Baattrup-Pedersen, A., **Graeber, D.**, Kallestrup, H., Guo, K., Rasmussen, J.J., Larsen, S.E., Riis, T. (2020):
Effects of low flow and co-occurring stressors on structural and functional characteristics of the benthic biofilm in small streams
Sci. Total Environ. **733** , art. 139331

http://dx.doi.org/10.1016/j.scitotenv.2020.139331

Effects of low flow and co-occurring stressors on structural and functional characteristics of the benthic biofilm in small streams

Annette Baattrup-Pedersen[1], Daniel Graeber[2], Helena Kallestrup[1], Kun Guo[3], Jes Jessen Rasmussen[4], Tenna Riis[3]

[1] Department of Bioscience, Aarhus University, Denmark

[2] Dep. Aquatic Ecosystem Analysis, Helmholtz-Centre for Environmental Research – UFZ, Brückstraße 3a, 39114 Magdeburg, Germany

[3] Department of Biology, Aarhus University, Denmark

[4] Norwegian Institute for Water Research, Gaustadalleen 21, 0349 Oslo, Norway

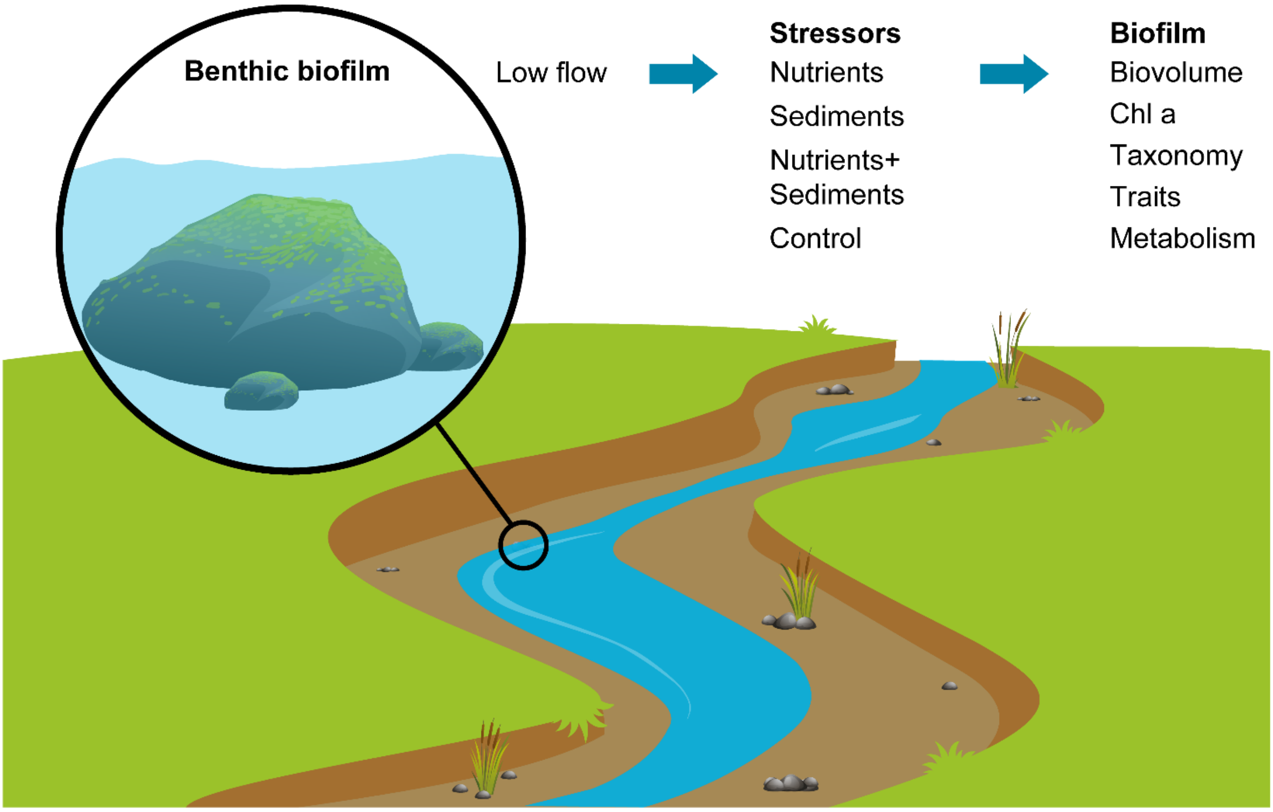
Keywords:

GPP, traits, biomass, nutrients, fine sediments, eutrophication

Highlights

- Small agricultural streams are severely affected by multiple stress
- Stress imposed by nutrients was less pronounced than stress imposed by fine sediments under low flow on the benthic biofilm
- Nutrient enrichment can mitigate effects of sediments on the structural and functional response of the benthic biofilm
- Stream benthic algae community and biofilm metabolism displayed similar resilience to stress imposed by low flow and co-occurring stress from nutrients and sediments on a short and longer time scale

31 Graphical abstract

32 

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Abstract

Low flow and co-occurring stress is a more and more frequent phenomenon these years in small agricultural streams as a consequence of climate change. In the present study we explored short and longer term structural responses of the stream benthic algae community and biofilm metabolism to multiple stress in small streams applying a semi-experimental approach. We hypothesized that i) a reduction in flow in combination with secondary stress (nutrients and sediments) have immediate effects on the benthic algae community in terms of biomass (chlorophyll a, biovolume), taxonomic and trait (lifeform and size distribution) compositions as well as on metabolism (GPP and CR), and ii) that changes in the benthic algae community persist due to altered environmental settings but that functional redundancy among benthic algae species provide a high level of resilience in metabolism (GPP and CR). Overall, we found that stress imposed by nutrients was less pronounced than stress imposed by fine sediments under low flow, and that nutrient enrichment to some extent mitigated effects of fine sediments. Fine sediment deposition mediated a decline in the fraction of erect algae and/or algae with mucilage stalks but this did not happen under co-occurring stress from both sediments and nutrients. Additionally, fine sediment deposition mediated a decline in GPP of the biofilm, but again this did not happen under co-occurring stress from nutrients. We conclude that 1) the benthic algae community and biofilm metabolism displayed similar resilience to stress imposed by low flow and co-occurring stress from nutrients and sediments on a short and longer time scale and 2) as structure–function adaptations may occur at several trophic levels in the biofilm, more research is needed to explore mechanisms underlying mitigating effects of nutrients in response to sediment deposition under low flow.

Introduction

Small streams are highly disturbed ecosystems. Clearance of natural riparian vegetation, high nutrient loads and pollution with other dissolved substances (e.g. contaminants) in combination with alterations in the hydrology and morphology of the streams create a wide range of stressors acting with a high spatial and temporal variability(Izagirre et al., 2009; Stelzer and Lamberti, 2001; Woodward et al., 2012). In small agricultural streams, low flow in combination with nutrient and sediment loads have a strong influence on stream structure and function (e.g., Townsend et al., 2008; Hering et al., 2015) and the importance of sediment loads will likely intensify in the coming years due to more variable flow regimes (e.g., Milly et al., 2005; IPCC, 2014) and more frequent high and low flow periods that will affect sediment dynamics in the streams. In agricultural catchments, fine sediments originates primarily from stream bank and bed erosion (Kronvang et al., 2013; Townsend et al., 2008) that settles down under low flow.

