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Resistance but not recovery is related to the role of specialist taxa in river communities submitted to hydric stress

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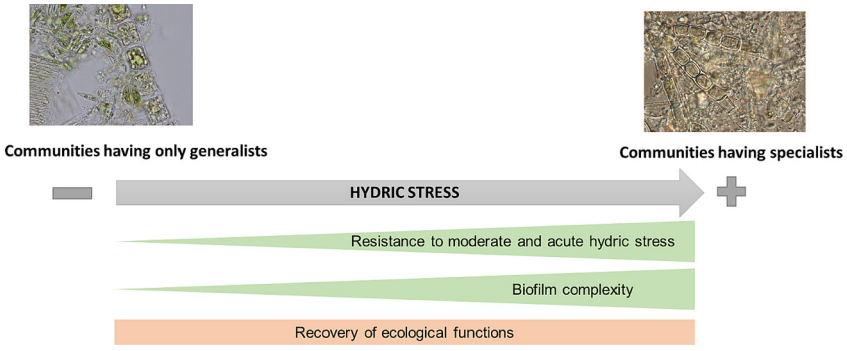
HIGHLIGHTS

- Hydric stress is a common expression of global change in river ecosystems.
- Effects of hydric stress were less important in communities having specialist taxa.
- Basal fluorescence and photosynthetic yield decreased more markedly when specialist taxa were absent.
- Metabolism remained similar under moderate hydric stress, but dropped by half under acute hydric stress.
- Specialists provided higher resistance to stress but failed to support recovery of ecological functions.

GRAPHICAL ABSTRACT



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ABSTRACT

One of the main effects of global change is the human interference in the global water cycle, which alters river hydrological dynamics and submits their biological communities to hydric stress. Hydric stress is a pulse disturbance with potential multiple effects on biodiversity and functions in river ecosystems. The presence of habitat specialists may support the response of biological communities to pulse disturbances, maintaining ecological functions more consistently than other communities only having generalists. We tested this general hypothesis in stream communities submitted to increasing hydric stress (normal conditions vs humidity vs desiccation). We used communities with variable proportion of specialist algal and cyanobacterial taxa and tested their resistance to hydric stress by analyzing potential changes on their number of species, biovolume, proportion of intact cells, and photosynthetic variables (basal fluorescence, photosynthetic yield). We also evaluated the recovery of ecological functions (net community primary production, community respiration, phosphorus uptake) once hydric stress conditions ended. Hydric stress caused a slight decrease in the number of species and biovolume of assemblages, but the proportion of intact cells did not significantly change because of the disturbance. Basal fluorescence and photosynthetic yield under hydric stress decreased more markedly in communities without specialist taxa, while communities with habitat specialists resisted better. Metabolism did not remarkably decrease under moderate hydric stress, but dropped by half under desiccation in all communities, having or not specialist taxa. Overall, specialist taxa did provide higher resistance to stress but did not support a distinct recovery of ecological functions. We suggest that this characteristic response is related to the high plasticity of biofilm structures.

1. Introduction

Biological communities are made up by a diverse set of specialist and generalist species, usually coexisting. Specialists are species with high

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specificity for habitats or resources, usually occurring under distinct environmental conditions (Futuyma and Moreno, 1988; Dolédec et al., 2000). Generalists are species which occur in a wide diversity of habitats and can use a variety of resources, therefore showing broad environmental tolerances (Clavel et al., 2011). Natural communities have a distinct level of specialization, according to the niche breadth of their species (Sexton et al., 2017) and to the environmental factors which influence their existence (such as disturbance events and environmental heterogeneity; Büchi and Vuilleumier, 2014). Habitats submitted to extreme environmental conditions may be optimal for specialists but are less suitable for generalists. The overall fitting of species to the main environmental gradient results in a specific degree of specialization which characterizes a community (Büchi and Vuilleumier, 2014).

Pulse disturbances (such as heatwaves, abrupt desiccation, or point-source contaminants) may lead to abrupt changes in environmental conditions which disproportionately affect the taxa with limited opportunities for adaptation (Harris et al., 2018; Ledger and Milner, 2015). Therefore, pulse disturbances perform therefore as environmental filters (Chase, 2007) and submit communities to a non-random process for the individuals' survival (O'Neill, 2016). In principle, the number and abundance of specialist taxa is one of the a priori factors substantiating consistent community responses to disturbances (Brown, 1995), particularly on the maintenance of community functions (Ledger and Milner, 2015). We may then expect that communities with high contribution of specialists would maintain ecological functions more consistently than communities having mostly generalists (Lyons et al., 2005; Bracken and Low, 2012).

River networks naturally include a large diversity of habitats and are submitted to a large array of environmental conditions, anticipating the occurrence of specialist species adapted to withstand the most extreme of them. One of these is water flow interruption, which causes hydric stress and challenges the survival of the less adapted organisms (Sabater et al., 2022). Testing the relevance of specialist taxa in communities submitted to hydric stress may be complicated when taxa simultaneously perform multiple functions, as it is the case of animal communities. However, photoautotrophs (algae and cyanobacteria) in biofilms have the common function of primary production in fluvial systems (Battin et al., 2016). Photoautotrophs occur on central and marginal areas of the streambed, mostly from middle sections to the mouth, and their distribution is habitat sensitive (Sabater et al., 2016). In photoautotrophic communities, generalist taxa co-occur with specialists (Reynolds, 2012), but the respective contribution of ones or the others depends on the long-term environmental conditions of the site. Specialists in biofilms may have significant contributions when stream systems are submitted to strong variation of water flow (Tornés and Sabater, 2010), extreme light (either low or very high) conditions (Sheath and Vis, 2015; Colls et al., 2021), or specific chemical characteristics (e.g., low pH; Pither and Aarssen, 2005). These specialist taxa may show specific morphological traits such as mucilage-building sheaths (Kann, 1978), physiological adaptations such as facultative dehydration, osmotic compensation (Holzinger and Karsten, 2013), or specific accompanying pigments (Colls et al., 2021), or even dormancy (Lennon and Jones, 2011), and reproductive strategies such as the production of resting stages (Souffreau et al., 2013).

