Pasqualini, J., Majdi, N., **Weitere, M.**, **Brauns, M.** (2025):
The contribution of the hyporheos to whole-stream invertebrate secondary production
Freshw. Sci. **44** (2), 170 – 186

https://doi.org/10.1086/735823

The contribution of the hyporheos to whole-stream invertebrate secondary production

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Abstract: The hyporheic compartment is a hot spot of numerous biogeochemical processes. Yet, its role in invertebrate mediated energy fluxes is unclear, due to limited studies assessing benthic and hyporheic production simultaneously. We sampled the meio- and macrofauna from the benthic (from surface to 5 cm depth) and hyporheic (from 5 to 15 cm depth) compartments of an agricultural and forested stream and quantified whole-stream and compartmental production. In the forested stream, 27% of whole-stream invertebrate secondary production occurred in the hydrologically well-connected hyporheic compartment and was sustained by early larval stages of macrofauna, mainly Diptera (28%) and Coleoptera (20%), as well as by the permanent meiofauna like Rotifera (12%), Acari (9%) and Copepoda (9%). In contrast, the agricultural stream had a lower hyporheic contribution (6%) likely because of fine sediment deposition that reduced the water exchange between the water column and the hyporheic compartment, creating anoxic conditions. Despite the large reduction in hyporheic contribution, whole-stream production was approximately threefold higher in the agricultural stream than in the forested one. Only few taxa sustained most of production, with the invasive species *Potamopyrgus antipodarum* (J. E. Gray, 1843) accounting for 42% of whole-stream production. Our findings provide support for the conclusions that (i) the whole-stream production is significantly greater in the agricultural than in the forested stream, this is most likely attributable to the enhanced availability of resources; whereas (ii) the relative importance of the hyporheic compartment decreases at the same time because of unfavorable habitat conditions caused by fine sediment clogging. Therefore, failure to include hyporheic compartment may result in a significant underestimation of whole-stream invertebrate secondary production in streams with permeable sediments. Given that the benthic and hyporheic compartments host different communities and exhibit different production rates both under natural and degraded conditions, each compartment provides a discrete set of information that cannot be inferred from the other. Consequently, for a comprehensive understanding of the overall functionality of the stream, it is necessary to sample the entire invertebrate length distribution present in both compartments.

Key words: stream functioning – meiofauna – clogging – agriculture – benthic – hyporheic

The hyporheic compartment is a hot spot of biogeochemical processes and serves several ecological functions including temperature regulation, degradation of contaminants, nutrient cycling, and habitat provisioning for invertebrates (e.g., Lewandowski et al. 2019, Boulton et al. 2010, Duff and Triska, 2000, Storey et al. 2004). However, its role for the whole-ecosystem invertebrate mediated energy fluxes is not fully understood because studies quantifying simultaneously benthic and hyporheic secondary production are scarce. Earlier studies suggested a limited contribution of the hyporheic compartment to whole-stream invertebrate production because abundance, biomass, and consequently productivity, of large invertebrates decreases with depth (e.g., Smock et al. 1992, Huryn 1996). Conversely, other studies that have included early larval stages of macrofaunal taxa (e.g., Collier et al. 2004, Reynolds and Benke, 2012, Wright-Stow et al. 2006) and the permanent meiofauna (Majdi et al. 2017, Tod and Schmid-Araya, 2009) showed that hyporheic invertebrates can contribute significantly to production. These apparently contrasting findings might reflect either intrinsic differences in the repartition of production among compartments in streams with different environmental conditions (e.g., pH, grain size distribution, nutrient concentration etc.), or could simply derive from an incomplete sampling of the entire invertebrate size-range in earlier studies.

The distribution of invertebrates between the benthic and hyporheic compartment is influenced by a multitude of environmental factors (Coleman and Hynes 1970, Dole-Olivier and Marmonier 1992, Peralta-Maraver et al. 2018, Dunscombe et al. 2018). Nevertheless, the characteristics of the sediment are amongst the most significant drivers (Williams et al. 1974, Strommer and Smock 1989, Strayer et al. 1997, Weigelhofer and Waringer 2003, Peralta-Maraver et al. 2018). Among the sediment characteristics, permeability and porosity are crucial, as they determine the access of invertebrates to the hyporheic compartment, while also regulating the rates of water exchange between the water column and the sediment (Sawyer and Cardenas 2009). Exchange rates, in turn, determine dissolved O_2 concentrations and the availability of food resources within the hyporheic compartment (Boulton et al. 1998, Hakenkamp and Morin, 2000, Strayer et al. 1997). Consequently, in highly permeable sediment, small-bodied invertebrates can occur at depths of up to 70 cm (Coleman and Hynes 1970, Reynolds and Benke 2012). However, the majority of them are found in the upper 15 cm (e.g., Williams et al. 1974). Such small-bodied invertebrates are expected to significantly contribute to whole-stream invertebrate production (Schmid-Araya et al. 2020) as they have higher biomass turnover rates compared to large-bodied taxa (Brown et al. 2004).

The permeability of the streambed can be reduced as a consequence of physical or biological clogging (Dubuis and De Cesare 2023). Such conditions may arise naturally in sandy or mud-silty streambeds, or may result from human activities such as agricultural land use that increase the deposition of fine sediment on the streambed as a consequence of runoff events (Allan et al. 1997). Most studies on the implications of fine sediment clogging have focused on invertebrate responses in the benthic compartment (e.g., Bo et al. 2007, Jones et al. 2012, Mathers et al. 2017). However, fine sediment deposition can drastically alter the environmental conditions in the hyporheic compartment, by diminishing water fluxes between the water surface and the hyporheic compartment (Buendia et al. 2013). This creates steep redox gradients and the rapid depletion of O_2, which, in turn, have negative effects on the abundance, biomass and affects assemblages of hyporheic communities (Smock et al. 1992, Boulton et al. 1998, Reynolds and Benke 2012, Mathers et al. 2014). Several studies acknowledge the detrimental effects of fine sediment on the structural and compositional attributes of benthic and hyporheic invertebrates (Jones et al. 2012, Mathers et al. 2014, 2017). However, there is still a lack of empirical understanding of how this affects invertebrate mediated functions, particularly those occurring in the hyporheic compartment, and how this, ultimately, affects whole-stream invertebrate production. In field conditions, this is further complicated by the fact that streams surrounded by agricultural land are not only exposed to fine sediment deposition, but often also have altered levels of light and nutrients (Tank et al. 2021). These conditions have been demonstrated to increase benthic macroinvertebrate secondary production compared to more pristine systems, by stimulating primary production and increasing food quality (Shieh et al. 2002, Wild et al. 2022). However, it is unclear whether the observed increase in production will be as pronounced if the hyporheic compartment is also included in whole-stream estimates. An increase in fine sediment deposition is likely to degrade hyporheic habitat conditions, potentially impairing hyporheic communities, and, consequently, invertebrate mediated functions. If the hyporheic compartment is a significant contributor to whole-stream production, this would imply that when comparing estimates of whole-stream production between agricultural and forested streams, the observed differences may be less pronounced.

The aim of this study was to investigate how the relative contribution of the benthic and hyporheic compartments varies in a forested and agricultural stream, and how this, in turn, affects whole-stream invertebrate production. Therefore, we sampled the macro- and meiofauna inhabiting the benthic and hyporheic compartments in a forested stream, with a highly-permeable sediment, and in an agricultural stream, with a reduced permeability due to fine sediment deposition. Specifically, our research aims were to 1) to compare environmental controls on benthic and hyporheic invertebrate communities in an agricultural vs. a forested stream, 2) to quantify the relative contribution of the benthic and hyporheic compartments to whole-stream production in both cases. We expect that the less permeable conditions in the hyporheic compartment of the agricultural stream should reduce the proportional contribution of the hyporheic compartment to whole-stream invertebrate secondary production.

METHODS

We conducted a field study from August 2019 to June 2020 in 2 headwater streams located in the Harz region in Germany. At each stream site, we concurrently sampled the benthic and hyporheic invertebrates and measured environmental characteristics of water column, benthic, and hyporheic compartments bimonthly over 1 y. We then calculated invertebrate secondary production and compared the relative contribution of the benthic and hyporheic compartments to whole-stream production in these streams.

