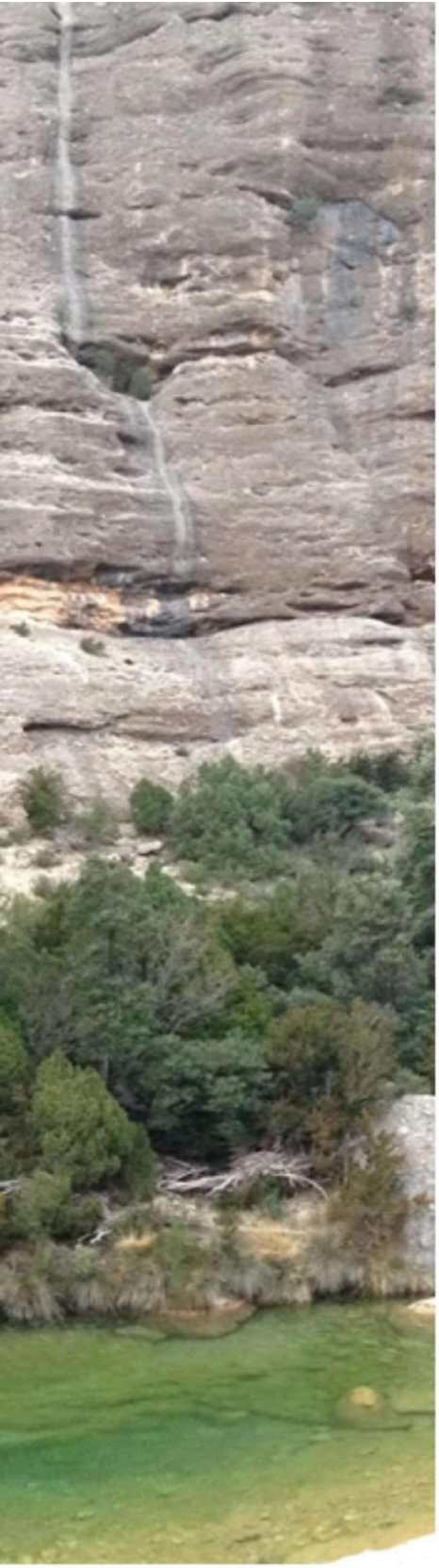
BIOFILM RESPONSES TO FLOW INTERMITTENCY IN MEDITERRANEAN RIVERS

Miriam Colls Lozano



DOCTORAL THESIS, 2020

Biofilm Responses to Flow intermittency in Mediterranean rivers

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Doctoral Thesis

Biofilm Responses to Flow Intermittency in Mediterranean Rivers

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Supervisors: Dr. Sergi Sabater Cortés and Dr. Vicenç Acuña Salazar

Thesis submitted in fulfilment of the requirements for the degree of: "Doctor of the University of Girona"

Dr. Sergi Sabater Cortés from the University of Girona and the Catalan Institute for Water Research, and Dr. Vicenç Acuña Salazar from the Catalan Institute for Water Research,

CERTIFY THAT:

The thesis entitled: "*Biofilm responses to flow intermittency in Mediterranean rivers*" presented by Miriam Colls Lozano to obtain a doctoral degree, has been complied under our supervision and meets the requirements to opt for an International Doctorate.

In witness whereof and for such purposes as may arise, the following certification in signed:

Dr. Sergi Sabater Cortés

Dr. Vicenç Acuña Salazar

Girona, 16 April 2020

"El fum dibuixarà
l'inici de la història
com una heura de joia
entorn del nostre cos
i plourà i farà sol
i dansarem a l'aire
de les noves cançons
que la terra rebrà.
Vindicarem la nit i la paraula DONA.
Llavors creixerà l'arbre de l'alliberament."

— 8 de març - Maria Mercè Marçal —

A totes aquelles dones que, amb el seu esforç i la seva vida, han fet possible que jo i moltes altres puguem arribar fins aquí.

Agraïments

Fa gairebé 5 anys vaig arribar a Girona. Una ciutat de la que, una Barcelonina com jo, en sabia ben poc. L'objectiu estava clar: fer el doctorat. Després d'aquests anys i de les experiències viscudes, Girona ja forma part de mi. Però són les persones que ens acompanyen al llarg del camí les que defineixen els llocs. Sense elles, els anys de tesis i Girona estarien buides de significat. En les properes línies, vull agrair a totes aquelles persones que, d'una manera o altre, han omplert aquest camí de records i significat.

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Ferran em passat tants bons moments junts que em costa saber per on començar. Al llarg de la tesis les coses no sempre han estat fàcils, ni com volia que fossin. Tot i així, tu has que ho semblin, sempre disposat a escolar-me i ajudar-me. No perdis mai aquest sentit de l'humor i energia que et caracteritza, amb el que ens ajudes a veure el costat positiu de la vida o, si més no, a relativitzar les coses que ens passen. Espero que tot el que hem començat durant aquesta anys, només sigui el principi de moltíssimes més coses que hem de passar junts. **Juan David** tu llegaste por sorpresa y… ¡casi nos acabamos casando! Gracias por ayudarnos a conocer nuevos lugares, maneras de pensar e ideas, abriendo siempre las puertas de tú casa de par en par. Se que para ti no siempre ha sido fácil estar aquí, pero a mí me ha gustado mucho compartir este tiempo contigo. A los dos, os quiero muchísimo y me ha encantado compartir esto años con vosotros. Sigo pensando, que nos deberíamos haber quedado en Porto....

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dinant, fent un cafè o simplement xerrant són el que fan el dia a dia amè i
Però a Girona no només han estat a l'ICRA...
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Publications List

Scientific publications included in this thesis

Colls, M., Timoner, X., Font, C., Sabater, S. & Acuña, V. (2019) Effects of duration, Frequency, and Severity of the Non-flow Period on Stream Biofilm Metabolism. *Ecosystems*; 22**,** 1393–1405. https://doi.org/10.1007/s10021-019-00345-1

Timoner, X., Colls, M., Salomón, S.M., Oliva, F., Acuña, V. & Sabater, S. (2020) Does biofilm origin matter? Biofilm responses to non-flow period in permanent and temporary streams. *Freshwater Biology*; 65: 514–523. https://doi.org/10.1111/fwb.13447

Colls, M., Timoner, X., Font, C., Acuña, V. & Sabater, S. (2020) Biofilm pigment composition as a footprint of dry periods and their severity in temporary streams. *Limnology and Oceanography*; Under Review.

Scientific publications derived from this thesis

Majdi, N., Colls, M., Weiss, L., Acuña, V., Sabater, S. & Traunsperger, W. (2020) Duration and frequency of non-flow periods affect the abundance and diversity of stream meiofauna. *Freshwater Biology*; Accepted.

Abbreviations List

µS: micro Siemens

A: Agricultural land use

ActChl: active chlorophylls

AFDW: ash free dry weight

ANOVA: analysis of variance

AT: average streambed temperature

BEF: biodiversity-ecosystem functioning relationship

Canth: canthaxanthin

CD: chlorophyll degradation products

CFI: comparative fit index

Chl-*a*: chlorophyll-a

ChlDeg: chlorophyll degradation products

cm: centimetre

CO_2: carbon dioxide

CR: community respiration

d: days

DA: daily streambed-to-air temperature amplitude ratio

DD: total duration of the non-flow period

dd^{-1}: degree $days^{-1}$

df: degrees of freedom

DOC: dissolved organic carbon

Ech: echinenone

exp: exponent

F: Fisher test

F: frequency of the non-flow period

Fr: forest land- use

FS: flow status

gnls: generalized nonlinear regression model

GPP: gross primary production

h: hour

HPLC: high-performance liquid chromatography

HS: hydrological status

Hz: hertz

IA: irrigated agricultural fields land-use

IndVal: community indicator value

IQR: interquartile range

k_{AT}: constant for weight average streambed temperature each day before sampling

k_{MT}: constant for weight maximum streambed temperature each day before sampling

k_{SR}: constant for weight solar radiation each day before sampling

l: litter

lm: linear model

m: meter

mg: milligram

min: minute

MJ: Megajoule

mm: millimetre

MnD: mean duration of the non-flow period

MT: maximum streambed temperature

Myx: myxoxanthophyll

n: sample size

NE: North-East coordinates

nls: nonlinear least squared

NM: net metabolism

NMDS: non-metric multidimensional scaling

$N\text{-}NH^{+}_{4}$: ammonium

$N\text{-}NO^{-}_{3}$: nitrate

O_2: oxygen

ºC: degree Celsius

P or PS: permanent stream (depending on the article)

p: *p*-value or probability value

PA: permanent streams in autumn

PAM: pulse-amplitude modulated

PCA: principal component analysis

$P\text{-}PO^{3-}_{4}$: phosphate

PQI: phaeophytization index

PrimCar: primary carotenoids

PS: Permanent streams in summer

PSII: Photosystem II

r: resilience

R: resistance

R^2: coefficient of determination

RMSEA: root mean square error of approximation

rpm: revolution per minute

RSE: residual standard error

RTC: streambed-to-air temperature change rate ratio

s: second

S: shrublands and grasslands land use

Scyt: scytonemin

SD: standard deviation SecCar: secondary carotenoids

SEM: structural equation model

SR: solar radiation

SRMS: standardized root means square residual

T or TS: temporary stream (depending on the article)

t: number of days before sampling period

T: temperature

TDA: temporary dry streams in autumn

TDS: temporary dry streams in summer

TFA: temporary flowing streams in autumn

TOC: total organic carbon

U: Mann-Whitney test

U: urban and industrial land use

UV: ultraviolet radiation

W: watts

WAT: weighted average streambed temperature

WMT: weighted maximum streambed temperature

WSR: weighted solar radiation

$\tilde{x}$: median

Y: photosynthetic yield

χ^2: chi-squared test

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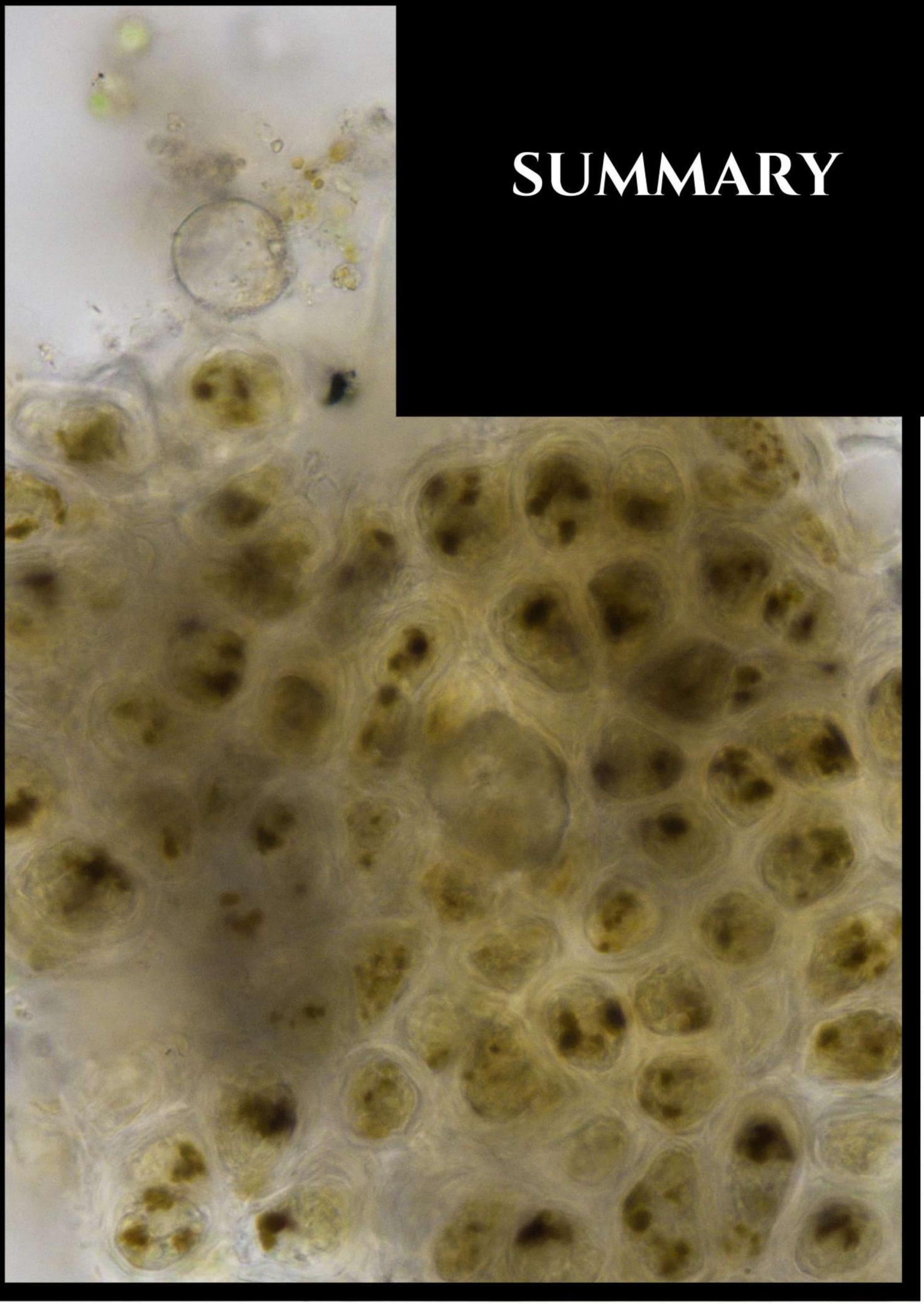
SUMMARY



Summary

Streams and rivers are dynamic ecosystems that play a key role in carbon and nutrient cycling, influencing terrestrial, lacustrine and marine ecosystems. Furthermore, fluvial ecosystems have provided ecosystem services that benefit humans for millennia and are essential to human well-being. Under flow conditions, water acts as a resource and habitat for biota, a vector for connectivity, and a determinant of the spatiotemporal distribution of species and processes. However, when water ceases to flow the longitudinal structure of streams and rivers is altered, which may have important implications for ecosystem functioning and service provision.

Streams that at times cease to flow at spatiotemporal scale along their course are called temporary streams. In river networks, headwater streams are generally temporary, and can account for more than 50% of the global fluvial network. Additionally, the intensification of anthropogenic uses and shifts in temperature and precipitation patterns caused by climate change are pressures that are increasing the number of temporary streams and lengthening their non-flow durations. These changes are altering fluvial ecosystem function and structure, as well as the ecosystem services that they provide. To effectively protect fluvial ecosystems adapting to global change, a detailed understanding of the effects that changes in hydrological regimes produce on their biodiversity and functioning is needed.

Organisms inhabiting temporary streams are directly affected by their hydrological regime, including the stream biofilm. Biofilms are associations of heterotrophic and autotrophic microorganisms co-habiting in a matrix of polysaccharides, exudates and detritus. They are of particular relevance in temporary streams because of their diversity, abundance, and key role in ecosystem processes. Therefore, understanding biofilm response to hydrological regime variability is a vital step in order to understand the implications of increasing non-flow periods on fluvial ecosystems.

Accordingly, the overall objective of this thesis was to investigate the effects of temporal components of the non-flow period (i.e. duration and frequency of the non-flow period) on fluvial ecosystems through stream biofilms, focusing on both the structure and functioning of their photoautotrophic community (i.e. algae and cyanobacteria), in order to understand and predict non-flow period effects on temporary and *new-temporary*

streams. To achieve this objective, detailed studies characterizing and analysing the effects of temporal components of the non-flow period (i.e. dry conditions) on stream biofilms were conducted at different scales. In Paper I, II and III of this thesis, I analysed how duration and frequency of the non-flow period influenced biofilm structure, physiology and functioning in 33 Mediterranean streams. Streams hydrology was monitored over one-year, based on previous information and continuous monitoring, streams flow regimes were characterized. Flow regime of selected streams ranged from permanent to ephemeral. The structure (photoautotrophic community composition), physiology (pigment composition) and functioning (community metabolism) of stream biofilm were characterized based on cobbles collected in each stream. Then, differences between permanent and temporary streams were analysed, as well as differences within temporary streams. In Paper IV of this thesis, I analysed the resistance and resilience of biofilms from permanent and temporary streams to dry conditions. This was achieved by exposing cobbles (collected from four permanent and four temporary streams) with intact biofilm to 31 dry day followed by 20 flowing days in artificial stream channels. Biofilm resistance and resilience were assessed at a structural (photoautotrophic biomass, and taxonomic composition of photoautotrophic community), physiological (pigment composition) and functional level (photosynthetic efficiency and community metabolism).

In Paper I, II, and III biofilm structure, physiology and functioning were negatively affected by the duration of the non-flow period, whereas frequency was not correlated with any analysed variable. These results highlight the non-flow duration above their frequency as an ecosystem driver in temporary streams, due to the limited stream biofilms capacity to withstand dry conditions. The exponential negative relationships between both the physiology and functioning of biofilm with the duration of the non-flow period also suggest an ecological zone-type threshold, with a transition from an aquatic to a terrestrial state after approximately 20-50 dry days. This transition from an aquatic to a terrestrial state was accelerated by the solar radiation and high stream temperatures to which biota was exposed during non-flow periods (severity of the non-flow period), indicating the importance of valley-floor form and riverine vegetation as a protective structure for temporary stream biofilms. Community analysis also highlighted the dominance of aerophyte and sub-aerophyte genera of cyanobacteria in temporary streams, whereas diatom genera characterized permanent stream communities. The dominance of

these cyanobacteria genera and the low relative abundance of diatoms in temporary streams under dry conditions suggest that the non-flow period acts as an environmental filter of photoautotrophic community composition, which reinforces the idea of a change of state from aquatic to terrestrial. This environmental filter decreased temporary stream α-diversity which, together with a reduction in active chlorophylls, drove a reduction in gross primary production. The observed cause-response relationship suggests that photoautotrophic organisms in temporary streams play an essentially singular role, and that when the photoautotrophic α diversity of temporary stream communities crosses a threshold, there is a sharp decline in autochthonous production. Because of the key position of stream biofilms in energy transfer and organic matter fluxes in fluvial ecosystems, changes in these communities might negatively affect ecosystem structure and functioning. Observed changes could lead to a reduction in the supply of autochthonous carbon downstream, promoting ecosystem heterotrophy and increasing CO_2 emissions. In addition, an increase in cyanobacteria in dry conditions could have important implications for fluvial food-webs, because they are less palatable for stream aquatic invertebrates and would thus reduce the autotrophic abundance base of food-webs.

Paper IV presents evidences that the hydrological history of temporary streams generates a pool of resistant species to dry conditions better than species pool from permanent streams. The observed lower structural resistance and resilience to the non-flow period of biofilms from permanent streams suggest that global change could have greater impact on *new-temporary streams*. However, the role that permanent pools or short flow events could play in the colonization or regrowth of stream biofilm once flow return remains unclear.

Overall, the results of this thesis demonstrate duration of the non-flow period as a key influence on the structure and functioning of biofilms in permanent and temporary streams. The results also suggest the importance of maintaining photoautotrophic stream biodiversity to preserve stream ecosystems functioning and highlight the importance of valley-floor form and riverine vegetation to protect these communities. Looking ahead, understanding the spatiotemporal effects of the non-flow period on stream biofilms is crucial to improve the management of fluvial ecosystems in response to global change.

Resum

Els rierols i rius són ecosistemes dinàmics que tenen un paper fonamental en els cicles del carboni i dels nutrients, influint així sobre els ecosistemes terrestres, lacustres i marins. A més, els ecosistemes fluvials han proporcionat serveis que han beneficiat als éssers humans durant mil·lennis, i que segueixen sent essencials per al benestar humà. En condicions de flux, l'aigua actua com a recurs i hàbitat per a la biota, com a vector de connectivitat i com a determinant de la distribució espacio-temporal de les espècies i els processos. No obstant, quan l'aigua deixa de fluir, l'estructura longitudinal dels rierols i rius es veu alterada, el que pot comportar importants conseqüències per al funcionament dels ecosistemes i els serveis ecosistèmics que aquests proporcionen.

Els rius que de manera més o menys periòdica deixen de fluir a escala espacio-temporal al llarg del seu curs s'anomenen rius temporals. A les xarxes fluvials, els rius de capçalera són generalment temporals y poden representar més del 50% de la xarxa fluvial mundial. Addicionalment, la intensificació dels usos antròpics i els canvis en els patrons de precipitació i temperatura causats pel canvi climàtic són pressions que estan incrementant el número de rius temporals i allarguen la duració dels període sense flux. Aquests canvis afecten la funció i l'estructura dels ecosistemes fluvials, així com els serveis ecosistèmics que proporcionen. Per protegir eficaçment els ecosistemes fluvials que s'estan adaptant al canvi global, es requereix una comprensió detallada dels efectes dels canvis globals en el règim hidrològic sobre la seva biodiversitat i el seu funcionament.

Els organismes que habiten rius temporals es veuen directament afectats pels canvis en el règim hidrològic, inclòs el biofilm fluvial. Els biofilms són associacions de microorganismes heteròtrofs i autòtrofs que cohabiten en una matriu de polisacàrids, exsudats i detritus. Aquestes associacions són especial rellevància en els rius temporals donada la seva diversitat, abundància i paper clau en els processos ecosistèmics. Per tant, la comprensió de la resposta del biofilm a la variabilitat del règim hidrològic és un pas vital per entendre les conseqüències del creixent període sense flux sobre els ecosistemes fluvials.

En conseqüència, l'objectiu general d'aquesta tesi va ser investigar els efectes dels components temporals del període sense flux (és a dir, la duració y la freqüència del període sense flux) als ecosistemes fluvials a través del biofilm, amb especial atenció tant a l'estructura com en el funcionament de la seva comunitat fotoautòtrofa (és a dir algues

i cianobacteris), per tal d'entendre i predir els efectes dels períodes sense flux sobre els rius temporals i els *nous rius temporals*. Per assolir aquest objectiu, es van realitzar estudi detallat caracteritzant i analitzant els efectes dels components temporals del període sense flux (és a dir, condicions seques) sobre el biofilm a diferents escales.. A l'Article I i II d'aquesta tesis, vaig analitzar com la durada i la freqüència del període sense flux van influir a l'estructura, la fisiologia i el funcionament de 33 rius mediterranis. La hidrologia dels rius es va monitorar durant un any, en base a la informació prèvia i la proporcionada pel monitoratge, es va caracteritzar el règim hidrològic dels rius. El règim hidrològic va variar de permanent a efímer. Es va caracteritzar l'estructura (composició de la comunitat fotoautòtrofa), fisiologia (composició de pigments) i el funcionalment (metabolisme de la comunitat) del biofilm fluvial a partir de còdols recollits a cada riu. Posteriorment, es van analitzar les diferencies entre els rius temporal i permanents, així com entre els rius temporals. A l'Article IV d'aquesta tesis, analitzo la resistència i la resiliència del biofilm de rius permanents i temporals a condicions seques. Això es va aconseguir exposant còdols (recollits de quatre rius permanents i quatre rius temporals) amb el biofilm intacte a 31 dies secs seguits de 20 dies amb flux a canals artificials. La resistència i la resiliència del biofilm es va avaluar a nivell estructural (biomassa fotoautòtrofa, composició pigmentaria i composició de la comunitat fotoautòtrofa) i a nivell funcional (eficiència fotosintètica i metabolisme de la comunitat).

A l'Article I, III i III l'estructura, la fisiologia i el funcionament del biofilm es van veure negativament afectats per la durada del període sense flux, mentre que la freqüència no es va correlacionar amb cap variable analitzada. Aquests resultats posen de manifest la durada de període sense flux per sobre de la seva freqüència com a impulsors dels ecosistemes dels rius temporals, donada la limitada capacitat del biofilm per resistir condicions seques. La relació exponencial negativa entre la resposta del biofilm i la durada del període sense flux també suggereix un llindar ecològica de tipus zona, amb una transició de l'estat aquàtic al terrestre després d'aproximadament 20-50 dies secs. Aquesta transició de l'estat aquàtic a un de terrestre va ser accelerada per la radiació solar i les altes temperatures (severitat del període sense flux), la qual cosa assenyales la importància tant de la morfologia del canal com de la vegetació ripària com a estructura protectora del biofilm de rius temporals. L'anàlisi de la comunitat també va posar de relleu el domini dels generes aeròfits i sub-aeròfits de cianobacteris als rius temporals, mentre que els gèneres de diatomees caracteritzaven les comunitats dels rius permanents.

El predomini d'aquests gèneres de cianobacteris i la baixa abundància relativa de diatomees als rius temporals en condicions seques suggereixen que el període sense flux actua com un filtre ambiental de la comunitat fotoautòtrofa, la qual cosa reforça la idea d'un canvi d'estat, de l'aquàtic al terrestre. Aquests filtre ambiental va disminuir la α-diversitat als rius temporals que, juntament amb la reducció de les clorofil·les actives, van impulsar la reducció de producció primària bruta. La relació causa-resposta observada suggereix que els organismes fotoautòtrofs en rius temporals tenen un paper essencialment singular, i que quan la α-diversitat fotoautòtrofa de les comunitats dels rius travessa un llindar, es produeix una forta davallada de la producció autòctona. A causa de la posició clau del biofilm en la transferència als fluxos de energia i matèria orgànica dels ecosistemes fluvials, els canvi en aquesta comunitat podrien afectar greument l'estructura i el funcionament dels ecosistemes. Els canvis observats podrien conduir a una reducció de l'oferta de carboni autòcton aigües avall, promovent l'heterotròfia de l'ecosistema i augmentat les emissions de CO_2. A més, un augment dels cianobacteris en condicions seques podrien tenir importants implicació important a les xarxes tròfiques, ja que són menys palatables pels invertebrats i, per tant, reduirien la base autòtrofa per a les xarxes tròfiques.

A l'Article IV, es presenten proves de que les condicions hidrològiques prèvies a les quals han estat exposats els biofilms dels rius temporals generaren un conjunt d'espècies capaces de resistir períodes més llargs i severs en condicions sense flux en comparació amb el conjunt d'espècies dels rius permanents. La menor resistència i resiliència estructural al període sense flux del biofilm dels rius permanents suggereixen que els efectes del canvi global podrien tenir un impacte major als *nous rius temporals*. Tanmateix, segueix sense estar clar el paper que podrien desenvolupar les basses permanents en la recuperació o colonització del biofilm un cop el flux retorni.

En general, els resultats d'aquesta tesis posen de manifest que la duració del període sense flux es una influencia clau en l'estructura i el funcionament dels biofilms de rius temporals i permanents. Els resultats també suggereixen la importància de mantenir la biodiversitat fotoautòtrofa dels rius per preservar el funcionament dels ecosistemes fluvials i remarquen la importància de la morfologia fluvial y la vegetació ripària per protegir les seves comunitats. De cara a endavant, la comprensió dels efectes espacio-temporals del període sense flux sobre el biofilm és crucial per millorar la gestió dels ecosistemes fluvials en resposta al canvi global.

Resumen

Los arroyos y ríos son ecosistemas dinámicos que desempeñan un papel fundamental en el ciclo del carbono y de los nutrientes, influyendo así en los ecosistemas terrestres, lacustres y marinos. Además, los ecosistemas fluviales han proporcionados servicios ecosistémicos que han beneficiado a los seres humanos durante milenios y que siguen siendo esenciales para el bienestar humano. En condiciones de flujo, el agua actúa como recuso y hábitat para la biota, como vector de conectividad y como determinante de la distribución espaciotemporal de las especies y los procesos. Sin embargo, cuando el agua deja de fluir, la estructura longitudinal de los arroyos y ríos se ve altera, lo que puede conllevar importantes consecuencias para el funcionamiento de los ecosistemas y los servicios que éstos proporcionan.

Los arroyos que de manera más o menor periódica dejan de fluir a escala espaciotemporal a lo largo de su curso se denominan arroyos temporales. En las redes fluviales, los arroyos de cabecera son generalmente temporales y puede representar más del 50% de la red fluvial global. Además, la intensificación de los usos antropogénicos y los cambios en los patrones de temperaturas y precipitación causados por el cambio climático son presiones que están aumentan el número de corrientes temporales y alargando la duración de los períodos sin flujo. Estos cambios están alterando la función y la estructura de los ecosistemas fluviales, así como los servicios ecosistémicos que proporcionan. Para proteger eficazmente los ecosistemas fluviales que se están ajustando al cambio climático, se necesita una comprensión detallada de los efectos de los cambios del régimen hidrológico en su biodiversidad y funcionamiento.

Los organismos que habitan en los arroyos temporales se ven directamente afectados por los cambios en el régimen hidrológico, incluido el biofilm fluvial. Los biofilms son asociaciones de microorganismos heterótrofos y autótrofos que cohabitan en una matriz de polisacáridos, exudados y detritus. Estas asociaciones son de especial relevancia en los arroyos temporales debido a su diversidad, abundancia y papel clave en los procesos ecosistémicos. Por consiguiente, la comprensión de la respuesta del biofilm a la variabilidad del régimen hidrológico es un paso vital para comprender las consecuencias de los crecientes períodos sin flujo en los ecosistemas fluviales.

En consecuencia, el objetivo general de esta tesis fue investigar los efectos de los componentes temporales del período sin flujo (es decir, la duración y la frecuencia del

período sin flujo) en los ecosistemas fluviales a través del biofilm fluvial, centrándose tanto en la estructura como en el funcionamiento de su comunidad fotoautótrofa (es decir, algas y cianobacterias), a fin de comprender y predecir los efectos de los períodos sin flujo sobre los ríos temporales y los *nuevos ríos temporales*. Para lograr este objetivo, se realizaron estudios detallados que caracterizaron y analizaron los efectos de los componentes temporales del período sin flujo (es decir, las condiciones secas) en el biofilm a diferentes escalas. En el Artículo I, II y III de esta tesis, analicé cómo la duración y la frecuencia del período sin flujo influyeron en la estructura y el funcionamiento del biofilm de 33 ríos mediterráneos. La hidrología de los ríos se monitoreó durante un año, en base a la información previa y la proporcionada por el monitoreo, se caracterizaron los regímenes hidrológicos de los ríos. El régimen hidrológico de los ríos varió de permanente a efímero. Se caracterizó la estructura (taxonómica de la comunidad fotoautótrofa), fisiología (composición de pigmentos) y el funcionamiento (metabolismo de las comunidades) del biofilm a partir de los cantos rodados recogidos de cada río. Posteriormente, se analizaron las diferencias entre los ríos permanentes y temporales, así como entre los ríos temporales. En el Artículo IV de esta tesis, analicé la resistencia y resiliencia del biofilm de ríos permanentes y temporales a condiciones secas. Esto se logró exponiendo cantos rodados (recogidos de cuatro ríos permanentes y cuatro temporales) con el biofilm intacto a 31 días secos seguidos de 20 días de flujo en canales artificiales. La resistencia y la resiliencia del biofilm se evaluaron a nivel estructural (biomasa fotoautótrofa, composición de pigmentos y composición taxonómica de la comunidad fotoautótrofa) y funcional (eficiencia fotosintética y metabolismo de la comunidad).

En el Artículo I, II y III la estructura, la fisiología, y el funcionamiento del biofilm se vieron afectados negativamente por la duración del período sin flujo, mientras que la frecuencia no se correlacionó con ninguna variable analizada. Estos resultados ponen de relieve la duración del período sin flujo por encima de su frecuencia como impulsor de los ecosistemas en los ríos temporales, debido a la limitada capacidad del biofilm para soportar condiciones secas. Las relaciones exponenciales negativa entre la fisiología y el funcionamiento del biofilm con la duración del período sin flujo también sugiere un umbral de tipo zona ecológica, con una transición del estado acuático al terrestre después de aproximadamente 20-50 días secos. Esta transición del estado acuático a uno terrestre fue acelerada por la radiación solar y las altas temperaturas (severidad del período sin flujo), lo que indica la importancia de la morfología de los ríos y la cubierta del bosque

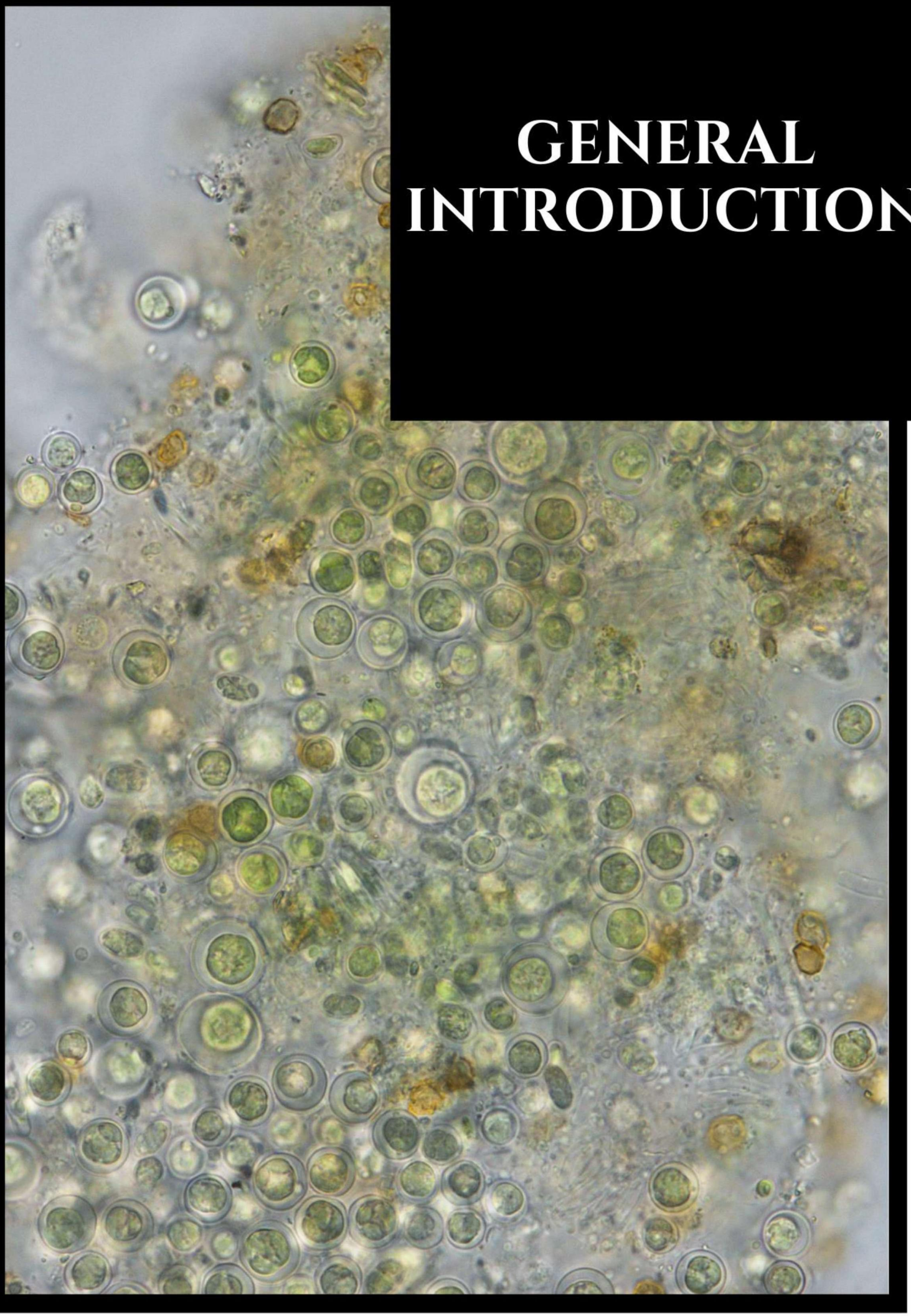
de ribera como estructura protectora del biofilm de ríos temporales. El análisis de las comunidades también puso de relieve el predominio de los géneros aerófitos y subaerófitos de cianobacterias en los ríos temporales, mientras que los géneros de diatomeas caracterizaron las comunidades de los ríos permanentes. El predominio de estos géneros de cianobacterias y la baja abundancia relativa de diatomeas en los ríos temporales en condiciones secas sugieren que el período sin flujo actúa como un filtro ambiental de la comunidad fotoautótrofa, lo que refuerza la idea de un cambio de estado del acuático al terrestre. Este filtro ambiental disminuyó laα-diversidad de los ríos temporales que, junto con la reducción de las clorofilas activas, impulsaron una reducción de la producción primaria bruta. La relación causa-respuesta observada sugiere que los organismos fotoautótrofos de ríos temporales juegan un papel esencialmente singular, y que cuando laαdiversidad fotoautótrofa de las comunidades de ríos temporales cruza un umbral, se produce una fuerte disminución de la producción autóctona. Debido a la posición clave del biofilm en las transferencias de energía y los flujos de materia orgánica en los ecosistemas fluviales, los cambios en estas comunidades podrían afectar gravemente a la estructura y el funcionamiento de los ecosistemas. Los cambios observados pondrían conducir a una reducción de la oferta de carbono autóctono aguas abajo, promoviendo la heterotrofia del ecosistema y aumentando las emisiones de CO_2. Además, un aumento de las cianobacterias en condiciones secas podría tener importantes implicaciones consecuencias para las redes tróficas fluviales, dados que son menos palatables para los invertebrados y, por lo tanto, reducirían la base autóctona de las redes tróficas.

En el Artículo IV se presenta pruebas de que las condiciones hidrológicas previas a las que han estado expuestos los biofilms de los ríos temporales generan un conjunto de especies capaces de resistir períodos sin flujo más largos y severos mejor en comparación con las especies de ríos permanentes. La menor resistencia y resiliencia estructural al período sin flujo del biofilm de ríos permanentes sugiere que los efectos del cambio global podrían tener un mayor impacto en los *nuevos ríos temporales*. Sin embargo, sigue sin estar claro el papel que podrían desempeñar las pozas permanentes en la recuperación o la colonización del biofilm una vez que el flujo regrese.

