Vol. 87: 47–60, 2021
https://doi.org/10.3354/ame01968

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online June 17

Differences in regulation of planktonic and epilithic biofilm bacterial production in the middle reaches of a temperate river

Kenji Tsuchiya[1,*], Ayato Kohzu[1], Victor S. Kuwahara[2], Shin-ichiro S. Matsuzaki[1], Masatoshi Denda[3], Kimio Hirabayashi[4]

[1]National Institute for Environmental Studies, Ibaraki 305-8506, Japan
[2]Soka University, Tokyo 192-8577, Japan
[3]Public works Research Institute, Ibaraki 305-8516, Japan
[4]Shinshu University, Nagano 386-8567, Japan

ABSTRACT: To clarify the governing factors of planktonic and epilithic bacterial production (BP) and to quantify their relative contributions to the carbon cycle, we investigated the seasonal variation and regulatory factors of planktonic and epilithic BP in the middle reaches of the Shinano River, Japan, ecosystem from February 2019 to May 2020. Sampling was conducted at 3 stations: upper stream riffle, upper stream pool, and lower stream riffle, where current velocity, water depth, and bed shear stress were distinct. Planktonic and biofilm BP ranged from 5.5 to 466 mgC m^{-3} d^{-1} and 2.9 to 132 mgC m^{-2} d^{-1}, respectively, showing clear seasonal variation. Biofilm BP was higher in the upper stream riffle than at the other stations, where no spatial variation in planktonic BP was observed. Generalized linear models suggest that BP was primarily regulated by water temperature. Additionally, planktonic BP was significantly correlated with dissolved organic carbon, suggesting carbon limitation. Biofilm BP showed no evidence of resource limitation (nutrients and organic matter), but was significantly explained by current velocity and station. The results suggest that although seasonality is dominant in biofilm BP variation, spatial differences are significant within the seasonal variability. Moreover, current velocity and bottom shear stress related to local geomorphologies such as riffles and pools affect substrate supply rate and biofilm formation processes, regulating biofilm BP variation. This study demonstrated different regulatory factors of planktonic and biofilm BP in the middle reaches of a temperate river.

KEY WORDS: Bacterial production · Biofilm · Shear stress · Riffle · Velocity · Dissolved organic carbon · DOC · Shinano River

1. INTRODUCTION

Inland waters receive an estimated 2.0–2.7 billion t of terrestrial carbon per year on a global scale (Battin et al. 2008, Aufdenkampe et al. 2011). In most streams and rivers, dissolved organic carbon (DOC) is the largest organic carbon pool (Karlsson et al. 2005, Allan & Castillo 2007). Heterotrophic bacteria are the main consumers of DOC, where the DOC is partly respired to CO_2 and partly transferred to bacterial biomass, which fuels the microbial food web (Azam 1998). Thus, knowledge of bacterial production (BP) rate dynamics is of significant importance for understanding biogeochemical cycles in lotic ecosystems.

Benthic biofilms play a significant role in the metabolic conversion and partial removal of biodegradable material in rivers and streams (Battin et al.

*Corresponding author: ktsuchiya@soka.gr.jp

2016). Algae in the biofilms account for the greatest part of primary production in low-order streams (Naiman 1983). Bacteria in the habitats of planktonic and epilithic biofilms can differ in trophic importance: planktonic bacteria are likely consumed by filter feeders, and biofilm bacteria are consumed by deposit feeders (Edwards et al. 1990, Hall 1995) and may behave differently as links or sinks (Pomeroy 1974). The balance between bacterial processes in these 2 habitats (planktonic and biofilm) can be an essential factor influencing biogeochemical cycling in rivers and streams. However, few studies have simultaneously assessed planktonic and biofilm BP in the same stream, and knowledge of quantitative contributions of biofilm bacteria to biogeochemical cycles in rivers and streams is limited (e.g. Ainsworth & Goulder 2000a,b, Kamjunke et al. 2015). These previous studies demonstrated that planktonic BP consistently varied along the course of a river (Ainsworth & Goulder 2000a,b) and varied based on water quality, such as DOC levels (Kamjunke et al. 2015), whereas biofilm BP did not show consistent variations along such gradients. The balance of epilithic biofilm and planktonic BP highly varied, and biofilm BP per m^2 was as high as that in 0.08–30 m^3 stream water planktonic BP (Ainsworth & Goulder 2000a,b, Kamjunke et al. 2015). Understanding, interpreting, and predicting biogeochemical cycles in rivers and streams is facilitated by identifying seasonal and spatial variability in the relative importance of planktonic and biofilm BP.

Planktonic and biofilm BP are regulated by different environmental factors, even when they occur in the same streams and rivers. For example, variation in biofilm BP was not explained by DOC in stream water, whereas planktonic BP was correlated to DOC concentration and quality in the Bode catchment area, Germany (Kamjunke et al. 2015). Further, planktonic BP is often correlated to bacterial abundance (BA), chlorophyll *a* (chl *a*), and primary production (e.g. Cole et al. 1988, Pace & Cole 1994). In contrast, relationships between biofilm BP and algal production/biomass are inconsistent; both positive relationships (Niyogi et al. 2003, Carr et al. 2005, Scott et al. 2008) and few or no relationships (Findlay et al. 1993, Fukuda et al. 2006) have been reported. Also, previous studies reported that biofilm BP was positively correlated to biofilm phosphorus (Kamjunke et al. 2015) and DOC in the water column (Niyogi et al. 2003), although results were not consistent across studies. Bacteria in the biofilm are likely able to compensate for changes in biofilm algal production and water column organic carbon by relying on the polysaccharide matrix for their carbon sources when algal products and external organic carbon are limited (Freeman & Lock 1995, Carr et al. 2005). In other words, biofilm BP may have the ability to equilibrate carbon sources, possibly masking the relationships between algal production and ambient resources.

Biofilm bacterial activity can be influenced not only by chemical or biological processes but also by physical conditions such as water velocity (e.g. Lau & Liu 1993, Sobczak & Burton 1996, Battin et al. 2003). Lau & Liu (1993) examined biofilm biomass accumulation under different flow rates (3.4–30 cm s^{-1}) in an experimental setting and found that biofilm accumulation was substantially reduced as flow shear stress increased and that maximum accumulation occurred under very low flow conditions. Sobczak & Burton (1996) compared epilithic bacterial biomass accumulation among a run, riffle, and pool in a temperate stream, and found that epilithic bacterial biomass increased rapidly with no significant differences among the 3 habitats throughout colonization. These 2 studies investigated variation in bacterial biomass (abundance). Higher water velocity and shallower water depth induce higher bottom shear stress (Harrison & Keller 2007), leading to frequent erosion and slough of biofilms (Lau & Liu 1993). Therefore, even though bacterial biomass increases rapidly under higher flow rate conditions, the higher shear stress reduces net biofilm accumulation through erosion and sloughing, suggesting that abundance-based investigations could not detect effects of flow rates (and shear stress) on biofilm BP. Battin et al. (2003) observed higher DOC uptake by biofilm microorganisms at high flow rates than at slow flow rates. This may be related to a thinner external boundary layer between the biofilm surface and water flow under higher current velocity, leading to higher organic matter supply from the water column to biofilm bacterial communities (Battin et al. 2003). Based on these results, we hypothesized that higher biofilm BP can be observed under higher flow rate conditions, even though the biofilm biomass may be relatively low. Streams and rivers are structurally heterogeneous environments, containing a mosaic of different habitat patches such as riffles and pools (Pringle et al. 1988, Hildrew & Giller 1994), and they exhibit different flow rates, water depths, and bottom shear stress. Thus, our hypothesis could be examined by comparing BP in riffles compared to pools and/or other riffles with different riverbed slopes and water depths. The examination of variability in bacterial responses to

different units or channels of streams and rivers will provide critical information for river management, development, and resources.

The objectives of the present study were to clarify the governing factors of planktonic and biofilm BP and to quantify their relative abundance in different habitats within a river ecosystem. To accomplish these objectives, we investigated seasonal variation in planktonic and biofilm BP in 3 habitats: (1) riffle and (2) pool in an upper stream, and (3) a downstream riffle in the middle reaches of the Shinano River, Japan. Although few studies have explicitly compared BP in riffle and pool habitats in the same stream, Rosenfeld & Hudson (1997) reported that BP was significantly higher in pools than in riffles in southern Ontario (Canada) streams. However, they considered BP in sediments within pool habitats, while BP on rocks (epilithic biofilm) was only considered in riffle habitats. In this study, we focused on epilithic biofilms in both riffle and pool habitats to examine our hypothesis.