Site selection

The forested stream (Drängetalbach, 51°48'21.02"N, 10°43'51.82"E) is surrounded by coniferous forest, has a natural hydromorphology with pool-riffle sequences, gravel bars, and woody debris (Jähkel et al. 2022) (Fig. S1-A, Appendix S1). Water column nutrient concentrations are relatively low (Table 1) and its streambed consisted of cobbles (range: 63-200 mm) and coarse gravel (range: 20-63 mm). In contrast, the agricultural stream (Asse, 51°55' 23.357" N, 11°1'53.443" E) is channelized, surrounded by herbaceous vegetation and alder on the river bank (Fig. S1-B, Appendix S1). The stream exhibits higher nutrient concentrations and conductivity in the water column (Table 1, Fig. 1), and the streambed is predominantly fine sand (range: 0.2-0.063 mm) and coarse silt (range: 0.063-0.02 mm). Water temperature is slightly higher in the agricultural stream than in the forested one (Table 1). In addition, the agricultural stream has a higher light exposure (Table 1), as the stream flows through open fields and has a less dense canopy cover compared to the forested stream which flows through a steep valley and exhibit a denser canopy cover. Conductivity, temperature, and pH were measured in the water column with a multimeter (Multi 3630 IDS SET F, Xylem Analytics GmbH, Weilheim, Germany). Light intensity was measured over 1 y by 5 light intensity data loggers (MX2202 Onset, Bourne, Massachusetts, USA) placed along the stream reaches.

Table 1 Environmental characteristics of the 2 studied streams. Values are annual means ± 1 SD of bimonthly measurements. Except discharge values which are annual means, minimum and maximum.

Variable	Forested	Agricultural
Land use (Forest % – Arable % – Other %)	100 – 0 – 0 [a]	12 – 85 – 3 [a]
DN (mg L^{-1})	2.2 ± 0.6	10.4 ± 2.0
N-NO_3^- (mg L^{-1})	1.9 ± 0.6	9.3 ± 2.3
N-NH_4^+ (mg L^{-1})	0.01 ± 0.01	0.06 ± 0.05
SRP (µg L^{-1})	7 ± 3.2	31 ± 15
DOC (mg L^{-1})	2.7 ± 0.7	2.6 ± 0.5
Dissolved O_2 (mg/L)	11.5 ± 1.5	10.0 ± 2.6
Chl *a* (µg/L)	< 1.1	4.0 ± 3.2
Discharge (L/s)	42 (5-196) [a]	23 (4-59) [a]
Water temperature (°C)	7.6 ± 3.2	9.3 ± 6.2
pH	7.9 ± 0.4	8.2 ± 0.1
Conductivity (µS/cm)	314 ± 37	1402 ± 155
Light (PAR)	40 ± 61 [b]	97 ± 148 [b]

[a] *Jähkel et al. 2022,* [b] *Jähkel A., Helmholtz Centre for Environmental Research, Magdeburg, Germany, personal communication.*

Environmental characteristics

We sampled the streams every 2 months over 1 y. On each sampling occasion (*n* = 6), we measured environmental variables related to water quality (i.e., nutrients and O_2) and sediment characteristics, as important drivers of invertebrate assemblages and productivity. We aimed to assess whether differences in nutrients, O_2 and sediment characteristics occurred between compartments (i.e., water column, benthic and hyporheic) within each stream.

Nutrients We followed the same sampling design in both streams. On each sampling occasion we collected 5 water samples from the benthic and hyporheic compartments and 1 from the water column. The need to collect a higher number of replicate samples from the sediment than from the water column arose from the higher variability in nutrient concentration within the former than in the latter (Hartwig 2016). A parallel study indicated that nutrient concentrations in the water column of both streams remained constant during each sampling date (Jähkel A., Helmholtz Centre for Environmental Research, Magdeburg, Germany, personal communication, Coefficient of Variation of nutrient concentration are in Table S1, Appendix S1).

In both streams, we collected 1-L of surface water and filtered it through 0.22-µm filters (Sartorius, Minisart Syringe Polycarbonate Filters). Samples were then transported at 4° C to the laboratory and DN, N-NO_3^-, N-NH_4^+, SRP, DOC concentration measured following standard protocols (DIN EN 1484, DIN EN ISO 11732, DIN EN ISO 13395, DIN EN ISO 15681-2). An additional 500 mL water sample was collected for chl *a* determination. The water was stored at 4° C, transported in the dark, and filtrated onto a glass microfiber filter (GFF: 47 mm diameter, 0.7 µm pore size, Whatman, England) in the laboratory on the same day. Chl *a* concentration was determined photometrically (DIN 38412-16).

To collect the pore water from the sediment of the agricultural stream, we extracted 5 sediment cores using a polyvinyl chloride corer (PVC) (UWITEC, 019011, Mondsee, Austria, inner diameter = 9 cm) pushed to a depth of 30 cm. After sealing the top with a rubber stopper, the core was extracted, and a second stopper placed underneath to prevent sediment loss. Cores were then cut into 0-5 cm and 5-15 cm sections, the sediment transferred to centrifuge tubes, and transported to the lab at 4°C. On the same day, the tubes were centrifugated (Allegra X-15R Centrifuge, Beckman Coulter, Krefeld, Germany) at 1500 rpm for 10 min to separate the pore water from the sediment. Extracted pore water was filtered and analyzed as previously described. Some samples lacked sufficient pore water volume to measure all parameters (*n* DN = 56/60, *n* N-NO_3^- = 56/60, *n* SRP = 60/60, *n* DOC = 58/60, *n* N-$NH4^+$ = 60/60).

For the forested stream, we used a different technique to extract pore water due to the cobble-gravel lithology. We planned to install 2 PVC tubes (1 cm inner diameter, screened at the bottom over 5 cm) at 5 locations a week before sampling. One tube was to reach 5 cm depth, the other 15 cm. However, due to the coarse lithology, we could not install a tube firmly at 5 cm depth, thus we only installed the tubes at a 15 cm depth. A week after the installation, pore water was retrieved using a peristaltic pump (Peristaltic pump 12 VDC, Eijkelkamp, Netherlands) at a constant rate of 1.8 mL/s, after discarding the first 20 mL. Collected pore water was filtered and stored as previously described.

To compare the nutrient concentration across compartments (i.e., water column, benthic and hyporheic), we averaged the measurements obtained from the 5 samples collected from each compartment during each sampling campaign, resulting in a final sample size of *n* = 6. Then, we used a linear mixed-effects model (function *lme,* package lme4, (Bates et al. 2015)) to examine the relationship between the measured nutrient concentrations and the compartments (i.e., water column, benthic, hyporheic). We set "*campaign*" as a random factor to account for lack of independency of water samples collected during the same sampling campaign. The model was formulated as: *nutrient concentration* ~ *compartment*, random = ~1 | *campaign*. When statistical significance was determined, we additionally ran a Tukey's Honest Significant Difference (Tukey's HSD) post hoc test to detect specific differences among the compartments. The normal distribution of residuals and homogeneity of variances of the data was visually inspected by plotting residuals vs fitted values.

Oxygen In both streams, we measured O_2 concentrations in the water column and sediment pore water. O_2 in the water column was measured with the Winkler method (DIN EN 25813) by collecting an additional 250 mL water sample. To assess O_2 in the pore water we employed 2 distinct approaches due to varying streambed lithologies. In the forested stream, we inserted a PVC tube (inner diameter = 1 cm) at 15 and 30 cm depth. A week after the installation, we collected pore water as previously described and measured O_2 using the Winkler method. In the agricultural stream, we retrieved an additional sediment core and determined the depth at which the sediment got anoxic (i.e., $O_2 < 0.5$ mg/L) by using a pre-calibrated Firesting needle (Oxygen Micro/Minisensor, sensor code: ZA7-521-197, Pyroscience, Aachen, Germany) attached to a house-made micromanipulator. The micromanipulator served to gradually insert the needle into the sediment. The O_2 concentration and the depth reached by the needle in the sediment were manually recorded. This procedure was repeated at 3 distinct spots within the same core during each sampling event.

Sediment Our aim was to characterize sediment grain size distribution in both streams.

