Phosphorus enrichment of stream ecosystems generally increases primary production in the benthos, but the consequences of eutrophication for the nutritional quality of periphyton for grazers are less clear. On short timescales, high phosphorus inputs may lead to reduced C:P ratios and high essential fatty acid contents of periphyton, which are both considered important determinants of food quality for grazers. However, nutrient enrichment may alter the taxonomic composition of periphyton and favor the growth of less palatable algal taxa. In this study, periphyton was grown under a gradient of dissolved phosphorus availability from 5 to 100 \( \mu \text{gP} / \text{L} \), to investigate eutrophication effects on periphyton taxonomy, C:N:P stoichiometry, and fatty acid composition. After 1 month, periphyton grown under oligotrophic conditions was mainly composed of diatoms (~86%). With increasing phosphorus availability, diatoms were gradually outcompeted by chlorophytes and cyanobacteria, which were the predominant taxon under eutrophic conditions. Unexpectedly, periphyton C:P ratios increased with greater phosphorus supply, from ~280 under oligotrophic conditions up to ~790 at 100 \( \mu \text{gP} / \text{L} \), reflecting a tendency of chlorophytes and cyanobacteria to produce more biomass per unit of assimilated phosphorus compared to diatoms. Periphyton content of essential polyunsaturated fatty acids relative to biomass followed a unimodal relationship with phosphorus availability and peaked at intermediate phosphorus levels, likely as a result of both taxonomic and nutrient effects. Our results demonstrate that phosphorus-driven eutrophication of freshwater ecosystems may worsen periphyton nutritional quality due to taxonomic sorting, which may further lead to lower growth and reproduction of herbivores.

**Key index words:** eutrophication; fatty acids; microalgae; nutrients; periphyton; stoichiometry; stream; taxonomy

**Abbreviations:** ALA, \( \alpha \)-linolenic acid; C, carbon; EPA, eicosapentaenoic acid; FA, fatty acid; HPLC, high-pressure liquid chromatography; LIN, linoleic acid; PUFA, polyunsaturated fatty acid; SAFA, saturated fatty acid

Benthic algae are the most nutritious food source for herbivores in stream ecosystems (Brett et al. 2017). Compared to allochthonous organic matter, algae provide higher relative amounts of essential nutrients (i.e., phosphorus and nitrogen) and polyunsaturated fatty acids (PUFAs), which are major determinants of food quality for grazers (Torres-Ruiz et al. 2007, Lau et al. 2009). However, periphyton nutritional quality is strongly influenced by environmental factors such as light intensity and nutrient availability (Hill et al. 2011), whose fluctuations may significantly alter the efficiency of energy transfer to consumers. In particular, low relative contents of nutrients and/or PUFAs in periphyton are known to constrain grazer growth and reproduction (Stelzer and Lamberti 2002, Guo et al. 2016a). Therefore, there has been growing interest in determining how abiotic resources affect benthic algal food quality in streams (Guo et al. 2016b).

