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Unicellular organisms in benthic habitats on different substrates in a first-order stream and their contribution to secondary production

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ABSTRACT: The abundances of bacteria, flagellates and ciliates were investigated over 13 mo in an unpolluted first-order stream. Four habitats on different substrates were integrated in our study: sediment, as well as the surfaces of dead wood, macrophytes, and leaf litter. Organismal biomass and secondary production in the habitats were calculated and the relative contribution to overall secondary production was estimated. We expected highest organismal densities on leaf litter in autumn and in the other 3 habitats in spring/summer. We assumed bacteria to be most abundant and ciliates to represent the highest biomass and dominate secondary production. Moreover, we hypothesized that dead wood and leaf litter would account for the largest share of total secondary production of all 4 habitats. In the stream's sediment, protozoan abundance showed a trend towards a seasonal pattern. Annual mean abundance, biomass and secondary production by all organismal groups were highest on dead wood. Ciliates made up the highest percentage of total annual biomass in sediment and on dead wood and leaf litter, whereas on macrophytes, bacteria had the highest percentage of total annual biomass. In all habitats, ciliates had the highest share of secondary production. Total organismal secondary production during the sampled year was 660.6 µg C cm^{-2}, of which 71% was contributed by organisms on dead wood. The contribution of dead wood to the annual mean habitat cover ratio at the sampling site was only 9%, but it still made up 34% of total unicellular secondary production. Our study clearly showed that the unicellular secondary production on dead wood in streams has thus far been highly underestimated.

KEY WORDS: Protozoa · Bacteria · Seasonal dynamics · Sediment · Dead wood · Macrophyte · Leaf litter

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1. INTRODUCTION

Flowing waters are heterogeneous and complex ecosystems influenced by spatial and temporal patterns (Townsend 1989, Winemiller et al. 2010). The submerged surfaces of streams, such as the sediment, macrophytes, as well as the dead wood and leaf litter entering the stream, establish a natural spatial heterogeneity and reveal different substrates for the development of habitats that can act as refuges for organisms by buffering unfavorable periods and conditions (Townsend 1989). Spatial and temporal patterns are closely connected to each other (Townsend 1989), as in streams located in temperate climate zones, seasonal dynamics influence factors including discharge, the input of organic material and the growth of macrophytes.

In freshwaters, 99% of microbial activity occurs within surface-associated communities (Bryers & Characklis 1982). Submerged surfaces are initially colonized by bacteria, which then serve as the basis for colonization by other organisms (Geesey et al. 1978). Those biofilms, which are assemblages of surface-associated microorganisms enclosed within

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an extracellular polymeric substance matrix (Lock et al. 1984, Donlan 2002), can be found on the surface of organic and mineral substrates (Risse-Buhl et al. 2012a) as well as in streambed sediments (Schmid-Araya 1994). In addition to bacteria and algae, stream biofilms contain microfaunal organisms (those passing through a sieve of 40 µm), including protozoans (Arndt et al. 2003, Weitere et al. 2018).

The biofilms that cover stream surfaces are hotspots of carbon turnover (Geesey et al. 1978, Weitere et al. 2018). Protozoans represent an important pathway for carbon transfer from bacteria to organisms at a higher trophic level (Marxsen 2006, Augspurger et al. 2008, Risse-Buhl et al. 2012b). Protozoan grazing can stimulate bacterial production, which leads to the enhancement of the carbon and energy flow through the system (Fenchel & Harrison 1976, Sherr & Sherr 1994). In addition, protozoans are potential prey, and therefore a possible carbon source, for higher trophic levels, including small metazoans (Schmid-Araya 1994, Reiss & Schmid-Araya 2011). However, little is known about the contribution of protozoans to secondary production (Reiss & Schmid-Araya 2010, Weitere et al. 2018), and especially their contribution in different habitats and the respective seasonal patterns in lotic systems should be explored.

Studies about the unicellular community in streams and rivers that include seasonal patterns have predominantly investigated protozoans or bacteria in the sediments (e.g. Baldock & Sleigh 1988, Schmid-Araya 1994, Packroff & Zwick 1998, Marxsen 2006, Reiss & Schmid-Araya 2008, 2010), whereas few studies also have integrated >1 habitat type and their seasonal patterns (Bott & Kaplan 1989, Sleigh et al. 1992). However, in studies investigating >1 habitat type, habitat-specific data were obtained from different streams rather than from the same stream. To our knowledge, only Sleigh et al. (1992) and Reiss & Schmid-Araya (2010) examined the secondary production of protozoans for ≥1 yr, even though, due to their short generation times and rapid population growth, protozoans can contribute considerably to the secondary production of a system (Reiss & Schmid-Araya 2010).

In the present study, we focus on the distribution of protozoans (ciliates and flagellates) and bacteria in 4 habitats on different substrates of a single first-order stream (Furlbach, Germany) over a 13 mo period. A unique dataset is presented on a spatial-temporal scale that allowed us to estimate the abundance, biomass and secondary production of the 3 organismal groups in these habitats in the course of over 1 yr. We were also able to estimate the relative contributions of the different habitats to the overall secondary production of a lotic system. The 4 habitats integrated in our study were the sediment and the surfaces on dead wood, macrophytes and leaf litter. Previous studies of different stream habitats showed that protozoan and bacterial densities are subject to seasonal variations (Sleigh et al. 1992, Packroff & Zwick 1998, Cleven 2004, Olapade & Leff 2004, Blatterer 2008), and similar results were expected for the habitats of the Furlbach. We assumed protozoan and bacteria densities in sediment as well as on the surfaces of dead wood and macrophytes to be higher in spring and summer than in winter months, whereas on leaf litter, the highest densities were assumed to occur in autumn. We also examined the relative contributions of bacteria, flagellates and ciliates to abundance, biomass and secondary production in the 4 habitats. Bacteria are the most numerous organisms in surface habitats (Giller & Malmqvist 2004) and this was predicted to be the case in the 4 investigated habitats of the Furlbach as well. In terms of biomass and secondary production, we predicted that ciliates would dominate because of their larger size relative to bacteria and flagellates. The surface habitats on organic substrates are known to be densely colonized by bacteria (Hieber & Gessner 2002) and protozoans (Bott & Kaplan 1989) that are directly connected to the decomposing processes and carbon transfer of a system. We therefore expected that, in terms of habitat, dead wood and leaf litter would account for the highest share of total secondary production in streams.

2. MATERIALS AND METHODS

2.1. Study site

The oligotrophic Furlbach stream is located in North Rhine-Westphalia, Germany. It originates in a protected area in Augustdorf, near the city of Bielefeld, from a marshy seepage spring where, after a few meters, a constantly flowing small stream is formed. The Furlbach is a reference stream of the German Federal Environment Agency (Pottgießer & Sommerhäuser 1999). Its dominant macrophytes are *Nasturtium officinale* (R.Br.) and *Berula erecta* (Huds.). Sampling for this study took place at an approximately 20 m reach of the Furlbach, 450 m downstream of the seepage spring, where the Furlbach is surrounded by broad-leafed trees including black alder *Alnus glutinosa*, downy birch *Betula pubescens*, sycamore maple *Acer pseudoplatanus* and hornbeam *Carpinus betulus*.