The effects of nutrients on benthic algae have been subject to extensive research, but still the relationship between nutrients and community structure is not well described. Generally, the biomass of the benthic algae community increases with increasing nutrient availability (e.g., Dodds et al., 2002) but community composition also changes due to differences in nutrient uptake rates, utilization and requirements for maximum growth rates (Aristi et al., 2016; Carrick and Lowe, 1988) . Additionally, increased deposition of sediments also mediate changes and species displaying particular traits such as motility can increase in abundance (Neif et al., 2017; Wagenhoff et al., 2013). Nutrients and sediments can interact in complex ways on the benthic algae community (Matthaei et al., 2010; Townsend et al., 2008), depending on the level of these stressors and their interactions we may see different outcomes. For example, one study reports compositional changes in the benthic algae community in response to low flow in combination with fine sediment deposition, whereas no significant changes occurred in combination with nutrient enrichment (Neif et al., 2017), whereas another study observed that nutrients were more important than fine sediments for community composition, even though both parameters influenced the response (Lange et al., 2016) .

In general, primary production (GPP) and community respiration (CR) of the biofilm community can be directly related to land use intensity with increasing GPP and CR with increasing urban and rural land uses (Bernot et al., 2010; Clapcott et al., 2016). It can be difficult to predict how changes in community composition affect GPP and CR, however, as structural patterns and processes are not necessarily linked. For example, the biomass of the benthic algae community is linked to GPP but the variation can be pronounced as structure and processes respond over different time scales (Fellows et al., 2006) . Whilst the biomass and composition of the algae community integrate environmental conditions over weeks, GPP and CR are immediate measures of the performance of the community (Fellows et al., 2006) . Under base flow conditions GPP is mainly controlled by light (Bernot et al., 2010; Young et al., 2008) , whereas under low flow GPP can be increasingly limited by mass transfer reductions (Arnon et al., 2013; Riis et al., 2017) . In addition, sedimentation of suspended fine sediments under low flow can cause an immediate reduction in GPP due to shading, whereas respiration may increase due to increased deposition of organic matter associated with the fine sediment particles (Fellows et al., 2006) . Furthermore, compositional changes in response to environmental stress may not to the same extent be reflected in stream functions due to redundancy and compensatory responses among species (Odum, 1985). For example, nutrient induced shifts in community composition may support alternate communities without affecting the total biomass. Recently it was demonstrated that nutrient enrichment strongly affected algal community composition in a field experiment in a shallow lake with some species reacting positively to increased nutrient availability i.e. *Fragilariaceae* and small diatoms, whereas other taxa reacted negatively (McCormick et al., 2019) . However, these compositional shifts did not lead to changes in the biomass of the community or in primary production (McCormick et al., 2019). Functional redundancy among species may in that way insure against loss of ecosystem functioning following compositional changes providing the system with a high level of resilience (capacity of the ecosystem to regain pre—disturbance characteristics). As a consequence, GPP and CR may be more resilient to environmental stress than community composition.

In the present study, we explored short and longer term structural responses of the stream benthic algae community and biofilm metabolism to multiple stress in small streams applying a semi-experimental approach. We followed the benthic algae community in terms of biomass, community composition and biofilm metabolism i.e. GPP and CR over four weeks in response to a flow reduction in combination with nutrients and siltation with fine sediments. The biofilm in small open-canopy agricultural streams is dominated by photoautotrophs (Tank et al. 2018, Riis et al. 2017) and we therefore expected a close coupling between the structural response of the benthic algae community and the functional response of the biofilm. Overall, we hypothesized that i) a reduction in flow in combination with secondary stress (nutrients and sediments) have immediate effects on the benthic algae community in terms of biomass (chlorophyll a, biovolume), taxonomic and trait (lifeform and size distribution) compositions as well as on metabolism (GPP and CR) and ii) that structural changes in the benthic algae community persist due to altered environmental settings but that functional redundancy among benthic algae species provide a high level of resilience in metabolism (GPP and CR).

Methods

Experimental stream flumes and treatments

We conducted the experiment in twelve outdoor flumes during summer 2014 in Denmark (56°4′N, 9°31′E). The flumes were 12 m long, 60 cm wide and 30 cm deep with various types of inorganic sediments to create natural run-riffle sequences. The stream flumes were fed from 1,000 L plastic feeder tanks with unfiltered water pumped from the nearby Lemming stream. The water output of the stream feeder pump was controlled by a frequency regulator (VLT Aqua Drive FC202 7.5 kW, Danfoss) and individual flow regulators installed at the pipes before the feeder tanks for fine adjustment of the flow. An additional recycling pump was installed at the end of each of the flumes to fine-tune the flow when needed. Please find details of the flumes in (Neif et al., 2017).

Before starting the experiment, 20 stones (tiles, side length c. 9 cm, height c. 2.5 cm, with a roughly textured surface) were introduced into two run habitats along the main flow path in each of the flumes. After two weeks of benthic algae colonization, the normal-flow phase was initiated with nutrient enrichment in six randomly chosen flumes (NP treatment). Nutrients were added with a 12-channel peristaltic pump (BVP-Process with a 12-channel CA pump head, Ismatec, Wertheim, Germany) from a central 600 L tank. We used fertilizer (SweDane NPK 21-3-10 and GrowHow NS 24-6, DLG, Copenhagen, Denmark) to enrich with dissolved inorganic nitrogen and phosphate to obtain a fivefold increase for dissolved inorganic nitrogen (on average 5.5 mg N/L in the nutrient enriched flumes compared to a background concentration of 1.1 mg N/L) and a sevenfold increase for phosphate (on average 0.064 mg P/L compared to 0.009 mg P/L) throughout the experiment.

After almost 4 weeks of normal flow, the low-flow phase was initiated. The flow was reduced to simulate a low flow event that can happen in small lowland streams in Central Europe during summer (Graeber et al., 2015, 2012; Hille et al., 2014) . The discharge was lowered from, on average, 5.2 L/s during the normal-flow phase to 1 L/s during the low-flow phase by switching off the recycling pumps and fine-adjusting the discharge with the frequency regulator of the pump feeding stream water into the system. The nutrient-enrichment treatments were continued in the low-flow phase to keep stable nutrient conditions.

At the start of the low-flow phase, fine sediment was added manually in six randomly chosen flumes, three without nutrient enrichment (FS treatment) and three with nutrient enrichment (NP + FS treatment). The fine sediment was pumped from the source stream into several carboys and manually introduced into the flumes until >90% fine sediment cover was reached. We also observed a continuous increase in the coverage of fine sediments during the low-flow phase in the non-fine sediment treatments as fine sediment transported with stream water settled within the stream flumes due to the low current velocities. However, the fine sediment cover was significantly lower in the non-fine sediment treatments than in the fine-sediment treatments (Neif et al., 2017).