Photoautotrophs in benthic systems are part of biofilms, which may be defined as self-contributed structures attached to a substratum, and whose architectural complexity and biological interactions increases under favorable circumstances (Sabater et al., 2016). Photoautotrophs account for a substantial fraction of total biomass in biofilms (Peterson, 1996), and their overall growth may become a limiting factor for the transfer of gases and nutrients from the water environment (Riber and Wetzel, 1987). By means of their complex structures, biofilms can buffer the external environmental influences and protect their components (Romaní et al., 2013). It is therefore obvious that the structure of the biofilm (as it may be accounted for by its biomass, architecture, or thickness) may play a role in the response of photoautotrophs to pulse disturbances. Complex biofilms may reinforce the role of specialists associated to hydric stress affecting river ecosystems. As an extreme case of biofilm complexity, stromatolitic biofilms (Sabater, 2000) resist complete dehydration for long periods of time and can recover their main functions soon after water returns to the system (Romani and Sabater, 1997). Structural complexity may therefore add to the role of specialist taxa in the overall response of stream biofilms to desiccation.

We here use biofilm communities grown under distinct conditions of light and water flow. We searched evidence on the role of specialists and generalists on the immediate resistance of each of these communities to hydric stress. We did so by evaluating the biofilm abilities to recover its ecological functions after the disturbance, through monitoring their net community primary productivity, community respiration, and phosphorus uptake. We considered the relative complexity of the biofilms (biomass, formation of structures), and their community structure (number of species, fraction of intact cells after disturbance, biovolume). We performed our experiments in microcosms, looking for a thorough control of the disturbance caused to the biofilms. Microcosms provide a steady state response (by definition, microcosms do not allow replacing community individuals which might be lost or dead). Even though microcosms do not fully reflect the complexity of biotic interactions and life cycles occurring in nature (Kröel-Dulay et al., 2022), their use allows to test the effect of short-term disturbances with a sufficient statistical power.

We test the general hypothesis that ecological functions are better preserved when specialist taxa have a significant presence in biological communities. We have designed an experimental set of increasing hydric stress, where communities under fully submerged (normal) conditions were compared to partially stressed (humidity) and complete stressed (desiccation) hydric conditions. Specifically, we assume that I) communities with high contribution of specialists show the lowest structural changes when they are submitted to hydric stress; II) photosynthesis decreases in intensity with hydric stress, but their decline is less pronounced when habitat specialists are present; III) once disturbance has ceased, ecological functions recover faster when habitat specialists are present. Our experimental approximation imitates conditions such as those experienced by stream benthic communities during episodes of water flow interruption, so it might value the relevance of specialist taxa on these conditions. Through testing these hypotheses, we aim to shed light on the relevance that specialists may have regarding global change impacts in river ecosystems.

2. Methods

2.1. Study sites

We selected communities from three 3rd order streams which were a priori comparable on their physiographical characteristics. The three streams were submitted to a similar Mediterranean climate and had similar channel and streambed structure. Two of the streams had permanent flow (Avencó, Brugent), and a third was intermittent (Mieres), but the three were at the basal stage of water flow at the time of the sampling (March–April 2021). One of the streams was highly shaded (Brugent), while the other two were partially shaded. The three streams drained calcareous watersheds and received low human interferences. Avencó (Av) and Brugent (Br) had the lowest nutrient concentrations, and Mieres (Mi) had higher nitrogen and DOC content and high-water hardness due to sulfates and calcium contribution (Table S1). Previous information on the physiographical characteristics of the three streams is available in Colls et al. (2019) and Tornés et al. (2021).

2.2. Experimental procedure

Acclimation. We collected epilithic biofilms in each of the three sites and transported them to the laboratory in less than 2 h. Once in the laboratory, we carefully removed grazers from the substrata, and the cleaned cobbles were moved to plastic trays filled out with stream water. From these, we selected communities showing similar F0 (basal fluorescence, see below) and size, and we placed them in an incubator (Radiber AGP-570) for a 3-day acclimation period. Those selected were placed in a rotatory plate to provide

constant movement, in conditions of darkness /light cycles of 12 h/12 h, air temperature of 15 °C, and light irradiance of 130–150 μmol photons m^{-2} s^{-1}. We replaced stream water in the trays two times per day during the acclimation period, to avoid nutrient depletion. We monitored the status of the photoautotrophic biofilms by measuring F0 daily; we considered steady F0 values as indication that no significant changes occurred during the acclimation period. We discarded those cobbles showing a notorious decline of F0 during the acclimation, looking for a maximal homogeneity at the onset of the experiments. After acclimation, the selected epilithic biofilms were transferred to the microcosms and assigned at random to each microcosm.

Microcosm setting. We used laboratory glass crystallizers as microcosms. Glass materials had been cleaned and sterilized to ensure that the potential responses of the epilithic photoautotrophic biofilms were not modified. Microcosms were 7 cm in diameter, 4 cm height, and were filled with 60 mL of stream water, 25 mL of sand sediment (>1 mm) on its bottom, and two colonized cobbles of equivalent size and form. The sand sediment had the function to buffer the water content in each microcosm. Sands had been previously incinerated in a muffle furnace (450 °C, 4 h) and washed to eliminate turbidity. All microcosms were placed in a rotatory plate to provide constant movement, in the same light and temperature conditions than during the acclimation period.

Experimental design. We performed separate experiments for each photoautotrophic biofilm community, with identical designs. Hence, we defined two treatments of hydric stress and a control (C, no hydric stress), each one with six replicates. The first treatment defined a moderate hydric stress (T1, humidity), which we forced by reducing the water content in the selected microcosms from 60 to 20 mL. This reduction left the cobbles partially emerged and their lower part submerged, so biofilm communities were occasionally splashed through the water movement. The second treatment simulated an intense hydrological stress (T2, desiccation), which we promoted by leaving the cobbles completely dry, though the sand sediment in the crystallizer remained wet. We replaced water in each microcosm once per day, by using a syringe to minimize disturbance of the microcosms.