Nutrient concentrations in streams have been increasing in the past few decades due to agricultural runoffs, wastewater discharge, and other anthropogenic activities, often leading to an increment in algal production known as eutrophication (Dodds and Smith 2016, Wurtsbaugh et al. 2019).
Elevated inputs of phosphorus (P) or nitrogen (N) may result in a high algal nutrient content relative to carbon (C), and thus low C:P or C:N stoichiometric ratios, as demonstrated in several laboratory studies with natural periphyton (Stelzer and Lamber蒂 2001, Cross et al. 2005, Fanta et al. 2010, Hill et al. 2011). A few studies have also shown that high phosphorus availability may lead to an increased proportion of PUFAs relative to total fatty acids in algal cells because high phosphorus concentrations aid the formation of PUFArich galactolipids and phospholipids, while at low nutrient availability, carbon is stored and accumulated in saturated fatty acids (SAFAs; Reitan et al. 1994, Guschina and Har- wood 2006, Hill et al. 2011). Therefore, phosphorus enrichment might improve the nutritional quality of periphyton for grazers by increasing its relative content of nutrients and PUFAs. However, eutrophica- tion is often associated with an increase in the proportion of periphytic cyanobacteria and/or chlorophytes, and a subsequent decrease in diatom abundance (Carrick and Lowe 1988, Leland and Porter 2000, Pan et al. 2000, Marcarelli and Wurts- baugh 2006, McCall et al. 2017). Diatoms are con- sidered a high-quality food source for aquatic herbivores because they are rich in long-chained PUFAs, particularly eicosapentaenoic acid (EPA, 20:5ω3), which serve a wide range of essential physiological functions for cell membranes and hormone regulation in animals (Brett and Müller-Navarra 1997, Lang et al. 2011, Twining et al. 2016). Chloro- phytes are poor in EPA and other long-chained PUFAs, but are considered of intermediate dietary quality because they contain high relative amounts of C18 PUFAs, particularly α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LIN, 18:2ω6), which also have important physiological roles for invertebrate growth (Torres-Ruiz et al. 2007, Taipale et al. 2013, Guo et al. 2016b). Cyanobacteria, on the other hand, are a low-quality food source because they are generally poor in PUFAs and sterols (von Elert et al. 2003, Brett et al. 2006, Burns et al. 2011). As a consequence, a shift in taxonomic composition caused by nutrient enrichment is likely to impact the fatty acid profile of periphyton, resulting in lower amounts of available essential PUFAs for herbivores. However, only a few field studies have investigated how nutrient enrichment affects periphyton fatty acid composition in streams, with complex and differing results, as nutrients were often interacting with other variables, such as light (Cashman et al. 2013, Guo et al. 2015, Whorley and Wehr 2016, Guo et al. 2016a).

While it is acknowledged that nutrient enrichment may increase algal nutrient content, little is known about the C:P stoichiometry of communities experiencing taxonomic shifts in response to eutrophication. Laboratory studies on algal monocultures have shown that diatoms tend to maintain lower C:P ratios than chlorophytes when grown under the same conditions (Ho et al. 2003, Quigg et al. 2003). As a consequence, positive effects of increasing P availability on periphyton relative P content, as expected from observations on stable communities, may be counteracted by negative effects, when chlorophytes and cyanobacteria are favored over diatoms along the nutrient gradient. A number of studies observed high variation in the relationship between P availability and periphyton C:P stoichiometry in natural streams, although no mechanistic explanations were provided (Fanta et al. 2010, O’Brien and Wehr 2010, Liess et al. 2012, Hiatt et al. 2019). Therefore, controlled experiments are needed to investigate how eutrophication affects periphyton C:P stoichiometry in relation to its taxonomic composition.

In this study, stream periphyton was grown under a gradient of dissolved P availability in laboratory flumes to test nutrient enrichment effects on periphyton food quality in terms of fatty acid composition and nutrient stoichiometry. We manipulated water P concentrations to mimic conditions in natural streams from highly oligotrophic to highly eutrophic. We tested the following hypotheses: (i) Diatoms would dominate the periphytic community at low P availability, while the fraction of chloro- phytes and cyanobacteria would increase with P availability; (ii) As a consequence of the change in taxonomic composition, periphyton content of essential PUFAs (particularly EPA) would decrease with increasing P availability; and (iii) Periphyton C:P ratio would not strongly correlate with P availabil- ity as two contrasting effects (i.e., direct P effect on stoichiometry of single populations vs. indirect P effect on stoichiometry of communities mediated by taxonomic sorting) would negatively interact.

**MATERIALS AND METHODS**

*Experimental set-up and procedure.* Each experimental unit consisted of a circular flume in a 22 × 27 cm (height × diam- eter) plastic bucket with a circular water current generated by a pump in the centre. On the bottom surface of each flume were nine 5 × 5 cm slots for tiles. The experiment consisted of five treatments of increasing dipotassium phosphate (K₂HPO₄) concentrations in the water: oligotrophic (5 µg P · L⁻¹), oligomesotrophic (25 µg P · L⁻¹), mesotrophic (50 µg P · L⁻¹), meso-eutrophic (75 µg P · L⁻¹), and eutrophic (100 µg P · L⁻¹), according to Dodds and Smith (2016). Each flume was filled with 6 L modified WC medium (Guillard and Lorenzen 1972; Table S1 in the Supporting Information) diluted in aerated tap water (1:15) with an adjusted P concentration according to the treatment. The average current velocity in the flumes was 14 cm · s⁻¹. Each treatment was replicated twice, resulting in a total of 10 flumes. Nine 4.7 × 4.7 cm unglazed ceramic tiles were placed horizontally in the tile slots on the bottom surface of each flume, arranged in a circle, as substrate for periphyton growth.