The sediment collection is described in detail in the next paragraph as it is associated with the invertebrate sampling. In the forested stream, sediment was sieved using a nested column of sieves (details in Pasqualini et al. 2023), while the sediment of the agricultural stream was analyzed with a particle analyzer by laser diffraction (CILAS 1190d, CPS USA) due to the very fine grain size of the sediment. Then, the 90, 50, and 10 percentiles (d90, d50, d10) of the grain size accumulation curves of the sediment were calculated. A paired *t*-test was run to test for differences in d90, d50 and d10 and fine sediment content (i.e., percentage < 2 mm) among compartments in each sediment sample. The *t*-test was paired to account for the non-independency of benthic and hyporheic samples collected from the same core.

To ensure uniform hydrological conditions in the hyporheic zone of the forested stream, we additionally installed to a depth of 15 and 30 cm 5 high-density polyethylene (HDPE, 4 cm outer diameter) piezometers screened at the bottom over a 5 cm range, in an area located 1-1.5 m from the area where the invertebrates where planned to be collected. We measured vertical head gradients (VHG) between surface water and piezometer water. We recorded only downwelling conditions. This procedure could not be conducted in the agricultural stream due to the displacement of non-cohesive fine sediment when hammering the piezometer into the sediment.

Invertebrates

At each stream site, we sampled the benthic (0-5 cm depth) and hyporheic (5-15 cm depth) invertebrate communities every 2 months along a 300-meter reach. On each sampling occasion ($n = 6$) at each stream, we collected 5 samples from 5 sites using a Surber sampler and 5 samples using a sediment corer. The sediment corer served two purposes: to collect smaller benthic invertebrates that were not retained by the Surber net; and to sample invertebrates in the hyporheic compartment. After extracting invertebrates from the sediment in the laboratory, secondary production was calculated for both the stream and compartments using Plante and Downing regression (1989).

Sample collection

In both streams, the Surber sampler (0.0625 m², 250-µm mesh) was placed on the sediment and the sediment was vigorously stirred to 5 cm depth. The collected material was rinsed on a white plastic tray to separate organic and inorganic fractions and the organic fraction preserved in 70% ethanol. A corresponding sample was collected with the sediment corer 3-7 d later at a distance between 0.5-1.5 m from the Surber sampling area, ensuring similar microhabitats (i.e., water depth, flow velocity, sediment characteristics, shading) to those sampled with the Surber sampler.

In the agricultural stream, we used a PVC corer (UWITEC, 019011, Mondsee, Austria, inner diameter = 90 mm) manually inserted in the sediment to a depth of 30 cm. The cores were extracted and cut into 2 parts (0-5 cm depth and 5-15 cm depth), stored in plastic bags, and transported frozen at –20°C to the laboratory. In the forested stream, we used a freeze-corer (UWITEC, freeze-corer type 1, Mondsee, Austria), which is the only quantitative method for collecting hyporheic invertebrates in cobble-gravel streambeds (Bretschko 1985). Freeze-corer tubes were installed to a depth of 45 cm, after the Surber samples were collected. After 3-7 d, the cores were extracted with a tripod, and the cores were cut into 2 segments (0-5 cm depth and 5-15 cm depth). The samples were transported at –20°C to the laboratory for subsequent processing. The details of the extraction with the freeze-corer are presented in Pasqualini et al. 2023.

Sample processing Invertebrates collected with the sediment corers were extracted using the flotation method described in the protocol by Traunspurger and Majdi 2017. After thawing, the sediment was mixed, weighed and a weighted subsample was sieved on a nested column of stainless-steel sieves (2-mm, 1.12-mm, and 20-µm). The fraction retained on the 20-µm sieve was collected and centrifuged at 1500 g for 5 min (Allegra X-15R Centrifuge, Beck-man Coulter, Krefeld, Germany) to remove excess pore water which was poured onto a 20-µm sieve. Ludox HS-40 (Sigma Aldrich, 420816), diluted to 1.14 g/mL, was added to the sediment. Mixing of the sediment and diluted Ludox solution was done by placing the samples in a mechanical vertical rotor (Reax 2, Heidolph Instruments GmbH and CO. KG, Schwabach, Germany) for 10 min at 20 rpm, and then centrifuging them at 800 g for 5 min to separate the organic from the inorganic fraction. Extracted invertebrates and the Ludox were poured onto the 20-µm sieve, and the extracted were preserved in a 4% formaldehyde solution with a few drops of Rose Bengal dye. This method enabled the extraction of both temporary and permanent meiofauna. Invertebrates collected with the Surber sampler and the corer were counted and identified under a stereomicroscope (Leica S8AP0, Wetzlar, Germany) with a 10-80x magnification. Identification of macrofauna was done to the lowest possible level, while permanent meiofauna was identified to the major group level (i.e., Rotifera, Nematoda, Copepoda, Ostracoda, Cladocera, Tardigrada, Acari). Subsampling was performed for Rotifera and Nematoda when more than 200 individuals were counted in the same sample. We measured the body length (BL) or head width (HW) of 30 randomly selected individuals from each taxon and sample. During the processing phase, we lost 1 benthic sample collected in August in the agricultural stream, and 1 hyporheic sample collected in October in the forested stream.

Data preparation We created a benthic sample by combining the benthic sample obtained from the Surber sampler with the upper 5 cm portion of the corresponding sediment sample collected with the corer. This step was crucial to include small-size invertebrates not retained by the Surber net. However, to avoid overestimation of production due to counting individuals twice, we corrected the abundance values of those benthic taxa which were collected simultaneously by both techniques. For this, we analyzed the length size distributions of taxa collected with both techniques and implemented a selection criterion (detailed in Pasqualini et al. 2023). Briefly, if the length distributions did not overlap, we considered the techniques complementary and summed abundances. However, if there was overlap, we recalculated the numerical abundances of individuals within the overlapping range. Our approach assumed that coring techniques provided a better estimate of small-size invertebrate abundance, while the Surber sampler was more accurate for larger ones.

Community composition analysis We aimed to assess differences in the composition of invertebrate communities among the compartments of the two streams. To visualize differences, we used non-metric multidimensional scaling NMDS (function *metaMDS*, package vegan version 2.6-4 (Oksanen et al. 2020)). This was supplemented by permutational multivariate ANOVAs PERMANOVA (Anderson 2006) (function *adonis2*, package vegan version 2.6-4 (Oksanen et al. 2020)) on Bray-Curtis similarity matrixes generated from Hellinger-transformed abundance data for macrofauna, and square-root transformed data for permanent meiofauna. To address the lack of independence between benthic and hyporheic samples retrieved from the same sediment core, we incorporated the blocking factor "strata = id" into the formula. The average contribution of each taxon to differences among compartments was evaluated with the *SIMPER* function (package vegan version 2.6-4 (Oksanen et al. 2020)). Data was checked to meet the assumption of homogeneity of multivariate dispersions with the function *betadisper* (package vegan version 2.6-4 (Oksanen et al. 2020)). The analyses were performed separately for the macrofauna and the permanent meiofauna fraction due to the different taxonomical resolution. All tests and analysis were performed in R (R version 4.3.2, R Project for Statistical Computing, Vienna, Austria).

Secondary production Macrofauna and permanent meiofauna secondary production were calculated separately for both compartments using Plante and Downing (1989) regression (P&D) (Eq. 1). While P&D regression is commonly used for permanent meiofauna (e.g., Majdi et al. 2017), it is less commonly applied for macrofauna. Usually, macrofauna estimates are obtained using the size frequency (SF) method following Hynes and Coleman (1968) and Hamilton (1969), with correction for cohort production intervals (Benke 1979). However, SF tend to overestimate production compared to P&D (Butkas et al. 2011). Given the prevalence of macrofauna in the benthic compartment and permanent meiofauna in the hyporheic compartment, the use of different methods could increase the differences between compartments. To prevent such artifacts, we applied the same method to both invertebrate fractions. Taxa with a mean annual abundance > 50 ind./m^2 were included in the calculation. For those, dry mass (DM) was calculated using published length–mass (Table S1, Appendix S2). Taxon-specific production P_i (g DM m^{-2} y^{-1}), was calculated based on mean annual taxon-specific biomass values B_i (g

DM/m^2), maximum mass per individual M_{max} (mg DM/ind.) and annual average temperature T (°C) measured in the water column:

$$\mathrm{Log}_{10}(P_i) = 0.06 + 0.79\mathrm{Log}_{10}(Bi) - 0.16\mathrm{Log}_{10}(M_{max}) + 0.05T. \quad \text{(Eq. 1)}$$

