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

Julia Pasqualini[1,3], **Nabil Majdi**[2,4], **Markus Weitere**[1,5], **and Mario Brauns**[1,6]

[1]Department of River Ecology, Helmholtz Centre for Environmental Research (UFZ), Magdeburg, Sachsen-Anhalt, Germany
[2]Laboratoire Arago, Réserve Naturelle de la Forêt de la Massane, Banyuls-sur-Mer, France

Abstract: The hyporheic zone has been recognized as a hot spot of numerous biogeochemical processes, yet its role in invertebrate-mediated energy fluxes is unclear. In this study, we sought to improve our understanding of the contribution of the hyporheic zone to whole-stream invertebrate secondary production relative to that of the benthic zone, as well as how agricultural land use affects the functionality and relative contributions of both zones to overall stream production. We sampled the meio- and macrofauna from the benthic (surface to 5-cm depth) and hyporheic (5–15-cm depth) zones of an agricultural and a forested stream and quantified their production in both zones. The benthic zone's contribution to whole-stream production was consistently higher than the hyporheic zone's contribution in both streams, but the hyporheic zone's contribution varied between streams. In the hydrologically well-connected hyporheic zone of the forested stream, a diverse community of Diptera, Coleoptera, Rotifera, Acari, and Nematoda sustained 31% of whole-stream production. Conversely, the hyporheic community in the agricultural stream, primarily composed of Diptera and Nematoda, contributed only 8% of whole-stream production. This smaller contribution is likely due to the presence of fine sediment in the agricultural stream, which hydrologically disconnected the hyporheic zone and created unfavorable habitat conditions (i.e., anoxia, elevated NH_4^+) for most taxa. In contrast, the benthic zone of the agricultural stream was extremely productive, with the invasive taxa *Potamopyrgus antipodarum* (J. E. Gray, 1853) accounting for 46% of whole-stream production. The increase in production was likely driven by higher resource quality and quantity as well as reduced fish predation. Overall, the co-occurrence of agricultural stressors altered the spatial distribution of production within the stream, leading to a disproportionate increase in the benthic zone and a reduction in the hyporheic zone. Given that the benthic and hyporheic zones play distinct roles in many different ecological processes, it is important to examine whether overall stream functioning can be sustained when imbalances in the contributions of both zones arise because of stressors.

Key words: ecosystem functions, meiofauna, clogging, agriculture, benthic, hyporheic, secondary production, macroinvertebrates, fine sediment, stream communities, nutrient pollution

INTRODUCTION

The hyporheic zone 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., Duff and Triska 1990, Boulton et al. 1998, Lewandowski et al. 2019). However, the contribution of the hyporheic zone to whole-ecosystem invertebrate-mediated energy fluxes remains poorly understood because of the limited number of studies that simultaneously quantified benthic and hyporheic secondary production. Early studies suggest that the hyporheic zone contributes little to whole-stream invertebrate production because of a decrease in abundance and biomass of large invertebrates with depth (Williams and Hynes 1974, Bretschko 1998). Conversely, more recent studies that included early larval stages of macrofauna (e.g., Wright-Stow et al. 2006, Reynolds and Benke 2012) and permanent meiofauna (Majdi et al. 2017) have shown that hyporheic invertebrates can substantially contribute to production. These contrasting findings could be explained by variation in sediment physical properties (i.e., permeability),

Email addresses: [3]julia.pasqualini@ufz.de; [4]nabil.majdi@espaces-naturels.fr; [5]markus.weitere@ufz.de; [6]mario.brauns@ufz.de
ORCID iDs: J. Pasqualini, https://orcid.org/0000-0002-8045-7873; N. Majdi, https://orcid.org/0000-0001-7052-4297; M. Weitere, https://orcid.org/0000-0002-5259-2293; M. Brauns, https://orcid.org/0000-0002-5012-9721

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resulting in differing hydrological and physicochemical conditions, or from earlier studies' incomplete sampling of the full invertebrate size range.

Sediment permeability defines the hydrologic interactions between the water column and the hyporheic zone (Tonina and Buffington 2009), determining its temperature, redox conditions, and nutrient concentration (Brunke 1999). These conditions strongly influence the distribution of invertebrates between the benthic and hyporheic zones (Williams and Hynes 1974, Strommer and Smock 1989, Strayer et al. 1997). For example, in permeable hyporheic zones, invertebrates are typically found within the upper 15 cm (Coleman and Hynes 1970, Reynolds and Benke 2012). However, if O_2 concentrations do not decrease steeply with depth, invertebrates can be found at depths up to 70 cm (Williams and Hynes 1974, Palmer 1990). The physical properties of the stream bed also act as a filter (Peralta-Maraver et al. 2018) that influences the distribution of meio- and macrofauna within the sediment (Vervier et al. 1992, Hakenkamp and Palmer 2000). The body size of invertebrates inhabiting the hyporheic zone is typically smaller compared with those found in the benthic zone (Stead et al. 2005b), which results in decreased abundance and biomass of larger invertebrates with increasing sediment depth (Coleman and Hynes 1970, Adkins 1997, Peralta-Maraver et al. 2018). However, early larval stage of macrofauna and permanent meiofauna (e.g., Rotifera, Nematoda, Copepoda) can navigate through the spaces between sediment substrate grains and spend a portion, or their entire life cycle, within the hyporheic zone. The permanent meiofauna is extremely abundant and has higher biomass turnover rates compared with macroinvertebrates (Brown et al. 2004), so despite its smaller size, it is expected to make a substantial contribution to whole-stream invertebrate production (Schmid-Araya et al. 2020).

Streambed permeability can be reduced through physical or biological clogging (Dubuis and De Cesare 2023), a process that can occur naturally in lowland streams or as a consequence of human activities. For example, agricultural land use increases fine-sediment deposition on the stream bed (Allan 2004). This fine-sediment deposition alters the environmental conditions within the hyporheic zone by reducing water flux, creating steep redox gradients, and rapidly depleting O_2 (Buendia et al. 2013). Studies have consistently shown negative effects of clogging on both benthic (e.g., Bo et al. 2007, Jones et al. 2012, Mathers et al. 2014) and hyporheic communities, resulting in reduced abundance and biomass and in altered community composition (Reynolds and Benke 2012, Mathers et al. 2014). Despite these well-documented impacts, our understanding of how clogging specifically affects invertebrate-mediated functions within the hyporheic zone and, consequently, whole-stream invertebrate production remains incomplete. Moreover, in agricultural streams, fine-sediment deposition rarely occurs in isolation but is often accompanied by other stressors, such as changes in light and nutrient levels (Tank et al. 2021). The co-occurrence of high nutrient and light levels has been shown to enhance basal resource quality and quantity (Shieh et al. 2002, Finlay 2011, Wild et al. 2022), which, together with reduced predation pressure in agricultural streams (Shieh et al. 2002, Wild et al. 2022), stimulates benthic production of a few tolerant taxa (Shieh et al. 2002, Finlay 2011, Wild et al. 2022). Nevertheless, it remains unclear whether a similar subsidy–stress response pattern (Odum et al. 1979) will also be observed in the hyporheic zone. Invertebrates might not be able to benefit from increased resource availability if essential habitat conditions (i.e., O_2 availability, pore space, resources) are compromised by the deposition of fine sediment.

This study aimed to understand the functional contributions of the benthic and hyporheic zones to energy fluxes mediated by invertebrates in streams under reference and impacted conditions. Therefore, we compared the relative contributions of the benthic and hyporheic communities to whole-stream invertebrate production in a forested stream with highly permeable sediment and an agricultural stream with reduced permeability due to fine-sediment deposition. Specifically, our research questions were the following: 1) How do nutrients and sediment characteristics, which are potential environmental controls on benthic and hyporheic invertebrate production, vary among zones within an agricultural and a forested stream? 2) What are the relative contributions of the benthic and hyporheic zones to whole-stream production in both streams? We expected that the less permeable conditions in the hyporheic zone of the agricultural stream would create unfavorable habitat conditions for the hyporheic community. As a consequence, we expected the relative contribution of agricultural hyporheic invertebrates to whole-stream secondary production to be lower than that of the forested stream, despite greater resource availability.