Natural periphyton was collected in the river Rhine in Cologne (50°54′25.5″ N, 6°58′41.7″ E) in August 2018 and...
PAR intensity above the flumes, produced by LED lamps, was measured at the water surface. The water in the flumes was replaced every 7 d with fresh medium of the respective P level. Each flume was shifted by one position to the right every 2 d, to exclude position effects and ensure equal average lighting in all flumes. The experiment ended on 3rd September 2018, 3 d after the last medium exchange. Periphyton was scraped off each tile and homogenized in 500 mL tap water, of which 40 mL were transferred into 5 mL extraction solvent (CH2Cl2/MeOH, 2:1 v/v) and stored at −20°C. Fatty acid methyl esters were extracted by b) adding 6 mL isohexane to each sample. The hexane phases were evaporated to dryness under a nitrogen stream and redissolved in 100 µL isohexane.

The fatty acid composition of the samples was subsequently determined via gas chromatography with a 6890N GC System (Agilent Technologies, Waldbronn, Germany) equipped with a DB-225 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) and a flame ionization detector. Instrument settings were as follows: injector and flame ionization detector temperatures were 200°C, the initial oven temperature was 60°C for 1 min, followed by a 120°C: min−1 temperature ramp to 180°C and a 50°C: min−1 ramp to 200°C; after 10.5 min at 200°C, the temperature was increased to 220°C at 120°C: min−1 and kept for 10 min. Helium with a flow rate of 1.5 mL min−1 was used as carrier gas. Fatty acid methyl esters were quantified by referring to the known amounts of internal standards and previously established calibration functions for each fatty acid.

DATA analysis. All data were checked for normal distribution with a Shapiro-Wilk’s test and for homogeneity of variances with a Levene’s test. Regression analyses were performed to test for relationships between P availability and periphyton taxonomic composition, stoichiometry, and fatty acid profile; for each analysis, the regression model that best explained the observed variance (i.e., with the highest R2 value) was selected. Statistical analyses were conducted in SigmaPlot (version 11, Systat). Type I error was set at 0.05 for significance level.

RESULTS

Periphyton initial conditions. Periphyton collected from the river Rhine was mainly composed of diatoms (87% ± 1.5% contribution to total chlorophyll a, mean ± SD), together with a small proportion of chlorophytes (11% ± 1%) and cyanobacteria (2% ± 0.5%; see Fig. S1 in the Supporting Information). No other algal classes were observed. Periphyton average molar C:N:P ratio was 160:13:1 (Table S2 in the Supporting Information).
fatty acid (FA) content was 40.62 ± 4.3 µg · mg C⁻¹ and total polyunsaturated fatty acid (PUFA) content was 9.25 ± 0.7 µg · mg C⁻¹ (Table S2). Eicosapentaenoic acid (EPA) was the most abundant PUFA at 3.75 ± 0.3 µg · mg C⁻¹, followed by linoleic acid (LIN, 1.60 ± 0.1 µg · mg C⁻¹) and α-linolenic acid (ALA, 1.55 ± 0.1 µg · mg C⁻¹; Fig. S2 in the Supporting Information).

**Periphyton taxonomic composition.** One month after inoculation, periphyton taxonomic composition was strongly affected by P availability (Fig. 1). Diatoms were the predominant algal group in the assemblages at 5 and 25 µg P · L⁻¹, on average constituting 86% and 67% of the total, respectively. At 50 µg P · L⁻¹ and above, diatom relative abundance was markedly lower (<13%), except in replicate B of the meso-eutrophic treatment (75 µg P · L⁻¹), where diatoms accounted for 60% of the periphyton community. For this reason, the same replicate is marked in grey in the remaining figures (Figs. 2–5; Fig. S3 in the Supporting Information), although it was not excluded from statistical analyses. Diatom abundance decreased exponentially with increasing P availability (nonlinear regression, two-parameter exponential decay equation, $R^2 = 0.67$, $P = 0.004$, Fig. S3a), whereas chlorophyte abundance peaked at 50 µg P · L⁻¹ (83% on average; Fig. S3b). The proportion of cyanobacteria increased exponentially from <1% at the lowest P availability to an average of 46% at 100 µg P · L⁻¹ (nonlinear regression, one-parameter exponential equation, $R^2 = 0.85$, $P < 0.001$; Fig. S3c).