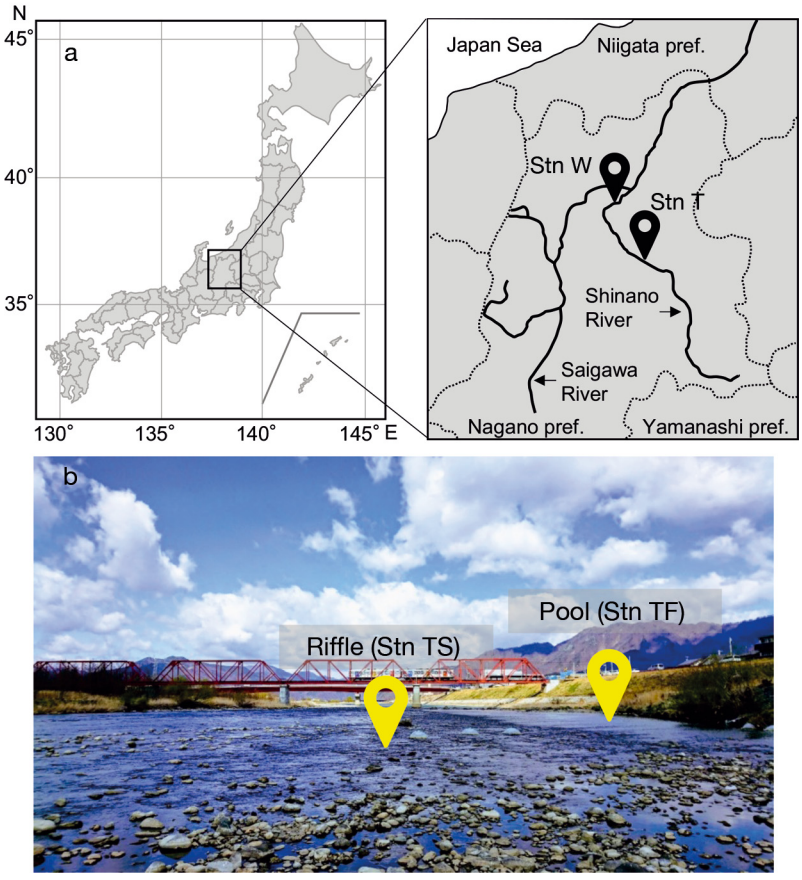

Fig. 1. (a) Location of sampling Stations Tokida (Stn T) and Iwano (Stn W) in the Shinano River, Japan. (b) Riffle (Stn TS) and pool (Stn TF) at Stn T

2. MATERIALS AND METHODS

2.1. Study site and sampling

The Shinano River is the longest river in Japan (length ca. 367 km: drainage area ca. 11900 km^2), running through Nagano and Niigata Prefectures and flowing north into the Japan Sea (Fig. 1). The study sites were located in the middle reaches of the Shinano River: Tokida (36°23′N, 138°15′E; 440 m above sea level, 258 km upstream from the river mouth; Stn T) and Iwano (36°34′N, 138°09′E; 350 m above sea level, 230 km upstream from the river mouth; Stn W) (Table 1). The slopes of the riverbed, river widths, and average water depths were 1/180, 250 m, and 0.4 m at Stn T and 1/1000, 450 m, and 1.2 m at Stn W, respectively. River water and stones in riverbeds were collected from February 2019 to May 2020 at riffle and pool sites in the Tokida area (Stns TS and TF, respectively; Fig. 1), and at riffle sites in the Iwano area (Fig. 1; see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a087p047_supp.pdf). Current velocities and water depths ranged from 50.9 to 62.8 cm s^{-1} and from 3.4 to 20 cm at Stn TS, 10.4 to 26.2 cm s^{-1} and 25.8 to 50.4 cm at Stn TF, and 40.0 to 59.6 cm s^{-1} and 11.8 to 41.8 cm at Stn W, respectively (Table 1, Fig. S2). River water was collected in a 2 l polycarbonate bottle (Nalgene), and 5 stones were collected in polyethylene bags (Unipack®, 0.08 mm thickness, SEISANNIPPONSHA) filled with ambient river water at each station. The major ellipse diame-

Table 1. Riverbed slope, sampling depth, and current velocity at Stns TS, TF, and W in the Shinano River, Japan (see Fig. 1), during the study period. Post hoc pairwise comparisons were conducted using Steel-Dwass tests, with $p < 0.05$ considered significant (indicated by different superscript letters within columns). Sampling depth and current velocity are shown as mean ± SD

Station	Riverbed slope	Sampling depth (cm)	Current velocity (cm s^{-1})
TS	1/180	13 ± 6^{a}	58 ± 4^{A}
TF	1/180	41 ± 10^{b}	19 ± 7^{B}
W	1/1000	24 ± 9^{c}	54 ± 5^{A}

ters of collected stones ranged from approximately 5 to 17 cm. The water and stone samples were brought to the laboratory in a cooler box within 3 h. We measured physicochemical conditions using portable meters: water temperature, dissolved oxygen (DO) (HQ30d, HACH), pH, electric conductivity (EC) (WM-22EP, DKK Toa), and current velocity (VE-10, Kenek). Unfortunately, a strong typhoon (category 5, Hagibis) in October 2019 caused a severe flood in the Shinano River and destroyed our Tokida stations (Fig. S1). After the flood, sampling at the riffle unit was resumed at a point ca. 300 m upstream of the original Tokida station. However, sampling at Stn TF was not conducted after November 2019 due to the development of deep water at the pool site, which was hazardous.

2.2. Sample preparations of river water

In the laboratory, river water samples were filtered onto pre-combusted (450°C for 4 h) glass-fiber filters (Whatman GF/F) and were analyzed for chl *a* measurement. Water samples were filtered through 0.22 µm polycarbonate membrane filters (Cyclopore, Millipore) and rinsed with Milli-Q water, and the filtrates were analyzed for nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, dissolved total nitrogen and dissolved total phosphorus) and DOC measurements. The bulk river water samples were analyzed for total nitrogen and phosphorus. River water was incubated with a 50 nM final concentration of [$^{15}N_5$]-2′-deoxyadenosine (^{15}N-dA, NLM-3895, Cambridge Isotope Laboratories) in the dark at *in situ* temperatures (3.0–22.6°C) ± 2°C for 1–3 h (Tsuchiya et al. 2015). After the incubations, the water was filtered onto 0.2 µm pore size polytetrafluoroethylene (PTFE) membrane filters (Omnipore, Millipore), and rinsed with 2 ml of 70% ethanol to quench bacterial metabolism and avoid DNA degradation. The filters were then analyzed for BP, BA, and community structure. All water samples and filters were stored at −20°C for up to 3 mo until further analysis.

2.3. Sample preparations of stones (epilithic biofilms)

River water was filtered through 0.45 µm pore size PTFE membrane filters (Omnipore, Millipore), and filtrates were poured into polyethylene bags (Unipack®, 0.08 µm thickness, SEISANNIPPONSHA), in which a stone was stored. The stones were incubated with a 50 nM final concentration of ^{15}N-dA in the dark at *in situ* temperature (3.0–22.6°C) ± 2°C for 1–3 h using an incubator (SLC-25A, Mitsubishi Electric Engineering) (Tsuchiya et al. 2015). After the incubations, 25 cm^2 of the stone surface were brushed using a toothbrush with a grid of 5 cm × 5 cm, and suspended in Milli-Q water. Biofilm suspension volumes were measured (~100 ml), and then 1–10 ml of the biofilm suspensions were filtered onto 0.2 µm pore size PTFE membrane filters and rinsed with 2 ml of 70% ethanol to quench bacterial metabolism and avoid DNA degradation. The filters were analyzed for measurements of BP, BA, and community structure. Also, 1 to 15 ml of the biofilm suspensions were filtered onto precombusted (450°C for 4 h) glass-fiber filters (Whatman GF/F) for chl *a* measurement of epilithic biofilms. All filters were stored at −20°C for up to 3 mo until further analysis.

2.4. Sample analysis

Nutrient concentrations were measured by using a continuous flow auto-analyzer (QuAAtro, BLTEC) in technical triplicates (Nojiri 1987, Otsuki et al. 1993). Chl *a* concentration was measured spectrophotometrically in biological duplicates (UV2550, Shimadzu) (Marker et al. 1980) after extraction in 99.5% ethanol. DOC concentration was quantified in technical triplicates with a TOC-V analyzer (Shimadzu) (Tsuchiya et al. 2019).