METHODS

We conducted a field study from August 2019 to June 2020 in 2 headwater streams, 1 forested and 1 agricultural, located in the Bode River catchment, central Germany. At each stream site, we concurrently sampled benthic and hyporheic invertebrates and measured environmental characteristics of the water column and the benthic and hyporheic zones bimonthly over 1 y ($n = 6$ sampling campaigns). We used linear modeling to compare environmental characteristics and multivariate analyses to compare invertebrate community composition between the zones within each stream. We calculated invertebrate secondary production and compared the relative contributions of the benthic and hyporheic zones to whole-stream production.

Site selection

To enable comparison of the contributions of benthic and hyporheic invertebrates to whole-stream production under different stream conditions, we selected 2 sites with different land-use, hydrological, and sediment characteristics. The forested stream (Drängetalbach: lat 51°48′21.02″N, long 10°43′51.82″E) is surrounded by coniferous forest and has a natural hydromorphology with pool–riffle sequences, gravel bars, and woody debris (Jähkel et al. 2022; Fig. S1A). This stream's water-column nutrient concentrations are relatively low (Table 1), and its stream bed consists of cobbles (range: 63–200 mm) and coarse gravel (range: 20–63 mm). In contrast, the agricultural stream (Asse: lat 51°55′23.357″N, long 11°1′53.443″E) is channelized and surrounded by herbaceous vegetation and alder on the riverbank (Fig. S1B).

We measured conductivity, temperature, and pH in the water column of both streams with a MultiLine® Multi 3630 IDS SET F multimeter (Xylem Inc.®, Washington, DC) every 2 mo over the 1-y study period, and we measured light intensity every 10 min over 1 y from May 2019 to August 2020 with 5 Hobo® MX2202 pendent light-intensity data loggers (Onset® Computer Corp., Bourne, Massachusetts) placed along the stream reaches (see below for nutrient sampling methods). In the agricultural stream, water-column nutrient concentrations, temperature, and conductivity were higher than in the forested stream (Table 1, Fig. 1A–J), and the stream bed was predominantly composed of fine sand (range: 0.063–0.200 mm) and coarse silt (range: 0.020–0.063 mm). In addition, the agricultural stream had higher light exposure (Table 1) because the stream flows through open fields and has less dense canopy cover compared with the forested stream, which flows through a steep valley and has denser canopy cover (Fig. S1A).

Table 1. Environmental characteristics of the studied streams in the Bode River catchment, central Germany. Values are annual means ±1 SD of measurements taken from samples collected in the water column every other month (August 2019–June 2020), with the exception of discharge values, which are annual means (minimum–maximum). DN = dissolved N, SRP = soluble reactive P, DOC = dissolved organic C, Chl *a* = chlorophyll *a*, PAR = photosynthetically active radiation. Land-use, discharge, and PAR data are from other sources[a, b].

Variable	Forested	Agricultural
Land use (forest %–arable %–other %)	100–0–0[a]	12–85–3[a]
DN (mg/L)	2.2 ± 0.6	10.4 ± 2.0
NO_3-N (mg/L)	1.9 ± 0.6	9.3 ± 2.3
NH_4-N (mg/L)	0.01 ± 0.01	0.06 ± 0.05
SRP (µg/L)	7 ± 3.2	31 ± 15
DOC (mg/L)	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] Source: Jähkel et al. 2022

[b] Source: A. Jähkel, Helmholtz Centre for Environmental Research, Magdeburg, Germany, personal communication

Environmental characteristics

We sampled the streams every 2 mo over the 1-y study period. 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 assemblage and productivity. We aimed to assess whether there were differences in nutrients, O_2, and sediment characteristics between zones (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 each from the benthic and hyporheic zones and 1 sample from the water column. We only collected 1 replicate sample from the water column because of its lower variability in nutrient concentration (Hartwig 2016). A parallel study indicated that nutrient concentrations in the water column of both streams remained constant during each sampling date (A. Jähkel, Helmholtz Centre for Environmental Research, Magdeburg, Germany, personal communication; see also CV of nutrient concentrations in Table S1).

In each stream we collected 1 L of surface water from the thalweg into two 0.5-L amber glass bottles (DWK square screw bottles, 238164454; Laboratory and Medical Supplies, Brigachtal, Germany). We filtered 0.5 L of the sampled water through 0.22-µm filters (Minisart® High Flow Syringe Filters, PES, 16532-Q; Sartorius AG®, Göttingen, Germany) and transported it at 4°C to the laboratory to measure dissolved N (DN), NH_4-N, NO_3-N, soluble reactive P (SRP), and dissolved organic C (DOC) concentrations following standard protocols DIN EN ISO 13395, DIN EN ISO 11732, DIN EN 1484, and DIN EN ISO 15681-2 (Beuth Verlag GmbH 1996, 2005, 2019a, 2019b). The additional 0.5-L water sample was used for Chl *a* determination. The water was stored at 4°C, transported in the dark, and filtered through a glass microfiber filter (47-mm diameter, 0.7-µm pore size; Whatman®, Buckinghamshire, UK) in the laboratory on the same day. We determined Chl *a* concentration photometrically (DIN 38412-16; Beuth Verlag GmbH 1985).

To collect pore water from the sediment of the agricultural stream, we extracted 5 sediment cores with a PVC corer (inner diameter = 86 mm; model 019013; UWITEC GmbH, Mondsee, Austria) pushed to a depth of 30 cm. After sealing the top with a rubber stopper, we extracted the core and placed a 2nd stopper underneath to prevent sediment loss. We sliced the cores into 2 sections (0–5-cm and 5–15-cm depths), transferred the sediment to centrifuge tubes, and transported the tubes to the lab at 4°C