Water temperature was measured every ten minutes in each flume with a temperature logger (HOBO Pendant UA-001, Onset, USA) and was, on average, 12.1°C with a minimum of 4.7°C and a maximum of 18.8°C. Average water temperature did not change significantly during the low-flow phase.

Benthic algae sampling and trait allocation

The density and composition of the benthic algae were analysed in all flumes the last week of the normal-flow phase (week 1) and weekly in the four weeks of low-flow treatment (week 2-5). First low-flow sampling was 1 week after low-flow onset (week 2). We sampled two stones from two run habitats on each of the five sampling occasions. Biofilm was scrubbed carefully from the upper side of two stones using a soft toothbrush and combined into one sample. The exact length and width of the stones were measured. The sample from the stones was divided into two subsamples for analysis of species composition and chlorophyll a, respectively, the latter using ethanol extraction of filter residues (Whatman glass microfiber filters, GF/C, 47 mm, GE Healthcare, Brondby, Denmark) according to Jespersen and Christoffersen, (1987).

The benthic algae slurry (50 mL) was fixed and preserved in a solution of acetic lugol (5%). Counting was conducted in the laboratory in random fields (mean of 150 fields was calculated for each sample), using an inverted microscope according to Utermöhl (1958). Based on this, the algal density was calculated as the number of counted individuals (a unicellular organism, a colony, a filament, or coenobium) divided by the area of the 150 fields. Algae were identified at 400× magnification to the lowest taxonomic level possible. Identification of the species was based on literature (Croasdale and Flint, 1986; Dillard, 1991; G.W. et al., 1982; Gary E., 1990; J. and K., 2005; K., 1982; Krammer and Lange-Bertalot, 1991, 1988, 1986) . Very concentrated samples were diluted to facilitate visualisation and counting of individuals. If samples were diluted, the dilution factor was included in the calculation of algal densities. The benthic algae biovolume was calculated by multiplying the density of each taxon by its respective volume. The cell volume was calculated from geometric models, according to the species-specific shape of the cells (Hillebrand et al., 1999; Sun and Liu, 2003) .

The life forms of the algae were categorised into one of five types based on literature (Guiry and Guiry, 2020; Passy, 2007; Passy and Larson, 2011; Schneck et al., 2011) : (1) adnate or prostrate (Achnanthidium, Cocconeis, Characium, Coleochaete and Chamaesiphon), (2) erect or with mucilage stalks or tubes (Cymbella, Eunotia, Fragilaria, Gomphonema, Ulnaria and Chroococcus), (3) motile (Cymatopleura, Gyrosigma, Navicula, Nitzchia, Pinnularia, Pandorina, Mallomonas, Cryptomonas, Euglena, Phacus and Trachelomonas), (4) metaphyton (Closterium, Cosmarium, Scenedesmus, Dictyosphaerium, Pediastrum and Tetraedron) and (5) filamentous (Planktolyngbya, Pseudoanabaena and Geitlerinema). The algae were categorised into three size classes depending on the length of algae cells: small: <30 µm, medium: 30–90 µm and large: >90 µm (Piggott et al., 2015) . Following Piggott et al. (2015), we considered unicellular organisms, colonies, filaments and coenobium as individuals, and used the average length of the longest axis of the individuals to define the size classes. Length was used instead of biovolume for the size classes to maximise the comparability of the size classes to earlier multiple-stress studies (e.g., Piggott et al., 2012, 2015) .

The biological production of oxygen via gross primary production (GPP), and consumption via community respiration (CR) in the biofilm present on stones were analysed in all flumes during the last week of the normal-flow phase and weekly during the low-flow phase. Change in DO over time in light and dark incubation were regarded as net biofilm production (NPP) and biofilm community respiration (CR), respectively, and gross primary production (GPP) was then calculated as the sum of NPP and CR (Cardinale et al., 2013; Reisinger et al., 2016). At each sampling occasion, we sampled two stones, not previously used for measurements, from two run habitats on each of the five sampling occasions. The stones were transported to the laboratory (<2 minutes away) and incubated in a hemispheric chamber filled with water from the specific flume. The chamber was mounted with an oxygen electrode (ProODO optical dissolved oxygen meter) and placed in a temperature-controlled water bath (15 ^{0}C) at 10 cm depth. Water inside the chamber was stirred using a magnetic stirrer placed underneath the chamber in the water bath. Illumination was provided at 263 µmol m^{-2} s^{-1} (PAR) by a halogen lamp (Philips HPI-T 400W, measured with Li-Cor Li-1400), which was positioned at 41.5 cm above the stones. Dissolved oxygen concentration (DO) and temperature was logged every 10 seconds. After at least 30 minutes of measurement or an increase in the oxygen concentration in the water of at least 0.30 mg L^{-1}, the light was turned off and the hemisphere covered with black plastic to measure respiration. We measured respiration for at least 30 minutes or until a decline in the oxygen concentration in the water was at least 0.30 mg L^{-1}.

Statistics

We only found very sparse occurrence of metaphyton (5 samples out of 60) and filamentous algae (0 samples out of 60) and these types were therefore omitted from data treatment.

Initially, a detrended correspondence analysis (DCA) was performed applying abundance data using R software (R Core Team, 2019) to summarise overall patterns in the algae community under the various treatment conditions over time. DCA 1 and DCA 2 scores were then used to describe community composition in further analysis.

We applied a Before-After-Control-Impact (BACI) design to assess the impact from low flow and co-occurring stress on structural and functional community characteristics. First, assumption for normality and homoscedasticity were checked by inspecting residual plots and data were log-transformed if necessary. The BACI design is preferred over a simple Before-After comparison as a change in the response may occur independently of any impact because of temporal effects. By establishing a control (where presumably no effect of the impact will occur), the temporal change that occurs in the absence of the impact can be measured. We used week 1 as *Before*, and week 2 as *After* to detect short term changes and week 1 as

Before and week 4 as *After* to detect longer term changes in the response of the benthic algae community. The BACI analysis was performed in a mixed model with site as random and treatment and period (Before/After) as fixed effects, i.e. a two-way ANOVA with random sites. The BACI effects were estimated and tested using contrasts in the SAS software, version 9.4 (SAS Institute, 2014) .

To compare structural and functional properties of the benthic algae community at a certain time period we conducted a one-way ANOVA. In case of significance we applied Dunnett's Method for post hoc pairwise comparisons to detect significant differences among treatments and control.

Results

Biomass and trait characteristics of the benthic algae community

The chlorophyll a content of the benthic algae community was not significantly affected by the nutrient treatment under normal flow (Fig. 1A; ANOVA; $F=2.59$; $P>0.05$), but, after one week of low-flow, we observed a significant negative effect of the combined effect of nutrients and sediments under low flow (BACI mixed model; $t=-2.32$; $P<0.05$; Table 1), but not of the single effects of either fine sediments or nutrients. At the end of the experimental period after four weeks of low-flow, however, no significant effects of low flow on the chlorophyll a content was observed in any of the treatments (Fig. 1A; Table 1), and the chlorophyll a content did not vary among treatments after four weeks of low flow either (Fig. 1A; ANOVA $F=1.89$; $P>0.05$).