2.3. Biofilm analyses

Algal community composition. We collected samples for community composition of photoautotrophic biofilms by scraping the superficial area of each cobble, and cells were preserved with a drop of formalin solution diluted to a final volume of 5 mL and stored in the dark until microscopical observation. We performed this analysis in four replicates per each treatment and stream. We identified alga and cyanobacteria to the lowest possible taxonomic level (genus or species) using light microscopy at 600× magnification (Nikon CS1, Tokyo, Japan). We counted at least 400 cells per replicate, and we visually separated cells into three categories according to the preservation of their chloroplasts. Cells having complete chloroplasts with greenish color were identified as live cells; cells with chloroplasts partially deteriorated or with pale color, were considered as impaired cells; and cells with vacuolated or absent chloroplasts, were considered dead. We performed the identification and counting within the first month after the collection of the sample, to ensure the best chloroplast preservation.

Knowledge of specialization is rather complete in biological groups such as insects or vertebrates, but much less in others (Colles et al., 2009). Knowledge of specialization in algae and cyanobacteria is scattered and restricted to some groups (e.g., diatoms), and restricted to a few environmental gradients (such as pH and eutrophication; Lindholm et al., 2018, Leboucher et al., 2019). In those cases, patterns may be then derived from large data sets (Fridley et al., 2007), and a proper attribution may be given to generalists and specialists in relation to the governing environmental gradient. Our data set had a limited size, and we could not produce such an analogous estimation. We therefore assigned specialization to the taxa present in our communities according to, i) their known distribution and ecological characteristics in small streams elsewhere (Kann, 1978; Sheath and Vis, 2015; Komárek and Anagnostidis, 1999), and ii) the occurrence of specific traits indicating the resistance to hydric stress, such as thick mucilage or thick cell walls (Sabater et al., 2017). According to this information, we designated as specialists some species in each stream (see below for the identity of the taxa). Once this was done, we estimated the biovolume of the cells counted, and the corresponding biovolume fraction of the habitat specialists and generalists. Biovolume was calculated from average sample dimensions for each taxon using the algorithms provided by Borics et al. (2021).

Algal biomass determination. Chlorophyll-*a* (Chl-*a*) was measured as the concentration of biofilm pigment extracts per surface area. We scrapped off biofilms from the cobbles and re-suspended them in filtered (0.22 μm) Mili-Q water, and then extracted pigments on 90 % acetone overnight in the dark and at 4 °C. Chlorophyll extraction was completed using a sonication bath, in which we left the samples for two minutes (SELECTA, Spain). We quantified Chl-*a* concentration spectrophotometrically using a Lambda UV/VIS spectrophotometer (U- 2000 Hitachi, Japan) following Jeffrey and Humphrey (1975).

In vivo fluorescence measurements. We monitored the basal fluorescence (F0) and the photosynthetic efficiency (Yeff) in each photoautotrophic community at 0, 24, 48 and 72 h. We measured in-vivo chlorophyll-*a* basal fluorescence (F0) and the effective quantum yield (Yeff) using a Diving PAM (Pulse Amplitude Modulated) underwater fluorometer (Heinz Wlaz, Effeltrich, Germany). We measured the two parameters four times in each community before the experiment started (0 h), and then every 24 h until the end of the experiment (72 h). Basal fluorescence (F0) is a surrogate of algal biofilm biomass (Corcoll et al., 2015), and the effective quantum yield (Yeff) accounts for the status of physiological activity of the algal community (Timoner et al., 2012).

Biofilm metabolism. We estimated metabolic biofilm rates (net community primary production and respiration) by measuring changes in oxygen concentration under light and dark conditions after 72 h. These measurements were performed with the photoautotrophic biofilms being placed in cylindrical acrylic chambers with a water volume 0.96 L. Each of the chamber was provided with submersible water circulation pumps and oxygen sensors (PreSens OXY-10mini, Regensburg, Germany) (Colls et al., 2019). We placed chambers in an incubator (Radiber AGP-700-ESP, Barcelona, Spain). Incubations generally lasted for 60 min, under conditions analogous as those for the microcosm experiments (air temperature of 15 °C and light irradiance of 140–150 μmol photons m^{-2} s^{-1}). Dissolved oxygen concentration inside the chambers was measured every 30 s and recorded, and then we derived the production or consumption rate after the initial slope of the corresponding measurements (Acuña et al., 2015). We first measured community respiration (CR) as the reduction of oxygen concentration in darkness, and consecutively the net community primary production (NCPP) as the oxygen concentration in the light. CR and NCPP were calculated as the slope of the linear regression of the oxygen concentration change vs incubation time, and in relation to the surface area colonized and incubation period. Metabolism measurements in the T1 and T2 biofilms were aimed to measure their recovery after water return, in comparison with the activity in the control mesocosms.

P-uptake capacity measurement. We estimated the uptake rate of soluble reactive phosphorus (SRP) on the biofilms (Proia et al., 2017). We performed these assays in each of the replicates immediately after the completion of the metabolism estimates. Measurements started with a controlled spike of SRP (see below), defined as to 4× the background SRP concentration in the respective stream waters. Addition of SRP was performed using a Na_2PO_4 stock solution (10 mM). Biofilms were then incubated for 30 min under the same controlled conditions of temperature, light, and turbulence as in the metabolism measurements. 30 mL of filtered water (0.45 μm glass fiber filters) aliquots were collected at the end of the incubation and stored at −20 °C until analyses. SRP ($P\text{-}PO_4^{3-}$) was determined by colorimetry after Murphy and Riley (1962), using a spectrophotometer (Alliance-AMS Smartchem 140, AMC, Frépillon, France). The phosphate uptake rate (U, $\mu gP cm^{-2}$ h^{-1}) was calculated as the difference between P_0 (the initial mass of P after the spike) and P_f (the final mass of P at the end of

incubation), in relation to the area covered by biofilm and the incubation period (Proia et al., 2017).

2.4. Data treatment

We tested for normality the structural and functional variables measured in the experiments using the Shapiro-Wilk test (Legendre and Legendre, 1998), and the homogeneity of variances using Levene's test and residual plots (Levene, 1960). When necessary, we transformed the data using log (x + 1), and we square-root transformed all proportional data. We performed separated analyses for each photoautotrophic biofilm. So forth, one-way ANOVA was used for Chl-a as a function of algal biomass, and repeated-measures analyses of variance (RM-ANOVA) were used for F0 and Yeff. Examining the temporal tendency of F0 and Yeff allowed us to test if the presence of specialist in these communities may slow down the photosynthesis decline under hydric stress. Data of algal biomass in Mieres were heteroscedastic, and therefore we replaced the one-way ANOVA by a Welch's ANOVA (Welch, 1951). When applicable, significant ANOVA and Welch's ANOVA results were subject to post-hoc paired comparisons (Tukey's HSD test and Games-Howell test respectively) to determine differences between treatments. Post-hoc paired comparison with Bonferroni adjustment was also applied for RM-ANOVA. Differences in the community composition, including the proportion of specialist and the proportion of intact cells between treatments for the three study communities were tested by non-parametric analyses of variance (Kruskal-Wallis test). Finally, we analysed the overall relationship between the proportion of specialist taxa and the response variables by means of linear regression analysis, considering the three photoautotrophic communities in each of the treatments.