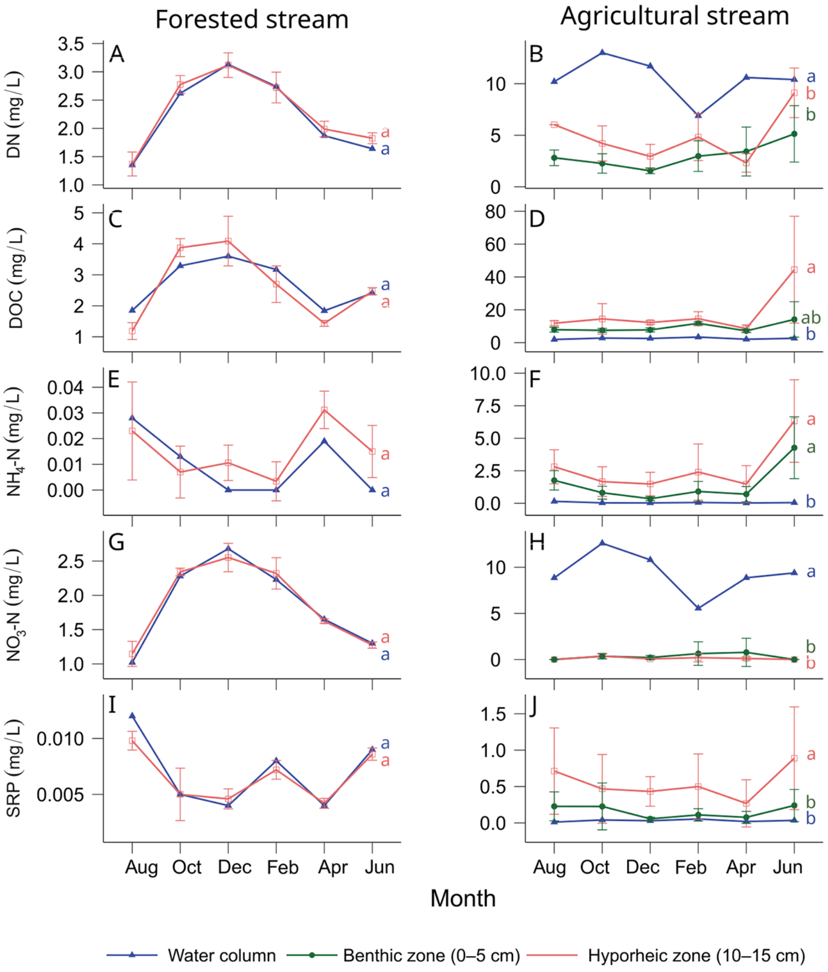

Figure 1. Nutrient concentrations were measured every 2 mo (August 2019–June 2020) in the water column and the benthic (surface to 5-cm depth) and hyporheic (5–15-cm depth) zones in a forested and an agricultural stream in the Bode River catchment, central Germany. Nutrients are dissolved N (DN) (A, B), dissolved organic C (DOC) (C, D), NH_4-N (E, F), NO_3-N (G, H), and soluble reactive P (SRP) (I, J). Different lowercase letters indicate differences between sampling zones within the same stream (Tukey's honestly significant post hoc test with p-values <0.05). Note the varying y-axis scales. Values measured in the benthic and hyporheic zones are means ± SD. In the water column a single sample was collected during each sampling campaign. Values in the benthic zone of the forested stream are missing because of the infeasibility of installing a tube firmly at a depth of 5 cm.

(Lagauzère et al. 2011). On the same day, we centrifuged the tubes on an Allegra X-15R Refrigerated Centrifuge (Beckman Coulter Life Sciences®, Brea, California) at 58.3 Hz (3500 rpm) for 10 min at 8°C to separate the pore water from the sediment (Dadi et al. 2023). We filtered, stored, and analyzed extracted pore water as described above for water-column samples. Some samples lacked sufficient porewater volume to measure all parameters (DN $n = 56/60$, NO_3-N $n = 56/60$, SRP $n = 60/60$, DOC $n = 58/60$, NH_4-N $n = 60/60$).

We used a different technique to extract pore water in the forested stream because of the cobble–gravel lithology (Dahm et al. 2007). We planned to install 2 high-density polyethylene (HDPE) tubes (1-cm inner diameter, 5-cm screened section at the bottom end) at 5 locations 1 wk before sampling, 1 at 5-cm depth and the other at 15-cm depth. However, because of the coarse lithology, we could not install a tube firmly at 5-cm depth. Thus, we only installed the tubes at 15-cm depth. We installed the HDPE tubes with an outer casing and a lost tip. After inserting the tube, we pushed the tip out and carefully removed the casing. After installation, we immediately pumped (developed) the tubes with a peristaltic pump (12 VDC; Royal Eijkelkamp, Giesbeek, The Netherlands) to ensure the screens were not blocked. One week after installation, we retrieved the pore water with the peristaltic pump at a constant rate of 1.8 mL/s, after discarding the first 20 mL. We filtered, stored, and analyzed the collected pore water as described above.

To compare the nutrient concentration across zones (i.e., water column, benthic, and hyporheic), we averaged the measurements obtained from the 5 samples collected from each zone during each sampling campaign, resulting in a final sample size of 6. In the forested stream the comparison was only made between the water column and the hyporheic zone. Then, we used a linear mixed-effects model (function lme, package *lme4*, version 1.1.35.5; Bates et al. 2015) in R (version 4.3.2; R Project for Statistical Computing, Vienna, Austria) to assess differences in measured nutrient concentrations among zones within each stream separately. We set campaign as a random factor to account for the lack of independence of water samples collected during the same sampling campaign. The models for each stream were formulated as nutrient concentration ~ zone, random = 1 | campaign. We visually inspected the normal distribution of residuals and homogeneity of variances of the data by plotting the residuals vs the fitted values. We additionally ran a Tukey's honestly significant difference (HSD) multiple comparisons post hoc test using the glht function from the *multcomp* package (version 1.4.26; Hothorn et al. 2008) in R.

Oxygen In both streams we measured O_2 concentrations in the water column and sediment pore water. We collected water from the water column in the thalweg and filled a 0.15-L glass bottle to the top (model 6315565; Th. Geyer, Warsaw, Poland). O_2 was determined in the laboratory with the Winkler method (DIN EN 25813; Beuth Verlag GmbH 1993). To assess O_2 in the pore water, we used 2 distinct approaches because of varying streambed lithologies. In the forested stream, we inserted an HDPE tube (inner diameter = 1 cm) to 15- and 30-cm depths. A week after the installation, we collected pore water as previously described and used the Winkler method to measure O_2. In the agricultural stream, we retrieved an additional sediment core (as described above) and determined the depth at which the sediment became anoxic (i.e., $O_2 < 0.5$ mg/L), as described in Koschorreck et al. (2003) and Lagauzère et al. (2011), by using a precalibrated FireSting® needle (Oxygen Micro/Minisensor, model OXF900PT-OI, sensor code: ZA7-521-197; Pyroscience® GmbH, Aachen, Germany) attached to a micromanipulator. The micromanipulator served to gradually insert the needle into the sediment. We manually recorded the O_2 concentration and the depth reached by the needle into the sediment. We repeated this procedure in at least 3 distinct spots within the same core during each sampling event.

Sediment We characterized sediment grain-size distribution and organic matter (OM) content in both streams at all 6 sampling campaigns (5 samples/campaign). The sediment collection is described in detail in the next paragraph because it is associated with the invertebrate sampling. In the forested stream, we sieved sediment with a nested column of sieves (details in Pasqualini et al. 2023), whereas we analyzed the sediment of the agricultural stream with a particle analyzer by laser diffraction (model 1190; Cilas, Orléans, France) because of the very fine grain size of the sediment. Then, we calculated the 90th, 50th, and 10th percentiles (D_{90}, D_{50}, D_{10}) of the sediment grain-size accumulation curves. We combusted 3 replicates from the <2-mm sediment fraction of each sample at 550°C to determine the OM content (to the nearest whole %) of the sediment by loss on ignition (Heiri et al. 2001).

To test for differences in D_{90}, D_{50}, and D_{10} among zones within each stream, we ran a Pearson's chi-squared test, and to test for differences in OM among zones, we ran a paired 2-tailed *t*-test separately for each stream in R. The *t*-tests were paired to account for the nonindependence 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 5 HDPE piezometers (4-cm outer diameter, screened at the bottom over a 5-cm range) to 15- and 30-cm depths in an area located 1 to 1.5 m from the invertebrate collection area. We measured vertical hydraulic head gradients between surface water and piezometer water. We recorded only downwelling conditions. This procedure could not be conducted in the agricultural stream because of the displacement of noncohesive 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 mo ($n = 6$) at 5 points along a 300-m reach. The locations of the sampling points were based on 2 criteria: 1) their representativeness in terms of stream conditions within the reach (i.e., substrate type, canopy cover, flow conditions) and 2) their accessibility for the freeze-corer in the forested stream. On each sampling occasion at each stream, we collected 5 samples with a Surber sampler and 5 samples with a sediment corer.

The sediment corer served 2 purposes: 1) to collect smaller benthic invertebrates that were not retained by the Surber net and 2) to sample invertebrates in the hyporheic zone. After extracting invertebrates from the sediment in the laboratory, we calculated secondary production for both streams and zones using Morin and Bourassa (1992) regressions.