En general, los resultados de esta tesis demuestran que la duración del período sin flujo es una influencia clave en la estructura y el funcionamiento del biofilm de ríos permanentes y temporales. Los resultados también sugieren la importancia de mantener

la biodiversidad fotoautótrofa de los ríos para preservar el funcionamiento de los ecosistemas fluviales y destaca la importancia de la morfología fluvial y la vegetación de ribera para proteger estas comunidades. Mirando hacia el futuro, la comprensión de los efectos espaciotemporales del período sin flujo en el biofilm es crucial para mejorar la gestión de los ecosistemas fluviales en respuesta al cambio global.

GENERAL INTRODUCTION



1) General Introduction

Among all of Earth's ecosystems, streams and rivers play a key role in global ecological systems due to their influence on terrestrial, lacustrine and marine ecosystems (Battin et al., 2009; Palmer & Ruhi, 2019; Raymond et al., 2013). The general characteristics of streams and rivers that influence the state and dynamics of Earth's ecosystems can be classified into those wherein water acts as a resource and habitat for biota, a vector for connectivity, and a determinant of the spatial and temporal distribution of species and processes (Pringle, 2003; Sponseller et al., 2013). Fluvial ecosystems have a longitudinal structure resulting from a gradient of changing environmental conditions that supply a multitude of habitats for biota from the headwaters to the mouth (Johnson et al., 1995, Vannote et al., 1980). In addition, terrestrial inputs, autotrophic instream production and downstream transport along these ecosystems supply energy for secondary biological production (Dodds et al., 1996). Thus, environmental conditions combined with sources of energy determine the structure and functioning of fluvial food-webs (Palmer & Ruhi, 2019). Changes in any of them, hydrological conditions or food-web resources, may affect the dynamic equilibrium of fluvial ecosystems, with consequences for the health of terrestrial, lacustrine and marine ecosystems and the ecosystem services they provide.

Historically, fluvial ecosystems have provided services attracting humans for millennia (e.g., fertile substrate for agriculture), especially in water-scarce areas (Schmutz & Sendzimir, 2018). Today they remain essential to human well-being, as they continue to provide important ecosystem services such as supporting nutrient cycling and primary production, supplying drinking water and regulating carbon sequestration (Flitcroft et al., 2019). The intensification of anthropic uses has caused an exponential increase in the ecological impacts rivers endure (Schmutz & Sendzimir, 2018). Dam building, river channelization and land-use change are some examples of human impacts currently affecting the morphology, hydrology, and aquatic biota of global streams and rivers (Elosegi et al., 2010, 2019; Elosegi & Sabater, 2013). In addition, shifts in precipitation patterns and rising temperatures caused by climate change are altering flow regimes and increasing extreme flow events, namely floods and non-flow events. Current and future global change scenarios predict that anthropogenic and climatic impacts will continue to increase (Döll & Schmied, 2012; Marx et al., 2017). Therefore, it is necessary to protect

and preserve fluvial ecosystems from present and future changes, which requires detailed understanding of the effects of flow regime changes on their biodiversity and functioning.

a) Flow Regime Variability

Flow regimes are defined by the temporal variability in stream discharge, which can be characterized by the quantity, timing, and variability of flow (Poff et al., 1997). Discharge can range from floods to non-flow events and govern stream and river geomorphology, water quality, and ecology (Datry et al., 2017). During flow periods, the river basin is connected, and water transfers matter, energy and organisms within the river network (Pringle, 2003; Sponseller et al., 2013). However, when water ceases to flow, hydrological connectivity is affected, potentially changing physical, chemical, and biological processes (Lytle & Poff, 2004). These non-flow periods can be characterized by their temporal components (i.e. duration, frequency, magnitude, and predictability) (Lake, 2003), and may occur due to natural or non-natural causes. Natural causes include freezing due to low temperatures and partial or complete drying of the streambed due to low precipitation, high temperatures, or geological factors (Datry et al., 2017). Human activities can also lead to flow intermittency through, for example, land-use changes, flow regulation or water abstraction (Döll & Zhang, 2010; Döll & Schmied, 2012).

Streams that at times cease to flow at spatiotemporal scale along their course are called temporary streams (Acuña et al., 2014). Depending on surface water temporal permanence, temporary streams can be classified into two categories: intermittent and ephemeral streams (Datry et al., 2017; Figure 1). Intermittent streams periodically alternate flow and non-flow conditions; they present flow conditions when the water-table is above the streambed level and non-flow conditions when the water-table is significantly below the streambed level. Ephemeral streams generally have the water-level below the streambed and their flow periods occur only briefly during and following rainfall. Temporary streams account for a significant proportion of the total number, and length of the world's rivers. In river networks, headwater streams are generally temporary, and can account for more than 50% of the global fluvial network (Datry et al., 2014). At a global scale, temporary streams are predominant in many regions, and they are more abundant in hyper-arid, arid, semiarid, Mediterranean, and dry-subhumid regions than in other climatic regions (Bonada & Resh, 2013). However, climate change models project significant river flow decreases during summer months, especially in Mediterranean

climate areas. This decrease could cause flow regime shifts from permanent to intermittent, and longer non-flow periods in temporary streams with strong changes on fluvial ecosystems (Döll & Schmied, 2012; Marx et al., 2017).

Figure 1.- Example of flowing intermittent and non-flow ephemeral Mediterranean streams: a) the Onyar intermittent stream, and b) the Joanetes ephemeral stream.

The Mediterranean climate spreads aver the Mediterranean Basin, coastal California, central Chile, the Cape region of South Africa, and the southwest and southern parts of Australia (Kottek et al., 2006; Figure 2). The Mediterranean climate is defined as a warm temperate climate, with warm and dry summers, and cold and wet winters. The annual precipitation ranges from 300 to 900 mm, mostly occurring during winter. Mean winter temperatures are typically mild (7–13ºC), whereas mean summer temperatures are commonly hot (14–25ºC; Miller, 1983). Despite interannual variability in precipitation, which defines dry, normal and wet years, the Mediterranean climate is generally

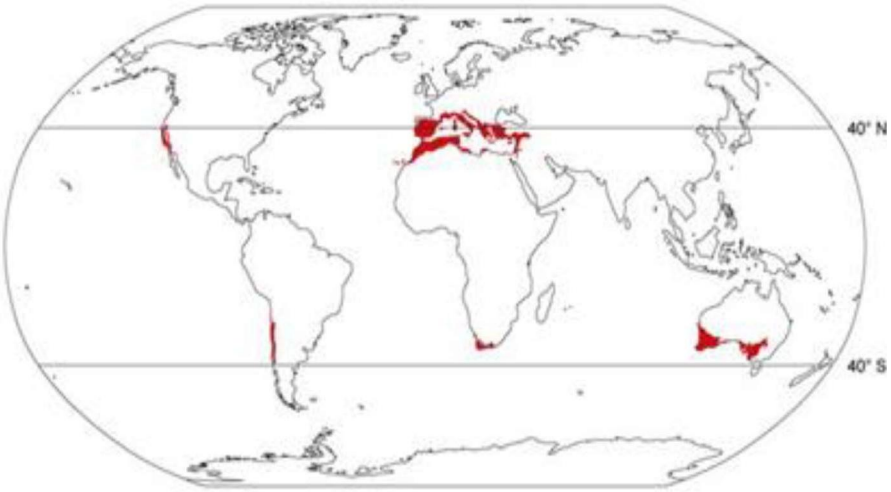


Figure 2.- Regions with a Mediterranean climate.

predictable and seasonal. Thus, during the dry season (i.e. summer), low precipitation and high evapotranspiration cause a decrease in the water-table level, which sometimes drops down below the streambed, potentially causing flow to cease.

During the non-flow period, aquatic habitats are generally reduced and disconnected (Boulton, 2003), creating a habitat mosaic with dry sediment and with or without connected or isolated pools. Under dry conditions, low moisture, direct effects of solar radiation and high streambed temperatures directly affect aquatic organisms (Timoner et al., 2012, 2014). In isolated pools, reductions in dissolved oxygen concentrations and pH shifts are some of the significant changes that occur (Gómez et al., 2017). These harsh environmental conditions typically increase in strength over time (Lake, 2003), and are a key selective force for streambed organisms (Stubbington et al., 2017). Continuous hydrological data is thus needed to characterize temporal patterns of non-flow periods (i.e. their duration, frequency, timing, magnitude and predictability), in order to analyse biotic response to intermittency (Datry et al., 2017), but are rarely collected on temporary streams (Zimmer et al., 2020). Recently, the number of studies on the effects of the temporal components of the non-flow period on aquatic biota has grown considerably, but they are still greatly outnumbered by studies categorizing the hydrological regime of temporary streams (i.e. intermittent or ephemeral); of the 154 articles used in this thesis related with temporary streams, 13% used temporal components of the non-flow period to analyse the biotic response to that disturbance (Figure 3).

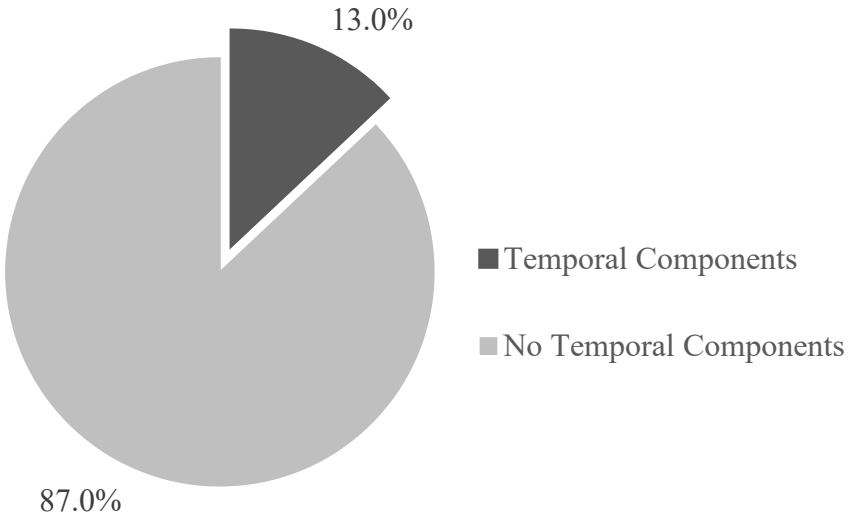


Figure 3.- Proportion of articles that analysed temporal components of the non-flow period versus those that categorized their hydrological regime.

b) The Biota of Temporary Streams

Organisms inhabiting temporary streams have developed different attributes or traits to avoid or not be affected (i.e. resist) the non-flow period (Bogan et al., 2017). A trait is here defined as a measurable life feature of an organism, used to characterize responses to environmental changes including community assembly, or the influence that organisms can have on ecosystem processes (Díaz et al., 2013; Violle et al., 2007). Temporary stream organisms possess multiple traits that can be classified as life-history, morphological, behavioural or physiological traits (Litchman & Klausmeier, 2008). Life-history traits are characteristics that affect the growth, reproduction, and survivorship of organisms. For example, some fishes and macroinvertebrates possess asexual reproduction or facultative dormancy (Bonada et al., 2007; Fenoglio et al., 2010). Morphological traits are related to the form and structure of organisms. For instance, microorganisms (e.g. bacteria, algae and cyanobacteria) produce resistant structures, such as cysts, spores, thickened cells or protective pigments that enable their survival during non-flow periods (Romaní et al., 2013; Timoner et al., 2014). Behavioural traits are related to the conduct of organisms facing conditions of stress, such as migration into the hyporheic zone or leaf packs as a refuge habitat by microorganisms or macroinvertebrates during dry conditions (Robson et al., 2008; Stubbington, 2012). Finally, physiological traits are constrained to a particular range of variation by the morphological traits but are more plastic and respond more quickly to environmental changes, such as flow intermittency. For instance, among photoautotrophic organisms cyanobacteria possess the ability to synthetize scytonemin, a specific pigment of this group, to protect them cells under dry conditions (Karsten & Holzinger, 2014; Takaichi, 2011). Since physiological traits, such as pigments, are one of the fastest responses to environmental changes, they could be used effectively to detect stressful conditions in the biofilm communities.

Biofilms are associations of heterotrophic and autotrophic microorganisms co-habiting in a matrix of polysaccharides, exudates and detritus (Sabater et al., 2016), colonizing multiple surfaces of streams and rivers. Stream biofilms are of particular relevance in temporary streams because of their diversity, abundance, and key role in ecosystem processes, driving biogeochemical cycles, processing and fuelling to higher trophic levels organic matter and nutrients (Battin et al., 2016; Sabater et al., 2007). The abundance and diversity of photoautotrophic (Cyanobacteria [blue-green algae], Chlorophyta [green algae], Bacillariophyta [diatoms] and Rhodophyta [red algae], Euglenophyta

[euglenophytes], Charophyta [charophytes]) and heterotrophic (bacteria, fungi, archaea, and protozoa) organisms depends on the substrate colonised and the environmental conditions. Epilithic biofilms (Figure 4a, c), grow on coarse substrates such as cobbles, and contain a greater proportion of photoautotrophic organisms (Romaní et al., 2008) than epipsammic biofilms (Figure 4b, d), which live attached to fine sediments such as sand and tend to be more heterotrophic (Timoner et al., 2012). The biotic differences are mainly attributed to the different physical conditions coarse and fine substrates offer. Inhabiting organisms in coarse substrates are more exposed to solar radiation than those who live in sand, promoting photoautotrophic growth under flow conditions. In contrast, fine sediments have a greater capacity to retain moisture and organic matter, but receive limited light, which promotes heterotrophic growth.

Generally, dry conditions associated to flow intermittency decreases the local (alpha) diversity and abundance of both autotrophic and heterotrophic organisms (Sabater et al., 2016; Stubbington et al., 2017). However, heterotrophic organisms may be more resistant

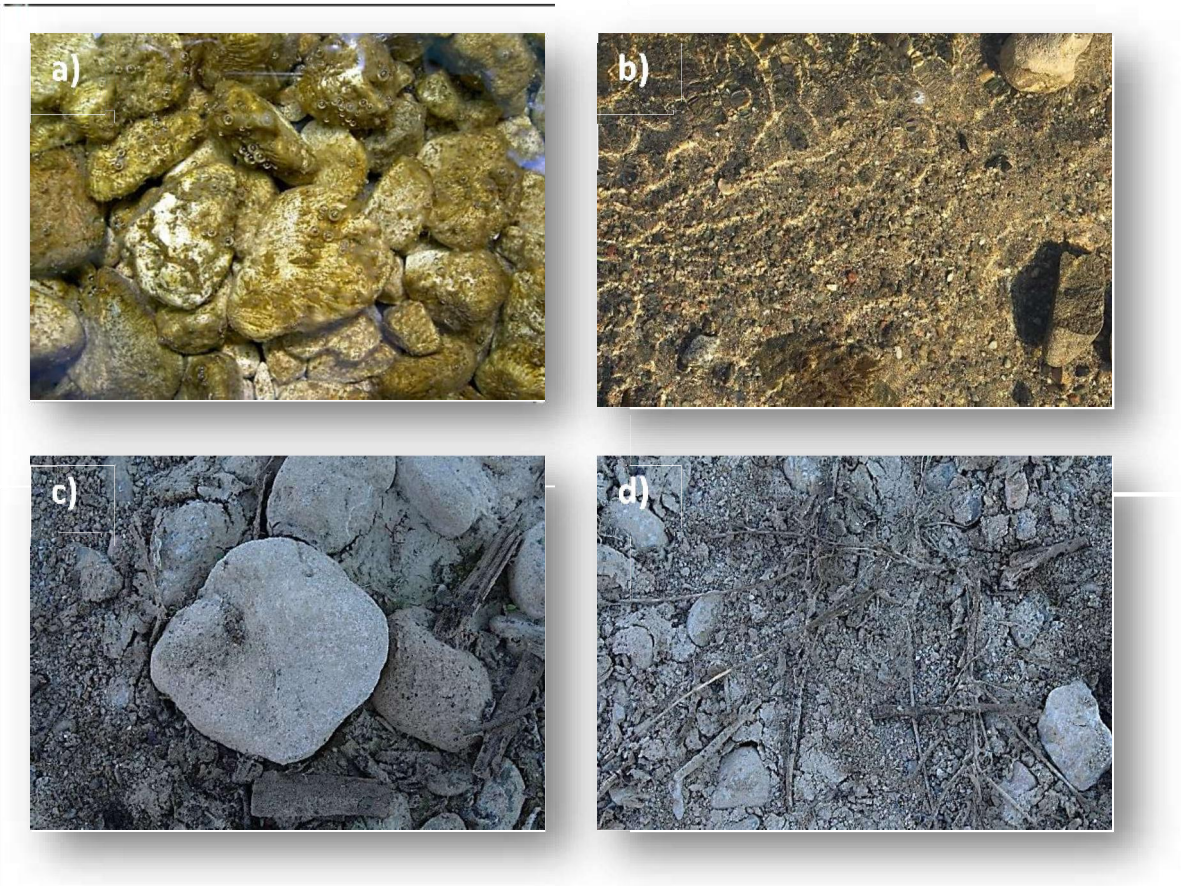


Figure 4.- Examples of epilithic and epipsammic biofilm. a) wet epilithic biofilm, b) wet epipsammic biofilm, c) dry epilithic biofilm, and d) dry epipsammic biofilm.

to dry conditions than photoautotrophic organisms (Timoner et al., 2012). It is now well established, from various studies, that the structural and physiological traits of certain groups and species make them better adapted to dry conditions than others. Diatoms, for instance, typically have low resistance to desiccation (Falasco et al., 2020; Tornés & Ruhí, 2013). However, some resistant diatoms, such as the genera *Cymbella or Gomphonema*, form stalks or tubes that host cells in protective filaments (Sabater et al., 2017), while others can migrate to deeper sediments to avoid desiccation (McKew et al., 2011). The rhodophyte *Hildenbrandia rivularis* or the chlorophyte *Gongrosira* resists long dry conditions due to their thick walls that confer them a high resistance to desiccation (Ledger et al., 2008). The genera *Oedogonium, Zygnema* and *Spirogyra* form resistant sexual eggs (zygospores) that remain dormant until flow returns (Sabater et al., 2016).

Generally, under dry conditions, algae and cyanobacteria reduce their chloroplasts size and their active chlorophylls start to degrade (Timoner et al., 2014). This decline in active chlorophylls, and the increase of their degradation products, may be accompanied by the synthesis of protective carotenoids (Takaichi, 2011). Protective carotenoids are produced in large quantities by algae and cyanobacteria under conditions of stress condition, such as dry conditions, to protect them cells (Belnap et al., 2004; Karsten & Holzinger, 2014; Timoner et al., 2014). There are two types of protective carotenoids: extracellular and intracellular (Sabater et al., 2017). Scytonemin is a protective pigment found in the extracellular polysaccharide sheaths of cyanobacteria, and protects cells from desiccation, high temperatures and solar radiation effects (Belnap et al., 2004; Timoner et al., 2014). Pigments such as echinenone, canthaxanthin, or myxoxanthophyll are found in green algae and cyanobacteria cells and protect photosynthetic apparatus and cell structures from harsh dry conditions (Karsten & Holzinger, 2014; Takaichi, 2011). Nevertheless, a systematic understanding of how temporal components of the non-flow period shape the biodiversity and abundance of biofilm communities and how this is reflected in pigment composition is still lacking.

c) Temporary Streams Metabolism

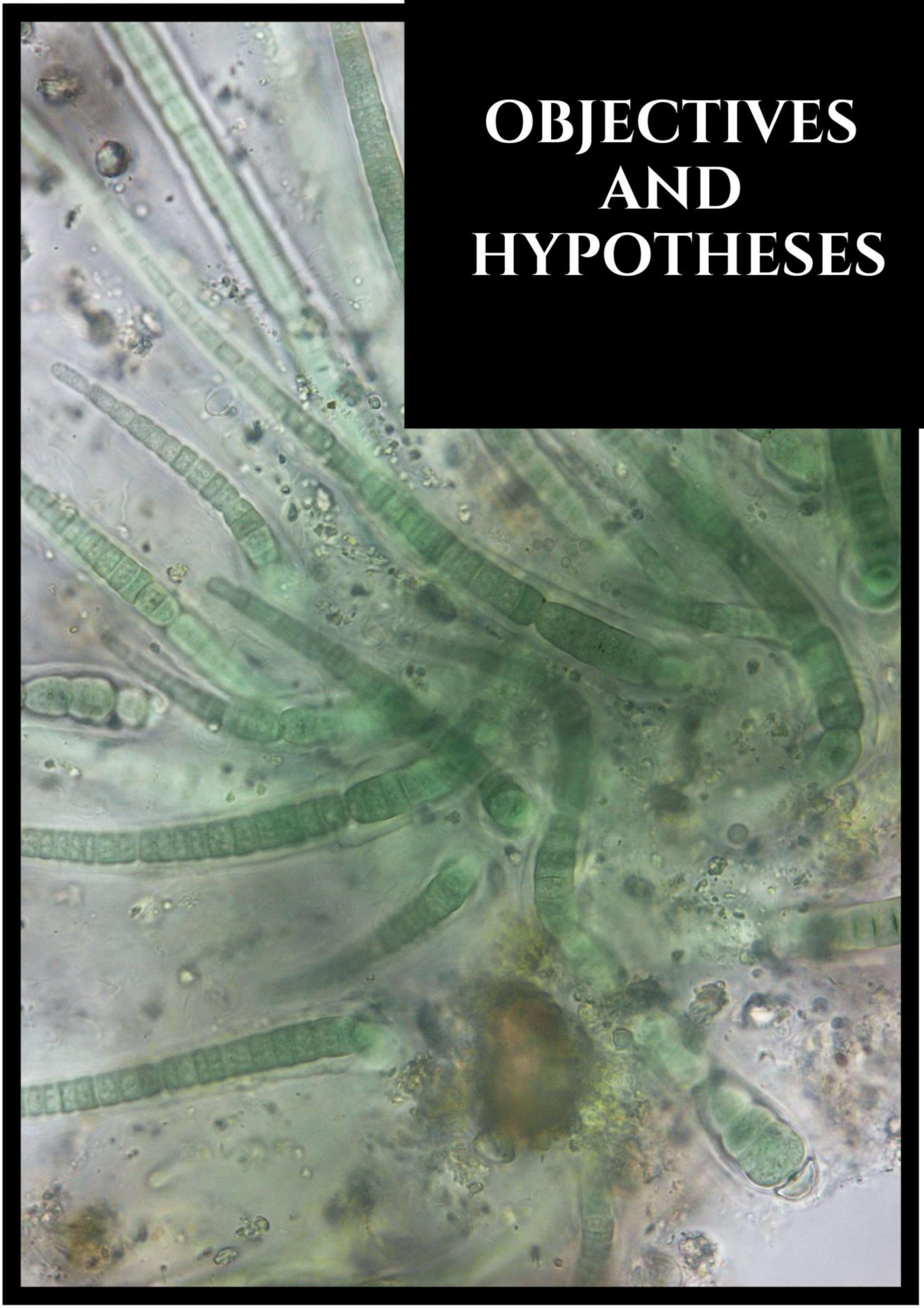
Alternation between flow and non-flow periods influences nutrient and carbon cycles in temporary stream ecosystems. During the non-flow period the transport of nutrients and materials ceases (von Schiller et al., 2017; von Schiller et al., 2011) and a diversity of substrates may accumulate on the dry streambed (Datry et al., 2018). For example, plant

litter is slowly accumulated and decomposed by photodegradation, heterotrophic microbes and invertebrate shredders (Austin & Vivanco, 2006; Foulquier et al., 2015), whereas stream biofilm may accumulate large quantities of bioavailable organic carbon and nitrogen due to the exudates and products of cell lysis (Romaní et al., 2017). Yet, there is an important knowledge gap regarding the temporal variability of the non-flow period and the implication for the ecosystem functioning, especially under dry conditions, (Gómez-Gener et al., 2016).

Stream biofilms are the main biotic group responsible for the metabolic pathways sustaining ecosystem functioning because of the multiple taxonomic kingdoms that they integrate (i.e. prokaryotes and eukaryotes; Sabater et al., 2007). Biofilms are at the base of stream food webs; their metabolic rates, gross primary production (GPP) and community respiration (CR), are important determinants of ecosystem biomass, trophic structure, and carbon and nutrient cycles (Tank et al., 2010). GPP represents the organic matter produced by photoautotrophic organisms within the fluvial ecosystems through photosynthesis. CR is a proxy for the total consumption of organic matter by autotrophic and heterotrophic organisms supplied from within (autochthonous) and outside of (allochthonous) an ecosystem. Biofilm metabolic rates are strongly correlated with flow intermittency (Sabater et al., 2016). Throughout the flow period, GPP is mainly defined by light and nutrient availability, and in Mediterranean climates there are two favourable periods for GPP. The most relevant period is in spring, because of the long daylight hours and scarcity of leaves on trees. The second period is in autumn, when leaves start to fall, and environmental conditions are still favourable (Romaní et al., 2013). However, floods are common in autumn, which can negatively affect biofilm development and functioning (Ylla et al., 2007). Conversely, biofilm metabolism is negatively affected by the harsh dry conditions during non-flow periods (Acuña et al., 2015). The ability of photoautotrophic organisms to carry out photosynthesis (photosynthetic efficiency) is positively correlated with moisture; under dry conditions, chlorophyll-a abruptly decreases, and consequently GPP immediately falls (Acuña et al., 2015; Timoner et al., 2012). In contrast, the heterotrophic component of stream biofilms has a higher capacity to withstand dry conditions, which increases the CO_2 released from these dry habitats by means of community respiration (von Schiller et al., 2014). At a temporal scale, Acuña et al. (2015) found that GPP and CR responded differently to increases in the non-flow period duration. Shifting balances between GPP and CR could reflect changes in the

biogeochemistry of temporary streams, which can in turn affect higher trophic levels. However, how biofilm metabolism behaviour reacts to the duration and frequency of non-flow periods in natural ecosystems has not yet been investigated in detail.

OBJECTIVES AND HYPOTHESES



2) Objectives and Hypotheses

The overall **objective** of this thesis was to investigate the effects of temporal components of the non-flow period on stream biofilms, focusing on both the structure and functioning of their photoautotrophic community, in order to understand and predict global change effects on fluvial ecosystems. More specifically, the purpose of this research was to answer the following questions:

- **i.** How do duration and frequency of the non-flow period affect biofilm functioning? (Paper I)
- **ii.** How do duration and frequency of the non-flow period affect the physiological response and composition of the photoautotrophic community under dry and wet conditions? (Paper II)
- **iii.** What environmental variables and structural factors determine biofilm functioning? (Paper III)
- **iv.** Does the resistance and resilience of biofilms to the non-flow period differ between permanent and temporary streams? (Paper IV)

In Paper I, I tackle the question of how temporal components of the non-flow period affect the GPP and CR of biofilm communities. I incubated dry streambed cobbles from different streams origins under standard conditions and analysed their response, according to the preceding hydrological conditions. In Paper II, I investigated the pigment composition of biofilms from different stream origins, to characterize responses in physiology and community composition to current flow status and stream's hydrological history. In Paper III, I looked into the causal relationships of community diversity and physiological status with GPP mediated by the temporal components of the non-flow period. I analysed the photoautotrophic community composition and, together with the data from Paper I and Paper II, I performed a structural equation model to analyse the causal relationships. In Paper IV, I exposed stream biofilms from permanent and temporary streams to the same durations of non-flow (i.e. dry conditions) and subsequent flow return in artificial stream channels. Resistance and resilience of both biofilm from both stream types were assessed at structural and functional level.

The main **hypothesis** I aimed to test was that the structure and functioning of stream biofilms would reflect temporal variability in the non-flow period in temporary fluvial

ecosystems. I also expect that the non-flow period would act as an environmental filter that generates important differences between permanent and temporary stream biofilms.

Particularly, I aimed to test the following hypotheses:

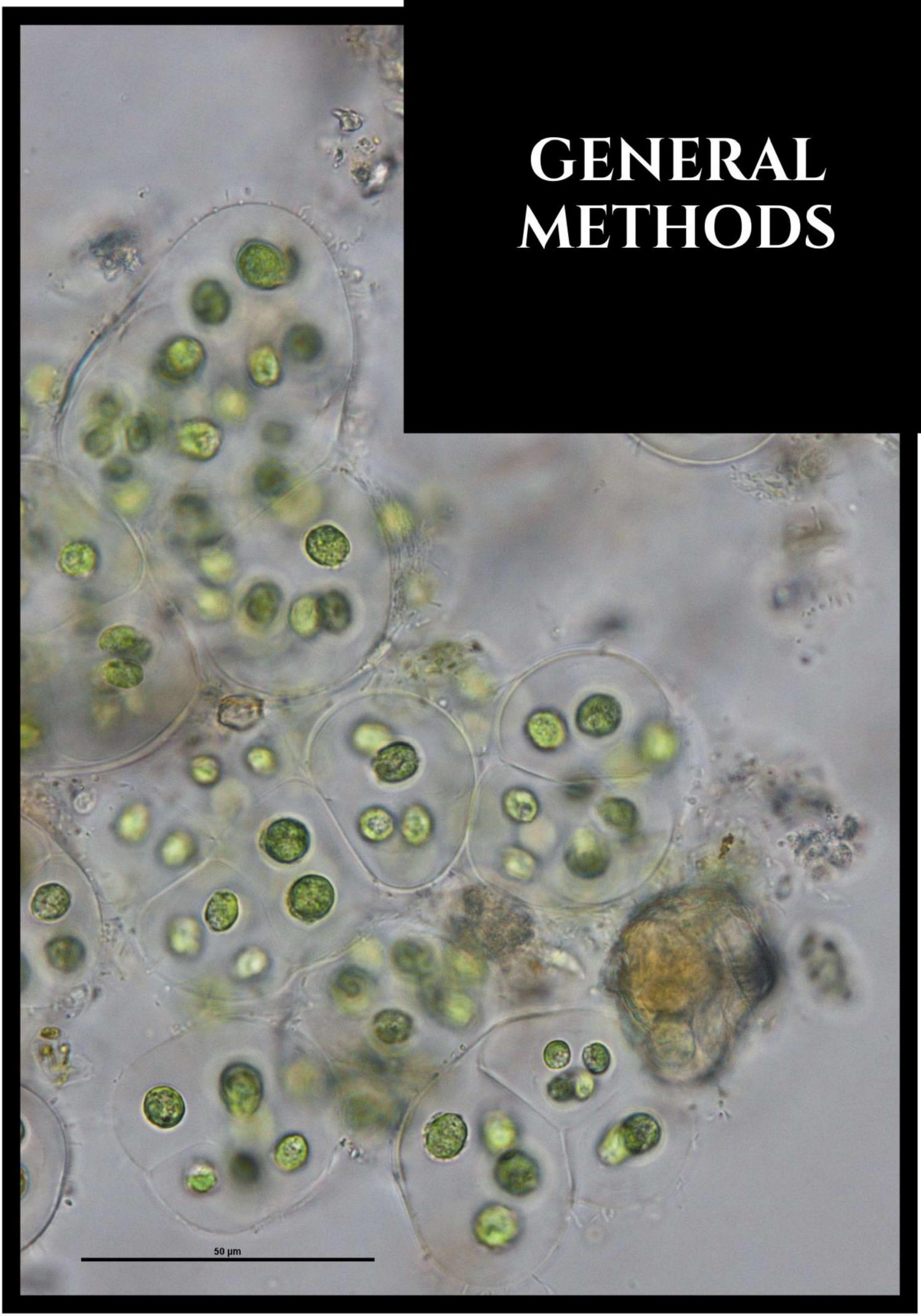
i. Longer and more severe non-flow periods reduce GPP and CR, although the effects are more pronounced for GPP because of its lower resistance to dry conditions.

ii. Longer and more severe non-flow periods produce greater changes in community physiology and composition of stream biofilms and extend their recovery periods after flow returns.

iii. The structure and physiology of photoautotrophic community varies as a function of non-flow duration; which is reflected in gross primary production.

iv. Permanent stream biofilms are less resistant and resilient to non-flow periods because of their poor adaptation to dry conditions because their hydrological history.

GENERAL METHODS



3) General Methods

This section provides a brief summary of the study site characteristics and the materials and methods used for the development of this thesis (Table 1). Detailed information on particular methods are provided in each of the chapters. The section is focused on methods to characterize the structure and functioning of stream biofilms, as it is a cross-cutting theme in the four papers that constitute the core of the thesis.

a) Study Sites and Hydrological Characterization

The work included in this thesis explored permanent and temporary stream biofilms, from Mediterranean Basin streams. To test my main hypothesis, the four papers of this thesis encompass two approaches; research for Papers I, II and III was carried out over the 2016 in the field, whereas Paper IV was conducted in mesocosms.

i) Field Work

The first three papers of this thesis derive from a field study conducted over a one-year period in 10 permanent and 23 temporary (n = 33) streams (Figure 5) across nine basins located in the North East of the Iberian Peninsula (Muga, Ter, Fluvià, Tordera, Besos, Llobregat, Foix, Francolí, and Ebre basins). Stream order range from 2 to 5, and their flow regime ranged from permanent to ephemeral. The streams sub-basins were mostly dominated by forests (Fr), followed by shrublands and grasslands (S), non-irrigated agricultural fields (A), irrigated agricultural fields (IA), and urban and industrial cover (U). Studied sub-basins included a range of mid-mountain altitudes, precipitation levels, and catchment areas; all of them were influenced by Mediterranean climate types, with a distinctly warm and dry summer season. The mean annual precipitation of study sub-basins ranges from 428 to 1093 mm, most of which falls during winter. Streams were selected to represent a range of non-impacted Mediterranean-climate streams with permanent to ephemeral water flow (Figure 6).

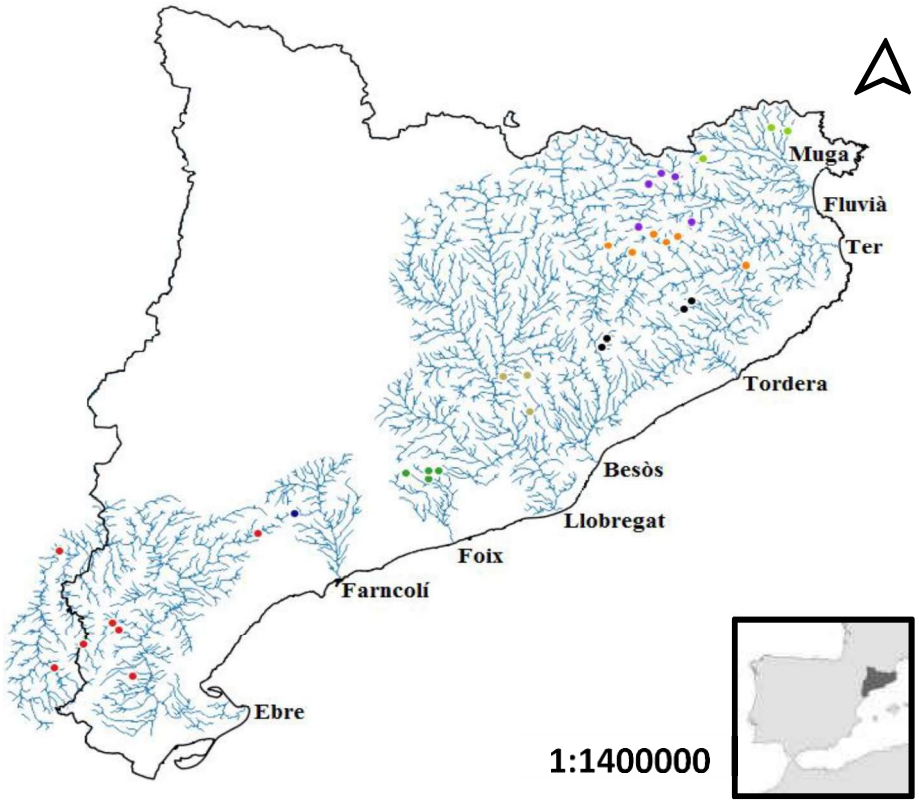


Figure 5.- Location of the sampling sites across nine basins (North-East of the Iberian Peninsula).

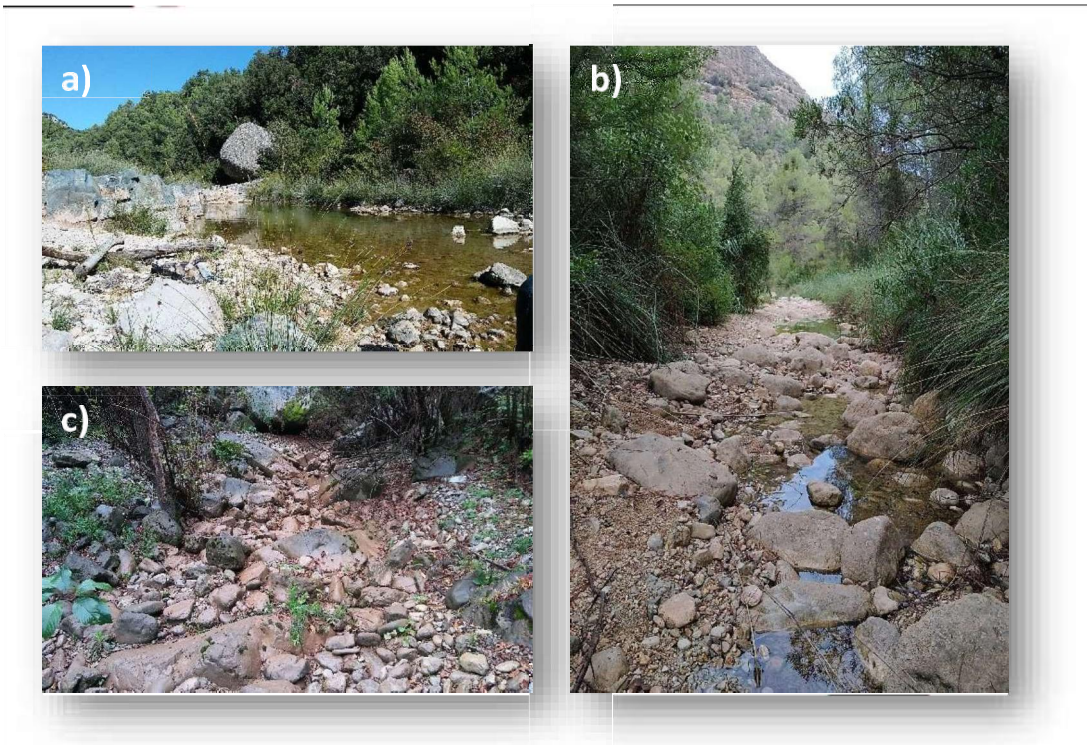


Figure 6.- Examples of sampling sites. a) the Siurana, a permanent river (Ebre basin), b) the Riera de la Vall d'Uixo, an intermittent stream (Ebre basin), and c) the Llierca, an ephemeral stream (Ter basin).