2.2. Sampling

Samples were collected monthly from April 2016 to April 2017. Physico-chemical data, including temperature (°C), O_2 (mg l^{-1}), conductivity (µS cm^{-1}) and pH, were determined from stream water once every sampling occasion and collected at the sampling site using a multi-probe (Multi 3430, WTW). Sediment, as well as the surfaces on dead wood, the macrophyte *N. officinale* and leaf litter, were sampled as representatives of the habitats in the Furlbach. Each habitat was sampled in 4 replicates, with 1 replicate taken approximately every 5 m along the stream. Stream water samples of 50 ml were collected in 50 ml sterile screw-cap bottles for analysis of nitrate and phosphate and kept frozen until analysis. All samples were stored in a cooling box and brought to the University of Bielefeld. Additionally, 5 l of stream water were collected in a plastic canister and filtered in the laboratory through 0.2 µm cellulose nitrate membrane filters (Whatman). This water is hereafter referred to as 'filtered stream water'.

2.2.1. Sediment

Sediment was sampled with a corer (3.3 cm diameter). Three cores per replicate were taken randomly from the upper 2 cm of the sediment from sandbanks and pooled in 100 ml PET bottles. In the laboratory, the samples were homogenized by gently stirring. One ml each for the counting/analysis of protozoa, bacteria, ash-free dry mass (AFDM) and chlorophyll *a* (chl *a*) was extracted from each replicate and transfererred to 2 ml microtubes using a graduated straw. Samples for protozoan counting were stored at 4°C in the dark, and those for analyses of bacterial abundance, AFDM and chl *a* were kept frozen until needed. Storage was the same for all habitat samples.

2.2.2. Surface of dead wood

The surface of dead wood was sampled using a brush sampler (2 cm diameter, for details see Peters et al. 2005). For each replicate, 2 adjacent samples from the same trunk were taken and pooled in 250 ml PET bottles. In the laboratory, the biofilm samples from the dead wood were suspended in filtered stream water to a total sample volume of 150 ml and then homogenized. One ml each for protozoan counting and bacteria analysis was taken with a pipette and transferred to 2 ml microtubes. For the analysis of AFDM and chl *a*, 20 ml each of the suspension were filtered onto pre-combusted (550°C, 7 h) and pre-weighed glass fiber filters (25 mm diameter, Whatman).

2.2.3. Surfaces of macrophytes and leaf litter

For the analysis of macrophyte surfaces, the upper parts of 5 to 8 plant stands of *N. officinale* completely covered by water were collected and carefully transferred under water into a white photo tray. For the analysis of leaf litter surfaces, 3 to 5 randomly chosen leaves per replicate were carefully transferred into a photo tray. Discs (1.6 cm diameter) were punched out from the collected macrophytes and leaves directly in the field and transferred to 25 ml plastic screw-cap bottles filled with approximately 20 ml of stream water. For counting protozoa, 10 leaf discs were cut out; for counting of bacteria and for AFDM and chl *a* analyses, 4 leaf discs each were cut out. In the laboratory, the surface of the 10 leaf discs for protozoan counting were carefully brushed from both sides with a bristle brush and the suspension then transferred to 50 ml screw-cap bottles. Samples with a final volume of 25 ml were prepared with filtered stream water.

2.3. Protozoan counting

Protozoans were counted in a counting chamber (Nagoette, 0.5 mm depth, 1.25 mm^3 volume) within 24 h after sampling. The sediment samples were prepared in filtered stream water filled to a final volume of 2 ml and vortexed for 30 s. Subsamples of 100 µl were taken from the overlaying water and transferred to the counting chamber. For samples prepared from dead wood, macrophytes or leaf litter, 100 µl subsamples were taken directly after the samples had been shaken. All samples for enumeration were analyzed by microscopy (Zeiss, Scope A1) at 100× magnification, scanning the whole chamber. To ensure identification of protozoans <10 µm, 400× magnification was used. Ciliates and flagellates were detected by their characteristic movements and counted according to the procedure of Gasol (1993), based on size classes (<10, 10–20, 20–30, >30 µm for flagellates and <25, 25–50, 50–75, 75–100, 100–125, 125–150, >150 µm for ciliates). Counting was repeated 3 times per sample.

2.4. Bacterial counting

Bacterial abundance was determined using the DAPI method of Porter & Feig (1980), modified by Schallenberg et al. (1989). Leaf discs were transferred to 50 ml screw-cap bottles and samples with a final volume of 25 ml were prepared in filtered stream water. Sediment and dead wood samples were kept in the microtubes. Each sample was diluted 1:30 with tetrasodium pyrophosphate, vortexed (5 s), sonicated (15 min), vortexed again (5 s), and a 200 µl subsample was taken and diluted with 1800 µl Millipore water. The diluted samples were stained with 100 µl DAPI (50 µl ml^{-1}) for 8 min and filtered onto black polycarbonate filters (0.2 µm, Nuclepore). Bacteria were then counted in 5 randomly chosen fields using an epifluorescence oil microscope (Zeiss, Axioplan2, 1000× magnification).

2.5. AFDM and chl *a*

Chl *a* and AFDM were measured separately for every sampled habitat. For the analysis of AFDM, samples from sediment, macrophyte and leaf litter were transferred to small pre-weighed ceramic pots (4 cm diameter); dead wood samples remained on the filters. All samples were dried (105°C, 24 h) and combusted (505°C, 7 h). AFDM was determined based on the differences in weight (µg cm^{-2}). For chl *a* analyses, the leaf discs of macrophytes and the leaf litter were transferred to 50 ml screw-cap bottles and samples of 25 ml were prepared using filtered stream water. The samples were vortexed for 10 s to detach the attached algae from the surface of the leaf discs, after which the leaf discs were removed and the algal suspension filtered onto glass fiber filters (25 mm diameter, Whatman). The filters prepared from the macrophyte and leaf litter samples as well as the filters prepared from the dead wood biofilm samples were transferred to 15 ml screw-cap bottles. The 1 ml sediment samples prepared in microtubes were directly transferred to 15 ml screw-cap bottles. The chl *a* content of all habitat samples was determined by extracting the samples overnight in 90% ethanol at 4°C in the dark and then analyzing the extracts spectrophotometrically without correcting for pheophytin (Stich & Brinker 2005).

2.6. Phosphate and nitrate

The total phosphate (PO_4) content of the habitat water was measured using the European Standard (ISO-6878, 2004), and the total nitrate (NO_3) content using the Aquanal-Plus test kit (Sigma-Aldrich).

2.7. Calculation of biomass and secondary production

After the protozoa and bacteria had been counted, their biomass was calculated and expressed as dry weight (dw, µg cm^{-2}). Protozoan biomass was calculated by assuming the nearest geometrical shape of each organism type (spherical for ciliates ≤ 50 µm, cylindrical for ciliates > 50 µm and cylindrical for flagellates of all size classes) and then applying a conversion factor of 0.17 pg dw μm^{-3} (Laybourn 1973) and a carbon (C) content of 0.1 pg C μm^{-3} (Borsheim & Bratbak 1987). For bacteria, the estimated cell volume was 0.125 μm^3 (Faupel et al. 2011) and a conversion factor of 1.09 g cm^{-3} and a wet weight ratio of 30% was used (Bakken & Olsen 1983).