Sample collection In both streams, we placed a Surber sampler (0.0625 m^2, 250-μm mesh) on the sediment and vigorously stirred the sediment to 5-cm depth. We rinsed the collected material on a white plastic tray to separate organic and inorganic fractions, and we preserved the organic fraction in 70% ethanol. We collected a corresponding sample with the sediment corer 3 to 7 d later at a distance between 0.5 and 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 additionally used a UWITEC PVC corer equipped with a steel crown (inner diameter = 86 mm; model 019013), which we manually inserted into the sediment to a depth of 30 cm. We extracted the cores and sliced them into 2 parts (0–5-cm and 5–15-cm depths), which we stored in plastic bags and transported frozen at −20°C to the laboratory. In the forested stream, we additionally used a freeze-corer (freeze-corer type 1; UWITEC), which is the only quantitative method for collecting hyporheic invertebrates in cobble–gravel stream beds (Bretschko 1985). We installed freeze-corer tubes to a depth of 45 cm after the Surber samples were collected. After 3 to 7 d, we extracted the cores with a tripod, sliced them into 2 segments, as described above, and transported them 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 We extracted the invertebrates collected with the sediment cores with the flotation method described in Traunspurger and Majdi (2017). After thawing, we mixed and weighed the sediment and sieved a weighted subsample on a nested column of stainless-steel sieves (2-mm, 1.12-mm, and 20-μm mesh sizes). We collected the fraction retained on the 20-μm sieve and centrifuged it at 25 Hz (1500 rpm) for 5 min (Allegra X-15R Refrigerated Centrifuge; Beckman Coulter Life Sciences) to remove excess pore water, which we poured onto a 20-μm sieve. We added Ludox® HS-40 colloidal silica (Sigma-Aldrich 420816; MilliporeSigma®, Burlington, Massachusetts), diluted to 1.14 g/mL, to the sediment. We mixed the sediment and the diluted Ludox solution by placing the samples in a mechanical vertical rotor (model Reax 2; Heidolph® Scientific Products GmbH, Schwabach, Germany) for 10 min at 0.3 Hz (20 rpm) and then centrifuged them at 13.3 Hz (800 rpm) for 5 min to separate the organic from the inorganic fraction. We poured extracted invertebrates and the Ludox solution onto the 20-μm sieve and preserved the extracts in a 4% formaldehyde solution with a few drops of rose bengal dye (Sigma-Aldrich 198250-5G; MilliporeSigma). This method enabled the extraction of both temporary and permanent meiofauna.

We counted the collected invertebrates and identified them by using keys listed in Haase et al. (2006) under a stereomicroscope (10–80× magnification; model S8AP0; Leica® Camera AG, Wetzlar, Germany). Identification of macrofauna was made to the lowest possible level, whereas permanent meiofauna were identified at the major group level (i.e., Rotifera, Nematoda, Copepoda, Ostracoda, Cladocera, Tardigrada, Acari). In samples containing >200 Rotifera or Nematoda ind., at least 200 ind. were counted, and counts were subsequently upscaled based on sample volume to estimate the total abundance.

We measured the head width (trichopterans) or body length (all other taxa) of 30 randomly selected individuals from each taxon and sample to later calculate biomass through published length–mass equations (see next section). 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.

We created a composite benthic sample by combining the benthic Surber sample with the upper 5-cm portion of the corresponding sediment core. This step was crucial for including small-sized 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 that were collected simultaneously by both techniques. To do so, we analyzed the size distributions (i.e., distributions of lengths) of the 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 sampling techniques complementary and summed abundances. However, if there was an overlap, we recalculated the numerical abundances of individuals within the overlapping range. Our approach assumed that coring techniques provided a better estimate of small-sized invertebrate abundance, whereas the Surber sampler was more accurate for larger ones.

Community composition analysis We used multivariate analyses to assess differences in the composition of invertebrate communities among the zones of the 2 streams. To visualize differences, we used nonmetric multidimensional scaling (NMDS; package *vegan*, version 2.6-4; Oksanen et al. 2025) with the default settings of the metaMDS function (i.e., 20 random starts and a maximum of 20 iterations). This analysis was supplemented by permutational multivariate analysis of variance (PERMANOVA; Anderson 2006) with the adonis2 function in the *vegan* package (perm = 999) on Bray–Curtis similarity matrices generated from Hellinger-transformed abundance data for macrofauna and square-root-transformed abundance 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. We evaluated the average contribution of each taxon to differences among zones with a similarity percentage (SIMPER) analysis with the simper function in the *vegan* package. We checked that data met the assumption of homogeneity of multivariate dispersions with the function betadisper in the *vegan* package. We performed these analyses separately for the macrofauna and permanent meiofauna fractions, because of their differing taxonomic resolutions, and separately for the 2 streams.

Secondary production Estimating production in both zones simultaneously posed challenges due to communities containing both macroinvertebrates and permanent meiofauna. These groups have different life cycles, and their production is usually estimated by applying different methods in streams (e.g., the size-frequency method [SF; Hynes and Coleman 1968, Hamilton 1969], regressions [Plante and Downing 1989], the instant-growth method [Reiss and Schmid-Araya 2010]). The application of different methods, however, can introduce biases that can inflate or mask real differences (Plante and Downing 1990). This limitation, coupled with the uneven distribution of macrofauna and meiofauna between the benthic and hyporheic zones, would prevent us from determining whether the observed differences between zones were due to actual differences between them—the aim of this study—or if they were artifacts of the methodological approach. Therefore, to prevent such artifacts, we decided to apply the Morin and Bourassa (1992) regression (hereafter the MB method; Eq. 1), developed for lotic invertebrates, to estimate the production of both macrofauna and meiofauna in the 2 zones. However, the use of regression models for estimating macroinvertebrate production (Morin and Bourassa 1992, Benke 1998) is considered to be a shortcut with reduced accuracy compared with methods such as the SF (Benke and Huryn 2007). Therefore, we assessed whether bias was introduced by the application of the MB method to the macroinvertebrate data by comparing estimates obtained via the MB method vs those obtained via the SF method corrected for cohort production intervals (Benke 1979). Details on the production calculations for the SF method are provided in Pasqualini et al. (2023). In terms of absolute values, we found that whole-stream invertebrate production differed between the 2 methods. Mean whole-stream production values estimated via the SF method were 1.98× higher in the forested stream and 1.96× higher in the agricultural stream than those estimated via the MB method (Table S2). However, the benthic and hyporheic zone contributions to whole-stream production were similar between the 2 methods, with a 6% (agricultural stream) and 9% (forested stream) difference in relative contributions (Table S3), indicating that our findings on zone contributions via the MB method are consistent with those obtained with the SF method.

We calculated secondary production separately for macrofauna and permanent meiofauna in each zone in each stream. Only taxa with mean annual abundance >50 ind./m^2 were included in the calculation. For included taxa, we used published length–mass equations (Appendices S1, S2) to calculate dry mass (DM). We calculated taxon-specific production with Eq. 1:

$$\log_{10}(P_i) = -0.75 + 1.01\log_{10}(B_i) - 0.34\log_{10}(M_{\text{mean}}) + 0.037T, \qquad \text{(Eq. 1)}$$

where P_i is taxon-specific production (g DM m^{-2} y^{-1}), B_i is mean annual taxon-specific biomass (g DM/m^2), M_{mean} is mean mass per individual (g DM/ind.), and T is annual mean temperature measured in the water column (°C). 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 S4, S5). For each combination, we estimated mean, SD, and 95% CI of taxon-specific production. We obtained zone and whole-stream production for each combination by summing mean taxon-specific production and propagating the error with the propagate function in the *propagate* package (version 1.0-6; Spiess 2018) in R. We calculated the relative contribution of each taxon to zone production by summing the means and determining the percentage contribution of each taxon. To assess differences in zone and whole-stream secondary production, we compared 95% CIs. Means with nonoverlapping CIs were considered to be 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 and hyporheic zone in the forested stream (Table S6, Fig. 1A, C, E, G, I). Conversely, there were differences in the agricultural stream (Table S7, Fig. 1B, D, F, H, J). Concentrations of DOC (Fig. 1D), NH_4-N (Fig. 1F), and SRP (Fig. 1J) were substantially higher in the hyporheic zone than in the water column (Tukey's HSD all $p < 0.001$; Table S8). Conversely, DN (Fig. 1B) and NO_3-N (Fig. 1H) concentrations were substantially lower in the hyporheic zone than in the water column (Tukey's HSD all $p < 0.001$; Table S8). 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 S9). In contrast, the sediment of the forested stream was oxygenated at least up to a depth of 30 cm during each sampling occasion (Fig. S2). Sediment D_{90}, D_{50}, and D_{10} did not differ among zones in either stream (Pearson's chi-squared test all $p \geq 0.2$; Table S10). In the forested stream, mean ± SD OM% did not differ among zones (paired t-test $p = 0.2$; Table S11) and was 4 ± 1% in the benthic zone and 4 ± 1% in the hyporheic zone. In contrast, the agricultural