For both BA and BP measurements, bacterial DNA was extracted from the filter sample using a commercial kit (Extrap Soil DNA Kit Plus ver.2, Nippon Steel & Sumikin Eco-Tech) according to the manufacturer's protocol. The efficiency of DNA extraction was considered to be 100% (Tsuchiya et al. 2019). For BP measurement, the ^{15}N-dA incorporation amounts were quantified in technical duplicates (Tsuchiya et al. 2015, 2020a). BA was determined by measuring 16S-rDNA concentrations of extracted DNA samples through real-time PCR assay (LightCycler, Roche) in technical duplicates according to the procedure described by Tsuchiya et al. (2020b). The 16S-rDNA concentration was converted to BA using a conversion factor of 0.31 cells (16S-rDNA copy)$^{-1}$ (Tsuchiya et al. 2020b).

2.5. Conversion of ^{15}N-dA incorporation to bacterial carbon production

For conversion of ^{15}N-dA incorporation rate to bacterial carbon production rate, we conducted 2 incubation experiments to obtain conversion factors: (1)

^{15}N-dA incorporation to cells produced for planktonic BP (CF_{cell}) and (2) ^{15}N-dA incorporation to ^{3}H-Leu incorporation for biofilm BP (CF_{Leu}). To obtain CF_{cell}, water samples collected in February, April, May, and June 2019 were filtered through 1 µm pore-size membrane filters (Nuclepore, Millipore) using a gentle vacuum. The water samples were incubated at *in situ* temperatures in the dark with ^{15}N-dA for 24 h. The ^{15}N-dA incorporation rate and bacterial cell numbers estimated from 16S-rDNA were measured by routine procedures described above. The factor for converting ^{15}N-dA incorporation to cells produced was determined as 8.80×10^6 cells (pmol ^{15}N-dA)$^{-1}$ (Fig. S3). For calculating planktonic BP, we used 20 fgC per bacterium as a cell-to-carbon conversion factor (Lee & Fuhrman 1987).

To obtain CF_{Leu}, we conducted a comparison experiment between ^{15}N-dA and ^{3}H-Leu incorporation rates. In this assay, due to technical difficulty in the treatment of radioisotopes (^{3}H-Leu) after the incubation of intact epilithic biofilms with the tracer, we used biofilm suspensions for the incubation. Stone samples for biofilms collected in May 2020 were brushed as described above, and the biofilm suspensions were diluted with filtered river water (0.45 µm pore-size PTFE membrane filters) by 1:4. Five ml (^{15}N-dA method) or 0.5 ml (^{3}H-Leu method) of the diluted water were incubated with final concentrations of 50 nM of ^{15}N-dA or 500 nM of ^{3}H-Leu (L-[4,5-^{3}H(N)]-, NET135H, Perkin Elmer), respectively. Each sample was incubated at 10, 18, and 26°C for 1–2 h. After incubation and quenching with the addition of ice-cold TCA (final concentration 5%), ^{3}H-Leu samples were centrifuged for 10 min at 14 000 × *g* at room temperature according to Smith & Azam (1992). The supernatant was removed by suction, and 1 ml of ice-cold 5% TCA was added to the tube. The centrifuging step was repeated, and the supernatant was then removed. One ml of ice-cold 80% ethanol was added to the tube, the centrifuging step was repeated, and the ethanol was then similarly removed. The tube was left to dry overnight at room temperature. One ml of scintillation cocktail (UltimaGold, Perkin Elmer) was added to the vial. After vortexing the vial, radioactivity was determined by a liquid scintillation counter (Wallac 1414, Perkin Elmer), and ^{3}H-Leu incorporation rates were calculated. The samples with ^{15}N-dA were quenched by adding 99.5% ethanol (final concentration >20%) at the end of incubation, filtered onto 0.2-µm PTFE membrane filters, and then stored at −20°C until further analysis. The ^{15}N-dA incorporation rates were quantified as described above. The CF_{Leu} was determined as 86.7 pmol ^{3}H-Leu (pmol ^{15}N-dA)$^{-1}$ (Fig. S4). The converted leucine uptake was converted into rates of bacterial carbon production of biofilm, assuming a conversion factor of 3.1 kg C mol^{-1} leucine (Kirchman 1993). In the present study, biofilm BP was obtained by incubation with 50 nM of ^{15}N-dA (final concentration). However, this concentration did not saturate the ^{15}N-dA incorporation rate (Fig. S5), leading to underestimation of biofilm BP. Therefore, we made a correction to the incorporation rate by converting ^{15}N-dA to ^{3}H-Leu incorporation rates by using the CF_{Leu}.

2.6. Calculation and statistical analysis

To examine the relative importance of epilithic biofilm chl *a*, BA, and BP in river ecosystems, we estimated equivalent depths (Z_{eq}) calculated as:

$$Z_{eq} = X_{biofilm} / X_{plankton} \quad (1)$$

where X represents chl *a*, BA, and BP of epilithic biofilms (g m^{-2} or g m^{-2} d^{-1}) or plankton (g m^{-3} or g m^{-3} d^{-1}), which estimated the height of overlying water column, with an area of 1 m^2, with the same activity or abundance as 1 m^2 of the stone surface. The relative contributions of epilithic biofilm chl *a*, BA, and BP were estimated as follows:

$$\text{Biofilm contribution (\%)} = X_{biofilm} / (X_{biofilm} + X_{plankton} \times \text{sampling depth}) \times 100 \quad (2)$$

where X represents chl *a*, BA, and BP of epilithic biofilms (g m^{-2} or g m^{-2} d^{-1}) or plankton (g m^{-3} or g m^{-3} d^{-1}).

Spatial differences in environmental parameters, planktonic and biofilm chl *a*, BA, and BP between riffle and pool habitats (Stn TS vs. TF, channel-unit scale), and bed slopes (Stn TS vs. W, segment scale) were determined by a 2-sided paired *t*-test throughout the study period. A generalized linear model (GLM), assuming a gamma distribution and log-link function, was used to examine the influences of station, water temperature, PO_4, dissolved inorganic nitrogen (DIN), DOC, planktonic chl *a*, and planktonic BA on planktonic BP, and station, current velocity, sampling depth, water temperature, PO_4, DIN, DOC, biofilm chl *a*, and biofilm BA on biofilm BP. Stations were transformed into dummy variables as they were categorical data. We compared and ranked all possible subset models based on Akaike's information criterion corrected for small sample size (AIC_C) ($n/K < 40$), where n and K represent the number of samples and variables, respectively. The goodness-of-fit of each model was measured by the Nagelkerke pseudo R^2,

which measures the proportion of variance explained by the model. Model-averaged estimates of the intercept and model coefficient were obtained as weighted-average estimates in each model, and the models were weighted with their Akaike weight. We determined the relative importance of each variable (relative variable importance [RVI]) by summing the Akaike weights of each model containing the factor (Burnham & Anderson 2002). The analysis was conducted using R software (version 4.0.3), 'MuMIn' package for model selection and averaging (Barton 2020), and 'piecewiseSEM' package for calculation of the Nagelkerke pseudo R^2 (Lefcheck 2016). In all statistical analyses, p = 0.05 was considered significant.

3. RESULTS

3.1. Spatial and seasonal variations

Water temperature ranged from 3.0 to 22.6°C during the study period and showed maxima in August at both stations (Fig. 2a). Water temperature was higher at Stn W than at Stn TS during each month (2-sided paired *t*-test, $p < 0.001$). DIN concentration was relatively high in winter and spring (December to May), and low in summer and autumn (June to November) (Student's *t*-test, $p < 0.05$ for Stns TF and W, $p < 0.01$ for Stn TS) (Fig. 2b). DIN concentration was higher at Stn W than at Stns TS and TF (2-sided paired *t*-test, $p < 0.001$). PO_4 concentration ranged from 0.024 to 0.091 mgP l^{-1} and did not show clear seasonal and spatial trends (Fig. 2c). DOC concentration ranged from 0.74 to 2.6 mgC l^{-1} and showed relatively high values in April to June (Student's *t*-test, $p < 0.05$ for Stns TS and TF, $p < 0.01$ for Stn W) (Fig. 2d). Although DOC concentration was highest at Stn TS in May 2019, no spatial differences were observed between stations.

Planktonic chl *a* concentration ranged from 2.0 to 19 µg l^{-1} (Fig. 3a). Planktonic chl *a* concentration did not show clear seasonal variations and was spatially similar among stations ($p > 0.05$). Planktonic BA ranged from 0.73×10^9 to 15×10^9 cells l^{-1} and increased in spring to early summer at all stations (Fig. 3b). Planktonic BP ranged from 5.5 to 466 mgC m^{-3} d^{-1} and showed maxima at all stations in May 2019 or 2020 compared to the other months (Student's *t*-test, $p < 0.001$, Fig. 3c). There were no significant spatial (station) differences in planktonic BA and planktonic BP ($p > 0.05$).