All studied streams were continuously monitored to characterize their daily hydrological conditions (i.e. flow or dry conditions), as well as environmental conditions (i.e. solar radiation and streambed temperature). Based on previous information and that provided by the monitoring, streams were classified according to their hydrological regimes in permanent or temporary. Temporal components of the non-flow period were characterized as the frequency and duration of the non-flow periods 30, 60, 90, and 150-d before each sampling campaign. Two sampling campaigns were performed, one in summer, when most temporary streams had non-flow conditions, and one in autumn, when flow had returned to some temporary streams. During the summer sampling campaign, epilithic biofilm samples were collected to analyse their functioning (Paper I) and structure (Paper II and III) in the autumn sampling campaign, samples were collected to analyse epilithic biofilm structure (Paper II).

ii) Mesocosms Experiment

Four permanent and four temporary streams were selected to carry out research presented in Paper IV of this thesis. Colonized cobbles with intact biofilms were collected at the beginning of autumn 2016 and transported to the laboratory (Figure 7a). The cobbles were immediately immersed in artificial streams of the Catalan Institute for Water Research indoor Experimental Stream Facility. A total of eight artificial streams were used, each containing cobbles from one stream (Figure 7b). After 7-d acclimatization period, the non-flow period started in all artificial streams; after 31 dry days, flow was simultaneously returned to all artificial streams for a 20-d period (Figure 8b).

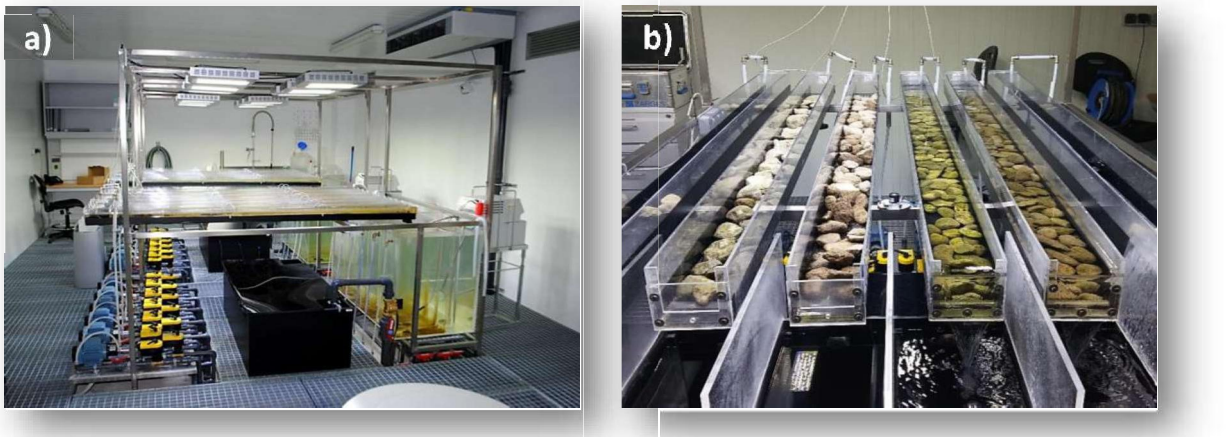


Figure 7.- (a) Experimental Stream Facility; (b) cobble distribution in the artificial streams.

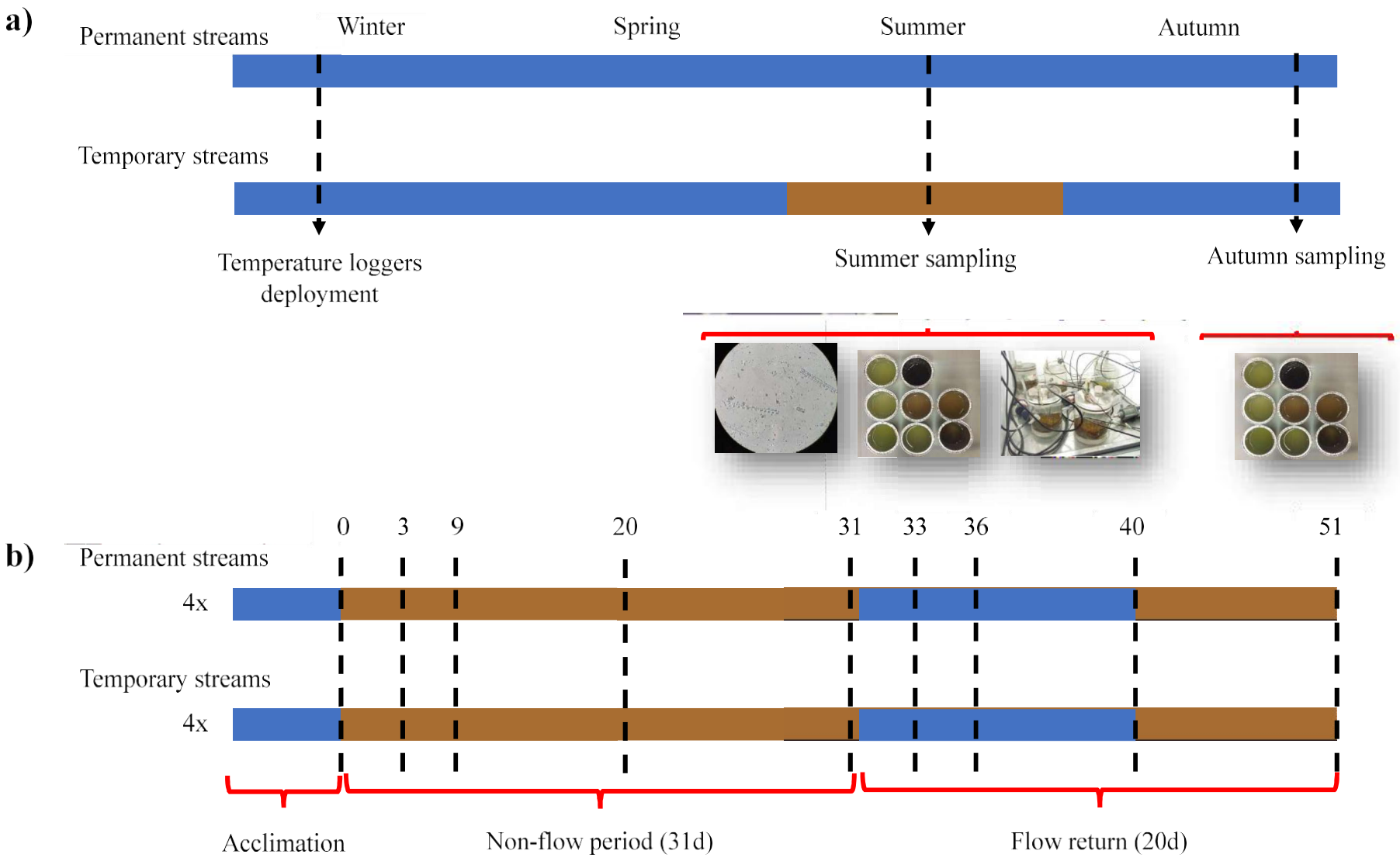


Figure 8.- (a) Field campaign design and measured parameters; (b) mesocosms experiment design.

b) General analytical methods

i) Biofilm Functional Parameters

- *Biofilm metabolism*

Community Respiration (CR) and Net Metabolism (NM) were assessed through changes in oxygen concentrations under light and dark conditions, respectively. Stream cobbles were placed inside cylindrical chambers, each fitted with a submersible water circulation pump and oxygen logger (PreSens OXY-10mini, Regensburg, Germany). Dissolved oxygen was continuously logged for 1 hour, under standard conditions of light and temperature (Figure 9). After the incubation, metabolic rates were calculated according to Acuña et al. (2008).

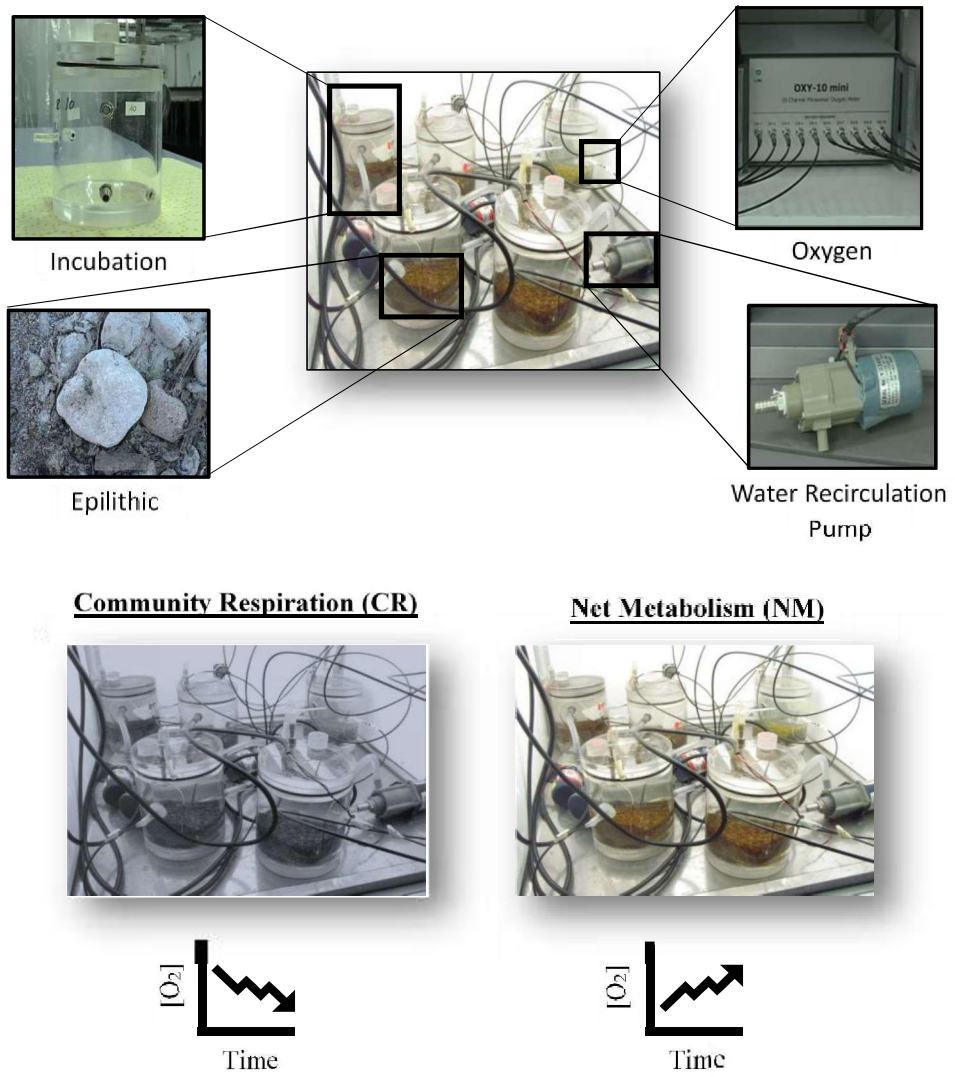


Figure 9.- Schematic view of the incubation process employed to determine biofilm metabolic rates.

- *Photosynthetic activity (Y_{eff})*

The efficiency of energy conversion within photosystem II (PSII) reaction centres was measured by means of pulse-amplitude modulated (PAM) fluorometry using a DIVING-PAM fluorimeter (Diving-PAM; WALZ, Effeltrich, Germany).

ii) Biofilm Structural Parameters

- *Ash-Free Dry Weight (AFDW)*

AFDW was used as a proxy of biofilm biomass. Each surface cobble was scraped to remove the biofilm, then the biofilm suspension (Figure 10) was dried (60ºC, to constant weight), weighted, combusted (450ºC, 4 h), and reweighed (Elosegi & Sabater, 2009).

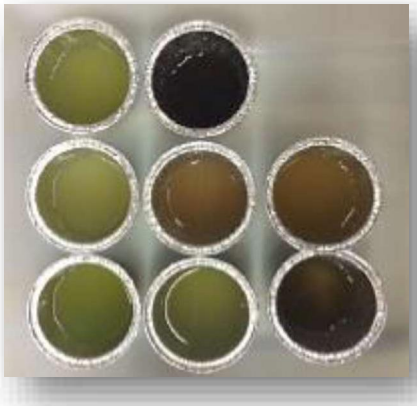


Figure 10.- Biofilm suspension before drying out.

- *Pigment analysis*

Each surface cobble was scraped to detach the biofilm, then the biofilm suspension was centrifuged, the supernatant removed, and remaining pellet was immediately frozen, then lyophilised and pigments extracted with 90% v/v acetone (4ºC, 12h). The analytical method used to total chlorophyll-*a* analyse was performed using spectrophotometer and the chlorophylls and carotenoids using high liquid performance analysis (HPLC), specifically:

Total chlorophyll-a (Chl-*a*) was used as a surrogate of photoautotrophic biomass. Total Chl-*a* concentration was estimated spectrophotometrically according to Elosegi & Sabater, (2009).

Chlorophyll and carotenoid composition and abundance analyses were used as a proxy of the physiological status and composition of stream biofilm communities. Biofilm pigments were determined using HPLC analysis (Figure 11) through the eluent gradient described by Buchaca & Catalan (2007), using a Waters HPLC 510 and Waters

Figure 11.- High liquid performance analysis (HPLC).

Photodiode array detector 996 (Waters) on a C18 column (5 µm, 250 × 4.6 mm, Spherisorb, ODS 1 Waters).

- *Community composition*

Photoautotrophic community composition was determined at the genus level using a light microscope (Nikon CS1, Tokyo, Japan), following the classification described by Wehr, Robert, & Kociolek (2015) Figure 12).

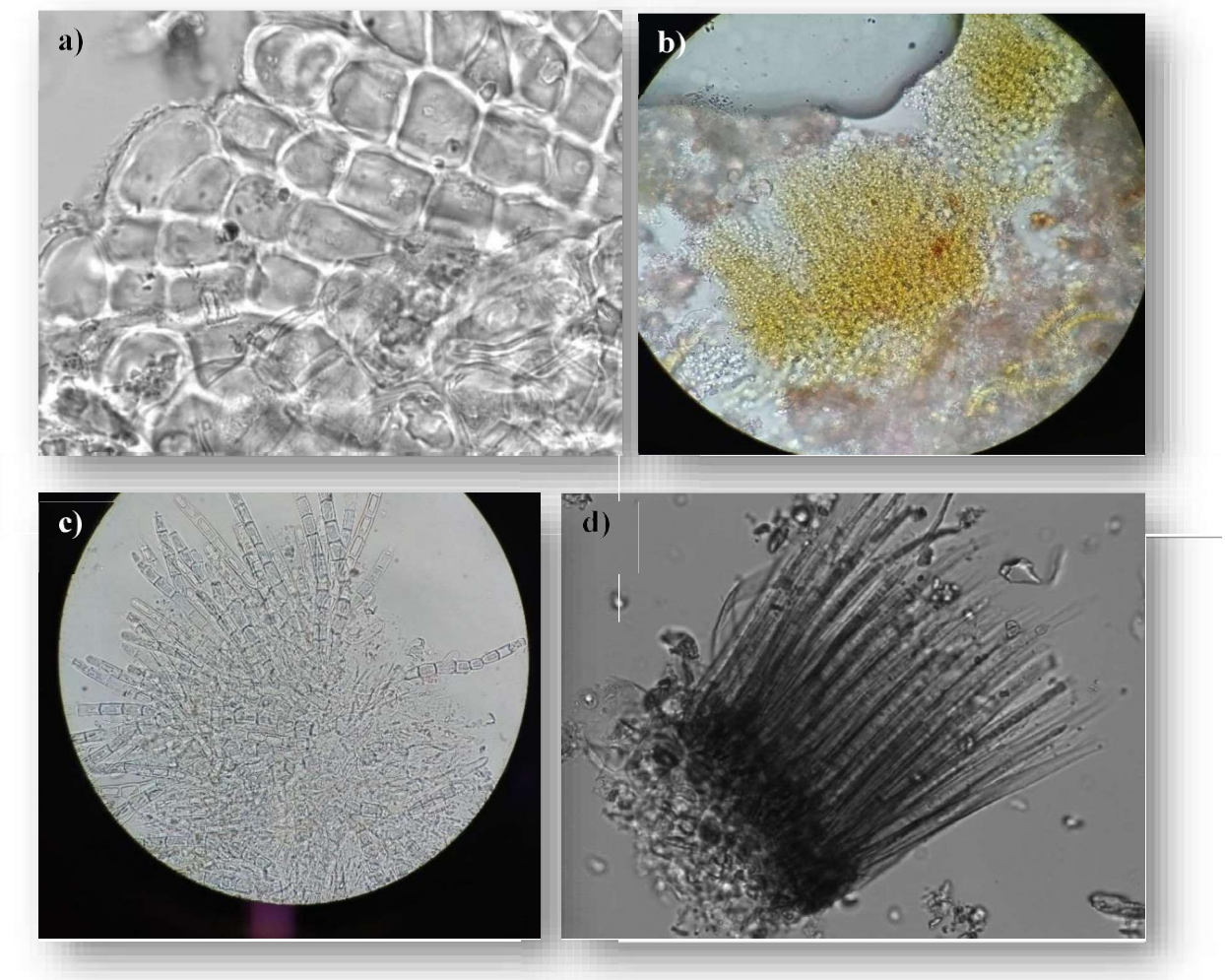
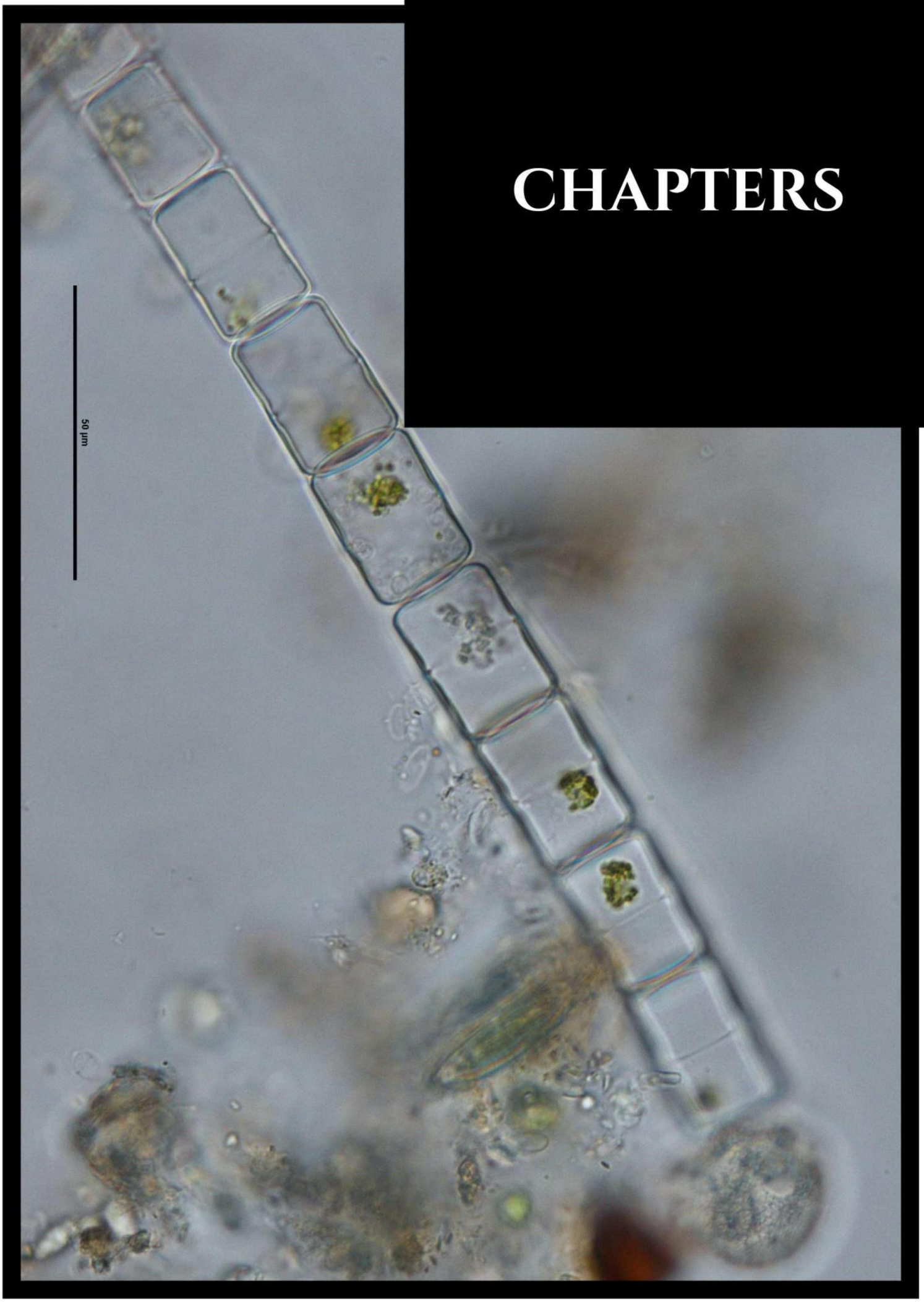


Figure 12.- Examples of photoautotrophic organisms identified in biofilm communities. a) Herbaudiella; b) Oncobyrsa; c) Audouinella; d) Rivullaria.

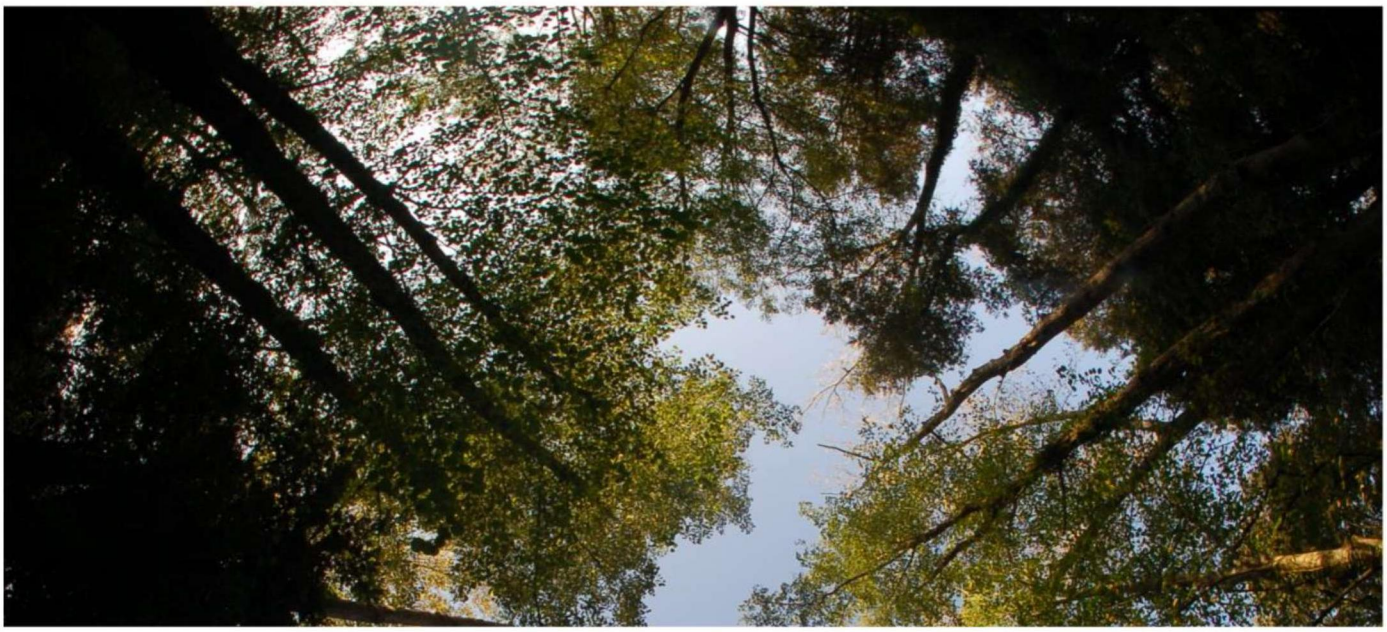
Table 1.- Summary of the methods used in this thesis.

	Field work			Mesocosms experiment
	Paper I	**Paper II**	**Paper III**	**Paper IV**
Hydrological characterization				
Biofilm metabolism				
Photosynthetic activity				
Ash-free dry weight				
Total chlorophyll-*a*				
Pigment composition				
Community composition				

CHAPTERS



Effects of Duration, Frequency, and Severity of the Non-flow Period on Stream Biofilm Metabolism



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Effects of Duration, Frequency, and Severity of the Non-flow Period on Stream Biofilm Metabolism

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Abstract

Temporary streams make up the majority of river networks in many regions around the world. While they are known to have non-flow periods, it is uncertain in what ways the temporal components of the non-flow period affect stream ecosystems. We analyzed how duration and frequency of the non-flow period influence the biofilm metabolism of 33 Mediterranean streams in NE Iberian Peninsula. Selected streams ranged from perennial to ephemeral, and their hydrology was characterized during a period of 150 days before the sampling. Cobbles were collected from the streams, for which the total biofilm biomass (ash-free dry mass and chlorophyll-*a*) and metabolism (Community Respiration and Gross Primary Production) were measured. Metabolic differences were observed between both permanent and temporary streams, as well as within temporary streams. Among these, the frequency of the non-flow period did not affect biofilm biomass or metabolism, but the duration did significantly decrease autotrophic biomass and Gross Primary Production. Severity of the non-flow period (solar radiation and maximum streambed temperature) also affected Gross Primary Production negatively. Thus, 80% of the observed Gross Primary Production variability among all temporary streams was explained by the total duration and the severity of the non-flow period. In contrast, Community Respiration in the streams was not affected by the temporal components of the non-flow period. Our results highlight the effects of different temporal components of the non-flow period on autotrophic and heterotrophic processes, indicating that longer durations of the non-flow period or high severity conditions might decrease Gross Primary Production promoting heterotrophy.

Keywords: temporary streams, duration, frequency, metabolism, severity, Gross Primary Production, Community Respiration

Introduction

Temporary streams are watercourses that cease to flow at some point in space and time along their course (Acuña et al., 2014). They are common in headwaters throughout the World, especially in temperate and dry regions (Döll & Schmied, 2012; Raymond et al., 2013) but their occurrence is growing as a consequence of Climate Change and increasing water abstraction (Döll et al., 2018; Marx et al., 2017) . These factors are causing permanent streams to start exhibiting non-flow periods and temporary streams to have longer and more frequent non-flow periods. The changes being produced are indeed relevant, for they have an impact on the ecological and societal values associated to temporary streams (Acuña et al., 2017; Steward et al., 2018).

It is well known that non-flow periods in temporary streams produce changes in the structure and function of stream communities. These can include changes such as decreases in invertebrate community abundances, as well as a reduction of the taxa richness (White et al., 2018; Wood et al., 2005) with higher presence of eggs, cysts or dormancy states (Williams, 1998); the reduction of extracellular enzyme and the slowdown of metabolism (Timoner et al., 2012) with the formation of spores, dormant cells, and protective carotenoids in the stream biofilm (Romaní et al., 2013; Timoner et al., 2014); or the decrease of available fresh C and N organic matter sources (Ylla et al., 2010) during the transition between the flow and the non-flow periods. However, temporary streams can include a wide variability of hydrological regimes, alternating between non-flow and flow periods. In these systems, the non-flow period occurs as a ramp disturbance that steadily increases in strength over time (Lake, 2003), in spite of the fact that non-flow periods may appear with a variety of durations and frequencies. Regardless of this variability, most studies so far have assessed the effects of non-flow periods without accounting for their temporal components, frequency and duration.

Datry (2012) and Schriever et al. (2015) showed that an increase of the duration of the non-flow period led to a decrease in both benthic and hyporheic macroinvertebrates density and taxonomic richness. Jaeger et al. (2014) showed that prolonged non-flow periods compromised fish populations because of the loss of habitat availability, and the consequent heightened risk of competition, promoting high local extinction probabilities and ultimately dampening metapopulation persistence and community structure. Acuña et al. (2015) identified that the increase of the duration of the non-flow period in a set of

artificial channels promoted biofilm heterotrophy. Finally, Datry et al. (2018) have recently highlighted the important roles of aridity, canopy cover, channel width and duration of the non-flow period on the amount and decomposability of plant litter. Overall, the non-flow period imposes evolutionary pressures that constrain stream communities and select species attributes (Poff & Ward, 1990); however, the spatiotemporal manifestation of these periods and their influence on ecosystem processes is still rather unknown (Acuña et al., 2017; Jaeger et al., 2014). Additionally, when water ceases to flow, and streambeds become completely dry, high air temperatures and the direct incidence of solar radiation can increase the harshness of the abiotic conditions on streambed. These two variables describe what is here understood as the "severity" component of the non-flow period, which may contribute to non-flow period's impact (Closs & Lake, 1994, 1996; Timoner et al., 2014). The combination of the temporal components and the severity of the non-flow period could also have further effects on the structure and functioning of the streambed communities.

We investigated the effects of the temporal components of the non-flow period (duration and frequency) and the severity of the non-flow period on stream biofilm. Specifically, we measured biofilm biomass and metabolism, because of its important role in organic matter dynamics and in the nutrient cycle in streams. Our hypotheses were: (i) Gross Primary Production (GPP) and Community Respiration (CR) would be higher in biofilms from permanent streams than those from temporary streams, due to the fact that temporary streams must endure non-flow periods that cause structural effects which probably affect biofilm metabolism; (ii) longer durations and higher frequencies of non-flow periods would reduce both GPP and CR, although the effects would be lower on CR because of its higher resistance to the non-flow period (Acuña et al., 2015), and (iii) high air temperature and the direct incidence of solar radiation during the non-flow period would exacerbate its effect on stream biofilms.

Material and Methods

Study Area and Streams

A total of 33 permanent and temporary streams distributed across nine basins in the NE Iberian Peninsula were selected for this study. Catchments were mostly dominated by forests (F) (PI. S. Table 1), followed by shrublands and grasslands (S). None of the less

common land uses, such as non-irrigated agricultural fields (A), irrigated agricultural fields (IA) and urban and industrial cover (U), showed important levels of anthropogenic impact in any of the studied catchments. The selected streams had orders from 2 to 5, and their flow regimes ranged from ephemeral to permanent. The study sites included a range of mid-mountain altitudes, mean precipitations, and catchment areas (PI. Table 1); all of them had a Mediterranean climate, with a distinctly warm and dry summer season. The annual precipitation in these streams ranges from 428 to 1093 mm, most of which falls during winter storms. Mediterranean climates have high seasonality and predictability (Cid et al., 2017; Tonkin et al., 2017). In these systems, summer rainfalls may not compensate water surface loss, causing them to stop flowing. We therefore adapted the sampling periods to midsummer (July 1st to August 1st), when most temporary streams experience the non-flow period.

PI. Table 1.-Information of the studied streams.

Code	GPS coordinates	Altitude	Precipitation	Catchment area	Stream
	WGS84	m	mm	km^2	
LL 01	41°35'0.57"N 1°59'5.63"E	328	732.00	10.99	T
LL 02	41°41'43.67"N 1°53'33.00"E	258	634.54	23.96	T
LL 03	41°41'46.46"N 1°59'1.67"E	468	692.00	8.68	T
BE 04	41°46'1.10"N 2°16'11.98"E	430	788.40	6.50	T
BE 05	41°47'35.79"N 2°17'29.77"E	540	748.40	19.35	T
FO 06	41°25'7.63"N 1°30'26.44"E	620	568.50	1.65	T
FO 07	41°25'13.53"N 1°35'32.05"E	422	578.12	1.05	T
FO 08	41°25'17.08"N 1°38'0.76"E	330	574.00	1.89	T
FO 09	41°23'52.63"N 1°35'37.29"E	320	590.07	26.87	T
TO 10	41°51'54.36"N 2°38'45.96"E	156	908.25	12.44	T
TO 11	41°51'55.61"N 2°35'35.62"E	290	874.60	49.06	P
TE 12	42° 5'20.95"N 2°35'18.08"E	385	1013.31	13.41	T

TE 13	42° 2'52.10"N 2°24'38.68"E	920	1063.67	3.71	P
TE 14	42° 4'18.70"N 2°32'29.11"E	386	1038.10	9.17	P
TE 15	42° 4'39.69"N 2°20'19.63"E	632	953.41	30.99	P
TE 16	41°59'14.51"N 2°50'15.69"E	81	815.88	7.71	T
TE 17	42° 6'34.82"N 2°29'19.67"E	530	963.42	13.32	T
FR 18	41°18'38.37"N 1° 5'2.51"E	583	469.82	28.93	P
FL 19	42°16'43.47"N 2°32'31.18"E	379	1017.41	42.63	T
FL 20	42°16'3.60"N 2°35'33.93"E	254	1043.01	172.23	T
FL 21	42° 7'37.99"N 2°38'26.65"E	266	997.71	13.78	T
FL 22	42° 7'28.45"N 2°26'29.72"E	476	1026.25	15.72	T
FL 23	42° 6'51.11"N 2°26'53.48"E	475	965.43	34.87	T
FL 24	42°14'56.07"N 2°29'30.32"E	496	962.86	6.26	P
MU 25	42°19'1.78"N 2°42'10.70"E	258	1093.24	44.93	P
MU 26	42°23'15.61"N 3° 3'6.24"E	105	841.83	13.53	T
MU 27	42°23'6.91"N 3° 1'59.28"E	88	868.00	48.69	T
EB 28	41°15'24.80"N 0°56'34.58"E	487	427.92	34.99	P
EB 29	40°52'11.42"N 0° 9'36.60"E	492	667.83	207.51	P
EB 30	40°50'6.20"N 0°27'9.60"E	85	709.63	29.17	T
EB 31	41° 0'5.81"N 0°23'4.08"E	225	561.01	70.51	T
EB 32	40°56'20.21"N 0°16'26.38"E	415	638.87	116.46	P
EB 33	41°13'30.33"N 0°11'51.45"E	170	540.29	1035.67	P

Hydrological Characterization and Flow Intermittency Metrics

Because water presence is strongly related with streambed temperature (Constantz et al., 2001; Stromberg et al., 2005), we used the daily variation in streambed and air temperatures to determine water presence in each sampling site's streambed. Streambed temperature in each sampling site was recorded at 30 min intervals using VEMCO Minilog (TR model, AMIRIX Systems Inc, Halifax, NS, Canada) temperature data loggers (5–35ºC, ± 0.2ºC). Before their deployment, all temperature data loggers were placed in a temperature controlled water bath to ensure their accuracy. Bath water temperature was successively adjusted to 5, 10, 15, 20, and 25ºC using a Cryo-Compact Circulator (Julabo CF-31, Seebach, Germany). Differences between measurements from the temperature data loggers and the water bath temperature averaged 0.095ºC, with maximum deviations of 0.4ºC. Therefore, no correction factor was applied to the data derived from the temperature data loggers, however they were grouped based on shown similarity and assigned to respective study streams. The temperature data loggers were placed inside protective stain less steel casings (c. 2.5 kg), which have shown minimal influence on instantaneous temperatures (± 0.1ºC) (Malard et al., 2001). Temperature loggers were deployed in all streams on the bottom of riffle areas. Air temperature of each studied stream was obtained from temperature data loggers that had been previously installed in the riparian zone, or from nearby meteorological stations (Servei Meteorològic de Catalunya; http://www.meteo.cat/). Furthermore, water level sensors (Solinst level-logger, Edge, Model 3001) were placed in 9 of the 33 sites, which provided data on water level and temperature. Once data had been obtained, the daily variations in streambed and air temperatures were characterized by two ratios: the daily streambed-to-air temperature amplitude ratio (DA) and the streambed-to-air temperature change rate ratio (that is, heating or cooling RTC). Both ratios were based on the relationship between the daily variations in streambed and air temperature; however, to reinforce results, each one assessed a different aspect of the temperature oscillations. DA was determined by the difference between the maximum ($\max(T)$) and the minimum ($\min(T)$) temperatures in the streambed and the air during a whole day and was calculated as follows:

PI. Equation 1.- Daily streambed-to-air temperature amplitude ratio.

$$DA_i = \frac{(\max(T) - \min(T))_{streambed}}{(\max(T) - \min(T))_{air}}.$$

RTC was determined as the ratio between the highest hourly temperature change rate in the streambed and the air, in each sampling site. Thus, to obtain RTC, it was necessary to calculate hourly temperature change rates ($|\Delta T|$) and to select the maximum rate of change ($max|\Delta T|$) for each day and at each site, in both the streambed and the air. Daily RTC was then calculated as follows:

PI. Equation 2.- Ratio of the streambed-to-air temperature change rates.

$$RTC_i = \frac{\max(|\Delta T|)_{streambed}}{\max(|\Delta T|)_{air}}.$$

Despite using both ratios, the occasional similarity between streambed and air temperature (particularly during autumn) could lead to erroneous interpretations of the stream's flow or non-flow state. This issue was overcome by considering the preceding and following 2 days. Thus, a moving average of the DA and RTC ratios was calculated, comprising a total of 5 days. This allowed dampening extreme values and achieving the best fit to water level data. The output value was named hydrological status (HS) and was given by:

PI. Equation 3.- Hydrological status.

$$HS_i = \frac{1}{10}\sum_{i=j-2}^{j+2}(DA_i + RTC_i).$$

Each daily HS value was standardized and classified as a flow status (FS), by making the following determinations: (i) when the value of HS was higher than a fixed threshold (see below), the studied stream was considered to have non-flow conditions (symbolized with a 1); and (ii) when the value was lower than the established threshold, the studied stream was considered to have flow conditions (symbolized with a 0). Threshold values were fixed previously on a monthly basis, based on the calibration of FS values against data from the 9 water level sensors distributed across the 9 studied basins, with an aim to check the goodness of fit of our method. The calibration was performed according to mean least squared error (MLSE) and R^2 values, resulting in an 81% correlation with the water level data. Departing from FS data, we characterized the temporality of the non-flow period through its duration and frequency. The frequency (F) was defined by the number of non-flow events, the total duration by the total number of dry days (DD), and the mean duration was the average of consecutive non-flow days ($MnD = DD/F$). These

temporal metrics were calculated for the periods of 30, 60, 90, and 150 days before sampling, to analyze the previous period affecting biofilm communities.