We run all statistical analyses in R free software (R Development Core Team 2021, version 4.0.5), and created figures with Sigmaplot (version 11.0, Systat Software, Inc. 2008). The complete data of algal communities is available at the Zenodo repository (DOI: https://doi.org/10.5281/zenodo.7220927).

3. Results

Effects of hydric stress on richness, relative abundance and biovolume, and biomass of the assemblage. The biofilm structure and the composition of the algal communities were different in the three streams (Table S2). Av was dominated by the encrusting cyanobacterium *Hydrococcus rivularis* Kütz. and the green alga *Sphaerobotrys fluviatilis* Butcher. Together with these taxa, a few diatoms (*Achnanthidium* sp. pl., *Cocconeis placentula* Ehr., *Gomphonema* sp. pl.) were also common. Br had a relevant proportion of the red alga *Audouinella* sp. and of several diatoms (*Achnanthidium* sp.pl., *Gomphonema* sp.pl., *Cymbella* sp. pl.). Mi was dominated by the cyanobacterium *Chroococcopsis fluviatilis* (Lagerh.) Komárek & Anagn. and the encrusting green alga *Gongrosira incrustans* (Reinsch) Schmidle, together with a few diatoms (*Gomphonema* sp.pl., *Cocconeis placentula*, *Cymbella* sp. pl.), and a small contribution of *Audouinella* sp. Altogether, we identified 19 algal and cyanobacterial taxa in Av, 26 in Br, and 20 in Mi.

Following the criteria outlined in the methods, we considered as specialists the taxa having thick cell walls, which included a few cyanobacteria, green and red algae. *Chroococcopsis fluviatilis* (Cyanobacteria) and *Gongrosira incrustans* (green algae) are colonial taxa resistant to hydric stress (Kann, 1978; Yang et al., 2019) and possess thick cell walls as the main resistance trait (Fig. 1). *Audouinella* sp. is a filamentous red alga which thrives under low light conditions in oligotrophic streams (Kann, 1978; Sheath and Vis, 2015), and has a large size and thick cell walls (Fig. 1), together with a pigmentary composition which allows adaptation to low light intensities (Sheath and Vis, 2015). The remaining cyanobacteria, green algae, and diatom taxa were considered generalist regarding their adaptation to hydric stress.

Habitat specialists were not present in Avencó and ranged between 1 and 22 % in Brugent and 56–74 % in Mieres (Table 1). Specialists had a high contribution to the biovolume, particularly in Brugent because of the large size of the red alga *Audouinella* sp. (Table 1).

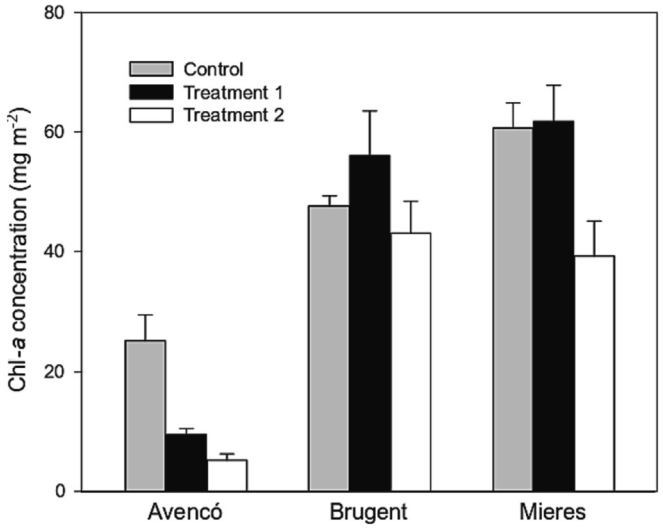

Fig. 1. Mean (± SE) (n = 5 for Av; n = 6 for Br, except T2 when n = 3, and Mi = 6) of chlorophyll-*a* concentration in the three different treatments after 72 h of exposure (Control, no hydric stress; Treatment 1, humidity; Treatment 2, desiccation) in the three study streams.

Increasing hydric stress caused changes in the number of taxa in the communities of the three systems. The number consistently decreased in Avencó, which shifted from 12.5 to 9.8–10, as well in Mieres, which decreased from 13.8 to 11.3–9.8 (Table 1). The decrease was more accentuated in T2 and affected the three communities (Table 1). The total biovolume of the assemblage also decreased with the acute hydric stress (T2), leading to a reduction by half in Avencó and Brugent, more moderate in Mieres (Table 1).

We also evaluated the proportion of intact cells remaining after 72 h (Table S2). We did not observe statistically significant changes between the control and the two treatments regarding cell integrity. The proportions remained between 75 % and 85 % of the total cells in Av, between 40 and 56 % in Br, and between 85 and 92 % in Mi. We did not detect statistically significant changes between habitat specialists or generalists in the proportion of damaged cells because of hydric stress.

Initial biomass (as chlorophyll-*a*) was lower in Av (25 ± 4.3 mg Chl-*a* m^{-2}) than in Br (47.6 ± 1.7 mg Chl-*a* m^{-2}) or Mi (60.6 ± 4.2 mg Chl-*a* m^{-2}). The chlorophyll-*a* concentration decreased after 72 h in the two hydric stress treatments, though with different intensities (Fig. 1). The reduction in T1 and T2 was very large in Av (79.2 %), and already statistically significant in T1 (ANOVA, $F_{2,12} = 22.63$, $p = 0.00008$). However, reduction of chlorophyll-*a* concentration was moderate in Br (10 %, not significant differences between T1 and T2 and the control) as well as in Mi, where the decrease was mostly occurring in T2 (35.3 %; Welch's ANOVA, $F_{2,\ 15} = 4.35$, $p = 0.046$) (Fig. 1). Biofilms were thin and simple in Av, thicker in Br, and thicker and complex in Mi. In this last stream carbonate deposition provided a tight structure to the biofilm. Even though we did not directly measure biofilm thickness, we rely on Chl-*a* as an indication of biofilm thickness since there is a close relationship between algal accumulation, chlorophyll-a concentration, and biofilm thickness (Sekar et al., 2004).