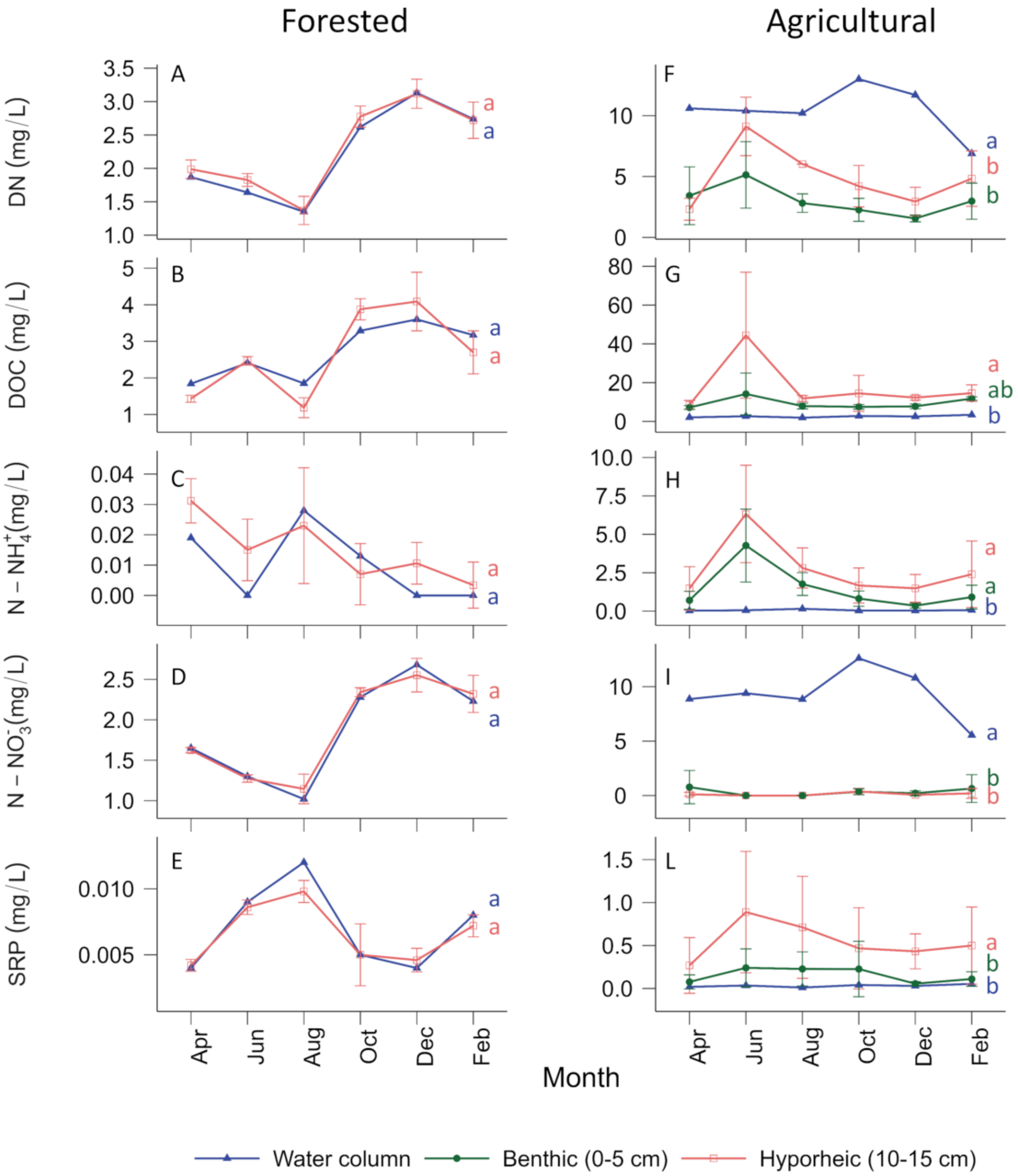
To account for spatial variability in abundance and biomass in our production estimates, we considered the 5 samples collected during each sampling campaign at each depth as replicates. We permuted these replicate samples using a randomized block design, generating all possible combinations across the 6 sampling campaigns (5^6 unique combinations, details in Tables S2-3, Appendix S1). For each combination, we estimated mean, median, SD, 95% CI of taxon-specific production. Compartmental and whole-stream production for each combination was obtained by summing mean taxon-specific production and propagating the error with the *propagate* function (package propagate version 1.0-6). The relative contribution of each taxon to compartmental production was calculated by summing the medians and determining the percentage contribution of each taxon. To assess differences in whole-stream and compartmental secondary production we compared 95% CI. Medians with nonoverlapping CI were considered significantly different (Cross et al. 2013, Brabender et al. 2016, Wild et al. 2022).

RESULTS

Environmental conditions

Nutrient concentrations did not differ among the water column, benthic and hyporheic compartment in the forested stream (Fig. 1A-E, Table S4 Appendix S1). Conversely strong differences occurred in the agricultural stream (Fig. 1F-L). DOC, N-NH_4^+, and SRP concentrations were substantially higher in the hyporheic compartment than in the water column (Tukey's HSD post hoc tests, Table S5 Appendix S1; Fig. 1G-H-L). Conversely, DN, N-NO_3^- concentrations were substantially lower in the hyporheic compartment than in the water column (Tukey’s HSD post hoc tests, Table S5 Appendix S1; Fig. 1F-I). In the agricultural stream, anoxic conditions (< 0.05 mg O_2/L) were recorded in the upper 5 cm of sediment on every sampling occasion (Table S6, Appendix S1). In contrast, the sediment of the forested stream was oxygenated at each sampling occasion at least up to a depth of 30 cm (Fig. S2, Appendix S1). Sediment d90, d50, d10, and fine sediment content did not differ among compartments in both streams (paired *t*-test, $p > 0.05$; data not shown).

Figure 0-1 Nutrient concentrations were measured bimonthly at various depths in the forested (A-E) and agricultural (F-L) streams. Different lowercase letters indicate significantly different values at the p < 0.05 level (Tukey's HSD post hoc test) within the same

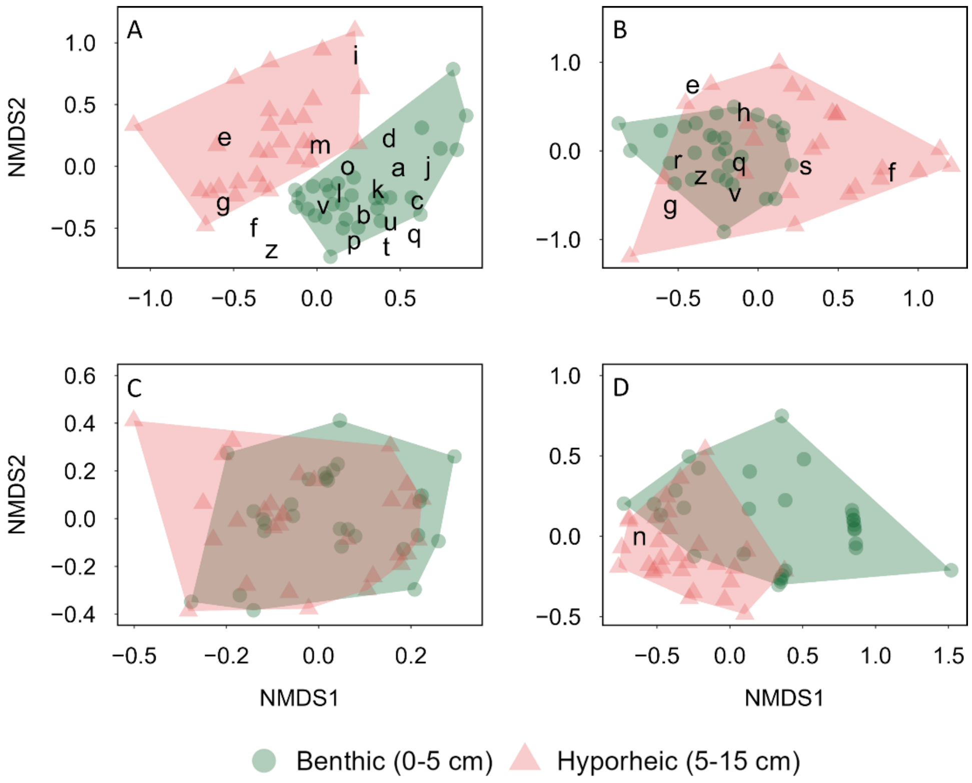


Invertebrates

Community composition NMDS ordination of macrofauna communities revealed marked differences in assemblage composition between the benthic and hyporheic compartments in both streams (Fig. 2A-B). In the forested stream, differences between benthic and hyporheic communities were significant (Fig. 2A, PERMANOVA, $F = 13.36$, $R^2 = 0.19$, $p < 0.001$). Ephemeroptera, Plecoptera, and Trichoptera were numerically more abundant in the benthic compartment than in the hyporheic compartment (Fig. 2A, Table 2). In contrast, Coleoptera and Diptera were more abundant in the hyporheic compartment and contributed to 35% to differences among compartments (SIMPER) (Fig. 2A, Table 2). In the benthic compartment of the agricultural stream, Diptera (8 taxa) and *Potamopyrgus antipodarum* [J. E. Gray, 1843] (Gastropoda) dominated the assemblage. Differences between benthic and hyporheic communities were significant (Fig. 2B, PERMANOVA, $F = 8.06$, $R^2 = 0.13$, $p < 0.001$) and reflected a lower abundance of Diptera larvae in the hyporheic compartment (SIMPER, 56% of variation attributable to Diptera larvae) (Fig. 2B, Table 3).