Secondary production by protozoa was calculated based on the relationship between body volume and generation time at a given temperature (Finlay 1978, Bergtold & Traunspurger 2005) and determined for 2 consecutive sampling occasions:

$$P_d = \frac{1}{G}\left(\frac{B_0 + B_t}{2}\right)2t \quad (1)$$

where P_d is daily production (µg C cm^{-2} d^{-1}), G is generation time, B_0 is initial biomass of a sampling period, B_t is final biomass of a sampling period and t is length of the sampling period in days. Secondary production by bacteria at a single sampling date was calculated based on the regression equation of White et al. (1991) for daily production in freshwater:

$$\text{Log}_{10}(P_d) = 0.43 + 1.00\text{Log}_{10}(\text{Abund.}) + 0.31T \quad (2)$$

where P_d is daily production (µg C cm^{-2} d^{-1}), Abund. is bacterial abundance at the time of sampling (individuals [ind.] $cm^{-2} \times 10^9$) and T is temperature at the time of sampling (°C).

2.8. Statistical analysis

All graphs were created using SigmaPlot (Systat Software, version 11). A Kruskal-Wallis rank sum test was performed to test whether habitat influenced the mean annual abundance, biomass and secondary production of ciliates, flagellates and bacteria. A nonparametric test was used because of the non-normal distribution of the data. As a post hoc test, Dunn's multiple comparison test was used because of differ-

ences in the sample sizes from the different habitats. The p-values were adjusted using the Holm-Bonferroni sequential correction procedure. Statistical analysis was performed using the computational environment R version 3.2.3 (R Development Core Team 2016). Dunn's test was performed using the R package dunn.test (Dinno 2017).

In Section 3, all mean data (n = 4 for monthly mean, n = 13 for annual mean for sediment and dead wood, n = 11 for leaf litter, n = 10 for macrophytes) are given with the respective standard derivation (±SD). For sediment, the 1 ml subsample volume used for quantification of organisms was referred to the total volume taken with every sediment sample and referred that to 1 cm^2 of the area sampled with the cores.

3. RESULTS

3.1. Physico-chemical analysis

The measured physico-chemical parameters from April 2016 to April 2017 are shown in Table 1. During that sampling period, the water temperature of the Furlbach was 5.9–11.3°C (Table 1), and flow velocity was 0.2–0.4 m s^{-1}. PO_4 values ranged between 21.0 and 43.1 µg l^{-1}, with an annual mean of 35.1 ± 6.2 µg l^{-1}. Annual mean NO_3 concentration was 13.9 ± 0.6 mg l^{-1}. Chl *a* and AFDM content varied among the 4 habitats, with the lowest chl *a* concentration determined on the surfaces of macrophytes and leaf litter (annual mean of 0.04 ± 0.03 µg cm^{-2} for both habitats). On the surface of dead wood, chl *a* concentration ranged between 0.3 and 6.2 µg cm^{-2}. The highest annual mean chl *a* concentration was measured in sediment (37.5 ± 59.6 µg cm^{-2}), with a peak in spring of 213.2 µg cm^{-2}. With an annual mean of 6.4 ± 2.3 µg cm^{-2}, AFDM values were also highest in sediment. Lowest annual mean AFDM values were found in the macrophyte samples (0.9 ± 0.3 µg cm^{-2}).

3.2. Protozoan and bacterial abundance, biomass and secondary production

Sediment, dead wood, macrophytes and leaf litter showed different occurrences of proto-

Table 1. Physico-chemical values of Furlbach stream from April 2016 to April 2017. Annual mean (±SD) values from 13 sampling occasions are shown in **bold**. Values of chl *a* and ash-free dry mass (AFDM) are shown as mean (n = 4) and were measured for every sampled habitat separately, except in the case of macrophytes from January 2017 to March 2017 and leaf litter from March 2017 to April 2017. Temp.: temperature; Cond.: conductivity; Sed.: sediment; Dw.: dead wood; Mph.: macrophytes; Ll.: leaf litter

	Temp. (°C)	O_2 (mg l^{-1})	Cond. (µS cm^{-1})	pH	Flow (m s^{-1})	PO_4 (µg l^{-1})	NO_3 (mg l^{-1})	Chl *a* (µg cm^{-2})				AFDM (µg cm^{-2})			
								Sed.	Dw.	Mph.	Ll.	Sed.	Dw.	Mph.	Ll.
2016															
Apr	7.3	10.3	357	7.3	0.2	24.0	14.0	93.1	5.5	0.1	0.1	5.6	2.5	0.9	4.4
May	9.1	8.6	366	7.4	0.2	21.0	12.6	213.2	6.2	0.04	0.1	9.1	3.0	0.7	4.1
Jun	10.3	7.4	401	7.4	0.2	37.8	14.0	26.8	3.1	0.02	0.1	8.9	2.7	0.6	4.3
Jul	11.3	9.2	379	7.5	0.2	37.4	14.1	11.2	3.9	0.001	0.04	7.6	1.6	1.6	4.2
Aug	10.8	7.8	381	7.4	0.3	32.1	14.7	7.0	2.7	0.02	0.03	7.2	3.0	0.6	4.3
Sep	10.4	7.6	385	7.4	0.3	37.0	14.1	6.0	1.1	0.02	0.03	6.4	4.0	0.6	4.8
Oct	8.4	7.6	389	7.4	0.2	33.9	14.3	6.5	0.9	0.1	0.04	2.0	1.6	0.8	5.5
Nov	6.6	8.2	390	7.4	0.3	43.1	13.1	7.6	1.5	0.04	0.01	5.2	3.3	0.8	3.6
Dec	8.8	7.7	389	7.3	0.3	38.6	13.9	4.8	0.3	0.03	0.01	2.2	1.7	1.0	5.6
2017															
Jan	5.9	8.8	395	7.5	0.4	40.0	14.5	4.2	1.1		0.04	6.7	2.7		5.7
Feb	8.0	8.1	384	7.4	0.4	37.1	14.2	9.7	0.6		0.03	6.5	2.2		5.7
Mar	7.7	8.3	383	7.4	0.2	38.3	13.9	26.5	0.9			9.5	2.7		
Apr	7.5	8.5	383	7.4	0.3	36.3	13.0	70.4	0.9	0.1		5.9	1.3	1.5	
	8.6 ± 1.7	**8.3 ± 0.8**	**383 ± 12**	**7.4 ± 0.1**	**0.3 ± 0.1**	**35.1 ± 6.2**	**13.9 ± 0.6**	**37.5 ± 59.6**	**2.2 ± 1.9**	**0.04 ± 0.03**	**0.04 ± 0.03**	**6.4 ± 2.3**	**2.5 ± 0.8**	**0.9 ± 0.3**	**4.7 ± 0.8**

zoans and bacteria throughout the 13 mo of sampling. In January, February and March 2017, the sampling of macrophytes, and in March and April 2017, the sampling of leaf litter was not possible because there were not enough plants/leaves to allow adequate sampling.

3.2.1. Sediment

Bacterial densities in sediment showed a slight decrease throughout the study, ranging between approximately $3.0 \times 10^6 \pm 9.9 \times 10^5$ (April 2016) and $3 \times 10^5 \pm 7.2 \times 10^4$ bacteria cm^{-2} (March 2017). Protozoans showed a trend towards a seasonal pattern, with highest abundances in spring 2016 and 2017 and lowest abundances during summer and winter (Fig. 1A). In December 2016, no ciliates were detected in the sediment. Protozoan biomass and secondary production reflected the seasonal abundance pattern. Thus, ciliate biomass and secondary production were highest in spring: up to 23.6 ± 26.8 µg dw cm^{-2} (recorded in May 2016) and 2.6 ± 2.4 µg C cm^{-2} d^{-1} (from May to June 2016), respectively (Fig. 1B,C). From July 2016 to February 2017, ciliate biomass was of the same order of magnitude as that of bacteria (Fig. 1B). Although flagellate biomass was lowest throughout the sampling period, except for March 2017 where it was as high as bacteria biomass (Fig. 1B), in spring 2016 and 2017 the daily secondary production of flagellates was higher than that of bacteria (Fig. 1C). Ciliate secondary production was highest of all 3 microfaunal groups during the 13 mo study period.