Severity Characterization of the Non-flow Period

The severity of the non-flow period in every studied stream was determined by the combined attributes of daily solar radiation below the canopy cover (SR_t), the daily maximum streambed temperature (MT_t) and the daily average streambed temperature (AT_t). SR_t (MJ m^{-2} d^{-1}) below the riparian canopy was estimated by filtering the data series of solar radiation above the canopy using light interception coefficients calculated with a HemiView canopy analysis software (version 2.1; Dynamax Inc., Houston, TX, U.S.A). Solar radiation data above the canopy cover were obtained from the nearest meteorological station to each studied stream (Servei Meteorològic de Catalunya; http://www.meteo.cat). HemiView was used to perform image analysis of hemispherical photography and to determine: (i) the proportion of the sky's hemisphere obscured by vegetation, (ii) the proportion of direct and indirect solar radiation received by the streambed from each direction, (iii) the site factor, and (iv) the Leaf Area Index (LAI). Hemispherical photographs of the canopy were taken during the samplings using a digital camera Nikon D-70s (NIKON Corporation, Tokyo, Japan) fitted to a 180° fisheye (Fisheye-NIKKOR 8mm; NIKON Corporation). Daily maximum streambed temperature (MT_t; degree days, dd^{-1}) and daily Average streambed Temperature (AT_t; degree days, dd^{-1}) were obtained from the data of the deployed temperature data loggers. To analyze the effect of time on each severity factor, each factor was weighted by the predefined periods analyzed before sampling (30, 60, 90, and 150 days):

PI. Equation 4.- Weighted Solar Radiation.

$$WSR = \sum_{t=1}^{t_f} SR_t * FS_t * exp^{-kSR*t}.$$

PI. Equation 5.- Weighted Maximum streambed Temperature.

$$WMT = \sum_{t=1}^{t_f} MT_t * FS_t * exp^{-kMT*t}.$$

PI. Equation 6.- Weighted Average streambed Temperature.

$$WAT = \sum_{t=1}^{t_f} AT_t * FS_t * exp^{-kAT*t}.$$

where WSR, WMT, and WAT were the weighted severity factors; SR_t, MT_t or AT_t were daily values of each severity factor; FS_t was the flow status of each day before the sampling (1 or 0 depending on if the studied stream had non-flow or flow conditions); *t* was the number of days before sampling; t_f were the considered periods before sampling (30, 60, 90, or 150); and K_{SR}, K_{MT}, K_{AT} determined the weight of each day before sampling ($0 \leq k \leq 0.2$). Its value was different for each severity factor. At the ecological level, *k* indicated the importance of the severity of the non-flow period on each sampled biological community; *k* values approximating 0 was indicative of severity being a timeless variable, whereas values around 0.2 implied that the severity metric should include the temporality factor.

Samples Collection

Several cobbles were collected at random from within 30 m of each stream's reach. Of these, five with similar sizes were selected, stored in a zip-bag, placed inside a fridge, and transported to the laboratory within 4h after collection. Once in the laboratory, zip-bags were opened and placed inside an incubator (Radiber AGP-700-ESP, Barcelona, Spain). The incubators were set to 21ºC and ensured complete darkness. Cobbles from the studied streams with flow conditions were also incubated with water containing 2.8 mg TOC l^{-1}, 1.7 mg $N\text{-}NO_3^-$ l^{-1}, 0.2 mg $N\text{-}NH_4^+$ l^{-1} and 0.003 mg $P\text{-}PO_4^{3-}$ l^{-1}. On the following morning, each cobble was moved to a cylindrical recirculating chamber, where its biofilm metabolism was measured (see below). After the metabolism measurements, cobbles were kept for independent measures of photosynthetic efficiency, total biofilm biomass and autotrophic biomass.

Biofilm Metabolism

Metabolism chambers were used to evaluate biofilm metabolism on the colonized cobbles (Acuña et al., 2008; Guasch et al., 1995; Tank et al., 2010). We used cylindrical recirculating chambers to estimate biofilm oxygen production and consumption. The chambers were made of acrylic glass (volume 0.96 L) and were equipped with a

submersible pump that recirculated water, avoiding the generation of low diffusion areas within the chamber. The incubations were carried out inside an incubator (Radiber AGP-700-ESP, Barcelona, Spain) at constant temperature (25°C) and standard nutrient concentration (2.8 mg l^{-1} TOC, 1.7 mg l^{-1} N-NO_3^-, 0.2 mg l^{-1} N-NH_4^+ and 0.003 mg l^{-1} P-PO_4^{3-}). Community respiration (CR) and net metabolism (NM) were measured for 60 min under dark and constant light conditions (168 ± 2 $\mu E \cdot m^{-2} \cdot s^{-1}$), respectively. Dissolved oxygen was logged at 15-s intervals with oxygen sensors (PreSens OXY-10mini, Regensburg, Germany). Metabolic rates were calculated as described by (Acuña et al., 2008). Gross primary production (GPP) was estimated as the sum of NM and CR. Additionally, photosynthetic yield (Y_{eff}) was measured for each cobble following incubation. Y_{eff}, which was measured with a portable pulse amplitude-modulated fluorometer under the same light conditions (Diving-PAM; WALZ, Effeltrich, Germany), was used to evaluate the efficiency of energy conversion in the photosystem II (PSII) reaction centres (Schreiber et al., 2002).

Characterization of Biofilm Structure

The colonized biofilm was scraped off each cobble with a toothbrush and placed in a solution of distilled water. The suspended material was divided into two aliquots, one for estimating ash-free dry weight (AFDW) and the other to estimate chlorophyll-*a* concentration (Chl-*a*). Biofilm material for the AFDW samples was dried (60°C, to constant weight), weighed, combusted (450°C, 4 h), after which the remaining ashes were reweighed. Samples prepared for the Chl-*a* analyses were centrifuged for 10 min at 4ºC and 2500 rpm and had their supernatant removed; they were then frozen (- 20°C), after which they were lyophilized and had the Chl-*a* extracted with 90% v/v acetone (4ºC, 12 h) (Steinman et al., 2017). The extraction of Chl-*a* from biofilm materials was completed by sonication (30 s, 360 W power, 50/60 Hz frequency, JP Selecta SA, Barcelona, Spain), further centrifugation (10 min at 4ºC and 2500 rpm), and spectrophotometric determination (Lambda UV/VIS spectrophotometer; U-2000 Spectrophotometer; Hitachi, Tokyo, Japan). Chlorophyll-*a* concentrations were estimated afterwards applying the Jeffrey & Humphrey (1975) method.

Data Analyses

Altitude, catchment area, mean precipitation, and land uses of the studied streams were determined from GIS layers using Quantum GIS (2.14.22) with GRASS (7.2.2). Data

normality was tested by the Shapiro-Wilk test (Shapiro & Wilk, 1965) with an assigned significance value of $p < 0.05$. When the data did not meet the assumption of the parametric tests, a Mann-Whitney test (U) (Mann & Whitney, 1947) was used as a non-parametric test to analyze the data differences. The relationships between biofilm structure and the duration and frequency of the non-flow period were fitted to a negative linear or decreasing exponential model using a Linear Model (lm) from the package "stats" or the Generalized Non-linear Regression Model (gnls) from the R package "nlme". The best fit was selected according to the values of the Fisher test (F), p value (p) and coefficient of determination (R^2). The relationship between biofilm metabolism and temporal metrics of the non-flow period was adjusted to a decreasing exponential model using the Nonlinear Least Squared (nls) R function. When the relationship was significant, the residuals (i.e. the difference between observed values and fitted values) were calculated. Following this, the relationships between the residuals and each severity factor were analyzed. The objective of this step was to determine whether the severity factors explained the variability of biofilm metabolism that had not been explained by temporal metrics. Once the significant severity factors had been determined, a nonlinear multiple regression was performed using the linear and nonlinear mixed effects models (nlme) R package. This was done to find the best fit between biofilm metabolism, the temporal metric of the non-flow period, and the severity factors, based on the residuals standard error (RSE) and R^2. The selected factors composing the severity variable were tested both individually and combined in order to assess their potential combined effects on the relationship between metabolism and the temporal components of the non-flow period (R Code PI 1). All analyses were performed for each of the considered periods before sampling (30, 60, 90, and 150 days), using RStudio (R Core Team 2016).

Results

Hydrology and Environmental Variables

Of the 33 studied streams, 11 were permanent (0 dry days) and 22 were temporary (with one or more dry days). Nutrient concentration in permanent streams ranged from 0.002 to 0.009 mg $P\text{-}PO_4^{3-}\ l^{-1}$, 0.002 to 0.018 mg $N\text{-}NH_4^{+}\ l^{-1}$ and 0.001 to 2.548 mg $N\text{-}NO_3^{-}\ l^{-1}$. In temporary streams, the period before sampling that provided the best fit with biofilm structure and biofilm metabolism was of 150 days (PI. S. Table 3). During this period, the total duration (DD) in the temporary streams ranged from 1 to 150 (being 150 the

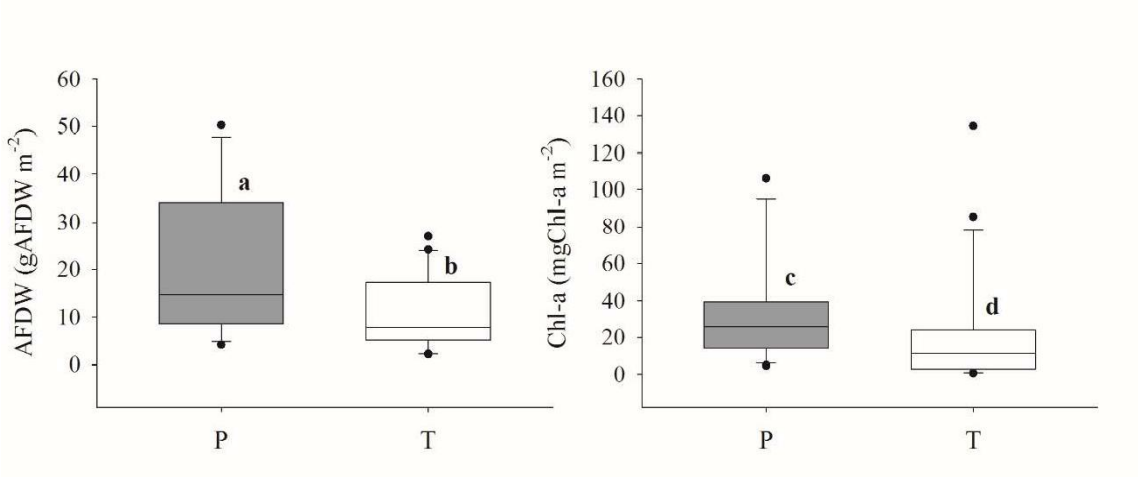
highest possible value, PI. Table 2). The non-flow period occurred mostly during the summer, though in some cases storms caused flow returns of up to 10 days long. Storms enhanced the frequency (F) of non-flow periods and decreased their mean duration (MnD). The 41% of the sites had open canopy cover, but other factors, such as river azimuth, affected the amount of solar radiation received by the streambed. Thus, studied streams with open canopy covers and North-South azimuths reached maximum daily solar radiation values of 31 MJ m^{-2} day^{-1} and minimum values of 18 MJ m^{-2} day^{-1}. Less exposed sites (with closed canopy covers and east-west azimuths), experienced minimum values of 2 MJ m^{-2} day^{-1} and maximum values of 16 MJ m^{-2} day^{-1}.

PI. Table 2.- Hydrology and severity of non-flow period in the studied streams.

Code	Hydrology			Severity			GPP ± SD	CR ± SD
	F	DD	MnD	SR	MT	AT	$mgO_2 \cdot m^{-2}$ min^{-1}	$mgO_2 \cdot m^{-2}$ min^{-1}
LL 01	1	150	150	3608.7	25.2	16.5	0.009 ± 0.01	-0.135 ± 0.08
LL 02	2	149	74.5	3604.23	25.6	15.7	0.325 ± 0.12	-0.562 ± 0.21
LL 03	3	50	16.7	781.9	25.78	12.2	0.053 ± 0.08	-0.480 ± 0.16
BE 04	1	41	41	808.3	24.6	20	0.103 ± 0.08	-0.347 ± 0.08
BE 05	1	32	32	128.9	44.2	20.9	0.006 ± 0.01	-0.210 ± 0.08
FO 06	8	120	15	2121.1	17.5	12.7	0.989 ± 0.69	-0.416 ± 0.35
FO 07	6	59	9.8	420.3	17.3	13.1	0.421 ± 0.14	-0.228 ± 0.09
FO 08	1	15	15	1432.6	22.8	18.6	0.964 ± 0.60	-0.515 ± 0.26
FO 09	1	47	47	452.3	30.5	22.7	0.048 ± 0.08	-0.818 ± 0.34
TO 10	2	4	2	25.8	23.5	19.2	0.478 ± 0.34	-0.291 ± 0.09
TE 12	1	2	2	4.2	13.5	12.9	4.133 ± 0.30	-0.480 ± 0.38
TE 16	2	11	5.5	43.3	28.9	20.5	1.335 ± 0.27	-0.405 ± 0.12
TE 17	8	107	13.4	569.9	19.3	10	1.307 ± 0.52	-0.353 ± 0.16
FL 19	6	138	23	1646.1	23.8	15.3	0.048 ± 0.05	-0.082 ± 0.06
FL 20	5	119	23.8	1750.6	25.5	15	0.059 ± 0.07	-0.305 ± 0.18
FL 21	4	11	2.8	23.8	26.7	21.2	1.552 ± 0.81	-0.732 ± 0.25
FL 22	1	66	66	529.9	36.8	21.2	0.569 ± 0.74	-0.243 ± 0.20
FL 23	11	102	9.3	779.4	21.4	13.9	1.518 ± 0.56	-0.350 ± 0.17
MU 26	8	52	6.5	988.9	22.8	18.8	0.873 ± 0.41	-0.658 ± 0.34
MU 27	1	20	20	471	50.1	27.6	0.191 ± 0.05	-0.293 ± 0.20
EB 30	1	150	150	3426	35.1	17.3	0.142 ± 0.20	-0.258 ± 0.06
EB 31	1	4	4	31.4	27.5	20.7	2.246 ± 0.75	-0.270 ± 0.08

Biofilm Biomass

Chl-*a* was higher in permanent streams (32.6 ± 27.9 mg Chl-*a* m^{-2}) compared to temporary streams (21.1 ± 33.0 mg Chl-*a* m^{-2}; PI. Figure 1). AFDW was also higher in permanent streams (20.6 ± 14.6 g AFDW m^{-2}) than in temporary ones (10.4 ± 7.7 g AFDW m^{-2}; PI. Figure 1). These differences were both statistically significant (Chl-*a*: $U = 61$, $p = 0.03$ and AFDW: $U = 64$, $p = 0.03$). AFDW in the temporary streams was only significantly related to the total duration (decreasing exponential: $F = 3.73$, $p = 0.04$, $R^2 = 0.28$), and was unaffected by both the mean duration ($F = 2.80$, $p = 0.09$, $R^2 = 0.23$) and the frequency of the non-flow period ($F = 0.35$, $p = 0.56$, $R^2 = 0.02$). While Chl-*a* was not affected by the frequency of the non-flow period ($F = 0.60$, $p = 0.45$, $R^2 = 0.03$), it was related to the duration of the non-flow period in a decreasing exponential way (MnD-Chl-*a*: $F = 10.95$, $p = 0.001$, $R^2 = 0.54$; DD-Chl-*a*: $F = 26.83$, $p < 0.0001$, $R^2 = 0.74$). The negative exponents of the exponential adjustments, indicated an abrupt decrease in total biofilm biomass and chlorophyll-*a* with the increase of the non-flow period's duration.

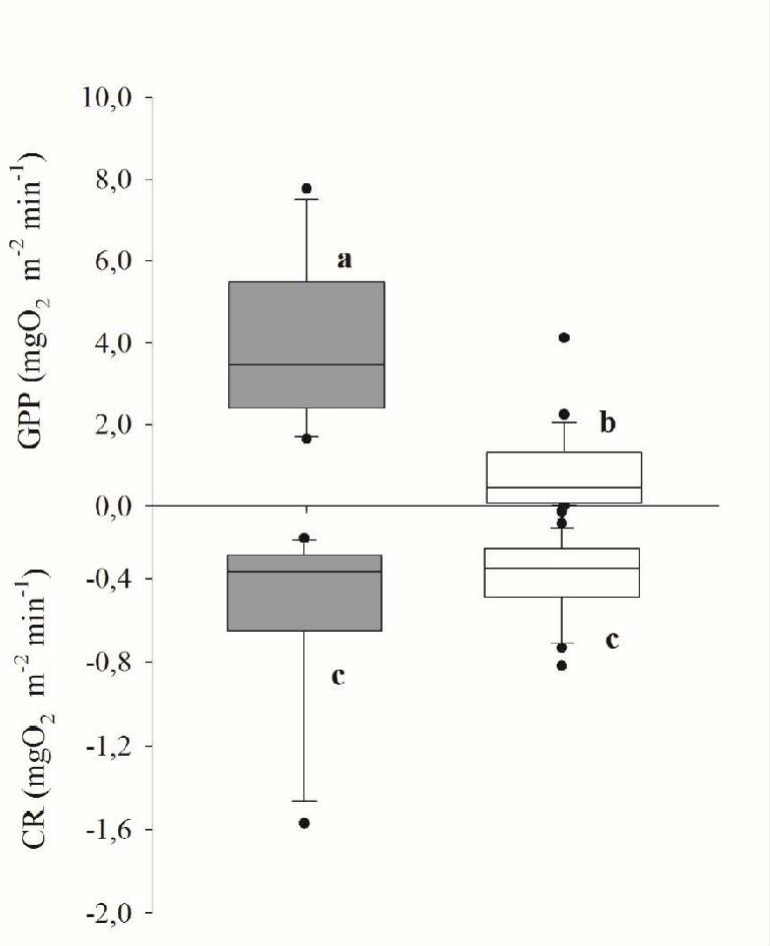


PI. Figure 1.- Structural parameters of stream biofilm (AFDW: Ash Free Dry Weight, Chl-a: chlorophyll-a) of Permanent (grey shading) and Temporary streams (white shading). Letters indicate cases in which structural parameters significantly differed between permanent and temporary streams.

Biofilm Metabolism

The Y_{eff} value was used to evaluate the efficiency of energy conversion in the PSII reaction center. Permanent streams also showed photosynthetic efficiencies almost doubling ($Y_{eff} = 549 \pm 53$) those of temporary streams ($Y_{eff} = 315 \pm 179$), reflecting the higher efficiency of energy conversion at the Photosystem II (PSII) reaction centers ($U = 25$; $p > 0.001$).

The CR values were similar ($U = 104$, $p = 0.5$) in permanent (-0.53 ± 0.42 mg O_2 m^{-2} min^{-1}) and temporary streams (-0.38 ± 0.19 mg O_2 m^{-2} min^{-1}; PI. Figure 2), whereas GPP was approximately six times higher (PI. Figure 2) in permanent (3.90 ± 1.97 mg O_2 m^{-2} min^{-1}) compared to temporary streams (0.79 ± 0.98 mg O_2 m^{-2} min^{-1}) and presented significant statistical differences ($U = 9$, $p < 0.001$). The metabolic variables were related to biofilm biomass in both permanent and temporary streams (PI. S. Table 2). CR was negatively related to AFDW (permanent: $F = 7.87$, $p = 0.02$, $R^2 = 0.47$; temporary: $F = 10.95$, $p = 0.004$, $R^2 = 0.35$). A negative linear relationship in permanent streams indicated that the gradual decrease of CR was associated with the decrease in total biofilm biomass. However, in temporary streams this relationship decreased exponentially. GPP also showed a positive linear relationship with Chl-*a* in both the permanent ($F = 5.82$, $p = 0.04$, $R^2 = 0.40$) and temporary streams ($F = 95.03$, $p < 0.001$, $R^2 = 0.83$); however, temporary streams had a higher coefficient of determination. The CR of temporary streams was not affected by the temporal components of the non-flow period (CR-DD: $F = 0.41$, $p = 0.53$, $R^2 = 0.02$; CR-MnD: $F = 2.31$, $p = 0.14$, $R^2 = 0.10$; CR-F: $F = 0.07$, $p = 0.80$, $R^2 = 0$). Similarly, GPP of temporary streams was not related to frequency of the non-flow period ($F = 0.12$, $p = 0.73$, $R^2 = 0$). However, GPP was negatively related to the mean duration of the non-flow period in an exponential way (GPP-MnD: $F = 8.09$, $p = 0.003$, $R^2 = 0.45$), and especially affected by total duration of the non-flow period also in a negative way (GPP-DD: $F = 16.79$, $p < 0.0001$ $R^2 = 0.68$; PI. Table 3; PI. Figure 3). To determine the period before sampling with the higher effect on biofilm communities, the relationship between GPP and DD was tested for the periods of 30, 60, 90, and 150 days before sampling. This exploration indicated that the best fit was obtained for the 150-day period.



PI. Figure 2.- Stream biofilm metabolism (GPP: Gross Primary Production and CR: Community Respiration) of Permanent (grey shading) and Temporary streams (white shading). Letters indicate the cases for which metabolic rates were significantly different between permanent and temporary streams.

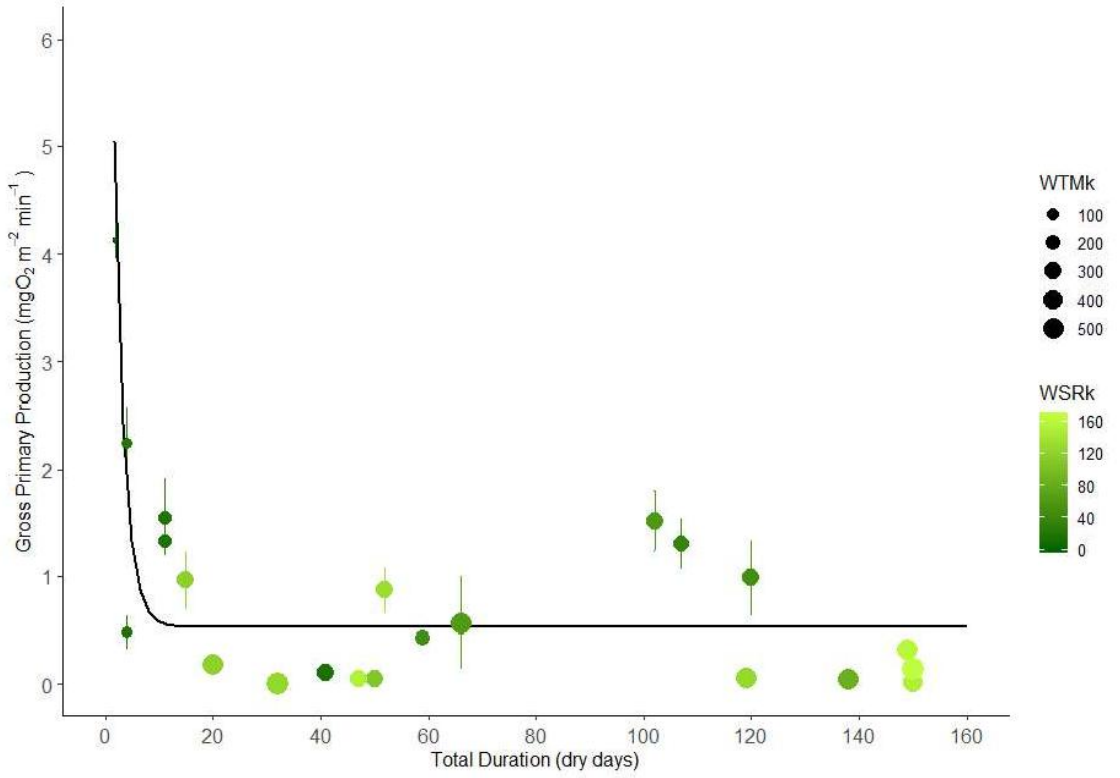
Severity of the Non-flow Period

The relationship between DD-GPP was significant and presented a higher R^2 value than the MnD-DD relationship. For this reason, the residuals of the observed and fitted valued of the linear regression between DD and GPP were calculated for the subsequent analyses with weighted severity factors (WSR, WMT, and WAT). Regression analyses showed that WSR and WMT were clearly correlated with biofilm metabolism, while the one performed for WAT was less clear. Given the high correlation between AT and MT (60%), only MT was selected as a severity factor. Conversely, the correlation between SR and MT was low (38%). SR and MT were therefore selected as severity factors and incorporated in the multiple regression models to test their effects together with DD. The relationship between GPP, DD, WSR, and WMT was tested for the 30-, 60-, 90-, and 150-day periods before sampling. The results showed that (i) the best fit was obtained

with the model that used the 150 days before sampling period (PI. S. Table 3), and (ii) the model with the two weighted severity factors (WSR and WMT) had the best fit with the observed GPP values (PI. Table 3). The *k* values were similar for WSR and WMT (k_{SR} = - 0.08; k_{MT} = - 0.08).

PI. Table 3.- Summary of the fitted models using generalized least squares method.

	Model	RSE	R^2
Any severity factor	$GPP = 0.53+10.72 \cdot exp^{(-0.54 \cdot DD)}$	0.58	0.68
One severity factor	$GPP = 1.08+14.42 \cdot exp^{(-0.77 \cdot DD)} -0.002 \cdot WSR$	0.50	0.78
	$GPP = 1.40+16.53exp^{(-0.90 \cdot DD)}-0.002 \cdot WMT$	0.49	0.79
Both severity factors	$GPP = 1.40+17.00 \cdot exp^{(-0.91 \cdot DD)}-0.001 \cdot WSR-0.002 \cdot WMT$	0.48	0.80



PI. Figure 3.- Relationship between GPP (mg O_2 m^{-2} min^{-1}) of temporary streams and DD (days): GPP = 0.53+10.72 exp(-0.54 DD), R^2 = 0.68. Bars indicate the standard error of 5 replicates (se); symbol size accounts for the weighted maximum streambed temperature (WMT) recorded during the non-flow period in each studied site, while color intensity indicates the weighted solar radiation (WSR).

Discussion

Total biofilm biomass and autotrophic biomass in permanent and temporary streams result from stream environmental conditions such as nutrient and organic matter, light availability, water velocity, and grazing pressure (Burns, 2001; Guasch et al., 1995; Tank & Webster, 1998; Sabater et al., 2002). The adverse conditions associated with the non-flow period in temporary streams also affect biofilm structure and metabolism (Sabater et al., 2016; Timoner et al., 2012). Our results show that temporary streams had lower mean total biofilm biomass and autotrophic biomass. This indicated that temporary stream biofilms were thinner than those from permanent streams. The structural differences between permanent and temporary stream biofilms probably caused the relevant metabolic differences, especially considering that metabolism was measured when the two types of stream biofilms were subjected to the same standard conditions of light, temperature and nutrients. Under these standard conditions, GPP values from the temporary streams were six times lower than those from the permanent streams. Furthermore, the temporary stream biofilms showed lower photosynthetic efficiency, a sign that the photosynthesis apparatus functioning was also hampered during the non-flow period. This result might be related to the lower fraction of active Chl-*a* in the algal communities of these biofilms, for the greater part of the Chl-*a* was either degraded or inactive as a consequence of the non-flow conditions. We believe that the lower Chl-*a* concentration, as well as the lower photosynthetic efficiency of the temporary stream biofilms, played crucial roles on their lower GPP values (Krause & Weis, 1991). Conversely, CR was similar in both kinds of streams in spite of the fact that the temporary streams had less biomass than the permanent ones. These results suggest that temporary stream community could be more tolerant to hydric stress, probably as a consequence of the higher resistance of biofilm heterotrophs to the non-flow period (Acuña et al., 2015; Romaní et al., 2013).

Beyond these general differences between permanent and temporary stream biofilms, we determined that the biofilm biomass and metabolism in temporary streams were explained by past hydrological conditions. Contrary to our hypothesis, neither biofilm biomass nor its metabolism were influenced by the frequency of the non-flow period. However, the duration of the non-flow period negatively affected biofilm, by reducing its biomass and metabolism. Overall, these results indicate that the duration of the non-flow period, rather

than their frequency, is the main abiotic variable affecting temporary stream biofilms. Specifically, total biofilm biomass was moderately affected by the mean and total duration of the non-flow period. In contrast, autotrophic biomass was strongly affected by the duration of the non-flow period; its abrupt decrease confirms that autotrophic biomass can oppose low resistance to the non-flow period (Acuña et al., 2015; Stanley et al., 2004; Timoner et al., 2012). However, it has not yet been determined how the duration of the non-flow period affects autotrophic structure (that is, by promoting the synthesis of resistance structures or protective pigments), or how it acts as a species filter for the autotrophs.

Regarding biofilm metabolism, GPP in temporary stream biofilms was strongly related (decreasing exponentially) to the total duration of the non-flow period. Overall, the high sensitivity of the autotrophic compartment, Chl-*a* and GPP toward desiccation could be a result of the damage inflicted to the photosynthetic apparatus (Gray et al., 2007; Karsten & Holzinger, 2014), either through dehydration or photoinhibition. Also, the negative exponential relationship between the total duration of the non-flow period and the GPP in temporary streams suggests that the greatest changes could have occurred in short-term periods (20–30 dry days) and that long-term changes would have been reduced. This trend had previously been reported for experimental streams (Acuña et al., 2015). As a consequence, streams with short non-flow periods showed large differences in their GPP values. These differences decreased rapidly in time, until they approached values of zero for long non-flow periods. This disturbance–response relationship probably represents an ecological threshold (Humphries & Baldwin, 2003) after which GPP presents residual values. In the present study, the best fit was obtained when considering a period of 150 days before sampling, thereby highlighting the importance of past hydrological conditions on present biofilm community. Thus, it might be assumed that the non-flow period is a drive of selection of certain attributes, and determine the presence and abundance of the most resistant algal species at long term, effectively shaping the resulting community (Lake, 2003). In contrast, the CR was not as affected by duration or frequency of the non-flow period. Community respiration was more resistant to the non-flow period, possibly as a result of the respiratory substrates remaining within the biofilms. Thus, organic matter (in the form of dissolved or particulate organic carbon) accumulated on the streambed, as well as within the biofilms during the non-flow period, would facilitate the fast respiration response after rewetting (Busch & Fisher, 1981;

Mulholland et al., 2001; Muñoz et al., 2018; Ylla et al., 2010). Also, resistance mechanisms of heterotrophs to water stress (Barnard et al., 2013; Timoner et al., 2012) could play a role in this pattern of response.

The effects of the duration of the non-flow period cannot be separated from those related with the severity of the non-flow period (that is, high air temperature and solar radiation). Of our studied streams, those with open riparian canopies and higher air temperatures experienced similar solar radiations and temperatures to those recorded in desert streams (Tait et al., 1994). Their values were about 26% higher than the ones found in sites with closed riparian canopies and colder temperatures. Therefore, streams with the same non-flow period duration could have considerably different conditions of severity, and thus, different impacts on GPP. In other words, similar non-flow period durations could have a higher or lower deleterious effects on the autotrophic components of the stream biofilm depending on the severity factor. Altogether, up to 80% of the observed variability of GPP could be accounted (PI. Table 3). This result suggests that high solar radiation during the non-flow period affects the photosynthetic apparatus both directly and indirectly, either by altering DNA or proteins, or by producing photooxidative stress (Sabater et al., 2016). Also, the occurrence of high temperatures could negatively affect autotrophs through the denaturalization of proteins in the thylakoid membrane (Geider, 1987). In brief, our results highlight the protective role of the riparian canopy cover in temporary streams since it might minimize the direct effects of ultraviolet and solar radiation. Interestingly, the *k* value of both severity factors indicated that the effects of severity of the non-flow period were not linear but determined by the most recent environmental conditions.

To conclude, our results show that it is the total duration and severity of the non-flow period, rather than the frequency at which they occur, what shapes biofilm biomass and metabolism in temporary streams, particularly in the case of the autotrophic compartment. Regarding the hydrological modeled predictions under climate change scenarios, longer non-flow periods could significantly decrease the GPP/CR ratio. In an ecosystem context, the energy flux would therefore move from an autotrophy-based to an allochthonous C-based situation, and biofilms might require allochthonous organic matter subsidies to sustain community respiration. In turn, this might cause changes in the distribution of biological assemblages (Gomi et al., 2002) and their connections within the river trophic web.

Supplementary material

Tables

PI. S. Table 1.- Land uses of catchment areas of studied streams.

Code	Land Uses (%)				
	U	A	IA	F	S
LL 01	0%	2.20%	0%	97.80%	0%
LL 02	0%	0%	0%	81.31%	18.70%
LL 03	0%	0%	0%	91.70%	8.33%
BE 04	0%	0%	0%	90.20%	9.80%
BE 05	0%	1.90%	0%	92.5%	5.63%
FO 06	0%	20.34%	6.78%	45.77%	27.11%
FO 07	0%	0%	0%	100%	0%
FO 08	0%	70.60%	0%	17.65%	11.77%
FO 09	1.35%	24.78%	1.80%	65.87%	7.21%
TO 10	0%	0%	0%	100%	0%
TO 11	0.49%	0%	0.74%	97.28%	1.48%
TE 12	0%	1.80%	0%	98.20%	0%
TE 13	0%	29.03%	0%	67.74%	3.23%
TE 14	0%	15.79%	0%	84.21%	0%
TE 15	0%	2.34%	0.39%	93.36%	3.91%
TE 16	0%	6.25%	0%	71.88%	21.88%
TE 17	0%	16.67%	0%	83.33%	0%
FR 18	0%	0%	3.77%	92.47%	3.77%
FL 19	0%	2.84%	3.69%	85.23%	8.24%
FL 20	0%	1.06%	1.34%	89.59%	8.02%
FL 21	0%	18.42%	0%	81.58%	0%
FL 22	0%	10.77%	3.08%	86.15%	0%
FL 23	1.04%	15.28%	3.13%	76.74%	3.82%
FL 24	0%	0%	0%	90.39%	9.62%
MU 25	0%	0%	0.54%	90.30%	9.16%
MU 26	0%	6.31%	0%	8.11%	85.59%
MU 27	0%	1.99%	0%	40.55%	57.46%
EB 28	0%	4.15%	0%	79.93%	15.92%
EB 29	0.06%	4.20%	7.28%	78.56%	9.89%
EB 30	0%	22.82%	1.66%	44.40%	31.12%
EB 31	0%	9.45%	18.04%	45.88%	26.63%
EB 32	0%	13.55%	6.95%	61.15%	18.35%
EB 33	0.15%	12.75%	21.42%	53.65%	12.03%

PI. S. Table 2.- Relationships between structural and functional variables of permanent and temporary streams.

	Permanent streams	Temporary streams
CR-AFDW	$CR_p = -0.13 - 1.95\,e^{-5} \cdot AFDW$ $R^2 = 0.47; F = 7.87; p = 0.02$	$CR_t = -0.29 - 7.74\,e^{-6} \cdot AFDW$ $R^2 = 0.22; F = 5.70; p = 0.03$
GPP-Chl-*a*	$GPP_p = 2.45 - 0.4\ \ Chl-a$ $R^2 = 0.39; F = 5.82; p = 0.04$	$GPP_t = 0.19 - 0.03\ \ Chl-a$ $R^2 = 0.83; F = 100.5; p < 0.0001$

PI. S. Table 3.- Summary of fitted the models for different time frames.