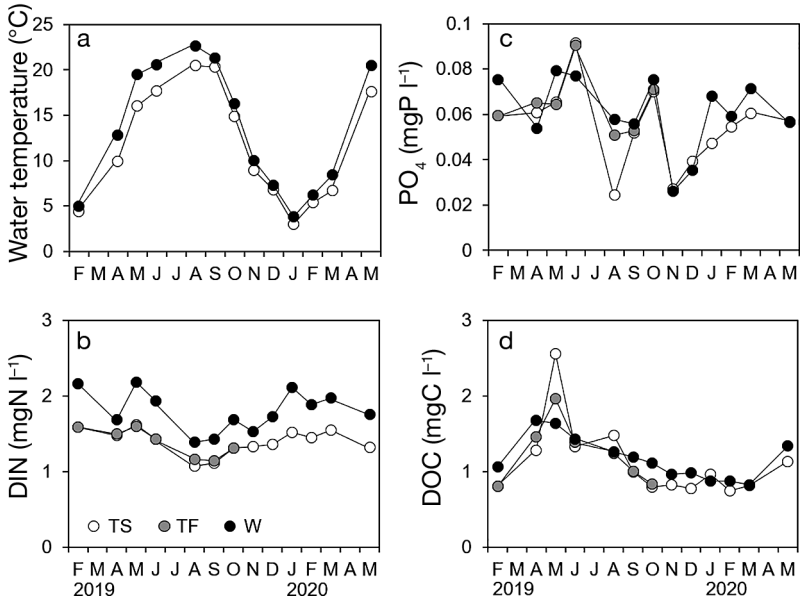

Fig. 2. Seasonal variation in (a) water temperature, (b) dissolved inorganic nitrogen concentration (DIN), (c) PO_4 concentration, and (d) dissolved organic carbon concentration (DOC) in the Shinano River during the study period. The depicted values of nutrients and DOC represent averages from 3 technical replicates; error bars are invisible because the symbols are larger than the error bars. Water temperature and DIN at Stn W were significantly higher than those at Stns TS and TF (2-sided paired *t*-test, $p < 0.001$). Since the water temperature of Stns TS and TF are the same, the data at Stn TF are not shown in panel a

In epilithic surfaces, biofilm chl *a* concentration ranged from 0.19 to 47 µg cm^{-2} and showed similar seasonal trends at all stations (Fig. 3d). A significant difference in biofilm chl *a* concentration between Stns TS and TF was observed ($p < 0.01$), whereas there was no significant difference between Stns TS and W ($p > 0.05$) throughout the study period. Biofilm BA ranged from 0.15×10^{12} to 35×10^{12} cells m^{-2} and showed similar seasonal trends, although Stns W and TS were higher in June and December 2019, respectively (Fig. 3e). Biofilm BA was not spatially different among stations throughout the study period ($p > 0.05$). Biofilm BP ranged from 2.9 to 132 mgC m^{-2} d^{-1}, and there was significantly higher BP in August and September at Stns TS and TF, and in September at Stn W compared to the other months (Student's *t*-test, $p < 0.001$, Fig. 3f). Biofilm BP was significantly different between Stns TS and TF ($p < 0.05$), and TS and W ($p < 0.01$) throughout the study period.

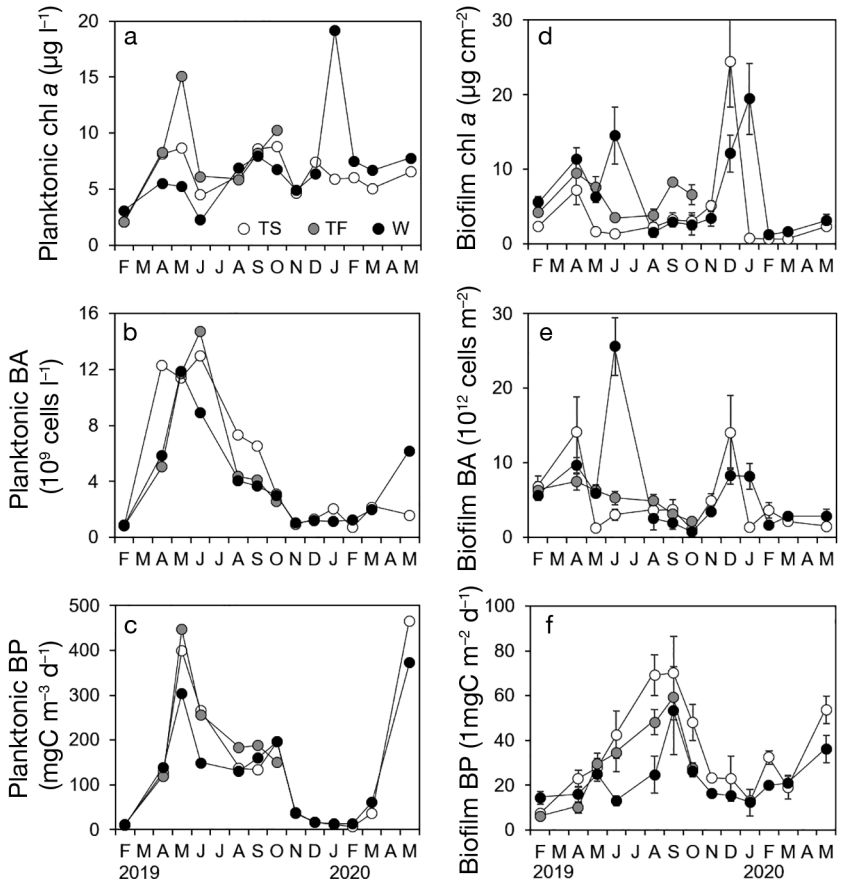

Fig. 3. Seasonal variation in chlorophyll *a* concentration (chl *a*), bacterial abundance (BA), and bacterial production (BP) of (a–c) river water and (d–f) epilithic biofilm in the river bed in the Shinano River during the study period. The depicted values for river water represent averages from biological or technical duplicates. Error bars for epilithic biofilm represent ±1 SE based on 4–5 stones collected at each station. There were significant differences in biofilm chl *a* between Stns TS and W (2-sided paired *t*-test, $p < 0.01$), and in BP between Stns TS and W ($p < 0.01$), and Stns TS and TF ($p < 0.05$)

The equivalent depth of biofilm (Z_{eq}) of chl *a*, BA, and BP ranged from 1.1 to 64 m, 0.11 to 10.7 m, and 0.066 to 5.9 m, respectively (Fig. 4a–c). In all variables, Z_{eq} showed relatively high values in winter, except for chl *a* at Stn W in June 2019. The Z_{eq} of BP reached a maximum in winter, up to 5.9 m at Stn TS. The Z_{eq} of chl *a* and BP were spatially different between Stns TS and TF ($p < 0.01$ for chl *a* and $p < 0.05$ for BP, paired *t*-test) throughout the study period, whereas no significant spatial difference in Z_{eq} was observed between Stns TS and W ($p > 0.05$).

Biofilm relative contributions of chl *a*, BA, and BP ranged from 88.4 to 99.6%, 52.1 to 99.1%, and 15.4 to 97.8%, respectively, during the study period (Fig. 4d–f). For chl *a*, relatively high contributions were maintained throughout the year (95.8 ± 3.2% on average). For BA and BP, the relative contributions showed relatively low values (73.2 ± 12.4% [SD] and 45.6 ± 21.5%, respectively) in spring to autumn (April to October) and higher values (93.4 ± 5.9% and 81.3 ± 9.6%, respectively) in winter (November to March).

3.2. Influences of environmental variables on planktonic and biofilm BP

In GLM analysis, water temperature, PO_4, DIN, DOC, and planktonic chl *a* were selected as explanatory variables for planktonic BP in the top 6 models showing ΔAICc < 2.0, which represents the difference in AICc between each model and the minimum AICc model (Model 1) (Table 2). The model-averaging analysis on planktonic BP revealed that water temperature and DOC had the largest RVI (Table 3). Except for DIN, all coefficients of the explanatory variables were positive (Table 3). For the analysis of GLM on biofilm BP, the explanatory variables were station, current velocity, water temperature, DIN, DOC, and BA in the top 4 models showing ΔAICc < 2.0 (Table 4). The model-averaging analysis on biofilm BP showed that water temperature and biofilm BA had the largest RVI, followed by DIN, station (TF and W), current velocity, and DOC (Table 5). The estimate coefficients of Stns TF and W were negative, implying that biofilm BP at Stns TF and W was lower than at Stn TS, and the result agreed with the results of the 2-sided paired *t*-test on biofilm BP between Stns TS and TF, and W. Biofilm BP was positively associated with current velocity and water temperature, and negatively associated with DIN, DOC, and biofilm BA (Table 5).