3.2.2. Surface of dead wood

Among the 4 habitats in the Furlbach, dead wood showed the highest seasonal fluctuations in bacterial and protozoan abundance (Fig. 1D). The highest bacterial ($5.6 \times 10^7 \pm 5.8 \times 10^6$ cells cm^{-2}), ciliate ($4.5 \times 10^3 \pm 8.4 \times 10^3$ ind. cm^{-2}) and flagellate ($7.6 \times 10^3 \pm 1.3 \times 10^4$ ind. cm^{-2}) abundances during the 13 sampling occasions of all 4 habitats were found on dead wood. While bacterial numbers tended to decrease throughout the sampling period, protozoan numbers were characterized by an irregular pattern. Flagellates

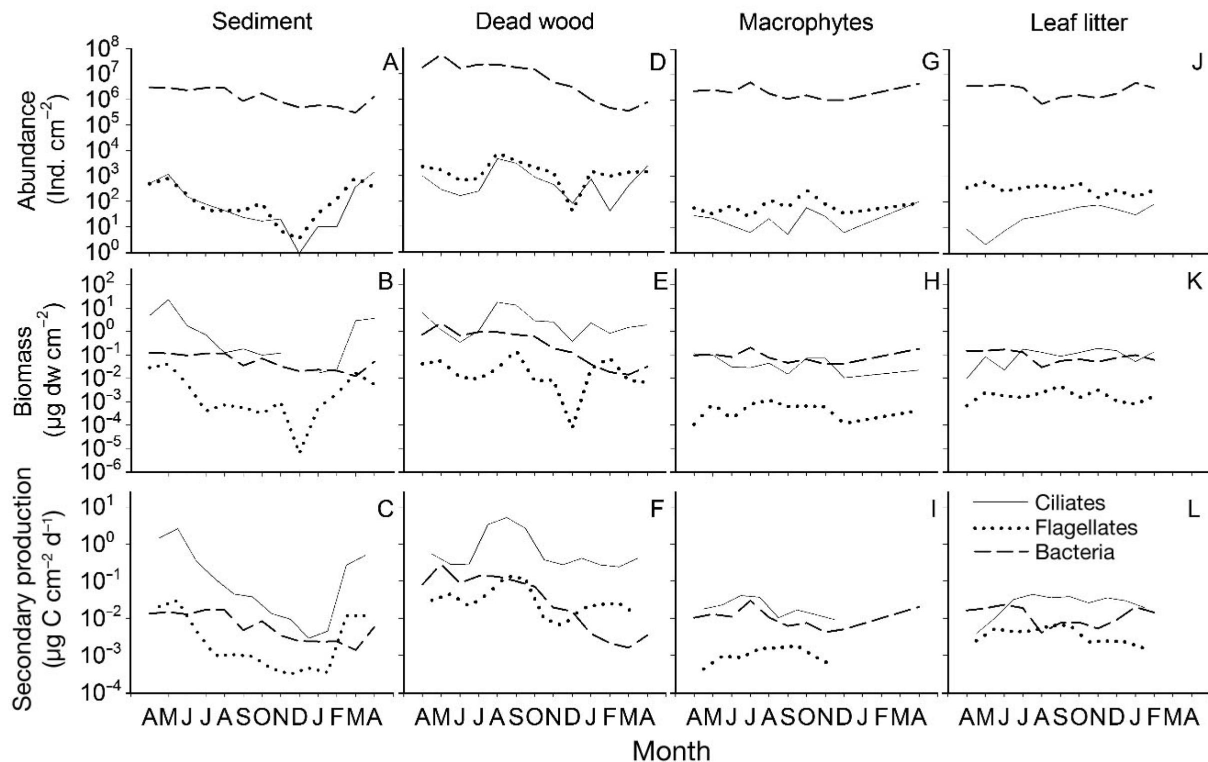

Fig. 1. Seasonal variation in abundance, biomass and secondary production of ciliates, flagellates and bacteria in 4 different habitats of Furlbach stream (A–C: sediment; D–F: dead wood; G–I: macrophytes; J–L: leaf litter) over 13 mo (April 2016–April 2017). The mean values of 4 replicates are shown on a logarithmic scale. Sampling of macrophytes in January–March 2017 and of leaf litter in February–April 2017 was not possible. dw: dry weight

and ciliates reached their highest numbers in August 2016 but peaks also occurred in April 2016 and 2017 as well as in January 2017. Flagellate abundance was higher than ciliate abundance at every sampling occasion except in December 2016, when the number of ciliates was slightly higher (Fig. 1D). Patterns of biomass and secondary production reflect the course of abundance. Flagellate biomass was lowest throughout the year, except for February 2017 (Fig. 1E). Bacterial biomass was higher than ciliate biomass in early summer 2016, whereas the rest of the year, ciliate biomass was higher, with a maximum of 17.7 ± 32.8 µg dw cm^{-2} in August 2016 (Fig. 1E). Ciliate daily secondary production was highest for all 13 mo of the study, reaching a peak in August/September 2017 of 5.2 ± 9.8 µg C cm^{-2} d^{-1}. Secondary production by flagellates was higher than that by bacteria in summer 2016 and from December 2016 until the end of the study (Fig. 1F).

3.2.3. Surface of macrophytes

Compared to the other habitats, protozoan numbers on macrophytes showed little fluctuation over the year, with abundances of 5 ± 4 to 101 ± 128 ind. cm^{-2} for ciliates and 24 ± 12 to 288 ± 189 ind. cm^{-2} for flagellates (Fig. 1G). Bacterial densities were in the range of $9.7 \times 10^5 \pm 4.2 \times 10^5$ to $5 \times 10^6 \pm 1.9 \times 10^6$ cells cm^{-2} (Fig. 1G) and bacterial biomass was higher than that of protozoans from May until October 2016 as well as from December 2016 until the end of the study period (maximum of 0.2 ± 0.08 µg cm^{-2} in July 2016, Fig. 1H). However, from April until November 2016, the highest secondary production on macrophytes was that of ciliates, with a peak in June/July 2016 (0.04 ± 0.03 µg C cm^{-2} d^{-1}, Fig. 1I). Flagellate biomass and secondary production were lowest throughout the study period (Fig. 1H,I).