The composition of the benthic and hyporheic permanent meiofauna did not significantly differ in the forested stream (PERMANOVA, $F = 8.06$, $R^2 = 0.008$, $p = 0.7$; Fig. 2C), but it did in the agricultural stream (PERMANOVA, $F = 4.27$, $R^2 = 0.007$ $p = 0.01$; Fig. 2D). In the agricultural stream, permanent meiofauna densities were considerably lower in the hyporheic compartment than in the benthic one, differences were especially large for Nematoda (Fig. 2D, Table 3).

Figure 2 NMDS ordination of macrofauna communities in the forested stream (A) and in the agricultural stream (B). NMDS of permanent meiofauna communities in the forested stream (C) and in the agricultural stream (D). Letters indicate the taxa identified with SIMPER that contributed the most to differences among zones. a Agapetus fuscipes, b Amphinemura spp., c Ancylus fluviatilis, d Baetis spp., e Ceratopogonidae, f Chironomidae, g Chironomini spp., h Elmis spp., i Esolus spp., j Glossosoma spp., k Ibisia marginata, l Leuctra spp., m Limnius spp., n Nematoda, o Nemoura spp., p Oligochaeta, q Orthocladiinae, r Potamopyrgus antipodarum, s Prodiamesa olivacea, t Sericostoma sp., u Simuliidae, v Tanytarsini spp., z Tanypodinae. NMDS = nonmetric multidimensional scaling.



Secondary production In the forested stream median and 95% CI annual whole-stream invertebrate production was 7.71 (6.21-9.18) g DM m^{-2} y^{-1} (Fig. 3A). 73% of production occurred in the benthic compartment and 27% in the hyporheic one (Fig. 3B). No taxa contributed to more than 12% of compartmental production (Fig. 4). In the benthic compartment, the taxa with the highest contribution to production were *Ancylus fluviatilis* (Gasteropoda:

Planorbidae) (12%), *Sericostoma* sp. (Trichoptera: Sericostomatidae) (10%), Chironomidae (Diptera) (7%) (Table 2). Most of production in the hyporheic compartment was sustained by early larval stages of *Esolus* sp. (Elmidae) (14%) and Chironomidae (12%), but also by representatives of the permanent meiofauna such as Rotifera (12%), Acari (9%), and Copepoda (9%).

In the agricultural stream median and 95% CI annual invertebrate production was 22.97 (9.54-36.33) g DM m^{-2} y^{-1} (Fig. 3A). 94% of production occurred in the benthic compartment and 6% in the hyporheic one (Fig. 3B). Benthic production was sustained by few taxa and *P. antipodarum* accounted for 52% of benthic production and 42% of whole-stream production (Fig. 4, Table 3), followed by *Pisidium* sp. (Bivalvia: Sphaeriidae) (7%) and Naididae (Clitellata) (7%) (Table 3). In the hyporheic compartment, Diptera (8 taxa) and Nematoda contributed respectively to 74% and 14% of hyporheic production.

Figure 3 Median and 95 % CI of annual whole-stream invertebrate production (A). Mean relative 766 contribution of the compartments to annual whole-stream invertebrate production (B). 767 DM = dry mass.

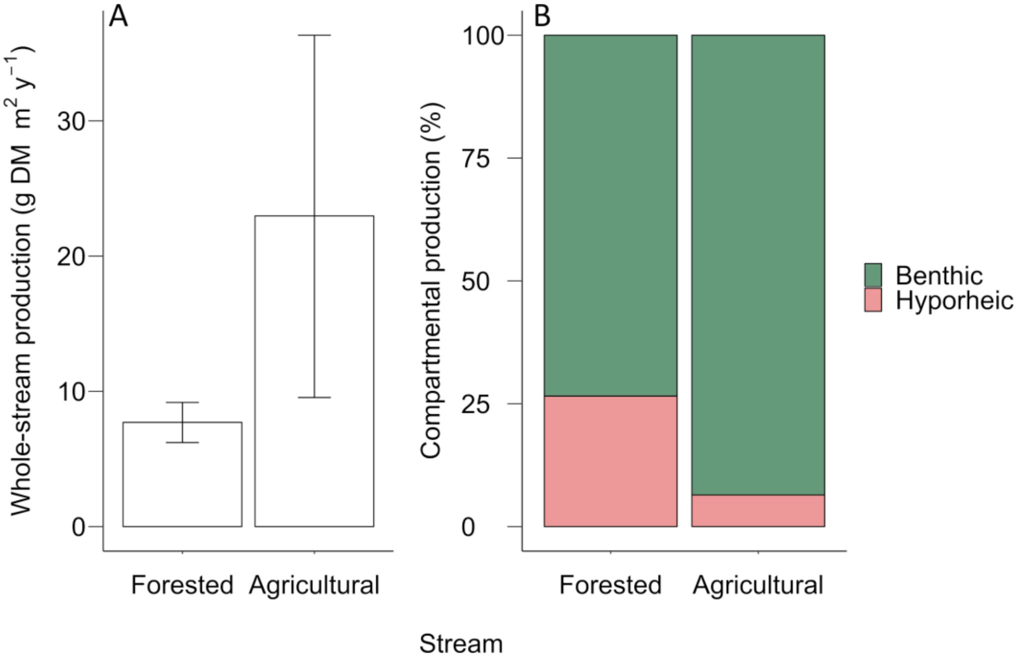
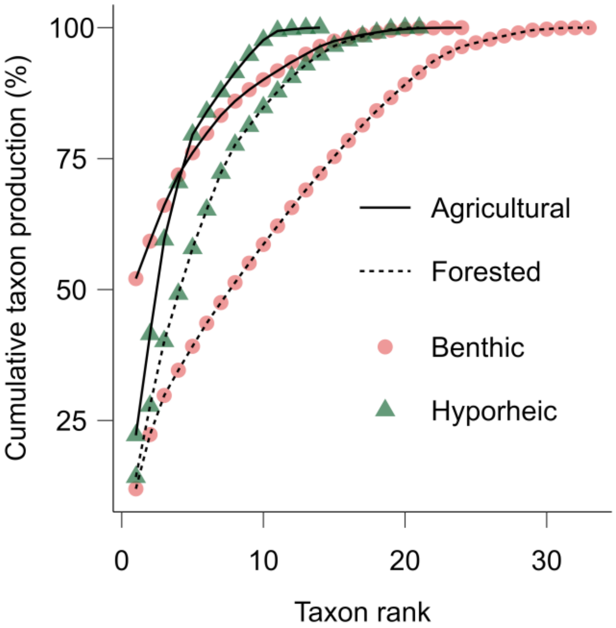


Figure 4. Cumulative percentage of taxa's contribution to whole-stream production in the benthic



DISCUSSION

The hyporheic compartment has been recognized as a hot spot for many ecosystem functions, but its role in invertebrate mediated energy fluxes has not been fully understood. In this study, we sampled the macro- and meiofauna inhabiting the benthic and hyporheic compartments of a forested and an agricultural stream, and quantified compartmental and whole-stream invertebrate secondary production.