4. DISCUSSION

The GLMs in the present study suggested that water temperature regulated both planktonic and biofilm BP (Tables 2–5). The fact that water temperature was a primary regulatory factor of bacterial activity is consistent with previous planktonic BP studies

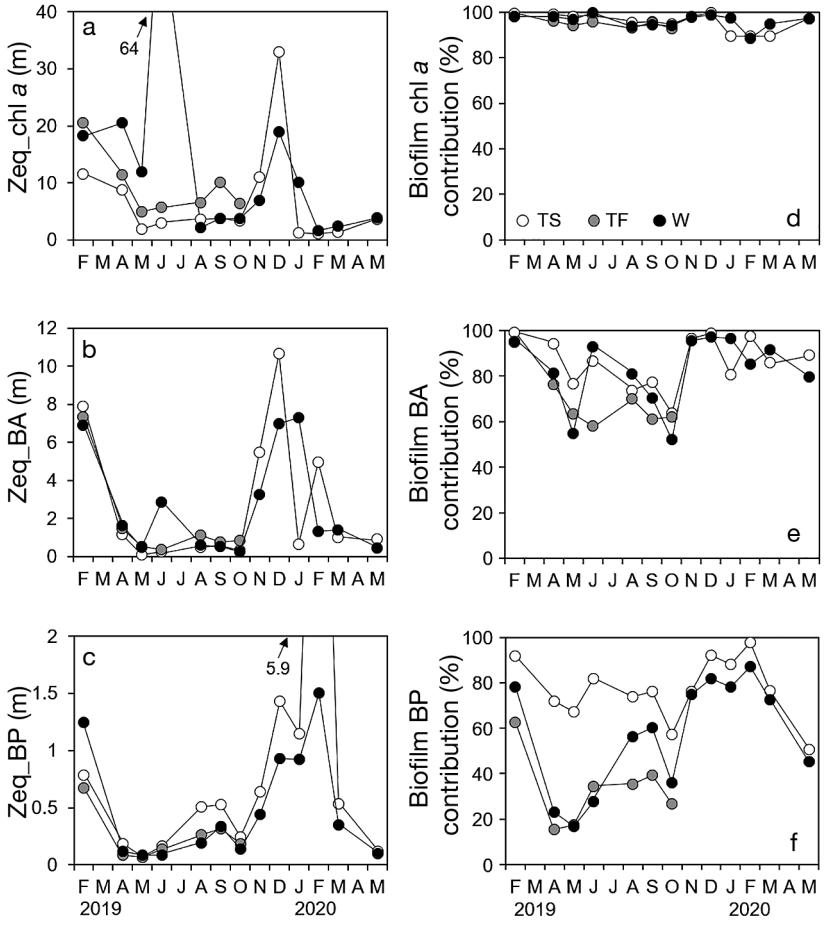

Fig. 4. Seasonal variation in depths (Z_{eq}) of biofilm chlorophyll *a* concentration (chl *a*), bacterial abundance (BA), and bacterial production (BP) equivalent to those of (a–c) water column (planktonic) and (d–f) biofilm relative contributions of chl *a*, BA, and BP in the Shinano River during the study period. Z_{eq} was calculated as $Z_{eq} = X_{biofilm} / X_{riverwater}$, where X represents chl *a*, BA, and BP of epilithic biofilms or river water. The relative contribution (%) was calculated as $X_{biofilm} / (X_{biofilm} + X_{riverwater} \times \text{sampling depth}) \times 100$

(e.g. Shiah & Ducklow 1994, Ainsworth & Goulder 1998, 2000a, Tsuchiya et al. 2020b). Contrary to planktonic BP, there are limited reports on the dependence of biofilm BP on water temperature (e.g. Kaplan & Bott 1989). No significant relationships between water temperature and biofilm BP were reported in the Ishite River (Fukuda et al. 2006), the River Tweed (Ainsworth & Goulder 2000a), and the River Swale (Ainsworth & Goulder 2000b). Other previous studies on biofilm BP did not report or test its dependency on water temperature (Carr et al. 2005, Scott et al. 2008, Kamjunke et al. 2015). Biological activity, such as biochemical reaction rates and metabolic rates, usually increases exponentially with temperature according to the kinetics described by the Boltzmann factor or the van 't Hoff–Arrhenius relation (Brown et al. 2004). Therefore, no relationship with water temperature suggested that other factors such as resource limitation, grazing pressure, and removal of biofilms by mechanical forces were more important for regulating biofilm BP in these ecosystems.

In addition to water temperature, biofilm BP was influenced positively by current velocity and negatively by station (compared with Stn TS), DIN, DOC, and biofilm BA (Tables 4 & 5).

Table 2. Selected generalized linear models (GLMs) for testing the effects of water temperature (WT), PO_4, dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and planktonic chlorophyll *a* (chl *a*) on planktonic bacterial production (BP) in the middle reach of the Shinano River. Shown are ΔAICc < 2.0, which represents the difference in Akaike's information criterion corrected for small sample size (AICc) between each model and the highest-ranked model (Model 1). Values for each explanatory variable represent the estimated coefficients (empty cells indicate variables that were not included in a particular model). R^2 and *Wi* indicate Nagelkerke pseudo R^2 and the Akaike weight for each model, respectively. Coefficients with $p < 0.05$ are shown in **bold**

Model	Explanatory variables for planktonic BP						AICc	ΔAICc	*Wi*	R^2
	WT	PO_4	DIN	DOC	Chl *a*	Intercept				
1	**0.155**	12.6		**0.818**		0.769	353.7	0	0.227	0.801
2	**0.154**	11.3		**0.724**	0.0556	0.564	354.0	0.31	0.195	0.817
3	**0.138**	**17.1**	−0.669	**0.943**		**1.61**	354.3	0.56	0.172	0.815
4	**0.161**			**0.779**	0.0592	**1.08**	354.5	0.79	0.153	0.796
5	**0.161**			**0.876**		**1.39**	354.7	1.00	0.138	0.777
6	**0.140**	**15.5**	−0.612	**0.850**	0.0492	1.34	355.1	1.37	0.115	0.828

Table 3. Model-averaged results (estimate) for the effects of water temperature (WT), PO_4, dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and planktonic chlorophyll *a* (chl *a*) on planktonic bacterial production (BP) in the middle reach of the Shinano River, using the top 6 models showing ΔAICc < 2.0. Results with *Z*-values > 2.0 are shown in **bold**. RVI: relative variable importance

Factors for BP	Estimate	SE	*Z*	RVI
WT	**0.152**	**0.022**	**6.76**	**1.00**
PO_4	13.8	7.3	1.81	0.71
DIN	−0.646	0.457	1.35	0.29
DOC	**0.827**	**0.321**	**2.47**	**1.00**
Chl a	0.0552	0.0318	1.66	0.46
Intercept	1.07	0.65	1.60	

Higher current velocity causes a thinner external boundary layer between the biofilm surface and water flow (Battin et al. 2003), inducing higher organic matter supply from the water column to biofilm bacterial communities. Moreover, biofilm BA showed the highest RVI as well as water temperature (Table 5). In the present study, biofilm BA and chl *a* were positively correlated (Spearman's rank correlation test, n = 33, rs = 0.73, p < 0.001), and there are positive relationships between biofilm thickness and chl *a* (Sekar et al. 2002, 2004, Kamjunke et al. 2012, Zhao et al. 2018), which suggested that biofilm BA and chl *a* could be used as a proxy for biofilm thickness. Therefore, higher BP was expected in thinner biofilm. The biofilm BP at Stn TS were significantly higher than those at Stns TF and W (Fig. 3, Table 5). Current velocity at Stn TS was higher than that at Stn TF, and sampling depth was shallower at Stn TS than at Stns TF and W (Table 1), which suggests that bottom shear stress at Stn TS was strongest among stations according to the calculation of Harrison & Keller (2007). High bottom shear stress can induce frequent erosion or slough of microorganisms from biofilms, decreasing net biomass accumulation and thickness in biofilms (Lau & Liu 1993). Biofilm formation is well known to be initiated by bacteria, and after a certain amount of bacterial mat foundation is developed, algae, protozoa, and meiofauna start to grow and dominate (e.g. Aizaki 1980, Lau & Liu 1993, Kathol et al. 2011, Majdi et al. 2012). Hence, biofilm BP would increase in earlier phases of the biofilm accumulation process (e.g. after the loss of biofilm caused by slough and feeding). In the present study, it was likely that the biofilm became thinner under high shear stress, and the high current velocity increased the supply rate of substrates to biofilm microorganisms, resulting in higher biofilm BP. Therefore, current velocity and bottom shear stress are significant regulatory factors of spatial variations of biofilm BP.