Effects of hydric stress on the photosynthetic variables. We measured the temporal variation of the basal fluorescence F0 and the effective quantum yield (Yeff) in each of the photoautotrophic biofilm communities (Fig. 2). F0 and Yeff maintained the original values after 72 h in the controls (except of a slight increase after 72 h in Mieres F0). F0 and Yeff declined in T1 and T2 in all biofilm communities, though showed separate patterns. F0 in Av declined by half in the T1 (RM-ANOVA, $F_{2,66} = 48.32$, $p = 1.19e^{-13}$) and by one third in T2 ($p = 0.001$). Yeff also declined in Av (RM-ANOVA, $F_{2,66} = 67.97$, $p = 2e^{-16}$), moderately in T1 (non-significant difference) but reaching values close to 0 after 24 h in T2 ($p = 0.0001$). F0 in

Table 1
Total number of taxa, number of habitat specialist taxa, proportion of habitat specialists in cell numbers, total counted biovolume in the assemblage, and biovolume fraction of specialists when present in the stream. Av, Br and Mi are acronyms corresponding the three studied streams, and C, T1 and T2 correspond to the control, humidity, and desiccation respectively. Data are averages and standard deviations (in italics; n = 4).

	Number of taxa		Number specialist taxa	%Abundance of specialists		Total biovol. (μm^3)		%Biovol. of specialists	
Av- C	12.5	±*1.5*	0	0		133,891	±*67,462*	–	–
Av-T1	9.8	±*1.9*	0	0		68,204	±*17,594*	–	–
Av- T2	10	±*1.2*	0	0		66,291	±*15,662*	–	–
Br-C	16.3	±*1.1*	1	21.2	±*11.9*	629,269	±*101,356*	74.2	±*10.1*
Br- T1	14.8	±*1.3*	1	4.8	±*4.1*	347,337	±*50,314*	29.7	±*18.3*
Br- T2	14.5	±*2.3*	1	11.4	±*9.0*	436,657	±*159,312*	51	±*25.0*
Mi- C	13.8	±*2.5*	3	69.2	±*21.1*	148,990	±*78,035*	27.7	±*1.9*
Mi- T1	11.3	±*2.3*	3	74.5	±*34.7*	180,257	±*39,130*	48.9	±*23.1*
Mi- T2	9.8	±*1.6*	3	74.4	±*27.4*	117,838	±*58,063*	37.7	±*25.6*

Br experienced a moderate, identical, decrease in T1 and T2, halving after 72 h (RM-ANOVA, $F_{2,66} = 14.96$, $p = 0.000004$). In this photoautotrophic biofilm, the effective quantum yield after 72 h remained similar to the initial C values in T1 but decreased steeply in T2 (RM-ANOVA, $F_{2,66} = 15.53$, $p = 0.000003$). Finally, F0 in Mi significantly decreased in T1 (RM-ANOVA, $F_{2,66} = 22.73$, $p = 0.00000003$), and less pronouncedly in the T2 ($p = 0.003$). The effective quantum yield in this photoautotrophic biofilm strongly decreased in T2, when values were very low after 72 h (RM-ANOVA, $F_{2,66} = 23.91$, $p = 0.00000001$).

Recovery of biofilm metabolism after disturbance. Net Community Primary Production (NCPP) per surface area in the control photoautotrophic biofilms was higher in Br and Mi than in Av, corresponding to their higher values of algal biomass (higher Chl-*a*). We measured the recovery rate of biofilm functions in T1 and T2 after biofilm substrata were returned to