3.2.4. Surface of leaf litter

Bacterial numbers on leaf litter ranged between $7.1 \times 10^5 \pm 2.0 \times 10^5$ and $4.9 \times 10^6 \pm 1.0 \times 10^6$ cells cm^{-2} and were lowest in summer and autumn 2016. Flagellate abundances showed very little seasonal variation, ranging between 149 ± 65 and 592 ± 243 ind. cm^{-2}. In May 2016, when flagellate numbers were highest, those of ciliates were lowest (2 ± 2 ind. cm^{-2}). Ciliate numbers increased up to 82 ± 58 ind. cm^{-2} in February 2017 (Fig. 1J). Ciliate biomass was higher than bacterial biomass from July 2016 until December 2017 and in February 2017 (Fig. 1K). From June 2016 until February 2017, daily secondary production by ciliates was higher than that by bacteria, up to 0.04 ± 0.04 µg C cm^{-2} d^{-1} (Fig. 1L). During the sampled year, flagellate biomass and secondary production were lowest (Fig. 1K,L), except for late summer

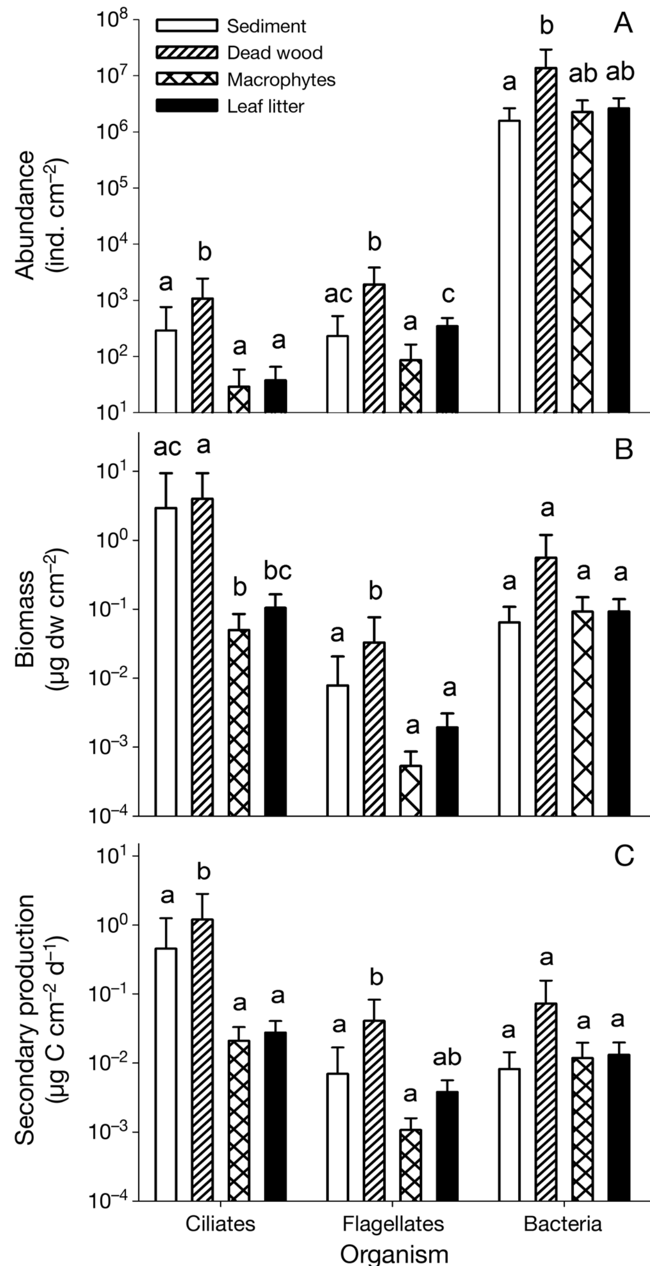

Fig. 2. Annual mean (±SD) (A) abundance, (B) biomass and (C) secondary production of ciliates, flagellates and bacteria in 4 different habitats of the Furlbach (sediment, dead wood, macrophytes, leaf litter) for the 13 mo from April 2016 to April 2017. Different lowercase letters above the bars indicate significant differences between the habitats (Dunn's test, $p < 0.05$), tested within 1 organismal group. dw: dry weight

Table 2. Kruskal-Wallis rank sum test for the influence of habitat on annual mean abundance, biomass and secondary production for ciliates, flagellates and bacteria. ns: not significant (p > 0.05)

Parameter	χ^2	df	p
Ciliates			
Abundance	20.064	3	<0.001
Biomass	23.677	3	<0.001
Secondary production	20.581	3	<0.001
Flagellates			
Abundance	26.099	3	<0.001
Biomass	20.558	3	<0.001
Secondary production	18.91	3	<0.001
Bacteria			
Abundance	78.205	3	<0.05
Biomass	69.517	3	ns
Secondary production	67.678	3	ns

2016 where it was in the same order of magnitude as bacterial secondary production.

3.3. Annual means of abundance, biomass and secondary production of ciliates, flagellates and bacteria in the four habitats

For ciliates, flagellates and bacteria in the 4 habitats, annual means of abundance, biomass and secondary production were highest on dead wood (Fig. 2). The Kruskal-Wallis rank sum test revealed an influence of habitat on the abundance, biomass and secondary production of ciliates and flagellates (*H*-test, all tested groups, p < 0.001, Table 2), but for bacteria, only on abundance (*H*-test, p = 0.049, Table 2). For bacterial abundance, only the difference between the surface of dead wood and sediment was significant (Dunn's test, p = 0.02). Ciliate and flagellate abundances were significantly higher on dead wood than on all other habitats (Dunn's test, all tested pairs, p < 0.05). This was also true for flagellate biomass and ciliate secondary production (Dunn's test, all tested pairs, p < 0.05). Moreover, the abundance of flagellates differed significantly between macrophyte and leaf litter surfaces (Dunn's test, p = 0.04). Ciliate biomass was significantly higher on sediment and dead wood than on macrophytes (Dunn's test, all tested pairs, p < 0.05) and on dead wood than on leaf litter (Dunn's test, p < 0.01). Flagellate secondary production was significantly higher on dead wood than on sediment and macrophytes (Dunn's test, all tested pairs, p < 0.01).

3.4. Contributions of the three organismal groups to abundance, biomass and secondary production in the four habitats

In all 4 habitats, bacteria accounted for 99.9% of the total abundance. In terms of total annual biomass, ciliates made up the highest relative proportion in the sediment, on dead wood and leaf litter: between 53 and 98% (Fig. 3). On macrophytes, bacterial biomass (65%) was higher than ciliate biomass (35%). The contribution of flagellates to annual biomass in all habitats was <1%, whereas for secondary production it was slightly higher, with a maximum of 9% on leaf litter (Fig. 3). In all habitats, ciliates had the largest impact on secondary production, accounting for 57% on macrophytes and nearly 97% in sediment (Fig. 3). The percentage contributed by bacteria was less for secondary production than for biomass at all 4 habitats and was largest (39%) on macrophytes (Fig. 3).

3.5. Contributions of the four habitats to total secondary production of Furlbach stream

In the Furlbach, the total sum of secondary production by ciliates, flagellates and bacteria at the 4 investigated habitats during the sampling period was

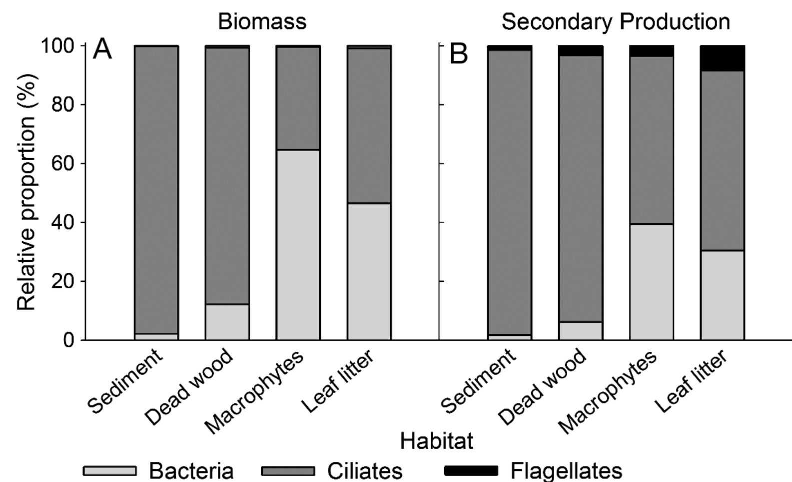

Fig. 3. Relative proportion of total (A) annual biomass and (B) secondary production contributed by ciliates, flagellates and bacteria in 4 different habitats of Furlbach stream (sediment, dead wood, macrophytes, leaf litter). Total annual biomass and secondary production were calculated as the sum of mean (n = 4) interval biomass and secondary production