The findings of this study indicate that the contribution of the hyporheic compartment to whole-stream production is significantly reduced in the agricultural stream, with clogged sediment, in comparison to the forested one, with more permeable sediment. Nevertheless, whole-stream production in the agricultural stream was approximately threefold higher than in the forested stream, with most of this difference reflecting increased production in the benthic compartment. Many factors regulate levels of invertebrate productivity streams, and it is challenging to disentangle the influence of individual components under field conditions. Nevertheless, by comparing our findings with those of previous studies conducted in low-order streams, we identify and discuss two main processes that may help to explain the observed patterns.

Resource availability is a major control of whole-stream invertebrate production

Several studies showed that nutrient enrichment and higher food quality considerably increase benthic macroinvertebrate production in agricultural (Shieh et al. 2002 and Wild et al. 2022), urban (Gücker et al. 2011), and detritus-based ecosystems (Cross et al. 2006) compared to reference conditions. However, it is unclear whether this increase is representative of the whole-stream, as these assessments are based on benthic macrofauna and do not include neither the hyporheic compartment, nor the full range of invertebrate sizes.

Our study is among the few that have included both compartments and the entire length distribution in estimating whole-stream production. Consequently, to contextualize our findings, we compare our whole-stream and compartmental production estimates with those obtained in studies with similar sampling strategies. Specifically, studies conducted in temperate low-order streams with different environmental characteristics but using comparable sampling strategies (i.e., included the whole invertebrate length distribution and the hyporheic compartment to various depths (Table 4)). Our whole-stream production estimates for the forested stream are comparable to those found in the Ems, Furlbach and Lone Oak, streams which have different substrates but relatively low nutrient concentrations (Table 4). While the whole-stream invertebrate production estimate for the sandy agricultural stream is comparable to values estimated for the gravel habitat of the Lambourn, a hypernutrified chalk stream sampled to a depth between 5 and 10 cm (Table 4).

Temperature can influence secondary production by stimulating invertebrate growth rates (Brown et al. 2004). However, differences in water temperature between these streams do not appear to be pronounced. Furthermore, similar production values are observed in streams with different streambeds, thus, large differences in whole-stream estimates may be attributable to differences in nutrient availability. This is consistent with previous conclusions based on benthic macroinvertebrates production alone (Shieh et al. 2002, Cross et al. 2006, Wild et al. 2022). Negishi et al. 2019 showed that benthic and hyporheic invertebrates assemblages responded differently to changes in water quality in a stream with a gravel bed. In this study, the co-occurrence of other stressors related to agricultural land use (e.g. fine sediment) prevents us from concluding that enhanced nutrients alone will stimulate differently benthic and hyporheic production. However, given the presence of different communities with different growth rates in the compartments (i.e. macro- vs. meiofauna), we expect that similarly enhanced nutrient availability will differentially affect invertebrate production, with further consequences for whole-stream production.

In the agricultural stream, 52% of benthic production was supported by the invasive species *P. antipodarum*. The success of *P. antipodarum* is attributable to a number of factors, including its ability to colonise fine substrates, feed on filamentous algae, tolerate a wide range of abiotic conditions, high fecundity, and its capacity to reproduce parthenogenetically (see review in Alonso and Castro-Díez 2008). Similar patterns of extremely high production of *P. antipodarum* have been observed in other streams with degraded environmental conditions (Hall et al. 2003, Alonso and Castro-Díez 2008), thus our result aligns with previous conclusion that agricultural practices may enhance stream production through the dominance of few generalist species (Wild et al. 2022). The overdominance of a single species can destabilize fundamental ecosystem processes such as secondary production, rendering these processes unstable and susceptible to collapse (Dolbeth et al. 2003, Hall et al. 2006, Wild et al. 2022). Our study, by including the hyporheic compartment, not only aligns with these conclusions, but also shows that the dominance effect is even more pronounced due to the simultaneous reduction in hyporheic functionality. This suggests that the susceptibility of these processes to collapse in the case of strong environmental perturbations may be even higher than expected.

Sediment characteristics are a major control of redistribution of invertebrate production between stream compartments

While resource availability appears to be the main driver of differences in whole-stream productivity, sediment characteristics have a significant influence on the establishment of the community in the hyporheic compartment, thereby affecting the relative contribution of the hyporheic compartment to whole-stream production. This pattern was clearly visible in our study. In the hyporheic compartment of the agricultural stream, the deposition of fine sediment on the streambed resulted in the sediment being disconnected from the water column, resulting in anoxic conditions (< 0.05 mg O_2/L) and elevated levels of N-NH_4^+ in the hyporheic compartment. These conditions impinge invertebrates over extended periods (Zhang et al. 2023), and it is likely that they have prevented the establishment of a resident hyporheic community. Indeed, in the agricultural stream, we consistently found lower abundance, biomass, and production of almost all taxa in the hyporheic compartment in comparison to the benthic compartment. We observed that the hyporheic community was mostly reduced to Diptera larvae and nematodes, altogether making up only 6% of the overall stream production. Dipterans have developed respiratory adaptations (e.g., synthesis of high-affinity respiratory pigments as haemoglobin) to cope with hypoxic or anoxic conditions, while nematodes are known to thrive under suboxic conditions (Taheri et al., 2014).

Given that no prior studies employed our sampling approach in streams with environmental conditions similar to those present in the agricultural stream, we are unable to compare hyporheic productivity estimates from the agricultural stream with previous research. Although we did not replicate our measurements in streams with similar conditions, we anticipate that the functionality of the hyporheic compartment will be similarly diminished in streams affected by biological or physical clogging because of the detrimental effects of fine sediment on the hyporheic compartment. This indicates that alterations in sediment permeability resulting from agricultural land use not only affect macroinvertebrate structural metrics (Bo et al. 2007, Jones et al. 2012, Mathers et al. 2017), but also substantially diminish the functionality of the ones inhabiting the hyporheic compartment. This necessitates the implementation of measures to preserve of the hyporheic compartment's role as a habitat, as it sustains vital ecosystem functions.

Unlike in the agricultural stream, the hyporheic compartment of the forested stream had similar water quality and O_2 concentration to the water column, indicating that the hyporheic compartment was well-connected with the surface water. In these conditions, we found a diverse and productive hyporheic community comprising early larval stages of Coleoptera and Diptera, as well as, more representatives of the permanent meiofauna, including Rotifera, Copepoda and Acari. This community collectively contributed 27% of the whole-stream production, stressing the need to include the hyporheos to yield accurate whole-stream production budgets

Our estimates of hyporheic productivity in the forested stream can be compared with the study of Majdi et al. 2017 performed in two sandy forested streams in north-western Germany (i.e., Furlbach and the Ems Table 4). This study sampled both the benthic and hyporheic compartments simultaneously, collected the entire invertebrate distribution, and assessed each compartment's relative contribution to production. Compared to this study, the productivity of the hyporheic community in our forested stream was within the range of values observed in the Furlbach and the Ems (Table 4). However, the relative contribution of the hyporheic compartment to whole-stream invertebrate production (27%) was approximately half compared to Furlbach and the Ems (48-51%) (Table 4). We attribute these differences to 2 main reasons. First, the differing substrate types present in the streams. The sediment of the forested stream was predominantly coarse gravel and cobble, in contrast to the sandy substrates of the Furlbach and the Ems. The presence of sand and the absence of a riffle-pool sequence have been found to favour meiofaunal size invertebrates (Poff et al. 1993). The differing substrate may therefore explain the lower relative contribution of benthic macroinvertebrates, and therefore of the benthic compartment to whole-stream production in the sandy streams, in comparison to the results obtained in this study. Furthermore, differences in the sampling strategy in the studies could have contributed to exacerbate observed discrepancies. While we employed both a Surber sampler and a corer, Majdi et al. (2017) solely employed a sediment corer which might have overlooked the contribution of larger and more mobile invertebrates such as Ephemeroptera and gammarids.