**Periphyton biomass and C:N:P stoichiometry.** Periphyton biomass expressed as particulate organic carbon (POC) followed a linear relationship with P availability (linear regression, $R^2 = 0.86$, $P < 0.001$; Fig. 2). Periphyton C:P ratio was the lowest at 5 µg P · L⁻¹ with an average value of 277, and it increased logarithmically with P availability, reaching an average value of 794 at 100 µg P · L⁻¹ (nonlinear regression, dynamic fitting, two-parameter logarithmic equation; $R^2 = 0.72$, $P = 0.002$; Fig. 3a). An analogous trend was observed with the N:P ratio (nonlinear regression, dynamic fitting, two-parameter logarithmic equation; $R^2 = 0.76$, $P = 0.001$; Fig. 3b). In contrast, periphyton C:N ratio was ~10 in all treatments, thus, it was not affected by P availability (linear regression; $R^2 = 0.0034$, $P = 0.9$; Fig. 3c).

**Periphyton fatty acid composition.** Periphyton total FA and PUFA content relative to C followed a unimodal relationship with P availability, both peaking at 50 µg P · L⁻¹ (nonlinear regression, dynamic fitting, three-parameter Gaussian equation; FA: $R^2 = 0.74$, $P = 0.008$, Fig. 4a; PUFA: $R^2 = 0.72$, $P = 0.01$, Fig. 4b). SAFA:PUFA ratio was the highest at 5 µg P · L⁻¹ and declined logarithmically with P availability (nonlinear regression, dynamic fitting, two-parameter logarithmic equation; $R^2 = 0.91$, $P < 0.001$; Fig. 4c). The nutritionally important fatty acids EPA, ALA, and LIN also showed a unimodal relationship with P availability, with EPA peaking at 25 µg P · L⁻¹ (nonlinear regression, dynamic fitting, three-parameter Gaussian equation; $R^2 = 0.61$, $P = 0.037$; Fig. 5a) and ALA and LIN at 50 µg P · L⁻¹ (nonlinear regression, dynamic fitting, three-parameter Gaussian equation; ALA: $R^2 = 0.63$, $P = 0.051$, Fig. 5b; LIN: $R^2 = 0.67$, $P = 0.02$, Fig. 5c).

**DISCUSSION**

In contrast to our expectations based on current knowledge, we observed negative relationships between increasing P availability and some essential nutritional quality variables of stream periphyton, at least in the higher ranges of nutrient
concentrations. While the patterns in fatty acid content along the P gradient were unimodal, with decreases in the transition from mesotrophic to oligotrophic treatment (5 µg P·L⁻¹), and followed a logarithmic curve along the P gradient (nonlinear regression, dynamic fitting, two-parameter logarithmic equation; C:P ratio: \( R^2 = 0.72, P = 0.002 \); N:P ratio: \( R^2 = 0.76, P = 0.001 \), while C:N ratio was not affected (linear regression; \( R^2 = 0.0034, P = 0.9 \)). Replicate B of the meso-eutrophic treatment, the only replicate in the higher end of the P gradient with a >50% diatom relative abundance (see Fig. 1), is marked in grey.

Fig. 3. Periphyton molar C:P (a), N:P (b), and C:N (c) ratios against P availability. Periphyton C:P and N:P ratios were the lowest in the oligotrophic treatment (5 µg P·L⁻¹) and followed a logarithmic curve along the P gradient (nonlinear regression, dynamic fitting, two-parameter logarithmic equation; C:P ratio: \( R^2 = 0.72, P = 0.002 \); N:P ratio: \( R^2 = 0.76, P = 0.001 \), while C:N ratio was not affected (linear regression; \( R^2 = 0.0034, P = 0.9 \)). Replicate B of the meso-eutrophic treatment, the only replicate in the higher end of the P gradient with a >50% diatom relative abundance (see Fig. 1), is marked in grey.

