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Experimental riparian forest gaps and increased sediment loads modify stream metabolic patterns and biofilm composition

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METHODS FOR OXYGEN DATA

Study site

We used 12 artificial channels at the Svartberget experimental facility (Laudon et al., 2021) in northern Sweden, 60km west of Umeå. We asked how sandy substrates, decreased riparian shading and nutrient additions affect biofilms (biomass and metabolic rates) and whole ecosystem metabolic rates (Figure 1). We chose to manipulate these three parameters as they represent typical physicochemical changes that follow final felling and site preparation in Sweden and other countries (Kreutzweiser & Capell, 2001; Kuglerová et al., 2021; Marttila et al., 2020). The artificial channels we used are 15m long and 20 cm wide, with flow through water from an adjacent, forest stream. Water depth varied from 3 to 11cm (top to bottom), with a slope of ~0.5cm per meter. Water discharge was constant at 1-2 L s^{-1} and water velocity was 0.1 m s^{-1}. Water to the artificial stream channels is continuously pumped from the forest stream using a bilge pump (Flygt KS 2610) to a 3000 L water collection tank, from which the water is then led to four 1000 L boxes, each feeding three of the channels (Figure 1). The residence time in the boxes is less than 30min. This setup ensures that water is well mixed in the tank and boxes so that all channels have similar water flow (discharge) and inlet chemistry (Appendix S1: Table S1). During the experiment, the channel water had high

dissolved organic carbon concentrations, (DOC; 20-30mg L^{-1}), low inorganic nutrient concentrations (average dissolved inorganic nitrogen; DIN=35µg L^{-1} and PO_4^{3-}=4µg L^{-1}) and was cold (average 11ºC), conditions typical for forest streams in the region (Laudon et al., 2021). We ran the experiment for 38 days from 1 August to 7 September 2022, with the tarp used to reduce light installed on August 3rd.

We coupled our chl *α* results from the artificial channel experiment with a snapshot field survey of chl *α* in 9 forest streams, upstream, within and downstream of recent clearcuts, in the county of Västerbotten within 1 h driving distance from the city of Umeå, northern Sweden (Table 1). The site conditions around the streams ranged from young (30-40 years) to mature (60+ years) forests in the upstream and downstream sites to clearcuts. The clearcuts range in buffer width from essentially no buffers (a few individual trees or high stumps) up to 15m wide buffers. Substrate conditions in the surveyed streams varied from no sand present to stream bottoms dominated by fine-grained particles. The sites were clearcut between 2014 and 2020 (Table 1).

Channel experiment set up

We used a 2 x 2 factorial design, with three replicates of each treatment. The two factors were substrate (sand vs stones as bottom substrate) and light (70 % shading tarp or no tarp, Figure 1). The sand (median diameter 0.2mm) and stones (median diameter 8cm) came from a nearby quarry, representing local, natural material, dominated by gneiss and granite. The substrate treatment tests for effects on biofilm growth and metabolism, but it does not test for disturbance effects such as burrowing or scouring because the bottoms were stable throughout the experiment. The sand sediment had no effect on nutrient concentration of the water in the channels (Appendix S1: Table S2) unlike in e.g. Pérez-Calpe et al. (2021). The tarp mimics stream light conditions in a mature forest stand or a wide buffer (>15 m, Chellaiah &

Kuglerová, 2021, Jyväsjärvi et al., 2022). There was no effect of the tarp on water temperature, and the average temperature was 11.0 (±0.06) in the shaded channels and 11.1 (±0.04) in the open channels. This enabled us to test for light effects without any confounding temperature effects, which is hard to achieve in natural streams as the two are closely coupled. For ease of installment and access to the channels, we fixed the tarp over three channels side-by-side instead of fixing it randomly, on individual channels. Nevertheless, each channel is treated as an independent replicate because the set up delivers well mixed water from the same source to each channel separately and the starting conditions for all channels did not differ (see Appendix S1: Table S1 and further below).

Incubation of NDS surfaces for community respiration

All NDS surfaces were incubated in the lab for estimates of community respiration (CR) and after that analyzed spectrophotometrically for chl α. Incubations were conducted 24h after collection using the modified dark bottle method (Johnson et al., 2009), where tubes are filled with oxygen saturated stream water, and dissolved oxygen (DO) saturation (YSI Pro ODO, Yellow Springs, USA) is measured pre and post incubation. NDS surfaces were incubated for 3h in dark at 12 °C, with an additional three blanks, treated the same way as the NDS samples, which were used to correct for any water column changes in DO. CR (μg O_2 cm^{-2} h^{-1}) was calculated as the background corrected DO consumption during dark incubations:

$$CR=(\Delta DO\times V)/(t\times A)$$

Where ΔDO is the difference in DO concentration before and after incubation, V the volume of the Falcon tubes, t the exact incubation time and A the area of the NDS surface. After incubations, all NDS surfaces were frozen. Chl α was later estimated following Steinman et al. (2007), including correction for pheophytins, using a JENWAY 6405 UV/Vis

spectrophotometer (Sheung Wan, Hong Kong). NDS surfaces were thawed and put in centrifuge tubes with 90 % acetone for 24 h prior to analysis of the extract.

Whole-channel metabolic estimates of GPP and ER

Each channel was equipped with a miniDOT (Precision Measurement Engineering Inc., USA) to record dissolved oxygen at 10-minute intervals. Metabolism was estimated using the single-station diel oxygen method approach where gross primary production (GPP) and Ecosystem Respiration (ER) was estimated using Bayesian inverse modelling (Hall & Hotchkiss, 2017). We used time series of dissolved oxygen (DO), water temperature, light (from lux loggers), as well as a prior for gas exchange rate coefficient (K) and channel depth (z). The main equation for GPP and ER was:

$$dDO/dt=(GPP+ER)/z+K(DOsat-DO)$$

The change in oxygen over time (O_2 m^{-3}) equals all oxygen produced by photosynthesis (GPP, g O_2 m^{-2} d^{-1}) minus all oxygen consumed by respiration of both autotrophs and heterotrophs (ER, g O_2 m^{-2} d^{-1}) and the rate of gas exchange between the water and air (K, d^{-1}). We modelled two parameters (GPP and ER) and used prior K based on nighttime regression analysis (following Rocher-Ros et al. 2020). We used an average K from nighttime regressions instead of daily values because these channels have stable flow and depths. Finally, we filtered data for erroneous model days by using the mean average error between the observed and the modelled DO concentrations. All days with a mean average error larger than 0.2 were removed (Lupon et al., 2019). When GPP is very low, a poor model fit can still produce a small error, thus all remaining days were also visually inspected to further exclude erroneous estimates. Following these guidelines, we removed 40 % of analyzed days across

all channels. We are aware that the footprint of the oxygen loggers might incorporate signals from the water tanks and/or the stream that feeds the channels, but the water is well mixed after being pumped through two different water tanks. If there still was a signal from the tanks, it would be the same for all channels and therefore it does not affect the treatment effects. We analyzed metabolic rates on the first days of the experiment (when there was no biofilm developed in the channels) and it revealed no GPP signal and only a weak ER signal (0-0.4 g O_2 m^{-2} d^{-1}). Nevertheless, absolute values of GPP and ER are not important for this study and should be used with caution if compared among studies. Metabolic rates are presented both as daily rates (figures in supplementary material) and cumulative rates (figures in manuscript). Cumulative rates were based on the days where there were data from at least one channel from each treatment (12 days out of 30).