Finally, our study is among the few that directly compares hyporheic versus benthic production by including both the macroinvertebrates and the permanent meiofauna. The first pioneer study which quantified stream meiofauna production was conducted by Stead et al. (2005) which demonstrated the meiofauna can contribute significantly, comprising 52% of benthic production. Despite this study was conducted in the benthic sediment (0-5 cm depth), its findings stimulated subsequent studies to expand the sampling boundaries and include the hyporheic zone in secondary production quantification. Nevertheless, since Stead et al. (2005) there has been a distinction between studies focusing on the hyporheic compartment and sampling the meiofauna and those sampling the benthic compartment and the macrofauna. However, both fractions are present in the compartments, thus sampling strategies should aim to include both in both compartments (Schmid-Araya et al. 2020). By applying combined sampling strategies, we showed that in both streams the majority of production occurs in the benthic compartment and is sustained by the benthic macrofauna. However, in permeable sediment, the meiofauna still contribute substantially to benthic and hyporheic production and must therefore be included in secondary production estimates (Tod and Schmid-Araya 2009, Majdi et al. 2017, Schmid-Araya et al. 2020, Pasqualini et al. 2023). Consequently, we advocate for a more comprehensive understanding of the entire stream ecosystem, through a holistic approach that encompasses not only the two compartments, but also the entire length distribution.

Broader implication

Our results bring further evidence that while resource availability is the major driver of production differences, sediment characteristics are the dominant factor controlling secondary production repartition between compartments within streams. We demonstrate that fine sediment deposition derived from agricultural land use, by strongly changing the environmental conditions in the hyporheic compartment, reduces the hyporheic compartment functionality. Given that fine sediment deposition and enhanced nutrients often co-occur in agricultural streams, and that agricultural streams are becoming increasingly common (Tank et al. 2021), it is likely that an increasing number of streams will experience a decline in hyporheic functionality coupled with an increase in benthic functionality, leading to unnaturally high production levels. Therefore, it is of the great importance to take immediate action to preserve both the functional and habitat role of both compartments by improving current agricultural practices. Land-use practices in the catchment exert a fundamental influence on the lotic ecosystem. Consequently, to improve stream's conditions, it is necessary to implement actions at the catchment scale. Nevertheless, local actions such as the establishment of riparian buffer zones, which simultaneously reduce nutrients (Hoffmann et al. 2009) and fine sediment inputs (Dunn et al. 2022), should also be implemented. These actions will not only improve habitat conditions for biodiversity, but will also improve streams functionality.

ACKNOWLEDGEMENTS

Author contributions: JP, MB, NM and MW conceived of the study and designed the field methods, JP conducted the fieldwork and analyzed the data. JP and MB led the writing of the paper with contributions from all authors. The authors thank S. Bauth, S. Willige, A. Kneur, H. Matthes, K. Reinmann, R. Degenhardt and S. Jolitz-Seif for their assistance with field and laboratory work. A. Hoff and the GEWANA department for analysis of nutrients in the laboratory. D. Graeber provided helpful comments on the sampling design.

This research was funded by the research program "Changing Earth – Sustaining our Future" of the German Helmholtz Association and was part of the Integration Platform 'Freshwater resources'. The authors declare no conflict of interest.

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FIGURE CAPTIONS

Fig. 1. Nutrient concentrations measured bimonthly in the compartments of the forested (A-E) and agricultural stream (F-L). Different lowercase letters indicate significantly different values at the $p < 0.05$ level (Tukey's HSD post hoc test) within the same stream. Please note the different scales on the y-axis. Values in the benthic compartment of the forested stream are missing because of the infeasibility to install firmly a tube at a depth of 5 cm.

Fig. 2. NMDS ordination of macrofauna communities in the forested stream (A) and in the agricultural stream (B). NMDS of permanent meiofauna communities in the forested stream (C) and in the agricultural stream (D). Letters indicate the taxa identified with SIMPER that contributed the most to differences among compartments. a *Agapetus fuscipes*, b *Amphinemura* spp., c *Ancylus fluviatilis*, d *Baetis* spp., e Ceratopogonidae, f Chironomidae, g *Chironomini* spp., h *Elmis* spp., i *Esolus* spp., j *Glossosoma* spp., k *Ibisia marginata*, l *Leuctra* spp., m *Limnius* spp., n Nematoda, o *Nemoura* spp., p Oligochaeta, q Orthocladiinae, r *Potamopyrgus antipodarum*, s *Prodiamesa olivacea*, t *Sericostoma* sp., u Simuliidae, v *Tanytarsini* spp., z Tanypodinae. NMDS = nonmetric multidimensional scaling.

Fig. 3. Median and 95 % CI of annual whole-stream invertebrate production (A). Mean relative contribution of the compartments to annual whole-stream invertebrate production (B). DM = dry mass.

Fig. 4. Cumulative percentage of taxa’s contribution to whole-stream production in the benthic and hyporheic compartments of the 2 streams.

Table 2 Secondary production, biomass and abundance of taxa collected in the forested stream. Values are annual medians and 95% CI. Values are rounded to nearest decimal except when <1. DM = dry mass.

	Abundance (ind./ m^2)		Biomass (mg/m^2)		Production (mg DM m^{-2} y^{-1})	
Taxon	Benthic	Hyporheic	Benthic	Hyporheic	Benthic	Hyporheic
Elmis sp.	70 (4 - 162)	0	2.4 (0.058 - 6)	0	33 (0 – 103)	60 (0 – 245)
Esolus sp.	388 (156 - 631)	665 (197 - 1295)	25.1 (6.7 - 47.2)	22.3 (6.5 - 39.9)	0	0
Limnius sp.	153 (43 - 339)	162 (19 - 319)	26.2 (9 - 47.3)	7.9 (0.047 - 19.9)	0	0
Oulimnius sp.	0	112 (0 - 562)	0	88.8 (0 - 444)	0	0
Ceratopogonidae	157 (13 - 422)	199 (55 - 369)	0.786 (0.035 - 2.7)	2 (0.138 - 4.3)	1220 (579 – 1941)	234 (36 – 453)
Chironomidae	3603 (1711 - 6979)	2057 (629 - 4953)	63.2 (22.6 - 125.1)	30.1 (6.1 - 83.1)	748 (220 – 1646)	520 (151 – 1273)
Chironomini sp.	539 (72 - 1552)	299 (38 - 771)	2.6 (0.103 - 9)	2.5 (0.082 - 7.7)	1069 (100 – 3117)	33 (0 – 92)
Ibisia marginata	80 (21 - 157)	0	41.2 (8.9 - 94.9)	0	0	0
Orthocladiinae	509 (279 - 777)	139 (0 - 565)	37.4 (12.5 - 79)	0.921 (0 - 3)	263 (131 – 424)	30 (0 – 74)
Simuliidae	102 (11 - 246)	0	20 (0.915 - 53.8)	0	0	0
Tanypodinae	874 (226 - 2246)	313 (42 - 697)	24.7 (6.8 - 57.6)	9.9 (0.897 - 28.4)	417 (97 – 921)	88 (0 – 215)
Tanytarsini	1581 (409 - 3342)	470 (162 - 804)	18.4 (3.8 - 42.8)	2.7 (0.322 - 5.5)	3628 (181 – 9550)	263 (4 – 1017)
Baetis sp.	295 (100 - 564)	104 (0 - 358)	34.7 (7.4 - 76.1)	0.95 (0 - 3.5)	0	0
Ephemerella mucronata	164 (22 - 392)	73 (0 - 310)	18.1 (0.825 - 42.7)	3.9 (0 - 15)	0	0
Habroleptoides confusa	78 (16 - 181)	0	34.3 (6.3 - 78.8)	0	0	0
Ancylus fluviatilis	277 (45 - 496)	0	174.1 (36.2 - 371.2)	0	0	0
Veliger (*Ancylus fluviatilis*)	308 (86 - 652)	76 (10 - 207)	30.3 (4.7 - 103.7)	6.9 (0.536 - 20.7)	0	0
Oligochaeta	137 (48 - 264)	0	41.7 (4.7 - 103)	0	339 (40 – 835)	0
Agapetus fuscipes	434 (111 - 1276)	64 (0 - 141)	29 (8.8 - 75)	4.4 (0 - 10)	0	0
Amphinemura sp.	406 (68 - 1210)	0	22.2 (5.2 - 45.7)	0	0	0
Anomalopterygella chauviniana	94 (5 - 259)	0	35.3 (5.5 - 85.3)	0	0	0
Drusinae	116 (0 - 533)	0	9.1 (0 - 39.8)	0	0	0
Glossosoma sp.	84 (19 - 163)	0	2.6 (0.447 - 5.5)	0	0	0