The biovolume of the benthic algae community was not significantly affected by the nutrient treatment under normal flow (Fig. 1B; ANOVA; $F=0.99$; $P>0.05$). After one week of low-flow, the biovolume was reduced in the sediment treated flumes (BACI mixed model; $t=-2.94$; $P<0.05$; Table 1) and sediment/nutrient treated flumes (BACI mixed model; $t=-2.40$; $P<0.05$; Table 1). At the end of the experimental period, no significant effect of low flow on the biovolume of the community was seen in any of the treatments (Fig. 1B; Table 1). The biovolume did not vary among treatments after four weeks of low flow either (Fig. 1B; ANOVA; $F=1.139$; $P>0.05$).

Community composition was analysed applying a detrended correspondence analysis (DCA). The eigenvalues for the first three DCA ordination axes were 0.54, 0.33 and 0.20, respectively. The benthic algae community did not vary significantly between control and nutrient treated flumes prior to the reduction in flow when applying DCA1 and 2 scores as community descriptors (Fig. 2AB; ANOVA; $F=0.990$ and 1.492, respectively; $P>0.05$). Under normal flow, the community was primarily dominated by small algae species with adnate/prostrate lifeforms accounting for 73%-96% of the total biovolume in the control flumes (control and sediment) and 55%- 80% in the nutrient enriched flumes. Significant changes occurred in community composition, lifeform, and to some extent in the size distribution of the algae in the community under to low flow (Fig. 2, 3 and 4; Table 1). After one week of low flow we observed a significant change in the composition of the algae community in the sediment treated flumes (expressed as DCA1; Fig. 2A; BACI mixed model $t=2.30$; $P<0.05$; Table 1). At the same time we observed a decline in the biovolume of adnate/prostrate algae species in the sediment treated flumes (Fig. 3A; BACI mixed model $t=-3.49$; $P<0.05$; Table 1) and sediment/nutrient treated flumes (BACI mixed model $t=-2.10$; $P<0.05$; Table 1), and in the biovolume of erect/stalked algae species in the sediment treated flumes (Fig. 3B; BACI mixed model $t=-2.43$; $P<0.05$; Table 1). The biovolume of small algae also declined in the sediment treated flumes (Fig. 4; BACI mixed model $t=-3.49$; $P<0.05$; Table 1) and nutrient/sediment treated flumes (BACI mixed model $t=-3.43$; $P<0.05$; Table 1). Some of these changes in lifeform composition and size distribution were transient, however, community composition (expressed as DCA1 and DCA2) became similar to those found in control flumes under normal flow conditions after four weeks of low flow (Table 1). Similarly, the biovolume of adnate/prostrate species in the sediment and sediment/nutrient treated flumes was similar to those found under in control flumes under normal flow conditions after four weeks of low flow (Table 1). Additionally, the biovolume of small algae species also reached levels similar to those found in control flumes under normal flow conditions after four weeks of low-flow treatments (Fig. 4; Table 1). In contrast, the biovolume of erect/stalked algae species continued to be lower in the sediment treated flumes after four weeks of low-flow (Fig. 3B; BACI mixed model t=-2.83; P<0.05; Table 1), and the biovolume of medium sized algae species was also lower after four weeks of treatment in the sediment treated flumes compared to control flumes under normal flow conditions (Fig. 4B; BACI mixed model t=-2.56; P<0.05; Table 1). The lifeform and size distribution of the benthic algae community did not vary among treatments after four weeks of low flow (Fig. 3 and 4; ANOVA; P<0.05).

GPP and CR

GPP, expressed as the primary production per unit area, varied among treatments under normal flow (Fig. 5A; ANOVA F=0.03; P<0.05) with significantly higher GPP in nutrient treated flumes compared to control flumes (144.7 mg DO m^{-2} h^{-1} compared to 103.5 mg DO m^{-2} h^{-1} in control flumes; Dunnett's Method LSD=3.676; P<0.05). After one week of low-flow GPP was reduced in the sediment treated flumes (Fig. 5A; BACI mixed model t=-2.09; P<0.05; Table 1), but not in nutrient and nutrient/sediment treated flumes (Table 1). Following the flow reduction, GPP increased over time in the sediment treated flumes (Fig. 5A) and, after four weeks of low-flow, GPP was at levels found in control flumes under normal flow conditions (Table 1). Still, however, GPP was higher in sediment/nutrient treated flumes than in control flumes after four weeks of lowflow (162.2 mg DO m^{-2} h^{-1} compared to 96.5 mg DO m^{-2} h^{-1}respectively; ANOVA F=9.04; P<0.05; Dunnett's Method LSD=14.72; P<0.05).

The CR also varied among treatments under normal flow (Fig 5B; ANOVA; F=10.9 P<0.05) with higher CR in nutrient and sediment/nutrient treated flumes compared to control flumes (Dunnett's Method LSD=9.55 and 13.36, respectively; P<0.05). CR responded strongly to low flow conditions (Fig 5B; Table 1). CR was reduced in both the sediment treated flumes, the sediment/nutrient treated flumes and in the nutrient treated flumes after one week of low flow (Table 1; t=-5.17, t=-2.56 and t=-4.48, respectively; P<0.05). After five weeks of low flow, CR was still lower in the sediment and nutrient treated flumes in comparison to control flumes under normal flow conditions (BACI mixed model t=-3.23, P<0.05 and t=-2.46, P<0.05, respectively; Table 1). Furthermore, after four weeks of low-flow, CR varied among treatments (Fig. 5B; ANOVA; F=11.13; P<0.05). CR was higher in the sediment/nutrient treated flumes (50.8 mg DO m^{-2} h^{-1}) compared to control flumes (29.4 mg DO m^{-2} h^{-1}; Dunnetts Method LSD=5.82; P<0.05).

Discussion

Short term effects of low flow and co-occurring stressors

We confirmed our first hypothesis that a reduction in flow had immediate effects on structural characteristics of the benthic algae community in terms of biomass (chlorophyll a, biovolume), taxonomic and trait (lifeform and size distribution) compositions as well as on biofilm metabolism (NPP and CR) and that the responses depended on co-occurring stressors. This is in accordance with previous findings reporting that increased loads of nutrients and fine sediments change the habitat template, leading to altered structural and functional characteristics of the biofilm (Atkinson et al., 2008; Clapcott et al., 2016). The nutrient enrichment scenario that we applied (on average 5.5 mg N L^{-1} in the nutrient enriched flumes compared to background concentration of 1.1 mg N L^{-1} and 0.064 mg P L^{-1} compared to 0.009 mg P L^{-1}) was in the range found in Danish agricultural streams (Wiberg-Larsen et al., 2012) whereas stress imposed from fine sediment deposition (>90% coverage) was in the high end compared to sediment characteristics in agricultural streams in Denmark (Baattrup-Pedersen et al., 2018) . Overall, we found that stress imposed by nutrient loads was less pronounced than stress imposed by fine sediments under low flow, and that nutrient enrichment to some extent mitigated effects of fine sediments. Thus, fine sediment deposition mediated a decline in the fraction of erect algae and/or algae with mucilage stalks but this did not happen under co-occurring stress from both sediments and nutrients. Additionally, fine sediment deposition mediated a decline in GPP, but again this did not happen under co-occurring stress from nutrients.