Table 2. Abundance, biomass, and annual secondary production of taxa collected in the benthic and hyporheic zones of the forested stream in the Bode River catchment, central Germany. Values are annual means and 95% CI of measurements taken every other month (August 2019–June 2020). Values are rounded to the nearest whole number, except for those <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 spp.	70 (4–162)	0	2 (0.1–6)	0	23 (1–55)	0
Esolus spp.	388 (156–631)	665 (197–1295)	25 (7–47)	22 (6–40)	221 (77–400)	248 (71–445)
Limnius spp.	153 (43–339)	162 (19–319)	26 (9–47)	8 (0.05–20)	143 (59–241)	83 (0.5–211)
Oulimnius spp.	0	112 (0–562)	0	89 (0–444)	0	348 (0–1828)
Ceratopogonidae	157 (13–422)	199 (55–369)	0.8 (0.03–3)	2 (0.14–4)	11 (1–36)	31 (2–66)
Chironomidae	3603 (1711–6979)	2057 (629–4953)	63 (23–125)	30 (6–83)	770 (322–1423)	394 (80–1094)
Chironomini spp.	539 (72–1552)	299 (38–771)	3 (0.10–9)	2 (0.1–8)	44 (2–129)	36 (1–111)
Ibisia marginata	80 (21–157)	0	41 (9–95)	0	172 (48–374)	0
Orthocladiinae	509 (279–777)	139 (0–565)	37 (12–79)	0.9 (0–3)	276 (106–555)	16 (0–51)
Simuliidae	102 (11–246)	0	20 (0.91–55)	0	129 (12–314)	0
Tanypodinae	874 (226–2246)	313 (42–697)	25 (7–58)	10 (0.9–28)	248 (85–548)	115 (10–330)
Tanytarsini	1581 (409–3342)	470 (162–804)	18 (4–43)	3 (0.3–5)	232 (55–502)	44 (5–90)
Baetis spp.	295 (100–564)	104 (0–358)	35 (7–76)	0.9 (0–3)	254 (62–496)	12 (0–45)
Ephemerella mucronata	164 (22–392)	73 (0–310)	18 (0.8–43)	4 (0–15)	152 (7–351)	36 (0–138)
Habroleptoides confusa	78 (16–181)	0	34 (6–79)	0	139 (36–297)	0
Ancylus fluviatilis	277 (45–496)	0	174 (36–371)	0	635 (163–1263)	0
Veliger (*Ancylus fluviatilis*)	308 (86–652)	76 (10–207)	30 (5–104)	7 (0.5–21)	275 (66–876)	80 (12–203)
Oligochaeta	137 (48–264)	0	42 (5–103)	0	221 (36–522)	0
Agapetus fuscipes	434 (111–1276)	64 (0–141)	29 (9–75)	4 (0–10)	212 (74–529)	36 (0–83)
Amphinemura spp.	406 (68–1210)	0	22 (5–46)	0	156 (47–296)	0
Anomalopterygella chauviniana	94 (5–259)	0	35 (5–85)	0	146 (23–294)	0
Drusinae	116 (0–533)	0	9 (0–40)	0	71 (0–188)	0
Glossosoma spp.	84 (19–163)	0	3 (0.4–5)	0	27 (5–51)	0
Hydropsyche spp.	86 (8–241)	0	78 (5–252)	0	285 (30–893)	0
Leuctra spp.	372 (116–903)	192 (9–508)	14 (6–22)	4 (0.01–13)	98 (48–150)	45 (0.2–152)
Nemoura spp.	382 (72–823)	89 (0–255)	5 (1–10)	0.6 (0–2)	61 (20–106)	10 (0–27)
Sericostoma spp.	89 (37–157)	0	157 (66–279)	0	432 (208–713)	0
Acari	970 (582–1807)	1408 (620–3017)	10 (3–24)	12 (2–31)	168 (63–390)	227 (45–591)
Alona	179 (9–543)	64 (9–130)	0.2 (0.01–0.6)	0.1 (0.004–0.2)	9 (1–22)	3 (0.3–7)
Copepoda	3939 (1543–8486)	5363 (2246–11,000)	5 (2–12)	6 (2–12)	173 (71–401)	205 (78–424)
Copepoda: Naupli	625 (214–1133)	1614 (719–3263)	0.002 (0.001–0.003)	0.004 (0.001–0.01)	0 (0.2–0.9)	1 (0.4–3)
Nematoda	82,000 (35,000–150,000)	120,000 (56,000–200,000)	2 (0.6–6)	2 (0.7–4)	266 (105–514)	295 (114–488)
Ostracoda	110 (14–280)	285 (21–614)	0.2 (0.007–0.4)	0.3 (0.005–0.5)	8 (0.6–18)	14 (0.5–25)
Rotifera	130,000 (62,000–230,000)	210,000 (100,000–350,000)	2 (1–3)	4 (2–6)	314 (164–466)	523 (232–835)
Tardigrada	6336 (2311–11,000)	15,000 (5307–43,000)	0.4 (0.1–0.6)	0.8 (0.3–2)	37 (15–60)	83 (29–224)

stream's OM% was substantially higher (paired t-test, $p <$ 0.001; Table S11) in the benthic zone (9 ± 3%) than in the hyporheic zone (6 ± 2%).

Invertebrates

Community composition NMDS ordination of macrofauna communities indicated marked differences in assemblage composition between the benthic and hyporheic zones in both streams (Figs S3A, B, S4A, B, S5A, B). In the forested stream, the benthic and hyporheic communities differed (PERMANOVA pseudo-$F = 13.4$, $R^2 = 0.19$, permutational-$p < 0.001$; Fig. S3A). Ephemeroptera, Plecoptera, Diptera, and Trichoptera were more abundant in the benthic zone than in the hyporheic zone (Table 2). In contrast, Coleoptera were more abundant in the hyporheic zone and, together with Diptera, contributed to 35% of differences among zones (SIMPER) (Table 2).

In the benthic zone of the agricultural stream, Diptera (8 taxa) and *Potamopyrgus antipodarum* (J. E. Gray, 1853) (Gastropoda) dominated the assemblage. As in the forested stream, the benthic and hyporheic communities differed (PERMANOVA pseudo-$F = 8.06$, $R^2 = 0.13$, permutational-$p < 0.001$; Fig. S3B), and the hyporheic zone of the agricultural stream had a lower abundance of Diptera larvae than the benthic zone (Table 3). SIMPER results indicated that 56% of variation was attributable to Diptera larvae.

The composition of the benthic and hyporheic permanent meiofauna did not differ in the forested stream (PERMANOVA pseudo-$F = 8.06$, $R^2 = 0.008$, permutational-$p = 0.7$; Figs S3C, S4C, S5C) but did differ in the agricultural stream (PERMANOVA pseudo-$F = 4.3$, $R^2 = 0.007$, permutational-$p = 0.01$; Figs S3D, S4D, S5D). In the agricultural stream, permanent meiofauna abundances were considerably lower in the hyporheic zone than in the benthic zone, and differences in abundance were especially large for Nematoda (Table 3).