Time (days)	**Model**	**RSE**	**R^2**	**kWSR**	**KMxT**
150	GPP = 1.40+17.00·exp$^{(-0.91\cdot DD)}$-0.001·WSR-0.002·WMT	0.48	0.80	0.08	0.08
90	GPP = 1.54+365·exp$^{(-2.47\ DD)}$-0.001·WSR-0.002·WMT	0.50	0.79	0.08	0.08
60	GPP = 1.53+2.60·exp$^{(-1.12\cdot DD)}$-0.001·WSR-0.002·WMT	0.50	0.79	0.07	0.08
30	GPP = 1.45+2.68·exp$^{(-2.0\ DD)}$-0.001·WSR-0.001·WMT	0.48	0.80	0.11	0.00

R Code PI 1

```
RSE0=1e+6
mod0=NULL
k01=-1
k02=-1
kk1<-seq(0,0.2,0.001)
kk2<-seq(0,0.2,0.001)
for(k1 in
 kk1){ WSRk=WSR%*%exp
 (-k1*t)
   mod=tryCatch(nls(GPP~a+b*WSRk+d*exp(-k*DD),      start=c(a=1.56,      b=-0.003,
d=335, k=2.44),
                algorithm = "port", upper=c(Inf, 0, Inf, Inf)), error=function(e) NULL )
 if(!is.null(mod)){
   residuals=GPP-predict(mod)
   RSE=sqrt(sum(residuals**2)/(22-4))
   if(RSE<RSE0) {RSE0=RSE
   mod0=mod
   k01=k1
   k02=-1}
 }
 mod=NULL
   for(k2 in kk2){
   TMk=TM%*%exp(-k2*t)
      mod=tryCatch(nls(GPP~a+c*TMk+d*exp(-k*DD),      start=c(a=1.56,      c=-0.002,
d=335, k=2.44),
               algorithm = "port", upper=c(Inf, 0, Inf, Inf)), error=function(e) NULL )
   if(!is.null(mod)){
     residuals=GPP-predict(mod)
     RSE=sqrt(sum(residuals**2)/(22-4))
     if(RSE<RSE0) {RSE0=RSE
     mod0=mod
     k01=-1
```

```
    k02=k2}
  }
  mod=NULL
      mod=tryCatch(nls(GPP~a+b*WSRk+c*TMk+d*exp(-k*DD), start=c(a=1.56, b=-
0.003, c=-0.002, d=335, k=2.44), algorithm = "port",
                   upper=c(Inf, 0, 0, Inf, Inf)), error=function(e) NULL )
  if(!is.null(mod)){
    residuals=GPP-predict(mod)
    RSE=sqrt(sum(residuals**2)/(22-5))
    if(RSE<RSE0) {RSE0=RSE
    mod0=mod
    k01=k1
    k02=k2}
  }
  mod=NULL
 }
}
```

Biofilm pigmentary composition as a footprint of dry periods and their severity in temporary streams

M. Colls, X. Timoner, C. Font, V. Acuña & S. Sabater

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Biofilm pigment composition as a footprint of dry periods and their severity in temporary streams

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Abstract

Water availability is a key environmental determinant of primary production in temporary streams, as it influences the physiology of photoautotrophic organisms and community composition of stream biofilms through its duration and frequency. Global change is leading to changes in the duration and frequency of dry periods, as well as the occurrence of dry periods in formerly permanent watercourses. The effects of dry periods on stream biofilm photoautotrophic organisms can be quantified by analysing the pigments of primary producers, because pigment composition responds to environmental stressors and community composition. However, previous studies have focused on the overall effects of dry periods, thus neglecting the specific effects of the duration and frequency of dry periods, as well as streambed environmental conditions (solar radiation and temperature) during these periods. Here, we assessed the effects of duration, frequency, and severity of dry periods on pigmentary composition and its recovery after flow resumption. The study was performed in 32 permanent and temporary streams across nine Mediterranean basins. The duration and severity of dry periods caused a decrease in active chlorophylls and an increase of protective carotenoids. Pigmentary composition after flow resumed was driven by short dry periods that interrupted flow resumption and modulated seasonal characteristics. Biofilms experiencing longer and more severe dry periods were least similar to those from permanent streams in terms of their pigmentary composition. Overall, the pigmentary composition reflected the stream's hydrological history and biofilm capacity to resist and recover after dry periods.

Keywords: green algae, cyanobacteria, diatom, carotenoid, chlorophyll, canthaxanthin, myxoxanthophyll, scytonemin

Using Structural Equation Modelling to Approach the Biodiversity - Ecosystem Functioning Relationships in Temporary Streams

Using Structural Equation Modelling to Approach the Biodiversity - Ecosystem Functioning Relationships in Temporary Streams

Introduction

Environmental conditions drive both biodiversity and functioning of aquatic ecosystems (Palmer & Ruhi, 2019). Any modifications occurring in the ecosystem may translate into biodiversity changes, changing their associated functions and modifying their ecosystem services (Naeem et al., 2009).

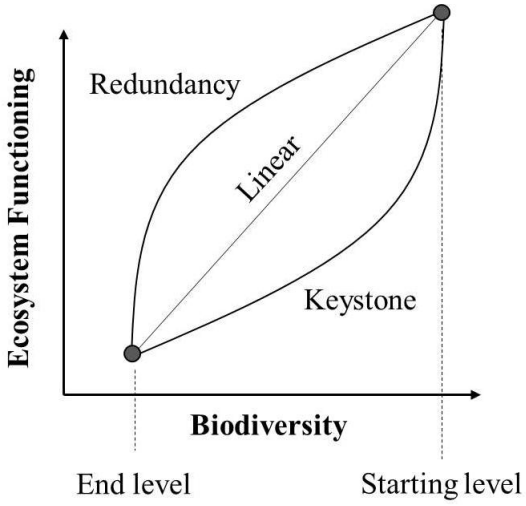
In permanent fluvial ecosystems, water performs as a resource and habitat for biota, a vector of connectivity, and a determinant of species, resources and processes distribution in space and time (Sponseller et al., 2013). Comparing temporary streams with other permanent may show different patterns on organisms life-history (Bonada et al., 2007; Bonada & Resh, 2013; Soria et al., 2017; Tornés & Ruhí, 2013b), and biogeochemical cycles (Palmer & Ruhi, 2019; Raymond et al., 2013; Schwalm et al., 2011). In temporary fluvial ecosystems, the non-flow period can differ on its duration and frequency (Lake, 2000). Flow and non-flow periods alternate, and shape community composition and ecosystem functioning. Accordingly, many aquatic taxa cannot stand the absence of water flow and disappear during these periods, while others have acquired physiological and behavioural adaptations to cope with desiccation (Bogan et al., 2017). Simultaneously, abiotic processes such as photodegradation, physical disruption, or the precipitation of solutes through evaporation become important, while maintaining some processes where microorganisms play an important role (von Schiller et al., 2017).

In both permanent and temporary stream ecosystems, microorganisms', such as algae (i.e. algae and cyanobacteria), bacteria, fungi and protozoa, co-habit in a matrix of polysaccharides, exudates and detritus called biofilms. Biofilms composition changes according to colonized substrates and environmental conditions (Romaní et al., 2013; Sabater et al., 2016). Environmental conditions change the composition and diversity of biofilms according to organism's capacity to withstand their effects (i.e. their resistance to change; Romaní et al., 2017; Sabater et al., 2017). For instance, diatoms are generally more sensitive to dry conditions than cyanobacteria (Belnap et al., 2004; Falasco et al., 2020; Timoner et al., 2014); leading to a reduction of α-diversity due to flow intermittency

(Tornés & Ruhí, 2013). In the heterotrophic realm, archaeal community composition is more affected than bacterial or fungi community composition (Gionchetta et al., 2020). Consequently, measures of the richness (i.e. number of species in an environment) or evenness (i.e. the relative abundance of the different species in an environment), provide information about the response of the biodiversity structure to any circumstance.

Biofilms play a crucial role in nutrient cycling's and fuelling energy to the upper trophic levels, affecting ecosystem metabolisms and nutrient cycles (Battin, et al., 2016; Sabater et al., 2016). Under light conditions, photoautotrophic organisms produce oxygen (i.e. gross primary production, GPP), and organic matter (i.e. autochthonous organic matter). The ability of autotrophic organisms to produce organic matter is correlated with the presence of active chlorophylls into their cells (Steinman et al., 2017). These photosynthetic pigments are a key component of the photosynthetic apparatus, where photoautotrophic organisms transform inorganic matter to organic matter through solar radiation. Thus, their abundance could be interpreted as a measured of photoautotrophic physiological status, since are growth-related (Paper II). Additionally, autotrophic and heterotrophic organisms continuously used and decompose internal and external inputs of organic matter, driving biogeochemical cycles and contributing to community respiration (CR) (Romaní et al., 2013). However, environmental conditions determine biofilm functioning either directly or indirectly, depending on structural and functional characteristics of organisms. Acuña et al. (2015) and Colls et al. (2019) already pointed out the importance of the duration of the non-flow period for the balance between photoautotrophic and heterotrophic processes of stream biofilm. Since photoautotrophic organisms are more sensitive than heterotrophic organisms to dry conditions (Timoner et al., 2012), increasing non-flow period reduced gross primary production, rather than community respiration. Similarly, Foulquier et al. (2015) found that microbial litter decomposition associated to fungal and bacterial communities was not affected by flow intermittency.

The linkage between community structure and their functions is analysed by the biodiversity-ecosystem Functioning (BEF) framework (Naeem et al., 2002). This aims to understand and predict how communities and ecosystem functioning respond to environmental changes (Bengtsson, 1998; van der Plas, 2019). A considerable amount of literature on BEF relationship come from terrestrial ecosystems, particularly grasslands (e.g. Cardinale et al., 2006; Hector, 1999; Tilman, 1997; see review: van der Plas, 2019).



PIII. Figure 1.- Three main hypothesis (redundancy, keystone and linear) in Biodiversity and Ecosystem Functioning (BEF) research. Adapted from Naeem et al. (2002).

In contrast, the nature and strength of BEF relationship are poorly understand in temporary streams, probably due to the lack of studies analysing both biodiversity and ecosystem functioning, specially under field conditions. We may expect that the BEF relationships may respond to one of three main hypotheses (PIII. Figure 1; Naeem et al., 2002). Redundancy hypothesis states that species are essentially redundant, and the loss of one species can be compensated by other species with similar functional traits. Keystone hypothesis states that species are essentially singular, suggesting a tipping point at which, if a diversity threshold is crossed, there will be a sharp decline in the ecosystem process. Finally, Linear hypothesis establishes that there is a direct relationship between the total number of species and the ecosystem process, and the loss or gain of species is directly reflected in the decrease or increase of the ecosystem process, respectively. With regard to community diversity, the absence of a simple relationship between species richness and ecosystem processes is the most likely possibility when one or a few species have strong ecosystem effects (i.e. would mean to discard linear hypothesis; Chapin III et al., 2000) Oppositely, an asymptotic relationshipevenness or α -diversity and ecosystem function could support keystone or redundancy hypothesis. Considering ecosystem functioning, those processes performed by a small number of organisms (i.e. narrow processes) could probably exhibit a keystone relationship (Haines-Young & Potschin, 2010). In this case, the response of these organisms, with particular functional capabilities, to flow intermittency could play a key role in the overall ecosystem response. Oppositely, processes which tend to be dependent upon a wider range of organisms, will

probably exhibit a linear or even redundancy relationship (Haines-Young & Potschin, 2010).

Although BEF relationship could be tested from linear or non-linear models under laboratory conditions, the integration of environmental factors derived from field studies requires the use of multivariate statistical methods. In addition, the existence of multiple cause-response relationship in natural ecosystems, and the existence of direct and indirect effects on pre-assumed causal relationship, limits the number of statistical techniques to be used. Structural equation modelling (SEM) is a multivariate statistical technique for testing a set of relationships between one or more independent variables and one or more dependent variables (Fan et al., 2016; Ullman & Bentler, 2003). Accordingly, SEM allows questions to be answered that involve multiple regression analyses of factors, such as BEF relationships. In this study, we analysed the nature and strength of BEF relationship mediated by temporal components of the non-flow period, using SEM and focusing on photoautotrophic organisms inhabiting temporary stream biofilms. Based on previous results (Paper I and II) we expect that the duration of the non-flow period (total or mean) drive structural and physiological changes. At structural level, we expect a decrease in the community diversity as the duration of the non-flow period increase (based on specific pigments' results from Paper II). Community physiology also will be affected by the duration of the non-flow period (Paper II). Both variables are closely related, since the community composition influence their ability to resist dry conditions and the capacity to survive under certain conditions will determine the physiological state of the community. So, we expect that both community diversity and physiology will determine community functioning. To sum up, we predict that the structure and physiology of photoautotrophic community varies as a function of non-flow duration; which will be reflected in gross primary production.

Materials and methods

Study area

A total of 32 streams, nine permanent and 23 temporary streams, distributed across nine basins in the NE Iberian Peninsula were selected for this study. Sub-basins characteristics were determined according to Corine land cover classification from GIS layers using Quantum GIS (2.14.22) with GRASS (7.2.2). Accordingly, stream sub-basins were mostly dominated by forests (F) followed by shrublands and grasslands (S), agricultural

fields (A) and urban and industrial cover (U; PIII. S. Table 1). The selected streams had orders from 2 to 5, and their flow regimes ranged from permanent to episodic. The study area has a mediterranean climate, with a distinctly warm and dry summer season. The annual precipitation ranges from 428 to 1093 mm, most of which falls during winter storms. Mediterranean climates have high seasonality and predictability (Tonkin et al., 2017). Summer rainfall may not compensate evapotranspiration, causing some streams stop to flow and streambed becomes dry. According to this seasonal pattern, selected streams were sampled in midsummer (July 1st-August 1st), the period of maximal drying across temporary streams.

Characterization of hydrological regime and temporal components of the dry period

We monitored streambed temperature every 30 min using VEMCO Minilog temperature data loggers (TR model, AMIRIX Systems Inc, Halifax, NS, Canada) in 23 of the 32 streams. Water-level sensors (Solinst level-logger, Edge, Model 3001), which recorded temperature and water level every 30 min were installed in the remaining nine streams. All loggers were deployed on riffle areas. Air temperature was obtained from loggers pre-installed in the riparian zone or from nearby meteorological stations (Servei Meteorològic de Catalunya; http://www.meteo.cat/). Hydrological regime (i.e. flowing or dry conditions) were daily characterized for each stream by comparing air and streambed temperatures, following (Colls et al., 2019). We characterized the hydrological regime during the 150-day period prior to sampling. The frequency and duration of the dry period were used as a metrics of temporal components of the dry period. Frequency (*F*) was described by the number of dry events, and duration was calculated as (i) the total duration of the dry period or total number of dry days (*DD*), and (ii) the mean duration of the dry period or the mean number of consecutive dry days ($MnD = DD/F$). These three variables were calculated for a period 150-d before sampling campaign based on previous studies (Paper I and Paper II), when 150-d period prior to sampling was higher than zero, we classified streams as temporary, whereas streams which did not dry were classified as permanent.

Sampling strategy

Five cobbles were collected randomly from a 50 m reach in each stream, stored in a sealed bag, refrigerated at 4°C and transported to the laboratory within 4 h after collection. Once

in the laboratory, zip-bags were opened and placed inside an incubator (Radiber AGP-700-ESP, Barcelona, Spain). The incubators were set to 21ºC and ensured complete darkness. Cobbles from the studied streams with flow conditions were also incubated with water containing 2.8 mg TOC l^{-1}, 1.7 mg N-NO_3^- l^{-1}, 0.2 mg N-NH_4^+ l^{-1} and 0.003 mg P-PO_4^{3-} l^{-1}. On the following morning, each cobble was moved to a cylindrical recirculating chamber, where its biofilm metabolism was measured (see Paper I). After the metabolism measurements, each cobble was scraped with a brush into 20 ml of filtered water (nylon 0.2 μm pore size) to remove biofilm, resultant biofilm suspension was divided in two equal parts in order to analyse photosynthetic pigment (see Paper II) compositions and photoautotrophic community compositions (see below). The scraped area of each cobble was wrapped tightly and carefully drawn in tin foil. The tin foil was cut out, and the equivalent surface of the scraped area was weighted. A linear regression used to calculate the total scraped area.

Water temperature, conductivity, dissolved oxygen, and pH were measured at each site using hand-held probes (WTW multiline 3310; YSI ProODO handled; YSI Inc., Yellow Springs, OH, U.S.A.). Three water samples were collected per site, filtered through glass fibre filters (Whatman's GF/F) and frozen at –20 °C until analysis. The concentration of nitrate was analysed by ion chromatography using a DIONEX C5000 (Dionex Corporation, Sunnyvale, CA, USA). The concentrations of ammonium and phosphate were determined colorimetrically using an Alliance-AMS Smartchem 140 spectrophotometer (AMS, Frepillon, France). Alkalinity was determined using a Metrohm 855 Titrosampler (Metrohm AG, Herisau, Switzerland).

Photoautotrophic biodiversity characterization

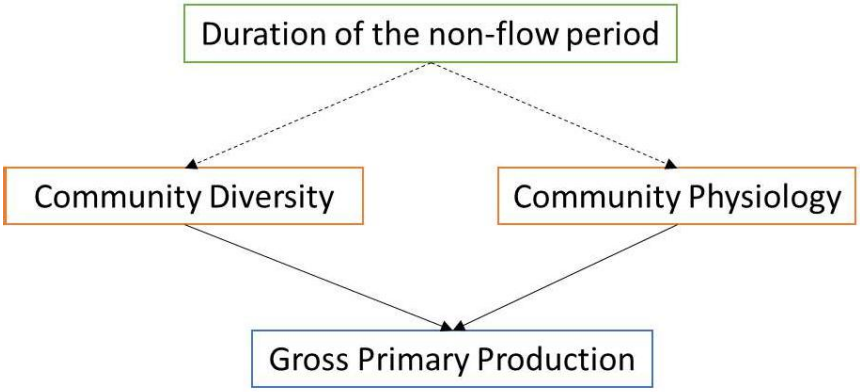
A composite photoautotrophic sample was created from the five cobbles collected from each stream, then each composite sample divided equally into two equal subsamples for the separate identification of diatoms on one hand, and non-diatom algae and cyanobacteria on the other. Diatom frustules were digested to clean organic material using sulfuric acid, dichromate potassium, and hydrogen peroxide. Slides were prepared using Naphrax (r.i. 1.74; Brunel Microscopes Ltd., Chippenham, UK). At least 400 valves were counted on each slide by using random fields under a Nikon Eclipse E600W light microscope (Nikon, Tokyo, Japan) equipped with Nomarski differential interference contrast optics at ×1000 magnification. Non-diatom algae (i.e. diatoms, green algae, red

algae, euglenophytes, and charophytes) and cyanobacteria were identified under light microscopy (Nikon Eclipse E600W) at a magnification of 1000, until were counted at least 400 cells. Algae (diatoms and non-diatom algae) and cyanobacteria were identified at the genus level using Krammer & Lange-Bertalot (1991–1997), and Lange-Bertalot (2001) for diatoms, and Wehr et al. (2015) for the remaining groups. The total abundance of each algal and cyanobacteria was converted to percentage, in order to calculate their relative abundance (%).

Data analyses

All statistical analyses were conducted in R v.3.4.0 (R Core Team, 2016). First, we examined the differences between the photoautotrophic communities of permanent and temporary streams. To that aim, we used non-metric multidimensional scaling (NMDS) of a Bray-Curtis distance matrix based on non-transformed abundance data to graphically represent patterns of photoautotrophic community composition in permanent and temporary streams, using the function "metaMDS" in R package "vegan" (Oksanen et al., 2016). The goodness of the representation in two reduced dimensions was checked using the degree of stress and the linear-R^2 and *p*-value of the Shepard plot. Photoautotrophic community composition patterns were explored using multivariate analyses. Non-metric multidimensional scaling was used to analyse the similarity in the species assemblages of permanent and temporary biofilms. Non-metric multidimensional scaling provided a two-dimensional graphical representation of the photoautotrophic genera clustering at stream type (i.e. permanent or temporary streams). An indicator species analysis was performed to identify the representative species of each cluster in the data set (IndVal; Dufrêne & Legendre, 1997) Only genera for which species' indicator $p < 0.05$ were selected as representative of permanent or temporary streams and added to the NMDS plot. Then, we analysed the differences each group (i.e. diatoms, green algae, red algae, euglenophytes, charophytes and cyanobacteria), richness, evenness, and α -diversity in permanent and temporary streams using Mann-Whitney tests (*U*). We used a structural equation modelling (SEM) to analyse causal relationships between structural and functional changes in photoautotrophic communities of temporary stream biofilms due to the non-flow period, following Grace et al. (2012), Kenny et al. (2014), and von Schiller et al. (2019). The main objective was to test which of the three hypotheses of the biodiversity-ecosystem functioning relationship (i.e. Redundancy hypothesis, Linear hypothesis, and Keystone hypothesis) holds true in temporary streams.

We first constructed a metamodel based on previous research (PIII. Figure 2). This metamodel considered GPP (Paper I) to be directly controlled by biological metrics representing the structure (richness, evenness or diversity) and physiology (active chlorophylls; Paper II) of photoautotrophic communities, which themselves depend on duration of the non-flow period (i.e. total or mean duration of the non-flow period). Based on previous studies, we predicted that community structure and physiology would have a positive effect on GPP (Paper I; Timoner et al., 2012, 2014), whereas the dry period duration would negatively affect both community structure and physiology (Tornés & Ruhí, 2013; Paper II) .



PIII. Figure 2.- Metamodel showing the predicted relationships of environmental factors (i.e. the non-flow period), and biotic factors (community structure, physiology and functioning). Blue box represents the response variable, orange boxes the endogenous variables, and green box the exogenous variable. Community structure is based on the three diversity indices, whereas physiology is based on the concentration of active chlorophylls. Hypothesized negative effects are indicated by dash arrows, and hypothesized positive effects are indicated by black arrows.

Then, we evaluated the linearity of all relationships using the "lm" function in the R package "stats" and applied square-root or log transformation as required. We fitted the metamodel using the "sem" function in the R package "lavaan" (Rosseel, 2012). The final models were accepted when $p > 0.05$ in the corresponding chi-squared test and the comparative fit index (CFI) > 0.95. The root mean square error of approximation (RMSEA) was not used as a goodness-of-fit measures due to few degrees of freedom and the small sample size in our models (Kenny et al. 2014), alternatively we used the standardized root mean square residual (SRMR), with values < 0.08 considered a good fit. Because the main objective was to test the three hypotheses of biodiversity-ecosystem functioning framework, the above SEM process was applied independently for the three diversity indices. Finally, we compared the three different models to select the best one.

Results

Hydrology and Environmental Variables

Of the 32 studied streams, nine permanent and 23 temporary streams. Nutrient concentration in permanent streams ranged from 0.002 to 0.009 mg $P\text{-}PO_4^{3-}$ l^{-1}, 0.002 to 0.018 mg $N\text{-}NH_4^{+}$ l^{-1} and 0.001 to 2.548 mg $N\text{-}NO_3^{-}$ l^{-1}. Temporary streams experienced a total number of dry days ranging from two to 150 during the non-flow period. Occasional summer storms caused episodic flow returns which favoured the alternance between non-flow and flow periods. Accordingly, the number of non-flow events ranged between one and 11, which decreased the mean duration of the non-flow period.

Ecosystem metabolism

GPP ranged from 1.65 to 7.77 mg O_2 m^{-2} min^{-1} in permanent streams, and was approximately six times higher (3.90 ± 1.97 mg O_2 m^{-2} min^{-1}) than in temporary streams (0.79 ± 0.98 mg O_2 m^{-2} min^{-1}; Paper I). GPP ranged from 0.01 to 4.13 mg O_2 m^{-2} min^{-1} (see detailed information in Paper I) in the temporary streams. The two groups of streams presented significant statistical differences ($U = 9$, $p < 0.001$). Measured community respiration in Paper I was not considered in the analysis since encompasses both autotrophic and heterotrophic compartments of stream biofilm, and heterotrophic structure was not analysed

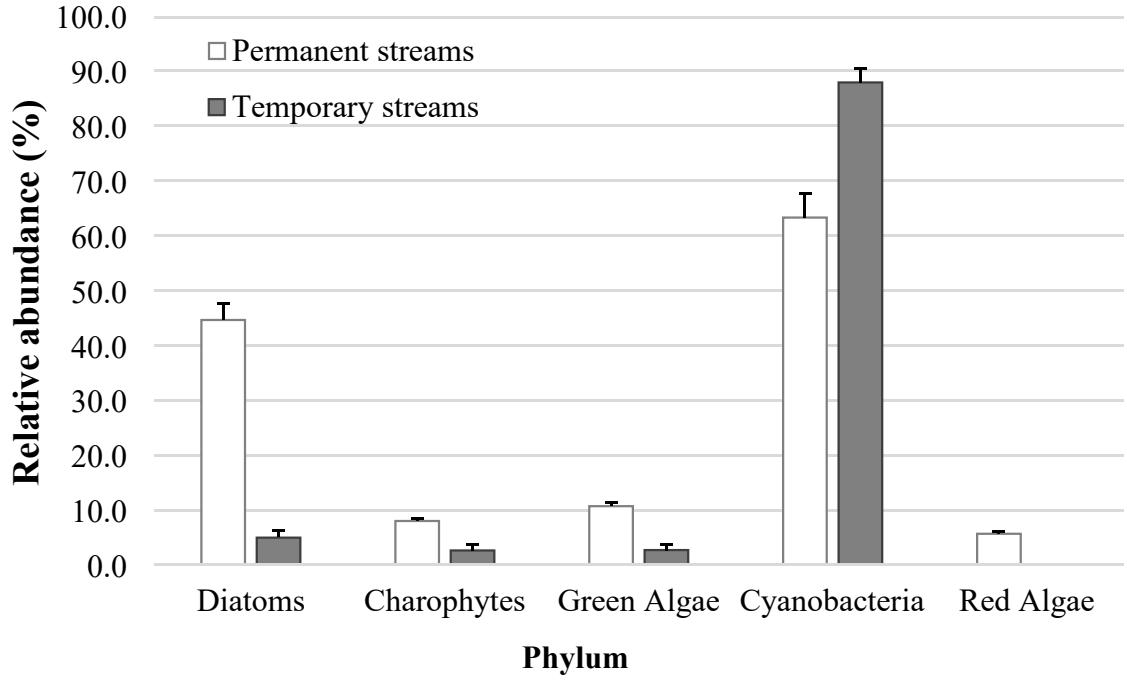
Community physiology

In Paper II pigmentary composition was grouped in four functional pigment classes (i.e. active chlorophyll, chlorophyll degradation products, primary carotenoids and secondary carotenoids). Of these four classes, active chlorophylls are those that are growth-related and directly related gross primary production, by means of photosynthesis, and that beside respond to flow intermittency. Accordingly, we decided to use this functional pigment class as a physiological measure. Active chlorophylls in permanent streams had a mean value of 1.47 ± 0.49 mg active chlorophylls cm^{-2} and were higher than in temporary streams ($U = 21$, $p = 0.002$), where had a mean value of 0.69 ± 0.70 mg active chlorophylls cm^{-2} (see detailed information in Paper II).

Community biodiversity

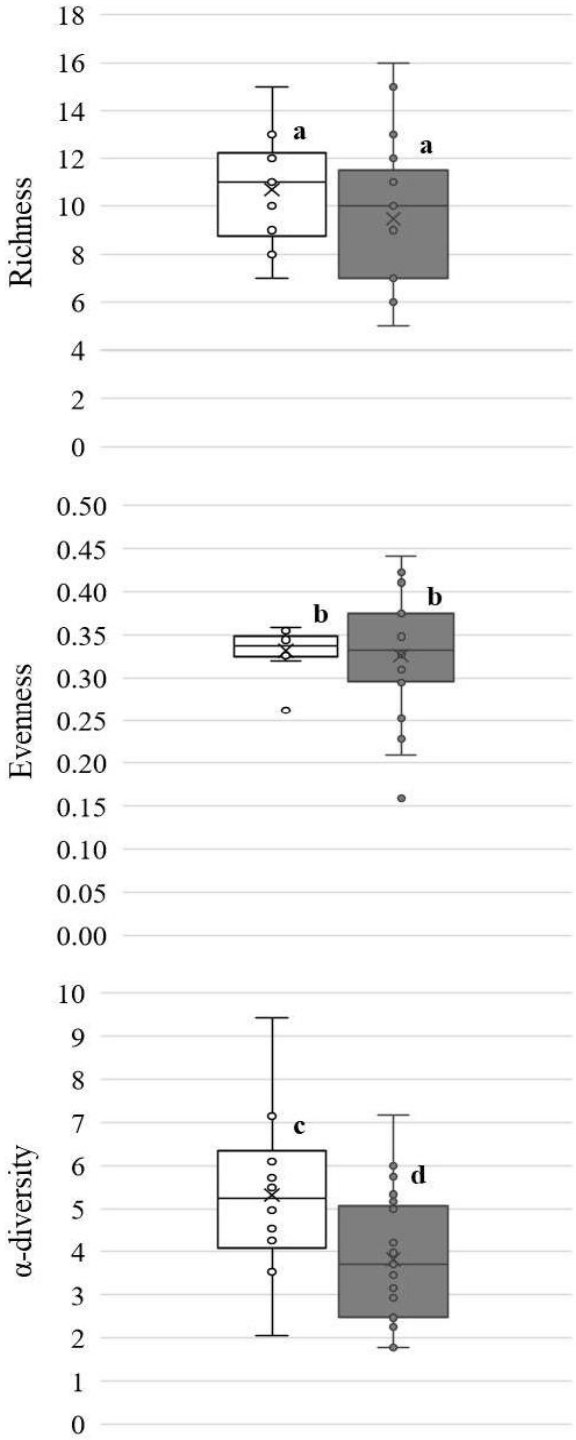
A total of 30 photoautotrophic genera were recorded in all studied streams (permanent streams: PIII. S. Table 2; temporary streams: PIII. S. Table 3). Permanent and temporary streams showed a distinct community composition, as it is evidenced by the non-metric multidimensional scaling ordination (i.e. permanent and temporary streams; k = 2; stress = 0.2). Also, the Shepard analysis showed that the assemblages were significantly different given a R^2 = 0.8. Diatoms, such as *Navicula*, *Nitzschia*, *Gomphonema*, *Encyonopsis* and *Rossithidium* were the indicator species from the photoautotrophic community of permanent streams. Cyanobacteria, including *Aphanocapsa*, *Gloeothece*, *Gloeocapsa* and *Pleurocapsa,* were indicators of the photoautotrophic community of temporary streams.

The relative abundance of diatoms and cyanobacteria in permanent streams accounted for 44.6% and 63.3% of community compositions, respectively (PIII. Figure 3). Green algae, red algae, and charophytes presented a similar low relative abundance (PIII. Figure 3). In temporary streams, cyanobacteria accounted for 87.9% of the photoautotrophic community composition; diatoms, charophytes, and green algae showed similar low relative abundances, and red algae were no present in temporary streams (PIII. Figure 3).



PIII. Figure 3.- Relative abundance of each genera in permanent and temporary photoautotrophic streams community.

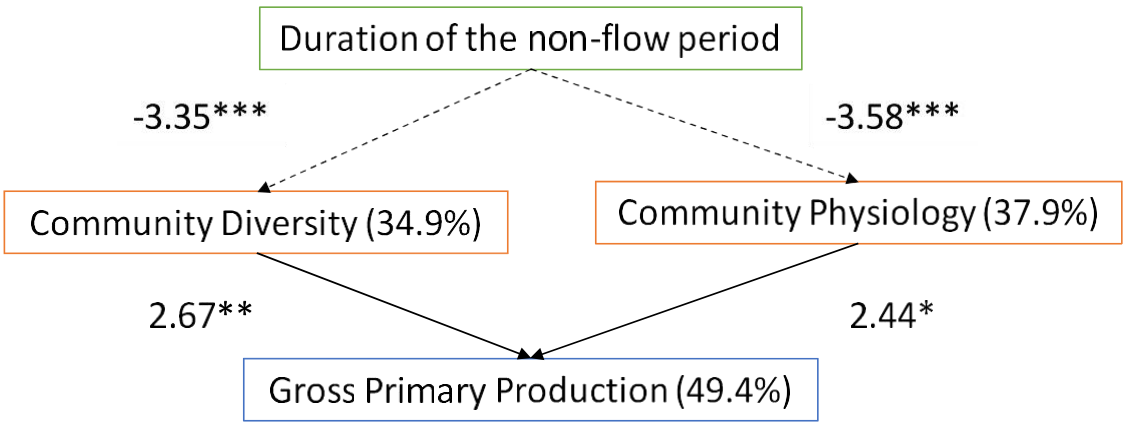
Overall, the genera richness ($H_{permanent} = 10.7 \pm 2.2$, $H_{temporary} = 9.5 \pm 3.7$, $U = 75.5$, $p = 0.22$), and evenness ($J_{permanent} = 0.33 \pm 0.03$, $J_{temporary} = 0.33 \pm 0.09$, $U = 104$, $p = 0.9$) were similar in permanent and temporary streams. Alpha diversity was higher in permanent than in temporary streams ($\lambda^{-1}_{permanent} = 5.3 \pm 1.9$, $\lambda^{-1}_{temporary} = 3.8 \pm 0.9$, $U = 57$, $p = 0.04$; PIII. Figure 4).



PIII. Figure 4.- Diversity indices in permanent (white) and temporary (grey) streams. Letters the differences or similitudes among streams.

BEF relationship mediated by temporal components of the non-flow period

According to the metamodel, the SEM revealed that community structure (i.e. community α-diversity and physiology) of photoautotrophic organisms changed in response to dry conditions, and that a causal relationship could be established between changes in the structure and those observed in the function ($\chi 2$ [2, n = 21] = 0.56, p = 0.76; CFI = 1.00; SRMR = 0.03). The final fitted model confirmed all predicted relationships when α-diversity and the mean duration of the non-flow period were considered (PIII. Figure 5). Specifically, the final model output (PIII. Figure 5) revealed that the mean duration of the non-flow period explained 34.9% of the variance in α-diversity and 37.9% of variance in active chlorophylls. Both variables, the α-diversity and active chlorophylls, contributed to the changes in GPP, explaining the 49.4% of their variability. The increase of the mean consecutive dry days reduced the mean species diversity in temporary streams and the active chlorophylls. The reduction in community diversity and active chlorophylls reduced GPP. Neither richness nor evenness were correlated with the duration and frequency of the non-flow period nor GPP, being impossible to perform the analysis with the SEM.



*PIII. Figure 5.- Final accepted structural equation model (SEM) showing all significant connections. Blue box represented the response variables, oranges boxes the structural measure (endogenous variables), and green box represent the significant environmental factor (exogenous variable). Negative effects are indicated by dash arrows, and positive effects are indicated by black arrows. Percentages indicate R^2 values; ***: p< 0.001; **: p < 0.01; *: p < 0.05.*

Discussion

Very often, laboratory and field ecological studies usually present complex data bases, are non-randomly distributed, hierarchically organized, and have spatial and temporal constraints (i.e. potential autocorrelations). SEM allows to process these data to determine the potential causal relationships between environmental factors and different biological aspects, such as community biodiversity, physiology and functioning. Some studies have already used SEM model to analyse causal relationship in ecology (e.g. Burdon et al. (2013); Eisenhauer et al. (2015); von Schiller et al. (2019)). However, SEM are not fully explored in ecological research (Fan et al., 2016), probably because of the large number of replicates (*n*) which are required to achieve the fully development of a complex SEM (i.e. including multiple interactions between exogenous and endogenous variables); which in fact involves a great sampling effort and budget. Accordingly, the abiotic drivers and the magnitude of their effect on biofilm function and structure reported here should be viewed with caution, because the small number of replicates which led to a simple model.

In our data set, diatoms were mainly present in permanent streams, while cyanobacteria were dominant in temporary streams. These results agree with previous observations (Acuña et al., 2015; Robson & Matthews, 2004; Timoner et al., 2014), and with Paper II, where specific pigments of diatoms and cyanobacteria contributed to explain differences between permanent and temporary streams. Specifically, temporary stream photoautotrophic community was dominated by aerophyte and sub-aerophyte genera. In other words, genera able to obtain their water supply from rain, dew, and atmospheric humidity (i.e. *Aphanocapsa*, *Gloeothece*, *Gloeocapsa* and *Pleurocapsa*). The identified genera of cyanobacteria are also present in biological soil crusts (Belnap & Eldridge, 2001). On which cyanobacteria are considered pioneer organisms (Hagemann et al., 2015) are highly resistant to desiccation. In contrast, diatoms are present in nearly all aquatic habitats, being important primary producers in streams and rivers (Potapova & Charles, 2002), but are sensitive to low moisture availability (Sabater et al., 2016). Thus, the dominance of aerophyte and sub-aerophyte cyanobacteria genera and the low relative abundance of diatoms in dry temporary streams suggest that the non-flow period selects taxa with resistance traits, such as cyanobacteria.

Temporary streams are usually considered to be 'low biodiversity ecosystems' at the local scale (Stubbington et al., 2017) , and therefore a lower α-diversity in temporary than in permanent streams cloud be expected. Accordingly, temporary streams presented low α-diversity than permanent ones. The observed low α -diversity of photoautotrophic organisms in temporary streams, indicated the strong negative effects of the non-flow period on biodiversity. However, the existent relationship between α-diversity, as well as active chlorophylls with the mean duration of the non-flow period in the SEM model suggest certain capacity from photoautotrophic organisms to resist dry conditions or a rapid response to flow return events that interrupt the non-flow period. Since few consecutive dry days were needed to reduce both physiological status and biodiversity, cyanobacteria with resistant traits to dry conditions were selected. However, a substantial proportion of the variance in α-diversity remained unexplained in the SEM, indicating that some important drivers were not characterized, possibly including the severity of the non-flow period or the habitat heterogeneity within the stream. The negative relationship between the mean duration of the non-flow period and active chlorophylls is consistent with the pigment composition results (Paper II), and reflects the damage inflicted by desiccation on photosynthetic apparatus. Finally,α -diversity and active chlorophylls positively affected GPP, indicating that photoautotrophic production was partially regulated by both the structure and physiology of stream biofilms.