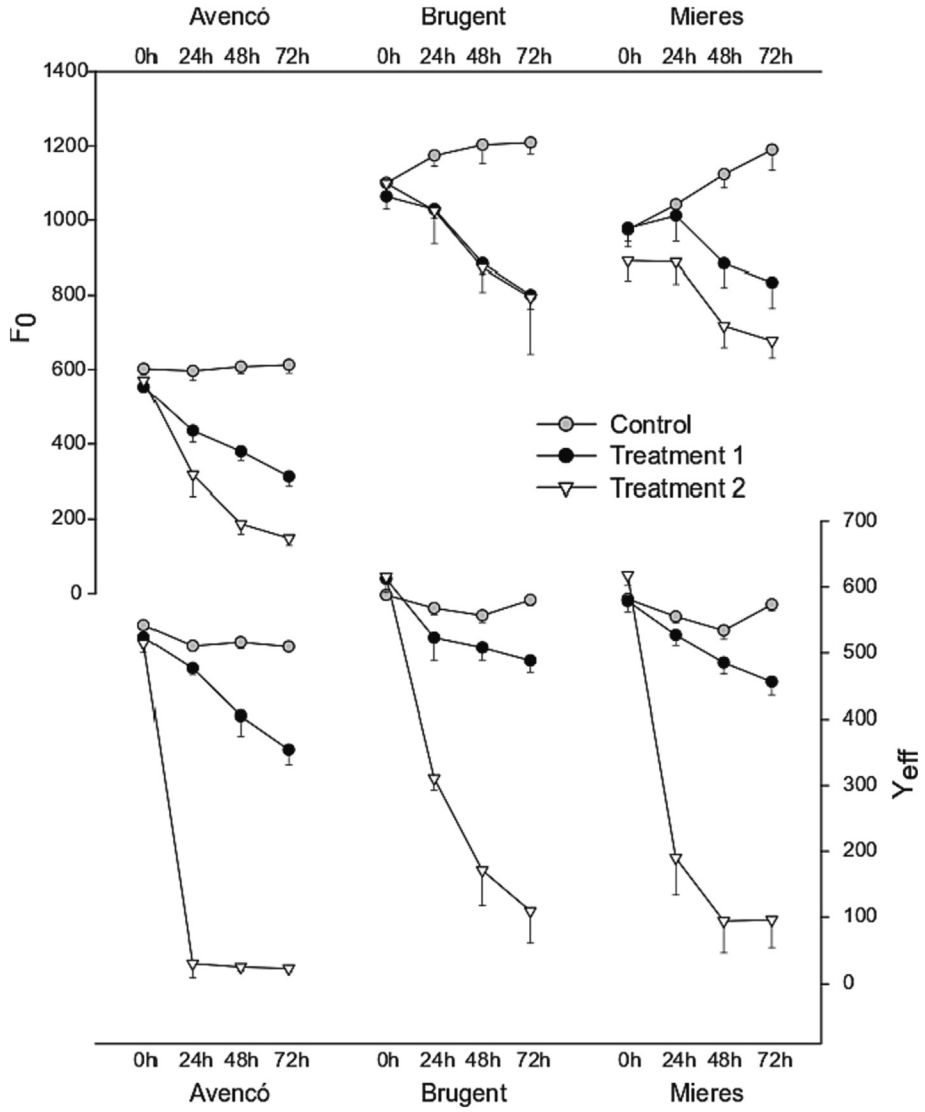

Fig. 2. Mean (±SE) (n = 6) of basal fluorescence (F0) and photosynthetic efficiency (Yeff), measured for the three distinct treatments (Control, no hydric stress; Treatment 1, humidity; Treatment 2, desiccation) and streams, at different experimental times (0, 24, 48 and 72 h).

normal hydric conditions. Recovery was estimated with respect to the values in the control mesocosms.

The recovery was not uniform among the three communities (Fig. 3). NCPP in Avencó accounted for 81 % recovery in T1 and 54.8 % in T2. NCPP was significantly lower in the two treatments (ANOVA, $F_{2,9} = 4.973$, $p = 0.0351$), this decrease being more pronounced in the T2 with respect to the control ($p = 0.029$). CR in this photoautotrophic biofilm approached a 100 % recovery in T1, but only a 36 % in T2 (statistically significant decrease; ANOVA, $F_{2,12} = 4.608$, $p = 0.032$). NCPP of the Brugent biofilms remained unaltered in T1 but decreased to a 52 % in T2 (though it was not statistically significant). CR in Brugent biofilms did not show significant changes between control and treatments. Finally, NCPP slightly enhanced in the T1 of Mieres (134 % recovery), but strongly decreased in T2 (49.4 % recovery), making the differences between T1 and T2 highly significant (ANOVA, $F_{2,15} = 15.91$, $p = 0.0002$). CR of Mieres initially increased in T1 (117% recovery) and finally decreased by half in T2 (44.6 % recovery). The differences between T1 and T2 were significant (ANOVA, $F_{2,15} = 35.65$, $p = 0.000002$).

Phosphorus exchange. The pattern of P exchange was similar in the three biofilm communities (Fig. 4), being positive (P uptake) in C and T1, and negative (P excretion) in T2 (ANOVA, $F_{2,12} = 62.02$, $p = 0.0000005$ (Av); $F_{2,12} = 14.7$, $p = 0.0006$ (Br), $F_{2,15} = 14.68$, $p = 0.0003$ (Mi)).

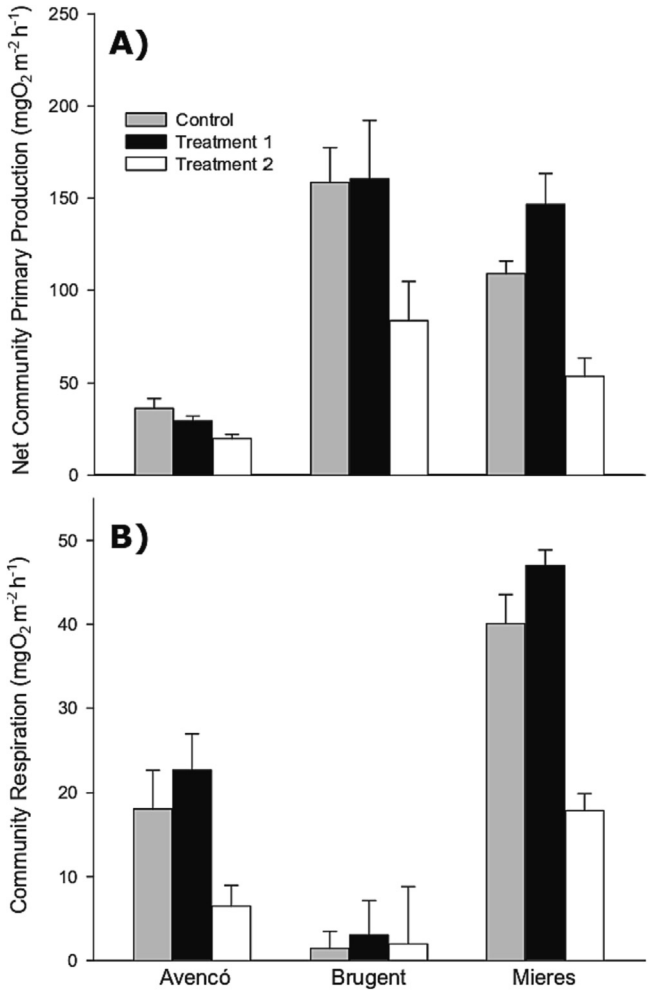

Fig. 3. A) Changes of Net Community Primary Production (NCPP) and B) Community Respiration (CR) expressed per surface area (mg O_2 m^{-2} h^{-1}) for the three different treatments and streams, after 72 h of exposure (Control, no hydric stress; Treatment 1, humidity; Treatment 2, desiccation). Bars represent averaged values and standard errors between replicates (n = 5 for Av CR and n = 4 for Av NCPP; n = 6 for Br CR and NCPP, except for T2, n = 3; n = 6 for Mi CR and NCPP).