We did not obtain similar results using chlorophyll a and biovolume as measures of biomass. The chlorophyll a content was only reduced in the sediment/nutrient treated flumes while the biovolume of the community was reduced in both the sediment and the sediment/nutrient treated flumes under low flow. The biovolume is often viewed as the most reliable measure to estimate algal biomass (Hillebrand et al., 1999) reflecting that chlorohyll a contents may change in response to a number of factors including both community composition (A., 1979; Baulch et al., 2009; Felip, 2000; Healey and Hendzel, 1975) , algal cell size (Felip, 2000) as well as environmental variability e.g. nutrients (Baulch et al., 2009; Healey and Hendzel, 1975) , temperature and light availability (Healey and Hendzel, 1975; LaBaugh, 1995; Thompson et al., 1999) . For example, temperature and light variability can be associated with a greater than threefold variability in cellular chlorophyll a content (Healey and Hendzel, 1975) . In our study, intensified light limitation in the sediment treated flumes may have mediated an increase in the cellular chlorophyll a content of the benthic algae as this can be a mean to adapt to lower light availability having consequences for the biovolume of the community.

Concomitant with changes in biomass in the sediment-treated flume under low flow, we observed a change in community composition (sediment treated flumes), a decline in the fraction of adnate/prostrate algae species (both sediment and sediment/nutrient treated flumes) and in the fraction of algae with erect and/or mucilage stalks (sediment treated flumes). A decline in the fraction of adnate/prostrate algae species in response to sediment load has also been observed previously (Piggott et al., 2015) and can be linked to the low-profile of the algae rendering them susceptible to partly or fully burial by siltation. The finding that the fraction of algae with erect and/or mucilage stalks also declined in response to fine sediment load contrasts previous findings(Piggott et al., 2015), however, a discrepancy that can link to differences in flow velocities in the two studies. Erect/stalked algae may therefore extend through the sediment layer thereby getting access to light which can compensate for reduced light availability, but at the same time, erect/stalked algae species can be more affected by low flow compared to adnate/prostrate algae species due to their larger area:volume ratio . Thus a large surface area:volume ratio can render these species more susceptible to suffer from diffusive limitation of substrates (Hein et al., 1995) than adnate/prostrate algae and more so under low flow conditions (Borchardt , 1996) and this may explain the lower biovolume of this lifeform. In the sediment/nutrient treated flumes, on the other hand, we saw no effect of low flow on the biovolume of erect/stalked species, but this finding may link to subsidiary effects of improved nutrient availability that may have compensated for a lower transfer of nutrients to the cells under low flow.

Finally, and in agreement with our first hypothesis we also observed that the functional properties (GPP, CR) of the biofilm responded to low flow and that the response depended on co-occurring environmental stressors. Specifically, we observed that GPP was negatively affected by sediment deposition after one week of low flow and that CR was negatively affected by sediment deposition and nutrient enrichment both alone and in combination. The finding that GPP was negatively affected by sediment under low flow may link to the observed reduction in the biovolume of the benthic algae community with fewer both adnate/prostrate and erect/stalked algae species. Interestingly, we observed that GPP was unaffected by sediment under elevated nutrient availability even though the chlorophyll a content and total biovolume was lower. This finding can link to an increase in biomass-specific production rates that have mediated an improved algal photosynthetic performance under nutrient enriched conditions due to higher cell enzymatic activity (Raven et al., 2014). The immediate decrease in CR in response to nutrient and sediment deposition, on the other hand, was unexpected. This finding likely reflect that the observed increase in CR in control flumes was more pronounced than the increase in the nutrient and sediment treated flumes and, consequently, the overall response turned out to be negative. Under normal flow, CR is likely to be mostly autotrophic (Riis et al., 2017), but increased shading of the biofilm due to enhanced sediment deposition under low flow may have caused a pronounced increase in CR in control flumes.

Longer term effects of low flow and co-occurring stressors

We could only partly confirm our second hypothesis that structural characteristics within the benthic algae community persist over time as opposed to metabolism in response to low flow and co-occurring stress. Thus, even though the benthic algae biomass was initially reduced in the sediment-treated flumes (sediment and sediment/nutrient) under low flow, no significant differences were observed in the biomass after four weeks of treatment. Community and trait characteristics of the community also approached pre-treatment conditions with an increase in the fraction of adnate/prostrate algae species, indicating that species with this lifeform re-established even though habitat templates were different. Consequently, the structural characteristics of the benthic algae community and biofilm metabolism both displayed a resilient response i.e. resembled pre-low flow conditions after four weeks of low flow. Only the biovolume of medium-sized species and erect/stalked species in the sediment treated flumes continued to be lower.

The observed response contrast findings achieved under non-flow conditions reporting that GPP of the biofilm community was more resilient to non-flow conditions in both permanent and temporary streams compared to structural features of the communities (biomass and community composition; Colls et al., (2019)). We maintained flow in our experiment although the flow velocity was very low and this may together with community characteristics explain the higher degree of resilience in community structure that we find in our study. The community was initially dominated by many small, adnate/prostrate algae species and even though changes occurred in response to low flow in species composition and the relative contribution of different lifeforms in the benthic algae community, the community remained dominated by adnate/prostrate algae species throughout the experimental period independent of treatment conditions. This lifeform is generally considered resilient towards disturbance (Schneck and Melo, 2012; Stevenson, 1996; Wu et al., 2019) and more so than other life forms e.g. motile and metaphytic life forms. Furthermore, species with this lifeform recolonize fast following a disturbance and may settle on the deposited sediments under low flow. This lifeform may also thrive when access to nutrients is limited which was the case in the sediment treated flumes (low in inorganic P and inorganic N) reflecting that prostate/adnate algae generally compete successfully in low resource-environments (Passy, 2007) .

GPP reached pre-treatment levels after four weeks of low flow in sediment treated flumes whereas GPP was higher in nutrient and sediment/nutrient treated flumes than in sediment treated flumes after four weeks of low flow at the end of the experiment. This finding support the conjecture of improved algal photosynthetic performance under nutrient enriched conditions in nutrient and sediment/nutrient treated flumes. On the contrary, we continued to see negative effects of single-stressors i.e. sediments and nutrients on CR after four weeks of low flow even though effects were less negative than after one week of low flow. We have no simple explanation to this finding, however, it shows that nutrient enrichment can mitigate effects of fine sediments on the functional response of biofilm on a longer term and this may link to a higher cell enzymatic activity that will enhance not only GPP as described above but also CR (Raven et al., 2014) . In addition, giving the multi-species nature of biofilm, further study regarding stressor-biofilm - function relationships need to include other organismal groups like bacteria and fungi to understand the underlying mechanisms.

