing ingestion rate (Cammen, 1980; Cummins and Klug, 1979). In conclusion, the present study demonstrated that agricultural land use affected benthic species growth and reproduction, as well as macroinvertebrate community composition via

altered food quality and oxygen demand in the sediment. These effects are partly driven by the C/N ratio (food quality), and partly by the degradability of the runoff sediment (sediment oxygen demand), both contributing to the observed biological effects. Thus, the present study indicated that agricultural land use affects lowland stream ecosystems via altered food quality and oxygen demand in the sediment.

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