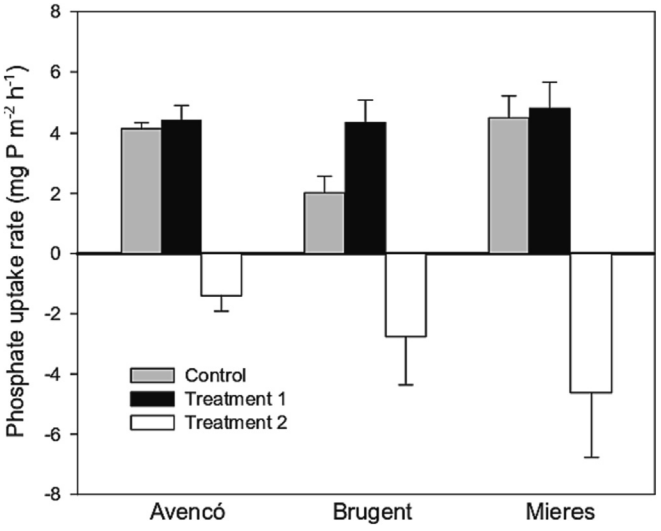

Fig. 4. Soluble reactive phosphorus uptake (or excretion) rate after 30 min of a controlled spike. Measurements obtained after 72 h of exposure for the three different treatments (Control, no hydric stress; Treatment 1, humidity; Treatment 2, desiccation) in the three study streams. Bars represent averaged values and standard errors between replicates (n = 5 for Av; n = 6 for Br, except in T2, n = 3; n = 6 for Mi).

This pattern implied a complete recovery of the P uptake in T1 in Av and Mi, and an increase of 215 % in Br, though differences were non-significant. P excretion was common in all T2 treatments, though this was lower in the Av community. Overall, the complete desiccation produced negative recoveries (−33.8 % in Av, −136 % in Br, and −102 % in Mi).

4. Discussion

We anticipated that resistance to hydric stress would be higher in communities having habitat specialists, and that the recovery of ecological functions after disturbance would be faster or more definite in these than in other communities only composed by generalists. We tested this general hypothesis using stream biofilms of different characteristics and variable contribution of habitat specialists, after submitting them to increasing intensities of hydric stress. Our results show that species loss was common to all communities experiencing hydric stress, and that both generalist and specialist taxa receded after the pulse disturbance. However, the photosynthetic ability of the biofilms resisted better when specialists were present, regardless their losses. The complexity of the biofilm (growth and structure) also played a role in this response, highlighting its role as refuge for organisms dwelling on it (Townsend and Hildrew, 1994). Though, this higher resistance did not translate into substantial advantages for the biofilm recovery of ecological functions.

We had a large proportion of habitat specialists in two of the photoautotrophic communities (Mieres and Brugent), which were those experiencing natural water flow contraction (leading to humid conditions or desiccation) or less favorable light conditions. Habitat specialists in these streams were cyanobacteria, green and red algae (Komárek and Anagnostidis, 1999; Kann, 1978), having traits enabling them to resist hydric stress (Sheath and Vis, 2015; Yang et al., 2019). The relevance of these taxa was not only related to their abundance (cell numbers) but also to their size (large biovolume) and occupation of the space in the biofilm microenvironment (Hill et al., 2009). As such, the relevance of *Chroococcopsis* in Mieres could be associated to their high number as well as to the protective biofilm onto which developed (see below); and that of *Audouinella* in Brugent was probably related to the large size and arbuscular structure of its filaments (Table 1), regardless of their relatively low cell number.

All photoautotrophic biofilm communities had a significant proportion of diatoms. Diatoms generally are habitat generalists with respect to hydric

stress because of their thin cell walls which account for high sensitivity to desiccation and ultimate cell death when this persists (Souffreau et al., 2010). However, a few diatom taxa are provided with thicker cell walls to resist long desiccation periods (Tornés et al., 2021). When present, these are subaerial taxa with low contribution to the overall diatom assemblage, both regarding the number of species and number of cells. It might be therefore anticipated that communities having a high proportion of diatoms would be the most sensitive to hydric stress and those having the least ability to recover once flow returns (Souffreau et al., 2013). The results of our experiments could not confirm this general response since we were unable to obtain direct evidence of the diatom sensitivity from our microscopical observations. On this regard, we could not assign a different proportion of viable (non-damaged) cells in the phototrophic assemblage between treatments or among the three stream biofilms. We assume that relying on visual inspection of the chloroplasts (Cox, 1996) may have masked such a response.

The relevance of habitat specialists in the response of photoautotrophic biofilms to hydric stress is highlighted when their corresponding fraction of biovolume is plotted against each one of the variables measured during the study, considering altogether the three communities (Fig. 5). There are three different types of response. A first one is the positive relationship (positive slopes) between response variables and specialist biovolumes. This is the case of chlorophyll-*a* concentration and F0 readings, which have higher values with the increasing proportion of specialists in both T1 (humidity) and T2 (desiccation), and more positive slopes than the control. A second type of response relates specialist biovolumes with response variables only with respect the Control (normal conditions) and T1 (humidity) but not with respect to T2 (desiccation). This is the case of the net community primary production and phosphate uptake rate, and (less markedly) of photosynthetic efficiency (Yeff), in which specialists allowed the maintenance of the variable values, but not any distinct increase. Finally, a third response is that the proportion of specialists does not affect the variable response, this being the case of community respiration, when specialists do not seem to play any substantial role.

Our experiments also showed that not only the proportion of habitat specialists (their biovolume), but also the lower or higher complexity of