Hydropsyche sp.	86 (8 - 241)	0	77.6 (5.1 - 252.3)	0	0	0
Leuctra sp.	372 (116 - 903)	192 (9 - 508)	13.8 (5.9 - 22.3)	3.8 (0.015 - 12.8)	0	0
Nemoura sp.	382 (72 - 823)	89 (0 - 255)	5.5 (1.3 - 10.3)	0.632 (0 - 1.7)	0	0
Sericostoma sp.	89 (37 - 157)	0	156.7 (66.4 - 278.6)	0	0	0
Acari	970 (582 - 1807)	1408 (620 - 3017)	10.1 (3 - 24.5)	11.6 (1.8 - 30.8)	92 (1 – 391)	2 (2 – 4)
Alona	179 (9 - 543)	64 (9 - 130)	0.233 (0.012 - 0.638)	0.071 (0.004 - 0.178)	43 (12 – 99)	0
Copepoda	3939 (1543 - 8486)	5363 (2246 - 1.1e+04)	4.8 (1.8 - 11.7)	5.6 (2 - 11.6)	547 (207 – 1109)	114 (29 – 252)
Copepoda: Naupli	625 (214 - 1133)	1614 (719 - 3263)	0.002 (0.001 - 0.003)	0.004 (0.001 - 0.01)	6 (2 – 14)	2 (0.1 – 5)
Nematoda	8.2e+04 (3.5e+04 - 1.5e+05)	1.2e+05 (5.6e+04 - 2e+05)	2.2 (0.647 - 5.9)	2.1 (0.734 - 3.6)	2619 (1302 – 4322)	898 (472 – 1464)
Ostracoda	110 (14 - 280)	285 (21 - 614)	0.176 (0.007 - 0.396)	0.264 (0.005 - 0.554)	169 (38 – 373)	59 (3 – 130)
Rotifera	1.3e+05 (6.2e+04 - 2.3e+05)	2.1e+05 (1e+05 - 3.5e+05)	2.3 (1.1 - 3.5)	3.6 (1.6 - 5.8)	312 (68 – 904)	98 (38 – 186)
Tardigrada	6336 (2311 - 1.1e+04)	1.5e+04 (5307 - 4.3e+04)	0.387 (0.136 - 0.651)	0.831 (0.291 - 2.2)	4 (0.7 – 10)	6 (1 – 13)

Table 3 Secondary production, biomass and abundance of taxa collected in the agricultural stream. Values are annual medians and 95% CI. Values are rounded to nearest decimal except when <1. DM = dry mass.

	Abundance (ind./ m^2)		Biomass (mg/m^2)		Production (mg DM m^{-2} y^{-1})	
Taxon	Benthic	Hyporheic	Benthic	Hyporheic	Benthic	Hyporheic
A. aquaticus	138 (0 – 358)	0	96 (0 – 220)	0	185 (0 – 405)	0
Gammarus pulex	94 (19 – 200)	0	210 (20 – 477)	0	543 (85 – 1166)	0
Elmis sp.	256 (0 – 893)	104 (0 – 437)	4 (0 – 12)	6 (0 – 31)	33 (0 – 103)	60 (0 – 245)
Apsectrotanypus trifascipennis	58 (19 – 107)	0	27 (11 – 43)	0	119 (49 – 180)	0
Ceratopogonidae	7218 (3581 – 1.1e+04)	1385 (169 – 3128)	95 (38 – 161)	16 (1 – 33)	1220 (579 – 1941)	234 (36 – 453)
Chironomidae	2471 (633 – 5336)	1715 (483 – 3300)	79 (18 – 186)	52 (14 – 136)	748 (220 – 1646)	520 (151 – 1273)
Chironomini sp.	3542 (1192 – 6841)	418 (0 – 957)	139 (10 – 423)	2 (0 – 6)	1069 (100 – 3117)	33 (0 – 92)
Orthocladiinae	242 (53 – 631)	163 (0 – 500)	52 (24 – 85)	2 (0 – 6)	263 (131 – 424)	30 (0 – 74)
Prodiamesa olivacea	201 (0 – 718)	135 (0 – 409)	75 (0 – 347)	55 (0 – 191)	280 (0 – 1276)	240 (0 – 768)
Tanypodinae	971 (208 – 2193)	202 (0 – 598)	47 (8 – 111.5)	10 (0 – 26)	417 (97 – 921)	88 (0 – 215)
Tanytarsini	4777 (1001 – 1e+04)	1256 (0 – 3838)	336 (10 – 1098)	15 (0 – 63)	3628 (181 – 9550)	263 (4 – 1017)
P. antipodarum	1092 (117 – 3384)	0	9574 (1323 – 2.8e+04)	0	14957 (2705 – 41319)	0
Naididae	142 (0 – 368)	0	624 (0 – 1628)	0	1294 (0 – 3101)	0
Oligochaeta	158 (24 – 339)	0	80 (5 – 217)	0	339 (40 – 835)	0
Pisidium sp.	213 (5 – 496)	0	528 (30 – 1284)	0	1143 (86 – 2641)	0
Limnephilus sp.	103 (3 – 275)	0	12 (0.662 – 31)	0	55 (4 – 131)	0
Acari	238 (53 – 596)	157 (111 – 282)	4 (0.008 – 11)	0.04 (0.03 – 0.06)	92 (1 – 391)	2 (2 – 4)
Alona	926 (166 – 2362)	0	1 (0.23 – 4)	0	43 (12 – 99)	0 (0 – 0)
Copepoda	5998 (2728 – 1e+04)	1512 (262 – 3342)	22 (8 – 52)	4 (0.4 – 10)	547 (207 – 1109)	114 (29 – 252)
Copepoda: Naupli	2813 (991 – 5630)	805 (162 – 1986)	0.035 (0.009 – 0.08)	0.009 (0 – 0.024)	6 (2 – 14)	2 (0.1 – 5)
Nematoda	8.4e+05 (3.6e+05 – 1.4e+06)	2.7e+05 (1.3e+05 – 4.6e+05)	21 (10 – 34)	7 (4 – 12)	2619 (1302 – 4322)	898 (472 – 1464)
Ostracoda	2082 (568 – 4158)	602 (160 – 1622)	5 (0.95 – 12)	2 (0.03 – 4)	169 (38 – 373)	59 (3 – 130)

Rotifera	7.3e+04 (1.7e+04 – 1.8e+05)	2.8e+04 (7961 – 5.9e+04)	3 (0.56 – 9)	0.89 (0.29 – 2)	312 (68 – 904)	98 (38 – 186)
Tardigrada	665 (90 – 1968)	1081 (125 – 2892)	0.04 (0.002 – 0.13)	0.06 (0.01 – 0.16)	4 (0.7 – 10)	6 (1 – 13)

Table 4 Stream characteristics and mean secondary production estimates from studies conducted in headwater streams with sampling strategies comparable to those used in this study. Nutrient and temperature values are annual means (± SD), except temperature data from the Lone Oak and Lambourn, for which temperature range is reported. To convert production values expressed in Carbon to dry mass (DM) a factor of conversion of 2 had been applied DM = Carbon*2. – no data.

Stream	Environmental characteristics				Production		
	Sediment/ habitat	$N\text{-}NO^-_3$ (mg/L)	$SRP/P\text{-}PO_4$ (µg/L)	Temperature (℃)	Benthic	Hyporheic	Total
					P (g DM m^{-2} y^{-1})		
Ems[1]	Sand	1.45 ± 0.6	25.9 ± 5.9	9.5 ± 1.32	2.54	2.62	5.16
Furlbach[1]	Sand	3.69 ± 2.82	5.32 ± 4.08	7.88 ± 2.11	5.7	5.22	10.92
Lone Oak[2]	Gravel to large cobbles	*Nutrient levels under limit of detection		4 - 15	5.22	–	5.22

Forested stream[3]	Coarse gravel-cobble	1.9 ± 0.6	6.3 ± 2.9 [5]	7.6 ± 3.2	5.66	2.05	7.7
Lambourn[4]	Gravel	Hypernutrified		7 - 15	–	–	22.55
Agricultural stream[3]	Fine sand	9.3 ± 2.3	31 ± 15 [5]	9.3 ± 6.2	21.47	1.47	22.93
Lambourn[4]	Gravel/macrophyte	Hypernutrified		7 - 15	–	–	64.99

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[1] Majdi et al. 2017, [2] Stead 2005, [3] This study, [4] Tod and Schmid-Araya 2009 * NA in the study. Information available in Reiss and Schmid-Araya 2008,[5] values are SRP not P-PO4