Conclusions

Based on our findings, we conclude that 1) the benthic algae community (biomass, species composition, lifeform and size) and biofilm metabolism (GPP and CR) displayed similar resilience to stress imposed by low flow in combination with co-occurring stress from nutrients and sediments on a short and longer time scale and 2) as structure–function adaptations may occur at several trophic levels in the biofilm, more research is needed to explore mechanisms underlying mitigating effects of nutrients on sediment deposition under low flow. For example, if the mechanism behind limited effects of the combined effect of fine sediment deposition and nutrient enrichment under low flow on primary production reflect a higher nutrient status of the benthic algae community then this may have cascading effects on the energy flow in the stream ecosystem.

Acknowledgments

This study was supported by the MARS project (Managing Aquatic ecosystems under water Resources under multiple Stress) funded under the 7th EU Framework Programme, contract no.6033378 and the Centre for Water Technology (watec.au.dk), Aarhus. Kun Guo was supported by China Scholarship Council (CSC no. 201708420157).

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Figure legends

Figure 1

Chlorophyll a content (a; mg m^{-2}) and ln transformed biovolume (b; mm^3 cells cm^{-2} times 1000) of the benthic algae community in experimental stream flumes (6 with background nutrient conditions and 6 with nutrient enriched conditions) one week before and weekly four weeks after a reduction in flow in the flumes. Low-flow onset marked by dotted line. Three different stressor treatments were applied under low flow: Nutrient enrichment (NP), Fine sediment deposition (FS) and a combination of fine sediment deposition and nutrient enrichment (FS+NP). The maximum, minimum and median of three replicate flumes are given.

Figure 2

DCA axes 1 and 2 values as descriptors for benthic algae community composition in experimental stream flumes (6 with background nutrient conditions and 6 with nutrient enriched conditions) one week before (W1) and weekly four weeks after a reduction in flow (W2-W5) with co-occurring stress. Three different stressor treatments were applied under low flow: Nutrient enrichment (NP), Fine sediment deposition (FS) and a combination of fine sediment deposition and nutrient enrichment (FS+NP). The maximum, minimum and median of three replicate flumes are given.

Figure 3

The biovolume (ln transformed; mm^3 cells cm^{-2} times 1000) of different lifeforms (adnate/prostrate; erect/stalked; motile) present in the benthic algae community in experimental stream flumes (6 with background nutrient conditions and 6 with nutrient enriched conditions) one week before (W1) and weekly four weeks after a reduction in flow (W2-W5) with co-occurring stress. Low-flow onset marked by dotted line. Three different stressor treatments were applied under low flow: Nutrient enrichment (NP), Fine sediment deposition (FS) and a combination of fine sediment deposition and nutrient enrichment (FS+NP). The maximum, minimum and median of three replicate flumes are given.

Figure 4

The biovolume (ln transformed; mm^3 cells cm^{-2} times 1000) of different algae sizes (small, medium, large) present in the benthic algae community in experimental stream flumes (6 with background nutrient conditions and 6 with nutrient enriched conditions) one week before (W1) and weekly four weeks after a reduction in flow (W2-W5) with co-occurring stress. Low-flow onset marked by dotted line. Three different stressor treatments were applied under low flow: Nutrient enrichment (NP), Fine sediment deposition (FS) and a combination of fine sediment deposition and nutrient enrichment (FS+NP). The maximum, minimum and median of three replicate flumes are given.

Figure 5

Gross primary production (a; GPP) and community respiration (b; CR) of the benthic algae community in experimental stream flumes (6 with background nutrient conditions and 6 with nutrient enriched conditions) one week before (W1) and weekly four weeks after a reduction in flow (W2-W5) with co-occurring stress. Low-flow onset marked by dotted line. Three different stressor treatments were applied under low flow: Nutrient enrichment (NP), Fine sediment deposition (FS) and a combination of fine sediment deposition and nutrient enrichment (FS+NP). The maximum, minimum and median of three replicate flumes are given.