Collectively, our results suggest that photoautotrophic organisms in stream biofilms are essentially singular, and the loss of α-diversity produces a change of status with a sharp decline in GPP (keystone hypothesis). Overall, longer non-flow periods decreased photoautotrophic taxa richness. Photoautotrophic microorganisms protect their cells through the synthesis of secondary carotenoids, but increasingly long non-flow durations ultimately reduce their biomass. Both diversity reduction and biomass reduction, are translated at the functional level as the reduction of photoautotrophic production. Thus, diverse, and more complex biofilms would be more productive than homogeneous and less developed biofilms, with implications for the structure and functioning of fluvial ecosystems.

Non-studied environmental variables should be considered to modify the observed results. For instance, GPP was measured under standardized laboratory conditions, and values were only potential, an increase in the water temperature used to measure biofilm metabolism could increase the community respiration (Acuña et al., 2008). This fact

highlights the need to extend our understanding of the nature and strength of BEF relationship. A deeper understanding of the BEF relationship could be crucial to improve our capacity to predict the effects caused by global change, and to improve the management of fluvial ecosystems.

Supplementary Material

PIII. S. Table 1.- Sub-basins of studied streams description.

Code	Coordinates	Altitude	Precipitation	Area	Land Uses (%)			
	(Lat., Long.)	m	mm	km^2	U	A	F	SG
LL01	41°35'0.57"N 1°59'5.63"E	328	732.00	10.99	0.00	2.20	97.80	0.00
LL03	41°41'43.67"N 1°53'33.00"E	258	634.54	23.96	0.00	0.00	81.31	18.70
LL05	41°41'46.46"N 1°59'1.67"E	468	692.00	8.68	0.00	0.00	91.70	8.33
BE06	41°46'1.10"N 2°16'11.98"E	430	788.40	6.50	0.00	0.00	90.20	9.80
BE07	41°47'35.79"N 2°17'29.77"E	540	748.40	19.35	0.00	1.90	92.5	5.63
FO08	41°25'7.63"N 1°30'26.44"E	620	568.50	1.65	0.00	27.12	45.77	27.11
FO09	41°25'13.53"N 1°35'32.05"E	422	578.12	1.05	0.00	0.00	100	0.00
FO10	41°25'17.08"N 1°38'0.76"E	330	574.00	1.89	0.00	70.60	17.65	11.77
FO11	41°23'52.63"N 1°35'37.29"E	320	590.07	26.87	1.35	26.58	65.87	7.21
TO12	41°51'54.36"N 2°38'45.96"E	156	908.25	12.44	0.00	0.00	100	0.00
TO13	41°51'55.61"N 2°35'35.62"E	290	874.60	49.06	0.49	0.74	97.28	1.48
TE17	42° 5'20.95"N 2°35'18.08"E	385	1013.31	13.41	0.00	1.80	98.20	0.00
TE19	42° 2'52.10"N 2°24'38.68"E	920	1063.67	3.71	0.00	29.03	67.74	3.23
TE20	42° 4'18.70"N 2°32'29.11"E	386	1038.10	9.17	0.00	15.79	84.21	0.00
TE21	42° 4'39.69"N 2°20'19.63"E	632	953.41	30.99	0.00	2.73	93.36	3.91
TE24	41°59'14.51"N 2°50'15.69"E	81	815.88	7.71	0.00	6.25	71.88	21.88
TE25	42° 6'34.82"N 2°29'19.67"E	530	963.42	13.32	0.00	16.67	83.33	0.00
FR27	41°18'38.37"N 1° 5'2.51"E	583	469.82	28.93	0.00	3.77	92.47	3.77
FL28	42°16'43.47"N 2°32'31.18"E	379	1017.41	42.63	0.00	6.53	85.23	8.24
FL29	42°16'3.60"N 2°35'33.93"E	254	1043.01	172.23	0.00	2.40	89.59	8.02

FL32	42° 7'37.99"N 2°38'26.65"E	266	997.71	13.78	0.00	18.42	81.58	0.00
FL33	42° 7'28.45"N 2°26'29.72"E	476	1026.25	15.72	0.00	13.85	86.15	0.00
FL34	42° 6'51.11"N 2°26'53.48"E	475	965.43	34.87	1.04	18.41	76.74	3.82
FL35	42°14'56.07"N 2°29'30.32"E	496	962.86	6.26	0.00	0.00	90.39	9.62
MU36	42°19'1.78"N 2°42'10.70"E	258	1093.24	44.93	0.00	0.54	90.30	9.16
MU37	42°23'15.61"N 3° 3'6.24"E	105	841.83	13.53	0.00	6.31	8.11	85.59
MU38	42°23'6.91"N 3° 1'59.28"E	88	868.00	48.69	0.00	1.99	40.55	57.46
EB42	41°15'24.80"N 0°56'34.58"E	487	427.92	34.99	0.00	4.15	79.93	15.92
EB45	40°58'48.19"N 0°24'24.60"E	316	532.10	8.93	0.00	11.08	18.72	70.20
EB46	40°50'6.20"N 0°27'9.60"E	85	709.63	29.17	0.00	24.48	44.40	31.12
EB48	41° 0'5.81"N 0°23'4.08"E	225	561.01	70.51	0.00	27.49	45.88	26.63
EB53	41°13'30.33"N 0°11'51.45"E	170	540.29	1035.67	0.15	34.17	53.65	12.03

PIII. S. Table 2.- Proportion of algae and cyanobacteria genera of the biofilms from permanent streams (n = 9). diatoms (Bacill), charophytes (Char), green algae (Chlo), cyanobacteria (Cyan), euglenophytes (Eugl), red algae (Rhod), ochrophytes (Ochr).

Phylum	Genera	EB33	EB34	EB29	FL24	MU25	FR18	TE14	TE15	TO11
Bacill	*Achnanthidium*	6	2	94	73	73	1	65	86	16
Bacill	*Adlafia*	0	0	0	0	0	0	1	0	0
Bacill	*Amphora*	31	0	0	0	0	1	21	1	32
Bacill	*Brachysira*	0	0	0	0	1	0	0	2	0
Bacill	*Caloneis*	0	0	0	0	1	0	0	0	1
Bacill	*Pulchella*	0	0	0	0	0	0	1	0	0
Bacill	*Cocconeis*	0	0	7	0	0	0	0	1	37
Bacill	*Cyclotella*	0	0	4	0	0	0	0	2	0
Bacill	*Cymbella*	0	2	24	9	12	0	0	7	0
Bacill	*Cymbopleura*	0	0	0	0	0	0	3	0	0
Bacill	*Delicata*	0	0	0	1	14	0	0	2	0
Bacill	*Denticula*	0	0	9	0	2	0	0	7	0
Bacill	*Diploneis*	0	0	0	0	0	0	1	0	0
Bacill	*Encyonema*	0	0	25	0	0	0	0	2	1
Bacill	*Encyonopsis*	0	7	15	15	36	0	1	38	0
Bacill	*Eolimna*	0	0	0	0	0	0	0	0	3
Bacill	*Epithemia*	1	0	0	0	0	0	0	0	0
Bacill	*Eucocconeis*	0	0	0	1	0	0	0	0	0
Ochr	*Eunotia*	0	0	0	2	0	0	0	0	0
Bacill	*Fallacia*	0	0	0	0	0	0	2	0	0
Bacill	*Fragilaria*	0	0	0	1	2	0	0	0	0
Bacill	*Geissleria*	0	0	0	0	0	0	0	0	3
Bacill	*Gomphonema*	1	13	5	67	4	0	5	4	0
Bacill	*Mastogloia*	0	0	0	0	0	0	0	0	0
Bacill	*Mayamaea*	0	0	0	0	0	0	0	0	1
Bacill	*Navicula*	0	0	2	4	12	0	8	12	4
Bacill	*Nitzschia*	0	0	3	1	0	0	2	3	2
Bacill	*Planothidium*	0	0	0	0	0	0	0	0	2
Bacill	*Reimeria*	0	0	0	0	0	0	0	0	3
Bacill	*Rhopalodia*	0	0	0	0	0	0	0	0	0
Bacill	*Rossithidium*	0	0	6	2	0	0	0	0	1
Bacill	*Sellaphora*	0	0	0	2	1	0	1	1	0
Bacill	*Simonsenia*	0	0	0	0	0	0	2	0	1
Bacill	*Ulnaria*	0	0	1	1	0	0	0	0	0
Bacill	*Cosmarium*	4	0	0	0	1	0	0	18	0
Char	*Mougeotia*	0	0	0	0	0	0	0	22	0
Char	*Bulbochaete*	0	0	0	12	2	4	0	0	0
Chlo	*Desmodesmus*	3	0	0	0	0	0	0	0	0

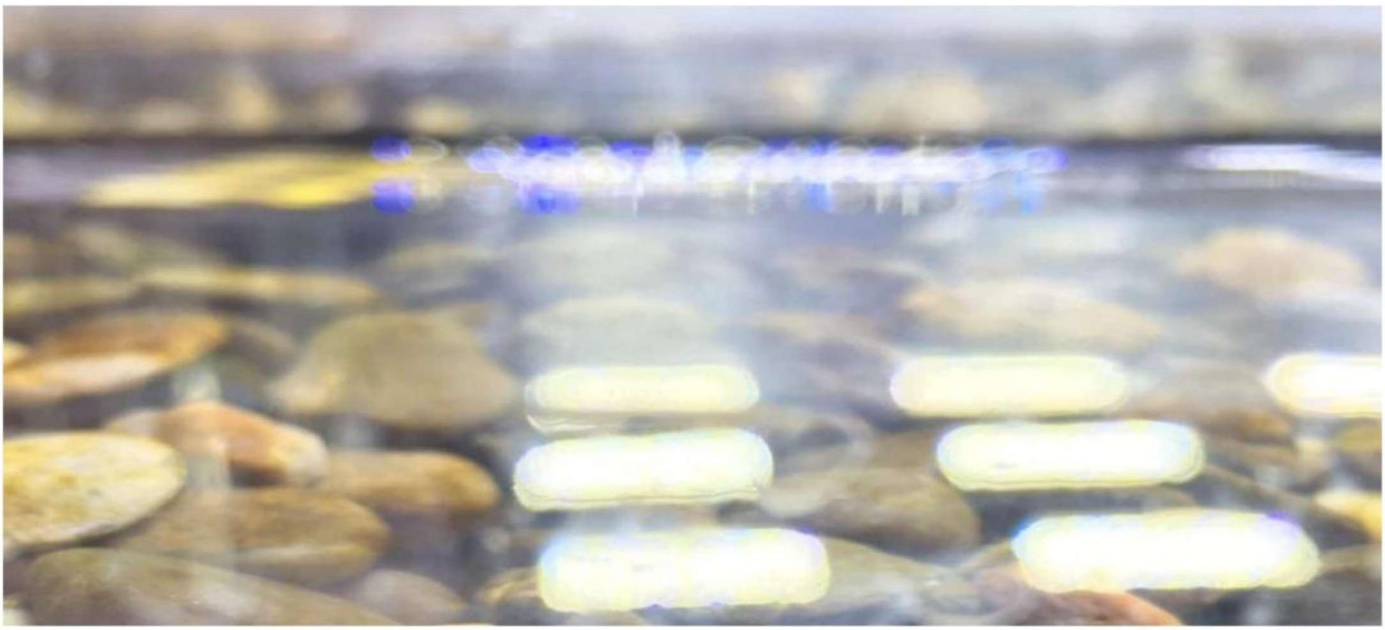
Chlo	*Oedogonium*	82	17	0	13	14	0	0	46	17
Chlo	*Pediastrum*	0	0	20	0	0	0	0	0	0
Chlo	*Spirogyra*	0	0	20	0	0	0	0	0	0
Chlo	*Cladophora*	0	0	0	31	0	8	0	0	0
Cyan	*Aphanocapsa*	18	0	0	109	0	0	104	0	79
Cyan	*Chroococal*	0	0	5	0	0	0	0	0	0
Cyan	*Chroococcus*	0	3	20	5	0	2	3	0	0
Cyan	*Gloeocapsa*	0	42	0	0	4	0	0	0	0
Cyan	*Gloeocapsopsis*	66	0	0	0	0	0	0	0	42
Cyan	*Gloeothece*	0	0	0	48	0	0	0	21	65
Cyan	*Leptolyngbya*	0	0	0	0	80	0	19	125	0
Cyan	*Lingbya*	0	78	0	0	0	10	0	0	0
Cyan	*Merismopedia*	0	0	79	0	0	0	0	0	0
Cyan	*Oncobyrsa*	147	25	26	0	0	0	0	0	0
Cyan	*Oscillatoria*	0	0	0	0	60	0	0	0	0
Cyan	*Phormidium*	0	3	0	0	0	0	0	0	0
Cyan	*Pleurocapsa*	14	125	0	0	0	13	0	0	89
Cyan	*Pseudanabaena*	0	84	11	0	11	54	162	0	0
Cyan	*Rivularia*	0	0	20	0	0	0	0	0	0
Cyan	*Tolypothrix*	26	0	0	0	36	0	0	0	0
Ochr	*Herbaudiella*	0	0	0	0	29	0	0	0	0
Rhod	*Audouinella*	0	0	0	0	0	192	0	0	28
Rhod	*Hildenbrandia*	0	0	0	0	0	115	0	0	0

PIII. S. Table 3.- Proportion of algae and cyanobacteria genera of the biofilms from temporary streams (n = 21). Diatoms (Bacill), charophytes (Char), green algae (Chlo), cyanobacteria (Cyan), euglenophytes (Eugl), red algae (Rhod).

Division	Genera	LL01	LL02	LL03	BE04	BE05	FO06	FO07	FO08	FO09	TO10	TE16	TE17	FL19	FL20	FL21	FL22	FL23	MU26	MU27	EB31	EB32
Bacill	*Cyclotella*	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacill	*Denticula*	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Bacill	*Diploneis*	0	1	1	0	0	0	2	1	0	0	0	0	0	0	0	0	0	4	0	0	0
Bacill	*Rhopalodia*	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0
Bacill	*Sellaphora*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	22	0	0	0
Bacill	*Fragilaria*	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	2	2	0	0
Bacill	*Nitzschia*	0	0	1	2	0	0	2	2	1	0	0	0	0	0	3	0	0	16	2	0	1
Bacill	*Encyonema*	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1
Bacill	*Encyonopsis*	0	3	10	1	0	2	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1
Bacill	*Achnanthidium*	1	8	18	17	13	4	10	30	1	5	1	4	5	1	5	2	1	3	6	0	4
Bacill	*Amphora*	0	0	0	5	0	0	1	15	0	2	0	0	0	0	4	1	0	0	0	2	5
Bacill	*Cocconeis*	0	0	0	18	0	0	0	1	0	8	1	0	0	0	1	3	0	0	5	1	0
Bacill	*Cymbella*	0	1	9	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1
Bacill	*Eolimna*	0	0	0	1	0	0	0	0	0	1	0	0	0	0	3	0	0	5	0	0	0
Bacill	*Epithemia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	11	0	0
Bacill	*Navicula*	0	5	2	8	0	0	3	6	0	1	1	0	1	0	4	1	0	7	2	1	1
Bacill	*Planothidium*	0	0	0	1	1	0	0	0	1	1	2	0	0	0	3	0	0	9	0	2	3
Bacill	*Reimeria*	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacill	*Rossithidium*	0	0	1	2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Bacill	*Gomphonema*	0	0	1	0	2	0	4	7	3	0	0	0	0	1	2	1	0	2	4	0	1
Bacill	*Rhoicosphenia*	0	0	0	1	0	0	0	3	7	0	0	0	0	0	1	0	0	0	0	0	0
Char	*Cosmarium*	9	0	9	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3
Char	*Mougeotia*	0	28	0	0	0	43	10	67	21	13	0	0	0	0	0	0	0	0	0	0	0
Chlo	*Bulbochaete*	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	2	0	0
Chlo	*Oedogonium*	0	0	22	25	22	0	8	0	22	0	0	0	0	45	0	0	0	41	5	0	0

Cyan	*Gloeothece*	0	0	4	0	0	56	70	0	87	34	233	30	40	0	38	18	25	0	0	11	0
Cyan	*Oncobyrsa*	151	0	0	0	160	82	0	0	0	0	0	32	0	101	0	92	0	0	0	294	50
Cyan	*Pleurocapsa*	21	184	134	45	0	40	10	45	34	69	0	124	142	0	35	0	95	21	4	18	78
Cyan	*Aphanocapsa*	114	0	61	247	49	48	37	0	73	29	69	0	81	143	125	195	89	112	0	45	0
Cyan	*Asterocapsa*	2	0	7	9	0	41	0	0	0	0	0	0	0	0	0	0	0	8	0	3	0
Cyan	*Chroococcus*	57	0	0	0	6	16	0	0	0	0	2	75	0	0	0	0	16	0	3	4	10
Cyan	*Gloeocapsa*	0	0	6	0	0	0	0	0	16	125	2	57	4	6	56	14	0	0	3	18	163
Cyan	*Gloeocapsopsis*	0	0	0	0	146	54	235	11	103	31	88	75	111	65	28	71	155	95	0	0	0
Cyan	*Oscillatoria*	0	0	16	4	0	0	4	120	0	0	0	0	0	36	0	0	0	44	9	0	33
Cyan	*Phormidium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	86	0	0	0	0	0	43
Cyan	*Pseudanabaena*	35	143	0	0	0	0	0	0	0	23	0	0	8	0	0	0	0	0	10	0	0
Cyan	*Rivularia*	0	0	0	0	0	0	0	0	8	55	0	0	0	0	0	0	0	0	199	0	0

Does biofilm origin matter? Biofilm responses to non-flow period in permanent and temporary streams



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Does biofilm origin matter? Biofilm responses to non-flow period in permanent and temporary streams

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Abstract

1. In some regions, climate change is increasing the variability of rainfall and the frequency of extreme events such as drought. Consequently, non-flow periods have grown in length and frequency, both in temporary and in formerly permanent streams. Water abstraction for human use may further prolong these dry periods.
2. We analysed the resistance and resilience of biofilms from permanent and temporary streams to non-flow conditions. This was achieved by exposing cobbles (collected from permanent and temporary streams) with intact biofilm to 31 days of non-flow, followed by 20 days of stream flow in artificial stream channels. Biofilm resistance and resilience were assessed at a structural (algal biomass, pigment composition and algae and cyanobacteria composition) and functional level (photosynthetic efficiency and community metabolism).
3. Algal taxa in biofilms from permanent and temporary streams differed throughout the experiment. Biofilms from permanent streams were less resistant to non-flow than those from temporary streams at structural level. Permanent stream biofilms also presented lower resilience at a structural level, but responded similarly to temporary stream biofilms at s functional level.
4. Our investigation shows how the non-flow period disturbed permanent stream biofilms, and suggests that temporary stream biofilms will have greater adaptive capacity as hydroperiod becomes shorter due to climate change.

Keywords: resistance, resilience, biofilm structure, biofilm functioning, climate change

Introduction

According to observations of climate variation during recent decades, and to the projections of global-scale climate models, there is an ongoing shift in the temporal variability of rainfall towards a higher frequency of extreme events such as supra-seasonal droughts, especially in arid and semi-arid zones (Döll & Zhang, 2010). Simultaneously, water abstraction and flow regulation have markedly altered river flow regimes, which is affecting river networks (Sabater et al., 2018). Altogether, non-flow episodes are becoming longer and more frequent in in some parts of the world (Pumo et al., 2016; Skoulikidis et al., 2017), and are beginning to affect permanent watercourses (Döll & Schmied, 2012). These changes in flow regimes are becoming increasingly common, and their consequences on river ecosystems are uncertain.

Previous studies have examined the effects of non-flow periods on vertebrate and invertebrate biodiversity (Datry et al., 2014; Ferreira et al., 2007), and on biofilm structure and function (Romaní et al., 2013; Sabater et al., 2016) of temporary streams. Others have observed specific adaptations to hydrological variability, such as life cycle coupling to flow and non-flow periods, strategies of dispersal, and respiratory traits (Bonada et al., 2007; Lytle & Poff, 2004). Resistance structures, such as cysts or thickened cell walls (Romaní et al., 2013), or the increase in protective pigments (Timoner et al., 2014) are also common adaptations of stream microorganisms. In addition, biofilms from temporary streams show high resilience after flow return, as a consequence of the development of several osmotic, photosynthetic, and enzymatic adaptive mechanisms (Robson et al., 2008; Robson & Matthews, 2004; Romaní et al., 2017), which allow them to recover their activity after short rewetting periods (Romaní & Sabater, 1997). There is an implicit assumption in the scientific literature that biological communities in temporary streams are better adapted to non-flow periods than those from permanent streams. This general assumption is induced by the fact that permanent streams have never suffered these disturbances and, therefore, the taxa should not necessarily have adaptations to non-flow periods. Consequently, the biota of permanent streams is expected to be less resistant to non-flow periods and less resilient after flow return than that of temporary streams. Thus, submitting permanent stream biota to non-flow periods may expose them to environmental conditions that limit their growth or survival (Wallenstein & Hall, 2012).

Biofilm function is central to stream ecosystem metabolism (Acuña et al., 2015), and the production and transformation of organic and inorganic matter (Battin et al., 2003; Sabater et al., 2017). However, biofilms from temporary streams are functionally constrained during non-flow periods, as they become affected by water stress, higher temperatures, and stronger solar irradiance (Colls et al., 2019). These situations mainly occur in temporary streams when precipitation shortfalls co-occur with high temperatures and an increase in evapotranspiration. However, global change is increasingly exposing permanent stream biofilms to these environmental conditions. Harsher environmental conditions require specialized or opportunistic organisms (Timoner et al., 2014) able to resist them in order to contribute to the biogeochemical and metabolic recovery of the system once the water flow returns (Acuña et al., 2007; Baldwin & Mitchell, 2000; von Schiller et al., 2011). Consequently, it is generally assumed that permanent streams, which would be deprived of adapted organisms, should show lower resistance to the non-flow period, and should take longer to recover after the return of the water flow.

To determine the veracity of these assumptions, we analysed the response of stream biofilms from permanent and temporary streams to the non-flow period. We hypothesized that the temporary stream biofilms, which include more tolerant taxa, would show higher resistance to desiccation at a structural and functional level because of their protective mechanisms. Similarly, we assumed that temporary stream biofilms would show higher resilience after the return of water flow and that their capacity for recovery would be reflected at a structural and functional level. Conversely, permanent stream biofilms would be more vulnerable to the non-flow period in terms of resistance and resilience. To test these hypotheses, an experiment was performed in artificial streams. Cobbles with intact biofilms collected from temporary and permanent streams were placed under laboratory conditions to analyse their resistance to the non-flow period (the community's capacity to withstand the disturbance) and their resilience after flow return (the community's capacity to regain pre-disturbance conditions). Resistance and resilience were assessed in terms of biofilm structure (algal biomass, pigment composition, and algae and cyanobacteria composition) and functionality (photosynthetic efficiency and community metabolism). The experimental duration of the non-flow period was established as a compromise between the natural duration of the non-flow period in the area where the cobbles were collected and the maximum duration that can be achieved

under laboratory conditions. This allowed monitoring biofilm responses under controlled conditions with the aim of extrapolating them to the natural environment.

Materials and methods

Study sites

Responses to the non-flow period were assessed in biofilms from four permanent streams (PS) and four temporary streams (TS). All the streams were located within the same geographical area (northeast of the Iberian Peninsula). They all shared a Mediterranean climate and had similar geology, and only differed in their hydrological regimes. The four PS and three of the TS streams were located in the headwaters of the Ter river basin, and the fourth TS was tributary of the Fluvià river. Mean monthly air temperatures in the stream sites oscillated around 7°C during winter and up to 20°C in late summer and early autumn, which was when the cobbles were collected. Mean annual precipitation ranged from 380 mm in the drier basins to 1200 mm in the most humid area. The streams were mainly surrounded by forest or by agricultural fields, and all showed low to moderate nutrient concentrations (PIV. Table 1). Water never completely stopped flowing in the PS. In the TS, water stopped flowing from mid-June to October, wherein TS were completely dry although occasional summer storms (70-100 mm) in early August briefly restored flow for up to 5 consecutive days (PIV. Table 1).

Cobbles colonized by intact biofilm (nearly 200 in each study site) were collected at the beginning of autumn (October 2016), when the TS were still dry. Permanent stream cobbles were kept submerged carefully in stream water in separate bags and taken to the laboratory, in order to avoid them hydric stress and unwanted biofilm detachment. Temporary stream cobbles were maintained dry and, also immediately transported to the laboratory, where they were transferred to flow conditions into the artificial streams.

PIV. Table 1.- Information on the eight studied streams. Nutrient concentration on the sampling day, duration and frequency of non-flow periods from 236 days before sampling until the sampling day, and land uses (F: Forest; A: Agriculture; U: Urban) and altitude, estimated with GIS layers with Quantum GIS GRASS 7.2.2.

	Basin	**Stream name and order**	N- NH_4^+	N-NO_3^{2-}	P-PO_4^{3-}	DOC	**Flow period**			**Altitude**	**Land use**
			($mg \cdot L^{-1}$)	($mg \cdot L^{-1}$)	($mg \cdot L^{-1}$)	($mg \cdot L^{-1}$)	**Duration**	**Frequency**	**Mean Duration**	(m)	(F; A; U)
Permanent	Ter	Ges (4)	0.042	1.034	0.004	0.876	236	1	236	490	96%; 3%; 1%
	Ter	Fornés (2)	0.002	0.144	0.002	0.686	236	1	236	490	97%; 3%; 0%
	Ter	Cogolls (2)	0.002	1.846	0.005	4.375	236	1	236	370	84%; 6%; 0%
	Ter	Llémena (2)	0.009	0.465	0.002	2.215	236	1	236	850	99%; 1%; 0%
Temporary	Ter	La Solana (2)	0	0	0	0	131	10	13.1	750	100%; 0%; 0%
	Ter	Vilardell (2)	0	0	0	0	127	10	12.7	670	96%; 4%; 0%
	Ter	Brugent (2)	0	0	0	0	104	10	10.4	560	83%; 17%; 0%
	Fluvià	Sant Miquel de Campmajor (2)	0	0	0	0	13	5	2.6	220	0%; 11%; 89%

Experimental set-up

The collected cobbles were immediately immersed into artificial streams of the Catalan Institute for Water Research indoor Experimental Stream Facility. A total of 16 artificial streams were used, each artificial stream containing cobbles from one particular stream. The four artificial streams containing biofilms from each one of the PS and the four ones with biofilms from TS were therefore considered replicates of each stream type. Each artificial stream consisted of an independent 2-m long methacrylate channel with a 50-cm^2 rectangular cross-section. We used a total of eight artificial streams, each of which operated as an independent system with a constant flow of 60 ml/s. Water for the artificial streams was collected from rainfall and was filtered through activated carbon filters. Water physicochemistry was monitored during each of the sampling days with flowing water conditions and presented values ranging between 9.1 - 9.2 mgO_2/L, 8.3 - 8.4 pH, and 414 - 423 µS/cm conductivity. Dissolved nutrient concentrations remained constant during the entire experiment: 0.006 - 0.01 mg/L $P\text{-}PO^{-3}_4$, 0.005 - 0.015 mg/L $N\text{-}NH^{+}_4$, 1.8 mg/L $N\text{-}NO^{-3}$ and 1 mg/L C-DOC. The cobbles were placed randomly along the artificial stream, with the upper side of cobbles facing up, mimicking the spatial configuration found in the field. Cobble dimensions ranged from 4 to 7 cm long and 2 to 3 cm high, to fit to the dimensions of the artificial streams. Water depth in the artificial streams ranged between 2.2 and 2.5 cm. Air room temperature was set at 21°C and a daily light/dark cycle of 14 hr/10 hr was simulated by LED lamps (Lightech, Girona, Spain). Light intensity was recorded every 10 min using four quantum sensors located across the array of artificial streams (sensor LI-192SA, LiCOR Inc), and reached 170 µ E m^{-2} s^{-1} at the surface of the artificial streams. Air temperature in the indoor Experimental Stream Facility was 20ºC and water temperature was 16ºC. Water temperature in each artificial stream was also recorded every 10 min during the entire experiment using VEMCO minilog (TR model, AMIRIX Systems Inc) temperature data loggers (5 - 35ºC, ±0.2ºC).

Experiment design

Before the start of the non-flow period, a 7-day acclimatization period allowed biofilms to adapt to the artificial stream environment; which was close to the mean duration of the flow period in the TS (9.7 flow days) during the dry phase (PIV. Table 1), when cobbles were collected. To compare PS and TS feasibly, we had to re-instate the flowing phase for these biofilms. Our rationale was that TS set the *reference* of the effects of the non-

flow on biofilm communities, and PS submitted to a temporary flow regime may have a different response pattern. Submitting the two to the same initial conditions required therefore maintaining the PS with flowing water and allow the TS to receive it. The non-flow period was initiated at the same time in all the artificial streams and lasted 31 days. During this period, flow ceased, water level dropped to zero, and the cobbles to progressively dried out until complete dryness. Then, flow was resumed and maintained during the following 20 days in all the artificial streams. All variables (see below) were measured simultaneously in all eight artificial streams. Chlorophyll-*a* (Chl-*a*) and photosynthetic efficiency were measured at time 0 (i.e. at the end of the acclimatization period and just before the non-flow period), and again after 3, 9, 20 and 31 days of non-flow conditions. These variables where measured again 3, 9 and 20 days after the flow was resumed (days 33, 36, 40 and 51 after the experiment commenced). Biofilm metabolism was measured before the onset of the non-flow period (day 0), and again after flow return (i.e. days 33, 36, 40 and 51 of the experiment), in order to preclude rehydrating the samples and interrupting their desiccation. Algae and cyanobacteria community composition and pigment composition were measured on three occasions: before the onset of the non-flow period (day 0), at the last day without flow (day 31), and at the end of the experiment (day 51).

Sampling and laboratory analyses

Gross primary production (GPP) and community respiration (CR) were measured as variables of biofilm metabolism. On each sampling day, three cobbles were randomly collected from the upper, middle and lower part of each artificial stream. They were then immediately placed together inside a metabolism chamber to homogenize potential within-stream variability and used to estimate the biofilm metabolism of each of the artificial streams. Net metabolism (NM) and CR were assessed by measuring the changes in mean oxygen values in cylindrical metabolism chambers (Acuña et al., 2008). The chambers were made of acrylic glass (PMMA; volume 0.96 L) and were recirculating by means of submersible water circulation pumps. The chambers were placed inside an incubator (Radiber AGP-700-ESP, Barcelona, Spain) that provided the same constant conditions of water temperature (21ºC) and light (168 ± 2 μE m^{-2} s^{-1}) as in the artificial streams. The incubations had a duration of 1 h in full darkness (to measure CR) and 1 h under light conditions (to measure NM). Dissolved oxygen concentration inside the

chambers was recorded at 30 s intervals with oxygen sensors (PreSens OXY-10mini, Regensburg, Germany). Metabolic rates were calculated according to Acuña et al. (2008). CR was computed as the reduction of oxygen concentration throughout the incubation, and NM as the increase of oxygen concentration along the incubation time. Gross primary production was estimated as the sum of NM and CR, both of which were expressed as $mgO_2\ m^{-2}\ min^{-1}$. After the metabolism measurements, the biofilms were immediately scraped off the cobbles with a brush and placed into 40 mL of filtered channel water (nylon 0.2 µm pore size). One biofilm suspension solution was obtained per cobble, to be used for subsequent measures (see below). The size of the area scraped was determined by wrapping the scraped area in tin foil and weighing it, and then transforming the weight to surface area using an appropriate empirical regression. After biofilm collection, the used cobbles were labelled with an elastic band and returned to their former place, in order to maintain the original hydraulic conditions of each artificial stream. On the sampling days in which artificial streams were under non-flow conditions and biofilm metabolism was not measured, three cobbles were collected per artificial stream and scraped using the same method as explained above.

Chlorophyll-*a* analyses were performed to measure the autotrophic biofilm biomass. Pigment composition, and algae and cyanobacteria community composition analyses were performed to measure structural and compositional changes. Photosynthetic efficiency (Y_{eff}) was measured as an approach of functional changes.

Chlorophyll-*a* and pigment composition analyses were performed on 8 ml of biofilm suspensions, which were divided into two aliquots. Both aliquots were centrifuged at 2500 rpm during 10 min at 4ºC, then the supernatant was removed, and the samples were lyophilised and stored at -80ºC until their analysis. Chlorophyll-*a* and pigments were extracted in 90% acetone during 12 hr in the dark and at 4ºC (Steinman et al., 2017). The complete extraction was ensured using sonication (30 s, 360 W power, 50/60 Hz frequency, JP Selecta SA, Barcelona, Spain) after which the samples were centrifuged at (804 g) during 10 min at 4ºC to separate the suspended biofilm from the extract. Chlorophyll-*a* was determined spectrophotometrically using a Lambda UV/VIS spectrophotometer (U-2000; Hitachi, Tokyo, Japan) by following (Jeffrey & Humphrey, 1975). Pigment composition was determined using HPLC analysis of the centrifuged and filtered extracts through Whatman Anotop filters (0.1 µm pore size, 25 mm diameter; Whatman International Ltd., Maidstone, England). Pigment samples were analysed using

the eluent gradient described by Buchaca & Catalan (2007), using a Waters HPLC 510 and Waters Photodiode array detector 996 (Waters) on a C_{18} column (5 µm, 250 x 4.6 mm, Spherisorb, ODS 1 Waters). The detector was set at 440 nm for carotenoids and 660 nm for chloro-pigments peak integration. Pigment standards (Chl-*a*, chlorophyll-*b*, chlorophyll-*c2*, chlorophyllide-*a*, diatoxanthin, fucoxanthin, canthaxanthin, lutein, neoxanthin, violaxanthin, zeaxanthin, echinenone, β-carotene, phaeophytin-*a* and pheophorbide-*a*; DHI Water and Environment, Denmark) were run individually at different concentrations and as a mixed standard to determine the retention time and calibration curves of each pigment. The different pigments were identified by comparing their retention times and absorption spectra. Pigments without available standards (scytonemin, pyridine, pheophorbide-b, dianoxanthin, diadinoxanthin, myxoxanthophyll, alloxanthin, canthaxanthin, allomer Chl-*a1*, allomer Chl-*a2*, epimer Chl-*a*, α-carotene, phaeophytin-*b*) were identified using a library of pigment spectra obtained from (Buchaca & Catalan, 2007). Peak areas were converted to concentrations using either the calibration curves or the extinction coefficients (Buchaca, 2005), which were related to the cobble surface area (cm^2). We determined the phaeophytization index (PQI = CD/*a*-phorbins) to evaluate the percentage of Chl-*a* degradation products, where Chl-*a* derivatives (CD) were calculated as the sum of the photosynthetically inactive Chl-*a* derivatives (chlorophyll-*a* allomer, chlorophyllide-*a*, pheophorbide-*a* and phaeophytin-*a1*, *a2*) and the *a*-phorbins were calculated as the sum of Chl-*a* and CD (Timoner et al., 2014).

Algal and cyanobacteria community composition was determined at the genus level by analysing a 5 mL biofilm suspension examined under a 600× microscope (Nikon CS1, Tokyo, Japan) following the classifications described by Wehr et al., 2015. The relative abundance of each genus was estimated using a semi-quantitative method based on cell abundance, where cells were ranked on the scale: (1very rare, ≤ 5%), 2 (> 5 to ≤ 20%), 3 (> 20 to ≤ 40%), 4 (> 40 to ≤ 60%), 5 (> 60 to ≤ 80%) and 6 (very abundant, > 80%). The relative abundance per genus in each artificial stream was estimated as the average between the three replicates (PIV. S. Figure 1).

Photosynthetic activity (Y_{eff}) was used as a non-destructive method to evaluate the functional changes in the autotrophic compartment of the stream biofilm because of the non-flow period. This parameter reflects the efficiency of energy conversion at the Photosystem II (PS II) reaction centres (Schreiber et al., 2002). A portable pulse amplitude modulate fluorometer (Diving-PAM; WALZ, Effeltrich, Germany) was used

to measure Y_{eff} and these measurements were performed in situ on every sampling day, using three intact cobbles from each artificial stream.

Data analysis

A natural logarithm transformation was applied to Chl-*a* and Y_{eff} in order to meet the assumptions of parametric Kurtosis tests. The differences between the biofilms from P and T streams before the non-flow period were tested using an analysis of variance (ANOVA). The effects of the non-flow period were then characterized by their Resistance (*R*) and resilience (*r)* (Grimm & Wissel, 1997; Holling, 1973; Uehlinger, 2000).

Resistance and resilience are clearly defined concepts (Lake, 2003; Stanley & Fisher, 1992), but estimating them is a complex issue, particularly in the case of resilience (Todman et al., 2016). Here, we used two different approaches: (1) the magnitude of the changes in each variable, calculated as the percentage of change; and (2) the rate or the velocity of the changes in each variable. These estimates provided complementary perspectives of the effects of the non-flow period on biofilms. The magnitude of the changes highlighted the intensity with which the non-flow period affected each studied variable, while the rate of change indicated the velocity at which the variable was reduced (resistance) or recovered (resilience). Thus, resistance (*R*) was calculated as (1) the percentage of reduction in each variable between day 0 (before flow interruption) and day 31 (the last dry day); and (2) the slope of the linear regression from day 0 to day 31, which comprised all the sampling days in between (i.e. days 3, 9 and 20). By contrast, resilience (*r*) was calculated as (1) the percentage of increase between day 31 (the last non-flow day) and day 51 (the last recovery day), and (2) the slope of the linear regression from the last dry day (day 31) to the last of the recovery days (i.e. day 51), which considered the sampling days in between (i.e. 33, 36, and 40) (Grimm & Fisher, 1989). Only slope values with coefficients of determination (R^2) > 0.75 were accepted as a valid for the calculation of resistance and resilience as the velocity of change (Acuña et al., 2015). Finally, the differences in resistance and resilience between PS and TS biofilms were tested using repeated-measures analysis of variance (ANOVA) of a fit linear mixed-effects model. The model included biofilm origin (PS versus TS) as a fixed factor, stream as a random factor nested within origin, and date as a fixed factor of repeated measures. This test was carried out using an F distribution and allowed including the daily changes of *R* and *r*, as

well as the variability within the groups (i.e. variability between biofilm from PS and between biofilm from TS), while avoiding response linearization.