Although DIN and DOC were selected as explanatory variables in GLMs (Tables 4 & 5), it is unlikely that they were resource-limiting factors due to their negative relationships. Moreover, DIN and station were employed as explanatory variables complementarily in GLMs (Table 4), and DIN concentration was always highest at Stn W in each month (Fig. 2), which may have affected the GLM analysis. The result agrees with previous studies (Carr et al. 2005, Kamjunke et al. 2015), which showed no significant relationships between biofilm BP and ambient nutrients or DOC. In biofilms, bacteria and algae symbiotically coalesce with each other through complex nutrient and organic matter exchange (Lock et al. 1984). Bacteria in biofilms can use the polysaccharide matrix and adsorbed organic matter as a carbon source when organic carbon in the water column is heavily depleted (Freeman & Lock 1995) although labile DOC (glucose) in the water column can enhance biofilm BP throughout biofilm colonization in amendment experiments (Sobczak 1996). Flexible use by bacteria of organic carbon sources derived from the water column and biofilm algae can mask the correlation between water quality and biofilm BP.

Regulatory factors of biofilm BP were different in our investigation to those in previous studies. Biofilm BP showed significant positive correlations to total

Table 4. Selected generalized linear models (GLMs) for testing the effects of station, current velocity, water temperature (WT), dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and biofilm bacterial abundance (BA) on biofilm bacterial production (BP) in the middle reach of the Shinano River, showing ΔAICc < 2.0. '+' symbols indicate that Station was selected as the explanatory variable for the models. Other details as in Table 2

Model	Explanatory variables for biofilm BP							AICc	ΔAICc	*Wi*	R^2
	Station	Velocity	WT	DIN	DOC	BA	Intercept				
1		**0.0073**	**0.0586**	**−0.704**		**−0.0324**	**3.39**	240.8	0	0.368	0.757
2	**+**		**0.0806**		**−0.320**	**−0.0326**	**2.98**	241.4	0.63	0.268	0.776
3			**0.0533**	**−0.703**		**−0.0321**	**3.81**	242.2	1.39	0.183	0.722
4	**+**		**0.0712**			**−0.0367**	**2.76**	242.2	1.42	0.181	0.746

Table 5. Model-averaged results (estimate) for the effects of station, velocity, water temperature (WT), dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and biofilm bacterial abundance (BA) on biofilm bacterial production (BP) in the middle reach of the Shinano River, using the top 4 models showing ΔAICc < 2.0. Results with *Z*-values > 2.0 are shown in **bold**. RVI: relative variable importance

Factors for BP	Estimate	SE	*Z*	RVI
Station TF	**−0.377**	**0.146**	**2.47**	**0.45**
Station W	**−0.448**	**0.121**	**3.53**	**0.45**
Velocity	**0.0073**	**0.0034**	**2.07**	**0.37**
WT	**0.0658**	**0.0140**	**4.60**	**1.00**
DIN	**−0.703**	**0.205**	**3.29**	**0.55**
DOC	−0.320	0.154	1.98	0.27
BA	**−0.0332**	**0.0113**	**2.80**	**1.00**
(Intercept)	**3.24**	**0.47**	**6.71**	

phosphorus in biofilm in the Bode catchment (Kamjunke et al. 2015), biofilm chl *a* in the River Tweed (Ainsworth & Goulder 2000a), biofilm chl *a* and biofilm BA in the River Swale (Ainsworth & Goulder 2000b), algal production in the National Capital Region, Canada, (Carr et al. 2005), biofilm BA in the Ishite Stream (Fukuda et al. 2006), and water temperature and current velocity in the middle reaches of the Shinano River. In the Ishite Stream, biofilm BP was measured based on the frequency of dividing cells method, which used BA to calculate BP; in other words, these variables were not independent. Thus, spurious correlations cannot be ruled out (Brett 2004). In terms of the difference in methodologies for BP measurement, Carr et al. (2005), Kamjunke et al. (2015), and our study used intact stones where the structure of the biofilm is maintained, whereas Ainsworth & Goulder (2000a,b) measured BP using biofilm suspensions, in which biofilm structure was not maintained. In the latter case, bacteria that normally reside in the deeper part of the biofilm (i.e. anaerobic or unproductive) can access the substrate in an aerobic environment in the incubation. This might have led to a positive correlation between microbial biomasses of algae and bacteria in the biofilms, which was not shown in our study and other studies (Carr et al. 2005, Kamjunke et al. 2015). In the future, it will be necessary to quantify the effect of different incubation methods on biofilm BP. In the present study, we were unable elucidate which environments in the river and which aspects of the biofilm microbial community induced the differences in limiting factors of biofilm BP among the ecosystems, due to the limited common variables and information. Extraction of the factors controlling the limiting factors will contribute to a better understanding of biofilm dynamics and the related biogeochemical cycle in river ecosystems.

Biological factors such as bioturbations, feeding, and excretion by invertebrates can also influence bacterial activity (e.g. Nascimento et al. 2012). Invertebrate grazing on biofilm algae generally leads to decreased algal biomass and area-specific productivity (Liess & Hillebrand 2004), which may decrease autochthonous substrate supply to bacteria. However, grazing can induce biofilms to be thinner and increase substrate supply rate (Battin et al. 2003), possibly enhancing bacterial activity. Mathieu et al. (2007) found that grazing by meiofauna (nematodes) on artificial diatom biofilm enhanced algal production. Those authors discussed that nematode activity could have increased porosity, enhancing light penetration and facilitating nutrient supply to microalgae. In this case, BP can be enhanced due to an increase in autochthonous substrates from biofilm algal communities. Moreover, faunal activities directly increased BP (Traunspurger et al. 1997, Mathieu et al. 2007, Nogaro et al. 2008), and mechanisms of the positive effects have been proposed: (1) grazing by meiofauna keeps the bacterial community in an active growth phase (Lillebø et al. 1999); (2) bioturbation by meiofauna provides electron acceptors (Kristensen 2000); and (3) fast nutrient return by meiofauna (Coull 1999). However, although biological interactions are predictable, to our knowledge, no study has directly examined the effects of meio- and macrofaunal activities on BP in epilithic biofilms in river ecosystems. The biological interactions in epilithic biofilms should be investigated for a better understanding of biogeochemical and microbial processes in river ecosystems.

The difference in regulatory factors between planktonic and biofilm BP (Tables 2–5) agrees with a previous study (Kamjunke et al. 2015), demonstrating that planktonic BP correlated with DOC concentration and quality. In contrast, DOC did not explain variation in biofilm BP in stream water. In the present study, planktonic BP and DOC showed their peaks simultaneously in spring (especially May) (Figs. 2 & 3), and there was a positive relationship between planktonic BP and DOC in GLM analysis (Tables 2 & 3), suggesting carbon limitation of bacterioplankton. DIN and PO_4 concentrations were more than 1.1 mgN l^{-1} and 0.024 mgP l^{-1}, respectively, suggesting that N and P were sufficient for planktonic BP (Gurung & Urabe 1999). Meanwhile, DOC/DIN and DOC/PO_4 ratios (molar) were 0.89 ± 0.33 (SD) and 55 ± 26, respectively, during the study period, and these ratios were relatively low compared to the Redfield ratio (C:N:P =

106:16:1). The results support the notion that planktonic BP is regulated by organic carbon sources, as shown by the GLM analysis.

Plankton chl *a* concentrations were higher at upstream sites than downstream in April, May, and June 2019 (Fig. 3). Usually, downstream increases in phytoplankton chl *a* concentrations can be expected due to algal growth (Ainsworth & Goulder 1998). However, a previous study conducted in the Shinano River demonstrated that planktonic algae and chl *a* did not monotonically increase downstream (Nakamoto & Yamamoto 1999) as was also shown in the present study. They suggested that planktonic algae settle on the riverbed and are consumed by aquatic organisms. In fact, the highest abundance of aquatic insects, especially caddisflies, was found midway between Stns T and W (Hirabayashi et al. 2016).