Table 3. Annual secondary production of ciliates, flagellates and bacteria in the 4 habitats of Furlbach stream as the sum of monthly secondary production from April 2016 to April 2017. % Cover ratio is the annual mean percent contribution of the area that was covered by the respective habitat; Habitat total is the sum of secondary production of all 3 organismal groups in 1 habitat; Furlbach total is the sum of secondary production of 1 organismal group at all 4 habitats; % Habitat is the percent contribution of 1 habitat to the total secondary production of the Furlbach; % Stream area is the percent contribution of the habitat to total secondary production in relation to % cover ratio

Habitat	% Cover ratio	Annual secondary production (μg C cm^{-2} yr^{-1})				% Habitat area	% Stream
		Ciliates	Flagellates	Bacteria	Habitat total		
Sediment	46.9	165.9	2.5	3.1	171.5	26.0	63.0
Dead wood	9.2	422.5	15.1	28.9	466.5	70.6	33.7
Macrophytes	35.0	5.0	0.3	3.7	8.8	1.3	2.4
Leaf litter	8.8	5.5	1.2	4.2	13.8	2.1	1.0
Furlbach total		601.9	19.1	39.7	660.6		

660.6 µg C cm^{-2} yr^{-1}; however, the proportions accounted by the habitats differed (Table 3). With 466.5 µg C cm^{-2} yr^{-1}, organisms on dead wood contributed 70.6%, followed by those in the sediment (26%, Table 3), whereas total secondary production on macrophytes and leaf litter together contributed <5% (Table 3). Protozoans together accounted for 94% (621 µg cm^{-2} yr^{-1}) of total secondary production, and bacteria for 6% (40 µg C cm^{-2} yr^{-1}, Table 3). However, the contribution of the habitats to total secondary production changed in relation to the annual mean area covered by each habitat in the investigated stream area. Here, sediment was the habitat with the highest proportion of secondary production (63%), followed by dead wood (34%), macrophytes (2%) and leaf litter (1%).

4. DISCUSSION

The present study is the first to demonstrate the different proportions and seasonal patterns of the abundance, biomass and secondary production of bacteria and protozoans in 4 habitats of a stream (sediment, as well as surfaces on dead wood, macrophytes and leaf litter). A comparison of those 4 habitats identified dead wood as the habitat with the highest annual mean values of all 3 parameters for bacteria, ciliates and flagellates. Table 4 summarizes the published studies (including the present study) that have investigated protozoans in natural stream habitats over a period of at least 1 yr (with monthly or more frequent sampling intervals), but unlike in our study, the other studies did not include dead wood as a habitat.

4.1. Unicellular communities in the four habitats

During our study, ciliate and flagellate densities in sediment ranged between 0 and 1394 and between 3 and 842 ind. cm^{-2}, respectively, and were thus in the lower range of previously reported densities (Table 4). In the studies listed in Table 4, ciliate and flagellate abundances in stream sediments were in the range of 0–33 200 and 0–2.29×10^7 ind. cm^{-2} respectively (Baldock & Sleigh 1988, Bott & Kaplan 1989, Sleigh et al. 1992, Schmid-Araya 1994, Packroff & Zwick 1998, Cleven 2004, Reiss & Schmid-Araya 2008). Bacterial densities in sediment ranging between 4.5×10^8 and 3.8×10^9 cells cm^{-2} were reported by Bott & Kaplan (1989) and Cleven (2004), but the bacterial numbers in the sediment of the Furlbach were lower. Other studies investigating bacterial densities in stream sediments, but without seasonal determinations, reported higher bacterial cell numbers than in the Furlbach as well (e.g. Fischer & Pusch 2001, Gücker & Fischer 2003).

On macrophytes, organismal numbers were lower in the Furlbach than in previous studies in other river systems. Sleigh et al. (1992) reported 200 ciliates, 1500 flagellates and 4.4×10^7 bacteria cm^{-2} on macrophytes in a chalk stream. In our study, the annual mean ciliate and flagellate density on macrophytes was 29 and 86 ind. cm^{-2}, respectively, while bacterial cell numbers ranged between 9.7×10^5 and 4.9×10^6 cells cm^{-2}. Although bacterial numbers were not as high as those reported by Sleigh et al. (1992), they fell well in the range determined in other studies (Hossell & Baker 1979, Baker & Orr 1986). Previous studies have shown that different aquatic submerged macrophytes can exhibit chemical defenses against the growth of biofilm on their leaves as well as against herbivory (Newman et al. 1996, Bolser et al. 1998, Bushmann & Ailstock 2006). Yeates & Esteban (2014) analyzed 3 different submerged macrophytes from the same stream reach considering their attached ciliate communities. They found that *Nasturtium officinale*, the same macrophyte investigated in our study, was associated with the lowest biofilm growth, ciliate abundances as well as ciliate species richness out of all 3 investigated plant species. Those authors discussed this result in terms of the plant's production of phenethyl isothiocyanate (PEITC), a herbicide. Moreover, Hempel et al. (2009) showed for

Table 4. Published studies (including the present study) that investigated protozoan and bacterial abundance, biomass and secondary production in different stream habitats over a period of at least 1 yr with monthly or more frequent sampling intervals. All values shown in original units. Dash = no data available. dw = dry weight

Study	Stream(s)	Habitat(s)	Abundance: Ciliates	Abundance: Flagellates	Abundance: Bacteria
Baldock & Sleigh (1988)	Itchen/Ober Water	Sediment	0–13 000 cm^{-3}	0–148 000 cm^{-3}	–
Bott & Kaplan (1989)	White Clay Creek	Sediment	5000–36 500 cm^{-2}	5.89×10^5–10.4×10^6 cm^{-2}	4.5×10^8 cm^{-2}
		Rocks	0–12 800 cm^{-2}	20 500–6.26×10^5 cm^{-2}	–
	Saw Mill Spring	Sediment	5000–33 200 cm^{-2}	89.6×10^5–2.29×10^7 cm^{-2}	3.8×10^9 cm^{-2}
		Leaf litter	795–1590 cm^{-2}	85 200–2.27×10^5 cm^{-2}	–
Sleigh et al. (1992)	Chalk streams	Sediment	3500 cm^{-2}	8000 cm^{-2}	–
		Macrophytes	200 cm^{-2}	1500 cm^{-2}	–
		Water column	0.7 cm^{-3}	65 cm^{-3}	–
Schmid-Araya (1994)	Oberer Seebach	Sediment	0–7351 dm^{-2}	0–452 000 dm^{-2}	–
Packroff & Zwick (1998)	Breitenbach	Sediment	0–4500 cm^{-3}	–	–
Cleven (2004)	Ladberger Mühlenbach	Sediment	0–895 cm^{-3}	0–491 000 cm^{-3}	0.2×10^9 cm^{-3}
Reiss & Schmid-Araya (2008, 2010)	Lone Oak	Sediment	ca. 50 000–900 000 m^{-2}	–	–
	Pant	Sediment	ca. 500 000–6 000 000 m^{-2}	–	–
Present study	Furlbach	Sediment	0–1349 cm^{-2}	3–842 cm^{-2}	2.9×10^5–2.9×10^6 cm^{-2}
		Dead wood	40–4538 cm^{-2}	40–7564 cm^{-2}	3.4×10^5–5.6×10^7 cm^{-2}
		Macrophytes	5–101 cm^{-2}	33–288 cm^{-2}	9.7×10^5–4.9×10^6 cm^{-2}
		Leaf litter	2–82 cm^{-2}	149–592 cm^{-2}	7×10^5–4.8×10^6 cm^{-2}