Fig. 4. Periphyton content of total fatty acids (FA, a), polyunsaturated fatty acids (PUFA, b), and SAFA:PUFA ratio (c) along the P gradient. Total FA and PUFA content followed a bell-shaped relationship with P availability (nonlinear regression, dynamic fitting, three-parameter Gaussian equation; FA: \( R^2 = 0.72, P = 0.008 \); PUFA: \( R^2 = 0.72, P = 0.01 \), while SAFA:PUFA ratio was the highest in the oligotrophic treatment and declined in a logarithmic fashion (nonlinear regression, dynamic fitting, two-parameter logarithmic equation; \( R^2 = 0.91, P < 0.001 \)). Replicate B of the meso-eutrophic treatment, the only replicate in the higher end of the P gradient with a >50% diatom relative abundance (see Fig. 1), is marked in grey.
eutrophic conditions, periphyton molar C:P and N:P ratios were the lowest in the oligotrophic treatment and increased continuously over the range from oligotrophic to eutrophic conditions. This is the first time that a positive relationship between P availability and periphyton C:P stoichiometry is observed, with periphyton relative P content decreasing rather than increasing under eutrophication. Previous studies have observed the opposite trend (Stelzer and Lamberti 2001, Fanta et al. 2010, Hill et al. 2011). We believe our results were mainly due to the observed taxonomic shift, which did not occur in the mentioned studies. At low P, the periphyton community was mainly composed of diatoms, whose relative abundance significantly declined with increasing P concentration. Chlorophytes were the predominant algal group in the mesotrophic treatment, while cyanobacteria abundance increased exponentially with P availability, as it is often observed in eutrophic systems (Dodds and Smith 2016). Diatoms may have tolerated P deficiency by minimizing biomass production, whereas chlorophytes and cyanobacteria may have built more biomass per unit of assimilated P, resulting in algal blooms under high P availability and relatively high C:P ratios in the equilibrium phase of periphyton succession. This reflects findings by Quigg et al. (2003) and Ho et al. (2003), in which chlorophytes had significantly higher C:P ratios than diatoms when grown under identical conditions. Therefore, eutrophication may result in a nutrient dilution effect under high algal productivity, and the high variance often observed between periphyton C:P stoichiometry and stream nutrient status (Fanta et al. 2010, O’Brien and Wehr 2010, Liess et al. 2012, Hiatt et al. 2019) may be explained by differences in taxonomic composition among sampling sites. Furthermore, diatoms are known to maintain lower N:P ratios than chlorophytes and cyanobacteria (Quigg et al. 2003, Weber and Deutsch 2010, Weber and Deutsch 2012, Hillebrand et al. 2013), which may also explain the positive relationship we observed between periphyton N:P ratio and P availability, even though N availability was kept constant in all treatments.

The fatty acid profile of periphyton was influenced by both taxonomic composition and P availability. Algal total FA and PUFA contents peaked in the mesotrophic treatment, where periphyton was dominated by chlorophytes. Although chlorophytes contain lower amounts of long-chained PUFA compared to diatoms, they are rich in C18 PUFA, particularly ALA and LIN (Torres-Ruiz et al. 2007, Taipale et al. 2013). The total FA and PUFA contents in the chlorophyte-dominated periphyton were higher than in the diatom-dominated periphyton likely as a result of the higher P availability, as P is needed for the synthesis of PUFA-rich galactolipids and phospholipids in algae (Reitan et al. 1994, Guschina and Harwood 2006, Hill et al. 2011). At the higher end of the P gradient, instead, the greater proportion of cyanobacteria resulted in a decrease in periphyton FA and PUFA contents, as cyanobacteria are generally poorer in PUFAs (Lang et al. 2016).
On the other hand, as predicted, periphyton relative EPA content was the highest at relatively low P availability, coinciding with a high diatom proportion in the community, and declined as diatoms were replaced by chlorophytes and cyanobacteria along the P gradient. EPA is one of the most important PUFAs for herbivore nutrition and occurs primarily in diatoms (Torres-Ruiz et al. 2007, Lang et al. 2011, Taipale et al. 2016). However, at the lowest P availability, periphyton EPA content was relatively low despite the high proportion of diatoms, likely as a result of nutrient stress. In this treatment, periphyton SAFA:PUFA ratio was the highest, as under P deficiency, diatoms tend to store carbon as SAFAs and limit the synthesis of PUFAs (Hill et al. 2011).