Secondary production In the forested stream, the mean (95% CI) for annual whole-stream invertebrate production was 9.30 (7.37–11.21) g DM m^{-2} y^{-1} (Fig. 2A). Mean benthic production was 6.41 (5.06–7.76) g DM m^{-2} y^{-1}, which corresponded to 69% of annual whole-stream invertebrate production (Fig. 2B). Mean hyporheic production was 2.89 (1.52–4.26) g DM m^{-2} y^{-1}, which corresponded to 31% of annual whole-stream invertebrate production (Fig. 2B).

Diptera and Plecoptera dominated benthic production in the forested stream, contributing 29% and 23%, respectively (Fig. S6). No single taxa contributed to more than 12% of benthic production (Fig. 3). Chironomidae (Diptera) had the highest contribution (12%), followed by *Ancylus fluviatilis* Müller, 1774 adults (Gastropoda: Planorbidae; 10%), *Sericostoma* spp. (Trichoptera: Sericostomatidae; 7%), and Rotifera (5%; Table 2). In the hyporheic zone, Coleoptera and Diptera similarly contributed to production, together accounting for 46% of hyporheic production (Fig. S6). Among the early larval stages of macrofauna, Chironomidae (14%), *Oulimnius* spp. (12%), and *Esolus* spp. (Elmidae) (9%) had the highest

Table 3. Abundance, biomass, and annual secondary production of taxa collected in the benthic and hyporheic zones of the agricultural stream in the Bode River catchment, central Germany, taken every other month (August 2019–June 2020). Values are annual means and 95% CIs. Values are rounded to the nearest whole number, except for those <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
Asellus aquaticus	138 (0–358)	0	96 (0–220)	0	233 (0–512)	0
Gammarus pulex	94 (19–200)	0	210 (20–477)	0	685 (107–1468)	0
Elmis spp.	256 (0–893)	104 (0–437)	4 (0–12)	6 (0–31)	42 (0–128)	76 (0–337)
Apsectrotanypus trifascipennis	58 (19–107)	0	27 (11–43)	0	151 (63–235)	0
Ceratopogonidae	7218 (3581–11,000)	1385 (169–3128)	95 (38–161)	16 (1.4–33)	1534 (730–2438)	298 (47–579)
Chironomidae	2471 (633–5336)	1715 (483–3300)	79 (18–186)	52 (14–136)	937 (276–2063)	673 (200–1664)
Chironomini spp.	3542 (1192–6841)	418 (0–957)	139 (10–423)	2 (0–6)	1347 (126–3892)	41 (0–116)
Orthocladiinae	242 (53–631)	163 (0–500)	52 (24–85)	2 (0–6)	332 (165–531)	39 (0–104)
Prodiamesa olivacea	201 (0–718)	135 (0–409)	75 (0–347)	55 (0–191)	353 (0–1620)	313 (0–1021)
Tanypodinae	971 (208–2193)	202 (0–598)	47 (8–111)	10 (0–26)	524 (121–1157)	115 (0–288)
Tanytarsini	4777 (1001–10,000)	1256 (0–3838)	335 (10–1098)	15 (0–63)	4575 (230–11,980)	337 (5–1350)
Potamopyrgus antipodarum	1092 (117–3384)	0	9574 (1323–28,000)	0	18,864 (3418–52,290)	0
Naididae	142 (0–368)	0	624 (0–1628)	0	1633 (0–3924)	0
Oligochaeta	158 (24–339)	0	80 (5–217)	0	427 (50–1070)	0
Pisidium spp.	213 (5–496)	0	528 (30–1284)	0	1442 (108–3340)	0
Limnephilus spp.	103 (3–275)	0	12 (0.7–31)	0	70 (5–169)	0
Acari	238 (53–596)	157 (111–282)	4 (0.008–11)	0.04 (0.03–0.06)	78 (1–214)	4 (4–6)
Alona	926 (166–2362)	0	1 (0.2–3)	0	58 (17–139)	0
Copepoda	5998 (2728–10,000)	1512 (262–3342)	22 (8–52)	4 (0.4–10)	631 (271–1142)	143 (32–303)
Copepoda: Naupli	2813 (991–5630)	805 (162–1986)	0.03 (0.01–0.08)	0.01 (0–0.02)	7 (3–15)	2 (0.2–5)
Nematoda	840,000 (360,000–1,400,000)	270,000 (130,000–460,000)	21 (10–34)	7 (4–12)	3122 (1698–4716)	1065 (615–1562)
Ostracoda	2082 (568–4158)	602 (160–1622)	5 (0.9–12)	2 (0.03–4)	203 (59–350)	74 (4–139)
Rotifera	73,000 (17,000–180,000)	28,000 (7961–59,000)	3 (0.6–9)	0.9 (0.3–2)	382 (86–1079)	117 (48–215)
Tardigrada	665 (90–1968)	1081 (125–2892)	0.04 (0.002–0.1)	0.06 (0.01–0.2)	6 (1–13)	9 (2–18)

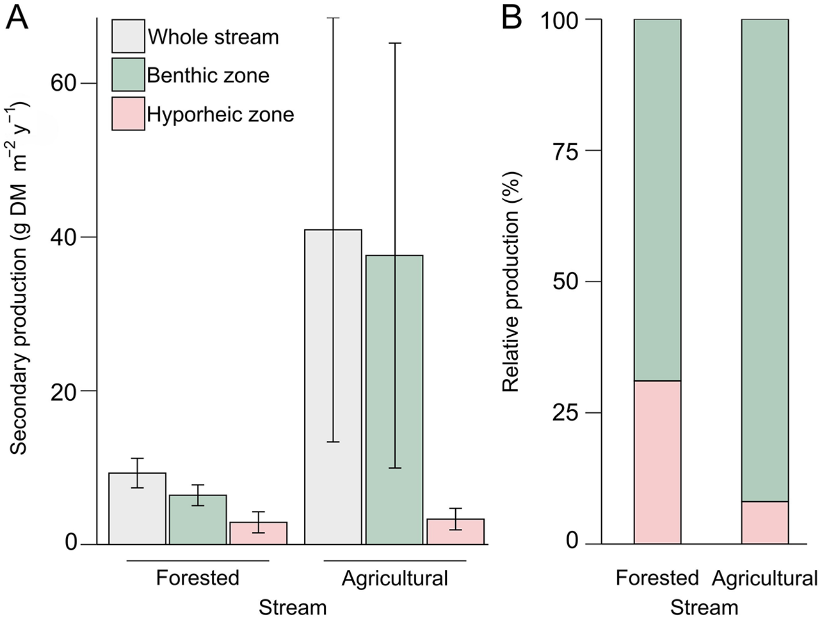

Figure 2. Mean and 95% CI of annual invertebrate production in the benthic (surface to 5-cm depth) and hyporheic (5–15-cm depth) zones in a forested and an agricultural stream in the Bode River catchment, central Germany (A). Mean relative contribution of the zones to annual whole-stream invertebrate production (B). DM = dry mass. Annual production was calculated from measurements taken every 2 mo (August 2019–June 2020).

contributions. Permanent meiofauna sustained 47% of hyporheic production (Fig. S6), with Rotifera (18%), Acari (8%), and Nematoda (10%) being the most productive taxa.