The Z_{eq} and relative contributions of biofilm chl *a*, BA, and BP to total (sum of planktonic and biofilm) measures were different among variables (Fig. 4). The relative contribution of biofilm chl *a* to the total exceeded 88.4% (95.8 ± 3.2% [SD] on average) during the study period, suggesting that epilithic biofilms were an important habitat for microalgae in the Shinano River. The Z_{eq} of chl *a* showed no correlation to water temperature ($r = -0.037$, $n = 33$, $p > 0.05$), and there was no seasonal variation in Z_{eq}. The relative contribution of chl *a* agrees with results from the Garonne River, France (99 ± 5%, Tekwani et al. 2013). In both rivers, diatoms were dominant (Okino 2006, Tekwani et al. 2013). For BA, although the Z_{eq} and relative contribution of biofilm BA showed relatively low values in spring to autumn (minimum of 0.11 m and 52.1%, respectively), the year-round (2.45 ± 2.92 m and 81.1 ± 14.3%, respectively) and winter averages (5.0 ± 3.2 m and 93.4 ± 5.9%, respectively) were high. Although there was no significant relationship between water temperature and biofilm BA ($r = -0.064$, $n = 33$, $p > 0.05$), planktonic BA significantly positively correlated to water temperature ($r = 0.55$, $n = 33$, $p < 0.001$). Low water temperature and DOC concentration in winter suppressed bacterioplankton production, possibly leading to lower values of planktonic BA. The Z_{eq} of BA significantly negatively correlated to water temperature ($r = -0.66$, $n = 33$, $p < 0.001$), suggesting higher relative importance of biofilm as a bacterial habitat in winter. In previous studies, Z_{eq} of BA was 1.6 ± 0.9 m (Ainsworth & Goulder 2000a) and 0.42 ± 0.37 m (Kamjunke et al. 2015), which is relatively low compared to the year-round average (2.45 ± 2.92 m) in the present study. However, these previous studies were conducted in spring to autumn (April, June, and October for the former and August for the latter), and the Z_{eq} of April–October was 0.79 ± 0.64 m, which was within the range of previous studies.

The Z_{eq} and relative contribution of biofilm BP varied widely from 0.0658 to 5.87 m and 5.4 to 97.8%, respectively, during the study period (Fig. 4). Values were relatively high in winter and low in spring/summer, and the Z_{eq} of BP was significantly negatively correlated with water temperature ($r = -0.47$, $n = 33$, $p < 0.01$), suggesting that the relative importance of biofilm BP changed along a gradient of water temperature. In particular, the Z_{eq} of April–October was 0.19 ± 0.13 m, which suggests that planktonic BP dominates in the river since mean depths at Tokida and Iwano stations were 0.4 and 1.2 m, respectively. The Z_{eq} of the present study (0.0658–5.87 m) was within the same range of values reported in previous studies: 0.08–5.4 m in the Bode catchment, Germany (Kamjunke et al. 2015), 0.12–30 m in the River Tweed, UK (Ainsworth & Goulder 2000a), and 0.51–2.2 m in the River Swale, UK (Ainsworth & Goulder 2000b). The ranges of biofilm BP variations are smaller than those of planktonic BP variations, supported by the ratio of coefficients of variance (CV) of planktonic to biofilm BP ($CV_{Planktonic}$ / $CV_{Biofilm}$ = 1.5 to 2.7, Table 6). The results demonstrated that biofilm BP was relatively stable compared to planktonic BP in the 4 temperate river/stream ecosystems, and the stability of biofilm BP could be maintained by supplying nutrients and organic matter from inside and outside biofilms.

Table 6. Planktonic and epilithic biofilm bacterial production (BP) in river and stream ecosystems. To convert leucine incorporation to BP, 3.1 kg C mol^{-1} leucine (Kirchman 1993) was used by Ainsworth & Goulder (2000a,b). CV: coefficient of variance

Study site	Season	Planktonic BP ($mgC\ m^{-3}\ d^{-1}$)	Biofilm BP ($mgC\ m^{-2}\ d^{-1}$)	$CV_{Planktonic}$/ $CV_{Biofilm}$	Reference
Tweed River, UK	Apr–Oct	0–394	7–89	2.7	Ainsworth & Goulder (2000a)
Swale River, UK	Jun–Sep	7–618	15–201	1.8	Ainsworth & Goulder (2000b)
Bode catchment, Germany	Aug	3–193	14–79	1.7	Kamjunke et al. (2015)
Shinano River, Japan	Year-round	6–466	3–132	1.5	Present study

In conclusion, the present study examined seasonal and spatial variation in planktonic and epilithic biofilm BP and revealed that regulatory factors differed between planktonic and biofilm BP. Both planktonic and biofilm BP were regulated primarily by water temperature. The GLMs suggested that organic carbon resources limited planktonic BP, and stoichiometric analysis supported this hypothesis. We found no evidence of resource limitation in biofilm BP in GLM analysis. It was likely that biofilm bacteria flexibly used organic matter and nutrients derived from the water column and biofilm algae, which could have masked the correlation between water quality and biofilm BP. Biofilm BP showed significant spatial variation at channel-unit (riffle and pool) and segment (different bed slopes) scales within the seasonal variability. The GLMs suggested that physical parameters such as current velocity and shear stress affected the spatial variability of biofilm BP through biofilm formation and substrate supply. The balance (Z_{eq}) of planktonic and biofilm BA and BP varied along with a gradient of water temperature, clarifying the substantial contributions of planktonic BP to carbon cycling during the more productive seasons in the middle reaches of the Shinano River. In contrast, especially in winter, the contribution of biofilm BP was high, and the biofilm became the primary productive habitat. This variability is mainly caused by the large variability in planktonic BP, while biofilm BP is relatively stable, as shown by comparison with data from 4 temperate river/stream ecosystems. It is unclear whether such a relationship can be extended to river ecosystems outside of temperate zones. For a future assessment of the impact on biogeochemical cycles, it is essential to estimate the balance between the biofilm and planktonic bacterial metabolisms in tropic and arctic regions that are highly vulnerable to climate change.

Acknowledgements. We thank Shinji Aoki for his support in comparison experiments using radioisotopes, and colleagues at the Chikuma River Group for their generous support and assistance. This study was partly supported by the Japanese Ministry of Land, Infrastructure, Transport and Tourism, the River Ecology Research Group of Japan, and Grants-in-Aid for Scientific Research (JP17J11577, 20K12140) of the Japan Society for the Promotion of Science.

LITERATURE CITED

Ainsworth AM, Goulder R (1998) Microbial organic-nitrogen transformations along the Swale–Ouse river system, Northern England. Sci Total Environ 210/211:329–355

Ainsworth AM, Goulder R (2000a) Downstream change in leucine aminopeptidase activity and leucine assimilation by epilithic microbiota along the River Swale, northern England. Sci Total Environ 251-252:191–204

Ainsworth AM, Goulder R (2000b) Epilithic and planktonic leucine aminopeptidase activity and leucine assimilation along the River Tweed, Scottish Borders. Sci Total Environ 251-252:83–93

Aizaki M (1980) Changes in standing crop and photosynthetic rate attendant on the film development of periphyton in a shallow eutrophic river. Jpn J Limnol 41:225–234

Allan JD, Castillo MM (2007) Stream ecology: structure and function of running waters. Springer, Dordrecht

Aufdenkampe AK, Mayorga E, Raymond PA, Melack JM and others (2011) Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Front Ecol Environ 9:53–60

Azam F (1998) Microbial control of oceanic carbon flux: The plot thickens. Science 280:694–696

Barton K (2020) Package 'MuMIn'. R package 1.43.17. https://cran.r-project.org/web/packages/MuMIn/index.html

Battin TJ, Kaplan LA, Newbold JD, Cheng X, Hansen C (2003) Effects of current velocity on the nascent architecture of stream microbial biofilms. Appl Environ Microbiol 69:5443–5452

Battin TJ, Kaplan LA, Findlay S, Hopkinson CS and others (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nat Geosci 1:95–100

Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packman AI (2016) The ecology and biogeochemistry of stream biofilms. Nat Rev Microbiol 14:251–263

Brett MT (2004) When is a correlation between non-independent variables 'spurious'? Oikos 105:647–656

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85: 1771–1789

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer-Verlag, New York, NY

Carr GM, Morin A, Chambers PA (2005) Bacteria and algae in stream periphyton along a nutrient gradient. Freshw Biol 50:1337–1350

Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems — a cross-system overview. Mar Ecol Prog Ser 43:1–10

Coull BC (1999) Role of meiofauna in estuarine soft-bottom habitats. Aust J Ecol 24:327–343

Edwards RT, Meyer JL, Findlay SE (1990) The relative contribution of benthic and suspended bacteria to system biomass, production, and metabolism in a low-gradient blackwater river. J N Am Benthol Soc 9:216–228

Findlay S, Howe K, Fontvielle D (1993) Bacterial–algal relationships in streams of the Hubbard Brook experimental forest. Ecology 74:2326–2336

Freeman C, Lock MA (1995) The biofilm polysaccharide matrix: a buffer against changing organic substrate supply? Limnol Oceanogr 40:273–278

Fukuda M, Matsuyama J, Katano T, Nakano Si, Dazzo F (2006) Assessing primary and bacterial production rates in biofilms on pebbles in Ishite Stream, Japan. Microb Ecol 52:1–9