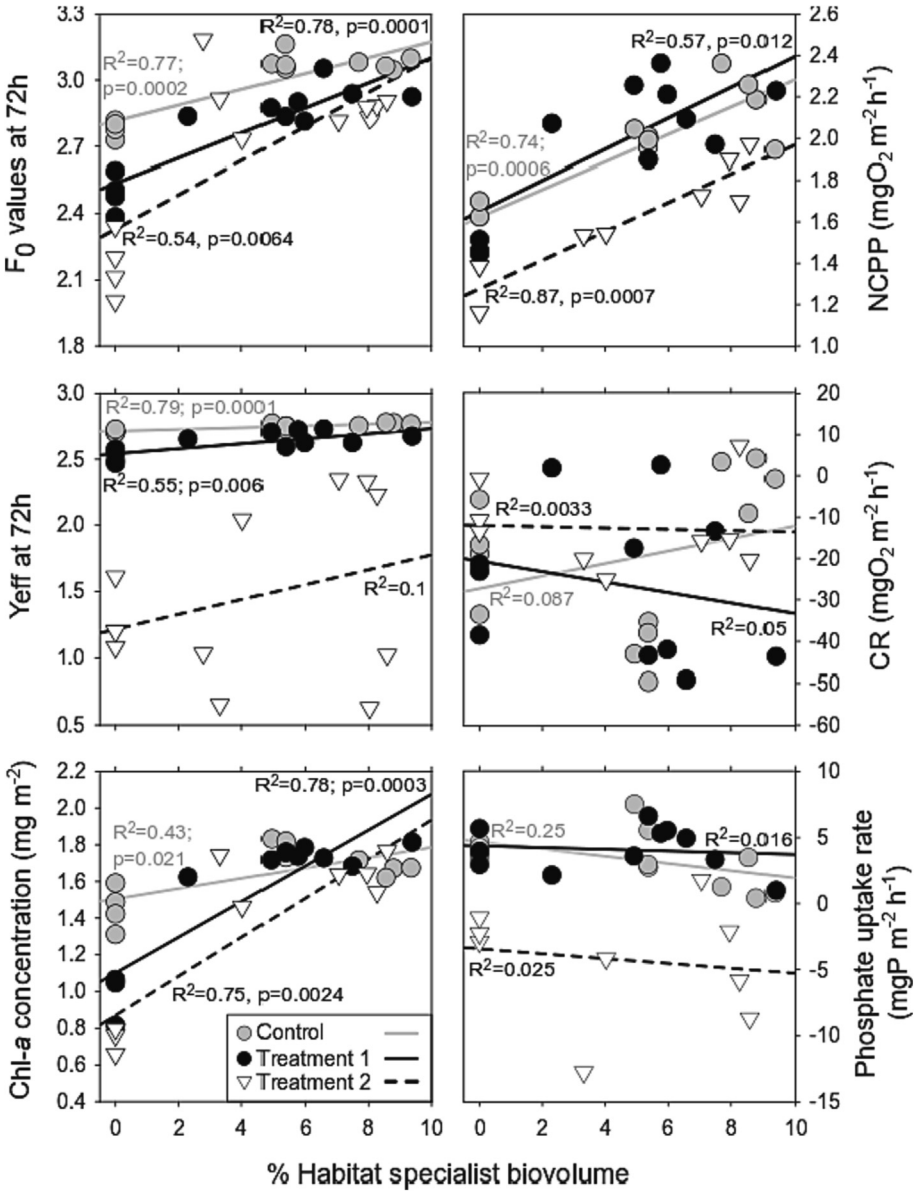

Fig. 5. Scatterplots showing the relationship between the fraction of habitat specialist biovolume (square-root transformed) against all response variables measured during the study (log transformed, except for CR and phosphate uptake rate). Coefficients of determination (R^2) are indicated for the three different treatments: Control, no hydric stress (grey line); Treatment 1, humidity (solid line) and Treatment 2 desiccation (dashed line). Only significant correlations are reported ($p \leq 0.05$). F0 values and Yeff are shown at the end of the experiment (72 h). Acronyms: NCPP, Net Community Primary Production; CR, Community Respiration.

the biofilm structure (biomass, thickness, carbonate precipitates) contributed to their resistance to hydric stress. The stream in which specialist taxa co-occurred with the most complex biofilm structures (Mieres) had the highest resistance to hydric stress, while those without one or the other had lower resistance. Such a combination was already apparent in the first stage of the hydric stress (water loss, high humidity conditions), and accounted for the pace of decrease in photosynthetic variables in the second (desiccation) stage, which was lower in the two streams having habitat specialists.

In a nutshell, the presence of habitat specialists or the complexity of biofilm structures did not provide substantial advantages to the recovery of ecological functions, except in the least severe hydric stress situation. The conditions of the T1 (humidity) did not strongly affect the functional responses in any of the three photoautotrophic biofilms and values remained similar as to those in the control conditions. The moderate hydric stress assured the humidity of the organisms but not their complete submersion, a situation which could favor the best adapted biofilm communities. Indeed, this treatment favored a relevant increase in the net community primary production and respiration in Mieres, where the biofilm was the most complex regarding biomass and thickness, and likely the best adapted to partial dehydration. However, biofilm functions were highly affected when hydric stress was extreme (T2, desiccation). Some of these functions were slightly reduced (community respiration), while others roughly decreased by half (net community metabolism) or even collapsed (phosphorus uptake). This pattern of response was common for the three biofilm communities, in what it might be described as a general loss of functionality associated to extreme hydric stress. Desiccation caused the apparent excretion of phosphorus, likely because of the cell lysis following dehydration (Potts, 1999), in a magnitude which was related to the biomass of each of the biofilms.

Finally, our observations may shed light on the chain of effects associated to global change which concern the community response to extreme disturbance. One of the main consequences of global change is habitat loss, which affects ecosystems such as coral reefs (Graham, 2007; Pratchett et al., 2012), grasslands (Botham et al., 2015; Assandri et al., 2019), and river ecosystems (Dudgeon et al., 2005). In particular, river habitats become altered through damming, channelization, alluvial sediment mining, or riparian canopy simplification. Headwaters and middle river reaches are narrowed, and meanders, lagoons, and oxbows are eliminated. Overall, this physical transformation adds to the increasing interference on the hydrological cycle, which favors basal flows and may lead to artificial water flow interruption (Sabater, 2008). These physical and hydrological modifications have both local (river reach) and regional (river network) impacts (Messager et al., 2021), and favor habitat homogenization at different spatial scales, with direct implications for biological communities. Water flow depletion and the direct loss of riverine habitats have a direct impact on the survival of biological communities (Dudgeon et al., 2005; Sabater et al., 2022), and lead to the homogenization of biota (Olden and Rooney, 2006) and the replacement of specialists by generalists (Clavel et al., 2011; Socolar et al., 2016). We may therefore assume that habitat simplification and loss may directly concern the relevance of specialists (Pandit et al., 2009), and potentially affect the overall resistance of communities to extreme events. It remains to be seen whether the idiosyncratic response we observed in photoautotrophic biofilms, which did not concern the overall recovery of ecological functions, is also of general application for other communities.

CRediT authorship contribution statement

LJ: Methodology, Formal analysis, Writing. **AF:** Methodology, Formal analysis, Writing Review and Editing **NB**: Methodology, Formal analysis. **SS:** Conceptualization, Methodology, Writing, funding acquisition.

Data availability

Complete data set will be provided upon the acceptance of the manuscript and stored at ZENODO.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.161952.

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