In the agricultural stream, the mean (95% CI) for annual whole-stream invertebrate production was 40.94 (13.34–68.55) g DM m^{-2} y^{-1}, which was ~4.4× higher than the forested stream (Fig. 2A). Production variability was also substantially higher in the agricultural stream, with ~14.4× wider 95% CI than the forested stream. Mean benthic production was 37.60 (9.95–65.23) g DM m^{-2} y^{-1}, which corresponded to 92% of annual whole-stream invertebrate production. Mean hyporheic production was 3.31 (1.91–4.71) g

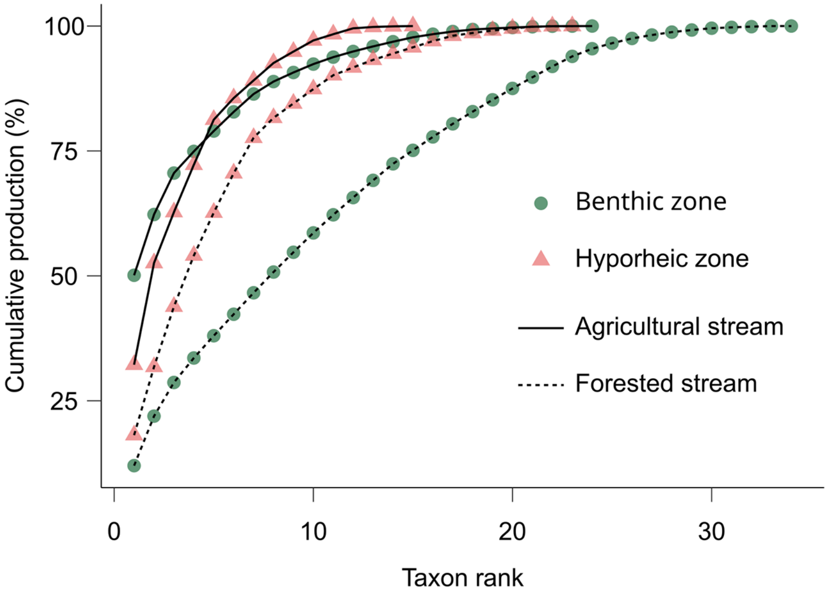

Figure 3. Cumulative percentages of the contributions of individual taxa to whole-stream production in the benthic and hyporheic zones of a forested and an agricultural stream in the Bode River catchment, central Germany, sampled over 1 y (August 2019–June 2020).

DM m^{-2} y^{-1} (Fig. 2A), which corresponded to 8% of annual whole-stream invertebrate production (Fig. 2B). Benthic production was sustained by only a few taxa, with *P. antipodarum* accounting for 50% of benthic production and 46% of whole-stream production (Table 3, Fig. 3), followed by Tanytarsini spp. (Diptera: Chironomidae; 12%) and Nematoda (8%; Table 3). In the hyporheic zone, Nematoda and Diptera (4 taxa) contributed to 31% and 53% of hyporheic production, respectively (Fig. S7).

DISCUSSION

The hyporheic zone has been recognized as a hot spot for many ecosystem functions, but its role in invertebrate-mediated energy fluxes remains understudied. In this study, we sampled the meio- and macrofauna from the benthic and hyporheic zones of a forested and an agricultural stream and quantified secondary production, both for the whole streams and within each zone. Our results demonstrate that although the benthic zone had the highest contribution to whole-stream production in both streams, the hyporheic zone's contribution was substantially larger in the forested stream (31%) than in the agricultural stream (8%). This difference was likely due to the presence of fine sediment in the agricultural stream, which hydrologically disconnected the hyporheic zone and created unsuitable habitat conditions (i.e., anoxia, elevated NH_4^+) for most taxa. These conditions likely compromised the establishment and survival of most taxa, hindering their contribution to secondary production. In contrast, benthic secondary production in the agricultural stream was high and likely the result of higher resource quality and quantity, coupled with reduced fish predation.

Sediment permeability drives the spatial distribution of invertebrate production

In the forested stream, the stream bed consisted of cobbles and coarse gravel, which facilitated water exchange between the surface and the hyporheic zone. The water exchange was evident from the synchronous fluctuations in nutrient and O_2 concentrations in the surface water and hyporheic zone throughout the year. This synchrony is similar to that reported for other streams with permeable sediment, where high exchange rates led to similar O_2 and DOC levels in the stream water and the hyporheic zone (Battin et al. 2003). The high degree of hydrological connectivity, combined with prevailing downwelling conditions, created favorable habitat conditions, allowing benthic invertebrates to colonize the hyporheic zone. In fact, the (macro)invertebrate community in the hyporheic zone was a subset of the benthic community and contributed to 16% of whole-stream production.

More than ½ of the hyporheic production in the forested stream was sustained by early larval stages of macroinvertebrates, with Coleoptera and Diptera having the highest contributions to production. Surprisingly, Coleoptera production was even higher in the hyporheic than in the benthic zone, reflecting high production values for *Esolus* spp. and *Oulimnius* spp., the 2 dominant Coleoptera taxa. The higher production of Coleoptera larvae in the hyporheic zone may be attributed to several advantages that the hyporheic zone offers over the benthic zone, including protection from predators, a stable food supply (e.g., detritus), and reduced shear stress (Boulton et al. 1998, Robertson and Wood 2010). Additionally, unlike Coleoptera adults, which rely on the plastron for respiration and must inhabit fast-flowing benthic zones, larvae possess gills that enable them to thrive in slower-flowing but still highly oxygenated environments (Elliott 2008). It is noteworthy that this pattern of higher hyporheic than benthic production is not exclusive to these 2 taxa but has been observed in species from other orders, such as *Olinga feredayi* (McLachlan, 1868) (Trichoptera; Wright-Stow et al. 2006) and *Leuctra tenuis* (Pictet, 1841) (Plecoptera; Dorff and Finn 2020). Similarly, *Leuctra major* Brink, 1949 has been reported to spend most of its life cycle in the hyporheic zone (Berthélemy 1968). For all other orders identified in this study, particularly Diptera, the 2nd-largest contributor to hyporheic production, the hyporheic zone appears to be important for production, but less so than the benthic zone. Our relative production estimates align with the findings of Reynolds and Benke (2012), who determined that ~40% of total chironomid production occurred in the hyporheic zone of a gravel–cobble stream. These results suggest that although the benthic zone is the primary habitat for most of the macroinvertebrate community and, consequently, the zone where most secondary production occurs, the hyporheic zone provides a critical habitat for taxa with larval stages that are particularly sensitive to shear stress and that spend a substantial portion or their entire life cycle within the hyporheic zone. Consequently, excluding the hyporheic zone in production estimations could lead to substantial underestimation of the contributions of these particular macroinvertebrate taxa.

The other ½ (i.e., 47%) of secondary production in the hyporheic zone of the forested stream was provided by permanent meiofauna, with Rotifera and Nematoda dominating hyporheic production. Our study is the first to report quantitative estimates of hyporheic permanent meiofauna production in oligotrophic forested streams with cobble–gravel sediment. The other studies that have included the meiofauna and found that it plays a relevant role in production were conducted in streams with different substrates (i.e., sand; Majdi et al. 2017) or in the benthic zone of an acidic stream (Stead et al. 2005a). Our estimates (i.e., 47% of hyporheic production and 20% of whole-stream production) are within the range of previously reported contributions (1–52%; Hakenkamp and Morin 2000, Schmid-Araya et al. 2020), supporting the notion that the permanent meiofauna is a numerically dominant component of the hyporheic community, with substantial contributions to secondary production (Hakenkamp and Palmer 2000).

In the agricultural stream, the stream bed was composed of fine sand and silt, which disconnected the hyporheic zone from the surface water, resulting in a porewater nutrient concentration that was largely uncoupled from streamwater fluctuations. Under these conditions, we observed lower abundance, biomass, and production of all taxa compared with the benthic zone. Secondary production in the hyporheic zone was ~11× lower than in the benthic zone, and Nematoda and Diptera sustained 84% of this secondary production. These results are likely the effect of anoxia and elevated levels of NH_4^+, which can be tolerated by only a few taxa, such as Diptera, Nematoda, Ostracoda, and Copepoda (Pacioglu 2010, Taheri et al. 2014). For instance, Diptera has developed respiratory adaptations, such as synthesizing high-affinity respiratory pigments like hemoglobin, to survive in low-O_2 environments (Grazioli et al. 2016). Nematoda, on the other hand, are generally tolerant and often dominate in environments with high organic pollution (Mösslacher 1998).