While we only manipulated P concentrations under constant background conditions, other factors should be taken into account when natural ecosystems are considered. Nutrient availability inevitably interacts with light intensity, which was kept moderate in our flumes but is typically variable in natural environments. High light intensity increases periphyton C:P and C:N ratios, especially at low nutrient concentrations, and dilutes algal PUFA content (Fanta et al. 2010, Hill et al. 2011, Guo et al. 2015). Together, both effects result in a lower nutritional quality of periphyton. Furthermore, while it is not uncommon that diatoms are replaced by chlorophytes and cyanobacteria when nutrient inputs increase, the taxonomic composition of stream periphyton is also influenced by several factors in concomitance with P availability. In particular, high water temperatures are known to favor the growth of chlorophytes and cyanobacteria over diatoms (Tilman et al. 1986, Marcarelli and Wurtsbaugh 2006). As a consequence, such a taxonomic shift from diatoms to chlorophytes and/or cyanobacteria following P enrichment does not occur ubiquitously. Instead, a change in diatom species composition may be observed under eutrophication, particularly, in cold waters (Winter and Duthie 2000, Sonneman et al. 2001, Lavoie et al. 2008, Korhonen et al. 2013).

Our results show that the maximum nutritional quality of stream periphyton, in terms of low C:P ratios and high PUFA content, is achieved at low-to-intermediate P availability. In highly oligotrophic conditions, nutrient stress will prevent the synthesis of PUFAs, as well as limit biomass production and thus food quantity for grazers; at high P availability, PUFA-poor taxa such as cyanobacteria may become dominant, and increased algal productivity will result in a lower nutrient content relative to carbon. Stream eutrophication will thus be detrimental to periphyton nutritional quality by reducing the relative amount of essential nutrients and PUFAs available to herbivores, which will compromise the efficiency of energy transfer to the upper trophic levels. PUFA- and nutrient-poor periphyton will lead to lower growth and reproduction rates of herbivores (Stelzer and Lamberti 2002, Fink and von Elert 2006, Guo et al. 2016a), which will in turn affect the survival and fitness of secondary consumers, such as fish. The fatty acid content of consumers has been widely observed to match the fatty acid composition of their food (Brett et al. 2006, Guo et al. 2016a), and essential PUFAs of phytoplankton and fish in lakes is often negatively correlated with lake trophic status (Ahlgren et al. 1996, Müller-Navarra et al. 2004, Taipale et al. 2016). Furthermore, the nutritional quality of periphyton may indirectly influence the strength of top-down control. Grazer behavior is known to affect periphyton community structure through selective feeding and nutrient regeneration (Mulholland et al. 1991, Rosemond et al. 2000, Beck et al. 2019); fluctuations in nutrient availability are likely to interact with top-down control by affecting grazer behavior, as in, e.g., compensatory feeding (Iannino et al. 2019). Future studies should focus on how long-term bottom-up effects would alter grazer growth and behavior, and how this would in turn influence periphyton biomass and nutritional quality.

Overall, we predict that the best periphyton nutritional quality for grazers is likely to be achieved in oligo- to mesotrophic environments, especially under moderate light intensity. With this study, we demonstrate for the first time that periphyton C:P ratio may increase under P enrichment due to a taxonomic shift toward dominance of chlorophytes and cyanobacteria, which exhibit a higher biomass productivity than diatoms. While nutrient enrichment may improve algal nutritional quality in the short term, our results highlight the importance of taxonomic sorting in a complex community, which can, in the long run, overlay physiological effects of single species.

We would like to thank Patrick Aurich and Suzette Soule for help with the experiment, and Maja Ilic for assistance with pigment analysis and Chemtax. This study was supported by the Deutsche Forschungsgemeinschaft (DFG), grant FI 1548/7-1 to Patrick Fink. Open access funding enabled and organized by Projekt DEAL.