[a]Annual mean values; [b]Dry weight; [c]Numbers m^{-2} stream floor; [d]Wet weight

the macrophyte *Myriophyllum spicatum* that the content of certain toxins might differ between older and younger plant parts, which resulted in higher influenced bacterial communities on the apices than on older leaves of *M. spicatum.* Generally, the production of toxin, and additionally the fact that we sampled only the upper, younger parts of *N. officinale* in the Furlbach, might be another reason for the comparatively low abundances of attached organisms we found in our study. Furthermore, in the Furlbach, *N. officinale* grows in dense plant cushions, so the upper plant parts are more strongly exposed to high flow velocities than the inner parts. In their study of a chalk stream, Baldock et al. (1983) found higher protozoan densities on the older than on the younger leaves of the investigated plants, whereby older plant parts were also present within the weed bed. It is very likely that the investigation of older plant parts from within the plant cushion of *N. officinale* would have shown higher protozoan densities, as the flow velocity in the inner part of the weed bed is lower than in the outer parts, where younger plants are located. The influence of flow on the colonization of habitats in streams is discussed below (2 paragraphs down).

Dead wood and leaf litter are habitats closely related to decomposing processes. On leaf litter, Bott & Kaplan (1989) counted up to 1590 ciliates and 2.27×10^5 flagellates cm^{-2}. These densities are much higher

Biomass			Secondary production		
Ciliates	Flagellates	Bacteria	Ciliates	Flagellates	Bacteria
2.06–9.82 µg cm^{-3} [a,b]	0.31–1.73 µg cm^{-3} [a,b]	–	–	–	–
147 mg C m^{-2} [a]	616 mg C m^{-2} [a]	–	–	–	–
51 mg C m^{-2} [a]	57 mg C m^{-2} [a]	–	–	–	–
208 mg C m^{-2} [a]	1.593 mg C m^{-2} [a]	–	–	–	–
14 mg C m^{-2} [a]	43 mg C m^{-2} [a]	–	–	–	–
61 mg m^{-2} [a,b,c] 4 mg m^{-2} [a,b,c] 0.001 mg m^{-2} [a,b,c]	10 mg m^{-2} [a,b,c] 0.9 mg m^{-2} [a,b,c] 1 × 10^{-4} mg m^{-2} [a,b,c]	– – –	12 g dw m^{-2} yr^{-1} [c] 3 g dw m^{-2} yr^{-1} [c] 2 × 10^{-4} g dw m^{-2} yr^{-1} [c]	4 g dw m^{-2} yr^{-1} [c] 0.03 g dw m^{-2} yr^{-1} [c] 5 × 10^{-5} g dw m^{-2} yr^{-1} [c]	70 g dw m^{-2} yr^{-1} [c] (assumed for whole stream)
0.669 mg m^{-2} [a,b]	0.14 mg m^{-2} [a,b]	–	–	–	–
1–169 µg cm^{-3} [d]	–	–	–	–	–
0–5.3 µg C cm^{-3}	–	–	–	–	–
ca. 0.25–2.5 mg C m^{-2} ca. 5–63 mg C m^{-2}	– –	– –	0.02 g C m^{-2} yr^{-1} 0.55 g C m^{-2} yr^{-1}	– –	– –
0–23.6 µg cm^{-2} [b]	6.1 × 10^{-6}–0.04 µg cm^{-2} [b]	0.01–0.12 µg cm^{-2} [b]	165.9 µg C cm^{-2} yr^{-1}	2.5 µg C cm^{-2} yr^{-1}	3.1 µg C cm^{-2} yr^{-1}
0.3–17.7 µg cm^{-2} [b]	7.3 × 10^{-5}–7.4 µg cm^{-2} [b]	0.01–2.31 µg cm^{-2} [b]	422.5 µg C cm^{-2} yr^{-1}	15.1 µg C cm^{-2} yr^{-1}	28.9 µg C cm^{-2} yr^{-1}
0.01–0.1 µg cm^{-2} [b]	1 × 10^{-4}–0.001 µg cm^{-2} [b]	0.04–0.20 µg cm^{-2} [b]	5 µg C cm^{-2} yr^{-1}	0.3 µg C cm^{-2} yr^{-1}	3.5 µg C cm^{-2} yr^{-1}
0.01–0.18 µg cm^{-2} [b]	7 × 10^{-4}–0.005 µg cm^{-2} [b]	0.03–0.17 µg cm^{-2} [b]	8.5 µg C cm^{-2} yr^{-1}	1.2 µg C cm^{-2} yr^{-1}	4.2 µg C cm^{-2} yr^{-1}

than those of equivalent habitats in the Furlbach. Bacterial cell numbers on Furlbach leaf litter were also lower than those determined at other locations (Suberkropp & Klug 1976, Bott & Kaplan 1989, Sleigh et al. 1992, Baldy et al. 1995, Hieber & Gessner 2002). On dead wood, however, the abundances of ciliates and flagellates were significantly higher than at all other habitats. The extensive biofilms that form on decomposing wood offer a highly colonizable habitat and a hotspot of microbial occurrence (Golladay & Sinsabaugh 1991, Sinsabaugh et al. 1991). Moreover, wood provides a more stable habitat than leaf litter and, with its slower breakdown, a durable substrate favoring biofilm formation in streams (Golladay & Sinsabaugh 1991, Tank & Webster 1998). This would explain the higher occurrence of protozoa and bacteria on wood than on leaf litter. Other studies also showed that the values of most microbial biomass and metabolic parameters were higher on the biofilms on wood than on leaves (Golladay & Sinsabaugh 1991, Sinsabaugh et al. 1991).

The lower numbers of protozoa and bacteria in the Furlbach compared to other streams could have been caused by the high flow velocities at the sampling site. Flow velocity ranged between 0.2 and 0.4 m s^{-1}, with an annual mean of 0.3 m s^{-1}. The influence of flow velocity on the total protozoan and bacterial community and especially on substrate colonization

was investigated by Risse-Buhl & Küsel (2009). They showed that biofilms were colonized more slowly at sites characterized by fast (mean flow velocity of 0.31 m s^{-1}) than by slow (0.09 m s^{-1}) flows, and that after 24 h, ciliate densities were 2–4 times lower and that slightly fewer species were found at fast-flow than at slow-flow sites. The flow velocity of the Furlbach is the same as that of the fast flowing site in the study of Risse-Buhl & Küsel (2009). Moreover, Furlbach is a stream which is poor in nutrients. Other studies suggest that the concentration of nutrients can have an influence on protozoan occurrence. For example, Baldock & Sleigh (1988) found higher ciliate abundances in nutrient-rich stream sites, and Reiss & Schmid-Araya (2008), who investigated ciliate abundances in 2 different streams, found higher protozoan abundances in the stream with the higher nutrient status. Also, decomposition processes and density of bacteria are connected to the nutrient status of a stream. Gulis & Suberkropp (2003) found decomposition and bacteria numbers on leaf litter to be significantly faster and higher in a nutrient-enriched (N and P) stream reach compared to a non-enriched reach.