Gurung TB, Urabe J (1999) Temporal and vertical difference in factors limiting growth rate of heterotrophic bacteria in Lake Biwa. Microb Ecol 38:136–145

Hall RO Jr (1995) Use of a stable carbon isotope addition to trace bacterial carbon through a stream food web. J N Am Benthol Soc 14:269–277

Harrison LR, Keller EA (2007) Modeling forced pool–riffle hydraulics in a boulder-bed stream, southern California. Geomorphology 83:232–248

Hildrew AG, Giller P (1994) Patchiness, species interactions and disturbance in the stream benthos. In: Giller PS, Hildrew AG, Raffaelli DG (eds) Aquatic ecology: scale, pattern and process. Blackwell, Oxford, p 21–62

Hirabayashi K, Ikutama E, Ohkawa K, Arai R, Nomura T, Tsukada M, Abe K (2016) Flight density of the aquatic insect fauna over the water surface in the middle reaches of the Shinano River, Japan, mainly among caddisflies (Trichoptera). Zoosymposia 10:203–213

Kamjunke N, Spohn U, Füting M, Wagner G, Scharf EM, Sandrock S, Zippel B (2012) Use of confocal laser scanning microscopy for biofilm investigation on paints under field conditions. Int Biodeterior Biodegradation 69:17–22

Kamjunke N, Herzsprung P, Neu TR (2015) Quality of dissolved organic matter affects planktonic but not biofilm bacterial production in streams. Sci Total Environ 506-507:353–360

Kaplan LA, Bott TL (1989) Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: effects of temperature, water chemistry, and habitat. Limnol Oceanogr 34:718–733

Karlsson OM, Richardson JS, Kiffney PA (2005) Modelling organic matter dynamics in headwater streams of southwestern British Columbia, Canada. Ecol Model 183: 463–476

Kathol M, Fischer H, Weitere M (2011) Contribution of biofilm-dwelling consumers to pelagic–benthic coupling in a large river. Freshw Biol 56:1160–1172

Kirchman DL (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology, Book 58. Lewis Publishers, Boca Raton, FL, p 509–512

Kristensen E (2000) Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426:1–24

Lau YL, Liu D (1993) Effect of flow rate on biofilm accumulation in open channels. Water Res 27:355–360

Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl Environ Microbiol 53:1298–1303

Lefcheck JS (2016) piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol Evol 7:573–579

Liess A, Hillebrand H (2004) Invited review: direct and indirect effects in herbivore–periphyton interactions. Arch Hydrobiol 159:433–454

Lillebø AI, Flindt MR, Pardal MÂ, Marques JC (1999) The effect of macrofauna, meiofauna and microfauna on the degradation of *Spartina maritima* detritus from a salt marsh area. Acta Oecol 20:249–258

Lock MA, Wallace RR, Costerton JW, Ventullo RM, Charlton SE (1984) River epilithon — toward a structural–functional model. Oikos 42:10–22

Majdi N, Mialet B, Boyer S, Tackx M and others (2012) The relationship between epilithic biofilm stability and its associated meiofauna under two patterns of flood disturbance. Freshw Sci 31:38–50

Marker AFH, Nusch EA, Rai H, Riemann B (1980) The measurement of photosynthetic pigments in freshwaters and standardization of methods: conclusions and recommendations. Arch Hydrobiol (Beih 14):91–106

Mathieu M, Leflaive J, Ten-Hage L, de Wit R, Buffan-Dubau E (2007) Free-living nematodes affect oxygen turnover of artificial diatom biofilms. Aquat Microb Ecol 49:281–291

Naiman RJ (1983) The annual pattern and spatial distribution of aquatic oxygen metabolism in boreal forest watersheds. Ecol Monogr 53:73–94

Nakamoto N, Yamamoto M (1999) Downstream change in water quality and algae along the Shinano River, Japan. In: Collection of Research Papers on River Beautification and Greening, Book 8. The River Foundation, Tokyo, p 1–25

Nascimento FJ, Näslund J, Elmgren R (2012) Meiofauna enhances organic matter mineralization in soft sediment ecosystems. Limnol Oceanogr 57:338–346

Niyogi DK, Lewis WM, McKnight DM (2003) Direct and indirect effects of mine drainage on bacterial processes in mountain streams. J N Am Benthol Soc 22:276–291

Nogaro G, Mermillod-Blondin F, Montuelle B, Boisson JC, Gibert J (2008) Chironomid larvae stimulate biogeochemical and microbial processes in a riverbed covered with fine sediment. Aquat Sci 70:156–168

Nojiri Y (1987) Progress in water quality analysis (3). J Groundw Hydrol 29:107–111

Okino T (2006) The nature of rivers created by floods: from the academic study of Chikuma River ecology. The Shinano Mainicni Shimbun, Nagano (in Japanese)

Otsuki A, Goma R, Aizaki M, Nojiri Y (1993) Seasonal and spatial variations of dissolved nitrogenous nutrient concentrations in hypertrophic shallow lake, with special reference to dissolved organic nitrogen. Int Ver Theor Angew Limnol Verh 25:187–192

Pace ML, Cole JJ (1994) Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. Microb Ecol 28:181–193

Pomeroy LR (1974) The ocean's food web, a changing paradigm. Bioscience 24:499–504

Pringle CM, Naiman RJ, Bretschko G, Karr JR, Oswood MW, Webster LWR Jr, Winterbourn MJ (1988) Patch dynamics in lotic systems: the stream as a mosaic. J N Am Benthol Soc 7:503–524

Rosenfeld JS, Hudson JJ (1997) Primary production, bacterial production, and invertebrate biomass in pools and riffles in southern Ontario streams. Arch Hydrobiol 139: 301–316

Scott JT, Back JA, Taylor JM, King RS (2008) Does nutrient enrichment decouple algal–bacterial production in periphyton? J N Am Benthol Soc 27:332–344

Sekar R, Nair KVK, Rao VNR, Venugopalan VP (2002) Nutrient dynamics and successional changes in a lentic freshwater biofilm. Freshw Biol 47:1893–1907

Sekar R, Venugopalan VP, Nandakumar K, Nair KVK, Rao VNR (2004) Early stages of biofilm succession in a lentic freshwater environment. In: Ang PO (ed) Proc Asian Pacific Phycology in the 21st Century: Prospects and Challenges. Springer, Dordrecht, p 97–108

Shiah FK, Ducklow HW (1994) Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Mar Ecol Prog Ser 103:297–308

Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar Microb Food Webs 6:107–114

Sobczak WV (1996) Epilithic bacterial responses to variations in algal biomass and labile dissolved organic car-

bon during biofilm colonization. J N Am Benthol Soc 15: 143–154

Sobczak WV, Burton TM (1996) Epilithic bacterial and algal colonization in a stream run, riffle, and pool: a test of biomass covariation. Hydrobiologia 332:159–166

Tekwani N, Majdi N, Mialet B, Tornés E and others (2013) Contribution of epilithic diatoms to benthic–pelagic coupling in a temperate river. Aquat Microb Ecol 69:47–57

Traunspurger W, Bergtold M, Goedkoop W (1997) The effects of nematodes on bacterial activity and abundance in a freshwater sediment. Oecologia 112:118–122

Tsuchiya K, Sano T, Kawasaki N, Fukuda H and others (2015) New radioisotope-free method for measuring bacterial production using [$^{15}N_5$]-2′-deoxyadenosine and liquid chromatography mass spectrometry (LC–MS) in aquatic environments. J Oceanogr 71:675–683

Tsuchiya K, Sano T, Tomioka N, Kohzu A and others (2019) Seasonal variability and regulation of bacterial production in a shallow eutrophic lake. Limnol Oceanogr 64: 2441–2454

Tsuchiya K, Sano T, Tomioka N, Kohzu A and others (2020a) Incorporation characteristics of exogenous ^{15}N-labeled thymidine, deoxyadenosine, deoxyguanosine and deoxycytidine into bacterial DNA. PLOS ONE 15:e0229740

Tsuchiya K, Tomioka N, Sano T, Kohzu A and others (2020b) Decrease in bacterial production over the past three decades in the north basin of Lake Biwa, Japan. Limnology 21:87–96

Zhao Y, Xiong X, Wu C, Xia Y, Li J, Wu Y (2018) Influence of light and temperature on the development and denitrification potential of periphytic biofilms. Sci Total Environ 613-614:1430–1437

Editorial responsibility: Rutger de Wit, Montpellier, France
Reviewed by: E. Buffan-Dubau and 1 anonymous referee

Submitted: October 28, 2020
Accepted: April 7, 2021
Proofs received from author(s): June 14, 2021