**AUTHOR CONTRIBUTIONS**

A.I. and P.F. designed the experiment; A.I. conducted the experiment; A.I., A.T.L.V., M.W., and P.F. analyzed the data; A.I. wrote the manuscript; A.T.L.V., M.W., and P.F. edited the manuscript.

Ahlgren, G., Sonesten, L., Bober, M. & Gustafsson, L. B. 1996. Fatty acid content of some freshwater fish in lakes of different trophic levels – a bottom-up effect? *Ecol. Freshw. Fish* 5:15–27.

Beck, W. S., Markman, D. W., Olesky, I. A., Lafferty, M. H. & Poff, N. L. 2019. Seasonal shifts in the importance of bottom-up and top-down factors on stream periphyton community structure. *Oikos* 128:680–91.

Brett, M. T., Bunn, S. E., Chandra, S., Galloway, A. W. E., Guo, F., Kainz, M. J., Kankaala, P. et al. 2017. How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshw. Biol.* 62:833–55.
Biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquat. Microb. Ecol. 71:165–78.

Taipale, S. J., Vuorio, K., Strandberg, U., Kahilainen, K. K., Järvinen, M., Hiltunen, M., Peltomaa, E. & Kankaala, P. 2016. Lake eutrophication and brownification downgrade availability and transfer of essential fatty acids for human consumption. Environ. Int. 96:156–66.

Tilman, D., Kiesling, R., Sterner, R., Kilham, S. S. & Johnson, F. A. 1986. Green, bluegreen and diatom algae: taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Arch. Hydrobiol. 106:473–85.

Torres-Ruiz, M., Wehr, J. D. & Perrone, A. A. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. J. N. Am. Benthol. Soc. 26:509–22.

Trench-Fiol, S. & Fink, P. 2020. Metatranscriptomics from a small aquatic system: microeukaryotic community functions through the diurnal cycle. Front. Microbiol. 11:1006.

Whorley, S. B. & Wehr, J. D. 2016. Connecting algal taxonomic information to essential fatty acid content in agricultural streams. Physiologia 55:331–42.

Windsch, H. S. & Fink, P. 2018. The molecular basis of essential fatty acid limitation in Daphnia magna: a transcriptomic approach. Mol. Ecol. 27:871–85.

Winter, J. G. & Duthie, H. C. 2000. Epilithic diatoms as indicators of stream total N and total P concentration. J. N. Am. Benthol. Soc. 19:32–49.

Wurtsbaugh, W. A., Paerl, H. W. & Dodds, W. K. 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. WIREs Water 6:e1373.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web site.

Figure S1. Taxonomic composition of periphyton inoculated into the flumes at the beginning of the experiment expressed as percentage contribution to chlorophyll a. Values are mean ± SD of n = 3 replicates.

Figure S2. Fatty acid composition of periphyton inoculated into the flumes at the beginning of the experiment. Values are mean ± SD of n = 3 replicates.

Figure S3. Relative abundance of diatoms (a), chlorophytes (b), and cyanobacteria (c) along the P gradient expressed as percentage contribution to total chlorophyll a. Diatom abundance declined exponentially with increasing P availability (nonlinear regression, two-parameter exponential decay equation, $R^2 = 0.67$, $P = 0.004$), whereas chlorophyte abundance peaked in the mesotrophic treatment (nonlinear regression, three-parameter Gaussian equation; $R^2 = 0.47$, $P = 0.1$). Cyanobacteria abundance increased exponentially with P availability (nonlinear regression, one-parameter exponential equation, $R^2 = 0.85$, $P < 0.001$). Replicate B of the meso-eutrophic treatment, the only replicate in the higher end of the P gradient with a >50% diatom relative abundance (see Fig. 1), is marked in grey.

Table S1. Chemical composition of the WC growth medium (from Guillard and Lorenzen 1972). All concentrations were subsequently diluted (1:15) for use in the experimental flumes. The dipotassium phosphate concentration was adjusted according to each treatment (see Materials and Methods).

Table S2. Molar C:P, C:N, and N:P ratios, total fatty acid (FA), and polyunsaturated fatty acid (PUFA) content of periphyton inoculated in the flumes at the start of the growing phase. Values are mean ± SD of n = 3 replicates.