Algal community composition patterns were explored using multivariate analyses. Non-metric multidimensional scaling was used to analyse the similarity in the species pools from PS and TS biofilms. Non-metric multidimensional scaling provided a two-dimensional graphical representation of the algal genera clustering at each sampling day (i.e. before the non-flow period, on the last dry day, and on the last recovery day). An indicator species analysis was performed to identify the representative species of each cluster in the data set, (INDVAL) (Dufrêne & Legendre, 1997). Finally, the differences in PQI were tested using an analysis of variance (one-way ANOVA). All analyses were considered significant at $p < 0.05$ and were performed using RStudio (R version 3.3.2 and RStudio 1.0.136).

Results

Biofilm characterization and metabolism before the non-flow period

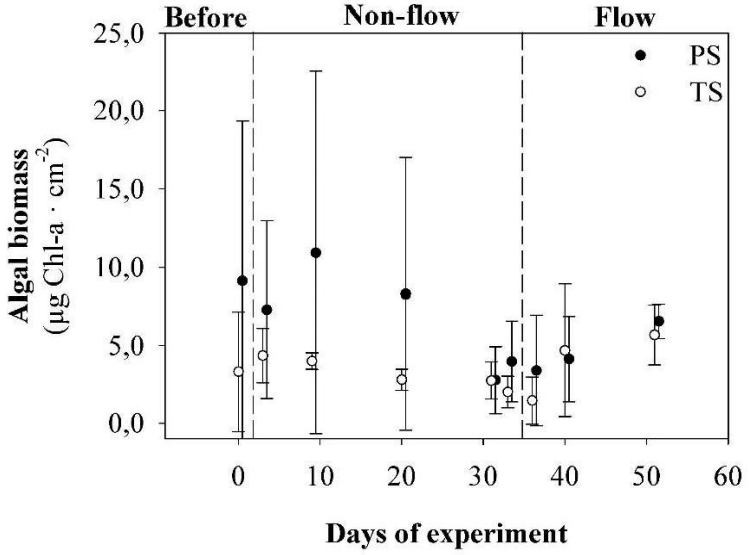
The species pool in PS and TS biofilms after 7 days of acclimation differed (PIV. S. Figure 1). Diatoms were significantly more abundant in the PS biofilms (PS: 59% versus TS: 29%, $p = 0.01$), whereas cyanobacteria were significantly more abundant in the biofilms from TS (TS: 50% versus PS: 26%, $p = 0.01$, PIV. S. Figure 1). Chlorophytes were similarly abundant in biofilms from both types of streams. ($p = 0.09$; PIV. S. Figure 1). The autotrophic biomass (expressed as Chl-*a*) was not significantly different between biofilms from PS (Chl-*a*: 9.1 ± 10.2 µg/cm) and from TS (Chl-*a*: 3.3 ± 3.8 µg/cm, $p = 0.33$; PIV. S. Table 1). Y_{eff} was statistically higher ($p = 0.048$) in biofilms from PS (PS: 0.37 ± 0.04 versus TS: 0.29 ± 0.05; PIV. S. Table 1). Biofilm metabolism (CR and GPP) was higher in biofilms from PS (CR: -0.67 ± 0.18 $mgO_2\ m^{-2}\ min^{-1}$, GPP: 3.79 ± 1.84 $mgO_2\ m^{-2}\ min^{-1}$) than in those from TS (CR: -0.27 ± 0.22 $mgO_2\ m^{-2}\ min^{-1}$, GPP: 1.66 ± 1.25 $mgO_2\ m^{-2}\ min^{-1}$; PIV. S. Table 3).

Biofilm responses to the non-flow period

Algae and cyanobacteria composition in the PS and TS remained distinct throughout the non-flow and flow return periods. The community composition of both biofilm types was separated by the non-metric multidimensional scaling ordination in the 3 tested sampling

days (0, 31 and 51 days; stress: 0.2, PIV. S. Figure 2), and the Shepard analysis showed that the assemblages were significantly different given a $R^2 = 0.77$. Cyanobacteria, such as *Aphanocapsa,* and *Calothrix,* and chlorophytes, such as *Chloroccocal* undetermined*, Ulothrix,* or *Palmella,* were the indicator species for the biofilms from TS (INDVAL > 0.5), which mostly included crustose and unbranched filament life forms. Bacillariophyta, such as *Cocconeis, Gomphonema, Navicula* and *Cymbella* were indicators of the biofilms from PS (INDVAL > 0.5), which mainly comprised by prostrate forms.

The non-flow period reduced the large variability among the permanent streams (PIV. Figure 1). Subsequently, among-stream variances were similar between the two stream types, so no difference in biomass was observed (PIV. S. Table 1). The rate of reduction in autotrophic biomass was also similar in the biofilms from PS (R = -1.23, R^2 = 0.87) compared to those from TS (R = -0.26, R^2 = 0.78). However, the repeated measures-ANOVA, which considered the within groups variability and each sampling day, showed that autotrophic biomass was statistically more resistant in TS (PIV. Table 2). Despite these differences, PQI was not significantly different between the two biofilm types (PS PQI = 56.2 ± 7.5%; TS PQI = 77.6 ± 9.7%; p = 0.15; PIV. Figure 2b). Accessory pigments, such as scytonemin, lutein, zeaxanthin, canthaxanthin and β-carotene, were substantially more variable in biofilms from TS than from PS (PIV. S. Tables 2).

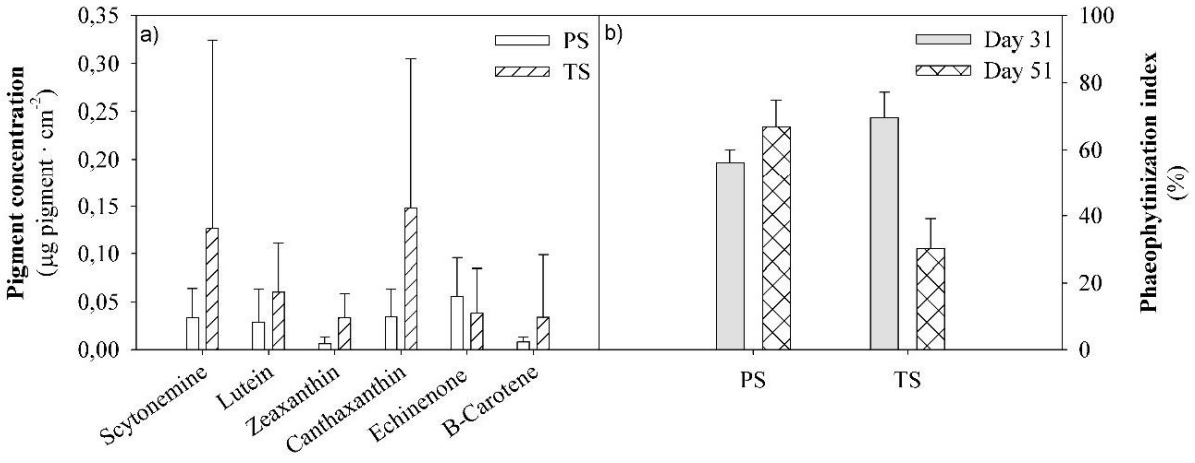


PIV. Figure 1.- Algal biomass (µg chlorophyll-a/cm) of the biofilms from the four temporary (TS) and the four permanent (PS) streams at each sampling day. Error bars indicate standard error.

PIV. Table 2.- Resistance and resilience of biofilms from permanent and temporary streams (origin) of algal biomass (chlorophyll-a) and photosynthetic efficiency (Y_{eff}). Differences and similarities and were tested by repeated measured analysis of variance (ANOVA) method (F statistic and p-values). Statistically significant results in bold.

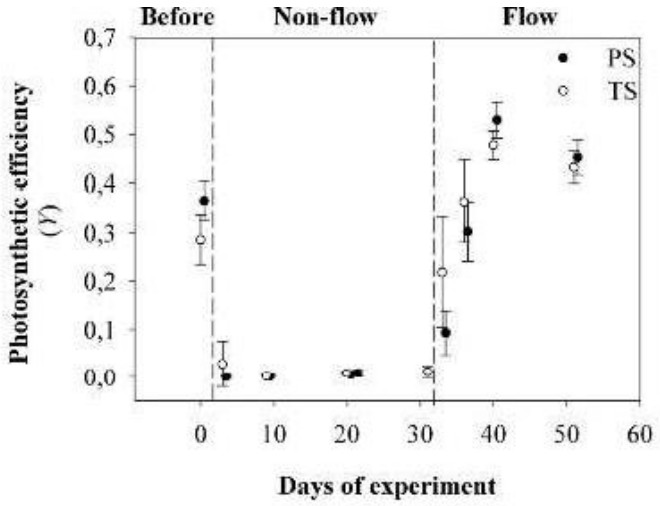
Resistance	**Chlorophyll-*a***	***Y_{eff}***
Origin	**F = 11.05** ***p* = 0.002**	F = 0.16 *p* = 0.70
Origin (Stream)	**F = 9.38** ***p* = 8.15e-6**	F = 0.04 *p* > 0.999
Resilience		
Origin	F = 0.15 *p* = 0.70	F = 0.68 *p* = 0.42
Origin (Stream)	**F = 4.8** ***p* = 0.002**	F = 0.34 *p* = 0.91

After 20 days of flow resumption, Chl-*a* in the biofilms from PS (6.6 ± 2.1 µg Chl-*a* cm^{-2}) became similar to that of TS biofilms (5.7 ± 2 .6 µg Chl-*a* cm^{-2}; PIV. S. Table 1). While the magnitude of recovery in the PS was lower than in TS (44.0 ± 0.1%, and 53.5 ± 0.4% respectively) and the rate of recovery was also lower in the biofilms from PS (r = 0.17, R^2 = 0.87) than in those from TS (r = 0.23, R^2 = 0.78). The repeated measures-ANOVA showed significant differences only in the cases when within-group variability was considered (PIV. Table 2), probably as a consequence of the low number of replicates and the high variability within groups. The PQI on the last recovery day was statistically lower (p = 0.02) in TS (PQI = 29.8 ± 6.6 %) than in PS biofilms (PQI = 66.9 ± 7.3%; PIV. Figure 2b). This index did not change significantly in the PS biofilms from the last dry day (p = 0.25; PIV. Figure 2b), indicating that active Chl-*a* in these biofilms had not recovered 20 days after flow return.



PIV. Figure 2.- (a) Pigment concentrations (µg carotenoid/cm²) and (b)phaeophytization index (%) of the biofilms from the four permanent (PS) and the four from temporary (TS) streams at the last non-flow day (Day 31) and the last recovery day (Day 51) with error bars showing the standard error.

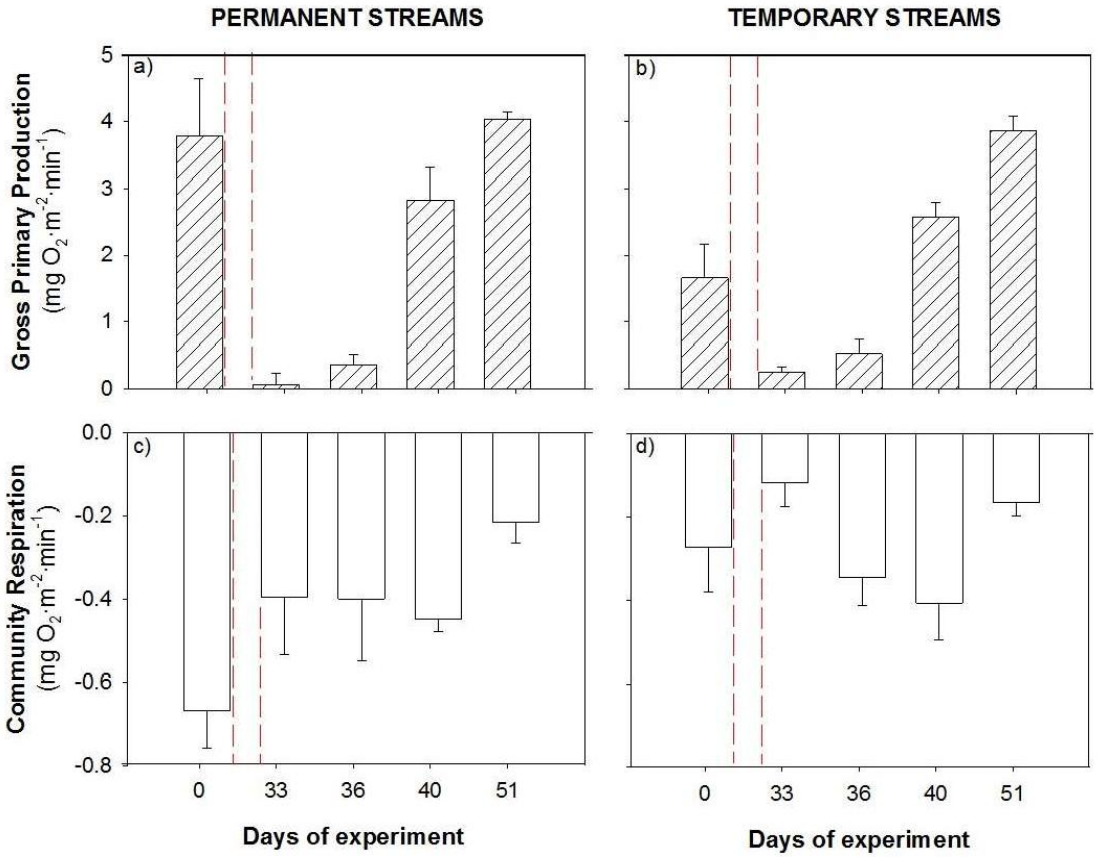
Y_{eff} showed a similar impact of drying in both biofilm types (PIV. Figure 3), showing a 98% decrease in the TS and a 96% decrease in the PS. The reduction rate was also similar in PS (R = -0.08, R^2 = 0.83) than in TS (R = -0.06, R^2 = 0.76), and repeated-measures ANOVA showed no significant differences (PIV. Table 2). The magnitude of recovery of Y after flow return was similar both biofilm types (26.8 ± 0.04% in PS, 34.7 ± 0.09% in TS) but was higher than before the non-flow period (PS t = 0 versus t = 31: p = 0.017; TS t = 0 versus t = 31: p = 0.003 respectively). The rate of recovery was slightly higher in biofilms from PS (r = 0.06, R^2 = 0.99) than in TS (r = 0.04, R^2 = 0.98), but the repeated-measures ANOVA did not show significant differences in Y_{eff} recovery between biofilms from both types of streams (PIV. Table 2).



PIV. Figure 3.- Photosynthetic efficiency (Y_{eff}) of the biofilms from the four temporary (TS) and the four permanent (PS) streams at each sampling day, with error bars indicating the standard error.

Effects of the non-flow period on biofilm metabolism

Both GPP and CR decreased after the non-flow period began. The magnitude of the impact on the GPP of biofilms from PS was higher (98.5%) than in those from TS (85.2%). Gross primary production was higher in biofilms from TS from the first day after flow resumption, but the rate of recovery was not significantly different between the two (PS: $r = 0.21$, $R^2 = 0.96$; TS: $r = 0.20$, $R^2 = 0.93$). Overall, GPP was more affected by non-flow than CR (PIV. Figure 4), despite the fact that CR also decreased in biofilms from both PS and TS after 31 days of non-flow conditions. The magnitude of the impact on CR was higher in biofilms from TS (57%) than in the PS (40%). After flow resumption, no clear CR resilience trend was observed in PS or TS (PIV. S. Tables 3).



PIV. Figure 4.- Gross primary production ($mgO_2\ m^{-2}\ min^{-1}$) of the biofilms from the four permanent (a), and the four temporary streams (b) before and after the non-flow period. Community Respiration ($mgO_2\ m^{-2}\ min^{-1}$) in permanent (c), and temporary (d) stream biofilms before and after the non-flow period. Vertical dashed lines indicate the onset of the non-flow period.

Discussion

The non-flow period acted as an abiotic disturbance (Chase, 2003), but differentially affected the biofilms from PS or TS. Effects were visible on the primary producers' community structure, as well as on biofilm metabolism; however, the resistance to the non-
flow period, and the ability to recover from it, was related to the biofilms' previous adaptation to desiccation (Acuña et al., 2015; Romaní et al., 2017; Sabater et al., 2017; Timoner et al., 2012). Overall, biofilms from PS showed differences in their resistance and resilience in terms of structure than those from TS, suggesting that drought-adapted biofilms respond better to drying than those from permanent streams.

Generally, the effects of the non-flow period on biofilm algal taxa during the experiment were modulated by the initial biofilm biomass and composition of each stream type, which is an expression of the past environmental effects to which they have been exposed (Lake, 2003; Steward et al., 2012). The TS used in the experiment received up to 10 periods of flow interruption before biofilm collection, with an average of 9.7 consecutive flowing days and a total duration of flow ranging from 13 to 131 days during the considered period (8 months in total). These biofilms therefore received continuous water flow interruptions which highly likely to determine their low initial biomass and particular biofilm structure (Lake, 2003). Even though the non-flow period finally depressed autotrophic biomass in both TS and PS equally, the resistance estimates show that TS offered higher resistance to the non-flow period. The higher initial biomass of the PS biofilms, and its physical attributes (e.g. specific surface or total volume), did not provide any advantage during the non-flow period. Also, although the origin-related differences in community composition persisted throughout the experiment, the non-flow period did not cause the two communities to converge.

Diatoms were far more abundant in biofilms from PS, while cyanobacteria dominated in those from TS. Diatoms have been reported to use a full range of drought refuge types (Robson et al., 2008) and are able to re-colonize stones after flow resumption, but also are highly sensitive to desiccation (Ledger et al., 2008; Romaní et al., 2013), since they become easily dehydrated. However, cyanobacteria have structural elements and an associated architecture (Sabater, 2000) that confers them higher resistance to desiccation (Potts, 1999; Romaní & Sabater, 1997; Kawecka, 2003). Apart from the taxonomic differences, TS groups showed traits which contributed to the higher non-flow resistance.

The prevailing encrusting life-form in the TS biofilms conferred a higher resistance to desiccation (Timoner et al., 2012), and probably enable a higher proportion of algal biomass to resist the non-flow period. Therefore, the structural architecture, prevailing life forms, and community composition bestowed the phototrophic biofilm community of TS with higher resistance and a greater ability to recover from the non-flow period. This type of response has been also seen in perennial regulated streams in areas submitted to water level fluctuations (Benenati et al., 1998; Blinn et al., 1998). Autotrophs inhabiting environments prone to desiccation have evolved to provide themselves with protection and damage repair mechanisms (Karsten & Holzinger, 2014), attributes that are not common in the phototrophic communities of PS. Despite the specific higher resistance in bulk chlorophyll described above, both stream biofilms accumulated similar concentrations of protective carotenoids, probably because of the higher variability within TS and small replicates number. These carotenoids protect the photosynthetic apparatus against abiotic disturbances (Sabater et al., 2018; Timoner et al., 2014), as well as against photodamage (Adams et al., 1993; Garcia-Pichel & Castenholz, 1991). These protective carotenoids do not only contribute to chlorophyll protection during the non-flow period, they also facilitate chlorophyll reactivation during flow return (Pietrasiak et al., 2013). Some of these structural differences between TS and PS could justify the lower ability to resist and recover of the latter under longer and severe non-flow periods. However, the way in which the non-flow period promotes the synthesis of these protective pigments in both stream types has not yet been determined.

Despite these structural differences, the cessation of water flow immediately affected the photosynthetic efficiency of both biofilm types. This was the most sensitive variable to desiccation, which suggests that the electron transport flux in the photosynthetic apparatus becomes quickly inhibited in these circumstances (Gray et al., 2007; Karsten & Holzinger, 2014). Photosynthetic activity was, however, quickly resumed after flow return in both types of stream biofilm; moreover, the recovery happened at the same speed. The fact that both types of biofilms showed equal photosynthetic efficiency resilience indicates that the rehydration of algal cells is the essential factor for the recovery of the electron transport flux, rather than the life forms or community composition of the biofilm.

The metabolism variables (GPP and CR) showed similar patterns in both PS and TS biofilms after the onset of the non-flow period. Reduction in GPP occurred in both biofilm

types, but was higher in the PS biofilms. Flow return facilitated the recovery of Gross primary production more immediately, and at a faster rate, in the biofilms from TS. GPP resilience was not significantly different between both types of biofilm, even though GPP was initially higher the TS biofilms. It remains to be seen if an increase of duration and frequency of non-flow period, or the severity of environmental conditions during the non-flow period (temperature and solar radiation; Colls et al. 2019), could change carbon incorporation and even determine the fate of upper trophic levels (Dodds et al., 1996).

Finally, our results confirm that biofilms from PS were less resistant and resilient to the non-flow period at a structural level (in terms of biomass and community composition). However, both types of biofilm showed similar functional (metabolism) responses. Because the duration and severity (Colls et al., 2019) of the applied non-flow period was moderate, longer or harsher non-flow periods could affect PS biofilms more severely, and cause more extensive changes in their functioning. Furthermore, repeated episodes of non-flow, or longer non-flow periods, could act as ramp disturbances (Lake, 2003), producing greater effects that would favour generalist species, which, in turn might react with more intense metabolic responses (Odum, 1985). Overall, our investigations highlight the necessity of extending our understanding of the responses of PS biofilms during non-flow periods. Since biofilms are key drivers of ecosystem metabolism and biogeochemical cycling, the mechanisms they use to respond to non-flow events will not only increase our ability to predict and manage the effects caused by global change, but will also help improve management of ecosystem processes related to biofilm functioning.

Supplementary Material

Tables

PIV. S. Table 1.- Chlorophyll-a (Chl-a) and photosynthetic efficiency (Y_{eff}) and standard deviation (SD) each sampling day (B: just before flow interruption, NF: under non-flow conditions and F: under flow conditions).

Biofilm origin	Flow conditions	Days of experiment	Chl-*a* ± SD (μg Chl-*a* · cm^{-2})	Y_{eff} ± SD
Permanent	B	0	9.12 ± 10.20	0.37 ± 0.04
	NF	3	7.30 ± 5.71	0.00 ± 0.00
	NF	9	10.94 ± 11.61	0.00 ± 0.00
	NF	20	8.29 ± 8.75	0.01 ± 0.00
	NF	31	2.77 ± 2.16	0.01 ± 0.01
	F	33	3.97 ± 2.60	0.09 ± 0.05
	F	36	3.39 ± 3.56	0.30 ± 0.06
	F	40	4.13 ± 2.75	0.53 ± 0.04
	F	51	6.56 ± 1.11	0.45 ± 0.04
Temporary	B	0	3.31 ± 3.86	0.29 ± 0.05
	NF	3	4.35 ± 1.76	0.03 ± 0.05
	NF	9	4.00 ± 0.53	0.00 ± 0.00
	NF	20	2.79 ± 0.69	0.01 ± 0.00
	NF	31	2.74 ± 1.19	0.01 ± 0.01
	F	33	2.01 ± 1.01	0.22 ± 0.11
	F	36	1.45 ± 1.52	0.36 ± 0.08
	F	40	4.68 ± 4.25	0.48 ± 0.03
	F	51	5.68 ± 1.91	0.43 ± 0.03

PIV. S. Table 2.- Pigment concentration (µg pigment/cm^2) in biofilm from permanent (PS) and temporary streams (TS).

Biofilm origin	Pigment	Concentration ± SD
Permanent	**Scytonemin**	0.033 ± 0.030
	Lutein	0.029 ± 0.035
	Zeaxanthin	0.006 ± 0.007
	Canthaxanthin	0.035 ± 0.029
	Echinenone	0.056 ± 0.041
	β-carotene	0.008 ± 0.005
Temporary	**Scytonemin**	0.126 ± 0.198
	Lutein	0.060 ± 0.051
	Zeaxanthin	0.034 ± 0.025
	Canthaxanthin	0.148 ± 0.157
	Echinenone	0.038 ± 0.046
	β-carotene	0.034 ± 0.065

PIV. S. Table 3.- Gross Primary Production (GPP; $mgO_2\,m^{-2}\,min^{-1}$) and community respiration (CR; $mgO_2\,m^{-2}\,min^{-1}$) of the biofilms from the four permanent (PS) and the four temporary (TS) streams each sampling day.

Biofilm origin	Days of experiment	CR ± SD ($mgO_2\,m^{-2}\,min^{-1}$)	GPP ± SD ($mgO_2\,m^{-2}\,min^{-1}$)
Permanent	0	-0.27 ± 0.22	1.65 ± 1.25
	33	-0.12 ± 0.12	0.25 ± 0.09
	36	-0.34 ± 0.14	0.52 ± 0.38
	40	-0.41 ± 0.18	2.57 ± 0.51
	51	-0.17 ± 0.06	3.86 ± 0.48
Temporary	0	-0.67 ± 0.18	3.79 ± 1.83
	33	-0.40 ± 0.28	0.06 ± 0.12
	36	-0.40 ± 0.30	0.35 ± 0.39
	40	-0.45 ± 0.06	2.83 ± 1.04
	51	-0.21 ± 0.10	4.04 ± 0.25

PIV. S. Table 4.- Proportion of algae and cyanobacteria genera of the biofilms from the four permanent (PS1, PS2, PS3 and PS4) and the fours temporary (TS1, TS2, TS3 and TS4) streams each sampling day (Day 0: just before non-flow; Day 31: the last non-flow day; Day 51: the last recovery day). Bacillariophyta (Bacill), Charophyta (Char), Chlorophyta (Chlo), Cyanobacteria (Cyan), Euglenophyta (Eugl), Rhodophyta (Rhod). Prostrate (P), Free living (F), Mucilage tubes (Mt), Mucilage pad (Mp), Stalked (S), Unbranched filamentous (Uf), Branched filamentous (Bf), Gelatinous (G), Crustose (C).

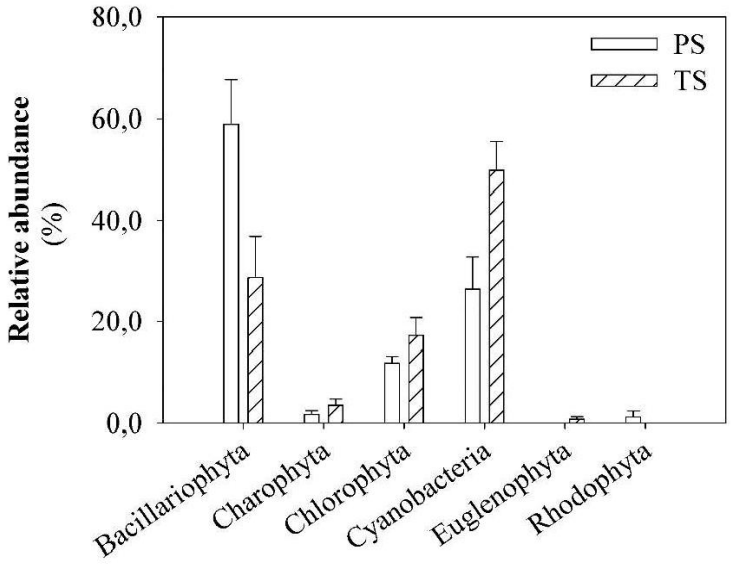
Div.	Genera	Life Form	Day 0								Day 31								Day 51							
			PS1	PS2	PS3	PS4	TS1	TS2	TS3	TS4	PS1	PS2	PS3	PS4	TS1	TS2	TS3	TS4	PS1	PS2	PS3	PS4	TS1	TS2	TS3	TS4
Bacill	Achnanthes	P	7.41	5.42	9.28	9.52	3.74	4.35	2.15	3.23	8.33	5.31	13.79	10.34	0.00	0.00	0.00	1.32	17.02	15.89	14.53	14.78	12.37	18.68	8.57	10.62
Bacill	Amphipleura	P	0.00	2.53	0.00	0.68	0.00	0.00	0.00	4.52	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.67	0.00	0.00	0.00	0.00	0.00	4.42
Bacill	Amphora	P	2.47	0.72	1.03	0.00	0.00	0.00	0.00	1.29	5.56	0.00	17.24	1.15	0.00	0.00	0.00	0.00	13.83	4.67	12.82	2.61	0.00	1.10	0.00	3.54
Bacill	Cyclotella	F	0.00	1.81	3.09	1.36	1.87	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.06	1.87	0.00	0.00	1.03	1.10	0.00	0.88
Bacill	Cocconeis	P	6.17	1.44	10.31	6.80	0.00	0.00	0.00	1.94	8.33	0.88	3.45	4.60	0.00	0.00	0.00	0.00	7.45	4.67	2.56	7.83	0.00	0.00	0.00	2.65
Bacill	Cymatopleura	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.80	0.00	0.00	0.00	0.00	0.00	0.00
Bacill	Cymbella	P	6.17	5.42	11.34	7.48	4.67	0.00	1.08	3.87	2.78	4.42	3.45	6.90	0.00	0.00	0.00	2.63	2.13	12.15	4.27	6.96	1.03	4.40	0.00	5.31
Bacill	Denticula	F	0.00	3.61	0.00	7.48	1.87	0.00	0.00	2.58	0.00	0.88	0.00	6.90	1.23	0.00	0.00	1.32	0.00	5.61	5.13	9.57	3.09	2.20	0.00	2.65
Bacill	Diatoma	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.80	0.00	0.00	0.00	0.00	0.00	0.88
Bacill	Diploneis	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	3.45	0.00	0.00	0.00	0.00	0.00	0.00	2.56	0.00	0.00	1.10	0.00	0.88
Bacill	Ellerbeckia	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.56	0.00	0.00	0.00	0.00	0.00
Bacill	Encyonema	Mt	7.41	1.44	0.00	0.00	0.00	0.00	1.08	2.58	8.33	1.77	27.59	4.60	0.00	0.00	0.00	1.32	3.19	6.54	0.85	0.00	0.00	3.30	0.00	2.65
Bacill	Epithemia	P	0.00	1.44	0.00	0.68	0.00	0.00	0.00	0.00	0.00	1.77	0.00	2.30	0.00	0.00	0.00	0.00	0.00	0.93	0.00	1.74	2.06	0.00	0.00	0.00
Bacill	Eunotia	P	1.23	1.44	0.00	0.00	0.93	0.00	0.00	0.65	0.00	0.88	0.00	2.30	0.00	0.00	0.00	0.00	0.00	5.61	0.85	0.87	1.03	1.10	0.00	0.88
Bacill	Fragilaria	Mp	6.17	1.81	1.03	2.04	0.00	0.00	1.08	2.58	5.56	0.00	3.45	0.00	0.00	0.00	0.00	0.00	0.00	6.54	0.00	0.00	0.00	0.00	0.00	0.00
Bacill	Frustulia	Mt	2.47	0.00	0.00	0.00	0.00	0.00	0.00	1.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.80	4.27	0.00	0.00	0.00	0.00	1.77
Bacill	Gomphonema	S	7.41	4.33	13.40	10.20	3.74	2.17	1.08	2.58	8.33	6.19	27.59	14.94	0.00	0.00	0.00	3.95	5.32	9.35	11.11	14.78	3.09	4.40	0.00	5.31
Bacill	Gyrosigma	F	2.47	0.00	0.00	0.00	0.00	0.00	0.00	1.29	5.56	0.00	3.45	1.15	0.00	0.00	0.00	0.00	1.06	0.93	4.27	1.74	0.00	0.00	0.00	0.00
Bacill	Halamphora	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88
Bacill	Hantzschia	F	0.00	0.00	0.00	0.00	0.00	0.00	2.15	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.00	0.00	0.00	0.00	2.86	0.88
Bacill	Luticola	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.88

Bacill	Melosira	Mp	3.70	0.00	0.00	0.00	0.00	0.00	1.08	0.00	2.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.26	0.00	0.85	0.00	0.00	0.00	0.00	0.00
Bacill	Meridion	Mp	0.00	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.85	0.00	0.00	0.00	0.00	0.00
Bacill	Navicula	P	9.88	3.97	8.25	4.76	2.80	0.00	1.08	3.87	8.33	3.54	13.79	4.60	0.00	0.00	0.00	2.63	7.45	7.48	5.98	2.61	1.03	2.20	0.00	6.19
Bacill	Neidium	F	0.00	1.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	1.06	4.67	1.71	0.00	0.00	0.00	0.00	0.00
Bacill	Nitzschia	Mp	7.41	3.97	4.12	1.36	1.87	4.35	1.08	4.52	5.56	2.65	13.79	0.00	0.00	0.00	0.00	3.95	8.51	6.54	2.56	2.61	2.06	1.10	6.67	7.96
Bacill	Planothidium	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacill	Pinnularia	F	3.70	2.53	0.00	0.00	0.93	0.00	0.00	2.58	0.00	0.88	6.90	1.15	0.00	0.00	0.00	1.32	2.13	4.67	3.42	0.00	0.00	1.10	0.00	1.77
Bacill	Reimeria	P	0.00	0.00	0.00	0.00	0.00	0.00	1.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.56	0.00	0.00	0.00	0.00	0.00
Bacill	Rhoicosphenia	S	3.70	0.00	8.25	0.68	0.00	0.00	0.00	0.00	0.00	0.88	3.45	3.45	0.00	0.00	0.00	0.00	3.19	0.00	4.27	0.00	0.00	0.00	0.00	0.00
Bacill	Rhopalodia	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.94	0.00	0.88	0.00	0.00	0.00	0.00	0.00	3.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.31
Bacill	Sellaphora	F	2.47	1.08	0.00	0.00	0.00	0.00	0.00	1.29	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	1.06	0.00	1.71	0.00	0.00	0.00	0.00	2.65
Bacill	Stauroneis	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.06	0.00	0.85	0.00	0.00	0.00	0.00	0.00
Bacill	Staurosirella	S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.03	0.00	0.00	0.00
Bacill	Surirella	F	4.94	1.08	0.00	0.00	0.00	0.00	1.08	0.00	5.56	1.77	3.45	8.05	0.00	0.00	0.00	1.32	2.13	2.80	0.85	0.87	0.00	0.00	0.00	0.00
Bacill	Ulnaria	Mp	2.47	3.97	0.00	0.00	0.00	0.00	0.00	1.29	0.00	0.88	0.00	1.15	0.00	0.00	0.00	1.32	1.06	5.61	0.85	1.74	0.00	0.00	0.00	4.42
Char	Ulothrix cf.	Uf	0.00	0.00	0.00	0.00	0.00	6.52	5.38	0.65	0.00	0.00	0.00	1.15	1.23	2.13	3.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Char	Closterium	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Char	Cosmarium	P	0.00	2.17	0.00	0.68	0.93	0.00	0.00	0.65	0.00	1.77	0.00	1.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Char	Spirogyra	Uf	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Char	Zygnema	Uf	0.00	0.72	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlo	Ankistrodesmus	F	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlo	Bulbochaete	Bf	0.00	1.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.00	1.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlo	Und. Chlorococcal sp1.	F	0.00	1.44	4.12	2.04	5.61	13.04	6.45	0.00	0.00	3.54	0.00	1.15	8.64	17.02	1.59	1.32	0.00	0.00	2.56	5.22	10.31	7.69	2.86	0.88
Chlo	Und. Chlorococcal sp2.	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.56	2.65	0.00	6.90	0.00	4.26	0.00	0.00	2.13	3.74	0.00	0.00	0.00	0.00	0.95	0.00
Chlo	Chlorococum	F	1.23	0.00	0.00	0.00	0.00	0.00	4.30	1.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00