Fig. 1

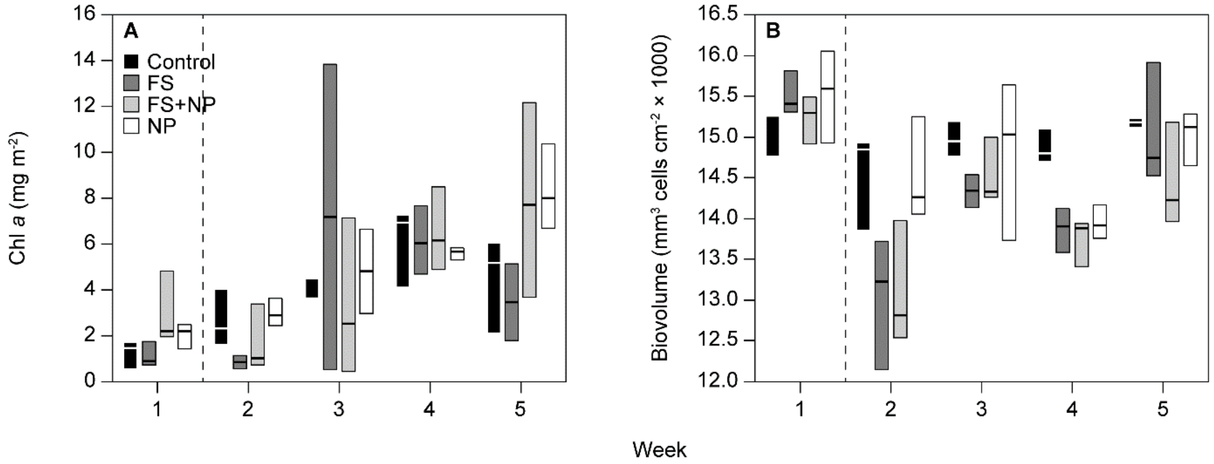

A
Control
FS
FS+NP
NP
Chl a (mg m-2)
B
Biovolume (mm3 cells cm-2 × 1000)
Week

Fig 2

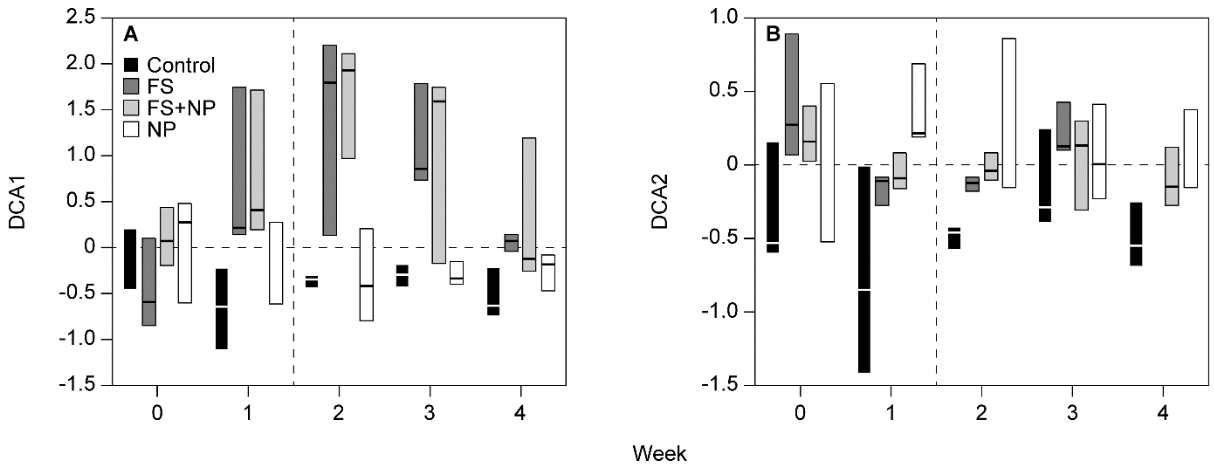
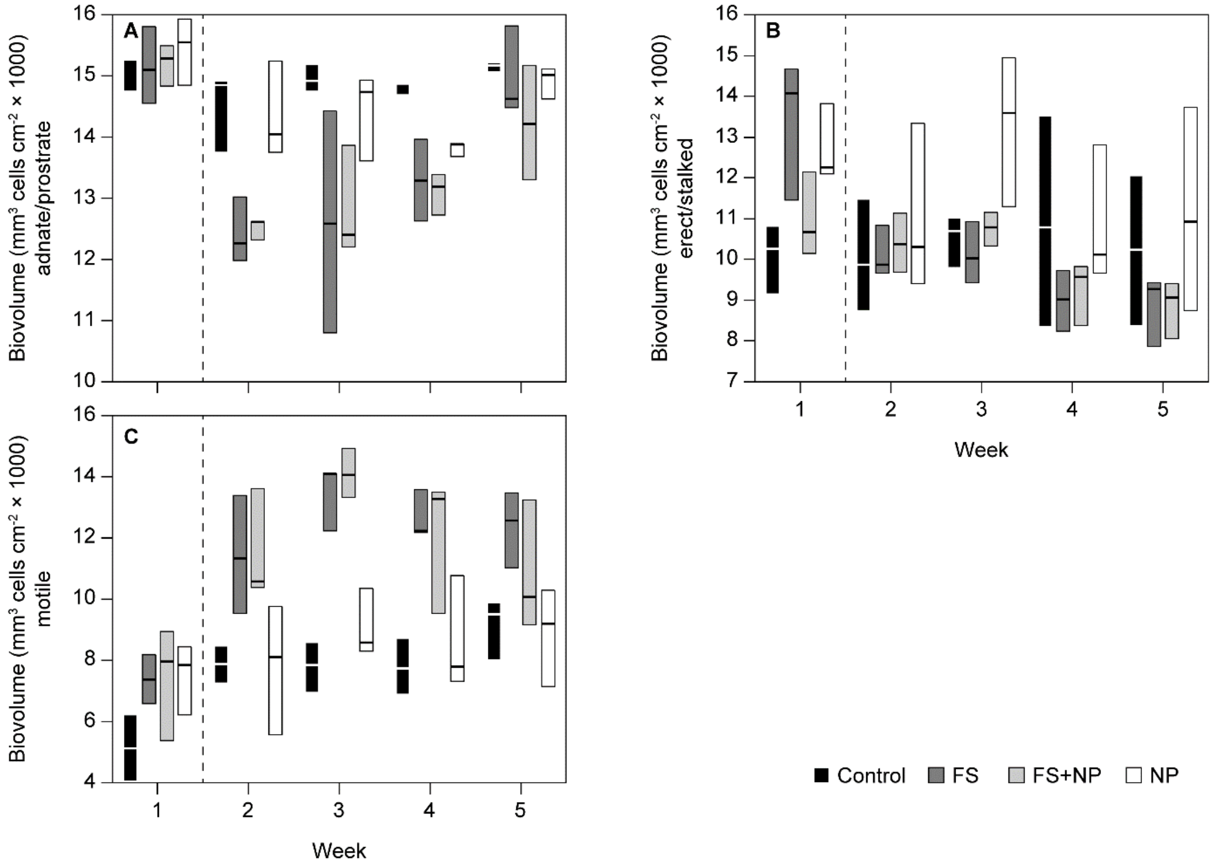
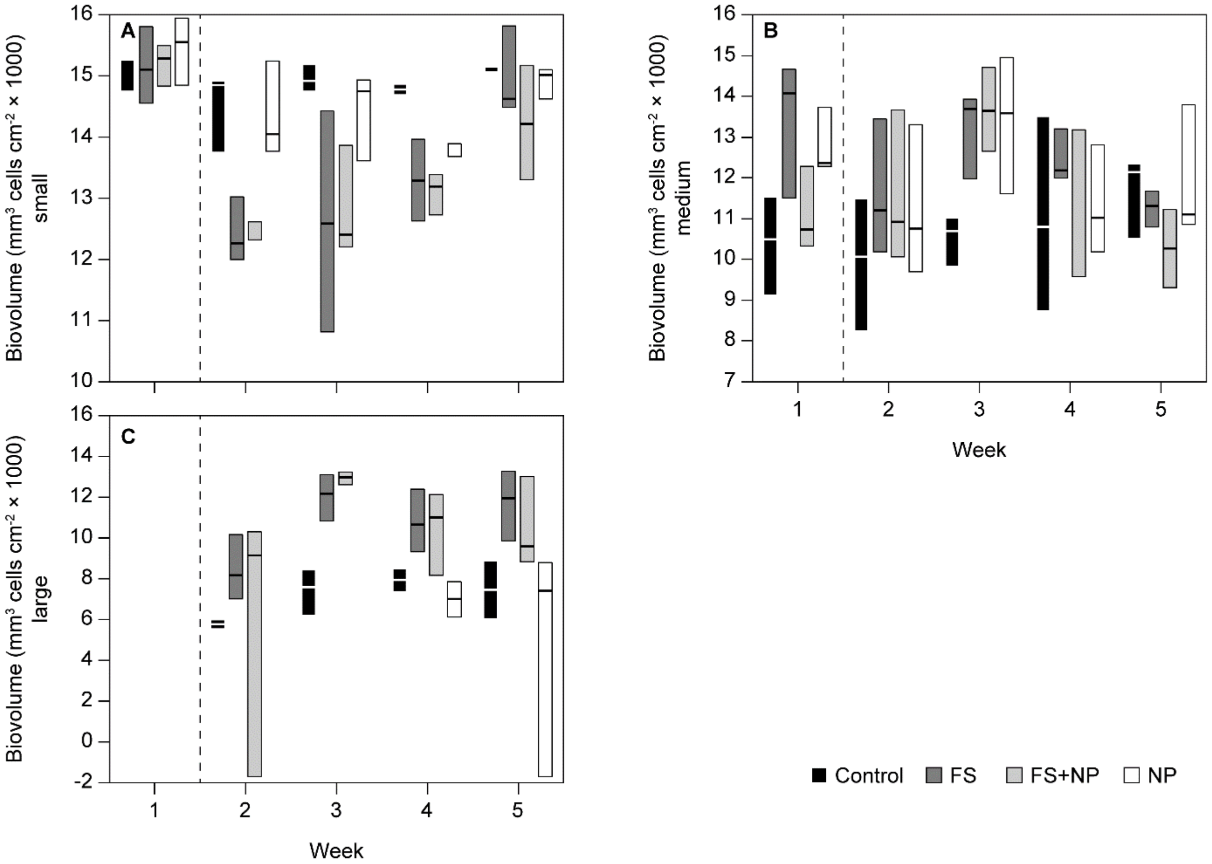