Another potential reason for the hyporheic zone's diminished contribution to production in the agricultural stream might be lower resource quality in the hyporheic than in the benthic zone. Unlike benthic invertebrates, which, in agricultural streams, can feed on abundant biofilm and fine benthic matter (Wild et al. 2022), hyporheic invertebrates must rely on OM as a basal source. The quality and quantity of this OM can strongly influence invertebrate secondary production (Junker and Cross 2014). Though we found a similar percentage of OM content in the fine-sediment fraction of the sediment (<2 mm) in the hyporheic zones of the agricultural and forested streams, the OM in agricultural hyporheic zones may have lower nutritional value due to reduced fungal activity under anoxic conditions (Cornut et al. 2010). Although not yet demonstrated for herbaceous vegetation inputs, which is the dominant particulate OM input in such streams (Wild et al. 2019), it is known that anoxic conditions can hinder fungal activity, strongly limiting leaf-litter decomposition (Cornut et al. 2010). Agriculture is known to alter the composition and temporal dynamics of OM in the benthic zone of streams (Wild et al. 2019), but it is unclear whether these alterations also occur in the hyporheic zone and how they affect whole-stream processing. Future research should investigate the impacts of agricultural land use on the quality and quantity of OM in the hyporheic zone.

To the best of our knowledge, our hyporheic estimates of production are the only estimates available for oligotrophic forested streams with coarse-gravel stream beds, and our estimates can be compared with only 2 other studies that reported separate production rates for the hyporheos. Smock et al. (1992) sampled the benthic and hyporheic macroinvertebrates of a sandy 1st-order stream in Virginia and reported that 65% of channel production occurred in the hyporheic zone. Majdi et al. (2017) sampled the macro- and meiofauna in both zones in 2 sandy streams in Germany and found that 48 to 51% of whole-stream production occurred in the hyporheic zone. Compared with these studies, our results (i.e., 31% in the forested, 8% in the agricultural) show lower relative contributions. Differences in substrate composition may partially explain these differences. Sandy streams, like those studied by Smock et al. (1992) and Majdi et al. (2017), are often associated with lower benthic macroinvertebrate production compared with coarser sediments (Entrekin et al. 2007). This lower production could be because the sediment grain size affects resource availability, thereby altering secondary production. In sandy-bottomed streams, benthic primary production can be severely limited (Scheidweiler et al. 2021) because the water current displaces the grains and makes the benthic zone a highly dynamic environment (Scheidweiler et al. 2021). Under these conditions, benthic invertebrate consumers may rely less on autochthonous production and more on detrital inputs as primary basal resources. However, these inputs can become buried from the frequent shifts of the sediment (Metzler and Smock 1990, Schofield et al. 2004), reducing their availability to benthic invertebrates while potentially making them accessible to hyporheic consumers. This mechanism, combined with the fact that sandy sediments tend to favor hyporheic meiofaunal organisms (Dole-Olivier and Marmonier 1992, Majdi et al. 2017) may explain why the hyporheic zone has a more prominent role in sustaining whole-stream production in streams with sandy sediments compared with streams with cobble–gravel sediments.

Differences in secondary production in the benthic zones of the 2 streams

Benthic production in the agricultural stream was 6× higher than in the forested stream. Our results fall within the typical range reported for low-order forested (Cross et al. 2006, Entrekin et al. 2007, Wallace et al. 2015) and agricultural streams (Shieh et al. 2002, Wild et al. 2022). Moreover, our results align with the common observation that agricultural streams have higher benthic production rates than pristine sites (Shieh et al. 2002, Finlay 2011, Wild et al. 2022).

Previous studies suggest that enhanced nutrients stimulate benthic primary production, leading to higher secondary production by increasing resource quality and quantity (Webster and Meyer 1997, Finlay 2011). A study conducted in the same region as this study showed that benthic macroinvertebrate production was 1.6 to 3.6× higher in agricultural than in forested streams because of both elevated nutrient levels and reduced fish predation (Wild et al. 2022). In this study, we did not directly measure primary production or fish predation pressure. However, P concentrations and light levels (Table 1) were high enough not to limit primary production, suggesting that an increase in autochthonous production could have contributed to the higher benthic production observed in the agricultural stream. Moreover, we found that the benthic community was characterized by an impoverished invertebrate community, with *P. antipodarum* accounting for 50% of benthic production and 46% of whole-stream production in the agricultural stream. *Potamopyrgus antipodarum* is known for its resistance to many predators because of its hard shell and solid operculum (Vinson and Baker 2008), and it can even survive fish ingestion (Rakauskas et al. 2016). Therefore, although reduced predatory pressure may have played a role in increasing production, it is likely not as strong a driver as nutrients, as found by Wild et al. (2022).

In contrast with the forested stream, where no taxa contributed more than 12% to benthic production, the dominance of a single species is frequently observed in degraded ecosystems (Shieh et al. 2002, Hall et al. 2003, Wild et al. 2022). Wild et al. (2022) found that *P. antipodarum*, *Gammarus pulex* (Linnaeus, 1758), Oligochaeta, and *Asellus aquaticus* (Linnaeus, 1758) were the primary contributors to secondary production in other agricultural streams in the same region. Though all of these species have similar ecological niches and can adapt their feeding strategies to available resources (e.g., Macneil et al. 1997), they tend to have different habitat preferences. For instance, *A. aquaticus* favors streams with rocky substrates covered with aquatic vegetation and organic material, offering protection from predators (Fišer et al. 2019). This preference for rocky substrates may explain why *A. aquaticus* was the dominant species in the gravel-bed agricultural stream sampled by Wild et al. (2022), whereas *P. antipodarum,* known for its preference for fine-sediment stream beds (Alonso and Castro-Díez 2008), was the dominant species in our study. These results suggest that despite variations in community composition due to secondary factors (e.g., habitat preferences or biotic interactions), agricultural streams exhibit a consistent pattern of increased benthic secondary production dominated by few tolerant species, often mollusks, suggesting functional redundancy (Wild et al. 2022).

Broader implications for whole-stream functioning

Our study contributes to the scant knowledge on the role of the hyporheic zone for overall stream functioning. Analogous with human metabolism, the benthic zone appears to function as the anabolic part of the stream ecosystem, supporting most processes involved in the production of new matter, such as gross primary production and secondary production (this study). Consequently, it is where the highest rates of nutrient assimilation are measured (e.g., NO_3^- uptake [Pasqualini et al. 2024], P uptake [Mulholland et al. 1997], NH_4^+ uptake [Hall et al. 2002]). Conversely, the hyporheic zone appears to contribute less to these processes. Instead, it plays a major role in the catabolic part of stream metabolism because it is where respiration is the highest (Naegeli and Uehlinger 1997) and recalcitrant OM is slowly degraded by the microbial (Boulton and Quinn 2000) or fungal community (Cornut et al. 2010), generating detritus, which serves as reservoirs of energy (Moore et al. 2004).

Under reference conditions it appears that the benthic and hyporheic zones may complement each other's contributions to maintaining whole-stream functioning; however, anthropogenic activities can alter these contributions. Agricultural stressors, such as nutrients and light, enhance the processing of new material, increasing benthic fluxes and resulting in higher gross primary production (Hall and Tank 2003, Bernot et al. 2010) and secondary production (Shieh et al. 2002, Finlay 2011, Wild et al. 2022) and increasing the role of the benthic zone in the decomposition of OM (Woodward et al. 2012). On the other hand, fine-sediment deposition appears to decrease the hyporheic zone functionality by reducing fungal decomposition (Cornut et al. 2010) and shredder activity (Herbst 1980, Danger et al. 2012), potentially diminishing the role of the hyporheic zone in OM processing.

A co-occurrence of stressors creates an imbalance by altering the spatial distribution of functions in streams. This imbalance raises several questions that need to be addressed by future studies. For example, can the benthic zone fully compensate for the reduced functionality of the hyporheic zone? Can hyporheic functionality be fully restored after it has been lost? Reducing stressors like nutrient pollution and light exposure may improve benthic conditions, but it remains uncertain whether the hyporheic zone can recover its original structure and function. Given our incomplete understanding of the hyporheic zone's functional role and its capacity for restoration, safeguarding it from clogging and further deterioration is imperative.

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