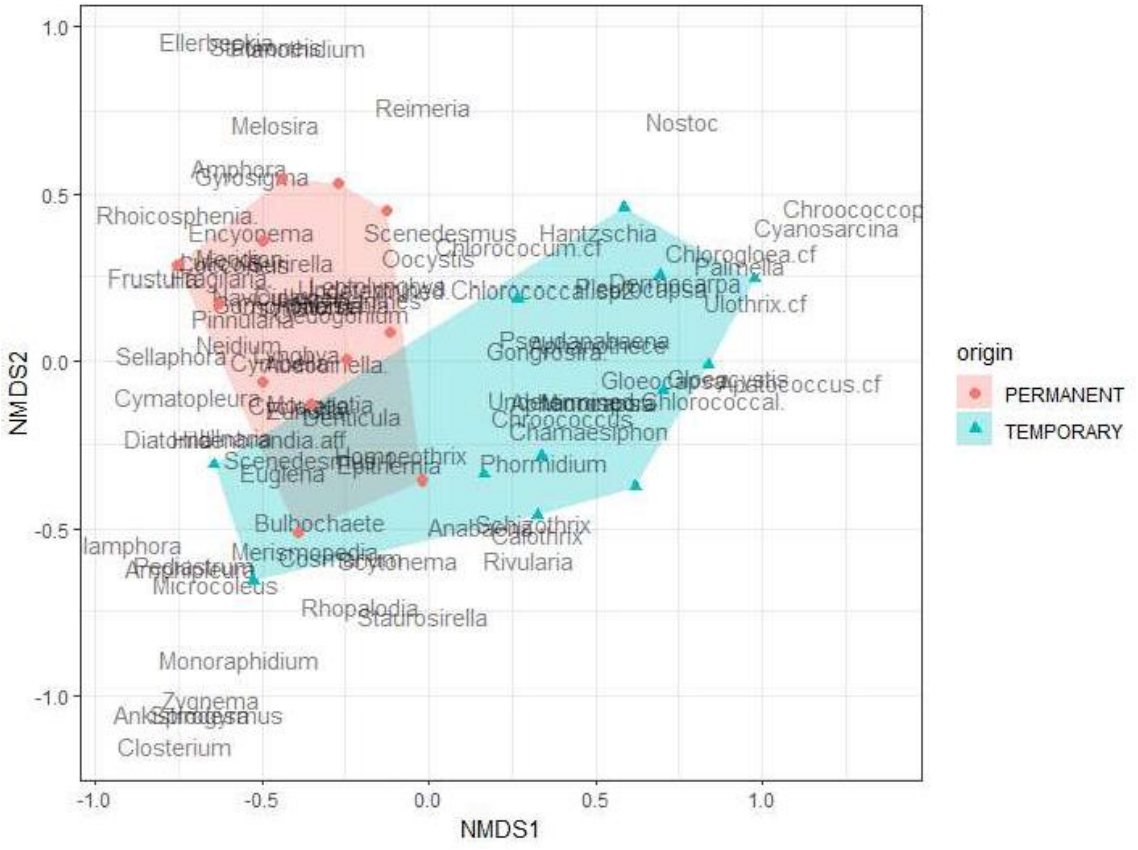
	cf.																								
Chlo	Scenedesmus	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.78	1.77	0.00	2.30	0.00	0.00	0.00	1.32	7.45	1.87	11.11	5.22	4.12	7.69	8.57	3.54
Chlo	Gloeocystis	G	0.00	0.72	0.00	0.00	0.00	2.17	4.30	1.29	0.00	2.65	0.00	0.00	0.00	6.38	1.59	0.00	0.00	0.00	0.00	0.00	1.03	0.00	0.00	0.00
Chlo	Gongrosira	C	3.70	0.00	6.19	6.80	10.28	0.00	3.23	0.00	11.11	7.96	0.00	12.64	8.64	14.89	14.29	5.26	4.26	1.87	0.85	15.65	7.22	4.40	7.62	0.00
Chlo	Microspora	Uf	0.00	2.17	0.00	0.00	0.00	2.17	0.00	0.65	0.00	4.42	0.00	2.30	0.00	0.00	4.76	0.00	0.00	1.87	0.00	0.00	0.00	0.00	0.00	0.00
Chlo	Monoraphidium	F	0.00	0.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlo	Oedogonium	Uf	0.00	1.08	0.00	2.72	1.87	0.00	0.00	0.00	5.56	2.65	3.45	4.60	1.23	0.00	0.00	0.00	1.06	0.93	3.42	2.61	0.00	0.00	0.00	0.88
Chlo	Oocystis	F	0.00	1.08	0.00	0.00	0.00	0.00	3.23	0.65	5.56	2.65	0.00	1.15	0.00	0.00	0.00	0.00	17.02	13.08	14.53	11.30	11.34	18.68	11.43	9.73
Chlo	Palmella	G	0.00	0.00	0.00	0.00	0.00	0.00	4.30	0.00	0.00	0.00	0.00	0.00	0.00	2.13	4.76	2.63	0.00	0.00	0.00	0.00	0.00	3.30	4.76	0.00
Chlo	Pediastrum	F	0.00	0.72	0.00	0.00	0.00	0.00	0.00	1.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.87	0.00	0.00	0.00	0.00	0.00	0.00
Chlo	Scenedesmus	F	2.47	2.89	0.00	0.00	1.87	0.00	1.08	1.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlo	Mougeotia	Uf	0.00	1.44	0.00	0.00	0.00	0.00	0.00	1.94	2.78	1.77	0.00	2.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyan	Apatococcus cf.	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.94	8.51	3.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.90	0.00
Cyan	Anabaena	Uf	0.00	0.00	0.00	0.00	1.87	0.00	1.08	1.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyan	Aphanocapsa	G	0.00	3.61	2.06	2.72	10.28	4.35	6.45	1.94	2.78	2.65	3.45	2.30	12.35	4.26	6.35	5.26	2.13	2.80	1.71	1.74	8.25	7.69	6.67	1.77
Cyan	Aphanothece	G	0.00	2.53	0.00	0.00	0.00	2.17	4.30	0.65	0.00	3.54	3.45	0.00	2.47	4.26	3.17	1.32	1.06	1.87	2.56	0.00	1.03	5.49	0.95	0.00
Cyan	Calothrix	Uf	0.00	0.72	0.00	0.68	1.87	0.00	0.00	1.94	0.00	2.65	0.00	0.00	2.47	2.13	0.00	7.89	0.00	0.93	0.00	0.00	1.03	3.30	0.00	2.65
Cyan	Chroococcus	G	1.23	2.17	2.06	2.04	6.54	10.87	3.23	1.29	5.56	8.85	0.00	3.45	7.41	4.26	1.59	3.95	2.13	2.80	0.00	3.48	4.12	3.30	1.90	0.88
Cyan	Chamaesiphon	Bf	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.70	0.00	0.00	0.00	1.06	0.00	0.00	1.74	5.15	4.40	0.00	0.00
Cyan	Chlorogloea cf.	C	0.00	0.00	0.00	0.00	0.00	0.00	6.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.17	1.32	0.00	0.00	0.00	0.00	2.06	0.00	4.76	0.00
Cyan	Chroococcopsis cf.	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95	0.00
Cyan	Cyanosarcina	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.52	0.00	0.00	0.00	0.00	0.00	1.03	1.10	1.90	0.00
Cyan	Dermocarpa	C	0.00	0.00	0.00	2.72	0.93	0.00	6.45	0.00	2.78	0.00	0.00	0.00	0.00	0.00	12.70	3.95	0.00	0.00	0.85	0.87	2.06	6.59	3.81	0.00
Cyan	Gloeocapsa	G	0.00	2.17	0.00	0.00	0.00	0.00	6.45	1.29	2.78	3.54	3.45	4.60	9.88	17.02	6.35	3.95	0.00	0.93	0.00	3.48	3.09	2.20	3.81	0.00
Cyan	Homoeothrix	Uf	0.00	2.17	5.15	6.80	9.35	4.35	0.00	2.58	0.00	2.65	0.00	1.15	1.23	0.00	0.00	3.95	0.00	5.61	2.56	5.22	0.00	0.00	0.00	1.77

Cyan	Leptolyngbya	Uf	0.00	1.44	0.00	0.00	0.00	0.00	0.00	3.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.32	2.13	1.87	9.40	2.61	0.00	4.40	5.71	7.08
Cyan	Lyngbya	Uf	0.00	2.53	6.19	4.76	0.00	0.00	1.08	3.87	0.00	0.00	3.45	6.90	0.00	2.13	0.00	0.00	1.06	0.00	0.00	2.61	0.00	0.00	0.00	0.00
Cyan	Merismopedia	G	0.00	1.81	0.00	0.68	0.00	0.00	0.00	0.65	0.00	1.77	0.00	1.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyan	Microcoleus	Uf	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.94	0.00	2.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.31
Cyan	Nostoc	G	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.10	4.76	0.00
Cyan	Oscillatoria	Uf	2.47	0.36	4.12	1.36	0.00	2.17	1.08	1.29	0.00	0.88	3.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyan	Phormidium	Uf	0.00	3.25	0.00	0.00	1.87	13.04	3.23	3.87	0.00	4.42	6.90	3.45	6.17	2.13	0.00	11.84	2.13	0.93	0.85	0.00	0.00	0.00	0.95	7.08
Cyan	Pleurocapsa	C	0.00	1.81	0.00	0.00	7.48	13.04	7.53	0.65	16.67	1.77	0.00	1.15	2.47	6.38	14.29	0.00	8.51	0.93	0.00	1.74	0.00	1.10	5.71	0.00
Cyan	Pseudanabaena	Uf	1.23	3.25	0.00	0.00	0.00	15.22	6.45	2.58	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	4.26	0.00	1.71	0.00	1.03	3.30	5.71	6.19
Cyan	Rivularia	Uf	0.00	1.08	0.00	6.80	9.35	0.00	0.00	4.52	0.00	0.88	0.00	0.00	13.58	0.00	0.00	10.53	0.00	1.87	0.00	1.74	14.43	0.00	0.00	5.31
Cyan	Schizothrix	Uf	0.00	3.61	0.00	0.00	7.48	0.00	0.00	0.65	0.00	5.31	0.00	4.60	13.58	0.00	0.00	13.16	0.00	8.41	0.85	5.22	15.46	5.49	0.00	2.65
Cyan	Scytonema	Uf	0.00	1.08	0.00	0.68	0.00	0.00	0.00	3.23	0.00	4.42	0.00	0.00	0.00	2.13	0.00	2.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.19
Eugl	Euglena	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.94	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.85	0.87	0.00	0.00	0.00	0.00
Rhod	Audouinella	Bf	0.00	0.00	0.00	2.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.35	0.00	0.00	0.00	0.00
Rhod	Hildenbrandia	C	0.00	0.00	0.00	2.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

PIV. S. Figure 1.- Relative abundance (%) of the main phyla observed before the non-flow period (after the acclimatization period in each artificial stream) of the biofilms from the four permanent (P) and the four temporary (T) streams, with error bars showing the standard error.



PIV. S. Figure 2.- Non-metric multidimensional scaling (NMDS) with the proportion of genus of the biofilms from the four permanent (PS) and the four temporary streams (TS). Red circles and blue triangles indicate the origin of the biofilm samples (from permanent and temporary streams respectively) analysed each sampling day (just before non-flow, the last non-flow day, and the last recovery day), respectively. The grey name tags indicate genus, and the polygons indicate the two groups in which genera were organized.



GENERAL DISCUSSION

5) General Discussion

In this thesis, I analysed the effects of the temporal components (i.e. duration and frequency) and severity of the non-flow period on temporary stream biofilms and the resistance and resilience of both permanent and temporary stream biofilms to the non-flow period, in the Mediterranean framework. Each of the presented papers examines different aspects of biofilm communities, encompassing their structure and functioning regarding their hydrological history and current flow status (i.e. flow or dry conditions) as key determinants of the biofilm response. Approaching this goal has required answering several challenges, which I detail below.

a) Mixing Statistical Approaches for a Holistic Understanding of Fluvial Ecosystems Dynamics

Ecosystem dynamics are the outcome of the ecological processes' integration at different scales, from community structure to physiology and functioning. Nowadays, recent improvements in data modelling provide us a deeper understanding of a wide range of ecological issues, including ecosystem functioning and ecological thresholds detection. The specific objective of this section to explain the reasoning beneath models' selections used in this thesis.

A variety of statistical methods can be used to understand natural ecosystems dynamics. The selection of the statistical methods partly depends on the dataset and the study objectives. Given that, in this thesis I used multiple linear and nonlinear regression models in order to encompass different scales, from organisms to the community. Nonlinear methods were used to understand physiological and functional changes within photoautotrophic community due to temporal components of the non-flow period. This small-scale approach allowed me to detect abrupt changes in the specific dynamic of a given biological variables (i.e. functional pigment groups and gross primary production). Contrastingly, multiple linear models (i.e. SEM) were used to get a broader view, understanding de causal relationships between community structure, physiology and functioning, mediated by non-flow period duration. For a holistic understanding of fluvial ecosystem dynamics, we thus need to use different statistical approaches, since each one allows us to understand the biological responses at different scales, improving our knowledges about temporary stream ecosystems' dynamics.

b) Using of Stream Biofilms under Dry Conditions

Traditionally, biological indicators were based on macroinvertebrates (Sarremejane et al., 2019), fish (Pont et al., 2007), macroalgae (Bermejo et al., 2012), or diatoms (Falasco et al., 2020). These organisms provide information about ecosystem structure, such as biodiversity, species distribution and abundance. In contrast, functional measurements, such as decomposition rate (Menéndez et al., 2019) and CO_2 emissions (Gómez-Gener et al., 2016), provide information about the processes that regulate carbon and nutrient cycles due to the joint activity of stream organisms (von Schiller et al., 2017). The combination of both structural and functional measurements at different scales thus provides an important basis of knowledge that could be used to improve our understanding and management of fluvial ecosystems, which could be achieved through biofilm (Burns & Ryder, 2001).

Streambed biofilms are species-rich and support a biodiverse range of microorganisms. Biofilms are attached to multiple surfaces of streambeds, provide a major energy source for fluvial food-webs and orchestrate several biogeochemical processes (Sabater et al., 2016; von Schiller et al., 2017). Short life cycles of microorganisms inhabiting biofilms allow them to respond rapidly to environmental changes, being the first to respond to disturbances (Burns & Ryder, 2001). All these attributes make biofilms a suitable monitoring tool to assess responses to environmental changes in fluvial ecosystems (Burns & Ryder, 2001), including flow intermittency. The results of this thesis support the used of biofilms as biological indicators, demonstrating that both the structure and functioning of stream biofilms reflect temporal variability of the non-flow period on fluvial ecosystems and generates important differences between permanent and temporary streams, supporting my main hypothesis. The prior hydrological conditions to which temporary stream biofilms were exposed had long-term effects on their structure (Paper III), physiology (Paper II) and functioning (Paper I), persisting despite flow resumption (Paper II). Both changes in community biodiversity and physiology, promoted by temporal components of the non-flow period, determined biofilm functioning (Paper III). Furthermore, the different hydrological history of permanent and temporary streams determined their resistance and resilience to the non-flow period (Paper IV). The main findings of the thesis, focusing on the results from the four papers

and the specific hypotheses of this thesis, their implications and other relevant aspects are discussed below.

c) Temporal Components of the Non-Flow Period and Biofilm Responses

Temporary streams are defined as those that cease to flow at spaciotemporal scale through their course (Acuña et al., 2014). This definition indirectly recognizes the importance of time range under consideration, since temporary streams cannot be classified through the hydrological conditions at a particular time. So, when streams are classified as permanent or temporary, their hydrological history is considered. However, no-differences in active chlorophylls (Paper II) nor community richness (Paper III) of permanent and temporary streams were observed, partly as a result of the large non-flow gradient within temporary streams. Nor there was any correlation between the structure (Paper II and III) nor functioning (Paper I) when short periods before sampling (i.e. 30- or 60-d periods) were considered. These results point out the importance of the hydrological history and, therefore, of considering large hydrological datasets to understand the community responses in temporary streams. Flow intermittency act as a driver of temporary fluvial ecosystems (Bonada & Resh, 2013; Datry et al., 2017; Lytle & Poff, 2004; Soria et al., 2017; Tornés & Ruhí, 2013), where current stream community is the result of their spatiotemporal variability.

Flow intermittency can be characterized by its spatial and temporal components. At temporal scales, non-flow periods can be characterized by their frequency and duration (total or mean duration) (Lake, 2003). Beyond being useful to classify streams (e.g. as permanent, intermittent or ephemeral), the relationship between measured biological variables (e.g. photosynthetic efficiency or active chlorophylls) and one or other non-flow metric provides information about the capacity of the biota to withstand dry conditions or their response velocity once flow returns. Certainly, if we are to progress and usefully compare the effects of temporal components of the non-flow period on biofilm responses, and consequently on fluvial ecosystems, we need to better understand these relationships. A negative relationship between a measured biological variable and the frequency of the non-flow period would mean a high sensitivity to non-flow periods itself, since by considering frequency we overlook the exposition time to dry conditions or flow absence. In other words, a decline in the measured biological variable, independently of the period

length, will reflect a very low capacity to withstand flow intermittency. Accordingly, some organisms, such as the vast majority of fishes, not posses' mechanisms to resist dry conditions and simply are not present in temporary streams (Kerezsy et al., 2017). In contrast, a negative relationship with the duration of the non-flow period (total or mean duration) would mean a certain capacity to withstand that period, since the response differs through time. In this thesis, biofilm structure (Paper III), physiology (Paper II) and functioning (Paper I) were strongly affected by the duration of the non-flow period, whereas frequency was not correlated with any analysed variable. These results highlight the relevance of non-flow duration as ecosystem driver in temporary streams, due to a certain biofilm capacity to withstand dry conditions (Sabater et al., 2017; Timoner et al., 2012). However, these results should be interpreted carefully due to certain limitations arising from the field studies. In that way, the high importance of duration over frequency of the non-flow period, and the low frequency variability within temporary streams (of 22 temporary streams, 10 experienced only one non-flow period) could have masked the physiological effects of increasing frequency. To encompass the entire potential effects of the frequency of the non-flow period on streambed organisms, laboratory experiments could be used in order to expose organisms to short non-flow event evenly distributed through the replicates or artificial streams.

In temporary streams, the duration of non-flow periods can be characterized as the total or the mean duration of the non-flow period. The total duration of the non-flow period is the total number of dry days over a specific time range, whereas the mean duration of the non-flow period describes the mean number of consecutive dry days. The correlation between a measured biological variable and one or the other non-flow duration metric provides detailed information about their resistance and resilience. A relationship with the total duration of the non-flow period reflects a cumulative effect on the biotic response, irrespective of whether dry days or flow lack were continuous or distributed between different non-flow events. In contrast, a relationship with the mean duration of the non-flow period could reflects that a certain number of consecutive dry days are needed to shape the biotic response or a rapid response capability when flow returns. Accordingly, the results of this thesis show that GPP (Paper I) was more sensitive than active chlorophylls (Paper II) or community diversity (Paper III) to dry conditions, due to their relationship with the total and mean duration of the non-flow period, respectively. Similar results have previously been recorded in field and laboratory conditions. Timoner

et al. (2012) observed that the photosynthetic efficiency abruptly decreased to zero at the beginning of the non-flow period, whereas residual values of chlorophyll persisted after 112 dry days. Similarly, Acuña et al. (2015) reported an exponential disturbance-response relationship between the duration of the non-flow period and GPP, but a sigmoidal disturbance-response relationship between the duration of the non-flow period and chlorophyll-a concentration. One possible explanation for the different response of the photoautotrophic physiology and functioning is that under dry conditions photoautotrophic organisms invest more energy in resistant strategies to protect their cells, such as the synthesis of secondary carotenoids (Paper II) or resistance structures, more than in maintaining their functioning, allowing their faster recovery (Acuña et al., 2015; Timoner et al., 2012). Violle et al. (2007) defined 'functional trait' as "morphological, biochemical, physiological, structural, phenological, or behavioural characteristics that are expressed in phenotypes of individual organisms and are considered relevant to the response of such organisms to the environment and/or their effects on ecosystem properties". Accordingly, the results of this thesis evidence that biofilm physical structure, and the plasticity of inhabiting microorganisms, perform functionally by determining the response of biofilms communities to flow intermittency. Beyond the observed differences between the physiological (Paper II) and functional (Paper I) biofilm response to dry conditions, the negative exponential disturbance-response relationships observed in both cases reflected a rapid response of the biofilm to dry conditions. Thus, the greatest changes in both biofilm physiology and functioning occur at short-term (i.e. within the first month or month and a half) and are progressively reduced at long-term. These results suggest an ecological threshold, i.e. a point after which a relatively small change in external conditions causes a rapid and pronounced change in the ecological response. After that, the ecosystem may no-longer be able to return to its state by means of its intrinsic resilience, which leads to rapid change in ecosystem health (Groffman et al., 2006). Specifically, the results suggest a zone-type threshold (Huggett, 2005), with a transition of the biofilm from the aquatic to the terrestrial state, after approximately 20-50 dry days; understanding the aquatic state as the one where organisms survival largely depends on water availability and terrestrial state as the one where organisms are able to obtain their water supply from rainfall, dew, or atmospheric humidity. This assumption is reinforced by dominance of aerophyte and sub-aerophyte genera of cyanobacteria in temporary streams (Paper III), which also are present in biological soil crusts (Belnap & Eldridge, 2001). On the other hand, the higher

abundance of cyanobacteria than of diatoms or green algae in temporary streams (Paper III) highlight the need to analyse the resistance and resilience of biofilm organisms exposed to dry conditions, with special attention on the potential different taxon-specific thresholds. At the reach scale, the non-flow period creates a habitat mosaic with dry sediment and connected or isolated pools. This spatial heterogeneity could also affect the threshold type, resulting, for instance, in a stepped threshold (Boulton, 2003). From the standpoint of ecosystem management and conservation, addressing the effects of habitat diversity on fluvial ecosystem is particularly important to operate within the heterogeneous environments of whole complex ecosystems, catchments or larger scales (Cardinale et al., 2004).

Beyond the duration and frequency of the non-flow period, its magnitude is also an important component. The field work presented here also highlights the importance of the severity of the non-flow period (Paper I and II), which could be understand as a measure of the magnitude of the dry conditions. The term *severity* has been used in this thesis to describe solar radiation and maximum stream temperatures to which biota are exposed under dry conditions. Biofilm responses across sampling sites with an equal duration of the non-flow period differed according to their severity; in streams with higher severity the effects of the dry conditions were more pronounced (Paper I and II; Figure 13). Thus, the severity of the non-flow period could accelerate the shift from the aquatic to the terrestrial-like state. There has been no detailed previous investigation about the severity of the non-flow period. Even so, Timoner et al. (2014) attributed physiological differences between stream biofilms to different light conditions during the non-flow period. Similarly, Zlatanovi et al. (2017) found a negative correlation between light availability in dry conditions and biofilm functioning. Karsten & Holzinger (2014) demonstrated solar radiation as an environmental filter on community composition of the alpine biological soil crusts. Overall, these results indicate that considering the severity of the non-flow period is necessary to characterize and understand its effects. Whereas, at the same time, they emphasize the role of catchment conditions, the valley-floor form (i.e. channel morphology) and riverine vegetation as a protective factor of fluvial ecosystems.

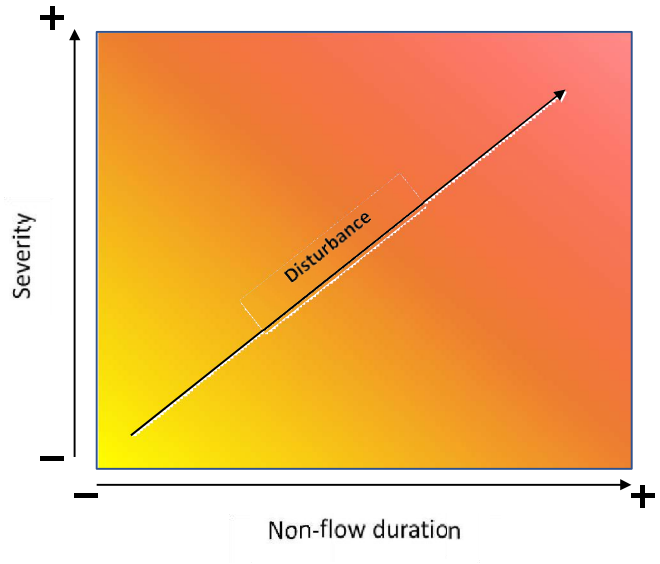


Figure 13.- Diagram representing the joint effect of the duration and severity of the non-flow period on fluvial ecosystems.

These results highlight the effects of the temporal components and severity of the non-flow period on stream biofilms. Longer and more severe non-flow periods produce higher changes on biofilm physiology and functioning, and act as an environmental filter of photoautotrophic community composition, supporting my first and second specific hypothesis. Gross primary production was greatly reduced by dry conditions, which limits autochthonous organic matter production and directly reduces net ecosystem metabolism (Acuña et al., 2015). Obtained results in Paper III helps to unravel the reasons for this reduction. Both physiological status and community diversity driven the reduction of gross primary production. Since chlorophylls are the photosynthetic pigments used to perform photosynthesis, their relationship with the gross primary production was predictable, as previous observed (e.g. Busch & Fisher (1981) and Paper I). However, the positive contribution of α-diversity to gross primary production highlight the key role of community diversity on the maintenance of autochthonous organic matter. Specially in headwater streams, which tend to be temporary (Datry et al., 2017), the role of photoautotrophic production plays a key function fuelling energy to higher trophic levels and driving carbon and nutrient cycles (Sabater et al., 2016). Accordingly, the reduction of photoautotrophic biodiversity, and consequent reduction of gross primary production, can reduce the fluxes of autochthonous organic matter and energy, endangering the dynamic equilibrium of fluvial ecosystems. Biodiversity has an intrinsic value, but to protect and preserve fluvial ecosystems properly, we need to understand which ecosystem

processes are most sensitive to biodiversity changes, as well as which organisms play a key role in maintaining certain processes. This knowledge will allow us to predict how global change may impact on ecological structures and processes.

Rather than previously occurring non-flow periods, short dry periods that interrupted flow resumption, and the low water temperature and low light availability in autumn (i.e. seasonal characteristics), limited photoautotrophic organisms' recovery (Paper II). Datry et al. (2014) and Boulton (2003) observed most marked results on macroinvertebrate assemblage composition when flow ceases than when flow returns, suggesting that colonist sources other than adjacent perennial reaches were important. Accordingly, the short dry periods that interrupted flow resumption may negatively affect the colonisation and slow the community recovery response. Low temperature and low light conditions limited the physiological and structural recovery due to growth dependence of temperature and light availability (Geider, 1987) and the specific requirements of some species (Tornés & Sabater, 2010). According to obtained results, the timing of flow return (i.e. when flow returns occurs) as well as how flow returns (i.e. at once or in stages) are important factors to consider to understand community recovery.

d) Fluvial Ecosystems under Global Change Scenarios

Understanding the adaptation mechanisms of stream biofilms to flow intermittency could play a vital role in predicting the response of stream and closely linked terrestrial food-webs to global change, due to the many processes of fluvial and terrestrial ecosystems depend on biofilm structure and functioning. This analysis needs to consider separately temporary and *new-temporary* streams, since prior hydrologic conditions could influence the structure and physiology of photoautotrophic organisms (Ledger et al., 2008). According to my fourth hypothesis, the hydrological history of temporary stream biofilms had generated a pool of resistant species to dry conditions better than species pool from permanent streams (Paper IV).

Previous studies analysing the effects of flow intermittency on stream biofilms have identified diatoms as the most sensitive algal class to dry conditions (Falasco et al., 2020; Timoner et al., 2014; Tornés & Ruhí, 2013). The results of this thesis support these findings, identifying diatoms as the characteristic organisms of permanent streams (Paper II, III and IV). On the other hand, dry conditions favour cyanobacteria (Robson & Matthews, 2004; Paper III and IV); the photoautotrophic organisms that seem to possess

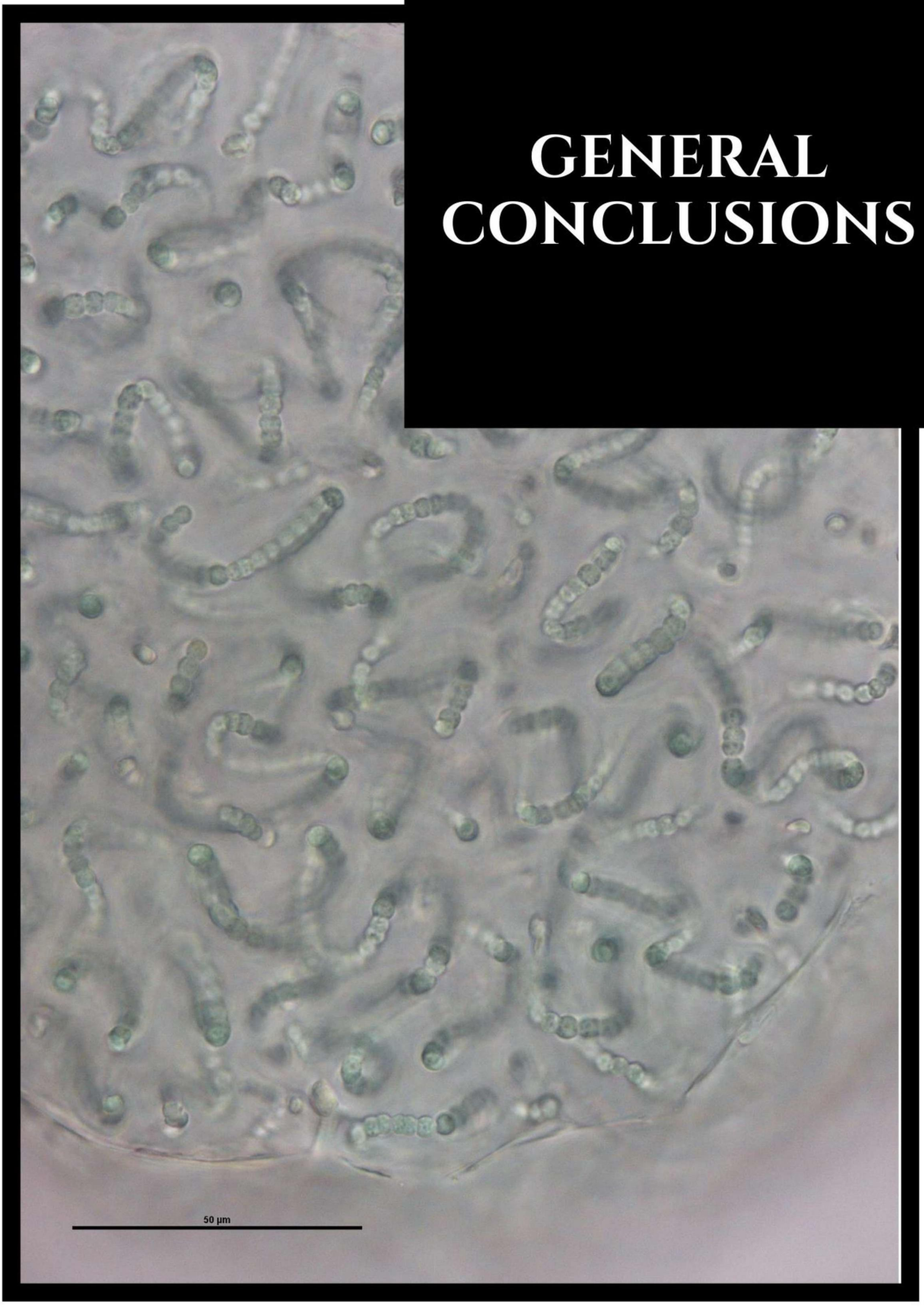
greater adaptative mechanisms to non-flow period duration and severity according to the results obtained in this thesis (Paper II, III and IV). Thus, longer and more severe non-flow periods or their occurrence in currently permanent streams could reduce the autotrophic relevance to the food-webs (Robson et al., 2008), compromising the higher trophic levels of stream ecosystems.

Permanent stream communities showed lower structural resistance and resilience to dry conditions than communities from temporary streams (Paper IV). Photoautotrophic organisms from temporary streams showed greater functional traits to resist dry conditions than those from permanent streams. However, no-differences at functional level were observed (Paper IV). One possible explanation could be the "mild" conditions to which biofilms were exposed in the mesocosms. Papers I and II show a threshold after approximately 20-50 dry days, depending on the severity of the non-flow period. Additionally, Paper III point out the physiology and community diversity as drivers of gross primary production. Accordingly, dry conditions to which biofilms were exposed in mesocosm may had little effects on the communities' physiology. Biofilms from temporary streams have functional traits to resist these conditions, while the higher initial biomass of biofilms from permanent streams, and its physical attributes (e.g. specific surface or total volume) could have provided certain protection to dry conditions. This may also explain the low resistance and resilience of biofilms from permanent streams. Aspin et al. (2018) observed that the non-flow period occurrence and the intensity of dry conditions modify invertebrate communities. Similarly, longer and more sever non-flow periods predicted under most climate change scenarios may modify the biofilm structure, physiology and functioning of new-permanent streams.

Some other factors not considered in the mesocosms experiment need to be highlighted, since they may also play an important role in biofilm recovery. Falasco et al. (2020), Robson et al. (2008) or Van Looy et al. (2019), suggest that the presence of permanent pools is essential for the recovery of stream biofilms in dry sediments. While, Ledger et al. (2008) point out the importance of frequency in the dynamic of photoautotrophic communities. Accordingly, to understand the resilience of temporary and new-temporary streams after dry conditions spatiotemporal heterogeneity needs to be included. So, an important challenge stands in documenting permanent pools importance in a context of significant spatiotemporal heterogeneity. For instance, by means of experimental scaling up from individual homogenous patches to large-scale. The understanding of the

spatiotemporal effects of non-flow conditions on stream biofilms could provide a relevant knowledge to predict and manage the effects caused by global change, as well as help to improve the management of fluvial ecosystems.

GENERAL CONCLUSIONS



6) General Conclusions

1. The non-flow duration drives the physiology, structure, and functioning of temporary stream biofilms. A threshold between 20-50 dry days causes biofilms to transition from an aquatic to a terrestrial state.

2. The severity of the non-flow period requires characterization to understand the effects of non-flow periods, because it exacerbates the effects of dry conditions and accelerates the transition from an aquatic to a terrestrial state.

3. Valley-floor form and riverine vegetation plays a protective role in temporary streams during the non-flow period, moderating temperatures and minimizing the direct effects of solar radiation on biofilms.

4. Biofilm functioning (i.e. gross primary production) has lower resistance to dry conditions than biofilm physiology (i.e. active chlorophylls), as shown by their relationship with the total and mean duration of the non-flow period, respectively.

5. Synthesis of secondary carotenoids plays a crucial role protecting photoautotrophic cells against desiccation, high temperatures and direct effects of solar radiation; these products have a key role on the adaptation of photoautotrophic organisms to dry conditions.

6. Non-flow periods act as an environmental filter, selecting taxa with traits promoting resistance to dry conditions. Diatoms are most sensitive to desiccation, and cyanobacteria are the most resistant.

7. Flow intermittency leads to a loss of α-diversity in photoautotrophic communities. This loss together with active chlorophylls reduction produces a sharp decline in gross primary production, potentially leading to changes in stream and wider food-webs.

8. Seasonal characteristics and short dry periods that interrupted flow resumption limit photoautotrophic recovery. Thus, environmental conditions during flow return are key factors of the biofilm structure and functioning, which probably influence resistance and resilience to supra-seasonal droughts.

9. Hydrological history act as an evolutionary force in temporary streams for species to adapt to flow intermittency. Biofilms from permanent streams were less resistant and resilient to flow interruption than those from temporary streams.

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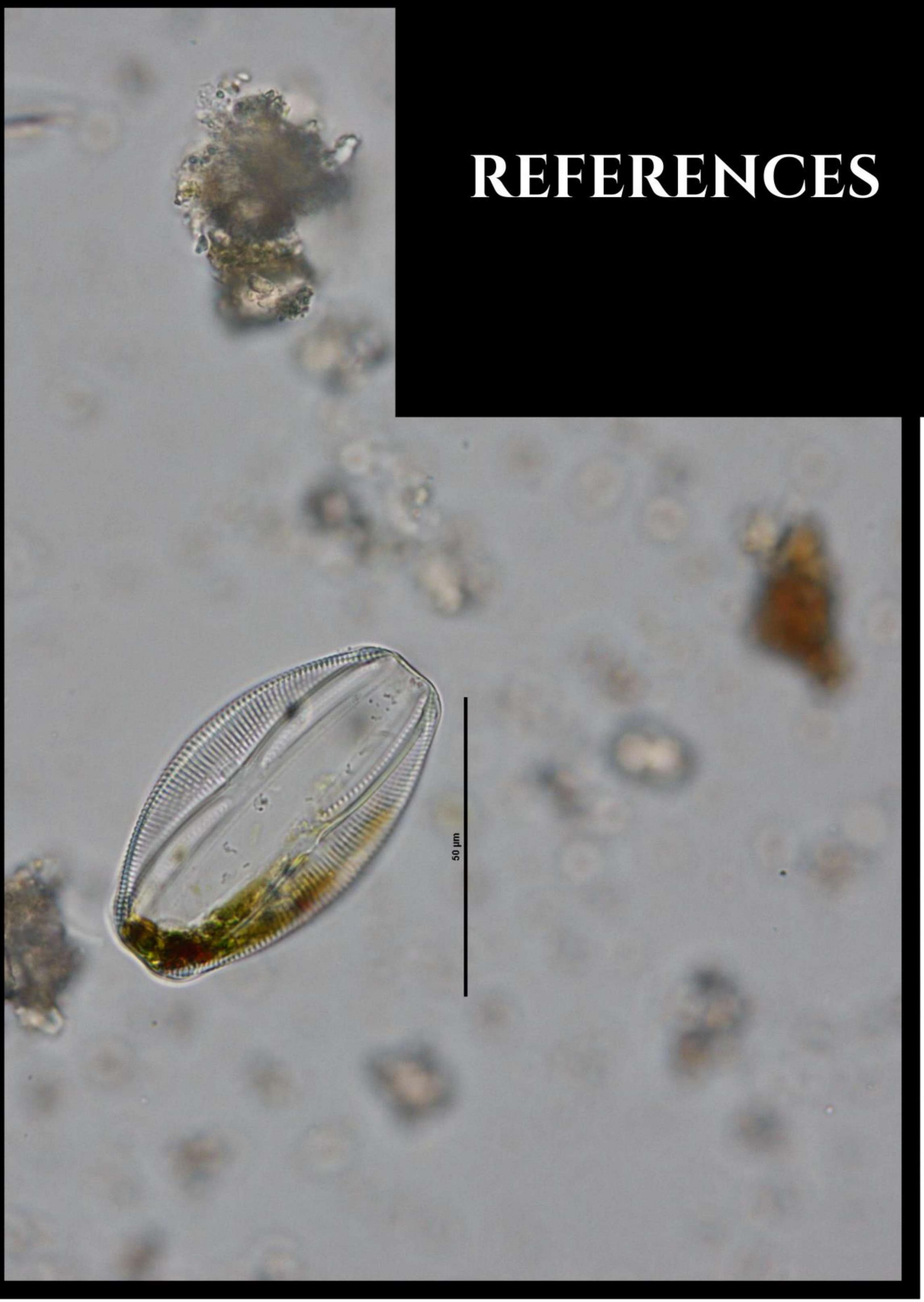

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