A
Control
FS
FS+NP
NP
DCA1
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
B
DCA2
1.0
0.5
0
-0.5
-1.0
-1.5
0
1
2
3
4
Week

Fig. 3


A
B
C
Biovolume (mm³ cells cm⁻² × 1000) adnate/prostrate
Biovolume (mm³ cells cm⁻² × 1000) erect/stalked
Biovolume (mm³ cells cm⁻² × 1000) motile
Week
Week
1 2 3 4 5
Control
FS
FS+NP
NP

657 Fig. 4



658

659

660

Fig. 5

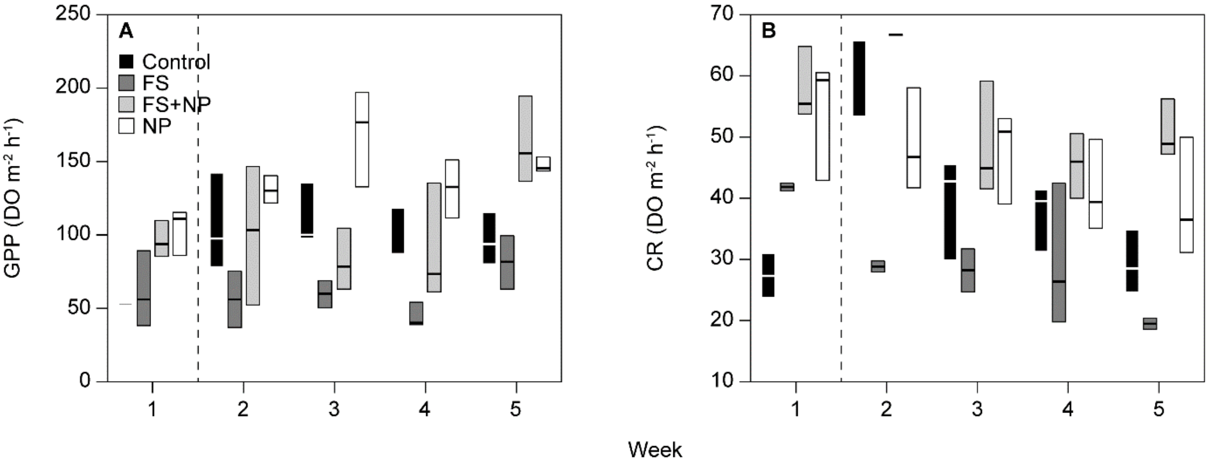

A
Control
FS
FS+NP
NP
GPP (DO m-2 h-1)
250
200
150
100
50
0
1
2
3
4
5
B
CR (DO m-2 h-1)
70
60
50
40
30
20
10
1
2
3
4
5
Week

Table 1. Results of BACI analyses applying the mixed procedure for the effects of low flow in combination with fine sediment addition and nutrient enrichment on structural (Chl a, algae biovolume, algae lifeform, algae size) and functional (GPP, CR) characteristics of the benthic biofilm. The table summarised statistics one week after (Week 1-2) and four weeks after (Week 1-5) low flow was introduced. Model estimates are given, together with t- and P-values. The biovolumes (mm³ cells / cm² / 1000), total and for the lifeform 'Motile' and 'Erect or with mucilage stalks', was ln transformed to reach residual normal distribution and homoscedacity prior to analyses. Significant models are in bold. BACI analyses could not be performed for the lifeform motile and the size class large due to data convergence problems. Significant results are highlighted in bold ($P<0.05$).

Parameter	Response variable	Secondary stressor	Week 1-2	t-value	P-value	Week 1-5	t-value	P-value
Biomass	Chl a	Sediments	-1.68	-1.37	0.1916	-0.88	-0.35	0.7380
		Nutrients	-0.45	-0.39	0.7017	3.11	1.32	0.2245
		Sediments/nutrients	**-2.69**	**-2.32**	**0.0346**	-1.64	0.69	0.5075
	Biovolume	Sediments	**-1.91**	**-2.94**	**0.0096**	-0.51	-1.03	0.3320
		Nutrients	-0.44	0.67	0.5119	-0.57	-1.15	0.2834
		Sediments/nutrients	**-1.56**	**2.40**	**0.0288**	-0.84	-1.70	0.1283
Composition	DCA1 (abund)	Sediments	**1.57**	**2.30**	**0.0350**	0.80	1.54	0.1426
		Nutrients	0.35	0.51	0.6163	0.00	-0.02	0.9668
		Sediments/nutrients	1.08	1.59	0.1312	0.46	0.89	0.3881
	DCA2 (abund)	Sediments	-0.13	-0.27	0.7874	-0.39	-1.02	0.3388
		Nutrients	0.60	1.26	0.2269	0.00	0.01	0.9920
		Sediments/nutrients	0.19	0.39	0.7074	-0.12	-0.31	0.7609
Lifeform	Adnate/prostate	Sediments	**-2.14**	**-3.49**	**0.0030**	-0.23	-0.36	0.7236
		Nutrients	-0.51	0.84	0.4137	-0.59	-0.93	0.3665
		Sediments/nutrients	**-2.10**	**-3.43**	**0.0034**	-1.03	-1.64	0.1207
	Erect or with mucilage	Sediments	**-3.23**	**-2.43**	**0.0411**	**-4.68**	**-2.83**	**0.0120**
		Nutrients	-1.66	-1.24	0.2485	-1.73	-1.05	0.3111
		Sediments/nutrients	-0.54	-0.41	0.6949	-2.28	-1.38	0.1856
Size classes	Small	Sediments	**-2.13**	**-3.49**	**0.0030**	-0.21	-0.33	0.7446
		Nutrients	-0.51	-0.84	0.4139	-0.57	0.91	0.3748

		Sediments/nutrients	**-2.10**	**-3.43**	**0.0034**	-1.02	-1.61	0.1265
	Medium	Sediments	-1.36	-0.82	0.4377	**-3.45**	**-2.56**	**0.0208**
		Nutrients	-1.09	0.66	0.5294	-2.15	-1.61	0.1276
		Sediments/nutrients	0.87	0.52	0.6170	-2.13	-1.59	0.1322
Metabolism	Gross primary productivity (GPP)	Sediments	**-62.22**	**-2.09**	**0.0410**	-26.34	-1.10	0.3060
		Nutrients	-26.98	-0.98	0.3580	-1.31	-0.06	0.9543
		Sediments/nutrients	-49.25	-1.79	0.1150	22.42	0.97	0.3665
	Community respiration (CR)	Sediments	**-42.99**	**-5.17**	**0.0002**	**-24.87**	**-3.23**	**0.0116**
		Nutrients	**-36.36**	**-4.48**	**0.0006**	**-17.56**	**-2.46**	**0.0408**
		Sediments/nutrients	**-21.30**	**-2.56**	**0.0236**	-9.77	-1.37	0.2104

Supplementary material

The stream flumes used for the experiment studying effects of low flow and co-occurring stressors on structural and functional characteristics of the benthic biofilm in small streams.