Annual mean flagellate numbers were somewhat lower than ciliate numbers in the Furlbach's sediment, whereas this was not the case in the other habitats. In sediment, flagellate abundance typically exceeds that of ciliates (Gücker & Fischer 2003). But a higher density of flagellates than ciliates was also shown by Eisenmann et al. (1998) for the Neckar River (Germany). In Furlbach, protozoans were counted alive within 24 h after sampling. Although the problem of sediment masking (Gasol 1993) should be less serious using live counting than preserved samples (Packroff & Zwick 1998), in the sediment samples, the size of the flagellates made it difficult to recognize these unicellular organisms between the sediment particles. Flagellates in the Furlbach sediment and in the other habitats were usually <10 µm in size (data not shown) but they were more easily distinguishable in the other habitats. Because the calculation of biomass and production is directly dependent on densities and on the detected size classes, then the contribution of flagellate biomass and production to total annual biomass and secondary production was understandably low in all 4 habitats.

4.2. Seasonal patterns

Other than predicted, only protozoans in the sediment exhibited a trend towards a seasonal pattern, in which abundances were highest in spring and lowest in winter. Sleigh et al. (1992) investigated the abundances of different ciliate and flagellate taxa over a period of 12 mo, and in sediment, they found the highest numbers in spring as well. Packroff & Zwick (1998) reported a seasonal pattern of ciliates, the abundance of which was lowest between December and January at 3 out of 4 sampling points. In our study, chl *a* values in sediment reached a peak in spring 2016 and 2017, consistent with the high abundances of microfaunal organisms. Indeed, chl *a* levels correlated with ciliate and flagellate densities (correlation coefficient of 0.875 and 0.823 for ciliates and flagellates, respectively; both $p < 0.001$, data not shown). A similar relationship was noted by Sleigh et al. (1992) for the surfaces of macrophytes. Olapade & Leff (2004) examined the biofilms on cobbles in a stream and reported a seasonal pattern for total bacterial densities. In their study, cell numbers declined to their lowest values in spring and summer but reached high values in autumn and winter. In the Furlbach, in none of the investigated habitats did bacterial densities show a clear seasonal pattern.

4.3. Contribution of bacteria, ciliates and flagellates to abundance, biomass and secondary production

Bacteria had the highest contribution to total abundance (>99%) in all 4 habitats. However, contrary to our expectations, on the surface of macrophytes, bacteria represented more biomass than ciliates did. Protozoan numbers on macrophytes were lower than in other studies (Sleigh et al. 1992), and in agreement with these low abundances, biomass of ciliates and flagellates were low compared to bacterial biomass at this habitat. However, annual secondary production at all 4 habitats of the Furlbach was largely that of ciliates, despite the higher biomass of bacteria on macrophytes. But generally, interpretations of the results should be carried out carefully because of different methods used to calculate secondary production (Bergtold & Traunspurger 2005). The regression by White et al. (1991) used in the present study seems to have underestimated bacterial production by many orders of magnitude. For example, annual bacterial secondary production of 3.1 µg C cm^{-2} yr^{-1} in the sediment of the Furlbach, which corresponds to 0.03 g C m^{-2} yr^{-1}, was much lower than that reported by other authors. Bott & Kaplan (1985) calculated a bacterial production of 26.4 g C m^{-2} yr^{-1} based on a phospholipid synthesis rate translation, and Marxsen

(2006) calculated 162.0 g C m^{-2} yr^{-1} in sediments according to a ^{14}C-labeled leucine incorporation method. However, total annual secondary production by ciliates in the Furlbach sediment (165.9 µg C cm^{-2} yr^{-1}, which corresponds to ~1.66 g C m^{-2} yr^{-1}) is in the range of values found in the literature, including those reported by Reiss & Schmid-Araya (2010) of <0.1 g C m^{-2} yr^{-1} at Lone Oak stream and 0.6 g C m^{-2} yr^{-1} at Pant stream (England), but is less than the 8.0 g C m^{-2} yr^{-1} reported by Marxsen (2006) in the Breitenbach (Germany).

4.4. Proportion of the four habitats to total secondary production

Total annual secondary production of bacteria and protozoans was 660.6 µg C cm^{-2} yr^{-1} whereby the amounts in the 4 investigated habitats differed. The surface of dead wood provided the highest share of secondary production cm^{-2} (71%), followed by sediment (26%), leaf litter (2%) and macrophyte (1%) surfaces. However, the percentages changed with respect to the stream area covered by the respective habitat. Here the contribution of sediment to total secondary production increased up to 63%, whereas dead wood decreased to 34%. Macrophytes and leaf litter together still accounted for <5%. Nevertheless, dead wood still contributed ⅓ of total unicellular secondary production even though only 9% of the sampled stream area was covered by that habitat.

In the chalk stream studied by Sleigh et al. (1992), total annual protozoan (ciliates, flagellates and amoebae) production was 15 g dw m^{-2} stream floor yr^{-1}, with different contributions by protozoans from sandy and stony sediments and those epiphytic on macrophytes. Applying the conversion factor used in the present study (per Borsheim & Bratbak 1987), this amount corresponds to 250 µg C cm^{-2} yr^{-1}. Combined protozoan production from sediment and macrophyte habitats in the Furlbach was, at 174 µg C cm^{-2} yr^{-1}, lower than the amount calculated by Sleigh et al. (1992); however, 10% of protozoan production in the latter study was that by amoebae, organisms not included in our investigation of the Furlbach. Moreover, in the chalk stream, macrophytes covered ~40% of the stream bed and protozoans epiphytic on these macrophytes accounted for ~30% of overall protozoan production. In the Furlbach, protozoan production on macrophytes with respect to the annual mean stream area covered by this habitat was among the lowest of the 4 habitats. This may have been due to the fact that the analyzed macrophyte, *N. officinale*, was highly abundant (≥40%) only on 5 of the 13 sampling occasions. At the other 8 sample collections, it was either hardly found or it covered only a small part of the stream bed at the sampling point. The irregular presence of the sampled macrophyte may be the reason for the small amount of production in this habitat.

Not included in the study of Sleigh et al. (1992) was biofilm on the surface of dead wood, but in the Furlbach, this was the habitat with the highest rate of secondary production and the second highest contributor with regard to the stream area covered by this habitat. Although we hypothesized that dead wood and leaf litter would account for the largest share of total secondary production, because decomposition processes dominate both of these habitats, this explanation was valid only in the case of dead wood.

5. CONCLUSION

Dead wood has been a highly under-represented habitat in terms of its contribution to energy budgets and energy transfer in streams, especially with respect to protozoans occupying this habitat. Studies on the composition of epixylon (wood biofilms) have integrated bacteria, algae and fungi (e.g. Couch & Meyer 1992). However, while protozoans are often mentioned as an important component of biofilms (Risse-Buhl et al. 2012a), the occurrence of these organisms on the surface of dead wood has rarely been investigated. In their study of the Satilla River, Benke et al. (1985) found the snag habitat to be the biologically richest habitat in terms of the diversity and production of invertebrates per unit of habitat surface. Our study clearly showed that this habitat can also be highly productive via its microorganisms. This may especially be true for small forested streams, where dead wood is a recurrent structural component.

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